BRIEF COMMUNICATION

Effects of water deficit and selenium on common buckwheat (*Fagopyrum esculentum* Moench.) plants

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Abstract

Two cultivars of common buckwheat (*Fagopyrum esculentum*), Pyra and Siva, were exposed to three treatments: water deficit (WD), foliar spraying by selenium (as Na_2SeO_4) (Se), and the combination of both. In WD-plants the stomatal conductance (g_s) was significantly lower, while WD+Se-plants of Siva had significantly higher g_s. None of the treatments resulted in significant differences of potential photochemical efficiency of photosystem 2 (PS2). A significantly higher actual photochemical efficiency of PS2 was obtained in Siva WD-plants and in Pyra Se- and WD-plants which was possibly due to improvement of plant water management during treatment. A significant interaction was observed between the effects of WD and Se on respiratory potential in Pyra. WD, Se, and the WD+Se combination resulted in shorter Pyra and Siva plants, with a reduced number of nodes. WD slightly negatively affected the yield per plant. The yield was highest in plants exposed to Se only. In Siva the number of seeds was triple while the average seed mass remained unchanged.

Additional key words: photochemical efficiency; photosystem 2; root; seed number and yield; shoot; stomatal conductance.

Plants are frequently subjected to periods of soil and atmospheric water deficit during their life cycle. The frequency of drought stress is likely to increase in the future, even outside today's arid/semi-arid regions (Chaves *et al.* 2002). Computer modelling shows that climate change is expected, not to reduce the average precipitation, but to increase the frequency of extreme events, *i.e.* heavy rainfall and drought (Albritton and Meira Filho 2001). Different plant species exhibit different sensitivities to water deficit (WD). In arid and semiarid regions, drought presents selection pressure resulting in a well adapted plant community, which is not the case in humid climates. In the latter, therefore, any change of ecological factors influences competitive relations among plants. Crops are even more vulnerable to different types of stress due to their breeding for high yield and cultivation outside their ecological boundaries (McKersie and Leshem 1994).

Selenium is an essential trace element for animals and humans (Tapiero *et al.* 2003) but its role in plants is still unclear (Hartikainen *et al.* 2000). Se added to wheat plants regulates the water status of plants under drought (Kuznetsov *et al.* 2003). In ryegrass, Se at low concentrations acts as an antioxidant and, at higher concentrations, as a pro-oxidant (Hartikainen *et al.* 2000). However, the role of Se as an essential micronutrient in higher plants is yet to be established (Terry *et al.* 2000).

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Abbreviations: DM – dry mass; ETS – electron transport system; F_0 – minimum fluorescence in the dark adapted leaf (all reaction centres are open); F_m – maximum fluorescence in the dark adapted leaf (all reaction centres are closed); F_v – variable fluorescence ($F_v = F_m - F_0$); F_m' – maximum fluorescence of an irradiated sample; F_0' – minimal fluorescence of an irradiated sample; F_v/F_m – potential quantum yield of PS2; INT – iodo-nitro-tetrazolium-chloride; PE – photochemical efficiency; PPFD – photosynthetic photon flux density; PS – photosystem; Se – selenium, SLA – specific leaf area, WD – water deficit.

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Changes in climate also require changes in agricultural practice. Different cultivars of common buckwheat and tartary buckwheat (*F. tataricum*), both staple crops, could become important alternative crops. Breeding and selection of buckwheat cultivars are not very advanced, compared to major cereals (Halbrecq *et al.* 2005), therefore buckwheat might exhibit great resistance to various stresses.

The aim of this study was to examine the response of two cultivars of common buckwheat (*Fagopyrum esculentum*), Pyra and Siva (most grown in central Europe), to WD, to addition of Se, and to the combination of the two. For this purpose, we monitored stomatal conductance (g_s), photochemical efficiency (PE) of PS2, and respiratory potential, coupled with morphological analysis.

