Biochemical changes in pear (*Pyrus communis* L.) depending on different phases of the dormancy

Göndör, M.[1], Honty, K.[1], Pedryc, A.[2], Hajdrik, I.[1] and Stefanovits-Bányai, É.[3]

BUESPA, Faculty of Horticultural Science, [1]Dept. of Fruit Science
[2]Dept. of Genetics and Horticultural Plant Breeding,
[3]Faculty of Food Science, Dept. of Applied Chemistry
H-1118 Budapest Villányi út 31., Hungary

Summary: Pear cultivars of variable frost tolerance were tested as for frost injuries suffered as a consequence of artificial freezing temperatures during the endodormancy as well as the ecodormancy. Damages were registered according to a visually defined scale, then peroxidase and polyphenol-oxidase activity was checked in buds, spurs and limbs. According to our results, 'Packham's Triumph' was the most frost tolerant cultivar. Regarding enzyme activity of both enzymes, the performance of cultivars displaying different susceptibility was also different in spurs as well as in buds. Results referring to the endodormancy were especially instructive. During the ecodormancy, data obtained at the same time indicated the differences existing between the developmental stages of dormancy in the respective cultivars.

Key words: pear cultivars, peroxidase, polyphenol oxidase, enzyme activity, dormancy, frost

Introduction

Plants are exposed during their development to different stresses either biotic-virus- or bacterial infection – (*Brisset* et al., 2000; *Sárdi* et al., 2000) or abiotic – drought, UV-radiation, heath, frost, heavy metals (*Stefanovits-Bányai* et al., 1998). Noxious radicals are induced by the stress factors (Foyer et al., 1994), which endanger the healthy growth of the plant by disturbing biochemical processes. Oxidative reactions harm finally the molecules of proteins, carbohydrates (*Sárdi* et al., 1996, 1999), lipids, and may start dangerous chains of reactions, which end with the death of the respective cells (*Salin*, 1987). Plants build up defence mechanisms, generally, which are able to eliminate the effect of the noxious radicals. The components of the defence mechanism are the stress-enzymes (superoxid dismutase, catalase, peroxidase and polyphenoloxidase (*Hegedűs* et al., 2001, 2004).

Pear is the fruit species one of the most susceptible to ecological stresses. Most problems are caused by the lack of precipitation, or the excesses of temperature (too high or too low). The average freezes of our winters, 10 or 15 °C, are though tolerated without damage, but drops below 20 or 25 °C, which are not rare as well, may cause serious injuries in fruiting branches and buds (*Filiti* et al., 1989; *Göndörné*, 2000). For the intense fruit production, it is indispensable to find ecologically stable cultivars. The main pear cultivars of the market represent large differences in tolerance to winter frosts (*Proebsting & Mills*, 1978; *Winter* et al., 1981; *Pieber*, 1985; *Iváncsics*, 2003). In an earlier attempt, studies around the end of endodormancy under field conditions gave information on the relative frost susceptibility of cultivars (*Göndör & G. Tóth*, 1998).

Changes in the activity of peroxidase, superoxid-dismutase, catalase, polyphenol-oxidase, etc. enzymes as consequences of biotic or abiotic stresses, are generally recognised phenomena (*Kwak* et al., 1996; *Lafuente & Martínez-Téllez*, 1997; *Rivero* et al., 2001). Present study endeavours to measure the frost tolerance in the generative buds as well as in the short bearing structures, the spurs, and to trace its relation to changes in biochemical parameters, as enzyme activities of peroxidase, polyphenol-oxidase.

Materials and methods

Plants used in the experiments

European cultivars (*Pyrus communis* L. – 'Packham's Triumph', 'Kaiser Alexander'), Japanese pear (*P. pyrifolia* Nakai - 'Hosui'), as well as hybrids of the two species 'Packham's Triumph' x 'Nijisseiki' (coded: NP), and 'Packham's Triumph' x 'Hosui' (coded: HP) have been scored as for their frost tolerance. Samples were taken in 2003 in the experimental station of the Faculty of Horticultural Science at Szigetcsép, during the endodormancy (February 10, 2003), afterwards during the ecodormancy (March 10, 2003). The development of pollen was checked by the microscopic study of microsporogenesis (The archesporium was interpreted as the sign of endodormancy, whereas phases of cell division related to microsporogenesis as ecodormancy).

