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Contents of polyamines during vernalization in wheat and the effect of zearalenone

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Abstract

The contents of endogenous free and conjugated polyamines, putrescine (Put) and spermidine (Spd), were determined during 9 week of vernalization (at 5 °C) in winter wheat seedlings cultivated on Murashige and Skoog media without (MS0) and with 2 mg dm^{-3} zearalenone (MSZEN). At the 4th week of chilling treatment, which is sufficient to induce generative development in 30 % of plants, the marked increase in free and conjugated forms of Put and free Spd were observed. The presence of ZEN in medium significantly accelerated the vernalization. About 20 % of plants treated with ZEN flowered already after 2 weeks and 40 % after 3 weeks of chilling. Significantly higher content of free Put was determined in roots grown on MSZEN compared with MS0 during the first 5 weeks of vernalization with maximum at the 4th week. After germination, a marked decrease in free Spd content was observed both in plants grown on MS0 and MSZEN. Application of ZEN significantly slowed down the Spd decline in leaves and roots during the first and second week of vernalization. The content of Spd and its conjugates decreased in vernalized plants after 1 week of cultivation at 20 °C.

Additional key words: flowering, polyamine conjugates, putrescine, spermidine, *Triticum aestivum.*

Introduction

Vernalization, defined by Chouard (1960) as "the acquisition or acceleration of the ability to flower by a chilling treatment" is an important control mechanism for many crop plants in temperate region. It prevents early transition to reproductive development which may be harmed by cold during winter. The molecular events of vernalization have been analyzed and described recently (*e.g.* Bastow *et al*. 2004, Sung and Amasino 2005, Kosová *et al*. 2008). However, the molecular network controlling vernalization is not yet described and understood in terms of physiological events (Michales and Amasino 2000, Filek *et al*. 2007). The physiological investigation of vernalization was mostly focused on phytohormones and various growth regulators. In our investigations we addressed the role of polyamines and zearalenone.

It has been suggested that low molecular mass polyamines, particularly diamine putrescine, triamine spermidine and tetramine spermine are involved in a wide range of physiological processes (Liu *et al.* 2007) including transition to reproductive development (Martin-Tanguy 2001). High contents of free and conjugated polyamines were associated with early stages of flower development in roses (Sood and Nagar 2005). High contents of free putrescine and spermidine were observed under inductive chilling conditions in olive trees (Malik and Bradford 2007). Increased spermine and spermidine contents occurred during the flower induction in the short-day plant *Pharbitis nil* (Zielińska *et al.* 2006). Bernier *et al.* (2002) proposed that polyamines, mainly putrescine, might represent one component of flowering induction pathway. The experiments with *Sinapis alba* showed a positive correlation between photoperiodic flower induction and augmentation of polyamines content

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Abbreviations: MS - Murashige and Skoog; ZEN - zearalenone.
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and decreased flowering response when their synthesis was inhibited (Havelange *et al.* 1996). Applewhite *et al.* (2000) succeeded to raise the flowering rate by spermidine treatment in *Arabidopsis* at short days.

It was reported that zearalenone (ZEN), the novel plant growth regulator, can be involved in the vernalization process (Meng *et al*. 1986, 1996) and flowering control (Fu *et al.* 1995). ZEN is a fungal metabolite produced by several species of *Fusarium*, mainly by *F. graminearum* and *F. culmorum*. These species are known to colonize maize, barley, oats, wheat, and sorghum (Bennett and Klich 2003). In the course of vernalization marked increase in the content of ZEN and its conjugates were observed in wheat and carrot. These results conducted Meng *et al*. (1992) to propose ZEN as one of the active factors in the control of the generative development of winter plants. In winter wheat ZEN application shortened markedly vernalization period as stated by Biesaga-Kościelniak (2001). Our earlier experiments showed that ZEN was accumulated to the higher degree in the root system than in the upper parts of wheat seedlings (unpublished results).

The aim of the present work was to study polyamine contents during winter wheat vernalization, to examine whether polyamines are related to vernalization process, and to establish the effect of zearalenone application on the content of polyamines during vernalization.