Plants were sown in sandy soil in pots on an outdoor research plot (Botanical Garden, University of Ljubljana, Slovenia), 320 m a.s.l., 46°35'N, 14°55'E) in the last week of June in 2004. After 5 weeks of growth, we reduced the watering of one half of the plants while control (C) plants were watered regularly. After 7 weeks of growth, half the C-plants and half the WD-plants were foliarly treated with a solution containing 1 g(Se) m^{-3} in the form of sodium selenate (Na_2SeO_4). Each of the two cvs. of buckwheat was therefore exposed to four different treatments: C (adequate amount of water, no Se), WD, C+Se (adequate amount of water, application of Se), and WD+Se. Morphological analysis was performed after plants had developed mature seeds, which was after 14 weeks of growth for Pyra and 16 weeks of growth for Siva. There were 50 plants per treatment.

Photochemical efficiency (PE) of photosystem 2 (PS2) was measured using a fluorometer *OS-500* (*Opti-Sciences*, Tyngsboro, MA, USA). The optimal quantum yield (F_v/F_m or potential PE) is a measure of the efficiency of energy conversion in PS2. F_0 and F_m are the minimal and maximal chlorophyll (Chl) *a* fluorescence yields in dark adapted samples, and F_v is the variable fluorescence, $F_v = F_m - F_0$. For dark adaptation, plastic leaf-clips were attached to the leaves for 20 min followed by measurement of F_v/F_m. Fluorescence was excited with a saturating beam of "white light" [photosynthetic photon flux density (PPFD) = 8 000 μmol m^{-2} s^{-1}, 0.8 s). The effective quantum yield of PS2 provides an estimate of the actual efficiency of energy conversion in PS2 and is defined as $(F_m' - F)/F_m' = \Delta F/F_m'$. F_m' is the maximal fluorescence of an irradiated sample and F is the steady state fluorescence (Schreiber *et al.* 1995). The effective quantum yield of PS2 was measured under saturating irradiance (1 800 μmol m^{-2} s^{-1}) at the prevailing ambient temperature by providing a saturating pulse of "white light" (PPFD = 9 000 μmol m^{-2} s^{-1}, 0.8 s) using a standard 60° angle clip. Measurements were performed on 8 plants per treatment.

g_s was measured on 6 plants per treatment with a portable *AP4* porometer (*Delta-T Devices*, UK) which measures the rate of water vapour diffusion through leaf surfaces. Chl fluorescence and g_s were measured at midday, when water deficit had its greatest effect on the plants.

Respiratory potential was measured as electron transport system (ETS) activity of mitochondria, as described by Packard (1971) and modified by Kenner and Ahmed (1975). A known fresh mass of leaves was crushed in a mortar in chilled 0.1 M sodium phosphate buffer (pH 8.4) containing 0.15 % (m/v) polyvinyl pyrrolidone, 75 μM $MgSO_4$, and 0.2 % (v/v) *Triton-X-100*, followed by ultrasound homogenization. The extract was centrifuged at 8 500×*g* for 4 min at 1 °C. An aliquot of the supernatant was added to the substrate solution [0.1 M sodium phosphate buffer, pH 8.4, 1.7 mM NADH, 0.25 mM NADPH, 0.2 % (v/v) *Triton-X-100*], and 20 mg 2-p-iodo-phenyl 3-p-nitrophenyl tetrazolium chloride (INT) in 10 cm^3 of bi-distilled water, and the mixture was incubated at 20 °C for 40 min. Absorbance of the resulting formazan was measured at 490 nm. ETS activity was calculated as the rate of INT reduction (Kenner and Ahmed 1975). Measurements were performed on 3 plants per treatment.

Morphological analysis was performed in the last week of September for Pyra and in the second week of October for Siva, when the plants had developed mature seeds. Height, basal diameter, number of nodes, leaf thickness, total leaf area, specific leaf area (SLA), plant biomass, number of seeds, average seed mass, and yield of seeds per plant were determined for 8 plants per treatment. For plant biomass, the sample plants were dried to constant mass at ambient temperature (14 d). When dry, underground and aboveground parts of the samples were weighed separately. Seeds were counted and also weighed separately.

