**Figure 1. Carbon isotope ratios correlate with phylogenetic relationships of *Kalanchoë* species.** Basal, group I Kalanchoës (Kitchingia section) have been found to possess C3-like carbon isotope signatures, whereas more derived group II and group III Kalanchoës generally have less negative 13C ratios although group II do show flexible CAM and thus large variations in 13C values. The most derived Kalanchoës in group III have the highest 13C ratios indicative of strong CAM. These species also have the most succulent leaves and inhabit the driest habitats in the south-west of their native Madgascar. This strong correlation between phylogeny and the strength of CAM physiology suggests that comparison of the genome sequences of these species will reveal genetic and epigenetic changes that correlate with strong CAM in group III species. The tree is representative only of the major groups within the genus *Kalanchoë* and was redrawn from the published *Kalanchoë* phylogeny [37].

**Figure 2. Examples of representative current and future model *Kalanchoë* species. A.** *K. gracilipes*. This species belongs to the most basal Kitchingia group within the genus (group I) and performs C3 photosynthesis in the well-watered state. Published 13C values for this species sampled from its native Madagascar indicate predominately C3 metabolism. **B**, **C**. *K. fedtschenkoi* and *K. laxiflora*. These intermediate species from group II perform CAM in their older leaves but C3 in their younger leaves. The two species shown are the main species being developed as models for CAM research, and both have genome sequencing efforts underway [5]. 13C values from wild collected material ranged from strong CAM to weak CAM (Fig. 1). Note the visible plantlets forming in the notches along the leaf margins of *K. laxiflora* at the base of the picture in C. The *K. laxiflora* plant also displays a large number of ripening seed pods held above the flowers (C). **D.** *K. millotii*, **E.** *K. beharensis* and **F.** *K. rhombopilosa.* These strong CAM species have the most succulent leaves of the species shown here. The published 13C values for wild collected material from Madagascar indicated that they rely predominantly on CAM in their native habitat (see Fig. 1 for 13C values).

**Figure 3. Transgenic experiments in *Kalanchoë fedtschenkoi* revealed that NAD-ME in the mitochondrion was the major malate decarboxylating enzyme functioning during the light phase of CAM.** Silencing of NAD-ME using an RNAi construct in transgenic *K. fedtschenkoi* diminished dark CO2 fixation, and greatly reduced light period malate decarboxylation. This indicated that decarboxylation of malate via NADP-ME in either the cytosol and/ or the chloroplast was not able to rescue the down-regulation of NAD-ME in these transgenic lines. The low flux through NADP-ME is indicated by the pale grey arrows in the diagram. Note that the CO2 liberated from malate in the mitochondrion would be refixed by Rubisco in the Calvin Benson cycle in the chloroplast but these details are absent from the diagram for clarity. Also note that several of the transporters are hypothesised rather than proven to mediate the indicated metabolite movements during CAM. The discovery that NAD-ME was the main route for malate decarboxylation in the light has focused research attention on several key questions concerning metabolite transport steps during the daily CAM cycle. Primary amongst these questions is the identity of the pyruvate transporter that exports pyruvate through the inner mitochondrial membrane following malate decarboxylation by NAD-ME. A mitochondrial pyruvate carrier (MPC) has recently been characterised from yeast, Drosophilla and human mitochondria, but the ability of this protein to export pyruvate from the mitochondrion is in doubt as it has only been demonstrated to function as a pyruvate import protein in yeast and animal mitochondria. Abbreviations: tonoplast dicarboxylate transporter (tDT), mitochondrial dicarboxylate carrier (DiC), NAD-malic enzyme (NAD-ME), NADP-malic enzyme (NADP-ME), mitochondrial pyruvate carrier (MPC), pyruvate orthophosphate dikinase (PPDK), Na+ pyruvate co-transporter (BASS2), PEP:Pi translocator (PPT), G6P:Pi translocator (GPT), chloroplast dicarboxylate transporter (DiT).