

Postharvest chilling-induced variations in cysteine protease and inhibitor gene expression in pineapple fruits (*Ananas comosus* (L) Merr.) under blackheart physiopathy

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Introduction

Pineapple is a commercially important tropical fruit used either for processing or as a fresh fruit in local or export markets. Chilling temperatures in the field and during postharvest storage induce a physiological disorder inside the fruit referred to blackheart. Cysteine proteases, an important group of plant cellular endoproteases, are involved in many aspects of plant physiology, development and stress responses. Previous studies have shown that cysteine proteases are responsible for cellular degradation in response to various abiotic stress. The aim of this study was to investigate the involvement of fruit bromelain, the major cysteine protease of pineapple fruit, and its natural inhibitor, cystatin in the development of postharvest chilling response inducing blackheart physiopathy. The studies concerned two varieties differing in their susceptibility to chilling stress.

Materials and methods

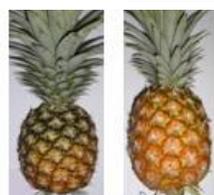


Figure 1: Smooth Cayenne and MD2 pineapple fruit varieties

Two pineapples varieties were chosen according to their susceptibility to blackheart. The susceptible variety Smooth Cayenne (SC), is currently the most exported one in the world whereas the tolerant MD2, is a recent variety with interesting organoleptic properties and resistance to several abiotic and biotic stresses. Pineapple fruits of uniform size were collected and harvested at the same commercial maturity stage (at least 12 Brix FAO, codex 2007). Fruits were submitted or not to postharvest chilling stress (Table 1), flash-frozen in liquid nitrogen and stored at -80 C until needed. Electrolyte leakages were measured at room temperature using a Fruit membrane injury test (FMI). Expression of a cysteine protease (fruit bromelain) and a cystatin genes were studied using q-PCR procedures with actin as a reference gene. Cysteine protease activities were measured in crude extracts prepared from pineapple fruit flesh.

Table 1 : treatments of pineapple fruits

Treatments
Control fruit, immediately used after harvesting : c
Fruit submitted to 10 days at 10 C then 10 days at 25 C : Tr

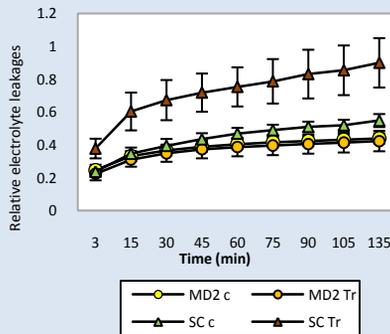


Figure 2: Time course of relative electrolyte leakage in pineapple fruits MD2 and Smooth Cayenne (SC) under control (c) or postharvest chilling stress (Tr) conditions.

Pineapple fruits exhibited distinct relative electrolyte leakage depending on the variety and the treatment applied (Fig. 2). Under control conditions, the susceptible Smooth Cayenne (SC), showed a significant higher rate of relative electrolyte leakage than the tolerant MD2 ($P < 0.05$). Thus cellular electrolyte leakages correlated well with blackheart susceptibility of the pineapple variety. This phenomenon was enhanced under postharvest chilling treatment ($P < 0.05$). Electrolyte leakages are related to fruit cell membrane damage, as shown in leaves (1), and this has been previously related to endoprotease activity in response to abiotic stress (2). Analysis of

Results and discussion

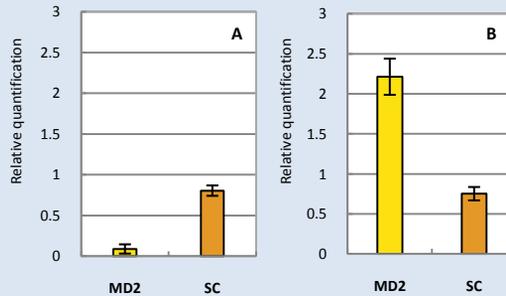


Figure 3: (A) fruit bromelain and (B) cystatin gene expression in MD2 and Smooth Cayenne (SC) fruits under postharvest chilling stress.

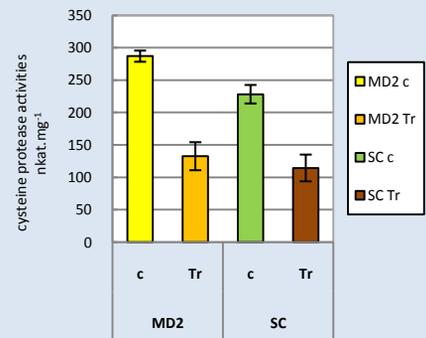


Figure 4 : Total cysteine protease activities in MD2 and Smooth Cayenne (SC) fruits under postharvest chilling stress.

transcript accumulation using qPCR showed that postharvest chilling stress induced a down regulation of fruit bromelain gene expression, especially in the resistant MD2 variety (Fig 3a). Chilling stress also resulted in an inhibition in fruit bromelain activity in both varieties, particularly in MD2 (Fig. 4). The trend was opposite in cystatin gene expression since an up-regulation was observed in the resistant MD2 variety whereas it was slightly down regulated in the susceptible SC variety (Fig. 3b). These results suggest an involvement of cystatin in postharvest chilling stress resistance of pineapple to blackheart physiopathy.

Conclusions: Postharvest chilling- stress induced a rise in electrolyte efflux in pineapple fruit, particularly in the blackheart susceptible variety SC. In the resistant variety MD2, gene expression of fruit-specific bromelain was reduced under postharvest chilling-stress, while cystatin inhibitor gene was up-regulated. Our results suggest that fruit bromelain and cystatin gene expression as well as membrane integrity could be involved in postharvest chilling tolerance at the cellular level.

References

- 1 Vasquez-Tello, A, Zuily-Fodil, Y, Pham Thi . A. T, and Vieira da Silva J. 1990. Electrolyte and Pi leakages and soluble sugar content as physiological tests for screening resistance to water stress in Phaseolus and Vigna species. J. Exp. Bot. (41).
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