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Research article

Proteomic study of 'Moncada' mandarin buds from on- versus off-crop trees

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ABSTRACT

A proteomic analysis of buds from mandarin trees with contrasting fruit load (on- and off-crop trees) was carried out during the onset of low-temperature induction. The aim of the study was to find out more about the molecular mechanism relating to alternate bearing in *Citrus* and its relationship with flowering. The 'Moncada' variety (Clementine 'Oroval' x 'Kara' mandarin), displaying remarkable behaviour in alternate production, was used in this study. From 2D DIGE gel, 192 spots were isolated: 97 showed increased expression in the off-crop buds as compared to the on-crop buds, while 95 exhibited enhanced expression in the on-crop buds versus the off-crop buds. These spots were identified by MALDI-MS or LC–MS–MS. The largest groups of proteins up-expressed in the off-crop buds were the proteins involved in carbohydrate and amino acid metabolism, and the proteins expressed in response to stimuli such as reactive oxygen species. The largest groups of proteins up-expressed in the on-crop buds were related to primary metabolism, oxidative stress and defence responses. Depending on their function, some of these proteins can stimulate the flowering, such as fructose-bisphosphate aldolase or leucine-rich repeat transmembrane protein kinase, while others can inhibit it, such as cytochrome c oxidase subunit II. Twenty-two other proteins with unknown functions were up-expressed in the on- or off-crop buds.

1. Introduction

Many *Citrus* cultivars are known to be alternate-bearing crops, especially late-ripening mandarin cultivars. This fact has significantly adverse economic impact [1]. Alternate bearing results from a reduced flowering production in the spring preceded by a heavy on-crop year [2,3]. In general terms, the longer the fruit-bearing period, the greater this suppression of flowering, which reduces the number of summer/fall shoots, thereby decreasing the number of nodes that can bear flowers for next spring [4,5]. Several factors determine this fruit load inhibition, such as environmental conditions, cultivar, number of fruits per tree and harvesting date [4].

In order to identify genes that regulate flower initiation in *Citrus*, specific homologues of *FLOWERING LOCUS T* (*CiFT*), *SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*CsSL1*), *APETALA 1* (*CsAP1*) and *LEAFY* (*CsLFY*) have been isolated and characterised in *Citrus* [6–9]. Likewise, our group studied the molecular basis of floral regulation by fruit load in the 'Moncada' hybrid mandarin, a strong alternate-bearing variety. We proposed that promoter gene *CiFT* in leaves and buds plays a pivotal role in inhibiting flowering as a result of crop load through a repressive mechanism. Moreover, the expression of *CsAP1* and *CsLFY* in leaves seems strongly modulated by fruit load [10,11]. Nishikawa et al. also demonstrated that fruit bearing suppresses the *CiFT* expression in vegetative shoots of the satsuma mandarin [12].

Furthermore, we recently performed a complementary proteomic study on up- and down-expressed proteins upon floral induction in *Citrus* leaves, and this study also approaches the issue of how fruit presence may affect protein levels [13]. The variety used for this analysis was the same as that utilised for our previous

Abbreviations: CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulphonate; 2D DIGE, two-dimensional difference gel electrophoresis; DTT, dithiothreitol; IPG, immobilised pH gradient; LC–MS–MS, liquid chromatography coupled with tandem mass spectrometry; MALDI-MS, matrix-assisted laser desorption/ionisation-mass spectrometry; pI, isoelectric point; PMSF, phenylmethylsulphonyl fluoride; Q-TOF, quadrupole time-of-flight mass spectrometer; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

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molecular studies, namely the 'Moncada' mandarin. According to this proteomic study, during the flowering-induction period, the primary metabolism is more active in the off-crop trees than in the on-crop ones. However during this same period, the up-expressed proteins in the on-crop leaves are related more to oxidoreductase activity as compared to the off-crop leaves. Moreover, other proteins with unknown functions have been isolated in 'Moncada' leaves, which might also be related to alternate bearing and flower induction. To clearly establish the proteins related to flowering regulation, and given the important role of buds in this process, as demonstrated in previous studies [11], a differential proteomic study of buds was also required. Accordingly, the current work has researched protein expression differences between the on-crop and the off-crop 'Moncada' buds during the same period. An ontology analysis was conducted to establish the molecular functions that are usually carried out by these differential bud proteins.

2. Results

2.1. Comparative proteome analysis

The aim of this work was to research the bud differential proteome from 'Moncada' mandarin trees with contrasting crops. In this study, we identified the protein spots which are up- and down-regulated in the off-crop buds as compared to the on-crop buds. To this end, bud samples from both the off- and on-crop trees were analysed by 2DE. Gels were of high quality and presented reproducible protein patterns among the replicates of the same samples (Fig. 1).

