SOLUTE TRANSPORT VIA XYLEM AND PHLOEM IN TWO WHEAT GENOTYPES DIFFERING IN DROUGHT SUSCEPTIBILITY

Feller U.[1,2], I. I. Vaseva[2], B. Yuperlieva-Mateeva[2]*

[1]*Institute of Plant Physiology and Oeschger Centre for Climate Change Research (OCCR), University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland*

[2]*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bldg. 21, 1113 Sofia, Bulgaria*

Summary: More frequent and more severe extreme events (e.g. flooding, drought, heat waves) were predicted from climate models for the next decades. Impacts of such events are relevant for crop productivity. Effects on a whole-plant level are the focus of this study. The influence of an extended drought period on solute allocation via the phloem was compared in two Bulgarian wheat varieties differing in drought susceptibility: "Sadovo" (drought-sensitive) and "Katya" (drought-tolerant). Young plants grown hydroponically on standard nutrient solution were transferred at the beginning of the experiment to fresh medium (controls) or to medium with polyethylene glycol 6000 (artificial drought). Radiolabeled solution (^{57}Co, ^{109}Cd, ^{134}Cs) was introduced via a flap into the lamina of the third or fourth leaf of young plants (youngest fully expanded leaf before stress treatment). Export and allocation of the radiolabel were monitored by gamma spectrometry. ^{57}Co, ^{109}Cd and ^{134}Cs are suitable radionuclides, since they are not metabolized and not released in gaseous form. The exported radionuclides were mainly found in the roots (30-50%) and in the younger leaves (30-60%), while only traces reached the older leaves indicating that only small quantities were transported back from the roots to the shoot via the xylem. A comparable percentage was transported from leaf 4 to leaves 5 and 6 in both varieties under control conditions. A much higher percentage was found in leaf 5 than in leaf 6 of "Katya" subjected to drought, while "Sadovo" was affected in the opposite direction. The results suggest that adaptations in the source/sink network are important for the drought susceptibility of wheat varieties.

*Corresponding author: urs.feller@ips.unibe.ch

INTRODUCTION

Climate models predict for the next decades more frequent and more severe drought periods in some regions as a consequence of Global Change (Frei et al., 2006; IPCC, 2012). Such extreme climate events may considerably decrease yield and represent a risk for agronomic production (IPCC, 2012). These perspectives represent a challenge for genotype selection, agronomic practices and plant breeding in order to avoid disastrous yield losses (Nair, 2014). Crop plants and varieties of a crop may differ considerably in their drought response (Tack et al., 2014). Several research teams addressed physiological drought responses of wheat varieties, since this may become a key issue in the future for some wheat producing regions in Europe as well as on other continents (Erdei et al., 2002; Guoth et al., 2010; Khan et al., 2013; Balla et al., 2014; Bencze et al., 2014). Bulgarian wheat varieties differing in drought tolerance are available (Boyadjieva, 1996; Vassileva et al., 2011). Physiological and morphological differences between some of these genotypes were reported during the past decade (Demirevska et al., 2008a,b; Vassileva et al., 2009; Simova-Stoilova et al., 2010; Grigorova et al., 2011a,b, 2012). Most of these characterizations refer to gene expression, hormone levels, protein pattern or metabolic activities in some parts of plants exposed to drought, while redistribution processes were so far not or only marginally considered (Vaseva et al., 2010; Vassileva et al., 2011; Feller and Vaseva, 2014). Effects of drought were reported for the sink structure in barley (Martinkova, 2013) and for dry matter accumulation in maturing wheat grains

(Plaut et al., 2004). The actual knowledge concerning drought effects on phloem transport in various plant species was reviewed recently in a broader context (Sevanto, 2014).

The export of solutes from a leaf via the phloem and the allocation to other plant parts can be investigated after introducing stable markers such as rubidium (Schenk and Feller, 1990) or radiolabels (Riesen and Feller, 2005) via a flap into a wheat leaf lamina. Cations such Rb^+, $^{134}Cs^+$ or radiolabeled heavy metals were found to be suitable in this context, since in contrast to ^{14}C- or ^{35}S-labeled compounds they are not metabolized and not released in gaseous form. ^{109}Cd is slowly exported from a labeled leaf lamina and reaches the roots and the youngest leaves via phloem (Riesen and Feller, 2005). From the roots it may be exported through xylem to the older leaves which are phloem sources themselves. Interestingly ^{57}Co transported to the roots via phloem is not (or is only in trace amounts) loaded into the xylem and delivered to transpiring leaves with the transpiration stream (Riesen and Feller, 2005). ^{134}Cs, like rubidium, is highly phloem-mobile and it is redistributed repeatedly (Schenk and Feller, 1990). The different translocation properties may be helpful to investigate effects of abiotic stresses on long-distance translocation.

