*The European Journal of Plant Science and Biotechnology* ©2010 Global Science Books

# Rapid Response Reactions of Buckwheat Plant Shoots to Changes in Sodium Chloride Concentration at the Root Zone and Blockage of Calcium Channels

**Natalia V. Budagovskaya**

Institute of Plant Physiology of the Russian Academy of Sciences, Botanicheskaya 35, Moscow 127276, Russia

***Corresponding author*: * postnabu@mail.ru**

## ABSTRACT

Use of a highly sensitive method (accuracy 0.07 μm) – laser interference auxanometry – made it possible to study rapid (min) and slower (h) response reactions of shoots of buckwheat plants (*Fagopyrun esculentum* Moench) under changing conditions of mineral nutrition (increase in NaCl concentration at the root zone and blockage of calcium channels). Addition of NaCl in increasing concentrations to the root zone of plants caused a rapid decrease in the growth rate of shoots. High NaCl concentration (100 mM) caused the growth of shoots to cease and caused their tissues to shrink as a result of dehydration. There were oscillations of plant stem elongation and shrinkage. When the calcium channel blocker verapamil was added at a mild concentration (6 mM) to the root zone it decreased the growth rate of shoots, first decreasing the shoot growth rate followed by its partial restoration. The growth of shoots was retarded and finally stopped after the addition of verapamil at a high concentration (12 mM). Verapamil not only slowed down the growth of plants, but also their development. Buckwheat plants from long-term experiments (10 days) with verapamil formed fewer leaves. They had shorter stems than control plants. Verapamil also induced a decrease in the turgor of stems. The root system of verapamil-treated plants developed poorly compared to non-treated plants.

**Keywords:** calcium channel blocker, growth rate, laser auxanometry, salinity

## INTRODUCTION

Saline soils occupy big territories around the world. These territories cause certain difficulties in crop production since most crop plants are glycophytes with low salt tolerance (Pitman *et al.* 2002). Plants grown under salinization subjected to functional disturbances, mainly related to water exchange and growth, result in decreased productivity (Vose 1983; Azaizeh *et al.* 2002; Gebauer *et al*. 2003). Plants grown on saline substrates also suffer from calcium deficiency due to inhibition of its uptake and transport (Lynch *et al*. 1985), which may cause disturbances connected with signaling and functional responses. Under natural conditions the level of salinization can change during vegetation as a result of alternation of dry and wet periods. Rain or watering of plants can decrease NaCl concentration at the root zone. Evaporation of soil moisture results in an increase of salt concentration (Armstrong *et al*. 1996). In the present work conditions of short-term salinization were created in experiments.

Use of a highly sensitive and non-destructive method – laser interference auxanometry – made it possible to study poorly known rapid (min) and slower (h) response reactions of whole plants to changes in mineral nutrition. This technique makes it possible to register the growth rate of plants under a continuous regime before and after changes in the concentration of mineral elements are made at the root zone or the introduction of biologically active compounds, and to investigate the transition of plants from one functional state to another (Budagovskaya and Guliaev 2001a; Budagovskaya *et al*. 2001b; Budagovskaya and Guliaev 2004; Budagovskaya 2007).

Previously changes in the functional state of buckwheat plants caused by the disturbance of mineral nutrition under low pH conditions and iron deficiency were studied (Budagovskaya 1998). The present paper deals with the study of the effect of an increase in sodium chloride (NaCl) concentration at the root zone on the growth rate of shoots of buckwheat plants. Growth dynamics of shoots reflects changes in the functional state of plants with increased NaCl concentration in the environment. In addition, experimental conditions for the disturbance of calcium transport in plants caused by the treatment with a calcium channel blocker – verapamil – were created, and the effect of this compound on the growth and development of buckwheat plants has also been studied.

