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TECHNICAL FOCUS

A diffusion approach to the electrolyte leakage from plant tissues

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The exchange of ions between plant tissues and the external solution in vitro exhibits prominent biphasic kinetics. This is generally ascribed to the different contribution of the two compartments – apoplast and symplast – involved in the process. In this regard, an electro-diffusion model of the leakage is proposed in the paper. On the basis of the balance of fluxes through the plasmalemma and the cell wall, a system of differential equations describing the ion concentration in the outer solution is found. For a wide range of the system's coefficients, its behaviour is well approximated by a previously obtained analytical function. The values of the function's parameters, derived from the fit with experimental data, correlate adequately with the water deficit conditions of the samples. Hence, these parameters may be used to characterize the physiological status of the investigated plants.

Introduction

The efflux of substances from plant tissues submerged in solution is widely used as a measure of the membrane permeability in the investigation of various stress conditions (Garty et al. 2000, Sailerova and Zwiazek 1993, Saltveit and Hepler 2004), factors of growth and development (Celikel and van Doorn 1995) and genotype specificity. In many cases, it is determined by the analysis of the conductivity of the solution in which the tissues are incubated. Thus, distilled water with samples in it gradually increases its conductivity owing to the leakage of ions from the internal cellular space under disturbed membrane permeability.

The conductometric assessment of the ionic content of liquid media offers some clear advantages as non-expensive and easily maintainable apparatus and possibilities for routine analysis in outdoor conditions. Furthermore, as with most of the electrochemical methods, this technique is accurate and sensitive enough and allows high reproducibility of the results. The use of conductometry for these purposes has not lost its currency in spite of the time elapsed from the first reported works (Dexter et al. 1932). This is revealed by the literature in which a permanent tendency for improvement of the method is observed (Premachandra et al. 1992, Vasquez-Tello et al. 1990).

At the same time, some questionable points exist. First, the lack of selectivity regarding the contribution of the different ions can be noticed. Evidently, conductometry itself is not able to ensure such an option. For a lot of studies, however, this obstacle can be overcome with the use of empirical relations between the conductivity of the external solution and the concentration of the basic ions (mostly K^+) flowing from the samples (Palta et al. 1977). Such relations can be obtained by independent measurements of the concentrations with the aid of additional methods (e.g. atomic emission spectroscopy). Another set of issues is connected with the results' interpretation in terms of physicochemical and electrochemical parameters of the cell membrane.

Abbreviations – PEG, polyethylene glycol

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Contribution of mineral nutrition to the response of barley seedlings to polyethylene glycol–induced mild water stress

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Summary

The effect of polyethylene glycol–induced osmotic stress on the activity of nitrate reductase, glutamine synthetase, and glycolate oxidase in leaves of young barley plants grown under two nutrient-supply regimes was studied. The activity of nitrate reductase gradually decreased after polyethylene glycol (PEG) application, while glutamine synthetase and glycolate oxidase were increased. It is speculated that the enhanced glutamine synthetase and glycolate oxidase activities are due to increased flux of metabolites through the photorespiratory cycle. Prominent increase in concentrations of free proline, reducing sugars, and free amino acids was observed. The possible contribution of these cellular solutes to the process of osmotic adjustment and the role of mineral supply is discussed. It is suggested that low N supply in combination with stress conditions switched the preferred osmolyte type from amino acids (N-containing) to sugars (C-containing).

Key words: glycolate oxidase / nitrate assimilation / osmolites / proline /water deficit

1 Introduction

Water and nitrogen (N) availability are among the major factors limiting plant growth and development. Drought may restrain nitrate acquisition by roots as well as restrict the ability of plants to reduce and assimilate nitrogen (*Frechilla* et al., 2000). Nitrate reductase (NR) is the first and rate-limiting enzyme in the nitrate-assimilation pathway (*Lillo* et al., 1996). Its activity was shown to decline in water-stressed leaves in a wide range of plant species (*Bandurska*, 1993; *Flores* et al., 2000; *Correia* et al., 2005). The regulation of this particular enzyme is related to several internal and external factors (*Vincentz* et al., 1993; *Lillo* et al., 1996). Nitrate is the primary signal inducing the transcription of NR genes. At post-translational level, NR activity is enhanced by sugars and repressed by glutamine and closely related end-products of N assimilation (*Li* et al., 1995; *Yaneva* et al., 2000; *Lillo* et al., 2004).

Glutamine synthetase (GS) is an important enzyme in higher plants involved in the re-assimilation of ammonium (NH_4^+) from photorespiration. Glutamine synthetase activity is indirectly related to osmotic adjustment since the synthesis of proline depends on glutamine (*Brugiere* et al., 1999; *Medici* et al., 2003/4). Glycolate oxidase (GO) is a key enzyme in the glycolate pathway of photorespiration that is enhanced in conditions of water deficiency, and this process represents an intersection of N and carbon (C) turnover (*Haupt-Herting* and *Fock*, 2002).

Physiological and biochemical strategies of plants to tolerate dehydration are objectives of interest in recent studies (*Bajji* et al., 2000; *Ghisi* et al., 2002). The appropriate provision with carbohydrates and amino acids in the required amounts must be achieved by efficient communication, regulation, and coordination of N and C metabolism (*Foyer* et al., 1998). The foliar concentrations of major cellular osmolytes such as free amino acids and sugars have been measured in plants grown under different N supply in order to gain insight into their possible action as signaling compounds (*Man* et al., 1999; *Correia* et al., 2005). In the present study, water deficit was imposed to plants grown under two mineral-supply levels to evaluate whether the relationships between N nutrition and the quantities of C- and N-containing compatible solutes were merely correlative or could be varied by causes other than dehydration. The aim was to determine the effects of mineral nutrition on the response of young barley plants to moderate water stress caused by PEG 8000. Polyethylene glycols (PEGs) are a family of neutral, osmotically active polymers often used in plant physiology to induce water deficit by lowering the osmotic potential of the nutrient solution and thus limit water availability for the plant (*Steuter* et al., 1981). The use of these osmotica gives very precise and reproducible results, which is more difficult to accomplish using soil cultures. The degree of stress was assessed by monitoring some aspects of the process of N assimilation, accumulation of solutes, and the development of water deficit.

2 Materials and methods

2.1 Plant material and imposition of stress

Barley (*Hordeum vulgare*, L.) plants from cultivar Odesskii, known for its adaptation to water-stress conditions in the field, were used. After surface sterilization, seeds were germinated for 48 h on wet filter paper in Petri dishes in a thermostat at 25°C in the dark. The seedlings were transferred to either 25% (reduced) or 100% (full strength) Knop nutrient solution for the experiments, the respective N levels being 2 and 8 mM N. Macronutrient composition at full strength was: 3.0 mM

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Changes in Foliar Proline Concentration of Osmotically Stressed Barley

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The amino acid proline is accumulated in plant tissues in response to a variety of stresses. The existence of two routes for its biosynthesis is well documented. However, little is known about the contribution of each pathway to the accumulation of free proline under stress conditions. In the present study young barley plants were subjected to osmotic stress by treating their roots with 25% polyethylene glycol. Prior to stress imposition roots were incubated for 24 h in nutrient solution containing proline or one of its metabolic precursors: glutamate and ornithine. Free proline quantity in the leaves was measured before and after stress. Relative water content (RWC) was used as a measure of the plant water status. Foliar proline levels showed a significant increase in ornithine- and proline-pretreated plants compared to the control. Nevertheless, no considerable changes in leaf RWC were observed. It was shown that before stress application only ornithine but not glutamate was immediately metabolized to proline. Under stress conditions, however, both precursors were converted into proline. The possible role of this amino acid in the processes of post stress recovery is discussed.

Key words: Barley, Osmotic Stress, Proline Precursors

Introduction

It is well known that one of the first symptoms of abiotic stress in plants is the accumulation of free proline in the tissues. Drought and salinity as well as suboptimal temperatures may all lead to the development of osmotic stress (Hsu *et al.*, 2003; Aziz *et al.*, 1998; Simon-Sarkadi *et al.*, 2006). It is assumed that increased proline content may be due to the following: enhanced biosynthesis, slow rate of degradation, and/or increased proteolysis (protein hydrolysis). In plant cells proline is synthesized from glutamate via Δ^1-pyrroline-5-carboxylate (P5C) in two consecutive reactions catalyzed by P5C synthetase and P5C reductase (Delauney and Verma, 1993). Apart from this metabolic route an alternative biosynthetic pathway is present in plants, which leads to proline from ornithine, involving the catalytic activity of the enzyme ornithine aminotransferase (OAT) (Mestichelli *et al.*, 1979). The amino acid arginine may also contribute to proline synthesis via ornithine and involving the enzyme arginase (Boggess and Stewart, 1976). The two main metabolic pathways (from glutamate and from ornithine) are spatially separated. Synthesis of proline from glutamate is located in the cytosol while the ornithine pathway is compartmentalized in mitochondria. Due to the existence of at least two biosynthetic pathways, the control of proline synthesis is rather complicated. Regulation is accomplished at two levels: enzyme activity and gene expression. Evidence exists for feedback regulation of the proline biosynthesis with the end product acting as an inhibitor of the reaction. Under stress conditions, however, this regulation is disturbed and despite the high level of proline its synthesis continues (Zhang *et al.*, 1995; Yoshiba *et al.*, 1997). In the present study we examined the contribution of two proline precursors (ornithine and glutamate) to stress-induced proline accumulation in leaves of young barley plants.

