BIOLOGIA PLANTARUM 54 (4): 753-756, 2010

BRIEF COMMUNICATION

The effect of 5-azacytidine on wheat seedlings responses to NaCl stress

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Abstract

The effect of 5-azacytidine (5-azaC) on the alleviation of damaging effects of NaCl treatment was studied in two wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance (salt-tolerant Dekang-961 and sensitive Lumai-15). The plants were pre-treated or not with 50 μM 5-azaC and then subjected to salt stress induced by 100 or 150 mM NaCl. Salinity caused reduction in biomass accumulation and increase in malondialdehyde content in root tissues in both cultivars, but less in pre-treated seedlings. The activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in the roots of both cultivars increased during salt stress, but the rate of increase was higher in Dekang-961. Plants treated with 5-azaC had higher root SOD, CAT and POD activities under salt stress than untreated plants. Content of 5-methylcytosine (5mC) decreased in both cultivars under salt stress, and the level of demethylation was higher in Dekang-961 than that in Lumai-15. Moreover, the degree of methylation was lower in both cultivars under salt stress after 5-azaC application compared to only salt-treated groups. These findings suggested that 5-azaC could protect plants from salt stress.

Additional key words: antioxidant enzymes, DNA methylation, lipid peroxidation.

In many areas of the world, salinity has serious impact on crops and represents the main constraint for agriculture (Munns 2002). Salinity imposes both ionic toxicity and osmotic stress to plants, leading to growth inhibition (Serrano and Rodriguez 2002, Zhu 2003) and production of reactive oxygen species (ROS), including the superoxide anion radical, hydroxyl radical, and hydrogen peroxide (Wang *et al.* 2003). These ROS can seriously disrupt normal metabolism through oxidative damage of lipids, proteins and nucleic acids (Apel and Hirt 2004). Plants have developed a number of antioxidant defense mechanisms to protect themselves against ROS. These mechanisms employ antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as low molecular mass antioxidants, including ascorbic acid, glutathione, and phenolic compounds (Noctor and Foyer 1998, Asada 1999). Plants with high contents of antioxidants, either constitutive or induced, have been reported to have greater resistance to the oxidative damage (Young and Jung 1999). Accordingly, the regulation of these antioxidants by an exogenous substance might mediate the plant tolerance to salt stress (Mutlu *et al.* 2009). Malondialdehyde (MDA) as the decomposition product of polyunsaturated fatty acids of biomembranes, shows great accumulation under salt stress (Aghaleh *et al.* 2009, Fedina *et al.* 2009). Environmental stresses such as salt stress (Kovarik *et al.* 1997, Dyachenko *et al.* 2006), aluminum (Choi and Sano 2007), heavy metals (Aina *et al.* 2004, Filek *et al.* 2008), cold (Lizal and Relichova 2001, Steward *et al.* 2002) and water stress (Labra *et al.* 2002) can cause an increase or decrease in cytosine methylation. The treatment with 5-azacytidine (5-azaC), a demethylating agent, replaces low-temperature treatment in several vernalization requiring plant species (Burn *et al.* 1993, Brock and Davidson 1994, Demeulemeester *et al.* 1999, Yong *et al.* 2003). The aim of this paper was to investigate the effect of 5-azaC application on responses of wheat seedlings to salt stress.

Received 1 April 2009, *accepted* 25 March 2010.

Abbreviations: 5-azaC - 5-azacytidine; 5mC - 5-methylcytosine; ANOVA - analysis of variance; CAT - catalase (EC 1.11.1.6); CTAB - cetyltrimethylammoniumbromide; MDA - malondialdehyde; NBT - nitroblue tetrazolium; POD - peroxidase (EC 1.11.1.7); ROS - reactive oxygen species; SOD - superoxide dismutase (EC 1.15.1.1); TBA - thiobarbituric acid; TCA - trichloroacetic acid.

Acknowledgements: This work was carried out with the financial support from the National Natural Science Foundation of China (No. 30821064) and the Chinese 111 Project (B06018).