In soil cultures the analyses of the roots is extremely difficult and often impossible. Furthermore the water potential in the soil may not decrease homogeneously causing patchiness. Polyethylene glycol can be added to the root medium in hydroponic cultures to lower the water potential for physiological experiments including analyses of the root system and the solute release from roots (Burlyn and Kaufmann,

1973; Steuter, 1981). The aim of the work presented here was to identify drought effects on the sink structure in wheat and to compare the solute allocation in two Bulgarian wheat varieties differing in drought susceptibility.

MATERIALS AND METHODS

Plant Material

The experiments were performed with two Bulgarian wheat (*Triticum aestivum* L.) varieties differing in drought susceptibility. "Sadovo" represents an established standard variety with good yield, while "Katya" is a more drought-tolerant variety (Boyadjieva, 1996; Vassileva et al., 2011).

Nutrient media and culture conditions

Dry seeds were germinated on tissue paper with tap water for 4 days in darkness and afterwards for 3 days in a light/dark cycle (14 h light, 300 μE m^{-2} s^{-1}, 22°C; 10 h darkness, 16°C). Afterwards the plants were grown hydroponically for one week on 1:2 diluted and then on full strength standard nutrient medium according to Page at al. (2012) under the light regime mentioned above. The full-strength nutrient medium contained 5.8 mM KH_2PO_4, 3 mM $MgSO_4$, 1.3 mM $Ca(NO_3)_2$, 0.88 mM KNO_3, 64 μM Fe-EDDHA, 4.93 μM H_3BO_3, 0.98 μM $MnCl_2$, 0.2 μM Na_2MoO_4, 0.1 μM $ZnSO_4$, 0.11 μM $CuSO_4$ and 0.05 μM $Ni(NO_3)_2$.

Experimental setup

One experiment was started with young plants (17 days old). "Sadovo" (2 plants) and "Katya" (2 plants) were incubated on the same pots with 150 mL standard nutrient medium (controls) or

150 mL standard nutrient medium plus 15 g PEG 6000 (+ PEG; artificial drought stress). The radiolabels (^{109}Cd and ^{57}Co) added to 0.5 mL standard nutrient medium were introduced via a flap into the lamina of the third-oldest leaf as described previously (Riesen and Feller, 2005). One set of plants was labeled at day 4 (collected at day 11; moderate stress) and another set of plants at day 11 (collected at day 18; severe stress). The nutrient solution was not replaced during the long stress phase, but in order to avoid nutrient depletion the following nutrient supply solutions were added to each pot (controls and + PEG) at day 7, day 11 and day 14: 2 mL 100 mM $Ca(NO_3)_2$, 2 mL 200 mM KNO_3 and 0.1 mL micronutrient solution (containing 1.96 mM $MnCl_2$, 9.86 mM H_3BO_3, 0.34 mM $ZnSO_4$, 0.4 mM Na_2MoO_4, 0.1 mM $Ni(NO_3)_2$ and 0.22 mM $CuSO_4$). Stressed plants did not receive additional water in the course of the experiment, while control pots were supplemented with 100 mL deionized water at day 7, day 11 and day 14. Four samples from separate pots were analyzed for each treatment (independent replicates).

Another experiment was started with older plants (29 d old). "Sadovo" and "Katya" were incubated in separate pots (1 labeled plant and 2 unlabeled controls) with 150 mL standard nutrient medium (controls) or on 150 mL standard nutrient medium plus 15 g PEG 6000 (+ PEG; artificial drought). The radiolabels (^{109}Cd, ^{57}Co and ^{134}Cs) added to 1 mL standard nutrient solution were introduced into the lamina via a flap of the fourth-oldest leaf at day 0 (start of the drought treatment) as described previously (Riesen and Feller, 2005). Controls were supplemented with 50 mL water per pot on day 3 and on

day 6. Plants were exposed to drought stress for 7 days. At the beginning of the recovery phase the old nutrient solution was replaced by 170 mL fresh standard nutrient medium in all pots. During the recovery water was added to control and former + PEG pots when the level of the media dropped below 70%. Transpiration was determined gravimetrically taking into account the extra volumes of water given to each pot. The water potential in "+ PEG" nutrient medium was calculated according to Burlyn and Kaufmann (1973). Four replicate pots for both genotypes and both treatments (controls, + PEG) were analyzed shortly before the end of the stress phase (day 6) and after the recovery phase (day 14).