Methods of research

The determination of the injuries in flower buds is essentially visual according to a numerical scale *(Figure 1)*, then the limbs sampled during the endodormancy were exposed in a climatic chamber for 24 hours to –25 °C-, while those collected in the ecodormancy were kept at –15 and –18 °C temperatures.

Fruit buds, spurs and limbs were tested for peroxidase activity (POD) by spectrophotometry in H_2O_2 substrate with ortho-dianidizine as choromegene reagent ($\varepsilon = 11.3$), at $\lambda = 460$ nm (*Shannon* et al., 1966).

Changes in polyphenol-oxidase (PPO) enzyme activity were determined with catechol at λ=420 nm by spectrophotometry (*Jen & Kahler,* 1974). Results are expressed in terms of U/mg protein, where the protein content was calculated according to *Bradford* (1976).

Results

Flower buds were cut longitudinally as described by *Göndörné* (2000) and shown in *Table 1.* 'Kieffer' and 'Packham's Triumph' and their hybrids proved to be rather frost tolerant, i.e. 86–100% of the buds remained healthy being in the endodormancy as well as in the ecodormancy. Japanese pear cultivar was more susceptible because totally killed buds were found in that cultivar during ecodormancy *(Table 1–3).*

Regarding changes in activity of peroxidase and polyphenoloxidase enzymes, all cultivars showed lowest values in the limbs, which could not be interpreted safely, therefore we had to rely on tests of spurs and buds.

In the check samples of *spurs,* the highest activity of **peroxidase** activity has been measured during the endodormancy *(Figure 2)* in 'Packham's Triumph'. Enzyme activity of susceptible cultivars was in all cases lower. Cold treatment of –25 °C lowered the values of enzyme activity in the resistant cultivars only ('Packham's Triumph'). In susceptible cultivars the frost increased the enzyme activity, though at different degrees. 'Kieffer', 'Kaiser Alexander' and 'Hosui' produced increasing values of enzyme activity, parallel with their increasing susceptibility. In the hybrids which frost tolerance was not previously scored, peroxidase activity did hardly change under the influence of the freezing treatment and remained at the originally low values comparable with those of 'Packham's Triumph'.

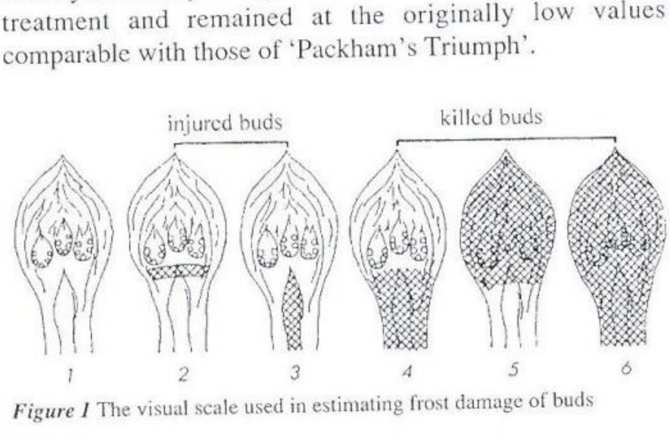

Figure 1 The visual scale used in estimating frost damage of buds

Table 1 Frost tolerance in flower buds of pear cultivars after a freezing treatment of –25 °C-during endodormancy (February, 2003)

Cultivars	Healthy buds %	Injured buds %	Killed buds %
Packham's Triumph	88	1.5	10.5
Kieffer	81	14	5
Kaiser Alexander	35	37	28
Hosui	60	8	32
NP 41	87	13	–
NP 14	100	–	–
HP 12	90	10	–

Table 2 Frost tolerance of fruiting buds of pear cultivars to a treatment of –15 °C freeze during ecodormancy (March, 2003)

