Jpn. J. Crop Sci. 63(2) : 368—369 (1994)

Short Report

# Gene Expression of a Kunitz-type Proteinase Inhibitor and Patatin in Response to Storage Temperature of Potato Tubers*

Kazutoshi YAMAGISHI, Koh NAGATANI, Takako FUKASE, Cristina MITSUMORI, Eliza Mitiko ISEJIMA and Yoshio KIKUTA
(*Laboratory of Crop Physiology, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan*)
Received October 2, 1993

バレイショ塊茎の貯蔵温度に応答したKunitz型プロテイナーゼインヒビターおよびパタチンの遺伝子発現：山岸和敏・永谷　工・深瀬孝子・三森クリスチーナ・伊勢島エリザ美智子・喜久田嘉郎（北海道大学農学部）
キーワード：遺伝子発現，温度，塊茎，Kunitz型プロテイナーゼインヒビター，パタチン，バレイショ（*Solanum tuberosum* L.）．
**Key words** : Gene expression, Kunitz-type proteinase inhibitor, Patatin, Potato (*Solanum tuberosum* L.), Temperature, Tuber.

The process of potato tuberization starts from the differentiation of an underground stem, called stolon, by cell division and expansion of a subapical meristem with concomitant accumulation of starch and proteins. The major proteins reserved in potato tubers are Kunitz-type proteinase inhibitor (PKPI), patatin (lipid acyl hydrolase) and proteinase inhibitor II[2,7,10]. Their roles are expected to supply amino acids when tubers are sprouting, and to be involved in defense mechanism against predators and pathogen attacks. Although those proteins and mRNAs were shown to accumulate as the tubers developed[3,4], little is known about their changes in harvested tubers. On tubers stored in cold cellars, it has reported that the dormant period could be prolonged[6] and the concentration of free sugars increased[5]. Thus, the storage temperature is expected to be an important factor regulating physiological conditions of harvested tubers. The present paper reports the effects of temperature on PKPI and patatin mRNA levels in intact potato tubers during storage.

## Materials and Methods

Potato tubers (*Solanum tuberosum* L. cv. Irish Cobbler) grown in the experimental field of Hokkaido University were harvested and stored in cellars at 4°C (cold) and 25°C (room temperature) in darkness. Those cellars were also used for temperature treatment of tubers. Heat treatment was performed in a convection incubator at 40°C in darkness.

Total RNA was isolated according to Shirras et al[9]. Northern blot analysis was performed as described by Sambrook et al[8]. The probes used for northern blot analysis were prepared from the cDNA clones encoding PKPI[11] and patatin[12].

## Results and Discussion

The influence of tuber storage temperature on PKPI and patatin mRNA levels is shown in Figs. 1 and 2. Little PKPI mRNA was found in field-grown tubers stored at 25°C for one month, but this mRNA was preserved in tubers stored at 4°C for one month (young tubers) even after 12 months (old tubers) after harvest. On the other hand, patatin mRNA levels were low in all tubers. Interestingly, when cold-stored tubers were transferred to the cellar at 25°C, PKPI and patatin mRNA levels increased within 48 h in both old and young tubers (Fig. 1). Transfer of 5 months-old tubers stored at 4°C to 25°C resulted in similar increases of the mRNA levels (data not shown). Subsequently, when the tubers were transferred to 40°C, those mRNA levels decreased within 24 h, but they were not affected by the transfer to 4°C and/or 25°C (Fig. 2).

* This work was conducted in the Research Center for Molecular Genetics, Hokkaido University, Sapporo, Japan, and was in part supported by Grant-in-Aid for General Scientific Research (B) 05454042 from the Ministry of Education, Science and Culture, Japan.