Materials and Methods

Barley seeds (cv. Pamina) were superficially sterilized with sodium hypochlorite (NaOCl) and germinated on wet filter paper in a thermostat (25 °C) in the dark for 24–48 h. Seedlings were grown in a phytostat chamber (12 h photoperiod, 25 °C, 60% relative humidity) on full strength Knop nutrient solution until the stage of the first fully developed leaf was reached. Macronutrient concentration was: 3.0 mM $Ca(NO_3)_2$, 1.5 mM KH_2PO_4, 2.0 mM KNO_3, 0.8 mM $MgSO_4$, 1.3 mM KCl. In the different variants 10-day-old barley

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Physiological Response of Wheat Seedlings to Mild and Severe Osmotic Stress

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In the present study the physiological status of two wheat (*Triticum aestivum* L.) cultivars subjected to polyethylene glycol-induced dehydration is evaluated. Wheat seedlings were exposed to either 8-d-long mild (15% PEG) or 24-h-long severe (30% PEG) osmotic stress by immersing their roots in PEG-supplemented Knop nutrient solution. Relative water content in the leaves and the levels of free proline, malondialdehyde, and hydrogen peroxide were chosen as indicative parameters corresponding to the degree of stress of the treated plants. Electrolyte leakage from leaf tissues of control and stressed plants was compared in terms of the common parameter Injury index used for characterizing cell membrane stability. In addition, a model test system was established for preliminary stress evaluation based on the kinetics of ion leakage. Short term exposure to higher concentration of PEG was considered to be more harmful than prolonged mild stress as judged by RWC, proline and hydrogen peroxide accumulation, and injury index. The two cultivars demonstrated more obvious dissimilarities under conditions of prolonged mild stress than under severe stress.

Keywords: electrolyte leakage, hydrogen peroxide, malondialdehyde, osmotic stress, polyethylene glycol, proline, wheat

Introduction

To maintain productivity while growing in unfavorable conditions is essential to all crop plants. Some physiological traits have been associated with yield potential (Richards et al. 2002; Hoffmann and Burucs 2005) suggesting that selection for such traits could be useful for screening physiologically superior genotypes to improve genetic yield gains. Drought is among the most damaging abiotic factors (Smirnoff 1998). Besides its direct impact on water status osmotic stress often harmfully affects plant cell membranes. Symptoms of these adverse processes include oxidation of unsaturated fatty acids, protein degradation, and the resultant loss of selective permeability of membranes (Smirnoff 1993; Hoekstra and Golovina 1999). To overcome or avoid lethal dehydration of their tissues plants react to low water potentials by increasing the intracellular concentration of 'compatible' solutes in a process known as osmotic adjustment (Serraj and Sinclair 2002; Larher et al.

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Plant Breeding

Molecular cytogenetic identification of a wheat-*Aegilops geniculata* Roth spontaneous chromosome substitution and its effects on the growth and physiological responses of seedlings to osmotic stress

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With 6 figures and 1 table

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Abstract

The karyotypic constitution of a wheat-*Aegilops geniculata* $2M^g$(2A) disomic substitution line, isolated from backcross progenies of a wheat-*Ae. geniculata* amphiploid, was determined using multicolour fluorescence *in situ* hybridization and genomic *in situ* hybridization. The ability of the $2M^g$ chromosome to compensate for wheat chromosome 2A was studied in relation to phenotypic traits and the growth and physiological responses of seedlings to polyethylene glycol (PEG)-induced osmotic stress. Plants of the substitution line had altered spike and seed morphologies, increased tillering, moderately reduced productivity and maintained better seedling growth in stress conditions. PEG treatment caused severe stress, resulting in considerable water loss from the leaves. Under this stress, the substitution line retained better leaf water status and suffered less oxidative damage than the wheat parent, as indicated by higher relative water content and lower malondialdehyde accumulation. These changes were consistent with more sustained membrane stability in the substitution line, evidenced by the lower value of the membrane injury index and the less-prominent increase in ion efflux from the apoplast.

Key words: drought — goat-grass — membrane stability — seedling growth — stress tolerance — *Triticum aestivum*

The *Aegilops* genus, which is closely related to *Triticum*, represents a large reservoir of valuable genes that could be exploited for wheat improvement (Schneider et al. 2008). Within the genus, *Aegilops geniculata* Roth (2n = 4x = 28, $M^gM^gU^gU^g$) has useful agronomic traits, such as good adaptability to extreme climatic conditions, especially to drought (Molnár et al. 2004, Schneider et al. 2008).

An essential step in the transfer of alien chromatin into wheat is the development of chromosome addition and substitution lines (Gale and Miller 1987). Up to now, one complete set of wheat-*Ae. geniculata* addition lines was produced by Friebe et al. (1999), and a few other addition lines were isolated by Landjeva and Ganeva (1999) and Stoilova and Spetsov (2006). Only the $5M^g$(5D) wheat-*Ae. geniculata* substitution line has been reported (Dhaliwal et al. 2002).

Detection and identification of alien chromatin introgressed into the wheat genome can be effectively accomplished using the molecular cytogenetic techniques fluorescence *in situ* hybridization (FISH) and genomic *in situ* hybridization (GISH). Multicolour FISH, using repetitive sequences such as pTa71, pSc119 and pAs1 or pAs1-like (Afa family) (described in Sepsi et al. 2008), allowed individual chromosomes of both wheat and *Aegilops* species to be distinguished (Mukai et al. 1993, Schneider et al. 2003, 2005, Badaeva et al. 2004, Molnár et al. 2011). With the aid of the GISH technique using differentially labelled total genomic DNA, it was possible to discriminate the U- and M-genome chromosomes in the allotetraploid species *Ae. geniculata* and *Ae. biuncialis* and to visualize the *Aegilops* chromatin in wheat-*Aegilops* introgression lines (Schneider et al. 2005, Molnár et al. 2011).

Wheat/alien substitution lines were used to study homoeologous relationships between wheat and related species and the ability of alien chromosomes to compensate for phenotypic and physiological traits (Molnár et al. 2007). The $5M^g$(5D) wheat-*Ae. geniculata* substitution line was used to transfer leaf rust resistance into bread wheat (Dhaliwal et al. 2002). However, no information is available on the ability of any *Ae. geniculata* chromosome to compensate for a wheat chromosome regarding drought tolerance traits.

Water deficit is one of the most widespread abiotic stresses, limiting wheat productivity in drought-prone environments. In continental climates with temporary early-season drought, the capacity of germinating seed to withstand water shortage near the soil surface and to develop adequate roots, coleoptiles and shoots for emergence is critical to early seedling growth (Richards et al. 2000). Much of the injury to plants experiencing drought stress, especially at early developmental stages, is associated with osmotic stress. At the cellular and subcellular levels, osmotic stress triggers various physiological responses, including excessive production of reactive oxygen species (ROS), leading to dysfunction of cell membranes (Smirnoff 1993). Oxidative stress is indicated by the accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) and is manifested as a loss of membrane selective permeability accompanied by ion leakage from the cells (Blum and Ebercon 1981). The ability to maintain cell membrane stability is one of the most characteristic physiological components of stress tolerance (Blum and Ebercon 1981). The degree of cell membrane disturbance is usually expressed as an injury index (Bandurska and Gniazdowska 1995) or is estimated by studying the kinetics of ion efflux (Kocheva et al. 2005).

The treatment of plant roots with solutions of high molecular weight polyethylene glycol (PEG) has been widely used to induce osmotic stress in hydroponic culture systems

Physiological Responses of Two Wheat Cultivars to Soil Drought

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Young plants of the two wheat cultivars Katya and Prelom, differing in their reaction to drought in the field, were grown in soil in pots, and their water status was assessed as well as the intensity of gas exchange, chlorophyll fluorescence, and accumulation of compatible solutes and hydrogen peroxide after 7 days of dehydration. It was established that cv. Katya displayed markedly better tolerance to soil drying in comparison with cv. Prelom. This was partly due to the more effective control of water balance, activity of the photosynthetic apparatus, and metabolic activity of leaves under stress. Consequently, lower amounts of hydrogen peroxide were accumulated and a lower membrane injury index was determined.