Cultivars	Healthy buds %	Injured buds %	Killed buds %
Packham's Triumph	100	–	–
Kieffer	20	80	–
Kaiser Alexander	100	–	–
Hosui	29	29	42
NP 10	100	–	–
NP 24	100	–	–
HP 4	12.5	87.5	–

Table 3 Frost tolerance of fruiting buds of pear cultivars to a treatment of –18 °C freeze during ecodormancy (March, 2003)

Cultivars	Healthy buds %	Injured buds %	Killed buds %
Packham's Triumph	86	14	–
Kieffer	–	100	–
Kaiser Alexander	43	57	–
Hosui	16.5	67	16.5
NP 10	100	–	–
NP 24	75	25	–
HP 4	–	100	–

In *buds,* enzyme activity was lower than in the spurs of the resistant cultivar, at the beginning *(Figure 3).* In buds of more susceptible cultivars, enzyme activity was always higher than in the spurs. Cold treatment did not change the enzyme activity in buds of 'Packham's Triumph', supposedly, the high enzyme activity of the spurs could prevent the damage of buds. In the susceptible cultivars, enzyme activity was lowered by the cold treatment. In the buds of hybrids, the values of enzyme activity were lower than in the spurs, however, the cold treatment did not change the enzyme activity of spurs neither of buds.

During the ecodormancy, enzyme activity was lower than during the endodormancy, except in 'Kaiser Alexander'. 'Packham's Triumph' produced the lowest activity of peroxidase, whereas all other, more frost-susceptible cultivars, especially 'Kaiser Alexander' had higher values of enzyme activity. In spurs, the enzyme activity has changed as a consequence of frost *(Figure 4),* in buds, increasing frost lowered the enzyme activity *(Figure 5).* As long as in the resistant 'Packham's Triumph', and unexpectedly, in the Japanese pear, 'Hosui', the enzyme activity in spurs was elevated after a cold treatment at –15 °C, whereas the activity was lowered after –18 °C, substantially, in the susceptible

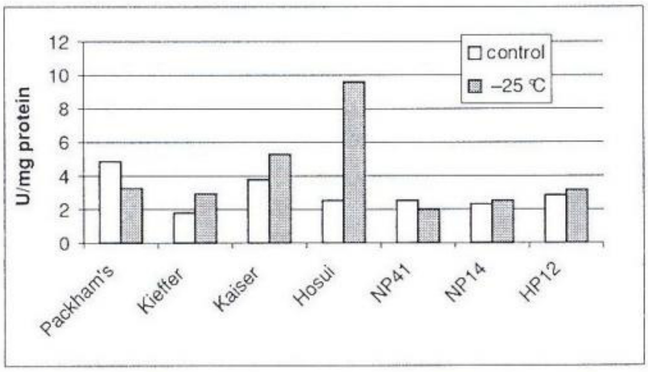

Figure 2 Peroxidase activity in pear spurs during the endodormancy (February, 2003)

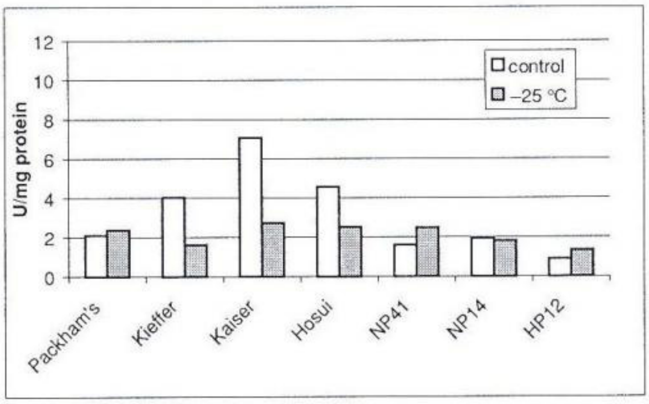

Figure 3 Peroxidase activity in pear buds during the endodormancy (February, 2003)