The significance of experimental effects was tested by one-way ANOVA (*SPSS 13.0*). Mann-Whitney U test (also *SPSS 13.0*) was used with data for which Levene's test showed statistically significant differences between variances.

In WD-plants the stomata were partly closed, so that g_s was significantly lower than in C-plants (Fig. 1*A*). Stomata closing contributed to reduction in water loss and maintenance of favourable water potential in the plant. The closing of stomata is the first reaction to drought in the majority of higher plants (Egert and Tevini 2002, Kawakami *et al.* 2006). The addition of Se mitigated the negative effect of WD in most cases but the effect was significant only in WD-plants of Siva. Similar findings were obtained by Kuznetsov *et al.* (2003) who concluded that Se regulates the water status of wheat plants by maintaining the content of water in their tissues sufficiently high.

Potential PE was 0.81±0.02 for Pyra and 0.80±0.02 for Siva. Different treatments of the plants did not result in significant changes of potential PE. The potential PE of PS2 of differently treated plants remained close to the theoretical maximum of 0.84 (Björkman and Demmig

1987), which indicates that WD and Se caused no disturbance to photosynthetic apparatus.

There were significant variations in actual PE of PS2 (Fig. 1*B*) between differently treated plants. We found a statistically significant increase, over C, in actual PE in Pyra WD+Se-plants and in Siva WD+Se- and WD-plants. The actual PE of PS2 (Fig. 1*B*) differed between differently treated plants. Actual PE of PS2 showed the presence of reversible stress in C-plants. The slight increase in actual PE of PS2 in WD-plants of Pyra and the statistically significant increase in actual PE in WD-plants of Siva, with and without Se, could be due to fact that during drought treatment WD-plants improve their resistance to water shortage (Larcher 2001), probably

