International Journal of Horticultural Science 2008, 14 (1–2): 41–44
Agroinform Publishing House, Budapest, Printed in Hungary
ISSN 1585-0404

Frost induced changes in enzyme activities and carbohydrate content in the spurs of some pear cultivars during the dormancy

Honty, K.,[1] Sárdi, É.,[2] Stefanovits-Bányai, É.[3] & Tóth, M.[1]

Corvinus University of Budapest, Hungary
[1]Faculty of Horticultural Science, Dept. of Fruit Science,
[2]Faculty of Horticultural Science, Dept. of Genetics and Horticultural Plant Breeding,
[3]Faculty of Food Science, Dept. of Applied Chemistry H-1118 Budapest Villányi út 35–43, Hungary

Summary: Frost tolerance of pear cultivars was checked after artificial cold treatment in 2003–2005. Limbs collected during the endodormancy were exposed in a climatic chamber for 24 hours to –25, –28 °C, while those collected in the ecodormancy were kept at –15 and –18 °C. Frost damages of buds were registered according to a visually defined scale, then peroxidase (POD), polyphenol oxidase (PPO) enzyme activities and carbohydrate contents were checked in buds and spur-part below the buds. POD activity of untreated control in tissue below buds was higher than in the buds, which were increasing continuously during the endodormancy and decreased at the end of the ecodormancy. During endodormancy, cold treatment of –25 and –28 °C effected different changes of enzyme activity in buds of the cultivars. In the ecodormancy, enzyme activities increased after a cold treatment of –15 °C, whereas the activities decreased significantly after –18 °C. 'Kaiser' – susceptible to frost – with its higher values of both enzyme activities marked out from other cultivars, which is correlated with its stress response. Changes in carbohydrate components – especially in glucose – of buds monitored well the different stress responses of tolerant and resistant pear cultivars induced by frost stress.

Key words: pear cultivars, peroxidase, polyphenol oxidase, carbohydrates, endodormancy, ecodormancy, frost-stress

Introduction

The Hungarian pear production has reduced significantly in the recent years. Among the reasons are diseases (e.g. fire blight), winter and spring injuries. The main commercial pear cultivars represent large differences in tolerance to winter frosts (*Filiti & Neri,* 1989; *Pieber,* 1985; *Iváncsics,* 2003; *Göndör & G. Tóth,* 1998).

During their development, plants are exposed to different biotic and abiotic stresses. Plants generally build up defence mechanisms, 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 polyphenol oxidase (*Hegedűs* et al., 2004). Changes in the activity of these enzymes are generally recognised phenomena (*Kwak* et al., 1996; *Lafuente & Martínez-Téllez,* 1997; *Saruyama & Tanida,* 1995). The cognition of biochemical and physiological changes induced by cold during dormancy can help us to select cultivars with suitable frost tolerance under Hungarian ecological conditions. Present study endeavours to measure the frost tolerance in the 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 and carbohydrate contents.

Materials and methods

European cultivars (*Pyrus communis* L. – 'Packham's Triumph', 'Kaiser'), Japanese pear (*P. pyrifolia* Nakai – 'Hosui'), and 'Kieffer' (*P. pyrifolia* x *P. communis*) have been scored for their frost tolerance. Samples were taken from the experimental station of the Faculty of Horticultural Science at Szigetcsép. The frost effect was checked 2 times in 2003 (10th February in the endodormancy, and 10th March, in the ecodormancy), 5 times in 2004 (12th January; 3rd, 10th, 17th February and 16th March), and 3 times in 2005 (3rd February; 3rd, 16th March). 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.

The limbs sampled during the endodormancy were exposed in a climatic chamber for 24 hours to –25 °C (2003), and –25, –28 and –30 °C (2004–2005), while those collected in the ecodormancy were kept at –15 and –18 °C (2003) and –18, –20 °C (2004, 2005) temperatures. After this frost treatment – as well as previously – the determination of the injuries in buds was checked visual according to a numerical scale (Göndörné, 2000).