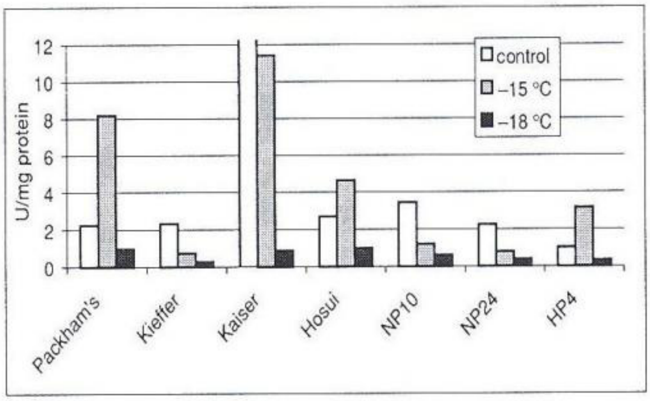

Figure 4 Peroxidase activity in pear spurs during the ecodormancy (March, 2003)

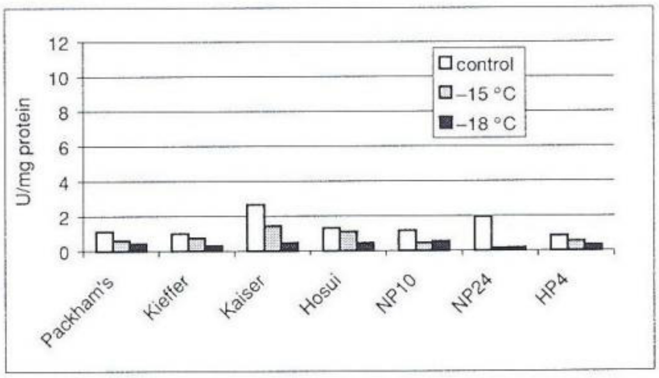

Figure 5 Peroxidase activity in pear buds during the ecodormancy (March, 2003)

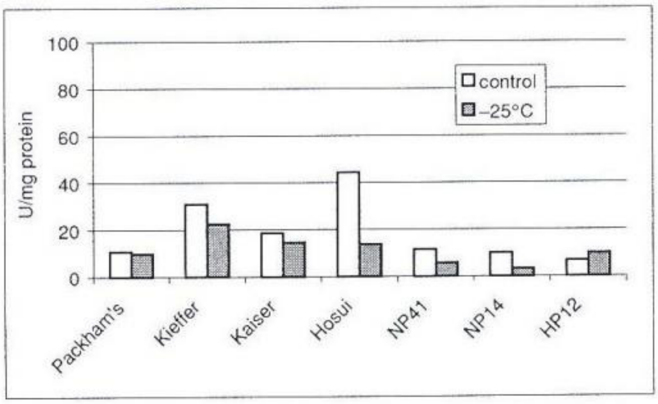

Figure 6 Polyphenoloxidase activity in pear spurs during the endodormancy (February, 2003)

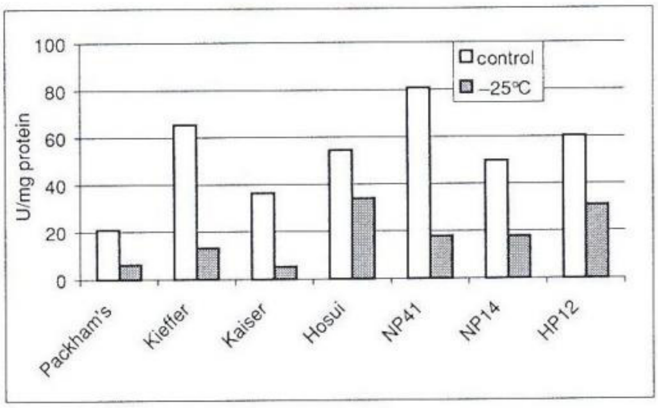

Figure 7 Polyphenoloxidase activity in pear buds during the endodormancy (February, 2003)

cultivar, 'Kaiser Alexander', spurs responded on the cold treatments by a continuous decrease of enzyme activity.

