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BRIEF COMMUNICATION

Effects of short-term soil flooding on stomata behaviour and leaf gas exchange in barley plants

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Abstract

Exposure of barley plants (*Hordeum vulgare* L.) to soil flooding for 2 to 24 h reduced the net photosynthetic rate and transpiration rate. Stomatal conductance also decreased in flooded plants. Stomatal closure started within 2 - 6 h and stomata remained closed up to 24 h of treatment.

Additional key words: *Hordeum vulgare*, net photosynthetic rate, stomatal conductance, transpiration rate.

The more common response to flooding is partial stomata closure within the first few hours of treatment. Stomata closed in response to waterlogging both in the presence of water deficit (Coutts 1981) and in its absence (Jackson and Hall 1987). Decreased leaf water potential (ψ) does not always accompany flooding injury, even in most cases ψ remain unaffected or increased in flooded plants (Bradford and Yang 1981). Recently Else *et al*. (2001) reported that soil flooding reduced stomatal conductance (g_s) and lowered transpiration (E), CO_2 uptake and leaf elongation in *Ricinus communis* within 2 - 6 h. The authors suggest that a co-ordination between above- and below-ground plant parts exists and must involve signal transmission from stressed root system to the shoot, and its subsequent perception and transduction into physiological responses. Jackson and Hall (1987) found that in pea plants, stomata begin to close approximately 24 h of the start of soil flooding with a parallel decrease in E and g_s. According to Bradford (1983) 24 h after the start of flooding of tomato plants photosynthetic rate (P_N) remained constant or declined at high intercellular CO_2 concentration (c_i). Author supposed that the observed low rate of photosynthesis was due to nonstomatal (biochemical) factors, such as inability for RuBP regeneration in Calvin cycle.

After prolonged flooding the root injuries caused restriction on the photosynthetic capacity by altering the biochemical reactions of photosynthesis. Pezeshki (1994) demonstrated that root hypoxia of baldcypress (*Taxodium distichum*) seedlings for 48 h caused 17 % decrease in the activity of ribulose-1,5-bisphosphate carboxylase (RuBPC). Recently we showed that flooding of barley plants for 72 to 120 h led to a noticeable decrease in rates of P_N and E. In treated plants c_i remained constant or even increased. A drop in the activity of RuBPC and of the activity of photorespiratory enzymes phosphoglycolate phosphatase and glycolate oxidase was also observed in flooded plants (Yordanova and Popova 2001). Changes in the activity of some antioxidative enzymes (superoxide dismutase, catalase and peroxidase) and some indices related to structural and functional membrane stability, as measured by electrolyte leakage, lipid peroxidation and increased level of hydrogen peroxide point that flooding led to oxidative stress that could be another reason for the observed inhibition of photosynthesis (Yordanova *et al*. 2003, 2004). All these data showed that soil flooding caused a decrease in photosynthesis but most probably different mechanisms are involved depending on the duration of treatment.

The aim of this study was to investigate the impact of short-term soil flooding on stomata function and morphology and on leaf gas exchange in barley leaves.

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Abbreviations: c_a - ambient CO_2 concentration; c_i - intercellular CO_2 concentration; E - transpiration rate; g_s - stomatal conductance; P_N - net photosynthetic rate; RuBPC - ribulose-1,5-bisphosphate carboxylase; ψ - water potential.

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Barley (*Hordeum vulgare* L. cv. Alfa) plants were grown for two weeks in soil in a growth chamber. The soil was classified as Eutric Fluvisol (pH 5.75). The environmental conditions were: irradiance 160 μmol m^{-2} s^{-1} PAR, 12 h-photoperiod, temperature 24 °C, and relative humidity of 60 %. When the plants were at the second to third- leaf stage half of the plants were flooded in the early morning by placing the pots inside larger glass containers filled with tap water to 25 mm above the level of the soil surface. Control plants remained well watered (60 % soil moisture) during the period of the experiment. Samples were taken 2, 6, 12, and 24 h after the start of flooding treatment. Each measurement was done independently.

Gas exchange measurements were performed by a portable photosynthesis system *Li-6000* (*Li-Cor*, Lincoln, USA). All measurements were taken on attached, mature leaves. Leaves of 5 - 6 plants (the first well-expanded leaf) were placed in a 250 cm^3 chamber. Irradiance was 870 μmol m^{-2} s^{-1} PAR, provided by a 500 W incandescent lamp fitted with reflector. Flow rate through the cuvette was 18 - 20 cm^3 s^{-1}, boundary layer resistance (r_a) was 0.8 s cm^{-1}. Leaf temperature was 26 ± 2 °C.

30 leaves per variant were used to obtain micro-relief replicas of stomata at the central part of adaxial and abaxial surface of the leaves. The replicas were used for direct counting and measuring of the stomatal density, length and width of stomatal aperture and evaporating surface leaf area.