Materials and methods

Sterile kernels of *Triticum aestivum* L. cv. Grana were surface-sterilized in 96 % ethanol (5 min) and 0.05 % $HgCl_2$ (3 min). After rinsing (5 times) in sterile water the kernels were germinated for 24 h in dark and isolated embryos were individually placed in Magenta vessels (*Sigma*, St. Louis, USA) containing Murashige and Skoog (1962) medium (MS0) or MS medium supplemented with 2 mg dm^{-3} ZEN (MSZEN). One half of the seedlings was cultivated under controlled vernalization conditions (5 °C, 9-h photoperiod, irradiance of 100 µmol m^{-2} s^{-1}) during 9 weeks and the other half was cultivated at 20 °C during 14 d at the same irradiance and photoperiod. Additionally, part of plants vernalized for 9 weeks was transferred to 20 °C for one week (9 + 1).

Plants were collected at 1-week intervals during and after vernalization and at 2-d intervals during growth at 20 °C. For analyses, 100 individual plants were chosen after discarding the non uniform specimens. The leaves (upper part of seedlings) were separated from the roots and fresh mass of these organs was determined. Further, the plant samples were freeze-dried and stored in liquid nitrogen.

To establish the vernalization requirement (days of chilling treatment) 100 plants were transferred from vernalization conditions (5 °C) to 20 °C in 1-week intervals and further cultivated up to the generative phase. The time and the percentage of flowering plants were recorded.

The tissues were ground in liquid nitrogen, extracted overnight at 4 °C with 1 cm^3 of 5 % perchloric acid (PCA) per 100 mg(f.m.), 1,7-diaminoheptane was added as an internal standard, and the extracts were centrifuged at 21 000 *g* for 15 min. Standards (*Sigma*) and PCA-soluble free polyamines were benzoylated according to the method of Slocum *et al*. (1989) and Cvikrová *et al.* (1999), and the resulting benzoyl-amines were analyzed by HPLC using a *Beckman-Video* (*Beckman Instruments*, Fullerton, CA, USA) liquid chromatograph equipped with a UV detector (monitoring the eluate at 254 nm) and a C_{18} *Spherisorb 5 ODS2* column (particle size 5 µm, column length 250 × 4.6 mm) according to the method of Slocum *et al*. (1989).

Statistical analysis of data involved two comparisons: between different times of sampling, between two types of media (MS0 and MSZEN) at the same time of sampling. Statistical differences were calculated on the basis of χ^2 ($P < 0.01$) test with respect to polyamine contents at 0 time of cultivation.

Results

Chilling treatment for 4 and 5 weeks induced flowering in 30 and 70 % of plants, respectively (Fig. 1). The presence of ZEN in medium significantly accelerated the process of vernalization. About 20 % of plants treated with ZEN flowered already after 2 weeks and 40 % after 3 weeks of chilling treatment.

The changes of dry mass were used to compare the growth rate of plants cultivated either under 5 or 20 °C. After 9 weeks of chilling treatment, the dry mass of leaves and roots equaled roughly that of plants cultivated for 14 d at 20 °C (Table 1). The seedlings at the end of vernalization treatment reached the same phenological growth phase as those cultivated at 20 °C for 14 d.

Content of Put (both free and conjugated forms) was much higher in vernalized plants in comparison to plants grown at 20 °C (Fig. 2). A pronounced maximum of free and conjugated Put occurred in leaves and roots (both on MS0 and MSZEN) after 4 weeks of chilling treatment and then continually decreased till the end of experiment. Significantly higher content of free Put was found in roots grown on MSZEN than on MS0 during the first 5 weeks of vernalization with maximum difference at the 4^{th} week. However, after 5 weeks the content of free Put was higher in roots grown on MS0 medium (Fig. 2). The

Table 1. Dry mass of leaves and roots of winter wheat seedlings cultivated at 5 °C and 20 °C on MS0 medium. Means ± SD from 100 plants.

