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Comprehensive elemental analysis of fruit flesh from European pear 'La France' and its giant fruit bud mutant indicates specific roles for B and Ca in fruit development

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ABSTRACT

The concentrations of macro- and microelements in fruits from European pear trees (*Pyrus communis* 'La France') were analyzed at seven developmental stages, ranging from two weeks after flowering to ripe fruits. Additionally, wild-type fruits (WT) were compared to a giant fruit bud mutant (GLaF) which sets 1.5 times bigger fruits compared to WT. With proceeding fruit development element concentrations relative to the dry biomass decreased. Differences in the rate and the degree of element concentration reduction were detected. Concentrations of B, K and Na were found to decrease at a slower rate and to a lesser degree compared to other analyzed elements. In comparison to WT fruits, most elements were detected at a lower concentration in GLaF fruits in early stage samples. During GLaF fruit development most element concentrations recovered, with the exception of B and Ca. Our results highlight common and differential element accumulation patterns in developing pear fruits. We suggest that B, K and Ca play key roles in pear fruit development and propose specific homeostasis mechanisms for these elements. Since GLaF fruits are more susceptible to corky spot disorder and B and Ca are important for the proper formation of the cell wall, we suggest that local B and/or Ca deficiency is responsible for corky spot disorder found in GLaF fruits.

1. Introduction

Fruit bearing trees provide an important contribution to the human diet. Among these, the most important species in terms of the harvested amount are apple (75.5 million tonnes) and pear (24 million tonnes) (www.faostat.org, data from 2011). For human nutrition fruits can be a source of calories and beneficial compounds (vitamins, antioxidants) as well as essential chemical elements. From a plant perspective, fruit tissues are metabolically active tissues, so it can be expected that plants exert some level of control over ion homeostasis in fruit tissues, as they do in other tissues. The concentrations of essential elements in living plant tissues are usually kept within a range that provokes neither deficiency nor toxicity symptoms. However, most fruits undergo drastic changes of fresh and dry weight, morphology and anatomy during development. In addition, the massive sugar accumulation in most fruits changes the osmotic conditions which in turn can affect mobility of bulk elements, such Ca or K. Also the homeostasis of micronutrients such as B, Cu, Fe, Mn or Zn is critical for reliable production of marketable fruits (Wojcik et al., 2008; El-Jendoubi et al., 2011).

We found a spontaneous branch mutant (bud mutant or sport) of European pear (*Pyrus communis* 'La France') in an orchard in Yamagata prefecture, Japan (Isuzugawa et al., 2014; Nashima et al., 2013a). The mutant branch, named 'Giant La France' (GLaF), sets *ca.* 1.5 times heavier fruits compared to wild-type (WT) branches, while the fruit sugar concentrations are comparable between WT and mutant fruits. *P. communis* 'La France' is diploid and consequently the ploidy level of its fruit flesh is 2C. Interestingly, although the ploidy of GLaF leaves is 2C, the ploidy level of its fruit flesh is 4C (Nashima et al., 2013a). Among others genes, cell cycle-related genes are differentially expressed in WT and GLaF fruits (Nashima et al., 2013b). Like other giant fruit mutants, also GLaF fruits suffer from bitter pit disorder, also called corky spot disorder (Isuzugawa

Abbreviations: GLaF, Giant 'La France'.

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et al., 2014; Raese, 1989). The reason for this might be the tendency of giant fruit mutants to have lower concentration of solutes and nutrients than corresponding WT fruits. Corky spot disorder is associated with mineral imbalances. Especially B and Ca deficiency was found to be associated with the occurrence of corky spots, but variable results have been obtained (Ferguson and Triggs, 1990; Ferguson and Watkins, 1989; Brun et al., 1985; Mason and Welsh, 1970).

The aim of this study was to analyze the ionome of pear fruits, including all essential macro- and micronutrients (except N), in a time-course series covering all principal developmental stages. Additionally, a comparative analysis of WT and GLaF fruits was performed to further characterize the physiology of this giant fruit bud mutant.

2. Materials and methods

2.1. Plant material

Pear trees of the commercial variant *P. communis* 'La France' were grown at Kaminoyama in Yamagata Prefecture in northern Japan according to local horticultural practice. WT and GLaF fruits were from different branches of the same tree. Fruit thinning was performed to leave one fruit per spur for each genotype. Fruits were sampled in 2010 at 20 May (0.5 month after flowering (MAF)), 4 June (1 MAF), 12 July (2 MAF), 10 August (3 MAF), 14 September (4 MAF), 14 October (5 MAF), 15 November (6 MAF) and in 2011 at 19 May (0.5 MAF), 6 June (1 MAF), 12 July (2 MAF), 10 August (3 MAF), 9 September (4 MAF), 14 October (5 MAF) and 9 November (6 MAF). For the 6 MAF samples fruits were harvest at 5 MAF and further incubated at 2 °C for 12 days and then at room temperature (15–20 °C) for 20 days for ripening. Fruit flesh tissues (receptacle) for elemental analysis were frozen in liquid N_2 immediately after harvesting and removal of the peel and core tissues (except for 2010/0.5 MAF samples where whole fruits were used and 2010/1 MAF samples where only the core was removed). For each developmental stage three replicate samples were prepared, each consisting of pooled tissues from ten (0.5 MAF) or five (1 MAF to 6 MAF) individual fruits each. After freezing, the samples were powdered using different mills (Automill TK-AM5-S, Tokken Inc., Kashiwa, Japan; Sample Mill SK-M2, Kyoritsu Riko Inc., Tokyo, Japan; or Multi-Blender Mill BLA-501, Nissei Inc., Tokyo, Japan), depending on the sample size.

