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# Mechanisms of drought resistance in common spinach (*Spinacia oleracea* L.) and New Zealand spinach (*Tetragonia tetragonoides* (Pall.) Kuntze) plants under soil dehydration

# Mechanizmy rezistencie k suchu v rastlinách špenátu siateho (*Spinacia oleracea* L.) a štvorbôčiku rozložitého (*Tetragonia tetragonoides* (Pall.) Kuntze) počas dehydratácie pôdy

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## ABSTRACT

In the pot experiments with commercial genotypes of common spinach (*Spinacia oleracea* L.) and New Zealand spinach (*Tetragonia tetragonoides* (Pall.) Kuntze) genotypic differences were studied in the capacity for osmotic adjustment occurred under progressive drought. The degree of plant drought tolerance was characterized through quantification of stomatal conductance, free proline and pigment contents. Water stress negatively affected the water regime of plants of all studied genotypes. Under the well-hydrated conditions, a non-significant genotypic difference was observed in the relative water content (RWC) (P=0.545) and osmotic potential ($\psi_S$) (P=0.637), respectively. The significant genotypic differences were observed in stomatal conductance (P<0.001). In the terminal phase of dehydration cycle, the statistically significant genotype differences (P<0.01) in RWC and $\psi_S$, as well as in the capacity for osmotic adjustment (P=0.032) was recorded. Moreover, a significant correlation between the osmotic adjustment and concentration of proline was measured in all genotypes ($r_P$=0.74, P=0.009). The results showed that proline is an important osmolyte in spinach during the progressive dehydration. It was also observed that the stomata of New Zealand spinach were very sensitive to dehydration. The concentration of chlorophyll pigments, especially Chl *b*, decreased and Chl *a*/ Chl *b* ratio increased under dehydration in all genotypes. Finally, drought resistance of spinach genotypes (especially 'Neptun') can be characterized by the mechanism of dehydration tolerance and plants of New Zealand spinach by the mechanism of dehydration avoidance.

**Keywords:** chlorophyll, drought, drought resistance, osmotic adjustment, proline, spinach, stomata

## ABSTRAKT

V nádobových vegetačných experimentoch s rastlinami genotypov špenátu siateho (*Spinacia oleracea* L.) a genotypu štvorbôčiku rozložitého (*Tetragonia tetragonoides* (Pall.) Kuntze, špenát novozélandský) boli počas postupnej dehydratácie študované odrodové rozdiely v kapacite pre osmotické prispôsobenie. Zároveň sme charakterizovali stupeň tolerancie na pôdne sucho prostredníctvom kvantifikácie prieduchovej vodivosti a obsahu prolínu a pigmentov. Narastajúci vodný stres negatívne ovplyvnil vodný režim rastlín všetkých genotypov. V dobre hydratovaných rastlinách sme zistili štatisticky nepreukazné genotypové rozdiely v hodnote relatívneho obsahu vody (RWC) (P=0,545) a osmotického potenciálu

($\psi_s$) (P=0,637). Signifikantné genotypové rozdiely boli pozorované v prieduchovej vodivosti (P<0,001). V terminálnej fáze dehydratačného cyklu sme zistili štatisticky vysoko preukazné genotypové rozdiely (P<0,01) v hodnotách RWC a $\psi_s$, ako aj v kapacite pre osmotické prispôsobenie (P=0,032). Pozorovali sme preukaznú koreláciu medzi kapacitou pre osmotické prispôsobenie a koncentráciou prolínu pri všetkých genotypoch ($r_P$=0,74, P=0,009). Potvrdzujeme, že v rastlinách špenátu ovplyvnených suchom je prolín významným osmolytom. Tiež bolo pozorované, že prieduchový aparát rastlín štorbôčiku rozložitého bol veľmi citlivý na dehydratáciu. Koncentrácie asimilačných pigmentov, najmä Chl *b*, počas dehydratácie poklesla vo všetkých genotypoch a pomer Chl *a* ku Chl *b* narástol. Záverom môžeme konštatovať, že genotypy špenátu siateho (najmä genotyp 'Neptun') sa vyznačujú mechanizmom tolerovania dehydratácie a na druhej strane rastliny štvorbôčiku rozložitého mechanizmom predchádzania dehydratácii.