Enzyme activity of **polyphenol-oxidase** was lower during the endodormancy in spurs *(Figure 6)* than in buds *(Figure 7),* without exception, in frost resistant as well as in susceptible cultivars. Spurs and buds, both proved the tendency that enzyme activity increased continuously along with increasing frost susceptibility of the respective cultivars. At –25 °C, the cold treatment lowered the enzyme activity in spurs and buds, but the reduction of activity was more expressed in the susceptible cultivars. During ecodormancy, enzyme activity of the check plants was lower than during the endodormancy. Both, frost resistant and susceptible cultivars had higher enzyme activity in buds than in spurs. Cold treatment increased the enzyme activity of spurs *(Figure 8)* in the resistant 'Packham's Triumph' and lowered that of the buds *(Figure 8).* In susceptible cultivars (except in Japanese one), buds and spurs, at different degrees, low temperatures lowered the enzyme activity. In the hybrids, –18 °C caused increased enzyme activity *(Figure 9).*

In the frost resistant cultivar, 'Packham's Triumph', POD-activity was lower in spurs, higher in buds, on the contrary, PPO-activity was higher in spurs and lower in buds, which is in conformity with the results obtained in grapes (*Stefanovits-Bányai* et al., 2003). The same tendency was hardly recognised in the susceptible cultivars.

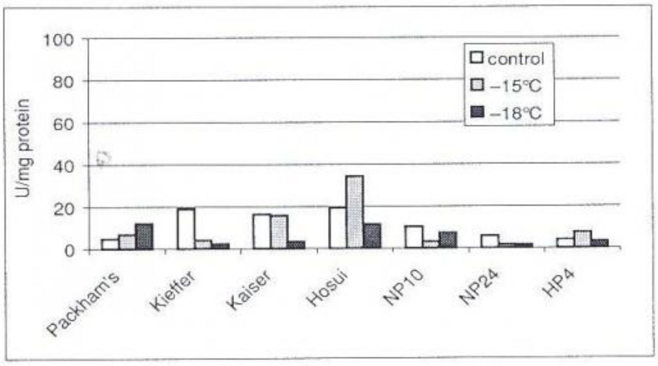

Figure 8 Polyphenoloxidase activity in pear spurs during the ecodormancy (March, 2003)

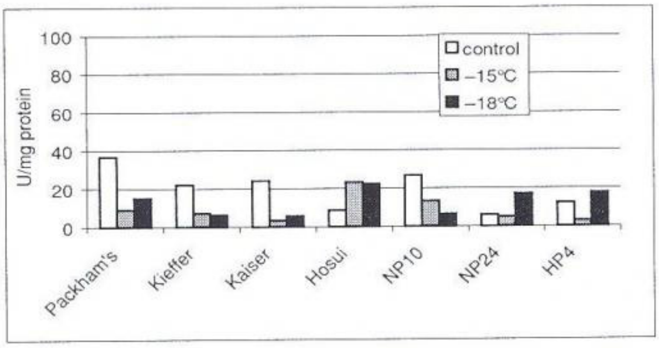

Figure 9 Polyphenoloxidase activity in pear buds during the ecodormancy (March, 2003)

Changes in the activity of both enzymes (POD and PPO) were much more equivocal as a response to freezing temperatures during the endodormancy than during ecodormancy. Our preliminary experiments allowed the conclusion that activity of the enzymes POD and PPO changed in spurs as well as in buds indicating well the level of susceptibility/resistance to freezing temperatures of pear cultivars.

Acknowledgements

This research was supported by grants from the Hungarian Scientific Research Fund (T 043279).

References

Bradford, M. M. (1976): A rapid and sensitive method for the quantification of micro-gram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem, 72: 248–254.

Brisset, M. N., Cesbron, S., Thomson, S. V. & Paulin, J. P. (2000): Acibenzoar-S-methil induced the accumulation of defense-related enzymes in apple and protects from fire blight. European Journal of Plant Pathology, 106 (6): 529–536.

Filiti, N. & Neri, D. (1989): Cold damage in fruit bud tissues of pear. Acta Hort. 26: 133–126.

Foyer, C. H., Lelandais, M. & Kunert, K. J. (1994): Photooxidative stress in plants. Physiol. Plant., 92: 696–717.