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Two cultivars of wheat (*Triticum aestivum* L.) were used: Dekang-961 (salt tolerant) and Lumai-15 (salt sensitive), obtained from Shandong Academy of Agricultural Sciences and Shandong Agricultural University, China. Seeds were surface sterilized with 0.1 % $HgCl_2$ for 3 min and then washed thoroughly with distilled water. The seeds (200 seeds of each cultivar; 25 seeds per Petri dish) were germinated for 3 d in the dark at 25 °C on filter paper soaked with distilled water. Selected seedlings were provided with 0 or 50 μM 5-azaC for 5 d, and later plants were grown in Hoagland solution containing 0, 100 or 150 mM NaCl without 5-azaC for another 5 d. Seedlings were grown under temperature of 25/15 °C (day/night), relative humidity of 65 - 75 % and a 16-h photoperiod with irradiance of 250 μmol m^{-2} s^{-1}.

Dry mass was measured after exposure of seedlings from each treatment at 105 °C for 15 min, then at 75 °C for 72 h. The content of MDA was measured according to Dhindsa *et al.* (1981) with some modification. Roots (1 g) were homogenized in 5 cm^3 of 0.1 % (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12 000 *g* for 5 min and 4 cm^3 of 20 % TCA containing 0.5 % (m/v) thiobarbituric acid (TBA) was added to 1 cm^3 of supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. Afterwards, the samples were centrifuged at 12 000 *g* for 15 min and absorbance of the supernatant was read at 450, 532 and 600 nm.

Roots (1 g) were ground to a fine powder using liquid nitrogen in pestle and mortar and extracted in 2 cm^3 extraction buffer (50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 1 % polyvinyl-pyrrolidone). The homogenate was centrifuged at 12 000 *g* for 15 min at 4 °C and the supernatants were used for the enzyme assay. SOD activity was determined according to the method of Giannopolitis and Ries (1977) using the photo-reduction of nitroblue tetrazolium (NBT) with some modification. The reaction buffer (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 μM NBT, 2 mM riboflavin and 0.05 cm^3 of the enzyme extract. Reactions were carried out at 25 °C, under irradiance of 300 μmol m^{-2} s^{-1} for 10 min. The absorbance was measured at 560 nm. One unit of SOD activity was assigned as the amount of enzyme that would inhibit 50 % of reaction. According to Jiang and Huang (2001) with some modification, CAT activity was determined by a decline in absorbance at 240 nm due to the decomposition of H_2O_2. Assay mixture (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 0.05 cm^3 of enzyme extract. One unit of activity was defined as the amount of enzyme required to decrease 0.1 unit of absorbance at 240 nm per min at 25 °C. POD activity was measured as the change of absorbance of 470 nm due to guaiacol oxidation according to the method described by Polle *et al.* (1994). The reaction mixture (3 cm^3) was composed of 50 mM potassium phosphate buffer (pH 7.0), 2.7 mM guaiacol, 2 mM H_2O_2 and 0.15 cm^3 of enzyme extract. One unit of activity was defined as the amount of enzyme required to increase 0.01 units of absorbance at 470 nm per min.

Roots were excised, frozen in liquid nitrogen and stored at -70 °C. DNA for HPLC was isolated following a modification of the cetyltrimethylammonium bromide (CTAB) extraction method by Murray and Thompson (1980). According to Demeulemeester *et al.* (1999), approximately 30 μg DNA was hydrolyzed to bases in 0.05 cm^3 of 70 % perchloric acid (100 °C for 1 h). The pH was adjusted to between 3 and 5 with 1 M KOH. The homogenate was centrifuged at 12 000 *g* for 5 min and the supernatant was prepared for HPLC. An aliquot of the filtrate (0.02 cm^3) was injected on the HPLC, using a column *ZORBAX Eclipse XDB-C18* (4.6 × 150 mm) (*Agilent*, Palo Alto, USA). The eluent (pH 4) contained 10 % methanol, 5 mM sodium pentanesulfonate and 0.2 % triethylamine, with a flow rate of 0.5 cm^3 min^{-1} and detection wavelength 273 nm. The concentrations of cytosine and 5-methylcytosine (5mC) in a sample were evaluated and the percentage of 5mC was calculated as (concentration of 5mC × 100)/(concentration of 5mC + concentration of cytosine).