Key words: Chlorophyll Fluorescence, Injury Index, Oxidative Stress

Introduction

Plants possess various mechanisms for tolerating, avoiding or resisting water deficit stress (Griffiths and Parry, 2002; Kramer and Boyer, 1995). Maintenance of a better leaf water content under stress correlates with certain metabolic changes in the cytosol leading to osmoregulation in cells (Handa *et al.*, 1983), changes in the plant phytohormone balance (Mittler, 2002), reorganization of the photosynthetic apparatus (Lawlor, 2002; Cornic and Fresneau, 2002), and various morpho-anatomical changes (Pankhurst and Loucks, 1972). The limitation of transpiration under stress is associated with reduced CO_2 absorption from the atmosphere by the leaf and leads to certain disturbances in the activity of the photosynthetic apparatus. Among them are the destructive role of excess light on the activity of photosystems I (PSI) and II (PSII), disturbed regulation of photochemical reactions of photosynthesis, and development of oxidative stress (Lawlor, 2002; Mittler, 2002; Bartosz, 1997). The reason for this is the decreased utilization of NADPH due to reduced CO_2 consumption. Directing the excess of electrons generated by PSII and I to other acceptors such as oxygen may be regarded as a protective mechanism for the photosynthetic apparatus against the excess energy when it is accompanied by scavenging of reactive oxygen species (ROS) (Lawlor, 2002; Cornic and Fresneau, 2002). Another mechanism is the dissipation of this energy in the form of heat from the light-harvesting complexes of PSI and II by means of the xanthophyll cycle (Neubauer and Yamamoto, 1992) and eventually by activation of photoinhibition processes (Cornic, 1994; Biehler and Fock, 1996; Cornic and Fresneau, 2002; Lawlor, 2002). Some of these photoprotective mechanisms may be assessed through changes in the chlorophyll fluorescence parameters. An important element of plants' protection mechanism against water stress is the metabolic turnover and synthesis of osmotically active substances (compatible solutes) that influence the water retention capacity of the cells (Stewart and Larher, 1980). Another mechanism counteracting stress is the reduction of the cell area and changes in the leaf morphology which confine the absorption of excessive light from the leaf. Cereal plants in general have drought tolerance mechanisms which may interrelate all of the above-mentioned mechanisms (Cattivelli *et al.*, 2008). Hence, the determination of differences in the physiological tolerance to water deficit stress

Abbreviations: PS, photosystem; ROS, reactive oxygen species; RWC, relative water content; SLA, specific leaf area.

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VERSITA

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Physiological and anatomical responses of wheat to induced dehydration and rehydration

Research Article

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Abstract: Hydroponically grown wheat seedlings of two prominent Bulgarian cultivars (Katya and Prelom) were subjected to 48 h osmotic stress with PEG 8000 and were then rehydrated. The degree of stress was evaluated by monitoring relative water content, lipid peroxidation level, and accumulation of free proline and hydrogen peroxide in the leaves. Anatomy and ultrastructure of leaf tissue were observed under light microscopy. After imposition of stress, drought tolerant cultivar Katya displayed higher free proline content and significantly lower malondialdehyde and peroxide concentration in leaves than in the leaves of susceptible cultivar Prelom. After 24 h of rehydration Katya showed better ability to restore leaf water status and an apparent tendency towards recovery, whereas Prelom sustained higher levels of hydrogen peroxide, lipid peroxidation products and free proline and markedly low relative water content. Here, we have uncovered some of the characteristics displayed by cultivar Katya that enable it to survive and recover from severe osmotic stress. Interestingly, there was congruence between our results and the high level of cultivar Katya drought tolerance observed in the field.

Keywords: *Hydrogen peroxide • Lipid peroxidation • Osmotic stress • PEG • Proline • Rehydration*

Abbreviations:

MDA – malondialdehyde;
PEG – polyethylene glycol;
ROS – reactive oxygen species;
RWC – relative water content.

1. Introduction

The ability to survive periods with low water supply is of great importance to all land plants. Possession of such a trait would be of practical use regarding crop plants and cereals in particular for they are main food resource worldwide. The preservation of plant functions at low plant water potential, and the recovery after water stress are the major physiological processes that contribute to the maintenance of high yield under drought periods [1]. In order to understand the mechanisms that enable plants to survive stress it is appropriate to study the reaction of tolerant species and compare it to other non-tolerant ones [2]. Drought causes various morphological, physiological and biochemical changes in plants. Among its harmful effects is the generation of highly reactive oxygen species (ROS). Some of them, hydrogen peroxide in particular, are also formed as products of normal cellular metabolism but when present at high concentrations may cause serious damage to biological molecules including lipid peroxidation, protein degradation and DNA nicking [3,4]. Free proline accumulates in many living organisms in response to a wide range of stresses such as water shortage, salinity, extreme temperatures, and high light intensity [5]. It participates in the complex cellular process of osmotic adjustment and is generally recognized as a stress marker [6]. The aptitude of some species to accumulate proline in high concentrations is often viewed as an adaptive trait towards dehydration [7,8]. The aim of the present work was to evaluate the reaction of two widespread

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An improvement of the diffusion model for assessment of drought stress in plant tissues

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The article discusses an improvement of a previously developed method for assessment of ion leakage from plant tissues as a gauge of membrane and cell wall performance under stressful environment. It employs conductometric measurements of the ion efflux from leaves and their quantitative interpretation by a theoretical model based on the laws of diffusion. Experimental data are readily fit with the model and results are in accordance with relative water content of dehydrated barley (*Hordeum vulgare*) seedlings of two distinct cultivars. Some new parameters obtained from fitting are proposed as reliable indicators of the leaf status. They appear to be helpful in further distinguishing the behavior of two separate cellular structures with respect to their electrolyte permeability. It is concluded that the established method based on the kinetics of ion leakage is adequate for evaluation of contrasting genotypes under normal and stress conditions. Furthermore, it could be used as a simple and powerful tool for routine analysis and screening for drought tolerance in crops.

Introduction

Drought induces a range of morphological, physiological and biochemical changes in plants and limits their growth and development. At the cellular level, a general stress response is the accumulation of ions and increased amount of metabolites which lower the osmotic potential. Ions are transported and relocated for homeostasis to be maintained, while compatible solutes are synthesized to protect proteins from dehydration and participate in osmotic adjustment (Ming et al. 2012). One of the harmful effects of desiccation is connected with the generation of reactive oxygen species (ROS) whose primary targets are cell membranes (Kuzniak and Urbanek 2000, Apel and Hirt 2004). ROS may cause lipid peroxidation which is often associated with changes in membrane permeability, composition and structure (Campos et al. 2003). A widely used technique for the assessment of membrane stability is the measurement of ion efflux from plant tissues (Roy et al. 2009, Sikder and Paul 2010). Increased electrolyte leakage is indicative of changes in membrane stability which is observed under various stress conditions including salinity (Pérez-López et al. 2009), drought (Guo et al. 2010), high temperatures (Corbineau et al. 2002), low temperatures (Campos et al. 2003, Repo et al 2004, Uemura et al. 2006) and is also suitable for assessment of root viability (Radoglu et al. 2007). The well-known Injury index has been used as a screening test for drought and salt tolerance in wheat (Bajji et al. 2002, Farooq and Azam 2006). However, this otherwise convenient parameter, does not distinguish the contribution of ions from specific cellular compartments to the entire amount of leakage. In a previous work (Kocheva et al. 2005), we developed a diffusion model which differentiated the involvement of the plasmalemma and the cell wall in the overall flux of ions. The aim of this work was to study the effect of soil drought on leakage kinetics parameters and its linkage

Abbreviations – MDA, malondialdehyde; PAR, photosynthetically active radiation; ROS, reactive oxygen species; RWC, relative water content.