**Kľúčové slová:** chlorofyl, osmotické prispôsobenie, prieduchy, prolín, rezistencia k suchu, sucho, špenát

## DETAILNÝ ABSTRAKT

Rast, produktivita a kvalita produkcie zeleniny je ovplyvňovaná mnohými environmentálnymi činiteľmi, pričom sucho sa zaraďuje medzi najvýznamnejšie. Suchovzdornosť rastliny môže byť potenciálne dosiahnutá dvoma základnými stratégiami, a to predchádzaním dehydratácii, alebo jej tolerovaním. V nádobových vegetačných experimentoch s rastlinami troch genotypov špenátu siateho (*Spinacia oleracea* L.; genotypy 'Neptun', 'Popey' a 'Matador') a rastlinami štvorbôčiku rozložitého (*Tetragonia tetragonoides* (Pall.) Kuntze, tzv. špenát novozélandský) pestovaných v skleníkových podmienkach sme počas postupnej (15-dňovej) dehydratácie študovali odrodové rozdiely v kapacite pre osmotické prispôsobenie (OA), ktoré je výsledkom aktívnej akumulácie osmoticky aktívnych látok v cytoplazme buniek. Zároveň sme charakterizovali stupeň tolerancie rastlín na pôdne sucho prostredníctvom kvantifikácie prieduchovej vodivosti a obsahu prolínu a asimilačných pigmentov v listoch. Práve koncentrácia asimilačných pigmentov v listoch rastlín špenátu je aj významným kvalitatívnym parametrom biomasy. Rastliny špenátu siateho ako celosvetovo populárnej listovej zeleniny, sa počas celej vegetačnej doby vyznačujú vysokými nárokmi na vlahu. Už v minulosti boli pozorované genotypové rozdiely v citlivosti rastlín špenátu na nedostatok vody. Na druhej strane rastliny štvorbôčiku rozložitého sa síce považujú za rastlinu s vysokým nárokom na vodu, ale vyššou schopnosťou tolerovať vyššiu teplotu. Cieľom práce bolo preto študovať dynamiku odpovedí vodného režimu rastliny a stabilitu asimilačných pigmentov rôznych genotypov špenátu siateho a rastlín štvorbôčiku rozložitého na prehlbujúci sa deficit vody v pôdnom substráte, ako aj charakterizovať genotypovú mieru rezistencie k suchu prostredníctvom reakcie OA a koncentrácie prolínu. V dobre hydratovaných rastlinách sme zistili štatisticky nepreukazné rozdiely medzi študovanými genotypmi v hodnote relatívneho obsahu vody v listoch (RWC) (P=0,545) a osmotického potenciálu ($\psi_s$) (P=0,637). Na druhej strane boli signifikantné genotypové rozdiely pozorované v prieduchovej vodivosti ($g_s$) (P<0,001). Narastajúci vodný stres počas postupnej dehydratácie negatívne ovplyvnil vodný režim rastlín všetkých genotypov. V terminálnej fáze dehydratačného cyklu sme zistili štatisticky vysoko preukazné genotypové rozdiely (P<0,01) v hodnotách RWC a $\psi_s$, ktoré boli realizované z dôvodu dosiahnutia rôznej kapacity pre OA. Najvyššiu úroveň OA dosiahli na konci dehydratačného cyklu rastliny genotypu 'Neptun' (0,66±0,11 MPa) a najnižšiu rastliny štvorbôčiku rozložitého (0,24±0,19 MPa). Zistili sme preukaznú koreláciu medzi kapacitou pre osmotické prispôsobenie a koncentráciou prolínu pri všetkých genotypoch od úrovne OA okolo 2,0 MPa (rP=0,74, P=0,009). Toto zistenie potvrdzuje, že aj pri rastlinách špenátu je akumulácia prolínu počas dehydratácie významnou osmoprotekčnou reakciou. Tiež bolo pozorované, že prieduchový aparát listov štorbôčiku bol veľmi citlivý na dehydratáciu. V listoch špenátu pokles RWC počas dehydratácie spôsobil pokles koncentrácie asimilačných pigmentov, najmä Chl b, čo sa prejavilo nárastom pomeru Chl *a* / Chl *b* z priemernej úrovne 2,62±0,33 v dobre hydratovaných rastlinách na úroveň 5,74±0,96 v genotype 'Matador' na konci dehydratačného cyklu. Rastliny špenátu novozélandského dosiahli na konci dehydratačného cyklu pomer Chl *a* / Chl *b* na úrovni 4,41±0,85. Záverom môžeme konštatovať, že zo sledovaných genotypov špenátu v experimente s postupnou dehydratáciou najvyššiu mieru rezistencie k suchu ukázal genotyp špenátu siateho 'Neptun'. Tento genotyp počas dehydratačného cyklu indukoval najvyššiu úroveň OA, čo umožnilo bunkám asimilačného aparátu udržať najviac vody (najvyššie RWC) a oddialiť degradáciu pigmentov a nástup senescencie listov pri súčasnej vysokej $g_s$ listu. Na druhej strane, rastliny štvorbôčiku rozložitého uplatňujú v suchovzdornosti najmä mechanizmus predchádzania dehydratácii.

## INTRODUCTION

Growth, productivity and quality of crop production are frequently affected by numerous environmental factors. Drought is one of the most important stress situations in arid and semi-arid production areas of the world (Kumar et al., 2012). Ability to adapt to conditions of water shortage requires changes at all hierarchy levels of a plant, from the molecular and biochemical to physiological and morphological modifications (Chaves et al., 2002; Lawlor and Tezara, 2009). It is well documented, that the drought resistance of plants is a polygenic complex trait (Munns, 2002). Hydration of plant cells and tissues directly as well as indirectly affects many physiological processes. The most sensitive processes to dehydration are cell elongation, stomatal conductivity and diffusion processes within the leaves, e.g. photosynthetic carbon assimilation (Chaves et al., 2002; Olsovska et al., 2016; Martin-StPaul et al., 2016). As it is well known from past few decades, plant drought resistance can be realized via two physiological ways, dehydration avoidance or tolerance (Levitt, 1980). The dehydration avoidance is achieved mainly through the reduced water loss by transpiration or better extraction of water from the soil by a robust root system. On the other hand, the dehydration tolerance allows to maintain water in the cell symplast. Osmotic adjustment is one of these mechanisms allowing cell adaptation to dehydration (Morgan, 1984). It is realized through an active accumulation of compatible solutes, e.g. proline (Hayat et al., 2012; Svetleva et al., 2012). The process of osmotic adjustment helps to maintain turgor of cells during the progressive reduction of tissue water potential, and it supports the activity of physiological processes, mainly photosynthetic exchange of gases (Wullschleger and Oosterhuis, 1991; Kovar et al., 2003).