## MATERIALS AND METHODS

Plant growth rate was measured by a laser interference auxanometer (LINA-EM3D, Russia), equipped with a helium-neon laser (632.8 nm). Sensitivity of the auxanometer was 0.07 μm. Growth parameters, and air and substrate temperature at the root zone were registered every 10 sec. Buckwheat (*Fagopyrun esculentum* Moench) cv. 'Molva' plants were used in the experiments. Plants were grown in washed sand in 9.5 cm diameter Petri dishes. Three days before the start of the experiments solitary plants were placed in 5 cm diameter Petri dishes filled with sand. Plants were watered daily with tap water (10 ml per 9.5 cm diameter Petri dishes, 3 ml per 5 cm diameter Petri dishes). NaCl solution (800 mM starting concentration) was introduced in 0.5 and 1.0 ml portions to the substrate at the root zone. Verapamil (ICN Biomedicals, Germany) was added at 30 mM (0.8 ml; starting solution) to the substrate at the root zone. Measurements of the growth rate of shoots were performed by an auxanometer at an air and substrate temperature of 22°C and air humidity of 64%. Experiments were carried out with 7-14 day-old plants. In long-term experiments 10 day-old plants were transferred to a solution of verapamil (0.9 mM) and to distilled water (control variant) for 10 days. Each experimental treatment was repeated 3-5 times. Five-10 plants were used per treatment.

**Received: 30 June, 2009. Accepted: 21 March, 2010.**

**Short Communication**

## RESULTS AND DISCUSSION

Plant growth rate was measured by an auxanometer for several hours in a continuous regime. The addition of NaCl (1.0 ml, 800 mM) to the root zone of buckwheat plants caused a progressive decrease in stem growth rate close to zero (**Fig. 1**). After that stem growth stopped and tissues shrank (negative values on the curve). Decrease in stem growth rate started after a small lag-period and proceeded, first rapidly, and later slowly. Further changes in the functional state of the plant are of special interest. The applied technique makes it possible to follow the character and time intervals of these changes. Five stationary states (5 time intervals) with different rates of elongation (plus) and shrinkage (minus) of tissues can be distinguished on the curve in 82-180 min interval: 1.26 µm/min for 12 min, 0.84 µm/min for 63 min, 0.42 µm/min for 7 min, -0.84 µm/min for 10 min and -1.26 µm/min for 7 min (mirror symmetry of the curve in positive and negative regions of coordinates). **Fig. 2** demonstrates the final time-frame of **Fig. 1**. There were oscillations in the studied processes of a period of 0.8-1.7 min. The transition from extension to shrinkage of tissues is step-wise: there is a change in the oscillation regime (2 positive and 2 negative regions). Transitions of the curve from the positive region through zero values to the negative region first close to zero and further to higher negative values indicate changes in the functional state of a buckwheat plant (i.e., transitions are triggered from one stationary state to another). Earlier similar transitional oscillation processes from extension to shrinkage of leaf tissues of cereal plants (oat, barley, rice) were revealed, caused by the introduction of NaCl to the root zone (Budagovskaya 2007). These oscillations were connected with water transport from roots to shoots and back (Budagovskaya 2007). According to Zyalalov (1981) oscillations in turgor pressure of parenchyma cells and diameter of the stem took place in the course of water transportation in plants indicating pulsations of cell volume. The work of Sherif (1977), Ushakova and Koltunova (1982) and Zholkevich (1991) demonstrated that absorbance, transport and transpiration of water by plants occur as an oscillation process within a period of a few minutes.

NaCl, introduced in high concentrations to the root zone of oat, barley and rice plants caused an efflux of water from leaves and shrinkage of their tissues (Budagovskaya 2007). Water transport was also reverted in isolated maize roots placed in NaCl solutions (Budagovskaya 2009). The rate of reversed water transport in roots was proportional to NaCl concentration in the environment. The shrinkage of stem tissues of buckwheat plants shown in **Figs. 1** and **2** may also point to a partial loss of water caused by the introduction of NaCl to the root zone. The dynamics of this process is demonstrated in **Fig. 2**. The rate of elongation of the stem first decreased, then elongation stopped and shrinkage of tissues proceeded at an increased rate. The addition of NaCl in lower concentrations (50 mM) to the root zone resulted in a decrease in buckwheat stem growth rate, but it did not fall to zero and was partially restored as was observed with leaves of barley and oat plants (Budagovskaya 2007).

Salinization caused a decrease in calcium uptake by plant roots and its transport, which could induce calcium deficiency and affect the functional activity of plants (Lynch *et al.* 1985). In the present work disturbance of calcium transport in buckwheat plants was also caused by the introduction of a calcium channel blocker – verapamil – to the root zone. Earlier, it was demonstrated in long- and short-term experiments that verapamil caused retardation of the growth of shoots and roots, slowed down the uptake of $CO_2$, $O_2$ evolution and transpiration in leaves in light and induced calcium deficiency in pea, maize, rice, wheat, sugar cane and pelargonium plants (Budagovskaya 1997; Budagovskaya and Guliaev 2001a; Budagovskaya *et al.* 2001b). In this work the effect of verapamil on the growth rate of buckwheat plants was studied.