2.2. Multi-element analysis

The concentrations of Ag, Al, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sr, Tl, V and Zn were analyzed. Frozen tissues were dried at 70 °C for two days and the dry weight was determined. Subsequently, samples were completely mineralized in 15 ml polypropylene tubes (Trueline, Nippon Genetics, Tokyo, Japan) by covering them with 3 ml 65% (v/v) HNO_3, incubation for two days at RT, followed by 3 h at 95 °C. After cooling down to RT 1.5 ml 30% (v/v) H_2O_2 was added and the temperature was increased to 70 °C again for 1 h. Before analysis each sample was filled up to a final volume of 10 ml with ultrapure water. Element concentrations were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) performed on an ICAP Duo 6500 system (Thermo Scientific, Yokohama, Japan). Samples were supplied to the plasma as an aerosol in Ar gas by a concentric nebulizer. The system was calibrated using Multi-element standard solution 5 for ICP (Sigma–Aldrich, St. Louis, MO, USA), with B manually added from a certified stock solution.

In some samples from 0.5 MAF and 1 MAF, regardless of genotype or year, very high concentrations of Co, Cr, Fe, Mn, Mo, Ni and V were detected. Based on not dissolvable residues exclusively in those samples we assumed this to be a contamination from the tools used for harvesting or homogenization and excluded these samples from further analyses. Consequently, results are based on fewer replicate for Fe, Mo and Mn and no error bars are shown in Fig. 4 for some samples.

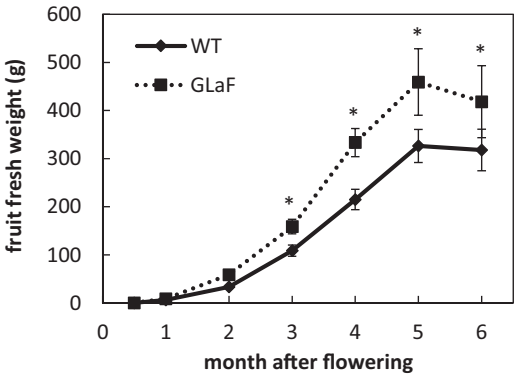

Fig. 1. Fruit fresh weight of developing WT pear fruits and a giant fruit bud mutant. Data-points show arithmetic means ± SD of WT and a giant fruit bud mutant (GLaF) developing fruits from the 2011 season (n = 11–20). Significant differences were calculated by a two-way ANOVA followed by Tukey's HSD test. Asterisks indicate $P < 0.001$.

2.3. Statistical analysis

Statistical analyses were performed using R. Non-linear regression was performed using the "nls" function and the formula $y = mx^b$, where y is element concentration and x is MAF. Two- and three-way ANOVA was performed using the "aov" function. Multiple comparisons of means were performed by the "TukeyHSD" function.

3. Results

3.1. Fresh weight of WT and GLaF fruits

The fresh weight of whole pear fruits (European pear, *P. communis* 'La France') from a giant fruit bud mutant branch (GLaF) and from WT branches was determined (Fig. 1). Fresh weight of GLaF fruits was higher than that of WT fruits at all analyzed developmental stages. From 3 MAF to 6 MAF fresh weight of WT and GLaF fruits showed a significant difference ($P > 0.001$). Between 0.5 MAF and 1 MAF fruit fresh weight approximately increased by a factor of 20 in both, WT and GLaF fruits. With proceeding fruit development the growth rate decreased and maximum fresh weight was reached 5 MAF, with 326 g for WT fruits and 459 g for GLaF fruits. After harvest and off-tree storage for ripening 6 MAF the fresh weight decreased slightly to 318 g for WT fruits and 418 g for GLaF fruits.

3.2. Element concentrations in WT fruits

We analyzed concentrations of 27 essential and nonessential elements commonly found in plants in the fruit flesh of developing pear fruits relative to the dry biomass. Of the 27 elements, the concentrations of Ag, Al, Be, Co, Ga, In, Pb and Tl and V were below the detection limit of our system in some samples, particular in those from later stage fruits. We were able to quantify the macroelements Ca, K, Mg, P and S (Fig. 2), the microelements B, Cu, Fe, Na, Mn, Mo and Zn (Fig. 3) and the non-essential elements Ba, Cd, Cr, Ni, Rb and Sr. The complete dataset can be found in Supplemental Table 1.

Supplementary Table 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2014.07.019.