5 °C [week]	Dry mass [mg plant^{-1}] leaves	roots	20 °C [d]	Dry mass [mg plant^{-1}] leaves	roots
1	0.62±0.25	1.05±0.17	2	0.26±0.08	0.43±0.11
2	0.98±0.33	1.35±0.18	4	4.32±0.12	2.04±0.32
3	1.20±0.21	1.45±0.15	6	7.19±0.45	2.95±0.26
4	4.45±0.28	2.30±0.29	8	11.75±0.62	4.09±0.48
5	8.24±0.42	3.15±0.36	10	12.93±0.51	4.89±0.60
6	10.62±0.17	3.81±0.30	12	16.35±0.60	5.05±0.43
7	12.07±0.35	4.45±0.42	14	17.65±0.54	5.56±0.40
8	14.22±0.39	5.01±0.31			
9	16.95±0.44	5.33±0.38			
9 + 1	18.24±0.45	5.96±0.41			

contents of Put and its conjugates determined in the roots grown on both media at 5 °C were higher than those in the leaves. At the end of cold treatment, amount of Put decreased and after one week of growth at 20 °C (9 + 1) reached the level comparable with that found out in plants cultured at 20 °C.

After germination and during the subsequent growth marked decrease in free Spd and its conjugate contents was observed. Contents of free Spd was higher in vernalized wheat plants in comparison with plants grown at 20 °C during the first 4 weeks of cold treatment and it was generally higher in leaves than in roots. No significant differences in Spd levels were observed in plants grown at 20 °C (Fig. 2). During the first week of vernalization the application of ZEN significantly increased the Spd contents in leaves and roots. On the contrary, content of free Spd was significantly higher in plants on MS0 with a peak at the 4th week of vernalization (Fig. 2). The content of Spd conjugates was higher in plants grown at 5 °C compared with plants grown at 20 °C in the beginning of vernalization only (Fig. 2). The content of Spd conjugates was rather low both in plants grown on MS0 and MSZEN. After one week of growth at 20 °C following the end of vernalization (9 + 1), the amounts of free and conjugated Spd were lower than those determined in plants grown for 14 d at 20 °C.

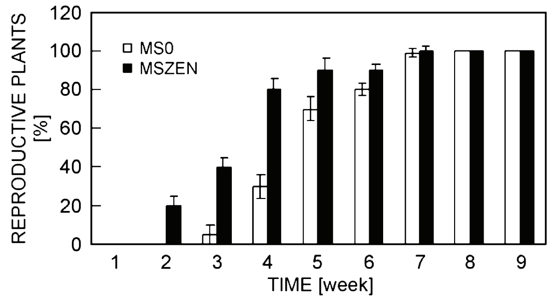

Fig. 1. The percentage of reproductive plants of winter wheat after 1, 2, 3, 4, 5, 6, 7, 8 and 9 weeks of vernalization (5 °C) of seedlings on MS media without (0) and with 2 mg dm^{-3} zearalenone (ZEN). Means ± SE, n = 100.

Discussion

Our data indicate that four weeks of cold treatment are sufficient to promote generative development in 30 % of winter wheat plants and the presence of ZEN in medium significantly accelerated the process of vernalization. Analysis of endogenous polyamines (PAs; Fig. 2) revealed significant fluctuations in their contents in leaves and roots during the cold treatment. Transient rise in Spd contents which was observed after 4 weeks of cold treatment coincided with the peaks of Put content both in leaves and roots. The second, much smaller increase in the contents of both polyamines occurred after 7 weeks of cold treatment. The peak of accumulation of PAs well correlates with the duration of cold treatment which is crucial for promotion of generative development. Keeping this in mind, we might conclude that this increase in PAs might coincide with the acquisition of reproductive potential. The continuous increase in contents of putrescine and its precursor agmatin during vernalization of several winter wheat cultivars was reported by Páldi *et al.* (1998).

There is firm evidence, however, that PAs play a positive role in flower induction (for review see Galston 1983). The participation of PAs in the flowering process has been strongly suggested by Malmberg and McIndoo (1983) and Caffaro and Vicente (1995). A sharp increase in Spd content in corms of *Polianthes tuberosa* at the early floral stage was recorded (Huang *et al.* 2004) and a close connection between PAs (Spd predominating) and reproductive development in *Arabidopsis thaliana* was described by Applewhite *et al.* (2000). A supporting evidence in the control of floral transition by PAs came from the use of inhibitors of Put, Spd, and Spm synthesis (Havelange *et al.* 1996).