Radionuclide quantification

After collecting the plant material the roots were rinsed in deionized water and the various plant parts were placed in plastic tubes and dried. The radiolabels were quantified with a   γ-spectrometer (1480 Wizard 3"; Wallac Oy, Turku, Finland). Four independent replicates were analyzed. Significant differences between "Sadovo" and "Katya" in the same treatment were identified with Student's t-test.

RESULTS

The export of ^{109}Cd and of ^{57}Co from the third-oldest (youngest fully expanded) leaf of young plants of two Bulgarian wheat varieties grown together on the same pots ("Sadovo", drought-suceptible and "Katya", drought-tolerant) under control conditions and under artificial drought is illustrated in Fig. 1. During the first labeling period (day 4 to day 11)

significant differences between "Sadovo" and "Katya" were observed for the controls, but not for the plants exposed to artificial drought (Fig. 1, left panel) indicating that the two varieties differ to some extent in their solute allocation to the roots of non-stressed plants. However, in both genotypes a higher percentage of the radiolabels ^{109}Cd and ^{57}Co was exported to the youngest leaves under drought than under control conditions while lower amounts of the tracers were detected in the root system. This drought effect was observed also during the second labeling period (although to a lesser extent) - when the stress was more severe as a consequence of water uptake (but not of PEG) from the nutrient medium causing a more negative water potential in the root zone (Fig. 1, right panel). It became evident that artificial drought altered the solute allocation in both varieties similarly in favor of the youngest leaves, while less solutes were transported to the roots.

For more detailed studies the two varieties were grown on separate pots (containing one labeled plant and two unlabeled controls) in order to detect tracer release from the roots into the medium. After the stress period (7 days treatment with PEG) all plant were transferred to fresh nutrient solution (recovery phase). Changes in the transpiration rates during the stress and the subsequent recovery are documented in Table 1. Although these values were initially slightly higher in the non-treated "Sadovo" plants they reached similar levels in the controls of both varieties at the end of the experiment. Under artificial drought the transpiration rate decreased in "Katya" more slowly than in "Sadovo" and re-increased more
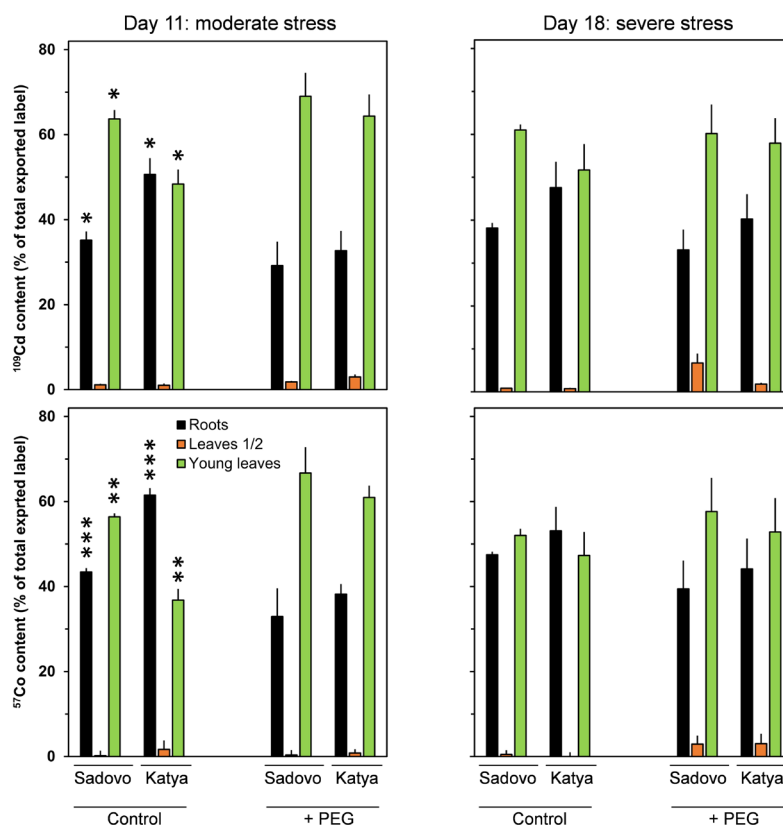
Figure 1. Redistribution of ^{109}Cd and ^{57}Co from leaf 3 to other parts of control and drought-stressed (+ PEG) wheat plants. At the beginning of the experiments plants were 17 days old (day 0). The radiolabels (^{109}Cd and ^{57}Co) were introduced via flap into the lamina of leaf 3 at day 4 (plants collected at day 11) or at day 11 (plants collected at day 18). Radiolabels were quantified in roots, the two oldest leaves together (Leaves 1/2) and in the young leaves (leaves 4, 5 and 6) by γ-spectrometry. The radiolabel retained in leaf 3 is not shown. Data are expressed in % of total exported label. Means+SE of 4 replicates are shown. Significant differences between "Sadovo" and "Katya" for the same treatment at P<0.05 (*), P<0.01 (**) and P<0.001 (***) are indicated.