Buds, spurs – tissues below the buds – and limbs were tested for peroxidase activity (POD) by spectrophotometry in

H_2O_2 substrate with ortho-dianidizine as choromegene reagent (ϵ = 11.3), at λ = 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. Separation and determination of carbohydrates was measured with overpressured layer chromatographic separations (OPLC, Mincsovics et al., 2003). OPLC separations were carried out on TLC and HPTLC silica gel 60 F_{254} precoated chromatoplates (Merck Co., Darmstadt, Germany) using acetonitrile: H_2O (85:15, V/V). Staining was performed by aniline- diphenyl amine – phosphoric acid reagent. For densitometric determination a Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan), λ=540 nm was used (*Sárdi* et al., 1996). Results were expressed in terms of μg/g.

Mean values were calculated from data measured on three replicates of each treatment, then standard deviation was determined.

Results

The visual examination of the damage suffered by the buds consecutively to frost-stress also confirmed that, among the cultivars researched, the 'Kaiser' is the most susceptible to low temperature both during endodormancy and ecodormancy. Out of our cultivars, 'Packham's Triumph' showed the lowest degree of frost damage as well as the least occurrence of death in every type of dormancy. According to information sourced from scientific publications, the 'Kieffer' is one of the less exacting cultivars as regards ecological conditions. Our observations showed 20 to 40% frost damage or death at –25 °C in endodormancy, while at temperatures of –28 °C and –30 °C the maximum rate of non-damaged buds was 20% – according to seasonal changes. In the ecodormancy period, buds kept at –18, –20 °C also significantly suffered. The reaction of Japanese pear cultivars ('Hosui', 'Nijisseiki') was similar: damage rate was 40 to 60% during endodormancy; after ecodormancy only about 20% of buds were intact.

Changes in enzyme activity

The measurements of enzyme activities (peroxidase: POD, polyphenol oxidase: PPO) carried out in 2003 showed the lowest values in limbs in all the cultivars. These low values did not allow valid conclusions.

The values of POD enzyme activity in non-treated controls of every cultivar were higher in spurs – in tissues below the buds – than in the buds themselves. POD activity in control buds increased during endodormancy and sank toward the end of ecodormancy. In all years (2003-2005) under study the most frost-susceptible 'Kaiser' significantly exceeded the values found in the other cultivars during the entire dormancy period *(Figure 1)*.

In endodormancy the reaction of frost-resistant (e.g. 'Packham's Triumph') or of other less frost-susceptible cultivars was comparable to cold treatments of –25, –28, –30 °C; their POD activity in the buds did not change or only insignificantly decreased. In tests carried out in 2004 the frost-sensitive cultivars (e.g. 'Kaiser') showed increased POD activity to the –25 °C frost at the end of endodormancy. The POD activity in the buds of all the researched cultivars went down following –28, –30 °C frost treatment *(Figure 2)*; this was attributable to a higher degree of tissue necrosis. POD activity varied somewhat during ecodormancy in both the frost-susceptible and the more frost-tolerant cultivars: –15 °C cold stress resulted slightly higher, while at –18 °C the POD activity diminished in buds.

In spurs, in the tissues below the buds the enzyme activity values observed in periods of endodormancy – although not uniformly – increased after a –25 °C frost effect, and went down approximately to the level of the controls at –28 °C *(Figure 3)*. POD activity of our cultivars, when in ecodormancy – similarly to buds – increased differently following a –15°C treatment: increases of 150% by the frost-resistant cultivars, 20% by the sensitive ones were observed. A –18, –20 °C frost effect provoked reduced activity (this diminution was marked in the more susceptible ones).

As opposed to POD, the PPO activity control values attained their minimum in the buds at the end of endodormancy, while during ecodormancy they increased. No such unequivocal trend was noticed in the tissues below the buds. At some of the cultivars we found stronger PPO control activity in ecodormancy than in endodormancy, at others, the opposite was observed.