As mentioned above, in our experiment the presence of ZEN in the medium accelerated the process of winter wheat vernalization. Similar results were described by Biesaga-Kościelniak (2001). After ZEN application the requirement of cold treatment in vernalization of wheat was shortened to approximately one half. The positive role of ZEN in plant development was first observed during the process of vernalization in wheat and carrot (Meng *et al.* 1986, 1992). The connection between ZEN and flower bud formation in *Nicotiana tabacum* was described by Fu *et al.* (1995, 2000).

The marked influence of ZEN on PAs contents within the first weeks of cold treatment was observed in our experiments. From the apparent correlation of Put content and enzymatic activities (Bertoldi *et al.* 2004) we might

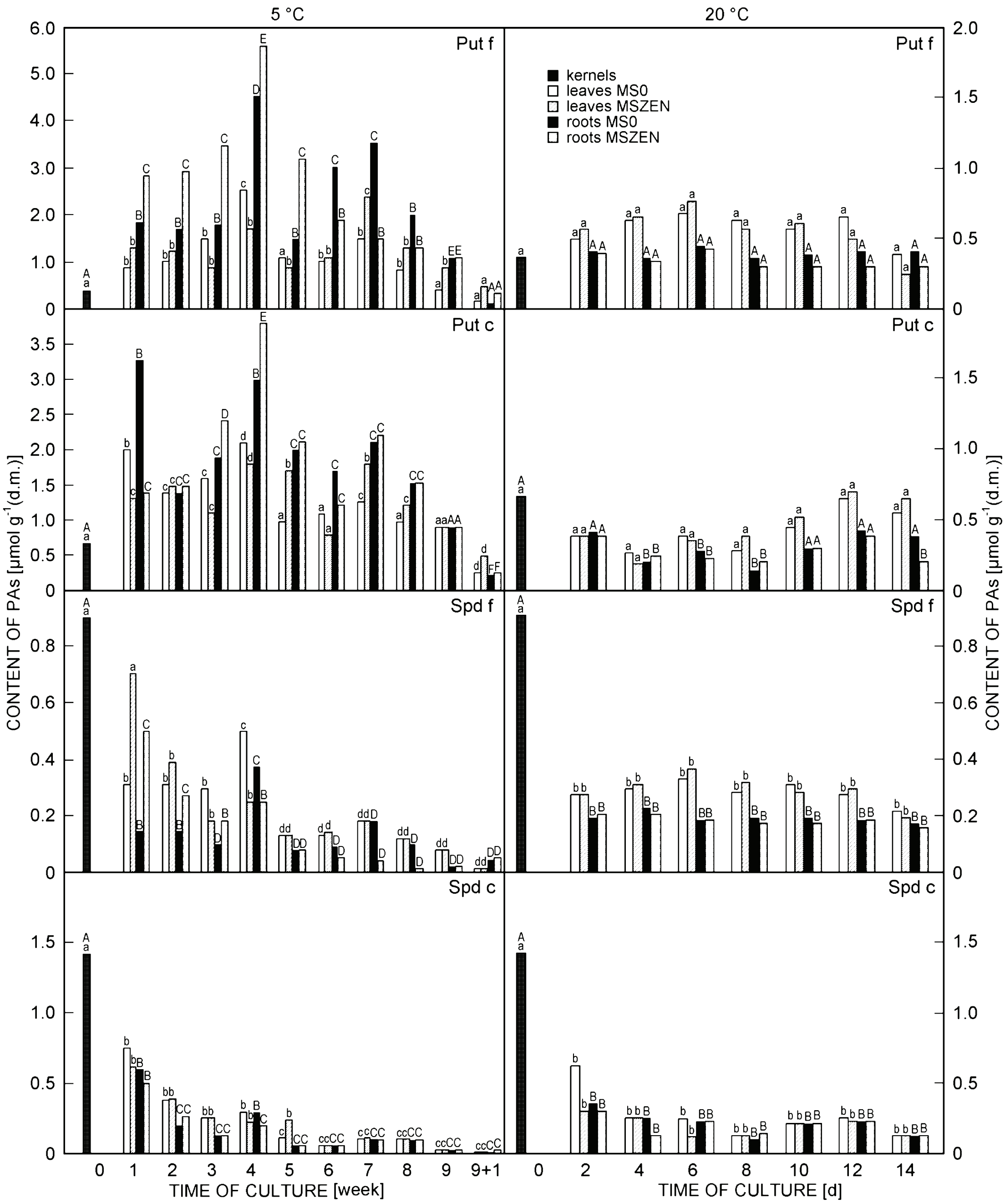

Fig. 2. Content of free putrescine (Put f), conjugates of putrescine (Put c), free spermidine (Spd f) and conjugates of spermidine (Spd c) in the leaves and roots of winter wheat seedlings during vernalization (5 °C) and 20 °C on Murashige-Skoog media without (0) and with 2 mg dm^{-3} ZEN. 0 - content of putrescine in winter wheat embryos. *Letters* denote significant differences ($P < 0.01$) between the same tissues (*small letters* - leaves, *capital* - roots) cultured on 0 and ZEN media.

assume that in roots ZEN might increase the PAs biosynthesis. Furthermore, we may hypothesize that high Spd contents in the leaves after application of ZEN resulted either from the increase in PAs biosynthesis, and/or by the decreased oxidative deamination of Spd in ZEN-treated plants. We suppose that high Spd content in the course of the first two weeks might coincide with acquisition of reproductive ability, as 20 % of plants treated with ZEN flowered already after 2 weeks of cold treatment (Fig. 1).

It is known that polyamines play important roles in responses to abiotic stresses (Kuznetsov *et al.* 2006). High polyamine contents were shown to be involved also in adaptations to low temperature (Hummel *et al.* 2004, Rácz *et al.* 1996). We are aware that part of the observed changes in the contents of PAs might be due to such an effect.

