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Research article

# Occurrence of a number of enzymes involved in either gluconeogenesis or other processes in the pericarp of three cultivars of grape (*Vitis vinifera* L.) during development

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ABSTRACT

It is uncertain whether the enzymes pyruvate orthophosphate dikinase (PPDK) or isocitrate lyase (ICL) are present in the pericarp of grape, in which they could function in gluconeogenesis. The occurrence of these and other enzymes was investigated in the pericarp of three cultivars of grape (*Vitis vinifera* L.). In particular, the abundance of the enzymes aldolase, glutamine synthase (GS), acid invertase, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), phosphoenolpyruvate carboxylase (PEPC), PPDK and ICL were determined during the development of the pericarp of the cultivars Cabernet Sauvignon, Chardonnay and Zibibbo. PPDK and ICL were not detected at any stage of development. Each of the other enzymes showed different changes in abundance during development. However, for a given enzyme its changes in abundance were similar in each cultivar. In the ripe pericarp of Cabernet Sauvignon, PEPC, cytosolic GS and aldolase were equally distributed between the vasculature and parenchyma cells of the flesh and skin. The absence or very low abundance of PPDK provides strong evidence that any gluconeogenesis from malate utilises phosphoenolpyruvate carboxykinase (PEPCK). The absence or very low abundance of ICL in the pericarp precludes any gluconeogenesis from ethanol.



## 1. Introduction

Many changes occur in grape berries during their development. For most cultivars the pattern of growth of the berry can be depicted as a double sigmoidal curve. Three stages of growth namely I, II and III can be distinguished. During stage I a large increase in the size of the berry occurs. In stage II the rate of increase in both the fresh and dry weights of the edible parts is lower. The onset of stage III coincides with the start of ripening. During ripening the rate of increase in both the fresh and dry weights of the edible parts is higher, and the increase in berry volume arises mainly from cell expansion (Coombe, 1992; Ollat et al., 2002). In addition, softening together with colour and compositional changes take place (Peynaud and Ribéreau-Gayon, 1971; Kanellis and Roubelakis-Angelakis, 1993; Scaglione et al., 2012). Physiological changes also occur and there is a marked reduction in photosynthetic capacity (Harris et al., 1971; Famiani et al., 2000; Palliotti and Cartechini, 2001). There is a large decrease in both titratable acidity and organic acid content. In contrast, there is a substantial increase in the content of both soluble sugars and, in red berry cultivars, anthocyanins (Kanellis and Roubelakis-Angelakis, 1993; Ollat et al., 2002). In most cultivars, the increase in sugar content is mostly a result of the accumulation of fructose and glucose (Boss and Davies, 2001; Ollat et al., 2002). The bulk of organic acid content of grape pericarp comprises malic and tartaric acid, however, the abundance of each of these is dependent on the cultivar (Ruffner, 1982).

There have been many investigations of enzymes involved in the metabolism of grape berries over the last 50 years (Meynhardt, 1965; Hawker, 1969a,b; Kanellis and Roubelakis-Angelakis, 1993; Famiani et al., 2000; Sweetman et al., 2009; Martínez-Esteso et al., 2013). We have previously investigated the changes in abundance of a number of enzymes involved in the metabolism of the flesh and seed of the grape Pinot Noir during development (Walker et al.,

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1999; Famiani et al., 2000). One aim of the present study was to determine whether changes in enzyme abundance, found in our previous study of Pinot Noir, were similar in other cultivars. A second aim was to determine whether the enzyme pyruvate orthophosphate dikinase (PPDK) was present in the pericarp of grapes. It is important to know whether this enzyme is present for the following reasons. In grape pericarp gluconeogenesis from malate occurs (Ruffner, 1982), and in plants this conversion can use either of two pathways (Fig. 1) (Leegood and Walker, 2003). One pathway utilises malate dehydrogenase in conjunction with PEPCK, and both these enzymes are known to be present in grape pericarp (Ruffner, 1982; Sweetman et al., 2009). The second utilises malic enzyme and PPDK, and although malic enzyme is known to be present in grape pericarp the presence of PPDK is less certain (Sweetman et al., 2009). Nevertheless, a recent study reported the presence of PPDK in grape pericarp (Martínez-Esteso et al., 2013). In the present study we investigated this further. A third aim of the paper was to investigate whether ICL was present in grape flesh. This is because there is evidence for the occurrence of aerobic fermentation with synthesis of ethanol in the pericarp of grape berries (Romieu et al., 1992; Tesnière et al., 1994; Terrier and Romieu, 2001) and is reported that its production is likely to be very common in grape berries in the vineyard (Romieu et al., 1992; Sweetman et al., 2009). This is also consistent with the finding that, starting from the inception of berry ripening, a strong increase of alcohol dehydrogenase transcripts occurs during ripening (Tesnière and Verriès, 2000; Sweetman et al., 2009). Tadege et al. (1999) reported the possibility for ethanol to be metabolised through the glyoxylate cycle, whose intermediates, such as malate and succinate, could be used for gluconeogenesis or to feed the TCA cycle and replenish biosynthetic intermediates (Fig. 2). In this, ICL is essential to produce the metabolites which could be used for gluconeogenesis. Therefore, we investigated the presence of ICL throughout development to evaluate if the described route is applicable to grape. The localisation of a number of enzymes in the grape berry has been investigated previously using immunohistochemistry and this is very important in order to relate structure and function in the developing fruit (Walker et al., 1999; Famiani et al., 2000). A fourth aim of the study was to investigate certain aspects of this localisation further using a different approach.