Exposure of barley plants to flooding caused a gradual decrease in P_N. 2 h after treatment the net photosynthetic rate declined by 19 % and reached over 40 % inhibition after 6 h, and then remained approximately constant (Fig. 1*A*). Transpiration rate was also decreased following the time of exposure of plants to flooding (Fig. 1*B*). The same tendency was observed and for the values of stomatal conductance (Fig. 1*C*). The results imply that flooding of barley plants for a short time (2 - 24 h) led to stomata closure and restricted supply of CO_2. This suggestion corresponds to the observed low values of c_i in flooded plants (Fig. 1*D*). In pea plants was found that stomatal closure occurred in the first few hours of flooding (Zang and Zang 1994). Authors proposed that the ABA transport from the older to the younger leaves or synthesis *de novo* was the reason for this closure. Another signal promoting stomatal closure might be the negative hydraulic message. Two hours after soil flooding of castor oil plants the root hydraulic conductance was strongly depressed and caused the decrease in leaf water potentials which was attributed by initial stomatal closure (Else *et al.* 2001). We found that soil flooding altered also stomata morphometric

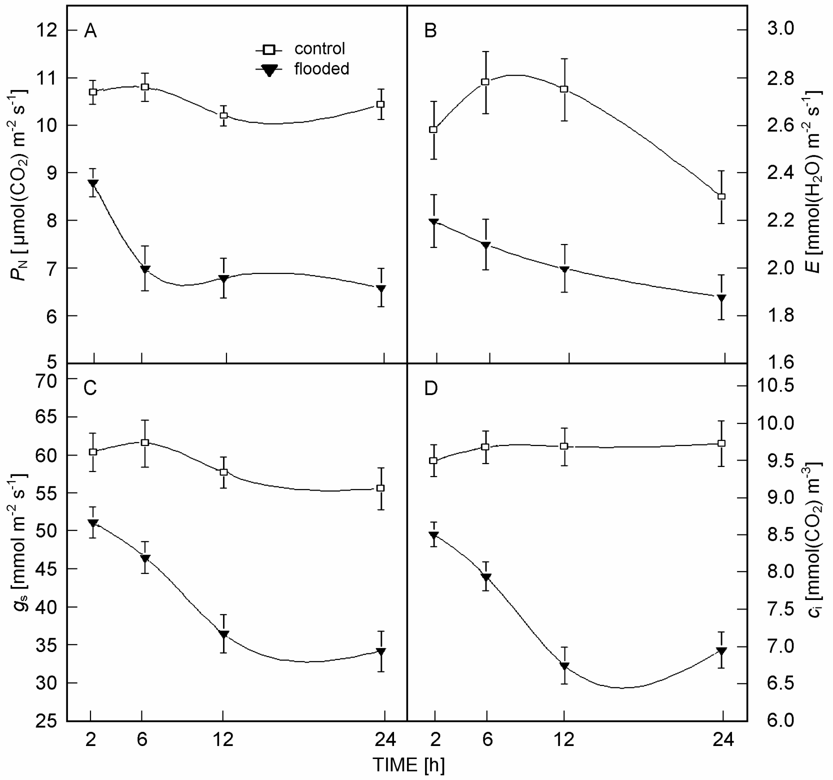

Fig. 1. Effect of short-term soil flooding on the gas exchange characteristics in barley leaves. P_N - net photosynthetic rate at 450 mg m^{-3} ambient CO_2 (*A*), E - transpiration rate (*B*), g_s - stomatal conductance (*C*), c_i - intercellular CO_2 concentration at 450 mg m^{-3} ambient CO_2 (*D*). Means ± SE, *n* = 4.

Table 1. Effect of short-term flooding on the stomatal density, stomatal aperture and evaporating surface of barley leaves. Means ± SE, *n* = 5.

	Time [h]	Treatment	Stomatal density [number mm^{-2}]	Stomata length [μm]	Stomatal width [μm]	Evaporating surface [μm^2]
Abaxial surface	2	control	43.72 ± 1.43	14.71 ± 0.50	2.76 ± 0.08	40.59 ± 1.38
		flooding	44.50 ± 1.30	15.81 ± 0.53	2.52 ± 0.11	39.84 ± 1.01
	6	control	44.41 ± 1.38	14.84 ± 0.82	2.75 ± 0.12	40.81 ± 2.22
		flooding	41.31 ± 1.89	14.34 ± 0.64	2.15 ± 0.09	30.83 ± 1.29
	24	control	43.37 ± 1.75	15.93 ± 0.55	2.27 ± 0.12	36.16 ± 1.13
		flooding	41.13 ± 1.76	14.55 ± 0.61	1.79 ± 0.09	20.67 ± 1.07
Adaxial surface	2	control	43.03 ± 1.40	15.73 ± 0.52	3.25 ± 0.19	54.37 ± 1.64
		flooding	44.06 ± 1.86	14.92 ± 0.42	2.50 ± 0.14	44.80 ± 1.10
	6	control	37.52 ± 2.99	14.25 ± 0.53	2.69 ± 0.21	38.33 ± 1.35
		flooding	38.90 ± 2.24	13.83 ± 0.92	2.02 ± 0.09	27.94 ± 2.83
	24	control	39.24 ± 2.06	13.81 ± 0.46	2.38 ± 0.14	32.87 ± 0.95
		flooding	38.90 ± 1.57	14.32 ± 0.50	1.65 ± 0.08	23.63 ± 0.83

parameters in barley plants (Table 1.). As could be expected, short-term flooding did not change the number of stomata at both the abaxial and adaxial leaf surfaces. Most significant was the width of stomatal aperture which decreased with the time of flooding. These changes reflected on the evaporation rate. 24 h after flooding the decrease of the surface area of evaporation was 57 % compared with the control. The dynamic of evaporating surface decrease coincided with the changes in all gas exchange parameters. These findings sustained the idea that despite of observed drop in photosynthetic rate, stomatal closure is one of the earliest shoot adaptive responses to soil flooding (Else *at al.* 1996, Jackson 2002).

In conclusion, we found that flooding of barley plants caused fast and strong stomata closure within the first few hours of the treatment. This was proved by the alterations of morphometric parameters of stomata and by low values of intercellular CO_2 concentration and stomatal conductance of treated plants. Data support the suggestion that the decrease in photosynthesis after short-term soil flooding is a result of indirect effect, mediated by stomata closure, causing a reduction of CO_2 supply.

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