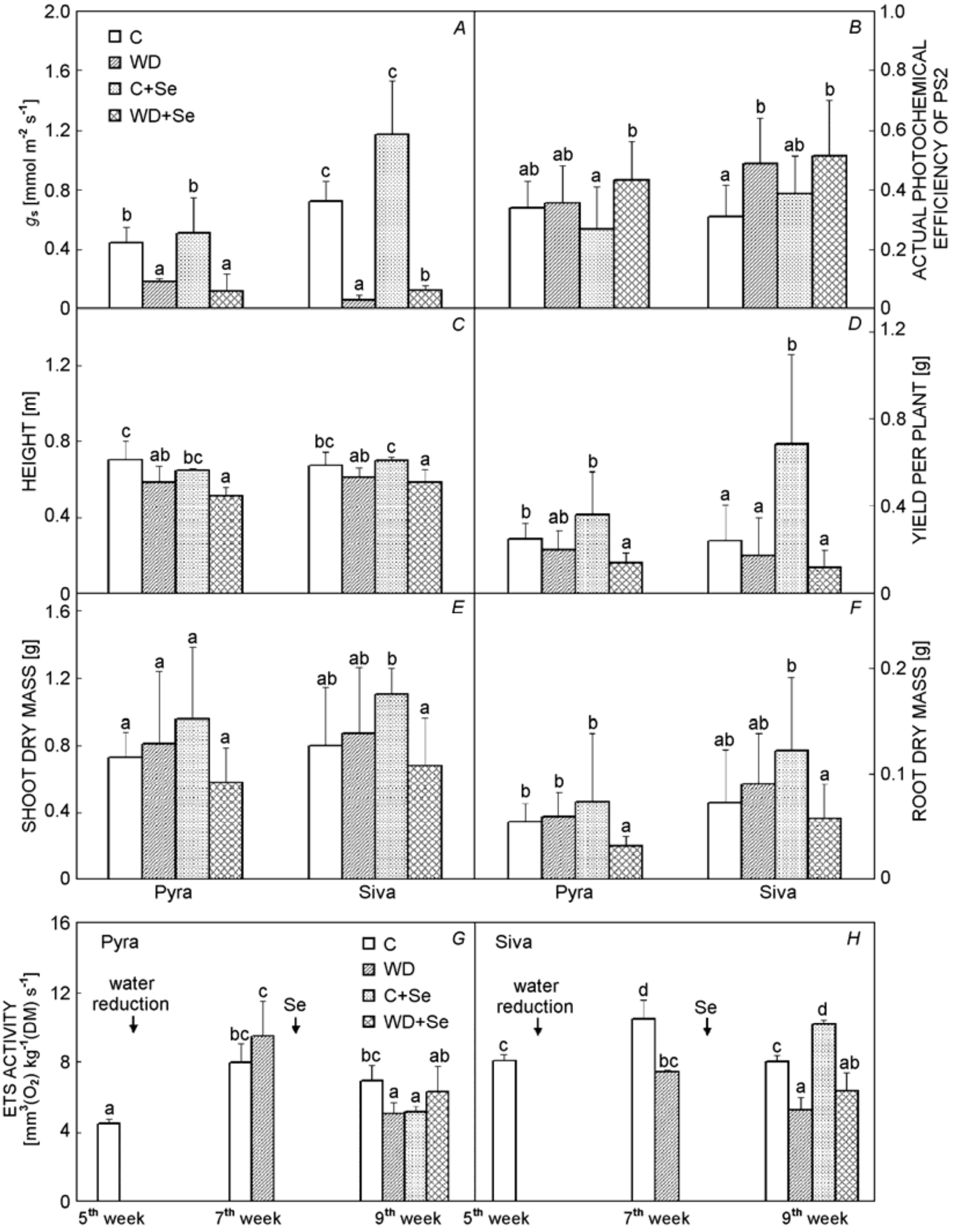

Fig. 1. Stomatal conductance, g_s (*A*), actual photochemical efficiency (PE) of photosystem 2 (*B*), plant height (*C*), yield per plant (*D*), shoot (*E*) and root (*F*) dry masses for cvs. Pyra and Siva affected by water deficit (WD) and Se or both in comparison to control (C) in the 9th week of growth. (*G, H*): ETS activities for differently old plants. Means + SD, $n = 6$ (g_s), $n = 8$ for actual PE and growth characteristics, and $n = 3$ for ETS. Columns not sharing the same letter are significantly different ($p<0.05$).

also due to more efficient regulation of stomata openings. Therefore they can be more successful under high temperatures and high irradiance during midday depression. The effect was even more evident in Pyra WD+Se-plants.

ETS activity (Fig. 1*G,H*) increased in the 7th week of growth and decreased in the 9th week of growth in both cultivars. The significant decrease in ETS activity was greatest in response to WD. Pyra Se-plants exhibited lower ETS activity but Siva Se-plants had significantly higher ETS activity, both relative to C-plants. A statistically significant interaction was obtained between WD and Se for ETS activity in Pyra (p=0.039). Se slightly increased ETS activity in WD-plants in both cultivars. ETS activity was highest in plants at the peak of the season, since they need additional energy during intensive growth in order to develop essential structural components as shown for *Fagopyrum esculentum* and *F. tataricum* by Breznik *et al.* (2005) and for *Potamogeton crispus* by Mazej and Gaberščik (1999). ETS activity is a measure of the metabolic potential of organisms and varies among different plant species (Germ *et al.* 2005). Thus, 'Siva' plants treated with Se exhibited an elevated metabolic potential regardless of the amount of water they received. A similar increase in ETS activity in young pea plants may reflect increased glutathione peroxidase activity in mitochondria (Smrkolj *et al.* 2006). Xue and Hartikainen (2000), Hartikainen *et al.* (2000), and Xue *et al.* (2001) found that Se exposure increased glutathione peroxidase activity in ryegrass and lettuce. However, WD might impair the flow of electrons in the respiratory chain.