Spinach belongs to the group of widely popular leafy vegetables with a high content of dietary valuable substances (Phillips and Rix, 1993). It is characterized by a high transpiration coefficient (Tc=680–865, and crop coefficient Kc=0.70–0.90; Piccinni et al., 2009) and shallow, extensive branching root system (Schenk et al., 1991). These traits resulted in high moisture requirements during the vegetative stage of growth, when plants produce their above-ground biomass in relatively short time (Pevná, 1985; Phillips and Rix, 1993). Still not so abundant, however increasingly more popular in the world becomes now New Zealand spinach (*Tetragonia tetragonoides* (Pall.) Kuntze), another leafy vegetable type. New Zealand spinach is distributed widely from the tropical to temperate areas (Matraszek, 2008). It is a day-neutral plant, so it can be cultivated for summer harvest (Phillips and Rix, 1993). Kato et al. (1985) has referred that New Zealand spinach is also successfully used as a medicinal plant. In comparison with common spinach, the New Zealand spinach is tolerant to higher temperatures, but it has similarly high moisture requirements (Yousif et al., 2010). Drought stress reduces leaf production, leaf area and leaf thickness in both vegetable species. A decrease of soil moisture reduces synthesis and / or accelerates degradation of chlorophylls. This process can finally limit the productivity, as well as dietary and sensoric quality of produced biomass in both common and New Zealand spinach species (Nishihara et al., 2001).

The objective of the work was to measure dynamic physiological responses of common spinach and New Zealand spinach genotypes under progressive soil dehydration, as well as to characterize their prevailing strategy of drought resistance based on the osmotic adjustment ability, stomatal conductance and free proline content quantifications.

## MATERIALS AND METHODS

In the pot vegetation experiment with controlled hydration of soil substrate three genotypes of common spinach (*Spinacia oleracea* L.; varieties ‘Neptun’, ‘Popey’ and ‘Matador’) and plants of New Zealand spinach (*Tetragonia tetragonoides* (Pall.) Kuntze) were tested. Plants (4 per pot) were grown in plastic pots (0.25×0.25 m; 2.8 kg substrate per pot) with TS-1 substrate (Klasmann Deilmann, Germany). The seed imbibition was carried out by immersion of seeds in water at 24 °C for 24 h in the dark. Plants were grown in the greenhouse conditions in spring 2013 (daily mean temperature 26°C and relative humidity 55%).

Soil moisture in each pot was controlled by gravimetric method and adjusted to the level of 70% of soil water capacity. In selected containers (five pots for each genotypes), 42-days after plant emergence the dehydration of soil and plants was induced by withholding water in the course of 15 days. Related physiological parameters were simultaneously quantified on the mature and fully developed leaves.

Relative water content (RWC; %) was determined by gravimetric method according to Barr and Weatherley (1962) with 4 h saturation of leaf samples in water at 4 °C in the dark. RWC was calculated as: RWC=[(FW-DW)/(SW-DW)]×100, where FW is fresh weight, DW is dry weight and SW is the weight after full saturation of leaf samples. The osmotic potential ($\psi_s$; MPa) of a leaf sample (Ø=6.0 mm) was determined by psychrometric method, using the psychrometric chamber C-52, as attached to hydrometer PsyPro (Wescor, USA). The humidity and temperature equilibration of leaf samples within the air space of a chamber was set up on 60 min and the chilling time on 16 sec. Calibration of C-52 chambers was done with KCl solutions. The osmotic adjustment (OA; MPa) was determined by means of full turgor method according to Ludlow et al. (1983). Osmotic adjustment was calculated as a difference between the $\psi_s$ of non-stressed and stressed plants, both calculated to the osmotic potential at full turgor ($\psi_s^{100}$) according to the formula: $\psi_s^{100}=\psi_s\times[(RWC- B)/(100-B)]$, where the component B represents apoplastic water fraction with the value of 20.0 as determined for the calculation. Measurements RWC and $\psi_s$ were taken for 5 plants using fully expanded mature leaves per genotype and treatment.

Stomatal conductance ($g_s$) on adaxial side of leaves was measured with diffussion porometer SC-1 (Decagon, USA). Level of $g_s$ was calculated as average of three measurement on different position of mature leaf blade (5th mature leaf counted from plant base). Concentration of free proline (Pro; μmol/g fresh weight) was analysed by ninhydrin method according to Bates et al. (1973) and determined spectrophotometrically (Jenway 6405; Bibby Scientific, UK) at 520 nm.