In buds in endodormancy we could ascertain that at –25 °C PPO activity increased, while at –28, –30 °C it diminished. The observed increases of activity were more marked in the frost-sensitive cultivars. In ecodormancy, PPO activity growth was observable in the Japanese pear cultivars under the effect of –15 °C caused cold stress, while in the others we measured diminution. The impact of -20 °C proved to be similar: it resulted in increased activity in the buds of Japanese cultivars and of Kaiser's, while no significant alteration occurred in the more resistant cultivars.

In tissues below the buds we noticed ongoing growth of PPO activity as a result of –28 °C frost effect, while this activity diminished after a –30 °C frost stress which is attributable to tissue necrosis *(Figure 3)*. By the most frost-resistant cultivars in ecodormancy consecutively to –18 °C treatment we observed increased PPO activity, while by the more sensitive cultivars no substantial alteration could be shown or decrease occurred.

Changes in carbohydrates

The results of our research have allowed the statement – confirmed also in the literature – that there are demonstrable correlations between the stress-induced changes in the concentration of carbohydrate fractions and the abiotic stress-tolerance capability of various plant species.

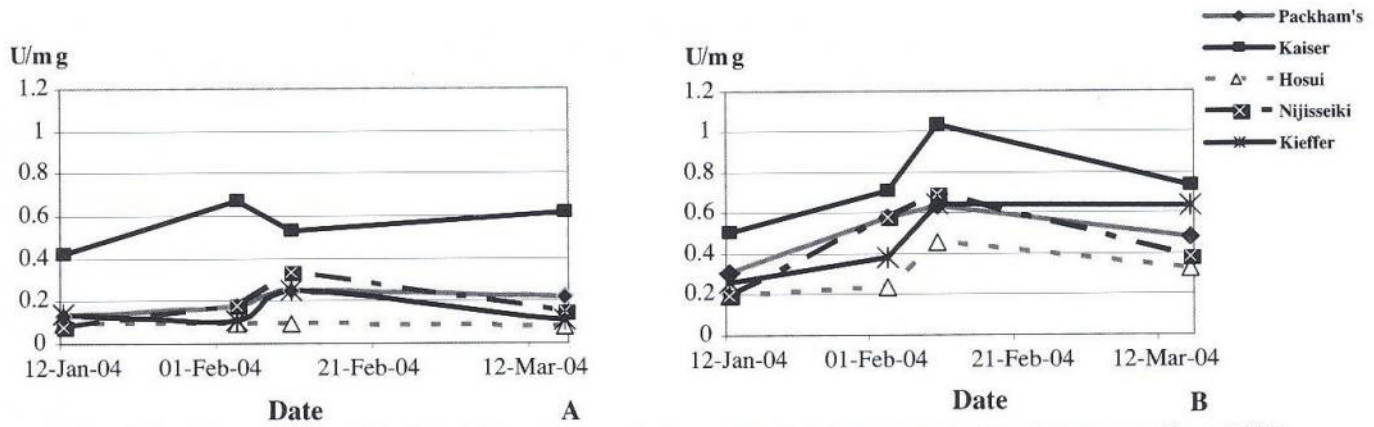


Figure 1 Peroxidase enzyme activities in buds (A) and in spurs, in tissues below buds (B) in the dormancy of some pear cultivars (2004)