Göndör M. & G. Tóth M. (1998): Evaluation of frost resistance and productivity of pear cultivars in Hungary. Proceedings of Eucarpia Symposium on Fruit Breeding and Genetics. Oxford. Acta Horticulturae, 484: 79–83.

Göndör Jné. (ed.) (2000): Körte. Mezőgazda kiadó, Bp.

Hegedűs A. Erdei S. & Horváth G. (2001): Comparative studies of H2O2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Science, 160: 1085–1096.

Hegedűs A., Erdei S., Janda T., Tóth E., Horváth, G. & Dudits D.: (2004): Transgenic tobacco plants overproducing alfalfa aldose/aldehyde-reductase show higher tolerance to low temperature and cadmium stress. Plant Sci., 166 (5): 1329–1333.

Iváncsics J. (2003): Körtefajták érzékenysége téli és tavaszi fagykárosodással szemben. Növényvédelmi Tanácsok, 12 (5): 14–16.

Jen, J. J. & Kahler, K. R. (1974): Characterization of polyphenol oxidase in peaches grown in the southeast. HortSci, 9: 590.

Lafuente, M. T. & Martínez-Téllez, M. A. (1997): Effect of the high temperature conditioning on ethylene, phenyalanine ammonia-lyase, peroxidase and polyphenol oxidase activities in flavedo of chilled Fortune mandarin fruit. J. Plant. Physiol, 150: 719–725.

Kwak, S. S. Kim, S. K., Park, I. J. & Lui, J. R. (1996): Enhancement of peroxidase activity by stress related chemicals in sweet potato. Phytochemistry. 43: 565–568.

Pieber, K. (1985): Winterfrostschäden in den Österreichischen Obstanbaugebieten. Erwerbsobstbau, 27 (10): 243–246.

Proebsting, E. L. Jr. & Mills, H. H. (1978): Low temperature resistance of developing flower buds of six deciduous fruit species. J. Amer. Soc. Hort. Sci, 103 (2): 192–198.

Rivero, R. M., Ruiz, J. M., García, P. C., López-Lefebre, L. R., Sánchez, E. & Romero, L. (2001): Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. Plant Science, 160 (2): 315–321.

Salin, M. L. (1987): Toxic oxygen species and protective systems of the chloroplast. Physiol. Plant., 72: 681–689.

Sárdi É., Velich I. Hevesi M. & Klement Z. (1996): The role of endogenous carbohydrates in the *Phaseolus-Pseudomonas* host-pathogene interaction.1. Bean ontogenesis and endogenous carbohydrate components. Hort. Sci. Hung. 28: 65–69.

Sárdi É., Velich I., Hevesi M. & Klement Z. (1999): Ontogenesis-and biotic stress dependent variability of carbohydrate content in snap bean (Phaseolus vulgaris L.). Z. Naturforsch. 54c: 782–787.

Sárdi É., Végvári A., Kerepesi I. & Stefanovits-Bányai É. (2000): Effect of natural infection of *Pseudomonas* on the peroxideses activities in bean (*Phaseolus vulgaris* L.). Plant Physiology and Biochemistry, 38: 224.

Shannon, L. M., Kay, E., Lew & J. Y. (1966): Peroxidase isozymes from horseradish roots. Journal of Biological Chemistry, 241 (9): 2166–2172.

Stefanovits-Bányai É., Sárdi É., Lakatos S., Zayan, M. & Velich I. (1998): Drought stress, peroxidase activity and formaldehyde metabolism in bean plants. Acta Biologica, 49. (2–4): 309–616.

Stefanovits-Bányai É., Varga I. & Balogh I. (2003): Biokémiai változások a szőlő különböző fenológiai fázisaiban, hazai szőlőajták esetében. SZAB Kertészeti Munkabizottságának Tudományos Ülése, Integrált Kertészeti Termelés, Szarvas, Szept. 17. Proceedings 269–275.

Winter, F., Janssen, H., Kennel, W., Link, H. & Silbereisen, R. (1981): Lucas' Anleitung zum Obstbau. Eugen Ulmer Stuttgart, 526.