References

Applewhite, P.B., Kaur-Sawhney, R., Galston, A.W.: A role for spermidine in the bolting and flowering of *Arabidopsis*. - Physiol. Plant. **108**: 314-320, 2000.

Bastow, R., Mylne, J.S., Lister, C., Lippman, Z., Martienssen, R.A., Dean, C.: Vernalization requires epigenetic silencing of FLC by histone methylation. - Nature **427**: 164-157, 2004.

Bennett, G.A., Klich, M.: Mycotoxins. - Clin. Microbiol. Rev. **16**: 497-516, 2003.

Bernier, G., Corbesier, L., Périlleux, C.: The flowering process: on the track of controlling factors in *Sinapis alba*. - Russ. J. Plant Physiol. **49**: 445-450, 2002.

Bertoldi, D., Tassoni, A., Martinelli, L., Bagni, N.: Polyamines and somatic embryogenesis in two *Vitis vinifera* cultivars. - Physiol. Plant. **120**: 657-666, 2004.

Biesaga-Kościelniak, J.: Zearalenone as a New Hypothetical Regulator of Plant Growth and Development. - Inst. Plant Physiol., Polish Acad. Sci., Krakow 2001.

Caffaro, S.V., Vicente, C.: Early changes in the content of leaf polyamines during the photoperiodic flowering induction in soybean. - J. Plant Physiol. **145**: 756-758, 1995.

Chouard, P.: Vernalization and its relations to dormancy. - Annu. Rev. Plant Physiol. **11**: 191-328, 1960.

Cvikrová, M., Binarová, P., Cenklová, V., Eder J., Macháčková, I.: Reinitiation of cell division and polyamine and aromatic monoamine levels in alfalfa explants during the induction of somatic embryogenesis. - Physiol. Plant. **105**: 330-337, 1999.

Filek, M., Biesaga-Kościelniak, J., Macháčková, I., Krekule, J.: Generative development of winter rape (*Brassica napus* L.) - the role of vernalization. - Int. J. Plant dev. Biol. **1**: 57-63, 2007.

Fu, Y.F., Han, Y.Z., Zhao, D G., Meng, F.J.: Zearalenone and flower bud formation in thin-cell layers of *Nicotiana tabacum* L. - Plant Growth Regul. **30**: 271-274, 2000.

Fu, Y.F., Li, H.Y., Meng, F.J.: The possible role of zearalenone in the floral gradient in *Nicotiana tabacum* L. - J. Plant Physiol. **147**: 197-202, 1995.

Galston, A.W.: Polyamines as modulators of the plant development. - Bioscience **33**: 382-388, 1983.