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DROUGHT STRESS

Changes in Water Status, Membrane Stability and Antioxidant Capacity of Wheat Seedlings Carrying Different *Rht-B1* Dwarfing Alleles under Drought Stress

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Keywords
antioxidant enzymes; cell membrane stability; DELLA proteins; drought; osmoregulation; reduced height genes; wheat

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Abstract

Water deficiency is a major constraint to wheat productivity in drought prone regions. The wheat DELLA-encoding height-reducing genes (*Rht*) are associated with significant increase in grain yield. However, the knowledge of their benefit in dry environments is insufficient. The objective of the study was to examine the effect of induced drought on leaf water content, level of oxidative stress, cell membrane stability, accumulation of osmoprotectants and activity of some antioxidant enzymes in wheat near-isogenic lines carrying the alleles *Rht-B1b* (semi-dwarfing) and *Rht-B1c* (dwarfing) in comparison with the tall control *Rht-B1a*. Six-day-long water deprivation was imposed at seedling stage. Plants carrying *Rht-B1c* and, to a lesser extent, those carrying *Rht-B1b* performed better under stress compared with *Rht-B1a* in terms of more sustained membrane integrity, enhanced osmoregulation and better antioxidant defence. These differential responses could reflect pleiotropic effects of the *Rht-B1* gene associated with the accumulation of the mutant gene product, that is, altered DELLA proteins, or might be related to allelic variations at neighbouring loci carrying candidate genes for proteins with a major role in plant water regulations and stress adaptation. These findings might be of importance to breeders when introducing *Rht-B1* alleles into wheat cultivars designed to be grown in drought liable regions.

Introduction

Water deficiency is a major stress factor affecting plants at different developmental stages and at various levels of their organization. In continental climate environments, autumn-sown wheat frequently experiences early season drought stress during seedling establishment. Such early setbacks can constrain the subsequent growth and development and may finally result in a substantial yield reduction (Blum 2011). At the organ and tissue level, the harmful consequences of drought include disturbed balance between water uptake by roots and loss of shoot water through transpiration flow, causing tissue water deficiency and associated osmotic stress. Generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) (Sairam and Saxena 2000, Apel and Hirt 2004, Singh et al. 2012), and accumulation of malondialdehyde (MDA), a secondary by-product of the oxidation of polyunsaturated fatty acids in cell membranes, are typical symptoms of the development of the accompanying oxidative stress (Selote and Khanna-Chopra 2006). Subsequent lipid peroxidation may disturb membrane integrity and thus lead to loss of selective permeability causing ion leakage from damaged tissues (Blum and Ebercon 1981).

The plant adaptive responses to drought are mainly focused to maintain water homeostasis and membrane stability, and limit the consequences of the developing oxidative stress. A general biochemical adaptation at the cellular level is the accumulation of organic solutes such as amino acids, sugars and polyols which improve osmotic potential and water uptake ability (Chen and Jing 2010, Sperdouli and Moustakas 2012). Besides acting as osmoprotectants, these low molecular weight and highly soluble substances may contribute for maintaining membrane integrity, stabilizing enzymes or proteins and minimizing oxidative damage (Matysik et al. 2002, Chen and Jing 2010). In addition, *Brief communication*

Variation in ion leakage parameters of two wheat genotypes with different *Rht-B1* alleles in response to drought

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The reaction to soil drying was evaluated in two *Triticum aestivum* near-isogenic lines carrying different alleles of the height-reducing gene *Rht-B1* based on an improved method for assessment of electrolyte leakage. The two lines were previously shown to differ in their physiological responses to induced water deficit stress. Drought was imposed for 6 days on 10-day-old seedlings. Ion efflux from leaves was measured conductometrically in multiple time points during the 24 h incubation period, and the obtained biphasic kinetics was interpreted according to a previously developed theoretical model proposing different leakage rates through the apoplast and the symplast. Most of the model parameters were able to properly differentiate the two closely related genotypes. The mutant *Rht-B1c* displayed lower and slower electrolyte leakage in comparison with the wild-type *Rht-B1a*. It was speculated that the *Rht* genes expressing defective DELLA proteins might be involved in water stress response through modulation of cell wall stiffness, which influences its capacity for ions retention, and also by their contribution to ROS detoxification, thus indirectly stabilizing cellular membranes. The presented analytical approach relating processes of ion and water flow in and out of the cell could be used for characterization of membrane and cell wall properties of different genotypes under normal and stress conditions.

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1. Introduction

Development of water deficit stress in plants is the common consequence of the unavailability of water to cells in conditions such as drought, high salinity and extreme temperatures (Hare *et al.* 1998; Sperdouli and Moustakas 2012). Cell membranes are the main targets of damage, which is generally associated with metabolic disturbances, leading to generation of reactive oxygen species (ROS) (Hare *at al.* 1998; Thapa *et al.* 2011; Kocheva *et al.* 2014b).

The most widely employed technique for assessment of membrane integrity is by estimating the Injury Index based on ion leakage from damaged plant tissues (Blum *et al.* 1997; Prášil and Zámečník 1998; Farooq and Azam 2006; Chipilski *et al.* 2012). Electrolyte leakage was used as a selection marker for differentiation of drought-tolerant genotypes, which tended to leak at lower rate compared to sensitive ones (Whitlow *et al.* 1992; Pelah *et al.* 1997; Bajii *et al.* 2002; Roy *et al.* 2009). However, inability to distinguish ion fluxes through different cellular compartments is one disadvantage of the method. This problem was recently solved by establishing a kinetic approach based on measurement of ion leakage from plant tissues at multiple time points (Kocheva *et al.* 2005). Diffusion processes between the apoplast and the symplast have been elucidated lately by introducing the rates of ion fluxes through cell walls and membranes, respectively, as promising parameters for evaluation of leaf water status (Kocheva et *al.* 2014a). The proposed improved model suggested that not only membrane permeability but also diffusion through the cell wall (apoplast) were influenced by dehydration.

Keywords. Cell wall; drought; electrolyte leakage; membranes; semi-dwarfing genes; wheat

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DROUGHT STRESS

Wheat Rht-B1 Dwarfs Exhibit Better Photosynthetic Response to Water Deficit at Seedling Stage Compared to the Wild Type

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Keywords
DELLA proteins; drought stress; dwarfing genes; leaf anatomy; *Triticum aestivum*

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Abstract

Wheat reduced height (*Rht*) genes encode modified DELLA proteins, which are gibberellin insensitive, accumulate under stress, restrain growth and affect plant stress response. The seedling reaction to soil water deficit regarding leaf gas exchange and chlorophyll fluorescence was compared in near-isogenic lines carrying the alleles *Rht-B1a* (tall), *Rht-B1b* (semi-dwarfing) and *Rht-B1c* (dwarfing) and was related to leaf water content and anatomy. Under drought, *Rht-B1c* line was characterized by less decreased CO_2 assimilation, delayed non-stomatal limitation of photosynthesis and higher instantaneous water use efficiency. The functional state of its photosynthetic apparatus was better preserved as evidenced by the less decreased actual quantum yield (Φ_{PSII}) and potential maximum quantum yield (F_v/F_m) of PSII, and the less increased quantum yield of non-regulated energy dissipation (Φ_{NO}). *Rht-B1b* line also tended to perform better than *Rht-B1a*, but differences were less pronounced. Although the leaves of both dwarf lines were smaller, thicker and more pubescent, their water content was not higher in comparison with the tall line. Nevertheless, in *Rht-B1c*, leaf thickness was less decreased and mesophyll cells were less shrunk under drought. The more effective performance of the photosynthetic machinery of dwarf lines under water deficit could be explained by a combination of morpho-anatomical and metabolic characteristics.

Introduction

Soil water deficit is a leading environmental challenge for the developing plant. It triggers various physiological responses among which changes in photosynthesis (Lawlor and Tezara 2009) and modifications in leaf anatomy (Vassileva et al. 2012). The adverse effects of this stress on photosynthesis lead to plant growth inhibition and eventually to substantial loss in yield. Wheat is rather sensitive to drought and genetically determined differences in resistance to water stress among genotypes exist (Molnár et al. 2004, Wu and Bao 2011a,b, Marcińska et al. 2013). One of the mechanisms for adaptive modulation of plant growth is mediated by DELLA proteins. They are nuclear transcriptional regulators with central role in gibberellin (GA) signalling. Under stress, these proteins accumulate and both restrain growth and increase plant survival (Achard et al. 2008). As DELLAs do not have a clearly identified DNA-binding domain, they are likely to regulate the expression of their target genes by interacting with other transcription factors. DELLAs are rapidly subjected to proteolysis after becoming part of a complex with GA and the GA–GID1 receptor (GA-INSENSITIVE DWARF1) (Sun 2010). In wheat, DELLAs are encoded by the wild counterparts of the reduced height (*Rht*) genes. Dwarfs with a broad range of plant height have been achieved through the introduction of mutant alleles at the *Rht-B1* and *Rht-D1* locus on chromosomes 4B and 4D, respectively. Mutations in these genes affect DELLAs binding to GID1, but not their transcriptional regulatory activity (Pearce et al. 2011). The result is size reduction at cellular, organ and whole plant level due to hampered GA-induced degradation of

Tzvetan Darakchiev, Yavor Chapanov

INVESTIGATION OF THE OSCILLATION OF THE VERTICAL AT OBSERVATORY "PLANA"

(S u m m a r y)

The main cases of the developed in the Central Laboratory for Geodesy of BAS methods for determination of the nontidal changes of the vertical at a given point from the Earth's surface by the permanent astronomical observations of the geographic latitude are described. Some essential results and conclusions of the investigation of the latitude changes and nontidal oscillations of the vertical at Geodetic observatory Plana for the period 1987-2001 are pointed out. Some of the nontidal changes of the latitude and vertical are explained by the earthquakes, long-period variations of the gravity and horizontal plate motion. The references include publications and other works of the authors, which are connected with the described methods.