rapidly in "Katya" than in "Sadovo" during the recovery phase. Water potential in the medium decreased gradually during the stress phase since the plants took up water (due to transpiration) but not the dissolved PEG (Table 2). The export of radiolabels from leaf 4 to other plant parts during stress and the subsequent recovery phase are documented in Fig. 2. The transport to the roots was not significantly different between the two genotypes during the stress period and recovery, but the allocation to the youngest leaves was significantly different between "Katya" and "Sadovo" under artificial drought. Most of the label in the shoot of "Katya" plants was detected in leaf 5 while in "Sadovo" leaf 6 was the strongest labeled leaf under stress. These results demonstrated that the relative sink strength in various young leaves of the two varieties was differently affected

Table 1. Transpiration rate of two wheat genotypes under artificial drought and during recovery. At the beginning of the experiment (day 0) the plants were 29 days old. After the stress phase (+ PEG) from days 0 to 7 plants were transferred to standard nutrient medium for the recovery phase (days 7 to 14). Means±standard errors of 4 independent replicates are shown.

Genotype	Transpiration rate [ml plant^{-1} d^{-1}]					
Treatment	Days 0 to 3	Days 3 to 6	Days 6 to 7	Days 7 to 9	Days 9 to 13	Days 13 to 14
Sadovo						
Control	8.33±0.27	8.45±0.32	14.51±0.59	13.79±0.69	16.41±0.22	16.77±0.61
+ PEG	5.05±0.41	2.70±0.28	2.21±0.40	5.26±1.09	7.82±0.61	8.77±1.40
Katya						
Control	6.76±0.41	7.57±0.57	11.46±1.23	11.25±1.15	15.62±0.52	16.63±0.56
+ PEG	4.20±0.35	2.88±0.26	2.63±0.63	5.78±0.73	8.44±0.61	10.24±0.82

Table 2. Water potential in the nutrient medium (+ PEG) during the stress phase. Means±standard errors of 4 independent replicates are shown.

Genotype	Water potential [MPa]			
	Day 0	Day 3	Day 6	Day 7
Sadovo	-0.16±0.00	-0.32±0.02	-0.56±0.08	-0.80±0.19
Katya	-0.16±0.00	-0.28±0.01	-0.47±0.04	-0.65±0.09

by drought. Such divergent allocation was consistently observed for the three applied radionuclides. No significant variances between "Sadovo" and "Katya" were detected after the recovery phase, although some trends consistent with the differences mentioned above were still detectable. Tillers of "Katya" plants appeared to be rather strong sinks in controls, while under stress and after the subsequent recovery phase only minor quantities of the radiolabels reached the tillers. Only trace amounts of the labels transported via the phloem to the roots reached the oldest leaves (leaves 1 to 3) via the xylem. As expected, ^{57}Co accumulated predominantly in roots indicating that a considerable percentage of the solutes exported from leaf 4 was transported via the phloem to the roots. The good mobility of ^{134}Cs allowed a continuing redistribution via xylem and phloem. ^{109}Cd was slowly exported from leaf 4 to other plant parts throughout the stress and recovery phases. The further redistribution of ^{109}Cd from the roots to leaves was intermediate between ^{57}Co and ^{134}Cs.