All data were statistically analysed by analysis of variance (*ANOVA*) and the mean differences were compared by LSD test, calculated at $P < 0.05$.

Dry mass of seedlings declined significantly in both cultivars with salinity stress being a little bit more prominent in sensitive Lumai-15 (Table 1). This decrease was largely prevented in seedlings that were previously treated with 5-azaC. Peroxidation of lipid membranes indicates oxidative damage at the cellular level under salt stress conditions (Hernandez *et al.* 2001, Sudhakar *et al.* 2001). MDA content was significantly higher in Lumai-15 than that in Dekang-961 under the control conditions (Table 1). Upon exposure to salinity, a significant increase in MDA content was found in both cultivars, and higher MDA content was in Lunai-15 (Table 1). The 5-azaC pre-treatment considerably reduced MDA content in both cultivars during NaCl treatment, thereby alleviating the damaging effects of salt treatment on membrane lipids (Table 1). Compared to Lumai-15, activities of SOD, CAT and POD were higher in Dekang-961 (Table 1). The activities of SOD, CAT and POD increased due to the increase of NaCl concentration in Dekang-961 and Lumai-15, but the rate of increase was significantly higher in the former (Table 1). Salt tolerance is often correlated with more efficient antioxidant system (Gueta-Dahan *et al.* 1997, Bor *et al.* 2003). Higher constitutive and/or NaCl-induced activities of SOD, CAT, and POD in Dekang-961 suggested better tolerance of Dekang-961 to salt stress when compared to Lumai-15. Supporting our results, higher constitutive and/or induced activities of antioxidant enzymes and lower levels of lipid peroxidation were found in tolerant wheat (Sairam *et al.* 2002), wild beet (Bor *et al.* 2003), and rice (Dionisio-Sese and Tobita 1998) cultivar under salt stress. 5-azaC pre-treatment significantly enhanced SOD, CAT and POD activities in both cultivars under control conditions as well as under NaCl stress (Table 1).

The mechanism how 5-azaC alleviated negative effects of salt stress and enhanced antioxidative enzyme activities

Table 1. 5-azacytidine effect on dry mass of the seedlings, MDA content, activity of SOD, CAT and POD and 5mC content in the roots of Dekang-961 and Lumai-15 under salt stress. 3-d-old wheat seedlings were pre-treated with 0 or 50 μM 5-azaC for 5 d, grown further for 5 d in Hoagland solution with various concentrations of NaCl (0, 100, 150 mM). The data present means ± SE of ten replicates in dry mass and three replicates in the other parameters. Different letters in columns indicate significant differences at $P < 0.05$.

