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Effect of vernalisation and azacytidine on the DNA methylation level in wheat (*Triticum aestivum* L. cv. Mv 15)

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ABSTRACT The aim of the research was to examine the changes occurring during vernalisation in the 5-methylcytosine content of the DNA in a winter wheat variety with a short vernalisation requirement after treatment with 5-azacytidine, and to determine the correlation between the quantity of 5-methylcytosine and flowering. The results indicated that both vernalisation treatment and 5-azacytidine, a compound causing demethylation, reduced the methylation of the DNA in the winter wheat variety Mv 15. The data also proved that although the 5-azacytidine treatment reduced the methylation of the DNA in unvernalised plants, this in itself was not sufficient to induce flowering. **Acta Biol Szeged 46(3-4):35-36 (2002)**

KEY WORDS

DNA nethylation
5-azacytidine
5-methylcytosine
HPLC
vernalisation
wheat

Investigations on the biochemical process of vernalisation speeded up considerably with the introduction of molecular biological methods. The turning point was the isolation of *Arabidopsis* mutants in which the expression of numerous genes was inhibited and certain processes were thus unable to take place. Genes responsible for vernalisation were identified in *Arabidopsis*, wheat and other plant species. The *ver203* gene was isolated from wheat and its primary structure was determined (Chong et al. 1997). The next step was the influencing of the expression of genes responsible for vernalisation using the antisense RNA technique (Chong et al. 1998), in the course of which it was found that by inhibiting the expression of the *ver203* gene heading could be inhibited even if the photoperiod was adequate. DNA modification plays an important role in regulating gene expression. The expression of methylated genes is completely inhibited, while these genes become activated and expressed as the result of demethylation.

In some temperate zone plants a specific period of cold treatment (vernalisation) and an adequate photoperiod are essential if flowering is to take place (Levy and Dean 1998). An important step in investigations on the mechanism of vernalisation was the observation that treatment with a demethylation agent, 5-azacytidine, caused certain plants to flower earlier, or reduced the cold requirement for vernalisation (Burn et al. 1993; Brock and Davidson 1994; Finnegan et al. 1998). In the course of vernalisation low temperature causes a similar reduction in the methylation of DNA, with a parallel increase in the activity of gene expression. In *Arabidopsis thaliana* vernalisation could be induced or partially replaced by 5-azacytidine and antisense RNA, which inhibits methylase activity (Metzger et al. 1990; Finnegan et al. 1998).

On the basis of the above, the aim of the present work was to study changes occurring during vernalisation in the 5-methylcytosine content of the DNA in a winter wheat variety with a short vernalisation requirement (*Triticum aestivum* L. cv. Mv 15) as the result of 5-azacytidine treatment, and to determine the correlation between changes in 5-methylcytosine and flowering.

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Materials and Methods

Plant material and growth conditions: Seeds of wheat cv. Mv 15 were sterilised and germinated for 72 h at 22°C in the dark. The seedlings were grown in Hoagland solution at 25°C for 4 months with 16/8-hour light-dark periodicity (1000 µE/cm^2). 5-azacytidine was added to the hydroponic solution for 9 days at a concentration of 50 µM L^{-1}.

Vernalisation was carried out at 2°C in the dark or in the light for 4 weeks. After the 72 h germination the seeds were transferred to 2°C and kept between filter papers in distilled water or 50 mM L^{-1} 5-azacytidine.

Isolation of DNA: This was carried out using the DNAzol™ kit. 1 g plant material was ground in liquid nitrogen and incubated for 5 min in 3 ml DNAzol and 9 µl RNAse. 3 ml chloroform was added and after 5 min the mixture was centrifuged at 12000 g for 10 min. The water phase was separated and mixed with 2.25 ml absolute ethanol. After 5 min of incubation, the samples were centrifuged at 5000 g for 4 min and the supernatant was discarded. The pellet was washed in DNAzol:ethanol (1:0.75) and centrifuged again at 5000g for 4 min. After adding 3 ml 70% ethanol, the samples were centrifuged (5000g, 4 min), after which the pellet was dried and resuspended in 700 µl TRIS-EDTA buffer pH 8.

Determination of 5-methylcytosine: DNA samples were hydrolysed for one hour at 95°C in 50 ml 70% perchloric acid. The pH was adjusted to between 3 and 5 with 1M KOH. After centrifugation the supernatants were stored and the pellets were washed twice with 200 µl distilled water. After combining the supernatants the samples were dried in a vacuum, redissolved in 250 µl sodium acetate (10 mM, pH 4) and filtered through a membrane with pore size 0.2 µm.