After the addition of verapamil (0.8 ml, 30 mM solution) the growth rate of the stem of buckwheat plant decreased during the first 1.5 h to 15% of the initial rate, remained at this level for 15 min and increased further during 18 min to 53% of the initial rate (**Fig. 3**). The increase in growth rate started within 1 h and 48 min after the addition of verapamil. The growth of the stem was retarded and then stopped after the addition on the next day of the same amount of verapamil (0.8 ml, 30 mM solution). Stem growth rate, similar to that when verapamil was initially added, could not be restored. In this case, an increase in verapamil concentration at the second addition caused a decrease in the growth rate of stem from 5 µm/min to zero for 1 h and 11 min. An analogous decrease in leaf growth rate to zero without a further increase was demonstrated earlier in experiments with the introduction of verapamil at high concentrations to the root zone of wheat and rice plants (Budagovskaya and Guliaev 2001a; Budagovskaya *et al.* 2001b).

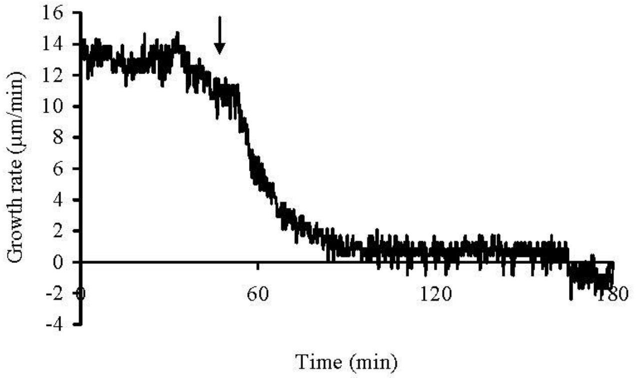


**Fig. 1 Effect of NaCl (100 mM) on the growth rate of stem of 9-day-old buckwheat plant.** ↓ - the moment of addition of NaCl (1.0 ml, 800 mM) to the root zone.

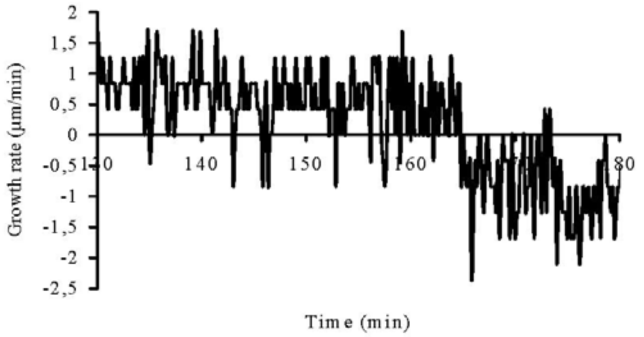


**Fig. 2 Effect of NaCl (100 mM) on the growth rate of stem of 9-day-old buckwheat plant.** Fragment of **Fig. 1** (130-180 min).

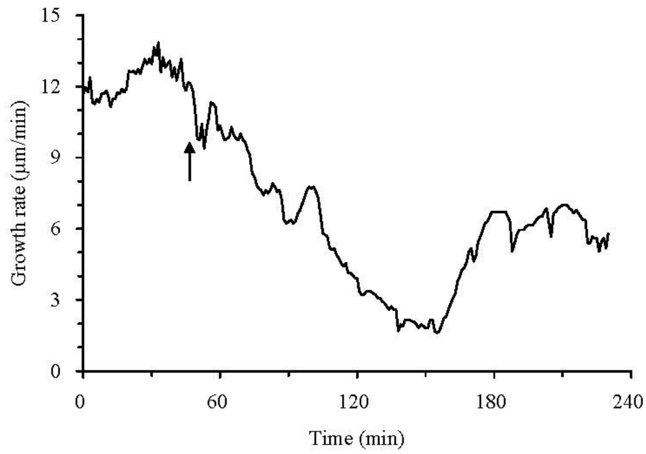


**Fig. 3 Effect of verapamil (6 mM) on the growth rate of stem of 12-day-old buckwheat plant.** ↑ - the moment of addition of verapamil (0.8 ml, 30 mM).