Cultivar	5-azaC [μM]	NaCl [mM]	Dry mass [mg seedling^{-1}]	MDA [nmol g^{-1}(f.m.)]	SOD [U g^{-1}(f.m.)]	CAT [U g^{-1}(f.m.)]	POD [U g^{-1}(f.m.)]	5mC [%]
Dekang-961	0	0	25.09 ± 1.65bc	11.20 ± 0.27g	11.25 ± 1.01efg	19.47 ± 1.39h	8.12 ± 0.77fg	27.37 ± 0.21a
	0	100	21.94 ± 2.16d	15.28 ± 0.40d	17.55 ± 1.10d	27.39 ± 1.29e	14.79 ± 1.41d	24.01 ± 0.13c
	0	150	21.84 ± 2.09d	19.44 ± 0.40b	21.31 ± 1.30c	32.16 ± 1.13c	15.12 ± 1.92d	23.05 ± 0.24d
	50	0	24.13 ± 1.96c	9.33 ± 0.33i	13.47 ± 0.97e	21.48 ± 0.85gh	11.04 ± 1.56e	22.16 ± 0.07e
	50	100	27.18 ± 1.70a	11.44 ± 0.58g	26.65 ± 1.46b	35.44 ± 1.22b	20.01 ± 1.41ab	19.07 ± 0.06g
	50	150	24.96 ± 2.92bc	12.72 ± 0.30f	30.68 ± 0.83a	40.22 ± 1.26a	22.22 ± 1.23a	17.89 ± 0.54h
Lumai-15	0	0	25.73 ± 2.03abc	12.59 ± 0.27f	9.44 ± 1.36g	17.22 ± 1.14i	6.37 ± 0.74g	25.91 ± 0.31b
	0	100	21.22 ± 1.83d	18.49 ± 0.45c	11.44 ± 0.89efg	22.25 ± 1.33g	9.63 ± 1.37ef	23.10 ± 0.72d
	0	150	20.31 ± 1.41d	23.14 ± 0.25a	12.62 ± 1.14ef	24.94 ± 1.39f	9.66 ± 1.35ef	22.30 ± 0.05e
	50	0	24.21 ± 2.12c	10.42 ± 0.76h	11.14 ± 1.36fg	19.59 ± 1.29h	8.36 ± 0.89fg	20.72 ± 0.07f
	50	100	26.24 ± 1.59ab	14.09 ± 0.21e	21.81 ± 1.26c	29.74 ± 1.54d	16.95 ± 1.16cd	18.63 ± 0.36g
	50	150	24.69 ± 1.46bc	15.44 ± 0.43d	24.88 ± 2.74b	34.96 ± 1.19b	18.33 ± 1.91bc	17.68 ± 0.47h

is not quite clear yet. In this study, HPLC approach was used to assess whether salt stress caused changes in DNA methylation. We found that the DNA methylation level decreased significantly in both cultivars with the increase of NaCl concentration, and the rate of decline was higher in Dekang-961 than that in Lumai-15. A higher demethylation was found in 5-azaC pre-treated plants and NaCl-stressed plants than in those only salt-stressed (Table 1).

It was shown that DNA methylation plays an important role in organizing the genome into trascriptionally active and inactive zones (Lee *et al.* 1998), and that the variation of methylation at specific sequences is often correlated to altered gene expression (Bender 1998, Paszkowski and Whitham 2001). A recent survey revealed that the pattern of DNA methylation spontaneously and reversibly changes during the life time of eukaryotes (Bird 2002). Hypomethylation can be then considered either a simple effect of salt stress or a precise defensive mechanism by which the cell regulates the gene expression. Choi and Sano (2007) found that, in healthy leaves grown under non-stressed conditions, NtGPDL (a glycerophosphodiesterase-like protein) was not expressed, and upon exposure to abiotic stress, its transcripts were induced and genomic locus was partly demethylated. This can be a good example indicating the close correlation between methylation and gene expression upon abiotic stresses. Since DNA methylation is closely linked to histone modification (Tariq and Paszkowski 2004, Fuchs *et al.* 2006), it is conceivable that demethylation might induce alteration of chromatin structure, thereby enhancing transcription. When 5-azaC was applied in addition to the cold treatment, the ratio of 5mC dropped lower than the level of methylation of plants only exposed to cold treatment (Horvath *et al.* 2003). We could speculate that demethylation induced by 5-azaC has led to increase in resistance to salt stress.

Heredity of epigenetic modification or acquired traits in response to environmental factors has long been speculated, and DNA methylation has been proposed as one of its promoters (Holliday 1993). Steward *et al.* (2002) have shown this by treatment of germinated rice seeds with 5-azaC; plants exhibited a global demethylation and altered phenotype at maturity and the acquired traits and demethylation patterns were inherited for up to at least six generations. Thus alteration of DNA methylation can induce changed expression of some genes, resulting in a new phenotype, both of which are heritable (Sano *et al.* 1990). It is proposed that environmental responses of plants could be partly mediated through alteration of the DNA methylation status.

References

Aghaleh, M., Niknam, V., Ebrahimzadeh, H., Razavi., K.: Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. - Biol. Plant. **53**: 243-248, 2009.