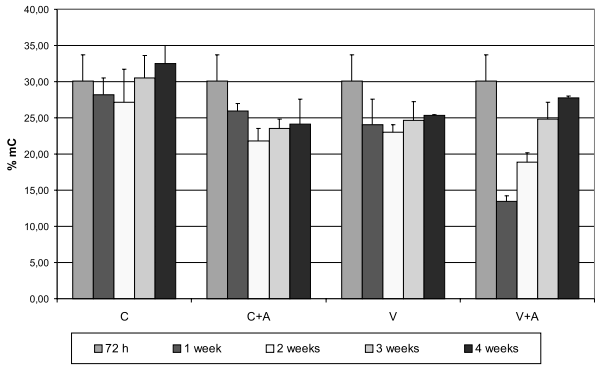

Figure1. Changes in the 5-methylcytosine content of DNA in the winter wheat variety Mv 15 as the result of vernalisation and of treatment with azacytidine. C: plants grown at room temperature, C+A: plants grown at room temperature and treated with 0.05 mM 5-azacytidine, V: vernalised plants (2°C), V+A: vernalised plants (2°C) treated with 0.05 mM 5-azacytidine.

Methylcytosine and cytosine were measured by HPLC according to Demeulemeester et al. (1999).

Results and Discussion

Changes in the quantity of 5-methylcytosine in the course of vernalisation were investigated in the winter wheat variety Mv 15, which has a 21-day vernalisation requirement. Changes in the 5-methylcytosine content were also traced in plants of the same age not exposed to cold but treated with 5-azacytidine. The effect of this treatment on the induction of flowering was also examined.

The 72-hour-old seedlings were grown at the required temperature and were sampled weekly. In the 72-hour-old seedlings the total quantity of 5-methylcytosine in the 72-hour-old seedlings was 30.07%. In the course of vernalisation the degree of methylation decreased by 6-7 % and then remained constant until the end of the vernalisation period. When 5-azacytidine was also applied, the proportion of methylated cytosine derivatives first dropped by 13.5 %, then gradually increased a little, but did not reach the degree of methylation of plants only given cold treatment even by the end of the vernalisation period.

In plants grown at room temperature the degree of methylation did not change substantially in the course of growth. Over the 4-week sampling period the quantity of 5-methylcytosine fluctuated between 28 and 30%. As the result of 5-azacytidine treatment the degree of methylation decreased, as observed in the vernalised samples, and the quantity of 5-methyl-cytosine was 23–25 %.

The results demonstrate unequivocally that both low temperature treatment (vernalisation) and the demethylation agent 5-azacytidine reduced the methylation of the DNA in the wheat variety Mv 15. Although treatment with 5-azacytidine reduced the methylation of the DNA in plants of this wheat variety which received no cold treatment, this was not sufficient in itself, without the necessary vernalisation period, to induce flowering.

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References

Brock RD, Davidson JL (1994) 5-azacytidine and gamma rays partially substitute for cold treatment in vernalising winter wheat. Environ Exp Bot 31:195-199.

Burn JE, Bagnall DJ, Metzger JD, Dennis ES, Peacock WJ (1993) DNA methylation, vernalisation, and the initiation of flowering. PNAS 90:287-291.

Chong K, Tan KH, Huang HL, Liang HG (1997) Sequence analysis of vernalisation-related cDNA clone from winter wheat. Acta Phytophysiol Sin 23:99-102.

Chong K, Bao SL, Tan KH, Liang TB, Zeng JZ, Huang HL, Xu J, Xu ZH (1998) Functional analysis of the *ver* gene using antisense transgenic wheat. Physiol Plant 102:87-92

Demeulemeester MAC, Van Stallen N, De Proft MP (1999) Degree of DNA methylation in chicory (*Cichorium intybus* L.): influence of plant age and vernalisation. Plant Sci 142:101-108.

Finnegan EJ, Genger RK, Kovac, K, Peacock, WJ and Dennis ES (1998) DNA methylation and the promotion of flowering by vernalisation. PNAS 95:5824-5829.

Levy YY, Dean C (1998) The transition to flowering. Plant Cell 10, 1973-1989.

Metzger JD, Burn J, Dennis ES, Peacock WJ. (1990) DNA methylation as the molecular basis for vernalisation. A hypothesis. Plant Physiol 94:68S.

Murray MG, Thompson WF (1989). Rapid isolation of high-molecular-weight plant DNA. Nucl Acid Res 8:4321-4325.