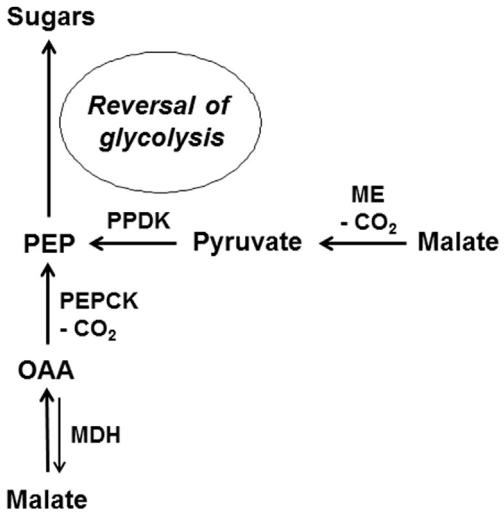


**Fig. 1.** Simplified scheme of gluconeogenesis from malate. OAA = oxaloacetate; PEP = phosphoenolpyruvate; MDH = malate dehydrogenase; PEPCK = phosphoenolpyruvate carboxykinase; ME = malic enzyme; PPDK = pyruvate orthophosphate dikinase.

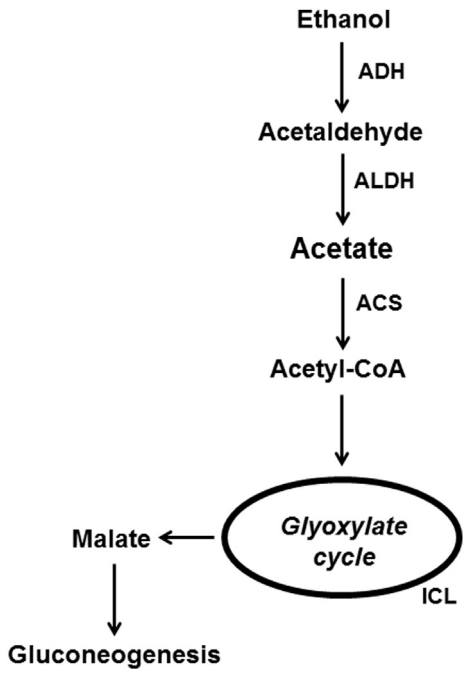


**Fig. 2.** Simplified scheme for potential gluconeogenesis from ethanol. Within the glyoxylate cycle, ICL is essential to produce the metabolites used for gluconeogenesis. Elaborated from Tadege et al. (1999). ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; ACS = acetyl-CoA synthetase; ICL = isocitrate lyase.

## 2. Materials and methods

### 2.1. Plant material

In 2005, healthy berries of grape (*Vitis vinifera* L.), from the cultivars Cabernet Sauvignon (wine cultivar), Chardonnay (wine cultivar) and Zibibbo (double purpose cultivar: table/wine), were collected at various times during development, from vines growing in the experimental vineyard of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia, in Deruta (PG) - central Italy. The stage of the fruit development was based on days after full bloom (AFB); full bloom is defined as 50% of flowers being open. Fruit samples were taken from several positions on the plant. In addition, both young and mature leaves of Cabernet Sauvignon were collected. At the beginning of the third week of August ripening berries were also collected from the cultivars Cardinal, Italia, Trebbiano Toscano, Muller Thurgau, Primitivo, Merlot, Verdicchio and Sangiovese and the rootstock 420 A (*Vitis berlandieri* × *Vitis riparia*). These vines were all growing in the same field of the aforementioned vineyard. All the vines were 7-year-old and trained to a spur pruned cordon.

Cucumber cotyledons were obtained by germinating seeds of cucumber (cv. Money Maker) sowed on 1% (w/v) agar and kept at 25 °C in the dark. Leaves of maize (cv. Golden Giant) were collected from plants growing in a greenhouse in Perugia, Italy.

### 2.2. Measurement of fresh and dry weights

For the cultivars Cabernet Sauvignon, Chardonnay and Zibibbo, the fresh weights of both whole berries and their pericarps were determined for 3 samples each consisting of 20 berries at various times during their development.

### 2.3. Preparation of a frozen tissue powder

For each cultivar, a frozen tissue powder was prepared to ensure that the sample was representative of the tissue. Seeds were