However, at lower verapamil concentrations the fall in growth rate of leaves of these plants (i.e. wheat and rice) and the subsequent increase was similar to that observed in buckwheat plant stems. The rise in the growth rate of leaves of wheat plants began within 2 h and within 3 h for rice plants after the addition of verapamil (Budagovskaya and Guliaev 2001a; Budagovskaya *et al.* 2001b). For buckwheat plants stem growth rate began to restore after almost 2 h after the addition of verapamil. A 2-3 h interval after the addition of verapamil was necessary for wheat, rice and buckwheat plants for the restoration of growth rate. The same time interval (2-3 h) was necessary for oat and barley plants for the restoration of growth rate of leaves to begin after the addition of NaCl in moderate concentrations (50 mM) to the root zone (Budagovskaya 2007). It is possible that this period of time is necessary for adaptation of plants (including *de novo* synthesis of protectors) allowing restoration of plant growth rate.

Long-term experiments demonstrated that verapamil slowed down not only the growth of buckwheat plants, but also their development. Plants placed into verapamil solution for 10 days not only had shorter stems, but also formed fewer leaves than control plants (**Fig. 4**). The stem length of experimental plants was 80-83% of control plants. Compared to control plants verapamil-treated plants had only cotyledonary leaves. They did not form first true leaves. The root system of verapamil-treated plants developed more poorly than that of non-treated plants (**Fig. 4**). Besides, verapamil induced a decrease in the turgor of plants, indicating a disturbance of water exchange. Stems of experimental plants wilted in contrast to those of control plants (**Fig. 5**). The effect of verapamil described for buckwheat plants in the present paper was observed previously for pea, maize and rice plants treated with verapamil; their shoots and roots developed poorly (Budagovskaya 1997; Budagovskaya and Guliaev 2001a).

The results obtained in this study characterize a rapid response reaction of buckwheat shoots to increased NaCl concentration at the root zone and the effect of a calcium channel blocker. Plant growth is an integral process and the change in growth rate reflects the change in the functional state of the plant. A decrease in growth rate under the effect of the mentioned stress factors to zero and subsequent shrinkage of shoot tissues as a result of their dehydration is a critical situation for plants and may lead to their death. Under moderate salinization (50 mM) and mild verapamil concentrations (6 mM) plant growth rate, which had decreased due to the effect of these stressors, was restored in a few hours.

The method of laser interference auxanometry can be used for the study of response reactions of the whole plant to changes in mineral nutrition and the action of biologically active compounds as well as for plant diagnostics.

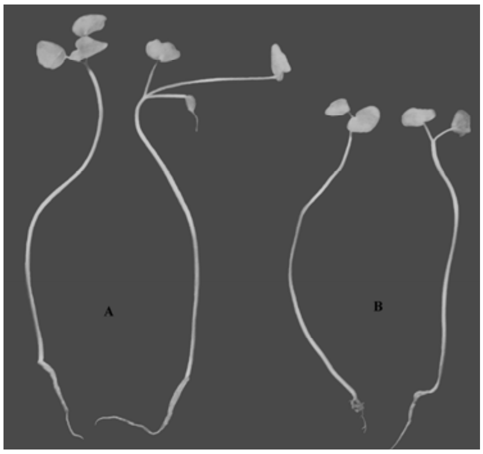


**Fig. 4 Effect of verapamil (0.9 mM) on the growth and development of 20-day-old buckwheat plants.** (**A**) Control plants; (**B**) experimental plants.

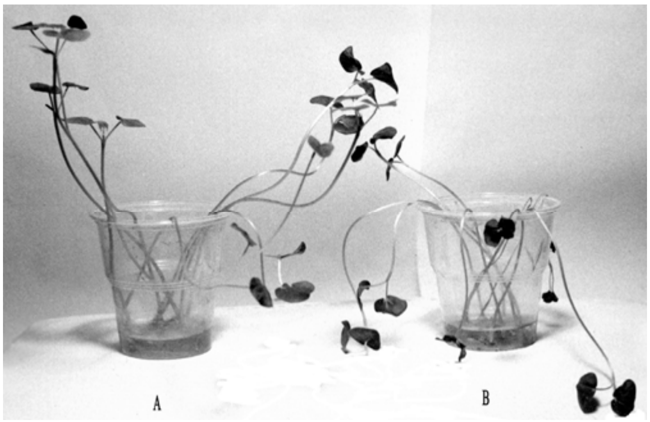


**Fig. 5 Effect of verapamil (0.9 mM) on the state of 20-day-old buckwheat plants.** (**A**) Control plants; (**B**) experimental plants.