Approximately 1,162 spots in the gel images from samples were resolved. To assess the global differences in the expression levels between the off- and the on-crop samples, gels were compared and quantified using the DeCyder™ Differential Analysis Software. Among all the proteins, 350 protein spots showed a significant quantitative differential accumulation (t-text < 0.05) between the on- and the off-crop samples. Next, 192 spots were confirmed to show a good match and sufficient volume for subsequent identification by mass spectrometry (Table 1). Among them, 97 displayed an increased expression in the off-crop samples as compared to the on-crop samples (Av ratio +), while 95 exhibited a decreased expression in the off-crop samples (Av ratio −).

2.2. Identification of differentially expressed proteins

We were able to manually excise all 192 proteins with a good match from a preparative 2DE gel to further identify 88 of them by MALDI-MS analysis and the other 104 proteins by LC–MS/MS analysis. Table 1 provides the spot number, the biological process involved for each protein together with: the putative protein name; the accession code; the organism upon which the protein was identified; the homologue in *Citrus clementina*, established by the database at www.phytozome.net (Phytozome v9.1); the homologue in *Arabidopsis thaliana*, established by the database at www.arabidopsis.org; the values for theoretical and experimental p*I* and molecular mass; the expression ratio and *p*-value; the MASCOT score; the sequence coverage and matched peptides. The Supplementary Table 1 (Table S1) provides peptide sequences and other data.

Some of these spots were identified as the same protein, which could account for the isoforms or post-translationally modified forms of these proteins (Table 1). Examples of identical proteins are: pyrophosphate-dependent phosphofructokinase alpha subunit for 223 and 225 spots; 6-phosphogluconate dehydrogenase for spots 398, 406, and 427 (Fig. S1); putative cytochrome c oxidase subunit II PS17 for spots 933, 967, 973, 998, 991, 1034, 1042, 1054, 1076, 1090, 1152, 1172, and 1214 (Fig. S1); miraculin-like protein for spots 837, 855, 890, 892, 981, and 1151; 5-methyltetrahydropteroyl triglutamate-homocysteine methyltransferase for spots 130, 144, and 149; putative thiolase for 517 and 518 spots (Fig. S1).

2.3. Classification of identified proteins

The identified proteins were classified into 10 groups according to the biological function involving each protein (Table 1): (i) primary metabolism (91 spots: 70 spots associated with photosynthesis and carbohydrate metabolism, 7 spots related to Krebs cycle, and 14 spots related to respiration); (ii) oxidoreductase activity (17 spots); (iii) stress/defence responses (12 spots); (iv) signal transduction (1 spot); (v) amino acid metabolism (25 spots); (vi) protein metabolism (18 spots); (vii) the mevalonate pathway (3 spots); (viii) flavonoid biosynthesis (1 spot); (ix) cell wall metabolism (2 spots); (x) other proteins (22 spots; the latter proteins are involved in unknown biological processes). The relative percentages of proteins in both the on-crop and the off-crop buds appear in Fig. 2.

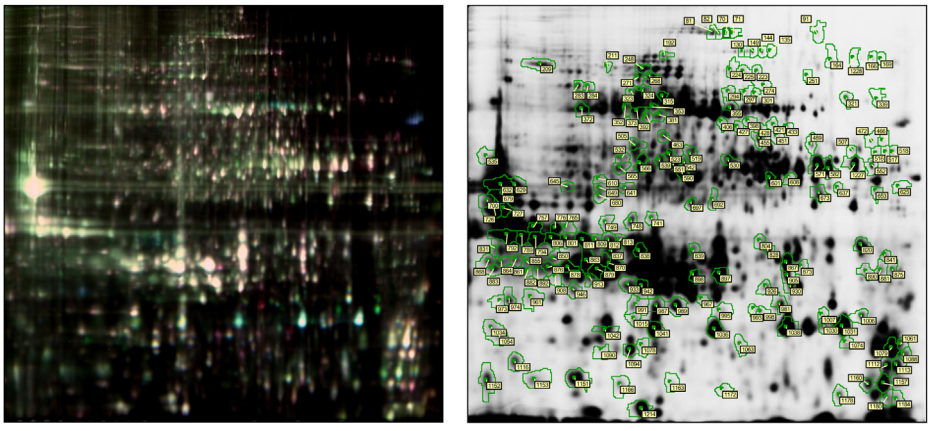

Fig. 1. A representative 2D DIGE gel of the proteins extracted from the 'Moncada' mandarin buds. Equal amounts (50 μg) of the on-crop buds sample (Cy5, red), the off-crop buds sample (Cy3, green) and the internal standard (Cy2, blue) were loaded on the same gel. (A) The proteins up-expressed in the off-crop buds appear in green, those down-expressed in the off-crop buds are shown in red, and unaffected proteins are depicted in yellow. (B) The proteins selected for the mass spectrometry analysis. Spot numbers are the same as for Table 1.