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EFFECTS OF *GINKGO BILOBA* L. EXTRACT ON THE SEED GERMINATION, SEEDLING GROWTH AND LEAF ANATOMY OF BARLEY UNDER SALINE CONDITIONS

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Abstract

Effects of *Ginkgo biloba* L. extract on the seed germination, seedling growth and leaf anatomy of barley under saline conditions were studied. In parallel with concentration rise, salt stress inhibited the seed germination and seedling growth of barley. The inhibitive effect of salt on the germination and coleoptile percentage was alleviated in varying degrees, and dramatically, by *Ginkgo biloba* application. However, it became ineffective in alleviating of salt inhibition on the radicle, coleoptile length, radicle number and fresh weight of barley seedlings. On the other hand, it was observed that *Ginkgo biloba* extract affected in different degrees the various parameters of leaf anatomy of barley seedlings, and this difference was statistically important.

Introduction

Nearly 20% of the world's cultivated area and half of the world's irrigated lands are affected by salinity (Zhu 2001). The salt-affected soils contain excess salts which affect plants by decreasing the osmotic potential of the soil solution (osmotic stress), interfering with normal nutrient uptake, inducing ionic toxicity, and associating nutrient imbalances (An *et al*. 2003). Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of production (Sairam and Tyagi 2004). In addition, it is evident that there are big changes in leaf morphology and anatomy of the plants growing in saline soils (Çavuşoğlu *et al*. 2008).

Ginkgo biloba L. (GB) is probably the oldest known tree species, dating back to 300 million years and it is often called the "living fosil" (Mariassyova 2006). More than 20 *Ginkgo* variations exist that differ in tree habitat and in shape, colour and size of leaves, the latter being the raw material for the pharmaceutical industry (Ellain-Wojtaszek *et al.* 2002). Especially the leaves of GB contain compounds possessing an antioxidant character (Zahradnikova *et al.* 2007). There have been isolated three main compounds from GB with an antioxidant activity-kaempferol, quercetin and isoharmnetin (Spence and Jane 1999). The antioxidant activity of a Ginkgo extract is determined mainly by flavonoids, which scavenge and destroy free radicals and the reactive forms of oxygen (Ellain-Wojtaszek *et al*. 2002). GB leaf extract is the most widely sold phytomedicine in Europe, where it is used to treat the symptoms of early-stage Alzheimer's disease, vascular dementia, premenstrual problems, peripheral claudication, altitude sickness and tinnitus of vascular origin (Sierpina *et al*. 2003).

Although there are many clinical reports on GB, unfortunately, the protective mechanisms of GB on salt stress in plants are stil poorly understood. The purpose of this study is to observe the influences of GB extract in reducing the inhibitive effects of salt stress on the seed germination, seedling growth and leaf anatomy of barley.

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Materials and Methods

Barley (*Hordeum vulgare* L. cv. Bülbül 89) seeds were used. Salt (NaCl) concentrations used were 0.0, 0.25, 0.275 and 0.30 M. *Ginkgo biloba* (GB) concentration used in the experiments was 0.5 ppm.

Germination experiments were carried out at a constant temperature (20°C), in the dark in an incubator. Barley seeds in adequate amount were pretreated in the beakers containing sufficient amount of distilled water (control, C) or aqueous solution of GB for 24 h at room temperature. At the end of this pretreatment, the solutions were filtered immediately and the seeds were dried in vacuum (Braun and Khan 1976). Twenty five seeds from every application were arranged into Petri dishes (10 cm diam.) lined by 2 sheets of Whatman No. 1 filter paper moistened with 7 ml of the salt solution. After sowing, Petri dishes were placed into an incubator for germination for 7 days. It was assumed that the radicle should be 10 mm long for germination. At the end of the 7th day, after determination of the final germination percentages, the coleoptile emergence percentages and radicle numbers were also recorded, and in addition, the fresh weights in mg/seedling were determined. All experiments were repeated four times.

The seedlings from the seeds germinated in the incubator at 20°C for 7 days were transferred to pots with perlite including NaCl solutions prepared with Hoagland recipe and were grown in a growth chamber for 20 days. Anatomical sections were taken from the second leaf of 20-day-old seedlings by a microtome, in 6-7 μm thickness. They were examined under a binocular light microscope (Olympus CX41) at 100 magnification. Stomata and epidermal cells in a 1-mm^2 unit area were counted to determine the stomatal index. These counts were made both in the lower and upper surfaces of each leaf 10 times as 3 replicates and the averages were calculated. After the determination of the number of stomata and epidermal cells in the leaf unit area, the stomatal index was estimated according to Meidner and Mansfield's (1968) method. Stomata width and length, epidermal cell width and length, leaf thickness and distance between vascular bundles were also determined. Statistical evaluation concerning all parameters was realized by using SPSS program according to DMRT.

