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Relationship between sorbitol and loquat

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SUMMARY – Loquat utilizes sorbitol as main carbohydrate metabolite. The data and information about the relationship between sorbitol and *in vivo* and *in vitro* morphogenesis of loquat, and molecular biology related to sorbitol are reviewed in this paper.

Key words: Sorbitol content, sorbitol metabolite, molecular biology, loquat.

RESUME – *"Relation entre sorbitol et nèfle". La nèfle utilise le sorbitol comme principal métabolite des hydrates de carbone. Cet article présente les données et l'information concernant la relation entre le sorbitol et la morphogenèse* in vivo *et* in vitro *des nèfles, ainsi que la biologie moléculaire liée au sorbitol.*

Mots-clés : *Teneur en sorbitol, métabolites du sorbitol, biologie moléculaire, nèfle.*

Introduction

At the beginning of 1970s, some authors reported about using sorbitol as carbon resource for cultures, and content and metabolite of sorbitol *in vivo* in Rosaceae family (Chong *et al.*, 1972). In 1977, Kawamata first determined that loquat, like other member of Rosaceae family such as apple, pear, cherry, apricot, plum and peach, contained sorbitol as the sugar component. From that on, the reports on sorbitol in loquat increased gradually. This paper reviews three aspects: *in vivo, in vitro* and molecular biology of sorbitol in loquat.

In vivo

Sugar accumulation during development of loquat fruit in cv. Tanaka was examined (Hirai, 1980). Sorbitol was the major soluble sugar in young fruit phase, which was characterized by growth in size and weight of the seed, while in fruit maturation phase, sucrose was the major component. The extra sugar, 90% of which accumulated within 2 weeks of maturation, is thought to be derived from other parts of the plant by starch hydrolysis.

Differences in sugar and other components of loquat fruits (cv. Mogi) picked at various stages of maturity were surveyed (Hamauzu *et al*., 1997). Loquat fruits were harvested at different maturity stages from stage 1 (green, small) through to stage 7 (yellowish orange, harvest maturity) and stage 8 (slightly over ripe). Fructose, glucose and sucrose were the dominant sugars; sorbitol was a minor one. Sucrose content decreased from stage 5 to stage 8; fructose was the dominant sugar at maturity stage 8.

Seasonal changes in the sugar content were followed throughout the development of loquat cv. Mogi fruits. Sucrose and fructose are the predominant sugars in mature fruits whereas fructose and sorbitol are the major ones in young fruits (Abnasan-Bantog *et al*., 1999).

During the developmental stages of loquat embryo *in vivo*, sorbitol increased gradually. Sorbitol in globular, torpedo-shape, and cotyledonary embryos were 5.7, 11.5 and 17.9 mg/g, respectively. It showed positive relationship between sorbitol and morphogenesis *in vivo* (Lin *et al.*, 1995, 1999).

Just before fruit colouring, fruits on loquat trees (cvs Tanaka, Toi, Mogi, Mizuho and Kusunoki) were placed in polyethylene bags with 25-30 mg granules with adsorbed ethylene. Ethylene decreased fruit acidity and increased sorbitol-6-phosphate dehydrogenase and NADP-malic enzyme activities (Hirai, 1982).

Ding *et al*. (1997) also examined the effects of polyethylene bag packaging and low-temperature storage on the physical and chemical characteristics of loquat fruits. Loquat fruits were packaged with different thicknesses of polyethylene film (PE-20, PE-30 and PE-50) and in perforated PE bags (as control) and stored at 5ºC. Total sugars did not vary markedly in any of the treatments, although sucrose decreased and sorbitol increased steadily during storage.

The anatomy of the sieve elements in the vascular bundle in loquat, and other member of rosaceous fruit trees such as peach, plum, apple, and so on, was observed by both optical and transmission electron microscopy (Nii, 1993). As a comparison, sieve elements of satsuma, persimmon and grape plants were examined. A distinguishing feature of the thickening portion of the sieve elements in the vascular bundles of the midrib, petiole, fruit stalk and pericarp of the rosaceous species appeared to be structural ingrowth of the cell wall, but only as a trace in the roots. From the anatomical observations, the ingrowth thickening in the sieve elements, referred to as nacreous wall formation, was completely different from the apparatus of the transfer cells. This sieve-element structure was a characteristic feature of the rosaceous fruit trees, in which sorbitol is the main photosynthetic product.

Nii *et al*. (1994) further studied anatomical features on the sieve elements and sorbitol content in various organs of Rosaceae fruit trees, focused on the nacreous cell wall in the sieve tube of the petiole, fruit stalk and root in loquat cv. Nagasaki-wase, and the soluble carbohydrate contents in these organs and in the leaves and fruits. The developmental degree of ingrowth of the cell wall in the fruit stalk was species-dependent; the ingrowth was thickest in loquat. The nacreous cell wall in sieve tubes of loquat roots was moderately thick; it was less thick in apple roots. Sorbitol was the predominant soluble carbohydrate in the lamina and petiole of all species. In the fruit stalk, sorbitol content varied between species. In ripe fruits, sugar composition also varied between species but sorbitol content was usually low. It is hoped that a relationship can be established between the degree of ingrowth of the nacreous cell wall and the soluble carbohydrate transported.

