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Review

Informed metabolic engineering of oil crops using control analysis

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ABSTRACT

Oil crops are a very important agricultural commodity. Demand for such oils is rising steadily (at more than 5% per year over the last half century). Although the majority of plant oils are used for food or animal feed, there is increasing interest in their use as renewable chemicals for industry. Because of the demonstrated demand for oils and finite agricultural land, attention is focussing on improving productivity. Genetic manipulation of crop plants needs a knowledge of the biosynthetic pathways concerned and how they are regulated. Although there are different ways to acquire much information, metabolic flux and metabolic control analyses are ways to provide quantitative assessments. In this review we describe our experiments using metabolic control analysis on important crops – oil palm, oilseed rape, olive and soybean. Such research provides information for future informed genetic manipulations and we give a successful example of this in oilseed rape (*Brassica napus* L.).

Contents

1. Introduction

Vegetable oils are a major agricultural commodity accounting for about US$156 billion p.a. for products (Jan. 2013 figures). Moreover, in some countries they are vital for the economy: in Malaysia, for example, palm oil is around 11.3% of the total value of the country's exports (MBOB review, 2011). Furthermore, consumption of vegetable oils has increased at about 5% annually for the last 40 years (Gunstone et al., 2007) and shows no sign of slowing down. Indeed, productivity in the context of finite cultivatable land is vital for food security. Although most plant oil is used for food or animal feed, there is an increasing interest in its use as renewable sources of chemicals which, in many cases, can be substitutes for the dwindling supplies of petrochemicals and which have less impact on climate change. For crops like oilseed rape about one third of the oil is already used for industrial purposes – a much higher percentage than for other major plant oils (Gunstone et al., 2007).

Whether one is interested in bulk oil production (such as for biodiesel) or increasing supplies of specialised oils, then it is clear that productivity is paramount. Some improvements can be achieved by growing specialised crops on marginal land with lower fertility and water content. On the other hand, better successes have been achieved by improving existing crops. For example, the best

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lines of oil palm grown under favourable conditions are capable of producing over 10 t of palm oil and 0.4 t of palm kernel oil per hectare (Gunstone et al., 2007). In addition, it should be possible to genetically engineer oil crops so that they direct more fixed carbon into oil. This approach is already producing soybean lines with significantly raised oil yields (H. Dumude and A. Kinney, personal communication) and which has been shown as a 'proof of concept' in tobacco leaves (Vanhercke et al., 2013).

So far as lipid biosynthesis is concerned, we understand most aspects of the pathways utilised except their regulation. In order to understand regulation of a complex metabolic pathway, one can gain information from classical biochemical studies of enzymes and intermediates (Perry and Harwood, 1993; Perry et al., 1999) and from mutants (Focks and Benning, 1998; Ruuska et al., 2002). The latter studies revealed the importance of transcription factors which have important influences on individual parts of storage oil biosynthesis (To et al., 2012).

However, flux control measurements are needed to provide an overview. Relative fluxes through competing pathways (Bates and Browse, 2011) and metabolic flux analysis (MFA) measurements (Hay and Schwender, 2011; Alonso et al., 2010) have been used recently. We have taken a different approach and used metabolic control analysis. MFA is needed to quantify fluxes whereas MCA is the analysis of influences on the values of these fluxes (see Fell, 1997). There are two major approaches in MCA: top-down and bottom-up. In the former, an overall view of the metabolic pathway is obtained and this method has another advantage in that specific ways of manipulating the activity of individual enzymes are not needed. For bottom-up analysis separate steps in the pathway are altered. Ultimately, top-down analysis of a pathway (by breaking down blocks of reactions into smaller and smaller groups) will give the same information as a bottom-up study which is able to look at all the constituent reactions.

2. Control analysis applied to plant lipid metabolism

The first application of MCA to plant lipid biosynthesis (in fact, its first application to lipid biosynthesis in any organism) was when we showed the importance of acetyl-CoA carboxylase for light-stimulated lipid synthesis in leaves (Page et al., 1994). It was well-known that leaf lipid synthesis was boosted some 20-fold in the light and, under these conditions, acetyl-CoA carboxylase controls 50–65% of the total carbon flux in barley and wheat plants. This was a bottom-up MCA because we used specific inhibitors of acetyl-CoA carboxylase, the graminicides (Walker et al., 1988).