Aina, R., Sgorbati, S., Santagostino, A., Labra, M., Ghiani, A., Citterio, S.: Specific hypomethylation of DNA is induced by heavy metals in white clover and industrial hemp. - Physiol. Plant. **121**: 472-480, 2004.

Apel, K., Hirt, H.: Reactive oxygen species: metabolism, oxidative stress, and signal transduction. - Annu. Rev. Plant Biol. **55**: 373-399, 2004.

Asada, K.: The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 601-639, 1999.

Bender, J.: Cytosine methylation of repeated sequences in eukaryotes: the role of DNA pairing. - Trends Biochem. Sci.

23: 252-256, 1998.

Bird, A.P.: DNA methylation patterns and epigenetic memory. - Genes Dev. **16**: 6-21, 2002.

Bor, M., Ozdemir, F., Turkan, I.: The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. - Plant Sci. **164**: 77-84, 2003.

Brock, R.D., Davidson, J.L.: 5-Azacytidine and gamma rays partially substitute for cold treatment in vernalization winter wheat. - Environ. exp. Bot. **31**: 195-199, 1994.

Burn, J.E., Bagnall, D.J., Metzger, J.D., Dennis, E.S., Peacock, W.J.: DNA methylation, vernalization, and the initiation of flowering. - Proc. nat. Acad. Sci. USA **90**: 287-291, 1993.

Choi, C.S., Sano, H.: Abiotic-stress induces demethylation and transcriptional activation of a gene encoding a glycerophosphodiesterase-like protein in tobacco plants. - Mol. Genet. Genomics **277**: 589-600, 2007.

Demeulemeester, M.A.C., Van Stallen, N.M., De Profit, M.P.: Degree of DNA methylation in chicory (*Cichorium intybus* L.): influence of plant age and vernalization. - Plant Sci. **142**: 101-108, 1999.

Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A.: Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. - J. exp. Bot. **32**: 93-101, 1981.

Dionisio-Sese, M.L., Tobita, S.: Antioxidant responses of rice seedlings to salinity stress. - Plant Sci. **135**: 1-9, 1998.

Dyachenko, O.V., Zakharchenko, N.S., Shevchuk, T.V., Bohnert, H.J., Cushman, J.C., Buryanov, Y.I.: Effect of hyper-methylation of CCWGG sequences in DNA of *Mesembryanthemum crystallinum* plants on their adaptation to salt stress. - Biokhimiya (Moscow) **71**: 461-465, 2006.

Fedina, I.S., Nedeva, D., Cicek, N.: Pre-treatment with H_2O_2 induces salt tolerance in barley seedlings. - Biol. Plant. **53**: 321-324, 2009.

Filek, M., Keskinen, R., Hartikainen, H., Szarejko, I., Janiak, A., Miszalski, Z., Golda, A.: The protective role of selenium in rape seedlings subjected to cadmium stress. - J. Plant Physiol. **165**: 833-844, 2008.

Fuchs, J., Demidov, D., Houben, A., Schubert, I.: Chromosomal histone modification patterns-from conservation to diversity. - Trends Plant Sci. **11**: 199-208, 2006.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutases. I. Occurrence in higher plants. - Plant Physiol. **59**: 309-314, 1977.

Gueta-Dahan, Y., Yaniv, Z., Zilinskas, B.A., Ben-Hayyim, G.: Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in citrus. - Planta **203**: 460-469, 1997.

Hernandez, J.A., Ferrer, M.A., Jimenez, A., Ros Barcelo, A.R., Sevilla, F.: Antioxidant systems and $O_2^{\bullet-}/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. - Plant Physiol. **127**: 817-831, 2001.

Holliday, R.: Epigenetic inheritance based on DNA methylation. - In: Jost, J.P., Saluz, H.P. (ed.): DNA Methylation: Molecular Biology and Biological Significance. Pp. 452-468. Birkhauser Verlag, Basel 1993.