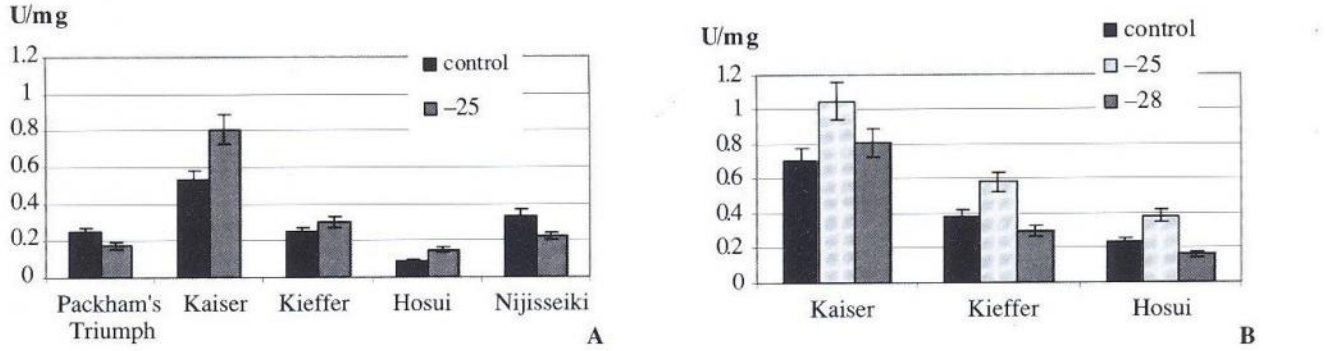


Figure 2 Changes in peroxidase activities in buds (A) and in tissue below buds (B) of pear cultivars after frost treatment (Febr, 2004)

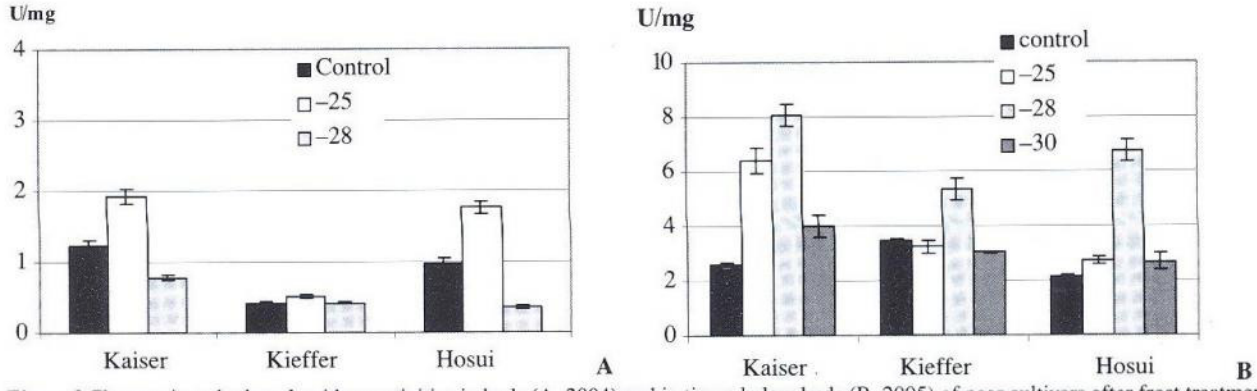
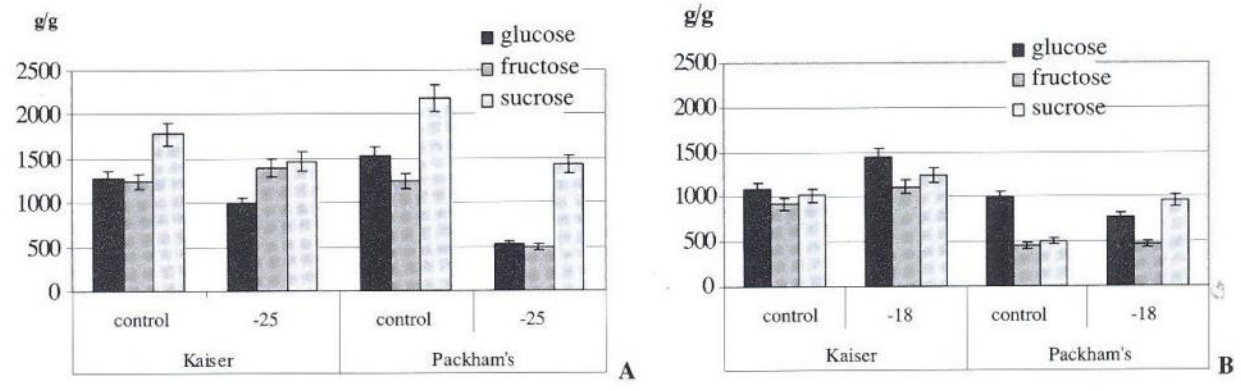