Results and Discussion

Results showed that GB application increased the germination and coleoptile percentage while it decreased the coleoptile length and fresh weight. In addition, it statistically showed the same values as the C regarding the radicle lenght and radicle number (Table 1). Çavuşoğlu *et al*. (2010) reported that GB partly reduced the germination percentage of *Vicia faba* seeds in distilled water medium. This result was not consistent with the present findings. Howewer, they also observed that GB did not show a meaningful effect on the radicle length of *V. faba* seedlings and this is consistent with the present findings. It can be said that GB can show different effects on seed germination and seedling growth depending on the plant species and the concentrations used.

Salt, in the paralellism of concentration increase, increased its inhibitive effect on all the examined growth parameters. For example, while C seeds germinated in distilled water medium showed 94 % germination on the 7th day, this value became 35, 24 and 18%, respectively in 0.25, 0.275 and 0.30 M salinity (Table 1). On the other hand, GB application markedly alleviated the inhibitive effect of salt stress on the seed germination. The seeds pretreated with GB demonstrated 77, 65 and 56% germination in the above mentioned salt levels. GB also continued its success on the coleoptile percentage. However, it was ineffective in alleviating salt inhibition on the radicle length, coleoptile length, radicle number and fresh weight of barley seedlings (Table 1). It is possible that GB may be effective in alleviating the inhibitive effect of salt on the seed germination by increasing nucleic acid and protein synthesis, by stimulating mitotic activity of

embryo, by providing stabilization of cell membranes or by raising antioxidant enzyme activities (Ellain-Wojtaszek *et al*. 2002, Zahradnikova *et al*. 2007).

Table 1. Various growth parameters of the seedlings from barley seeds germinated in saline conditions for 7 days.

NaCl (M)	Pretreatment (ppm)	Growth parameters					
		Germination (%)	Coleoptile (%)	Radicle length (mm)	Coleoptile length (mm)	Radicle number	Fresh weight (mg/seedling)
0.0	C	*94±4.0^{g}	93±2.0^{g}	104.0±1.7^{d}	131.9±2.7^{h}	4.8±0.0^{b}	401.9±2.7^{h}
	GB	100±0.0^{h}	97±2.0^{h}	104.9±1.4^{d}	110.7±1.2^{g}	4.8±0.2^{b}	379.7±1.2^{g}
0.25	C	35±3.8^{c}	34±2.3^{c}	58.4±2.0^{c}	38.4±1.9^{f}	4.4±0.1^{a}	208.4±1.9^{ef}
	GB	77±2.0^{f}	75±2.0^{f}	55.9±2.2^{bc}	35.9±1.3^{e}	4.4±0.2^{a}	205.9±1.3^{e}
0.275	C	24±3.2^{b}	24±3.2^{b}	56.0±2.7^{bc}	26.0±2.3^{cd}	4.4±0.1^{a}	196.0±1.3^{cd}
	GB	65±2.0^{e}	64±3.2^{e}	47.0±1.8^{ab}	19.9±1.7^{b}	4.3±0.1^{a}	189.9±1.7^{b}
0.30	C	18±2.3^{a}	18±2.3^{a}	45.5±1.7^{ab}	24.3±1.4^{c}	4.3±0.2^{a}	194.3±1.4^{c}
	GB	56±3.2^{d}	53±3.8^{d}	44.3±2.9^{a}	15.8±1.0^{a}	4.2±0.3^{a}	182.8±2.0^{a}

*The difference between values with the same letter in each column is not significant at 0.05 (± SD).

GB greatly affected the leaf anatomical structures of *Hordeum vulgare* seedlings grown under normal conditions (Figs 1, 2 and 3). In distilled water medium, GB pretreatment increased the number, width and length of stomata in the upper surface; the epidermal cell length in the lower surface; the epidermal cell width and stomatal index in both surfaces; and the distance between vascular bundles in comparison with the C seedlings. GB application reduced the width and length of stomata in the lower surface; the epidermal cell length in the upper surface in comparison with the C seedlings (Table 2).