From Table 3 it can be concluded that about 50% of the ^{109}Cd exported from leaf 4 reached the roots where most of it was retained. Only minor ^{109}Cd quantities were released into the medium and taken up by the unlabeled plants or transported via the xylem to the oldest leaves. The data for the two varieties were very
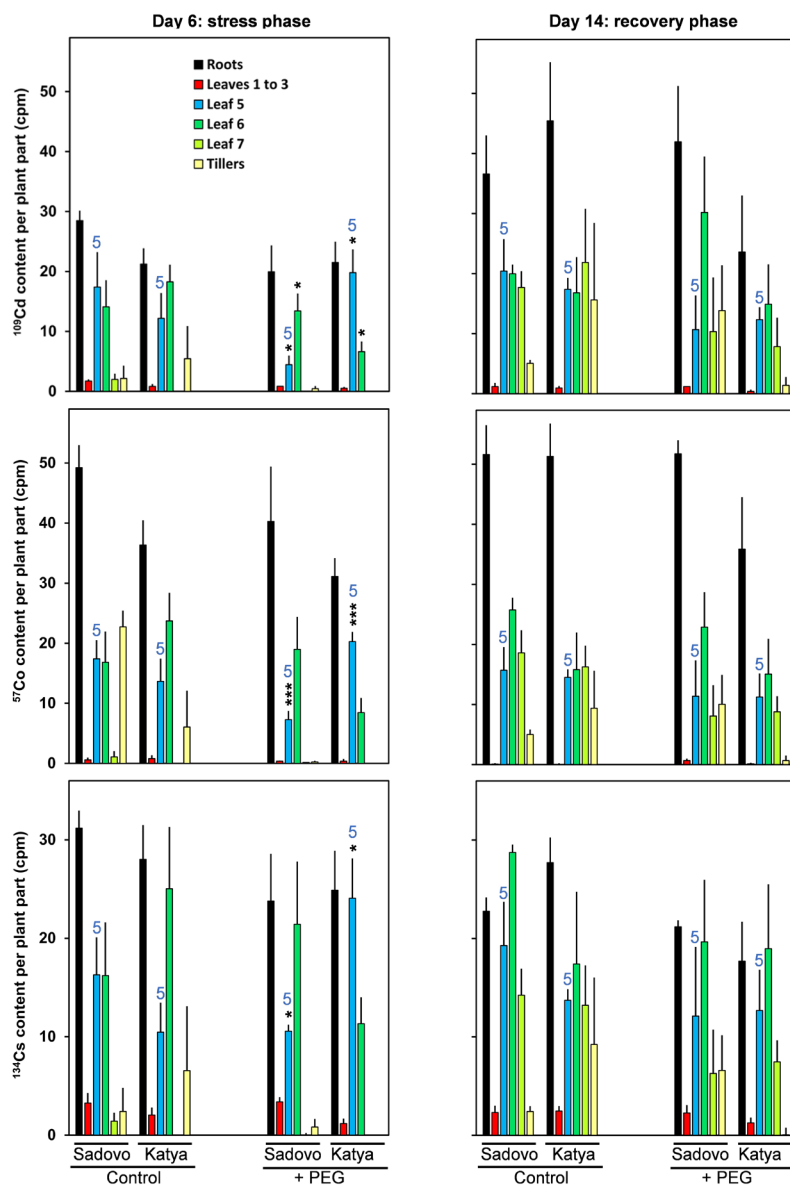
Figure 2. Redistribution of ^{109}Cd, ^{57}Co and ^{134}Cs from leaf 4 to other parts of control and drought-stressed (+ PEG) wheat plants. At the beginning of the experiments the plants were 29 days old. Drought stress lasted for 7 days and then all plants were transferred to standard nutrient medium (recovery phase). The radiolabels (^{109}Cd, ^{57}Co and ^{134}Cs) were introduced via flap into the lamina of leaf 4 at day 0 (start of the drought treatment). Radiolabels were quantified in roots, the three oldest leaves together (Leaves 1 to 3), leaf 5, leaf 6, leaf 7 and tillers by γ-spectrometry. The radiolabel retained in leaf 4 is not shown. Leaf 5 (blue columns) is labeled with "5" to improve legibility. Data are expressed in cpm (counts per minute). Means+SE of 4 replicates are shown. Significant differences between "Sadovo" and "Katya" for the same treatment at P<0.05 (*) and P<0.001 (***) are indicated.