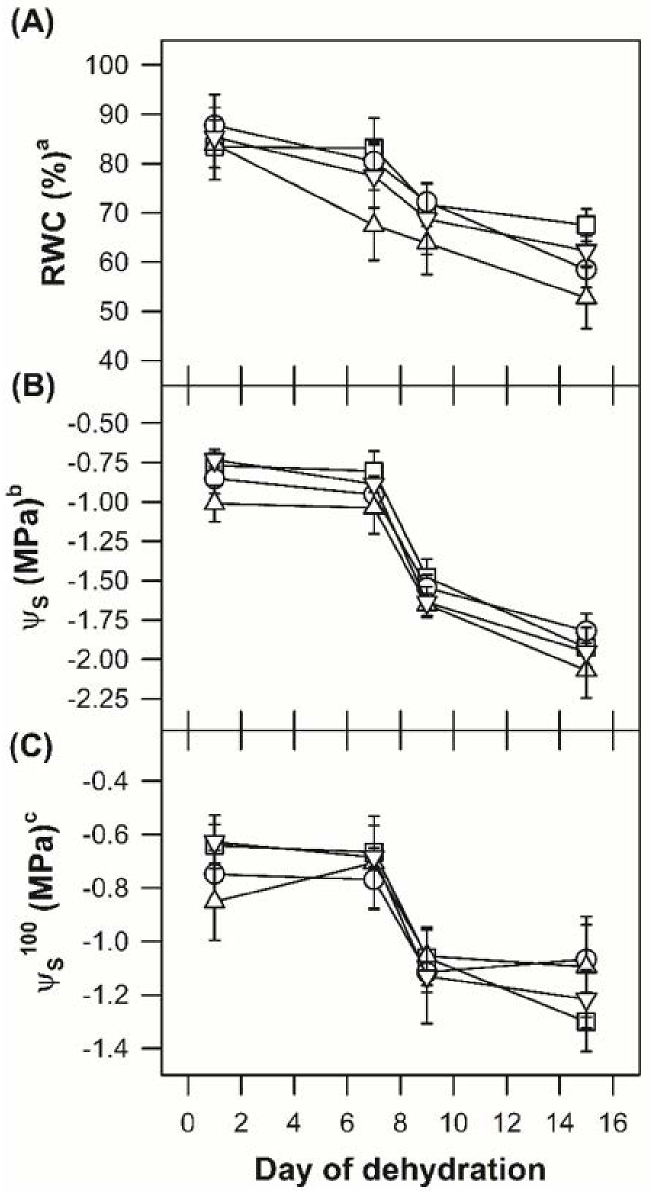
The concentration of pigments (chlorophyll a, chlorophyll b and total carotenoids; mg/m$^2$ leaf area) was measured in 80% acetone extract according to Porra et al. (1989).

Samples for biochemical analysis were taken for 5 plant using mature leaves per genotype and treatment.

Experimental setup of plant cultivation was full randomized. Briefly, forty pots with plants were before dehydration cycle start divided into two groups (well-watered and water stress), five pots for each genotypes and treatment. The data represent means±SE. The experimental data were statistically analysed by the Tukey HSD post hoc test using the Statistica 10 software (StatSoft, 2011).

## RESULTS AND DISCUSSION

Under natural conditions of cultivation of horticultural plants the drought is a very frequently occurring phenomenon, which can limit their growth, productivity and yield quality (Kumar et al., 2012). Phillips and Rix (1993) and Yousif et al (2010) reported in their studies, that plants of common spinach and New Zealand spinach are considered as thermo- and drought-tolerant. However, it is well known that strategies for drought resistance are multi-genetically related, with a high degree of phenotypic plasticity (Munns, 2002). In experiment the well hydrated plants showed a statistically non-significant genotypic differences in relative water content (RWC) and osmotic potential ($\psi_s$) of mature leaves (85.11±5.35% and -0.84±0.14 MPa), respectively (Figure 1A and 1B). Similar values of RWC and $\psi_s$ in leaves of well- watered spinach plants were observed in the experiment of Yousif et al. (2010). Moreover, non-significant genotypic differences in concentration of free proline in the cell symplast was observed, with the mean value of 0.11±0.04 μmol/g (Figure 2B), and significant differences ($P<0.001$) in stomatal conductance ($g_s$) between the leaves of common spinach and New Zealand spinach (681±36 mmol/m$^2$s for common spinach and 752±24 mmol/m$^2$s for New Zealand spinach, respectively) (Figure 3A). The mature leaves of well hydrated New Zealand spinach plants showed significantly (P<0.022) higher concentration of chlorophylls (Chl *a+b*) compared to leaves of common spinach plants (511.25±24.91 vs. 483.55±24.62 mg/m$^2$) (Figure 5C), which resulted in significantly dark green colour of the leaves.