Salinity of the medium caused changes in the leaf anatomical characters of seedlings (Figs 1, 2 and 3). 0.25 M salinity stimulated the epidermal cell number in the lower surface; the epidermal cell width and epidermal cell length in both surfaces; and the leaf thickness and distance between vascular bundles in the seedlings non-pretreated with GB in compared to C. This salt level decreased the stomata length in the lower surface; the stomata number, stomata width and stomatal index in both surfaces. As for 0.275 M salinity, it increased the epidermal cell width in the upper surface; the epidermal cell number in the lower surface; the epidermal cell length in both surfaces; and the leaf thickness and distance between vascular bundles. This salinity reduced the stomata length in the lower surface; the stomata number, stomata width and stomatal index in both surfaces. 0.30 M salinity increased the epidermal cell number in the lower surface; the epidermal cell width and epidermal cell length in both surfaces; and the leaf thickness and distance between vascular bundles. The mentioned salinity decreased the stomata number, stomata width, stomata length and stomatal index in both surfaces (Table 2). On the other hand, it was reported previously that salt stress caused positive or negative effects on the leaf anatomical parameters of barley seedlings (Çavuşoğlu *et al.* 2007, 2008). These observations indicate that barley leaves acquire succulent (for example, in the upper surface the increase in epidermal cell width; in the lower surface the decrease in stomata number) properties (Strogonov 1964).

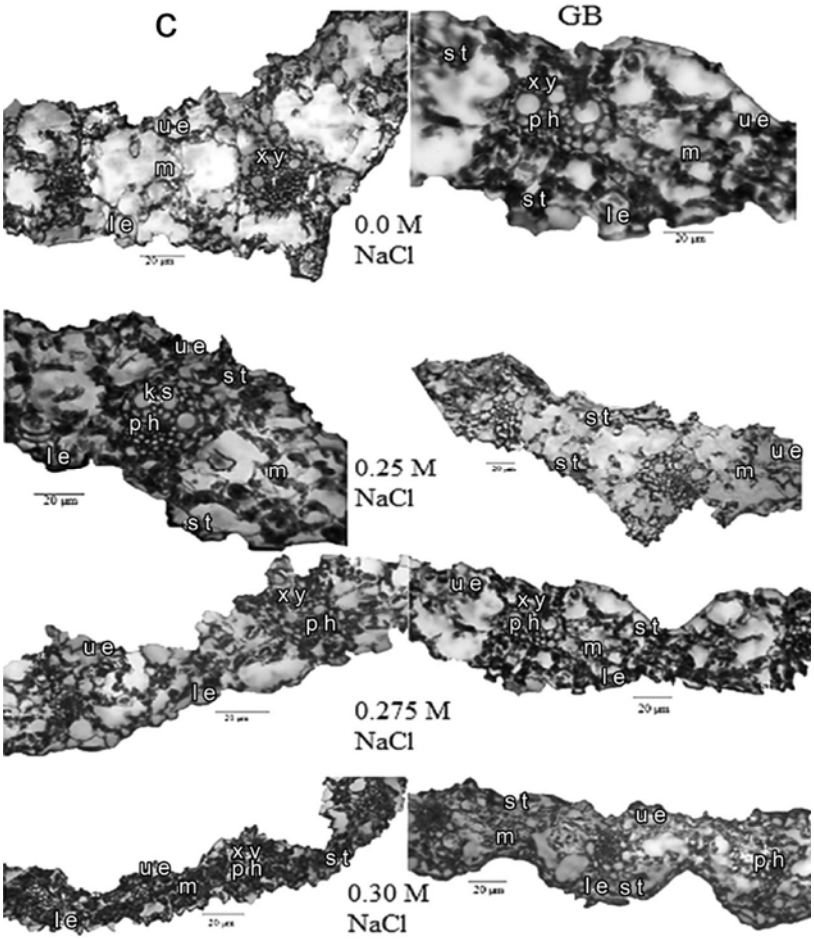

Fig. 1. Leaf cross sections from distilled water and GB pretreated barley seedlings grown in various concentrations of NaCI at 25°C (le: lower epidermis, ue: upper epidermis, m: mesophyll, st: stomata, xy: xylem, ph: floem).