Horvath, E., Szalai, G., Janda, T., Paldi, E., Racz, I., Lasztity, D.: Effect of vernalisation and 5-azacytidine on the methylation level of DNA in wheat (*Triticum aestivum* L., cv. Martonvasar 15). - Plant Sci. **165**: 689-692, 2003.

Jiang, Y.W., Huang, B.G.: Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. - J. exp. Bot. **52**: 341-349, 2001.

Kovarik, A., Koukalova, B., Bezdek, M., Opatrny, Z.: Hypermethylation of tobacco heterochromatic loci in response to osmotic stress. - Theor. appl. Genet. **95**: 301-306, 1997.

Labra, M., Ghiani, A., Citterio, S., Sgorbati, S., Sala, F., Vannini, C., Ruffini-Castiglione, M., Bracale, M.: Analysis of cytosine methylation pattern in response to water deficit in pea root tips. - Plant Biol. **4**: 694-699, 2002.

Lee, Y.W., Broday, L., Costa, M.: Effects of nickel on DNA methyltransferase activity and genomic DNA methylation levels. - Mutat. Res. Genet. Toxicol. Environ. Mutagenesis **415**: 213-218, 1998.

Lizal, P., Relichova, J.: The effect of day length, vernalization and DNA demethylation on the flowering time in *Arabidopsis thaliana*. - Physiol. Plant. **113**: 121-127, 2001.

Munns, R.: Comparative physiology of salt and water stress. - Plant Cell Environ. **25**: 239-250, 2002.

Murray, H.G., Thompson, W.F.: Rapid isolation of high molecular weight DNA. - Nucl. Acids Res. **8**: 4321-4325, 1980.

Mutlu, S., Atici, Ö., Nalbantoglu, B.: Effects of salicylic acid and salinity on apoplastic antioxidant enzymes in two wheat cultivars differing in salt tolerance. - Biol. Plant. **53**: 334-338, 2009.

Noctor, G., Foyer, C.H.: Ascorbate and glutathione: keeping active oxygen under control. - Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 249-279, 1998.

Paszkowski, J., Whitham, S.A.: Gene silencing and DNA methylation processes. - Curr. Opin. Plant Biol. **4**: 123-129, 2001.

Polle, A., Otter, T., Seifert, F.: Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.). - Plant Physiol. **106**: 53-60, 1994.

Sairam, R.K., Rao, K.V., Srivastava, G.C.: Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolytes concentration. - Plant Sci. **163**: 1037-1046, 2002.

Sano, H., Kamada, I., Youssefian, S., Katsumi, M., Wabiko, H.: A single treatment of rice seedlings with 5-azacytidine induces heritable dwarfism and undermethylation of genomic DNA. - Mol. gen. Genet. **220**: 441-447, 1990.

Serrano, R., Rodriguez, P.L.: Plants, genes and ions. - EMBO Rep. **3**: 116-119, 2002.

Steward, N., Ito, M., Yamakuchi, Y., Koizumi, N., Sano, H.: Periodic DNA methylation in maize nucleosomes and demethylation by environmental stress. - J. biol. Chem. **277**: 37741-37746, 2002.

Sudhakar, C., Lakshmi, A., Giridarakumar, S.: Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. - Plant Sci. **161**: 613-619, 2001.

Tariq, M., Paszkowski, J.: DNA and histone methylation in plants. - Trends Plant Sci. **6**: 244-251, 2004.

Wang, W.X., Vinocur, B., Altman, A.: A plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. - Planta **218**: 1-14, 2003.

Yong, W., Xu, Y., Xu, W., Wang, X., Li, N., Wu, J., Liang, T., Chong, K., Xu, Z., Tan, K., Zhu, Z.: Vernalization-induced flowering in wheat is mediated by a lectin-like gene VER2. - Planta **217**: 261-270, 2003.

Young, C.B., Jung, J.: Water deficit-induced oxidative stress and antioxidative defenses in rice plants. - J. Plant Physiol. **155**: 255-261, 1999.

Zhu, J.K.: Regulation of ion homeostasis under salt stress. - Curr. Opin. Plant Biol. **6**: 441-445, 2003.