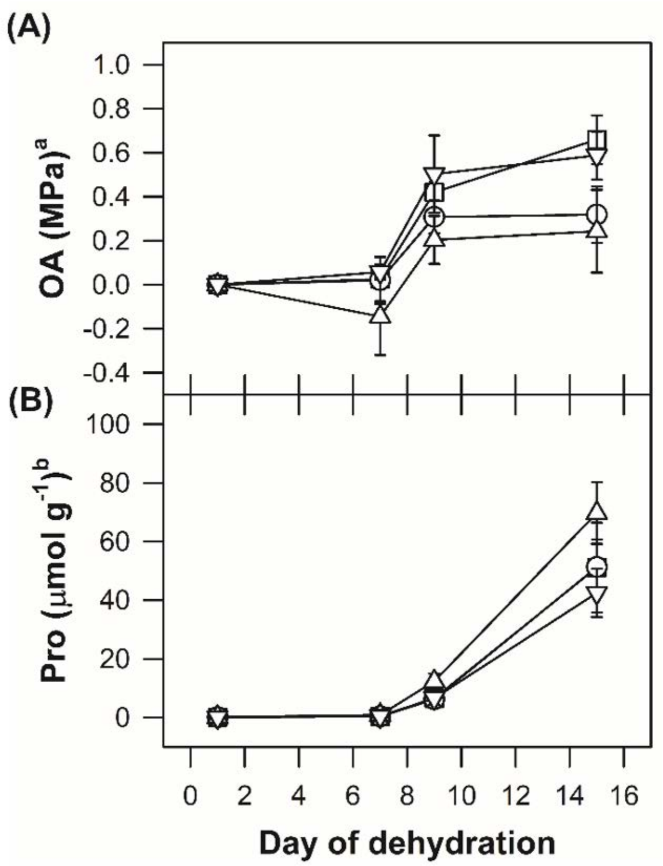


Symbols: ´Neptun´ – square, ´Popey´ – circle, ´Matador´ – triangle down, ´New Zealand spinach´ – triangle up. Each point represents mean ± standard deviation, n = 5

Symboly: ´Neptun´ – štvorec, ´Popey´ – kruh, ´Matador´ – spodný trojuholník, ´špenát novozélandský´– vrchný trojuholník. Každý bod reprezentuje priemer so štandardnou odchýlkou, n = 5

**Figure 1.** Dynamics of A) relative water content (RWC), (B) osmotic potential (ψs), and (C) osmotic potential at full turgor (ψs$^{100}$)

**Obrázok 1.** Dynamika (A) relatívneho obsahu vody (RWC), (B) osmotického potenciálu (ψs) a (C) osmotického potenciálu v plnom turgore (ψs$^{100}$)



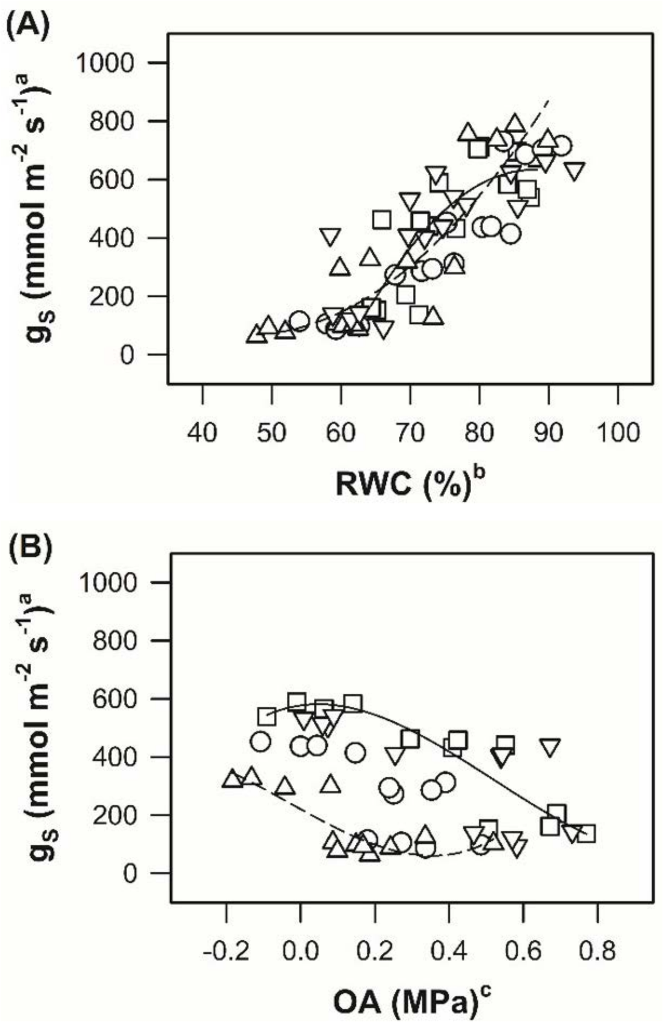
Symbols: ´Neptun´ – square, ´Popey´ – circle, ´Matador´ – triangle down, ´New Zealand spinach´ – triangle up. Each point represents mean ± standard deviation, n = 5

Symboly: ´Neptun´ – štvorec, ´Popey´ – kruh, ´Matador´ – spodný trojuholník, ´špenát novozélandský´– vrchný trojuholník. Každý bod reprezentuje priemer so štandardnou odchýlkou, n = 5

**Figure 2.** Dynamics of (A) capacity for osmotic adjustment (OA), and (B) proline content (Pro)

**Obrázok 2.** Dynamika (A) capacity pre osmotické prispôsobenie (OA) a (B) obsahu prolínu (Pro)

During the dehydration cycle the continual declines of RWC and ψs were observed (Figure 1) with significant (P<0.01) genotypic differences. In terminal phase of applied water stress the lowest value of RWC was recorded in New Zealand spinach (52.75±3.31%) and the highest value in genotype ‘Neptun’ (67.53±3.31%). Similarly, a decrease of ψs was observed (Figure 1B). This kinetics of RWC and ψs declines under soil dehydration agrees with the observation of Yousif et al. (2010). Thus, it can be assumed that different kinetics of RWC decline in leaves of common spinach and New Zealand spinach may be resulted from varying size and distribution of hydraulic conductivity of root tissues, leaf area size, sensitivity of stomata to the water stress and rate of transpiration, respectively (Kage et al., 2004). The osmotic potential at full turgor ($\psi_s^{100}$), which takes account for an active accumulation of osmotically active substances, was mostly decreased in genotype 'Neptun' (-1.30±0.11 MPa, Figure 1C). This leads to a significant increase (P<0.001) of osmotic adjustment (OA) in the genotype (0.66±0.11 MPa), as compared with New Zealand spinach (0.24±0.19 MPa, Figure 2A).