GB pretreatment increased the stomata width in the upper surface; the epidermal cell length in the lower surface; the stomata number, stomata length and stomatal index in both surfaces; and the leaf thickness in comparison with the C seedlings grown in 0.25 M salinity. This pretreatment reduced the epidermal cell length in the upper surface; the epidermal cell number and epidermal cell width in the lower surface; and the distance between vascular bundles. In 0.275 M salinity, GB application increased the stomata number, stomata width, stomata length and stomatal index in both surfaces. This application decreased the epidermal cell number in the lower surface; the epidermal cell width and epidermal cell length in both surfaces; and the leaf thickness and distance between vascular bundles. As for 0.30 M salinity, GB increased the epidermal cell number in the upper surface; the stomata number, stomata width, stomata length and stomatal index in both surfaces. It reduced the epidermal cell number in the lower surface; the epidermal cell width and epidermal cell length in both surfaces; and the leaf thickness and distance between vascular bundles (Table 2).

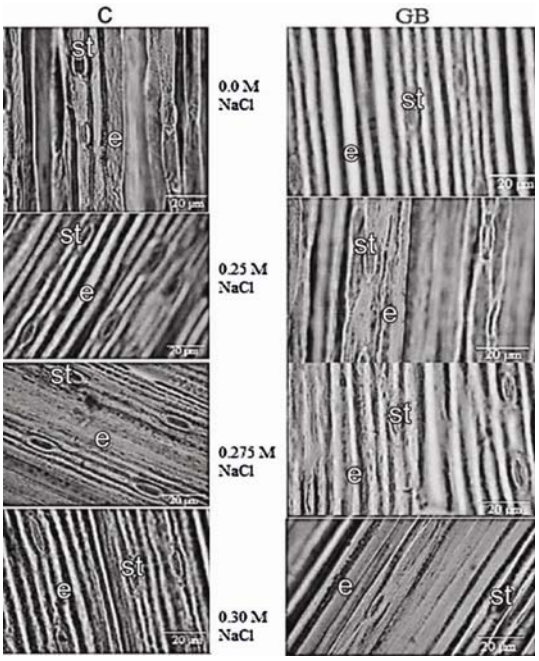

Fig. 2. Leaf lower surface sections from distilled water and GB pretreated barley seedlings grown in various concentrations of NaCI at 25°C (e: epidermis, st: stomata).

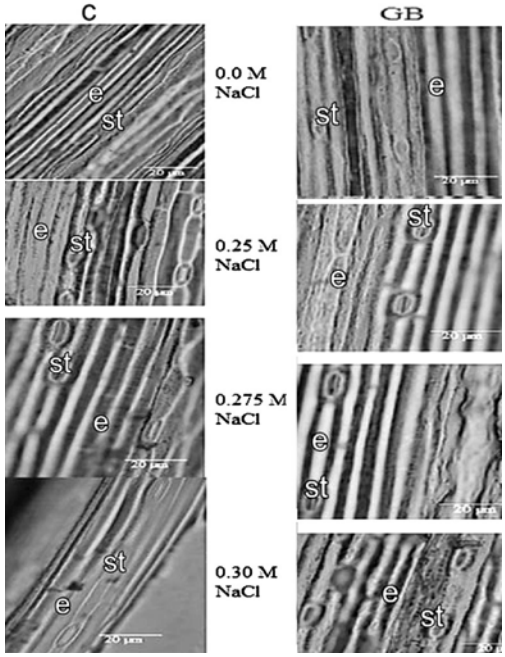

Fig. 3. Leaf upper surface sections from distilled water and GB pretreated barley seedlings grown in various concentrations of NaCI at 25°C (e: epidermis, st: stomata).

GB pretreatment makes water and food transport easy by reducing the distance between vascular bundles in 0.25, 0.275 and 0.30 M NaCl. Moreover, the mentioned application provides adaptation to saline conditions by increasing the leaf thickness in 0.25 M salinity and so decrease transpiration and water loss. In addition, it can lead to the same aim by causing a reduction of leaf area as a result of decreasing epidermal cell width and epidermal cell length of both surfaces of the leaves in all the salt levels.

Table 2. Some parameters of leaf anatomy of barley seedlings grown for 20 days in various concentrations of NaCl at 25°C after GB pretreatment.