Figure 3 Changes in polyphenol oxidase activities in buds (A, 2004) and in tissue below buds (B, 2005) of pear cultivars after frost treatment



Bars mean standard deviation

Figure 4 Changes in different carbohydrate contents in buds of pear cultivars in the endodormancy (A: Jan, 2004) and in the ecodormancy (B: March, 2004)

In function of the given conditions surrounding our measurements, glucose, fructose and sucrose were the main carbohydrate components in the plant samples, and we detected lesser concentrations of raffinose.

The carbohydrate fractions in the buds of the researched cultivars – as measured in the first samples taken during endodormancy (in January) showed more divergent alterations under the effect of cold stress than in later periods. In the frost-tolerant 'Packham's Triumph' the three major carbohydrate components (glucose, fructose, sucrose) substantially diminished compared to the values observed in the controls. In the buds of sensitive cultivars (e.g. 'Kaiser') the changes observed in the glucose and sucrose levels were far smaller, while the concentration of fructose slightly increased. In later samples the frost-induced responses showed generally divergent movements in the sensitive, respectively the resistant cultivars. In the buds of sensitive cultivars the effect of –25 °C resulted in substantially higher glucose and fructose levels, and they increased at –28 °C. Though not uniformly in the various cultivars, sucrose levels also tended to grow. In the buds of the frost-susceptible cultivars glucose concentration in ecodormancy increased at –18 °C, while in the resistant ones the glucose level showed lesser diminution *(Figure 4)*. As regards the fructose level, no marked change occurred in any of the genotypes.

Discussion

In analysing the changes in enzyme activity caused by frost stress, we found that the enzyme activity (POD) in tissue below the bud exceeded the levels measured in buds whatever the period or the type of treatment. We also stated a stronger correlation between the severity of the cold stress and the plant's endogenous rhythm and the change occurring in enzyme activity in bellow the bud tissues. The changes in enzyme activity also indicated that tissues below the buds suffered smaller frost damage than the buds themselves. The generally observed ascending enzyme activities were able to fulfil a protective function. We conclude therefore that the plant's mechanism of resistance to oxidative stress can be located under the bud; they are thus more marked in the tissues from where buds, after apparent death, can yet re-emerge. Indeed, the tissue appeared the more significant for long term plant survival. Our study of buds confirmed the differences in frost-sensitivity thanks to the examination of enzyme activity as the change in enzyme activity was in several instance different from the resistant cultivars.

Among the plant components under study it is the glucose measured in the buds, that is, the quantitative changes occurring under the effect of frost that can best be related to the frost-tolerance capabilities known on the basis of the experience of growers and breeders.

Acknowledgements

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

Many thanks Pedryc Andrzej for his help.

References

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

Göndör, M. & G. Tóth, M. (1998): Evaluation of frost resistance and productivity of pear cultivars in Hungary. *Acta Horticulturae.* 484: 79–83.

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

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. *Hort Sci,* 9: 590.

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.* 1996. (43): 565–568.

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.

Mincsovics, E., Ferenczi-Fodor, K. & Tyihák, E. (2003): Overpressured Layer Chromatography. In: Sherma, J. and Fried, B. (Eds.), Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, Third Edition, pp. 175–205.

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

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

Saruyana, H. & Tanida, M. (1995): Effect of chilling on activated oxygen-scavenging enzymes in low temperature-sensitive and -tolerant cultivars of rice (*Oryza sativa* L.). *Plant Science.* 109: 105–113.

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