Table 3. Total export of [109]Cd to the roots of two wheat genotypes under artificial drought and during recovery. At the beginning of the experiment (day 0) the plants were 29 days old. The label was introduced via a flap into leaf 4. After the stress phase (+ PEG) from days 0 to 7 plants were transferred to standard nutrient medium for the recovery phase (days 7 to 14). Means±standard errors of 4 independent replicates are shown.

Genotype	[109]Cd content [% of total exported label]				
Treatment	Roots	Leaves 1 to 3	Controls Roots	Controls Shoots	Total plus medium
Sadovo (day 6; stress phase)					
Control	41.6±3.2	2.6±0.6	4.4±0.1	1.0±0.3	50.8±3.0
+ PEG	46.4±5.4	2.3±0.5	3.2±0.8*	0.6±0.3	53.5±4.8
Katya (day 6; stress phase)					
Control	35.4±3.5	1.7±1.0	2.1±1.2	0.4±0.2	40.0±4.1
+ PEG	42.8±1.7	1.0±0.4	0.8±0.7*	0.4±0.2	48.1±0.7
Sadovo (day 14; recovery phase)					
Control	33.4±2.6	1.0±0.4	5.1±0.9	0.7±0.2*	40.7±3.6
+ PEG	35.9±2.8	1.2±0.3	5.8±0.6	0.9±0.3	44.3±3.5
Katya (day 14; recovery phase)					
Control	37.6±3.9	0.9±0.4	3.9±0.8	0.3±0.1*	42.7±2.8
+ PEG	36.6±2.7	0.6±0.6	5.1±0.7	1.3±0.1	43.6±2.8

*Significant differences between Sadovo and Katya at P=0.05.

Table 4. Total export of [57]Co to the roots of two wheat genotypes under artificial drought and during recovery. At the beginning of the experiment (day 0) the plants were 29 days old. The label was introduced via a flap into leaf 4. After the stress phase (+ PEG) from days 0 to 7 plants were transferred to standard nutrient medium for the recovery phase (days 7 to 14). Means±standard errors of 4 independent replicates are shown.

Genotype	[57]Co content [% of total exported label]				
Treatment	Roots	Leaves 1 to 3	Controls Roots	Controls Shoots	Total plus medium
Sadovo (day 6; stress phase)					
Control	52.7±2.7	0.7±0.4	3.5±0.5	0.2±0.6	59.2±2.7
+ PEG	52.2±6.6	0.5±0.1	2.6±0.9	0.2±0.6	63.2±4.2
Katya (day 6; stress phase)					
Control	42.0±5.2	1.0±0.8	2.1±0.8	0.0±0.1	49.3±6.1
+ PEG	46.6±3.0	0.5±0.5	0.3±0.7	0.1±0.3	56.6±2.0
Sadovo (day 14; recovery phase)					
Control	40.9±3.3	0.1±0.1	3.6±0.8	0.5±0.2	48.1±4.1
+ PEG	46.3±4.4	0.6±0.3	3.9±0.9	0.3±0.4	55.5±3.0
Katya (day 14; recovery phase)					
Control	44.9±2.8	-0.1±0.2	2.9±0.6	-0.3±0.2	50.8±3.1
+ PEG	46.0±1.5	0.2±0.2	3.9±1.7	0.7±0.3	57.1±3.4

similar. The strong retention of ^{57}Co in the roots can serve as an indicator for the total export to the root system (Table 4). Obtained data confirmed that 50 to 60% of the ^{57}Co exported from leaf 4 was transported to the roots. No major differences between the two varieties or between control and stressed plants were detected regarding the redistribution of this label as well. There was not any considerable increase in the release of ^{109}Cd and ^{57}Co to the medium provoked by the artificial drought indicating that both radionuclides were well retained in the plants.

DISCUSSION

The technique used to investigate the sink structure in cereals appears to be convenient to elucidate impacts of drought and other abiotic stresses. The use of suitable tracers allows detailed studies including genotype comparisons. With stable markers such as rubidium the procedure can be used easily for field studies (Schenk and Feller, 1990). Since drought experiments including analyses of the roots are in most cases not possible in soil culture, artificial drought caused by PEG is an adequate alternative, but the drought stress should progress slowly to allow sufficient time for plant responses. Impacts of water deficit stress on root development in wheat genotypes were reported recently (Mori and Inagaki, 2012). Root growth under drought stress is important to access new soil regions with more favorable conditions and may cause alterations in the sink pattern within the root system. Furthermore the production of abscisic acid (ABA) in roots and its translocation

via the xylem to the leaves, its levels in the aboveground parts of the plant as well as the response of the leaves to ABA signal may be genotype-specific (Mohamed and Ismail, 2009; Vaseva et al., 2010). The elucidation of drought impacts on the sink structure within the root system remains to be evaluated in the future. The methods described here can serve as a basis for such experiments (Feller and Vaseva, 2014).