NaCl (M)	Pre-treatment (ppm)	Epidermal cell number		Epidermal cell width (µm)		Epidermal cell length (µm)		Stomata number	
		Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
0.0	C	*11.8±2.1^{a}	9.1±2.2^{b}	8.7±1.3^{a}	9.2±1.2^{ab}	25.0±2.3^{ab}	24.7±1.8^{a}	5.7±1.1^{e}	5.1±1.0^{d}
	GB	10.5±2.2^{a}	9.0±2.0^{b}	10.2±1.8^{b}	10.7±1.6^{b}	24.0±2.1^{a}	28.5±1.0^{bc}	8.2±2.1^{f}	5.0±1.0^{d}
0.25	C	11.5±1.3^{a}	11.2±1.4^{c}	10.0±1.6^{b}	10.5±1.9^{b}	29.2±2.4^{c}	29.7±1.1^{c}	3.6±1.1^{bc}	3.8±1.1^{b}
	GB	10.4±2.3^{a}	8.3±1.7^{a}	10.0±1.6^{b}	9.2±1.6^{ab}	26.2±1.1^{b}	30.2±1.4^{d}	5.2±1.4^{de}	4.3±1.1^{c}
0.275	C	10.6±1.4^{a}	14.0±1.6^{d}	10.0±1.6^{b}	9.5±2.2^{ab}	29.2±1.1^{c}	29.2±2.4^{c}	3.4±0.9^{ab}	3.2±0.7^{a}
	GB	11.0±2.0^{a}	8.1±1.6^{a}	9.2±1.6^{ab}	8.5±1.2^{a}	24.2±1.0^{a}	25.0±1.3^{a}	4.4±1.1^{cd}	3.6±0.6^{ab}
0.30	C	11.7±1.9^{a}	14.9±2.3^{d}	9.2±2.0^{ab}	11.0±2.4^{b}	26.2±1.7^{b}	27.0±2.3^{b}	2.6±0.7^{a}	3.2±0.7^{a}
	GB	13.7±2.2^{b}	8.1±1.9^{a}	8.7±2.1^{a}	8.2±2.0^{a}	24.7±1.7^{a}	24.5±1.2^{a}	4.7±1.0^{d}	4.4±0.9^{c}

Table contd. (right side)

Stomata width (µm)		Stomata length (µm)		Stomatal index		Leaf thickness (µm)	Distace between vascular bundles (µm)
Upper	Lower	Upper	Lower	Upper	Lower		
13.5±1.7^{b}	16.5±3.1^{d}	38.1±2.6^{b}	47.1±4.2^{c}	34.2	34.0	67.8±1.3^{b}	122.7±1.2^{a}
15.8±2.1^{c}	14.2±2.0^{c}	41.1±3.3^{c}	41.8±4.1^{b}	40.8	36.2	68.1±2.3^{b}	154.8±2.6^{e}
12.7±1.6^{ab}	14.2±2.0^{c}	38.3±2.3^{b}	37.3±1.8^{a}	23.7	26.5	76.3±2.1^{d}	161.0±2.0^{f}
17.8±2.5^{d}	14.6±1.4^{c}	40.6±3.9^{c}	47.8±4.9^{c}	32.7	33.2	93.1±1.7^{f}	148.7±1.0^{c}
11.7±2.1^{a}	11.8±1.9^{ab}	37.5±2.2^{b}	38.0±2.2^{a}	21.1	18.4	78.5±1.8^{e}	167.1±1.8^{g}
18.6±2.2^{de}	16.1±1.5^{d}	41.1±3.2^{c}	41.8±4.3^{b}	28.8	29.7	75.0±1.4^{d}	155.3±1.4^{e}
12.2±1.9^{ab}	11.2±1.7^{a}	35.3±1.6^{a}	36.1±2.3^{a}	19.0	16.5	70.2±1.0^{c}	150.7±2.9^{cd}
19.8±2.2^{e}	12.7±1.7^{b}	42.2±4.3^{c}	43.3±4.4^{b}	25.7	35.2	65.9±2.2^{a}	135.5±2.6^{b}

*The difference between values with the same letter in each column is not significant at 0.05 (± SD).

It is clear that adverse effects of salt stress on the seed germination, seedling growth and leaf anatomy of barley were significantly improved by exogenous application of GB. The mechanisms by which salinity inhibits growth are complex and controversial. Moreover, they may vary according to species and cultivar. A universal mechanism has not been established yet. Although the causes of salinity have been characterized, our understanding of the mechanisms by which salinity prevents plant growth is still rather poor. This work may serve to provide new conceptual tools for designing hypotheses of salt tolerance in plants.

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