In vitro

The effects of different carbohydrate sources (D-sorbitol, sucrose, D-fructose, D-glucose and D-galactose) on loquat (*Eriobotrya japonica*) embryo culture were examined. The result showed that only callus transferred into the medium supplemented with 3% sorbitol as carbohydrate resource shoot could differentiate (Lin *et al*., 1995).

As an osmotic agent for protoplast isolation, sorbitol was less efficient than mannitol, though it could become more efficient at a higher mass fraction up to 16%. The highest frequency of loquat protoplast cell mitosis was observed when the protoplasts were cultured in MS liquid medium supplemented with 10% sucrose and 5% sorbitol; the effect of sucrose on protoplast culture was stronger than that of sorbitol or mannitol (Lin *et al*., 1996, 1997).

Isolated protoplasts are cultured to mitosis easily. However, it was very difficult for the callus derived from protoplast to differentiate when it was cultured in MS or modified MS medium without sorbitol. During callus's transfer, some callus became brown. The rest callus were transferred into the medium supplemented with same plant growth regulators but different carbohydrate. The experimental results showed that only callus transferred into the medium supplemented with 3% or 5% sorbitol shoot could differentiate (Lin *et al*., 1996, 1997).

In order to understand why sorbitol could promote the callus differentiate, concentration of sugar and sorbitol dehydrogenase activity in various callus cultured in various carbon resources were tested. The results showed that content of sorbitol and activity of sorbitol dehydrogenase in the callus cultured in medium with sorbitol were high than both common callus and brown callus. From the fact that both higher content of sorbitol and activity of sorbitol dehydrogenase were positively related with organogenesis, it suggested that higher activity of sorbitol dehydrogenase might be beneficial to increasing of sorbitol and then further promote organogenesis.

Molecular biology

Some related enzymes such as sorbitol-6-phosphate dehydrogenase have been surveyed. The activity of sorbitol-6-phosphate dehydrogenase both in leaf and in fruit increased before the increase of the sorbitol content, suggesting that sorbitol metabolism is regulated by sorbitol-6-phosphate dehydrogenase (Hirai, 1979, 1981, 1983).

Sorbitol-6-phosphate dehydrogenase was purified from loquat leaves and characterized, and its activity was detected in the leaves of apricot, peach, *Rhaphiolepsis indica, Sorbus aucuparia*, quince, *Photinia glabra* and *Spiraea thunbergii*. It is suggested that it catalyses sorbitol synthesis from glucose-6-phosphate during photosynthesis in rosaceous plants (Hirai, 1981).

The result of study of molecular biology in loquat is helpful to other member of rosaceae family such as apple. Nucleotide and deduced amino acid sequences are presented for the NADP-S6PDH cDNA (DDBJ/EMBL/GenBank accession number D11080), isolated from a cDNA library, constructed from apple seedlings, by screening with the antibody against NADP-S6PDH purified from loquat leaves. It is expressed as a mRNA of approximately 1.4 kb and the (G + C) content is 46.4% in the coding region. The protein has an ORF of 310 amino acids (Mr 34 900) with dimers consisting of 2 equally sized subunits (Kanayama *et al*., 1992).

Seasonal changes in activities of enzymes associated with sorbitol metabolism were followed throughout the development of loquat cv. Mogi fruits (Abnasan-Bantog *et al*., 1999). Sorbitol dehydrogenase (SDH) [L-iditol 2-dehydrogenase] activity remained at a low level until 19 May when it and sorbitol-6-phosphate dehydrogenase (S6PDH) activity increased dramatically with sugar accumulation; the levels reached a plateau on 26 May. The above rapid sugar uptake into the fruit was attributed to the increased unloading of sorbitol and sucrose from 19 to 26 May induced by the prominent rise in SDH activity from 23 May.

Gene expression of NAD+-dependent sorbitol dehydrogenase and NADP+-dependent sorbitol-6-phosphate dehydrogenase during development of loquat fruit was studied by Abnasan-Bantog *et al*. (2000). The aim of this study is to clarify the roles of NAD+-dependent sorbitol dehydrogenase (NAD-SDH) and NADP+-dependent sorbitol-6-phosphate dehydrogenase (S6PDH) in fruit development, and the regulatory mechanism(s) underlying their expression during development using loquat as the study material. The cDNA of NAD-SDH, cloned from loquat fruit, consisted of 1572 bp and contained an open reading frame of 1023 bp capable of encoding a protein of 371 amino acids. The deduced amino acid sequence has 97.8% identity to that of an apple fruit. The activity of NAD-SDH is a function of fruit development, i.e. the increase in protein of this enzyme synchronized consistently with that of activity showing no posttranslational modification throughout the developmental stages. Furthermore, NAD-SDH activity correlated with the mRNA levels, indicating that the key regulatory step of the activity is at the transcriptional level. The increase in NAD-SDH with fruit development plays a dominantly important role during fruit maturation and sugar accumulation. The trend of S6PDH activity during fruit development paralleled that of NAD-SDH activity. It correlated with protein and mRNA levels revealing that the regulation of the activity is mainly at the transcriptional level like NAD-SDH. However, the role of S6PDH in fruit is not yet clear.

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