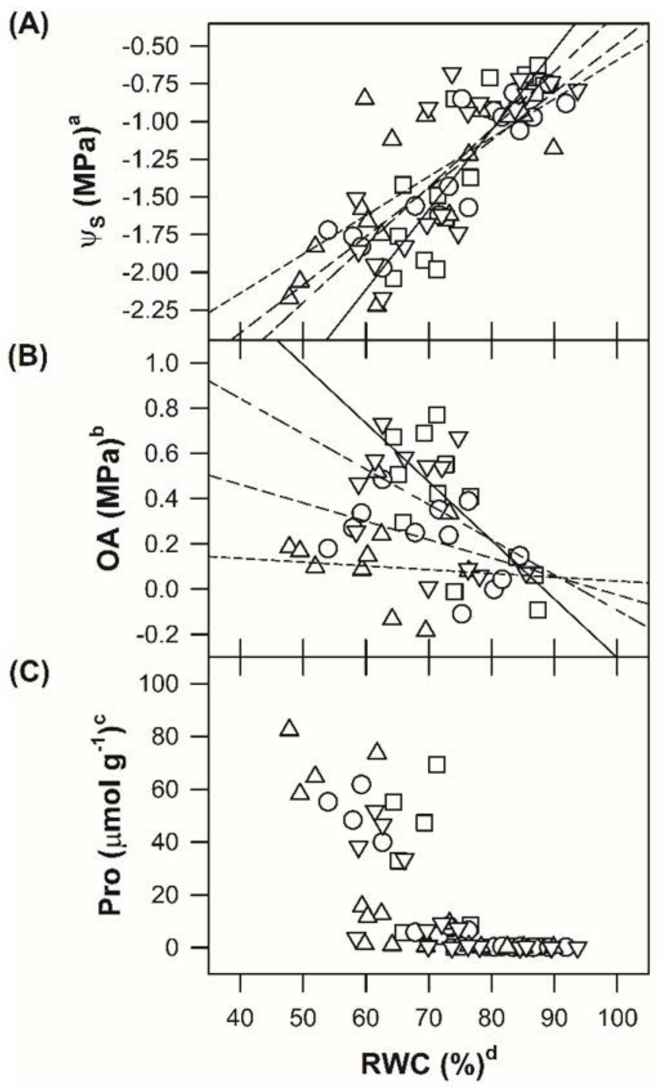
Symbols: ´Neptun´ – square and solid line, ´Popey´ – circle, ´Matador´ – triangle down, ´New Zealand spinach´ – triangle up dashed line

Symboly: ´Neptun´ – štvorec a plná čiara, ´Popey´ – kruh, ´Matador´ – spodný trojuholník, ´špenát novozélandský´ – vrchný trojuholník a prerušovaná čiara

**Figure 3.** Changes in stomatal conductance (gs) in relation to (A) relative water content (RWC) and (B) capacity for osmotic adjustment (OA) of genotypes under drought stress
**Obrázok 3.** Zmeny v prieduchovej vodivosti (gs) vo vzťahu k (A) relatívnemu obsahu vody (RWC) a (B) kapacite pre osmotické prispôsobenie (OA) genotypov počas sucha

Stomata closure and reduction of transpiration rate are a general response of plants to drought (Chaves et al., 2002; Luo et al., 2017). The progressively induced drought stress resulted in a slower bi-phasic decline of $g_s$ in all spinach genotypes, however mostly evident in genotype 'Neptun'. On the other hand, in New Zealand spinach the $g_s$ continually decreased with high slope of regression (Figure 3A). In the terminal phase of drought the New Zealand spinach genotype reached the lowest value of $g_s$ (84.1±17.3 mmol/m$^2$s) among all studied genotypes. On the contrary, genotype 'Neptun' reached the highest value of $g_s$ (164.1±29.9 mmol/m$^2$s, P<0.001).

The genotypic differences in water use at common spinach and New Zealand spinach can be seen from the modifications of regression slopes between $\psi_s$ and RWC (Figure 4A). Many authors consider the RWC decrease up to 70% as mild dehydration (Morgan, 1984; Lawlor and Tezara, 2009; Olsovska et al., 2016). When used the regression type of relationship, it was observed that at 70% RWC the genotype 'Neptun' reached $\psi_s$ of -1.59 MPa and OA of 0.47 MPa. In New Zealand spinach the $\psi_s$ was -1.37 and OA 0.08 MPa, respectively. The data can confirm that New Zealand spinach plants had under progressive dehydration a low capacity for the OA and their RWC drop was resulted from the loss of free water fraction by transpiration. Both inter- and intra-specific differences in capacity for OA (relationship between the $\psi_s$ and OA to RWC, respectively; Figure 4B) are accompanied by different mechanisms of phenotypic adaptations during active responses of plants to dehydration, as postulated by Zhang et al. (1999), Chimenti et al. (2002), and Kovar et al. (2003). It is well documented that OA is the mechanism important for the maintenance of cell turgor and for delay of full closure of stomata (Zhang et al., 1999). Thus, the decline of RWC at the first phase of drought in New Zealand spinach is accompanied with the high stomatal conductance, followed by a considerable reduction of $g_s$, as RWC dropped under 80% (dashed line in Figure 3A). Distinct advantages of higher capacity for OA keeping stomata open in genotype 'Neptun' can be seen from Figure 3B (solid line).