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КИНЕТИКА НА ЕЛЕКТРОЛИТНОТО ИЗТИЧАНЕ ОТ РАСТИТЕЛНИ ТЪКАНИ. ДИФУЗИОНЕН МОДЕЛ

Изтичането на вещества от растителни тъкани, потопени в разтвор, се използва широко като мярка за мембранната пропускливост при изследване на различни стресови условия, фактори на растеж и развитие, генотипни особености и т.н. В много случаи то се определя с помощта на кондуктометрични техники за анализ на проводимостта на разтвора, в който тъканите се оставят да престоят за различен период от време. Така, дестилираната вода, в която първоначално се поставят образците, съвсем естествено постепенно повишава проводимостта си поради навлизането в нея на йони от вътреклетъчното пространство под действие на концентрационния градиент при нарушена нормална мембранна проницаемост.

Кондуктометричното измерване на йонното съдържание в течни среди предлага редица удобства, като: евтина и лесна за обслужване апаратура, възможности за експресна рутинна оценка, работа в извънлабораторни условия. Същевременно, както повечето електрохимични методи, кондуктометрията е достатъчно чувствителна и прецизна, с много добра повторяемост на резултатите. Въпреки че е изминало доста време от първите докладвани работи в тази насока [1], методът не е изгубил своята актуалност. Това се вижда от литературата, в която се наблюдават тенденции към усъвършенстване чрез използването на по-прецизни протоколи, отчитащи особеностите на конкретното изследване [2; 3]. Наред с това, разбира се, съществуват и някои проблеми. На първо място това е липсата на селективност по отношение на приноса от различните йони, която кондуктометрията сама по себе си не е в състояние да даде. За много изследвания пречката може да бъде преодоляна с използването на емпирични зависимости, свързващи проводимостта на външния разтвор с концентрацията на основните видове йони (най-вече K^+), изтичащи от образците [4]. Тази връзка се получава от независимо определяне на въпросните концентрации с допълнителни методи (като напр. атомно-емисионна спектроскопия).

Друг немалък кръг въпроси е свързан с интерпретацията на резултатите по отношение на физикохимичните и електрохимичните параметри на клетъчната мембрана. Очевидно, кондуктометричните данни представят една обобщена характеристика на електролитните течове от вътреклетъчното пространство, без да казват нещо съществено за проницаемостта и трансмембранния потенциал, които ги определят. Това внася известна неяснота и усложнява коректната оценка на състоянието на мембраните.

Независимо от споменатите затруднения и дискусионни моменти, кондуктометричното дефиниране на йонните утечки от растителни тъкани предлага ценна информация за мембранните изменения, дължащи се на различни въздействия на околната среда, стареене или третиране в лабораторни условия [5; 6]. В редица проучвания тази информация съществено допълва общата картина на физиологичното състояние на растенията [7; 8].

Наред със специфичната електропроводимост, получавана директно от измерванията, в

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ИЗПОЛЗВАНЕ НА ПЕГ-ТЕСТ ЗА ФИЗИОЛОГИЧНА ОЦЕНКА НА СУХОУСТОЙЧИВОСТТА НА ЕЧЕМИКА

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Резюме

Кочева, К., Г. Георгиев. 2007. Използване на ПЕГ-тест за физиологична оценка на сухоустойчивостта на ечемика

Засушаването е едно от основните въздействия на средата, които ограничават растежа, развитието и продуктивността на ечемика. Изясняването на физиологичните и биохимични механизми на адаптация към стрес е основен подход при изучаване на сухоустойчивостта на културните видове. Основни метаболитни реакции са осмотично приспособяване, регулация на водообмена, активност на фотосинтетичния апарат. Лабораторните скрининг методи за изследване на физиологичните механизми на устойчивост на растенията имат известни предимства пред полските опити поради своята експресност, възможност за повторения и надежност. Освен това, при тези тестове се избягва взаимодействието между стресовете, което често пъти се наблюдава в природата. В предложената опитна постановка воден стрес се постига чрез третиране на корените на 10-дневни ечемични растения с разтвор на полиетиленгликол. Степента на водния дефицит се охарактеризира с величината относително водно съдържание. За оценка на физиологичното състояние са изследвани някои морфологични, биохимични и биофизични параметри на стресираните образци: биометрични показатели, количество натрупани осмолити, степен на увреждане на клетъчните мембрани. На базата на получените резултати се дискутира модел за оценка на физиологичното състояние на сорта ечемик и неговата приложимост в селекционната практика.

Ключови думи: Воден стрес – ПЕГ – осмотично приспособяване – пролин – клетъчна мембранна стабилност

Abstract

Kocheva, K.V. and G.I. Georgiev. 2007. The use of PEG-test for physiological evaluation of drought-tolerance in barley

Drought is a major abiotic factor which limits growth, development and productivity of crop plants and barley in particular. Clarification of physiological and biochemical mechanisms of stress adaptation is a basic approach towards selection for higher drought-tolerance. Metabolic reactions such as osmotic adjustment, cell membrane stability, water relations, and activity of the photosynthetic apparatus are of great importance. Laboratory screening techniques for evaluation of physiological mechanisms of plant resistance have certain advantages compared to field studies. They allow precise regulation of the degree of stress treatment and avoid interaction between stress factors, which often occurs in natural conditions. In the present study water stress was induced by treating the roots of 10-day-old barley seedlings with polyethylene glycol. This osmoticum simulates dehydra-

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PARAMETERS OF CELL MEMBRANE STABILITY AND LEVELS OF OXIDATIVE STRESS IN LEAVES OF WHEAT SEEDLINGS TREATED WITH PEG 6000

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Summary. Wheat (*Triticum aestivum* L.) seedlings of cv. Chinese Spring (CS) and hybrid Line 73 (breeding material based on a wide cross CS x *Aegilops geniculata* Roth) were grown for 7 days on a half strength Hoagland nutrient solution and afterwards transferred to 15 % PEG 6000. Changes in the parameters of cell membrane stability (electrolyte leakage kinetics), level of oxidative stress (MDA and hydrogen peroxide content) and leaf water status assessed by RWC during 5-days (mild) and 8-days (strong) water stress were investigated. The longer stress duration caused stronger dehydration. RWC of the leaves was strongly reduced in both genotypes. The level of water stress in the parental genotype correlated with greater lipid peroxidation (assessed as higher content of MDA) and reduced membrane stability (higher electrolyte leakage from damaged tissues) in leaves. These disturbances in the parental genotype corresponded to higher concentration of hydrogen peroxide of which Line 73 showed lower values. The selected genotype Line 73 disclosed higher tolerance to osmotic stress with PEG 6000 in laboratory conditions as assessed by the studied parameters.

Keywords: electrolyte leakage; H_2O_2; osmotic stress; polyethylene glycol; wheat.

Abbreviations: MDA – malondialdehyde; PEG – polyethyleneglycol; RWC – relative water content.

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Aegilops geniculata chromosome introgressions into bread wheat and their effects on plant physiological responses to abiotic stress

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The wild relative of wheat *Aegilops geniculata* Roth (syn. *Ae. ovata* L.) is an allo-tetraploid species (2n=4x=28, genome formula $M^gM^gU^gU^g$) native to the Mediterranean region, Southern Europe, the Aegaeis, North Africa, southern parts of Crimea, Near and Middle East, and western arc of the Fertile Crescent (van Slageren 1994). It is a potentially important source of genes for improving wheat earliness, disease resistance and tolerance to abiotic stress, including drought and mineral deficiencies (Zaharieva et al. 2001; Neelam et al. 2010). The establishment of wheat-alien chromosome substitution lines allows the study of genetic effects of the individual alien chromosomes in wheat background and their ability to compensate missing wheat chromosomes for phenotypic and physiological traits (Molnár et al. 2007). This work describes the production of wheat-*Aegilops geniculata* chromosome substitution lines, their cyto-molecular characterization, and the physiological responses of seedlings to induced osmotic stress and Fe-deficiency stress.