In previous studies drought effects on shoots of crop plants were mainly investigated with respect to leaf properties such as ultrastructure (Vassileva et al., 2009; Grigorova et al., 2012), photosynthesis (Flexas and Medrano, 2002; Vassileva et al., 2011), respiration (Vassileva et al., 2009 and 2011; Grigorova et al., 2012) or accumulation of stress-related proteins (Demirevska et al., 2008a; Grigorova et al., 2011a,b; Vaseva et al., 2010). Most of these investigations were focused on the fully expanded leaves, neglecting the effect of drought on newly developing leaves. The active biomass per plant and its structure can be considerably altered by drought (Feller and Vaseva, 2014). More detailed studies concerning solute allocation via the phloem should be envisaged since the sink structure within the shoots of the two wheat varieties with differing tolerance was unequally affected by the applied artificial drought as evident from the reported here experiments. The stress phase itself is important, but not sufficient to evaluate the overall stress impact. Besides the developmental stage of the plants at the beginning of the stress phase, stress progression and the duration of the subsequent recovery phase contributes

to the overall performance of plant species or varieties (Vassileva et al., 2011).

CONCLUSIONS

Impacts of abiotic stress on the sink structures of cereals should not be neglected in the context of Global Change. The performance of various organs may be affected differently by the stress. The time, duration and intensity of abiotic stress phases must be considered as well as the recovery phases for the evaluation of overall stress effects. Elements (e.g. rubidium), stable isotopes or radionuclides of metals introduced into a defined leaf via a flap are suitable for such investigations, since they are not metabolized, not released in gaseous form and can be accurately quantified by atomic absorption spectrometry, emission spectrometry, mass spectrometry or γ-spectrometry. Such techniques represent helpful tools for genotype selection or tests with newly bred varieties. More detailed investigations addressing responses of various parts of the root system and analyses of sink structures in wheat plants during the reproductive phase remain a challenge for future experiments.

ACKNOWLEDGEMENTS

REFERENCES

Balla K, S Bencze, P Bonis, T Arendas, O Veisz, 2014. Changes in the photosynthetic efficiency of winter wheat in response to abiotic stress. Centr Eur J Biol, 9: 519–530.

Bencze S, Z Bamberger, T Janda, K Balla, B Varga, Z Bedo, O Veisz, 2014. Physiological response of wheat to elevated atmospheric CO_2 and low water supply levels. Photosynthetica, 52, 71–82.

Boyajieva D, 1996. A study on the wheat productivity criteria for the breeding of drought tolerant cultivars. Cereal Res Commun, 24: 299–305.

Burlyn EM, MR Kaufmann, 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol, 51: 914–916.

Demirevska K, L Simova-Stoilova, V Vassileva, U Feller, 2008a. Rubisco and some chaperone protein responses to water stress and rewatering at early seedling growth of drought sensitive and tolerant wheat varieties. Plant Growth Regul, 56: 97–106.

Demirevska K, D Zasheva, R Dimitrov, L Simova-Stoilova, M Stamenova, U Feller, 2008b. Drought stress effects on Rubisco in wheat: changes in the Rubisco large subunit. Acta Physiol Plant, 31: 1129–1138.

Erdei L, I Tari, J Csiszár, A Pécsváradi, F Horváth, M Szabó, M Ördög, L Cseuz, M Zhiponova, L Szilák, J, Györgyey, 2002. Osmotic stress response of wheat species and cultivars differing in drought tolerance: some interesting genes (advices for gene hunting). Acta Biol Szeged, 46: 63–65.

Feller U, II Vaseva, 2014. Extreme

climatic events: impacts of drought and high temperature on physiological processes in agronomically important plants. Front Environ Sci, 2: 39. doi: 10.3389/fenvs.2014.00039.

Flexas J, H Medrano, 2002. Drought-inhibition of phothosynthesis in C_3 plant: stomatal and non-stomatal limitation revisited. Ann Bot, 89: 183–189.

Frei C, R Scholl, S Fukutome, R Schmidli, PL Vidale, 2006. Future change of precipitation extremes in Europe: intercomparison of scenarios from regional climate models. J Gephys Res-Atmos 11: D06105.