2.1. Examination of olive oil synthesis

The olive tree is a very ancient crop with over 4000 years of cultivation (Harwood and Aparicio, 2013). The oil is produced in the fleshy mesocarp of the fruit and is one of the few edible oils which is normally processed by mechanical means without the use of solvents. Although olive oil production only represents 2% of total vegetable oils, it is highly prized, as part of the 'Mediterranean diet'. The best quality extra virgin olive oils are sold at some twenty-times the price of regular kitchen oils.

Clearly for a perennial tree, it is difficult to perform rigorous biochemistry, so we elected to use tissue cultures. These were shown to mimic the developing fruits in all major aspects and to produce triacylglycerols enriched in oleic acid (Williams and Harwood, 2000). We applied top-down control analysis to the cultures using single manipulation (i.e. one variant of the experimental conditions) with exogenous oleate (Ramli et al., 2002a, 2002b) (see Fig. 1).

Calculation of the block control coefficients revealed that values for Block A were 0.57 for single manipulation (Table 1). Thus, the majority of flux control resided in the production of fatty acids and one would be advised to target these reactions in a first attempt to increase oil yields.

Because bromooctanoate was selective for diacylglycerol acyltransferase (DGAT) (within the Block B reactions), we also gained specific information about this enzyme. For olive cultures, DGAT has a flux control coefficient of 0.74 within the lipid assembly reactions and, therefore, might be a good target for over-expression (Ramli et al., 2005)

2.2. MCA in oil palm

Oil palm is the world's most productive oil crop with an oil yield per hectare of about 10-times that of oil seed crops (Gunstone et al., 2007). Recently it overtook soya bean as the world's major oil crop. Again, because palm is a tree, we used tissue cultures to study lipid biosynthesis. Once more, control experiments showed that the cultures reproduced fruit metabolism well (Ramli et al., 2002a) and gave activities at a similar level to the ripening fruits (Ramli et al., 2009). We were able to use

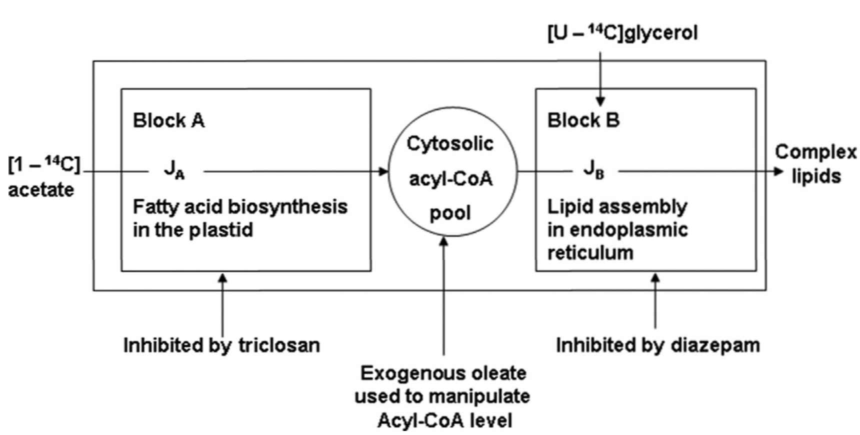

Fig. 1. The simplified system which we used for our MCA studies. [1-^{14}C] Acetate is used to specifically (> 98%) label fatty acids. Block A reactions (in the plastid) include acetyl-CoA synthase, acetyl-CoA carboxylase, fatty acid synthase reactions, stearoyl-ACP desaturase, acyl-ACP thioesterases and acyl-CoA synthase. The system intermediate is the cytosolic acyl-CoA pool. The latter will be modified by addition of polyunsaturated or very long chain fatty acids through the activity of desaturases and elongases in the endoplasmic reticulum. Block B reactions of lipid assembly include the Kennedy pathway enzymes as well as ancillary proteins such as PDAT (phospholipid:diacylglycerol acyltransferase). Radioactivity from [U-^{14}C]glycerol enters the pathway directly with < 1% labelling fatty acid chains. On the other hand complex acyl lipids are labelled indirectly from [1-^{14}C] acetate through the various acyltransferase reactions. See Ramli et al. (2002b) for further discussion of the theory behind the flux control experiments and Weselake et al. (2009) for remarks about the different reactions involved in triacylglycerol synthesis. The inhibitors shown are as used for oilseed rape. J_A and J_B represent the fluxes through each block of reactions. (Taken from Tang et al., 2012 with permission from the authors and New Phytologist.)