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ION LEAKAGE AND LEAF ANATOMY OF BARLEY PLANTS SUBJECTED TO DEHYDRATION

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Summary. In the present study the physiological status of two barley (*Hordeum vulgare,* L.) cultivars subjected to dehydration was evaluated. Ten-days-old plants were exposed to soil drying by withholding irrigation. The degree of stress was assessed by comparing leaf water content and the levels of malondialdehyde and hydrogen peroxide of treated and control plants. Cell membrane stability was evaluated based on electrolyte leakage from leaf tissues. It was ascertained that besides the commonly used Injury index leakage kinetics gave additional information about the changes in ion concentrations in leaf symplast and apoplast which could be used for preliminary stress evaluation of contrasting genotypes. The examined cultivars differed in their water content which corresponded to the extent of membrane injury caused by dehydration. Exposure to drought caused a significant decrease both in leaf thickness and total area of mesophyll cells. The most obvious change in leaf anatomy was the disappearance of vacuoles in mesophyll cells which contributed to the leakage of the major part of electrolytes during the fast phase of the kinetics. The involvement of the cell wall and the vacuole in osmotic stress response are briefly discussed.

Key words: barley; drought stress; electrolyte leakage; hydrogen peroxide; leaf anatomy; malondialdehyde; vacuoles.

INTRODUCTION

Drought causes various morphological, physiological and biochemical changes in plants. One of the harmful effects of desiccation is connected with the generation of reactive oxygen species such as hydrogen peroxide and products of lipid peroxidation, such as malondialdehyde, whose primary targets of damage are cell membranes (Kuzniak and Urbanek, 2000; Fu and Huang, 2001; Király and Czövek, 2002). Electrolyte leakage from plant tissues is widely used for the assessment of stress impact on cell membrane stability (Bajji et al., 2002; Repo et al., 2004; Farooq and Azam, 2006; Roy et al., 2009). Solutes leak both from the symplast (cytosol) and the apoplast (including cell walls, xylem elements and extracellular spaces). The largest compartment in the cytosol of a mature plant cell is the vacuole which may occupy more than 80% of the total cell volume. Due to their

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DROUGHT AS A CHALLENGE FOR IMPROVED SCREENING OF CEREAL GENOTYPES FOR SUSTAINABLE PLANT PRODUCTION

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Abstract

A bilateral project between the Institute of Plant Physiology and Genetics at the Bulgarian Academy of Sciences and The Slovak University of Agriculture in Nitra entitled 'Drought as a challenge for improved screening of cereal genotypes for sustainable plant production' was funded by the Bulgarian Ministry of Education, Youth and Science and the Slovak Research and Development Agency, The main goal of the Project is to study drought tolerance mechanisms in wheat and barley as important crops and to offer confident criteria for evaluation and screening of genetic resources under unfavorable climate conditions. The relationship between leaf water deficit, structural changes in leaves, cell membrane stability, osmolytes accumulation, development of oxidative stress and photosystem II efficiency in some of the most widely used Bulgarian and Slovak wheat and barley genotypes under drought conditions will be investigated. On the basis of the obtained new data, a model for drought resistance in cereals would be established. It might facilitate breeders in their pursuit of drought tolerance and sustainable plant production.

INTRODUCTION

Climate change evokes a need for better characterization of plant responses to stress and deeper understanding of physiological mechanisms related to adaptation and productivity under adverse conditions. Soil drought is the abiotic factor which most seriously limits growth and development of crop plants and negatively affects their yield. Clarification of physiological and biochemical mechanisms of stress adaptation is a basic approach towards breeding for higher drought tolerance in crops. Metabolic reactions such as osmotic adjustment, cell membrane stability, plant water status under stress, and their relation to the activity of the photosynthetic apparatus are among the most common parameters for selection of tolerant plant genotypes (Zivcak et al., 2008; Chipilski et al., 2012). In order to better identify the aspects of drought tolerance, an integration of methodological approaches and investigation of plant responses to stress on multiple levels of organization (from molecular to organ and whole-plant level) is necessary. Laboratory screening techniques for evaluation of physiological mechanisms of plant resistance have certain advantages compared to field studies. They allow precise regulation of the degree of stress treatment and avoid interaction between stress factors, which often occurs in natural conditions.

Wheat and barley are the most important cereal crops in many countries. In this regard, a bilateral project between the Institute of Plant Physiology and Genetics at the Bulgarian Academy of Sciences and The Slovak University of Agriculture in Nitra entitled 'Drought as a challenge for improved screening of cereal genotypes for sustainable plant production' has been funded by the Bulgarian Ministry of Education, Youth and Science and the Slovak Research and Development Agency. The main goal of the Project is to study drought tolerance mechanisms in cereals and to offer confident criteria for evaluation and screening of genetic resources under unfavorable climate conditions. Some of the most widely distributed Bulgarian and Slovak wheat and barley genotypes will be used for scanning the phenotypic variation in response to drought stress. An interdisciplinary approach will be applied and new methods and innovative technologies will be implemented. The research will be of particular importance to breeding practice and will render better understanding of

УПРАВЛЕНИЕ И УСТОЙЧИВО РАЗВИТИЕ 3/2012 (34) MANAGEMENT AND SUSTAINABLE DEVELOPMENT 3/2012 (34)

СЪДЪРЖАНИЕ НА ПРОЛИН И ПИГМЕНТИ В ЛИСТА ОТ ЧЕРВЕНА ВЛАСАТКА (*FESTUCA RUBRA* L.) И АНГЛИЙСКИ РАЙРГАС (*LOLUIM PERENNE* L.) ПРИ РАЗЛИЧНИ ПОЧВЕНИ И ПОЛИВНИ УСЛОВИЯ

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Резюме

При създаването на тревни площи е важно до каква степен използваните видове са толерантни към засушаването. Важно е и до каква степен те могат да бъдат максимално отзивчиви към наличието на вода. У нас найчесто се използват смески от червена власатка (*Festuca rubra* L) и английски райграс (*Lolium perenne* L). Добавянето на строителни отпадъци към почвата дава възможност да се имитират почвените условия в зелените площи. Изследването на водното съдържание при различни условия на средата допринася за по-пълно разбиране на необходимостта от водна обезпеченост. Свободния пролин в листните тъкани е индикатор за наличието на стрес. Увеличеното му съдържание корелира с намаляването на относителното водно съдържание. Съдържанието на фотосинтетични пигменти в листата е показател за общото физиологично състояние и в частност за фотосинтетичната активност на растението. С получените резултати се потвърждава, че тези параметри могат успешно да бъдат използвани като индикатори за състоянието на видове, широко използвани в практиката.

Ключови думи: относително водно съдържание, пролин, хлорофил, антропогенни почви, червена власатка, райграс, урбанизирани територии.
Key words: relative water content, proline, chlorophyll, anthropogenic soils, fescue, ryegrass, urban areas.

Увод

Засушаването е едно от основните въздействия на средата, което ограничава водообмена на културните и диви растения и инхибира растежа, развитието и разпространението им. Степента на въздействие на недостига на вода в почвата зависи силно от свойствата на почвения субстрат [2]. Сред факторите, влияещи негативно върху тези свойства на почвите са процеси на урбанистично замърсяване. Влошеното плодородие и водообмен на такива почви изисква както оптимален избор на използваната флора, така и поддържане на определен режим на водоснабдяване. За оценка на реакцията на растенията към неблагоприятен воден режим на почвата широко се използват няколко физиологични параметри. Сред тях е относителното водно съдържание на листата - важен физиологичен параметър за състоянието на водообмена в условия на воден стрес и характеристика на степента на засушаването [5, 9]. Съдържанието на свободен пролин и концентрацията на хлорофилните пигменти в листата, използвани като маркер на фотосинтетичния статус на растенията, са сред метаболитите с важна адаптационна роля в растенията към воден стрес [4, 8, 12]. Редица изследвания показват, че съществува висока степен на корелация между изброените физиологични параметри и нивото на засушаване в растенията, което предполага тяхното използване при оценка на толерантността на растителния вид [3, 6, 10, 12].

Целта на настоящото изследване бе да се установи връзката между нивото на водоснабдяване, състав на почвения субстрат и промените в съдържанието на свободен пролин, концентрация на хлорофил а и b и относителното водно съдържание в листа на два растителни вида, широко използвани при изграждане на зелени площи на силно урбанизирана среда, - червена власатка (*Festuca rubra* L.) и английски райграс (*Lolium perenne* L.).

Описание на опитната постановка

Настоящите изследвания са проведени с два растителни вида – червена власатка (*Festuca rubra* L.) и английски райграс (*Lolium perenne* L). Растенията са отглеждани като съдов почвен опит с използване на алувиално-ливадна почва без примеси и такава с 25% добавени примеси (строителни отпадъци). Прилагани са два поливни режима – 80% и 50% ППВ, от които 80% ППВ могат да бъдат разглеждани като оптимални условия на отглеждане на тревите (контрола). Растенията са отглеждани в продължение на един вегетационен сезон.