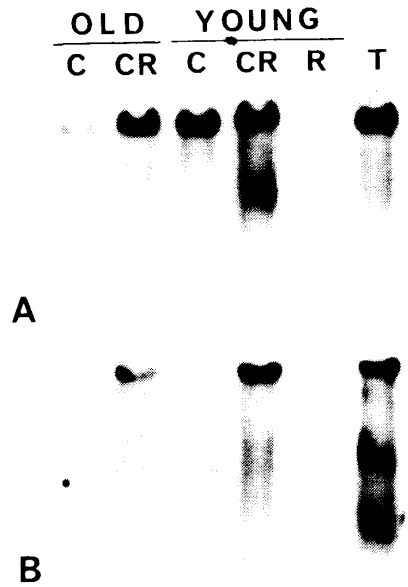


Fig. 1. Influence of storage temperature on PKPI (A) and patatin (B) mRNA accumulation in potato tubers. Field-grown tubers were harvested in 1991 and stored in a cellar at 4°C (C) for 12 months (OLD). Tubers harvested in 1992 were stored in cellars at 4°C (C) or 25°C (R) for one month (YOUNG). Then both of cold-stored tubers were transferred to a cellar at 25°C for 48 h (CR). Total RNAs (20 $\mu$g each) were analyzed by northern blot hybridization. Lane T corresponds to 10 $\mu$g total RNA isolated from developing tubers, *in vitro*.

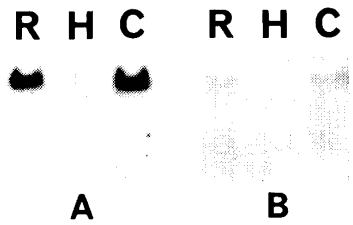


Fig. 2. Influence of heat and cold stress on PKPI (A) and patatin (B) mRNA accumulation in potato tubers. Cold-stored tubers (4°C for 5 months after harvested in 1992) were transferred to a cellar at 25°C for 48 h, followed by transfer to cellars at 25°C (R) and to 4°C (C), or to a convection incubator at 40°C (H) for 24 h, respectively. Total RNAs (20 $\mu$g each) were analyzed by northern blot hybridization.

Although PKPI and patatin mRNAs are expected to degradate after harvest of tubers, the degradation of PKPI mRNA may be somewhat prevented in cold-stored tubers. In addition, cold-stored tubers might possess transcriptional activities which were similar to those in developing tubers, because PKPI and patatin mRNA levels increased after the transfer of cold-stored tubers to 25°C. The increase in mRNA levels is expected to be accompanied by *de novo* synthesis of PKPI and patatin proteins, both of which could play an important role of defense mechanism during the tuber storage. In contrast, heat stress was shown to reduce PKPI and patatin mRNA levels. Slow heating stress (similar to our experiment) was demonstrated to reduce not only patatin mRNA level in immature tubers after harvest but also the mRNA levels of ubiquitin and heat shock protein which usually increased in rapid heating condition (heat shock)[1]. Since stored tubers are more likely to be exposed to slow heating stress than to heat shock, the most of tuber mRNA might disappear in heat condition. However, it is not clear whether slow heating repressed the gene expression or promoted degradation of mRNA.

The present findings and relevant data revealed that PKPI and patatin mRNA levels were affected by storage temperature of tubers. Further studies will be devoted to elucidation of a molecular mechanism which regulates the mRNA accumulation in potato tubers under post-harvest conditions.

## References

1. Belknap, W. et al. 1990. In Vayda, M.E. and W. D. Park eds., The Molecular and Cellular Biology of the Potato. Redwood Press Ltd., Melksham. 89—95.
2. Bryant, J. et al. 1976. Biochemistry 15 : 3418—3424.
3. Hannapel, D.J. 1991. Physiol. Plant. 83 : 568—573.
4. Hendriks, T. et al. 1991. Plant Mol. Biol. 17 : 385—394.
5. Isherwood, F.A. 1976. Phytochemistry 15 : 33—41.
6. Lindblom, H. 1970. Potato Res. 13 : 159—166.
7. Racusen, D. et al. 1980. J. Food Biochem. 4 : 43—52.
8. Sambrook, J. et al. 1989. Molecular Cloning : A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 7.43—7.52.
9. Shirras, A.D. et al. 1984. Planta 162 : 353—360.
10. Suh, S.G. et al. 1990. Plant Physiol. 94 : 40—45.
11. Yamagishi, K. et al. 1991. Plant Mol. Biol. 17 : 287—288.
12. Yamagishi, K. et al. 1993. Plant Mol. Biol. 21 : 539—541.