Grigorova B, V Vassileva, K Klimchuk, I Vaseva, K Demirevska, U Feller, 2012. Drought, high temperature and their combination affect ultrastructure of chloroplasts and mitochondria in wheat (*Triticum aestivum* L.) leaves. J Plant Interact, 7: 204–213.

Grigorova B, I Vaseva, K Demirevska, U Feller, 2011a. Combined drought and heat stress in wheat: changes in some heat shock proteins. Biol Plant, 55: 105–111.

Grigorova B, II Vaseva, K Demirevska, U Feller, 2011b. Expression of selected heat shock proteins after individually and combined drought and heat stress. Acta Physiol Plant, 33: 2041–2049.

Guoth A, D Benyo, J Csiszar, A Galle, F Horwath, L Cseuz, L Erdei, I Tari, 2010. Relationship between osmotic stress-induced abscisic acid accumulation, biomass production and plant growth in drought-tolerant and –sensitive wheat cultivars. Acta Physiol Plant, 32: 719–729.

IPCC, 2012. Summary for policymakers. In: Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptations. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change, Eds. CB Field, V Barros, TF Stocker, D Qin, DJ Dokken, KL Ebi et al., Cambridge University Press, 1–19.

Khan J, S Khan, MA Khetran, Amanullah, N Sadiq, M Islam, A Hanan, A Aziz, 2013. Tijaban-10 a drought tolerant and high yielding wheat variety for rainfed/Sailaba areas of Balochistan. Pak J Bot, 45: 1357–1362.

Martinkova J, V Hejnak, F Hnilicka, 2013. Effect of water deficit on sink strength of spring barley (*Hordeum vulgare* L.) ears. Res Crop, 14: 62–71.

Mohamed HE, GSM Ismail, 2009. The role of abscisic acid in the response of two different wheat varieties to water deficit. Z Naturforsch Section C, 64: 77–84.

Mori M, MN Inagaki, 2012. Root development and water-uptake under water deficit stress in drought-adaptive wheat genotypes. Cereal Res Commun, 40: 44–52.

Nair PKR, 2014. Grand challenges in agroecology and land use systems. Front Environ Sci, 2: 1. Doi: 10.3389/fenvs.2014.00001.

Page V, RM Blösch, U Feller, 2012. Regulation of shoot growth, root development and manganese allocation in wheat (*Triticum aestivum*) genotypes by light intensity. Plant Growth Regul, 67: 209–215.

Plaut Z, BJ Butow, CS Blumenthal, CW Wrigley, 2004. Transport of dry matter into developing wheat kernels and its contribution to grain yield under post-anthesis water deficit and elevated

temperature. Field Crops Res, 86: 185–198.

Riesen O, U Feller, 2005. Redistribution of nickel, cobalt, manganese, zinc and cadmium via the phloem in young and maturing wheat. J Plant Nutr, 28: 421–430.

Schenk D, U Feller, 1990. Rubidium export from indivdual leaves of maruring wheat. J Plant Physiol, 137: 175–179.

Sevanto S, 2014. Phloem transport and drought. J Exp Bot, 65: 1751–1759.

Simova-Stoilova L, I Vaseva, B Grigorova, K Demirevska, U Feller, 2010. Proteolytic activity and cysteine protease expression in wheat leaves under severe soil drought and recovery. Plant Physiol Biochem, 48: 200–206.

Steuter AA, 1981. Water potential of aqueous polyethylene glycol. Plant Physiol, 67: 64–67.

Tack J, A Barkley, LL Nalley, 2014. Heterogeneous effects of warming and drought on selected wheat variety yields. Clim Change, 125: 489–500.

Vaseva II, BS Grigorova, LP Simova-Stoilova, KN Demirevska, U Feller, 2010. Abscisic acid and late embryogenesis abundant protein profile changes in winter wheat under progressive drought stress. Plant Biol, 12: 698–707.

Vassileva V, C Signarbieux, I Anders, U Feller, 2011. Genotypic variation in drought stress response and subsequent recovery of (*Triticum aestivum* L.). J Plant Res, 124: 147–154.

Vassileva V, L Simova-Stoilova, K Demirevska, U Feller, 2009. Variety-specific response of wheat (*Triticum aestivum* L.) leaf mitochondria to drought stress. J Plant Res, 122: 445–454.