Материалите са събирани от един опит с по 3 повторения за всеки от изследваните показа-

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A SIMPLE IMPEDIMETRIC DEVICE FOR IN SITU ANALYSIS OF PLANT TISSUES

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(*Submitted by Academician A. Petrov on March 5, 2013*)

Abstract

Construction and major features of a set-up suitable for impedance analysis of plant tissues are disclosed in the paper. The measuring device is based on a simple scheme operating in conjunction with the PC sound card. While many programmes running directly on sound card are capable of simulating impedance analyzer, their performance is not consistent when chemical or biological systems are explored. The main reason is a relatively low input impedance of the card itself. An addition of coordinating preamplifier between the sample and the audio input of the PC drastically improves the implementation of the set-up. On the other hand, a specially designed measuring head gives possibilities for straightforward assessment of plant tissue's electrical parameters in situ at various ambient conditions. Some examples of impedance analysis of intact leaves are given and their behaviour in regard to a preliminary imposed desiccation is briefly discussed.

Key words: impedance analysis, leaf desiccation, plant water relations

Introduction. Drought and freezing are the basic abiotic factors limiting plant growth and development. They cause various morphological, physiological and biochemical changes in plants. As far as water and ions are ubiquitous participants in these phenomena, it is clear, that electrochemical methods of investigation would be of a primary interest. Indeed, to date many such techniques are used for evaluation of the stress consequences. Conductometric measurement of electrolyte leakage from plant tissues, for example, is widely accepted for assessment of cell membrane injury [1–3]. Electrochemical impedance spectroscopy (EIS),

ELECTROLYTE LEAKAGE AND K^+ IONS CONTENT IN THE LEAVES OF WHEAT PLANTS SUBJECTED TO POLYETHYLENE GLYCOL TREATMENT

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ABSTRASCT

The effect of PEG-induced osmotic stress on relative water content and electrolyte leakage from the leaves of two contrasting wheat (*Triticum aestivum*, L) cultivars was compared in the study. Drought tolerant cv. Katya retained higher water content in the leaves after imposition of osmotic stress in comparison with cv. Prelom. Higher amount of potassium ions tended to leak from the leaves of stressed cv. Prelom plants and the overall electrolyte leakage from the leaves of this cultivar was also greater in comparison with cv. Katya. It was concluded that capacity for better water retention in the tolerant cv. Katya could be connected with the retention of potassium ions which participate in many cellular processes and represent the major osmotic solute in plant cells. Lower electrolyte leakage from stressed Katya leaves in comparison to Prelom was indicative of less damaged cellular membrane permeability which could be connected with superior protective mechanisms.

Key words: *electrolyte leakage, polyethylene glycol, potassium ions, wheat.*

Wheat is a major and widely distributed crop which is often exposed to climatic adversities. Among the abiotic factors affecting yield and quality of production drought is one of the most frequent. Owing to the complex nature of drought tolerance, the problems related to plants ability to withstand water deprivation require investigation at many different levels. Study of physiological processes in plants under suboptimal or stress conditions could contribute to our knowledge of metabolic plasticity and could reveal some of the mechanisms involved in overcoming the effects of unfavorable environmental conditions. Induction of stress in laboratory conditions has certain advantages over field experiments as controlled growing conditions offer better reproducibility of the results and the possibility for separate evaluation of a particular limiting factor thus avoiding the interference of stressors. The use of polyethylene glycol (PEG) solutions for the induction of osmotic stress provides an option for precise control of the degree of dehydration within a wide range of osmotic potentials (Filek et al. 2012).

Cellular membranes are among the first targets of water stress and plant dehydration. Commonly, changes in membrane organization and composition lead to enhanced electrolyte leakage which could be determined by conductometric measurement. Ion leakage from plant tissues is widely used as a measure of cell membrane stability in the assessment of the impact of various stress factors. It is measured by common techniques for analysis of conductivity of the solutions in which tissues are immersed (Bajji et al. 2002). Thus the conductivity of distilled water in which the samples are submerged naturally increases owing to the flow of ions from the intercellular spaces driven by concentration gradient in the process of diffusion. When the normal membrane permeability is disturbed an increased leakage occurs. The implementation of conductometry as a highly sensitive and precise method is justified for assessment of ion leakage from plant tissues since it offers valuable information of membrane damages caused by various environmental factors (Filek et al. 2012). In numerous investigations this data substantially expands the view of the plant overall physiological status. Empirical dependences exist which connect conductivity of the outer solution to the concentration of main ion species (mostly K^+) flowing out of plant tissues (Palta and Li, 1980). Potassium is the most abundant ion in the plant cell which is related to its multiple functions such as osmoticum, role in

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EFFECT OF SAMPLE PREPARATION ON PARAMETERS OF ELECTROLYTE LEAKAGE KINETICS FROM *TRIFOLIUM* LEAVES

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(*Submitted by Academician V. Golemansky on May 9, 2014*)

Abstract

The paper employs a recently developed method for assessment of electrolyte leakage kinetics from plant leaves based on conductometric measurement of ion efflux and its quantitative interpretation by a theoretical model. Experimental data were readily fit with the model and variation of obtained parameters was explored with respect to sample preparation. The presented analytical approach relating processes of ion and water flow in and out of the cell could be used for characterisation of membrane and cell wall properties under normal and stress conditions. However, sample preparation should be chosen with caution, especially in cases where tissue damage due to excision could mask the expected stress injury.

Key words: electrolyte leakage, cell walls, membranes

Introduction. Measurement of the amount of electrolyte leakage from plant tissues is a long-standing method employed in plant physiology studies for evaluation of the functional status of cellular membranes (membrane permeability) under various environmental stresses [1–4]. The commonly used Injury index calculates the degree of damage in treated samples as related to untreated ones thus giving only relative values for estimation of harmful consequences of the imposed stress on cell membranes [5]. Comparison of untreated samples is not possible using the Injury index, because damage is expressed as percentage of the control [6]. The inability to accurately distinguish ion fluxes could be pointed out as another weakness of the method. These problems were recently solved by establishing the kinetic approach for analysis of diffusion processes based on conductometric

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INTERACTION OF CHLORSULFURON TREATMENT AND IRON DEFICIENCY OR EXCESS IN YOUNG PEA PLANTS

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Summary: Nutrient stress may modify plant reaction to herbicide treatment. On the other hand, herbicides might induce or aggravate nutrient disorders. The separate and combined effects of chlorsulfuron (ClS) and iron supply on growth, photosynthetic pigment content and functional state of the photosynthetic apparatus, assessed by chlorophyll fluorescence measurements, were compared. Young pea plants were grown hydroponically in a growth chamber, supplied with iron, ranging from complete deficiency to excess (0, 2- optimum concentration, 10 or 50 $mg.l^{-1}$ Fe) and sprayed with ClS (0. 10^{-6} or 10^{-5}M). Both Fe deficiency and strong Fe excess reinforced the inhibitory effect of ClS on shoot and root biomass, while low Fe excess (10 $mg.l^{-1}$ Fe) slightly mitigated the herbicide effect on growth, but affected negatively PSII activity. Separately, Fe deficiency and ClS decreased the chlorophyll and carotenoids content. Due to a concentration effect, when both factors were in combination the chlorosis of Fe deficient plants as well as the imbalance between the pigments were less pronounced. Fe deficiency was the individual stress with the strongest negative effect on the photosynthetic apparatus when estimated by chlorophyll fluorescence. In combination with ClS, this effect was softened. The negative effect of ClS on the fluorescence parameters was evident at 10^{-5}M and was reinforced when combined with 50 $mg.l^{-1}$ Fe. Individual stressors as well as their combinations caused an increase in electrolyte leakage from leaf tissue, which was an indicator for the occurrence of cell membrane injury. It was concluded that the effect of ClS depended not only on its concentration, but also on Fe supply.

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Keywords: Chlorsulfuron; combined stress; Fe deficiency; Fe excess; herbicides; *Pisum sativum* L.

Abbreviations: ALS – acetolactate synthase; Chl. – chlorophyll; ClS – chlorsulfuron; DM – dry matter; DMC – dry matter content; Fe – iron; IGA – index of growth alteration; PSII – photosystem II.