Havelange, A., Lejeune, P., Bernier, G., Kaur-Sawhney, R., Galston, A.W.: Putrescine export from leaves in relation to floral transition in *Sinapis alba*. - Physiol. Plant. **96**: 59-65, 1996.

Huang, C.K., Chang, B.S., Wang, K.C., Her, S.J., Chen, T.W., Cho, C.L., Liao, L.J., Huang, K.L., Chen, W.S., Liu, Z.H.: Changes in polyamine pattern are involved in floral initiation and development in *Polianthes tuberosa*. - J. Plant Physiol. **161**: 709-713, 2004.

Hummel, I., El Amrani, A., Gousbet, G., Hennion, F., Couée, I.: Involvement of polyamines in the interacting effects of low temperature and mineral supply on *Pringlea antiscorbutica* (Kergeuelen cabbage) seedlings. - J. exp. Bot. **55**: 1125-1134, 2004.

Kosová, K., Prášil, I.T., Vítámvás, P.: The relationship between vernalization- and photoperiodically-regulated genes and the development of frost tolerance in wheat and barley. - Biol. Plant. **52**: 601-615, 2008.

Kuznetsov, V., Radyukina, N.L., Shevyakova, N.I.: Polyamines and stress: biological role, metabolism and regulation. - Russ. J. Plant Physiol. **53**: 583-604, 2006.

Liu, J.H., Kitashiba, H., Wang, J., Ban, Y., Moriguchi, T.: Polyamines and their ability to provide environmental stress tolerance to plants. - Plant Biotechnol. **24**: 117-126, 2007.

Malik, N.S.A., Bradford, J.M.: Different flower-inducing conditions elicit different responses for free polyamine levels in olive (*Olea europaea*) leaves. - J. jap. Soc. hort. Sci. **76**: 205-209, 2007.

Malmberg, L., McIndoo, J.: Abnormal floral development of a tobacco mutant with elevated polyamine levels. - Nature **305**: 623-625, 1983.

Martin-Tanguy, J.: Metabolism and function of polyamines in plants: recent development (new approaches). - Plant Growth Regul. **34**: 135-148, 2001.

Meng, F.J., Han, Y.Z., Fu, Y.F., Guo, F.L.: Zearalenone in higher plants. - Flowering Newslett. **22**: 54-57, 1996.

Meng, F.J., Han, Y.Z., Que, Y.M., Wang, H.: Zearalenone, a key substance controlling plant development. - In: Karssen, C.M., Van Loon, L.C., Vreuggdeunilcedes, D. (ed.): Advances in Plant Regulation. Pp 291-297. Kluwer Academic Press, Dordrecht 1992.

Meng, F.J., Que, Y.M., Zhang, S.Q.: Zearalenone-like substance in winter plants and its relation to vernalization. - Acta bot. sin. **28**: 622-627, 1986.

Michales, S.D., Amasino, R. M.: Memories of winter: vernalization and the competence to flower. - Plant Cell Environ. **23**: 1145-1153, 2000.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Páldi, E., Janda, T., Szalai, G : Quantitative changes in polyamines during vernalization in wheat (*Triticum aestivum* L.). - Növénytermelés **47**: 613-622, 1998.

Rácz, I., Kovács, M., Lasztity, D., Veisz, O., Szalai G., Páldi, E.: Effect of short-term and long-term low temperature stress on polyamine biosynthesis in wheat genotypes with varying degrees of frost tolerance. - Plant Physiol. **148**: 368-373, 1996.

Slocum, R.D., Flores, H.E., Galston, A.W., Weinstein, L.H. : Improved method for HPLC analysis of polyamines, agmatine and aromatic monoamines in plant tissue. - Plant Physiol. **89**: 512-517, 1989.

Sood, S., Nagar, P.K.: Xylem and phloem derived polyamines during flowering in two diverse rose species. - J. Plant Growth Regul. **24**: 36-40, 2005.

Sung, S., Amasino, R.M.: Remembering winter: toward a molecular understanding of vernalization. - Annu. Rev. Plant Biol. **56**: 491-508, 2005.

Zielińska, N., Kęsy, J., Kopcewicz, J.: Participation of polyamines in the flowering of the short-day plant *Pharbitis nil*. - Plant Growth Regul. **50**: 149-158, 2006.