Symbols: ´Neptun´ – square and solid line, ´Popey´ – circle and short dash line, ´Matador´ – triangle down and long dash line, ´New Zealand spinach´ – triangle up and short-short dash line

Symboly: ´Neptun´ – štvorec a plná čiara, ´Popey´ – kruh a krátka prerušovaná čiara, ´Matador´ – spodný trojuholník a dlhá prerušovaná čiara, ´špenát novozélandský´ – vrchný trojuholník a krátko- krátko prerušovaná čiara

Linear regressions for (A): ´Neptun´ y=-5.291+(0.0529x), $r_P$=0.863, P<0.001; ´Popey´ y=- 3.679+(0.0319x), $r_P$=0.870, P<0.001; ´Matador´ y=-4.117+(0.0383x), $r_P$=0.782, P<0.001 and ´New Zealand spinach´ y=-3.168+(0.0257x), $r_P$=0.712, P=0.002

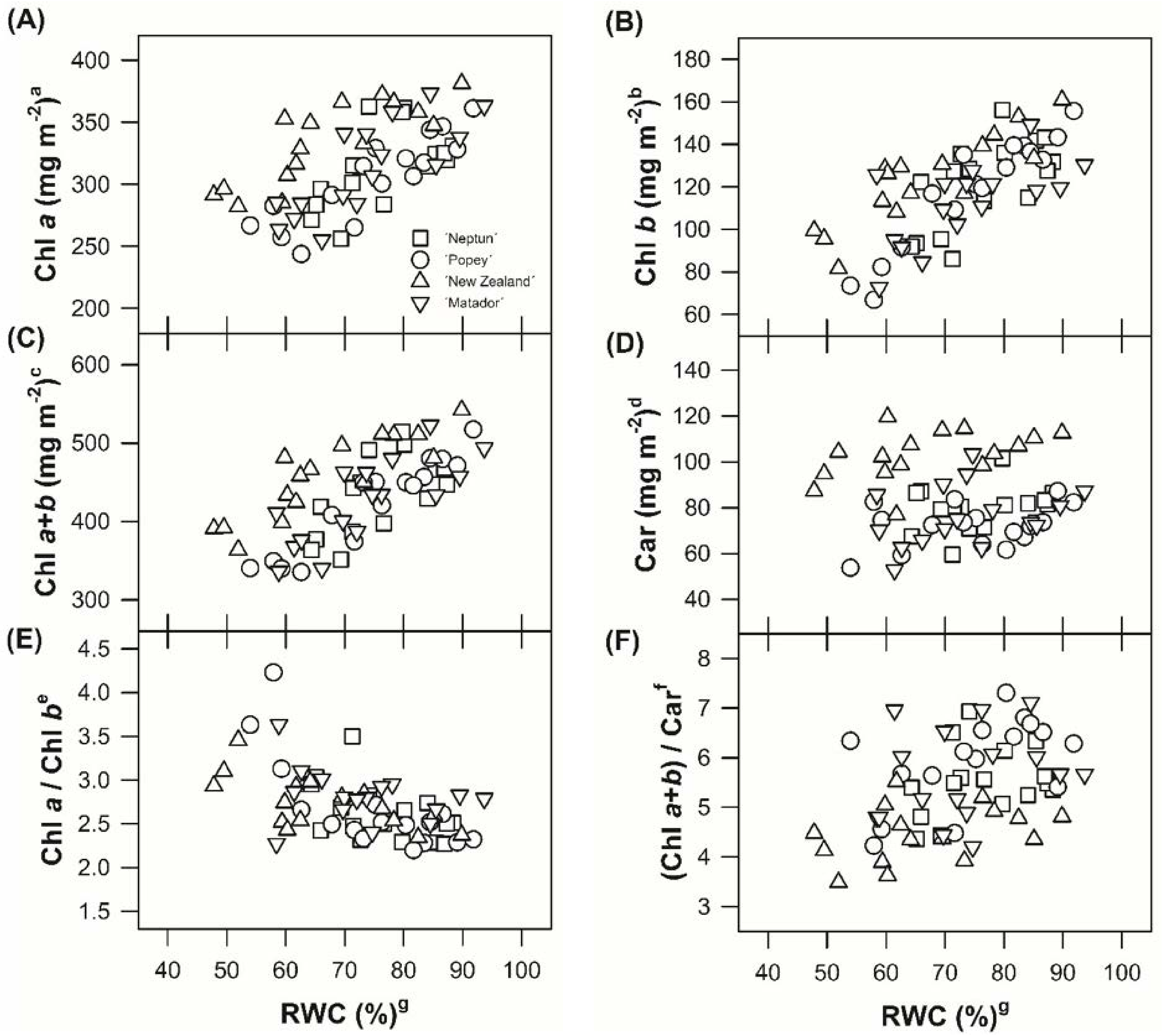
Linear regression for (B): ´Neptun´ y=2.287-(0.0259x), $r_P$=-0.728, P=0.007; ´Popey´ y=0.788- (0.0081x), $r_P$=-0.472, P=0.121; ´Matador´ y=1.465-(0.0156x), $r_P$=-0.489, P=0.107 and ´New Zealand spinach´ y=0.203-(0.0017x), $r_P$=-0.064, P=0.845