## ACKNOWLEDGEMENTS

The author is grateful to V. I. Guliaev for technical assistance.

## REFERENCES

**Amstrong ASB, Rycroft DV, Tanton TW** (1996) Seasonal movement of salts in naturally structured saline sodic clay soils. *Agricultural Water Management* **32**, 15-27

**Azaizeh H, Steudle E** (1991) Effect of salinity on water transport of excised maize (*Zea mays* L.) roots. *Plant Physiology* **97**, 1136-1145

**Budagovskaya NV** (1997) Effect of calcium channels blocker and growth regulators on the state of photoautotrophic and heterotrophic plant tissues. *The FASEB Journal* **11**, 1435

**Budagovskaya NV** (1998) Changes in the state of photoautotrophic and heterotrophic organs of buckwheat plants at iron deficiency and low pH. *Fagopyrum* **15**, 1-7

**Budagovskaya NV, Guliaev VI** (2001a) Effect of calcium channel blocker on growth dynamics of plants studied by laser interference auxanometry. In: Horst WJ, Schenk MK, Bürkert A, Claassen N, Flessa H, Frommer WB, Goldbach H, Olfs H-W, Römheld V, Sattelmacher B, Schmidhalter U, Schubert S, Wirén NV, Wittenmayer L (Eds) *Plant Nutrition: Food Security and Sustainability of Agro-Ecosystems through Basic and Applied Research*, Kluwer Academic Publishers, The Netherlands, pp 204-205

**Budagovskaya NV, Alekhin VI, Guliaev VI** (2001b) Effect of calcium channel blockage on functional activity of plants. *European Journal of Biochemistry* **268 (Suppl 1)**, 102

**Budagovskaya NV, Guliaev VI** (2004) Antioxidant induced changes in leave growth rate of plants. *Agriculture* **2**, 92-96

**Budagovskaya NV** (2007) Interaction of plant shoots and roots: dynamics and stability. In: Belousov LV, Voeikov VL, Martinuik V (Eds) *Biophotonics and Coherent Systems*, Springer, New York, pp 213-223

**Budagovskaya NV** (2009) Disturbance in water transport in roots of maize plants under salinization. In: *Proceedings of VIII International Symposium "New and Non-Traditional Plants and Perspectives of their Utilization*" (Vol II), Moscow, Russia, pp 62-66

**Gebauer J, El-Siddig K, Salih AA, Ebert G** (2003) Effect of different levels of NaCl-induced salinity on growth, photosynthesis, leaf chlorophyll concentration and ion distribution of *Adansonia digitata* L. seedlings. *Journal of Applied Botany* **77**, 103-107

**Lynch J, Lauchli A** (1985) Salt stress disturbs the calcium nutrition of barley (*Hordeum vulgare* L.) *New Physiol*ogy **87**, 351-356

**Pitman MG, Lauchli A** (2002) Global impact of salinity and agricultural ecosystems. In: Lauchli A, Luttge V (Eds) *Salinity: Environment-Plants Molecules*, Kluwer Academic Publishers, The Netherlands, pp 3-20

**Sherif DW, Sinclair R** (1973) Fluctuations in leaf water balance with a period of 1 to 10 minutes. *Planta* **113**, 215-228

**Ushakov VY, Koltunova IR** (1982) On pulsating character of transpiration and water supply to plant leaves. *Proceedings of the Russian Academy of Sciences* **266**, 766-768

**Vose PB** (1983) Rationale of selection for specific nutritional characters in crop improvement with *Phaseolus vulgaris* L. as a case of study. *Plant and Soil* **72**, 351-364

**Zholkevich VN** (1991) Root pressure. In: Waisel Y, Eshel A, Kafkafi U (Eds) *Plant Roots. The Hidden Half*, Marcel Dekker Inc., New York, pp 589-603

**Zyalalov AA** (1984) *Physiology-thermodynamical Aspect of Water Transport in Plant*, Nauka, Moscow, 135 pp