Both Pyra and Siva plants were shorter than controls when exposed to WD, Se, or to a combination of the two (Fig. 1*C*). Disturbances in plant growth are associated with WD in the vegetative stage (Desclaux *et al.* 2000). Highest yields per plant (Fig. 1*D*) were obtained when plants were exposed only to Se—especially in Siva. However, this effect of Se was not found in WD-plants that had the lowest yields. Se increased shoot dry mass (DM) (Fig. 1*E*) of C-plants. Shoot and root DM values were the smallest in Se+WD plants. A statistically significant interaction was observed between WD and Se for root DM in Siva (p=0.022). Se+WD lowered basal diameter, number of nodes, leaf thickness, and average seed mass (Table 1), while there were no statistically significant differences in SLA [mean SLA for Pyra was 253±26 $\mathrm{cm^2\,g^{-1}}$(DM)] and number of seeds in Pyra. In Siva, Se+WD treatment reduced basal diameter, number of nodes, number of seeds, and seed mass. Leaf thickness was reduced when the investigated plants were exposed to WD, but not to the combination of WD+Se. There were no statistically significant differences in SLA [mean SLA for Siva was 229±49 $\mathrm{cm^2\,g^{-1}}$(DM)]. We also found a statistically significant interaction between WD and Se for leaf thickness (p=0.029) and number of seeds (p=0.0001). A similar effect of Se was observed for pumpkins (*Cucurbita pepo*) (Germ *et al.* 2005). However, Se foliar spraying did not counteract the effect of WD on yield per plant.

Table 1. Basal diameter [cm], number of nodes, leaf thickness [µm], number of seeds, and average seed mass [g] in cvs. Pyra and Siva at water deficit (WD) and Se treatments. C = control. Means ± SD, n = 8, except for leaf thickness where n = 12. Values not sharing the same letter are statistically different at p<0.05.

		Basal diameter	No. of nodes	Leaf thickness	No. of seeds	Seed mass
Pyra	C	0.30 ± 0.04 ^{ab}	7.5 ± 1.3 ^{b}	170 ± 26 ^{bc}	9.7 ± 2.1 ^{a}	0.024 ± 0.004 ^{b}
	WD	0.31 ± 0.04 ^{b}	6.5 ± 1.1 ^{ab}	163 ± 25 ^{c}	12.4 ± 7.3 ^{a}	0.022 ± 0.005 ^{b}
	C+Se	0.31 ± 0.05 ^{b}	7.4 ± 0.7 ^{ab}	192 ± 34 ^{ab}	18.4 ± 13.5 ^{a}	0.022 ± 0.007 ^{b}
	WD+Se	0.27 ± 0.03 ^{a}	6.0 ± 0.8 ^{a}	148 ± 14 ^{a}	10.1 ± 5.7 ^{a}	0.017 ± 0.005 ^{a}
Siva	C	0.32 ± 0.06 ^{b}	8.6 ± 0.9 ^{ab}	239 ± 32 ^{b}	11.9 ± 6.9 ^{a}	0.019 ± 0.002 ^{b}
	WD	0.30 ± 0.04 ^{ab}	8.0 ± 1.1 ^{a}	210 ± 22 ^{a}	10.0 ± 7.5 ^{a}	0.017 ± 0.004 ^{ab}
	C+Se	0.30 ± 0.05 ^{ab}	9.6 ± 1.2 ^{b}	241 ± 32 ^{b}	36.8 ± 24.6 ^{b}	0.019 ± 0.003 ^{b}
	WD+Se	0.26 ± 0.03 ^{a}	7.8 ± 0.9 ^{a}	246 ± 14 ^{b}	6.4 ± 2.9 ^{a}	0.014 ± 0.003 ^{a}

The growth promoting effect of Se, also observed in ryegrass, was partly a consequence of anti-oxidative effects, which counteracted the senescence processes (Hartikainen *et al.* 2000). The stimulatory effect of foliar application of Se on growth is known for lettuce (Xue *et al.* 2001), potato (Turakainen *et al.* 2004), and green tea (Hu *et al.* 2003). However, the growth promoting effect of Se was not observed in buckwheat plants exposed to WD, since those plants had the smallest shoot and root DM.

We conclude that foliar application of solution containing 1 g(Se) $\mathrm{m^{-3}}$ is beneficial to regularly watered buckwheat, as revealed from photochemical efficiency of PS2 and biomass production in both cultivars. Biomass production was not increased when Se treatment was applied under WD. Both buckwheat cultivars were equally tolerant under WD.

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