**Figure 4.** Changes in (A) osmotic potential (ψs), (B) capacity for osmotic adjustment (OA) and (C) proline content (Pro) in relation to relative water content (RWC) of genotypes under drought stress

**Obrázok 4.** Zmeny v (A) osmotickom potenciáli (ψs), (B) v kapacite pre osmotické prispôsobenie (OA) a v (C) obsahu prolínu (Pro) vo vzťahu k relatívnemu obsahu vody (RWC) genotypov počas sucha

Osmotic adjustment responding to different stress situations is realized through an active accumulation of osmotically active substances (Morgan, 1984). Accumulation of free proline (Pro) in cell symplast plays an important role in OA (Hayat et al., 2012). In experiments with well-hydrated plants, statistically non- significant genotypic differences in the accumulation of Pro was observed, with mean value of 0.11±0.03 µmol/g of fresh leaf weight (Figure 2B). At the end of the dehydration cycle, a significant increase (P<0.001) of Pro concentration was found. The highest value of Pro content was reached in New Zealand spinach (69.73±10.55 µmol/g). However, this concentration was reached at 52.7% RWC. When was matematically converted this absolute concentration to the full turgor status, the $Pro^{100}$ concentration reached 36.81±7.21 µmol/g. The fastest accumulation of Pro was induced in genotype ‘Neptun’ (from ~78% RWC) and the slowest in New Zealand spinach genotype (~63% RWC) (Fig. 4C). Induction of Pro accumulation in leaf tissues under stress conditions at RWC values below 80% RWC was observed by Di Martino et al. (2003).

Is well known, that dehydration of the plant tissue may enhance the structural dis- integration of photosynthetic apparatus (Lawlor and Tezara, 2009). In this experiment, significant (P<0.001) degradation of photosynthetic pigments (Figure 5), mainly chlorophyll *b* (Chl *b*) were observed (Figure 5B). Faster degradation of Chl *b* in the spinach leaves under drought resulted in the increase of Chl *a* to Chl *b* ratio (Figure 5E). This response reflects physiologically a faster induction of light- harvesting antenna complex proteolysis, which results in both the decrease of radiation use efficiency, and perturbations of photosynthetic carbon assimilation processes (Lawlor and Tezara, 2009; Olsovska et al., 2016). Arunyanark with co- workers (2008) showed that chlorophyll stability during drought stress might be a criterion of the selection for drought resistance. Photosynthetic activity in many crops has been shown to be closely linked with chlorophyll content (Epron and Dreyer, 1993). Drought affected chlorophyll content in many crops, e.g. spinach plants (Ekincia et al., 2015).



Symbols: ´Neptun´ – square, ´Popey´ – circle, ´Matador´ – triangle down, ´New Zealand spinach´ – triangle up.

Symboly: ´Neptun´ – štvorec, ´Popey´ – kruh, ´Matador´ – spodný trojuholník, ´špenát novozélandský´ – vrchný trojuholník.

**Figure 5.** Changes in (A) chlorophyll *a* content (Chl *a*), (B) chlorophyll *b* content (Chl *b*), (C) chlorophyll *a+b* content (Chl *a+b*), (D) total carotenoids content (Car), (E) Chl *a* to Chl *b* ratio and (F) Chl *a+b* to Car ratio in relation to relative water content (RWC) of genotypes under drought stress

**Obrázok 5.** Zmeny v (A) obsahu chlorofylu *a* (Chl *a*), (B) obsahu chlorofylu *b* (Chl *b*), (C) obsahu chlorofylu *a+b* (Chl *a+b*), (D) obsahu celkových karotenoidov (Car), (E) pomeru Chl *a* ku Chl *b* a (F) Chl *a+b* ku Car vo vzťahu k relatívnemu obsahu vody (RWC) genotypov počas sucha

However, Zhang and co-workers (2014) did not observed any changes in chlorophyll content in spinach as affected by water deficit. It is so apparent that degradation of green pigments (and the loss of green colours of leaves) in addition to genetic predisposition is dependent on the magnitude and duration of drought stress.

## CONCLUSIONS

Spinach is globally popular leafy vegetables, however, it is relatively water- consuming plant. From this point of view, it is very topical to study its drought tolerance mechanisms for a better knowledge of its biomass production under intensive cultivation systems. Based on the results of experiment is clear that the highest degree of drought tolerance was determined in genotype 'Neptun'. This genotype induced the highest level of osmotic adjustment, allowing to maintain most water in cell symplast under drought (the highest value of RWC), delay the degradation of photosynthetic pigments (Chl *a+b*) and onset of leaf senescence, while maintaining a high $g_s$ of leaves. On the other hand, New Zealand spinach had very sensitive stomata in condition of soil dehydration and a weak osmotic adjustment capacity, indicating the drought avoidance as prevailing mechanism of drought resistance under induced drought.

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