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IMPLEMENTATION OF A KINETIC MODEL FOR EVALUATION OF LEAF ION LEAKAGE FROM SUNFLOWER (*HELIANTHUS ANNUUS*) PLANTS SUBJECTED TO HIGH ZINC AND LEAD CONCENTRATIONS

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Summary: In the present study, the effect of excess Zn and Pb ions added to the nutrient solution of hydroponically grown sunflower plants was studied in order to evaluate their impact on the properties of leaf tissue by employing an electrolyte leakage kinetics model. Based on the behavior of model parameters, it was demonstrated that treatment with excess Zn had a stronger effect on leaf leakage kinetics than Pb. This could be attributed to the higher mobility of Zn ions which were more readily transported towards the leaves in comparison to Pb. Ion fluxes through different subcellular compartments, namely apoplast (including cell wall) and symplast (comprising the vacuole and cell membranes) were used to explain the changes in ion leakage kinetics as reflecting the functional status of cellular membranes.

Citation: Kocheva K., M. Chavdarova, E. Gesheva, S. Doncheva, G. Georgiev, 2015. Implementation of a kinetic model for evaluation of leaf ion leakage from sunflower (*Helianthus annuus*) plants subjected to high zinc and lead concentrations. *Genetics and Plant Physiology*, 5(1): 23–28.

Key words: Electrolyte leakage; Pb; metal toxicity; sunflower; Zn.

INTRODUCTION

Metal toxicity is a common abiotic factor experienced by land plants worldwide. In contrast to organic pollutants, heavy metals cannot be biologically or chemically degraded, and thus may accumulate locally or be transported over long distances (Gonzalez-Mendoza et al., 2009). The effect of toxic concentrations of metals on plant metabolism is of particular importance for growing crop plants in contaminated areas (Verbruggen et al., 2009). Sunflower is a major crop which is also known for its ability to accumulate toxic concentrations of different elements from the substrate (Krystofova et al., 2009). There is an increasing interest in heavy metal detoxification in plants and assessing the fluxes of harmful ions across cell membranes is especially important

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ИЗСЛЕДВАНЕ НА ПРОМЕНИТЕ ПРИ ДЕХИДРАТАЦИЯ В ЛИСТА ОТ ОБИКНОВЕНА ПШЕНИЦА (*TRITICUM AESTIVUM*, L.) ЧРЕЗ ЕЛЕКТРОХИМИЧНА ИМПЕДАНСНА СПЕКТРОСКОПИЯ

ВЪВЕДЕНИЕ

Непрекъснато засилващият се интерес към използване на електрохимичните методи за изследване на процесите в биологични обекти се определя от съществената роля, която играят електрохимичните процеси в живата клетка. От друга страна, бързото развитие на аналитичната електрохимия в последно време снабди учените с нови мощни средства за извличане на информация за тези процеси на молекулно ниво. Поради редица свои преимущества като чувствителност, неинвазивност, сравнително проста и нескъпа апаратура, тя си извоюва едно много широко приложение особено в интердисциплинарните научни направления. Сред електрохимичните методи за анализ съществено място заема импедансният подход. Прилагането на електромагнитно лъчение върху образец с оглед оценка на неговата електрохимична характеристика определя този подход като електрохимичен и спектроскопичен едновременно, ако измерването се провежда при различни честоти на възбуждащия сигнал. Това формулира направлението като електрохимична импедансна спектроскопия (ЕИС) [1]. Чрез ЕИС могат да бъдат измервани диелектричните и електричните свойства на дадена среда в даден момент, като функция от честотата на електромагнитното лъчение, с което бива облъчвана. По този начин могат да се изследват честотнозависимите пасивни електрически свойства (т.е. импеданса или комплексната проводимост) на различни системи. Веднага трябва да се обърне внимание, че изследването на импеданса на химични и биологични системи изисква поспециално отношение най-малко поради две причини – стремежа към „невариращо“ въздействие (за да не се променят параметрите в хода на измерването) и по същество нелинейния отговор на системата. Напоследък тези подходи се обогатиха и с по-модерни техники на базата на нови методи и устройства, които доведоха до появата на сканиращата ЕИС и импедансната томография.

Разработването на все по-информативни и лесни за обслужване физични методи за изследване на физиологичното състояние на живи обекти от дълги години се радва на заслужено внимание от страна на специалистите в областта. Без съмнение експерименталните методи, даващи възможност рутинно да се определят различни състояния на живата клетка от животински, микробиален и растителен произход в организми, поставени при стресови условия, без да се нарушава целостта на обекта, стават все по-привлекателни за изследователите. От особено значение са такива методични подходи при изследване на растителни обекти, подложени на различни видове абиотичен или биотичен стрес [2, 3]. Изследванията с електрохимични подходи, включително използването на техники на ЕИС при изучаване на физиологията и екологията на растенията, са особено важни, тъй като растенията като отворена термодинамична система са по-тясно обвързани с континуума почва–растение–атмосфера, за разлика от други живи организми [4].

Електрохимичните методи за оценка на водно-солевото състояние на клетките са особено ефективни при изследвания върху растения, тъй като тяхното тяло съдържа до 90 % вода и йони. Наистина немалко методи, базирани на електрохимични принципи, са били успешно използвани досега във физиологията и биохимията на растенията за лабораторни и полеви изследвания. Без претенции за изчерпателност можем да споменем на първо място амперометричните методи и устройства за определяне на кислородното отделяне и газообмена при фотосинтезата [5, 6]. Също така кондуктометричният метод за оценка на мембранната стабилност на клетките по електролитното изтичане от различни тъкани [7, 8] и установките, базиращи се на измерване диелектричните свойства на образците, са често използвани във физиологията при изучаване на стреса в растенията [9 – 12]. Последният споменат подход фактически може да се разглежда като разширение на кондуктометрията в комплексната област на електромагнитните измервания. В този смисъл отговорът на системата под формата на импеданс (комплексно съпротивление или еквивалентния му адмитанс, комплексна про-

PEG 8000 INDUCED OSMOTIC STRESS CAUSES DIFFERENTIAL EFFLUX OF METAL CATIONS FROM WHEAT LEAVES

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ABSTRASCT

Osmotic stress induced by incubation of young wheat (*Triticum aestivum*, L) plants roots in PEG 8000 solution led to reduction in leaf relative water content and caused increased ion leakage from the leaves. Higher amounts of metal caions tended to leak from the leaves of stressed compared to untreated plants and the overall electrolyte leakage from PEG-treated samples was also greater. It was concluded that the capacity for better water retention could be connected with the retention of potassium ions, which participate in many cellular processes and represent the major osmotic solute in plant cells. Enhanced electrolyte leakage from stressed leaves could be indicative of altered membrane permeability or could be connected with specific protective mechanisms involved in the process of osmotic adjustment at the cellular level.

Key words: *cell membranes, electrolyte leakage, metal cations, polyethylene glycol, wheat.*

Water deficit severely inhibits plant growth and development thus limiting production and performance of cultivated plants. Dehydration causes wilting and decrease in cell turgor and thus contributes to the increasing of ion concentration in the cytosol. When plants are exposed to water deprivation certain inorganic ions and so called compatible solutes tend to accumulate in the cytosol in a process known as osmotic adjustment (Filek et al., 2012; Hare et al., 1998). These osmolytes lower cellular osmotic potential, allow turgor maintenance and protect biomacromolecules against destabilizing effects of oxidative stress.

Study of physiological processes in plants under suboptimal or stress conditions could contribute to our knowledge of metabolic plasticity and could reveal some of the mechanisms involved in overcoming the effects of unfavorable climatic conditions. A common and widely applied alternative to measuring drought stress response in the field is to study the reaction of plants to polyethylene glycol (PEG)-induced osmotic stress under laboratory conditions. The use of PEG solutions for the induction of osmotic stress provides an option for precise control of the degree of dehydration within a wide range of osmotic potentials (Filek et al., 2012).

Plasma membranes are the first receptors of stress and they can protect the cell through modifications that affect perception and rigidity of cellular structures (Farooq and Azam 2006). Changes in membrane organization and composition lead to enhanced electrolyte leakage which could be determined by conductometric measurement (Bajji et al., 2002). Increased ion leakage has long been used as an indicator of stress impact on cell membrane permeability (Prášil and Zámečník, 1998). The implementation of conductometry as a highly sensitive and precise method is justified for assessment of ion leakage from plant tissues since it offers valuable information of membrane damages caused by various environmental factors (Farooq and Azam 2006; Roy et al. 2009). In numerous investigations this data substantially expands the view of the plant overall physiological status.

Wheat (*Triticum aestivum*, L.) is an important and widely distributed crop which is often exposed to the environmental adversities. Investigating plant stress responses is essential for understanding the mechanisms leading to tolerance and adaptation. The aim of the present study was to assess the effect of osmotic stress on ion accumulation and leakage in young wheat plants and their relation to membrane functioning under normal conditions and dehydration.