

Whole genome expression and hydraulic architecture of diploid and doubled diploid citrus seedlings in intra and interspecific contexts

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SUMMARY

Genome expression in polyploid is usually considered to be quite different depending on the genetic context. While it is admitted that autotetraploids resulting from polyploidization events within species do not present major changes of genes expression, allotetraploid which result from hybridization between divergent species or lines were showed to present very important changes of expression compared to the diploid (2x) parental lines (genomic shock). In this work, we investigated the root citrus genome expression using custom Agilent Citrus microarray (4x44K format) of different couples of 2x and doubled diploid (4x) in absence of stress: *Poncirus*, Cleopatra Mandarin as well in the Cleopatra Mandarin × *Poncirus* intergeneric hybrid. We also evaluated the plant hydraulic architecture of this plant material. Our goal was to evaluate if any change in root genome expression related to polyploidy could lead to plant hydraulic architecture changes.

Index terms: citrus, polyploidy, root.

Expressão genômica e arquitetura hidráulica de plântulas diplóides e duplo diplóides de *Citrus* intra e interspecíficos

RESUMO

A expressão do genoma em poliploides geralmente é considerada como sendo bastante diferente, dependendo do contexto genético. Embora seja admitido que os autotetraploides resultantes de eventos de poliploidização dentro de espécies não apresentam grandes mudanças de expressão de genes, todos os alotetraploides resultantes da hibridação entre espécies divergentes ou linhas apresentaram mudanças de expressão muito importantes em comparação com as linhas parentais diploides (2x) parentais (genomic shock). Neste trabalho, investigamos a expressão do genoma de raiz de citros usando microarranjos, plataforma Agilent personalizada para Citrus (formato 4x44K) de diferentes duplas de 2x e duplos diploides (4x) na ausência de estresse: *Poncirus*, tangerina

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Cleópatra, e também híbrido intergenérico de Cleópatra × *Poncirus*. Também avaliamos a arquitetura hidráulica da planta. A meta foi avaliar se qualquer alteração na expressão do genoma de raiz relacionada à poliploidia poderia levar a alterações na arquitetura hidráulica da planta.

Termos de indexação: citrus, poliploide, raiz.

INTRODUCTION

Tetraploids may be allopolyploids or autotetraploid and may result either from sexual reproduction via $2n$ gametes or by duplication of the entire nucellar chromosome set. Allopolyploids inherit different subgenomes after interspecific hybridization whereas subgenomes of autotetraploid arise from the same species. Currently, genome expression changes in allotetraploids are considered to be more strongly affected by genome hybridization than by genome ploidy changes. Thus, interactions among genomes are considered to play an important role in the development of new phenotypes in allopolyploids. Studies aimed at identifying changes in genome expression pattern of autotetraploid are less numerous than for allotetraploids. Investigation in potato, using microarrays showed that gene expression changes were very limited (Stupar et al., 2007). These results suggest that doubled diploids, that derive all of their alleles from a single species, may undergo fewer alterations in their regulatory networks, resulting in very limited changes of gene expression between diploid and doubled diploid genotypes (Stupar et al., 2007). Genome doubling can produce autotetraploid (with intraspecific diploid) but also allotetraploid (from interspecific diploid). Gene expression changes observed between diploid and doubled diploid genotypes could be attributed to nuclear dosage and ploidy-driven cellular modifications leading to physiological changes such as cell size, division rate, or organellar composition but also to modifications of instable regulation network or epigenetic marks in interspecific hybrids. In addition, the genome doubling confers better adaptability to various environmental stresses included a higher tolerance to salinity (Saleh et al., 2008; Mouhaya et al., 2010; Ruiz et al., 2016) and water deficit (Allario et al., 2013). In this work, using microarrays we investigated the genome expression in root of diploids Trifoliolate Orange, Cleopatra mandarin, Intergeneric hybrid Cleopatra mandarin × Trifoliolate orange and their respective doubled diploid. Also we investigated the hydraulic architecture

of these plants. Our goal was to verify if the phenotypic differentiation due to polyploidy could lead to changes of the plant hydraulic architecture.

MATERIALS AND METHODS

Plant material

Plant material: Seeds from diploid ($2x$) Trifoliolate orange (*Poncirus trifoliata*) and Cleopatra mandarin (*Citrus reshni*), $2x$ intergeneric hybrid Cleopatra mandarin × Trifoliolate orange (*Citrus reshni* × *Poncirus trifoliata*) and their respective $4x$ plants were collected from trees of the INRA/CIRAD germplasm collection of San Giuliano, France. Seeds were planted in substrate (sand, turf, and peat, 1:1:1 by vol.) in a greenhouse for one year. The ploidy status of $2x$ and $4x$ plants was checked and confirmed by flow cytometry (Partec I).

Genome expression

When plants were 18 months old, secondary root samples were harvested and RNA was extracted. Four independent biological replicates were harvested at the same time. Samples were stored at -80°C and total RNA purification and a quality assessment were performed as previously described by Brumos et al. (2009). Gene expression profiles in root were generated by using custom Agilent Citrus microarray ($4x44\text{K}$ format). Labeling and hybridizing each sample to 3 of 4 separate microarrays. Agilent's microarrays include the Spike-In Kit that consists of a set of 10 positive control transcripts optimized to anneal to complementary probes on the microarray, minimizing self-hybridization or cross-hybridization. The concentrated Agilent One Color RNA Spike-Mix stock was diluted with buffer provided with the kit. Slides were scanned in an Agilent Microarray Scanner (G2565BA) according to the manufacturer's protocol.

Signal data were collected and analyzed with dedicated Genspring Software following the Agilent protocol.

Hydraulic architecture

Using a HPFM (High Pressure Flow Meter) Carbon efficiency of root (CERT), Specific conductances ($K_{\text{total leaf}}^1$, $K_{\text{total cross sectional}}^1$ are, $K_{\text{root leaf}}^1$) were measured in the different genotypes except 2x Cleopatra mandarin that was not included in the experimental design. Measurements were taken at ambient temperature according to Tyree et al. (1995). The stem was cut at 3 cm above the soil surface, the stump was connected to the HPFM with a water-tight seal.

Statistical analyses

Hydraulic data are expressed as means \pm SE and were analysed using SigmaPlot version 12, from Systat Software, Inc., San Jose California USA). T test was applied to detect statistical difference between 2x and 4x belonging to the same genotype (t-test, $P \leq 0.05$).

RESULTS AND DISCUSSION

Root genome expression

Root gene expression was investigated by comparing 2x and 4x couples. Just a few genes presented a significant differential expression between 2x and 4x Trifoliolate Orange (data not showed). In Cleopatra, mandarin very limited number of genes ($< 0.1\%$) presented a gene expression change > 2 fold (Figure 1). These results suggest that in these doubled diploid derive all of their alleles from a single species and may undergo fewer alterations in their regulatory networks, resulting in very limited changes of gene expression between 4x and diploid genotypes as previously proposed by Stupar et al. (2007). However, large changes of expression were observed when comparing the 2x intergeneric hybrid Cleopatra mandarin \times Trifoliolate orange and its respective 4x, 2826 genes being over expressed and 2378 gene being down-regulated (expression change > 2 fold). This suggests that this doubled diploid resulting from an intergeneric cross, behaves as an allotetraploid regarding it genome expression. Those changes in gene expression are potentially advantageous in allopolyploids because an increase in heterozygosity may be at the origin

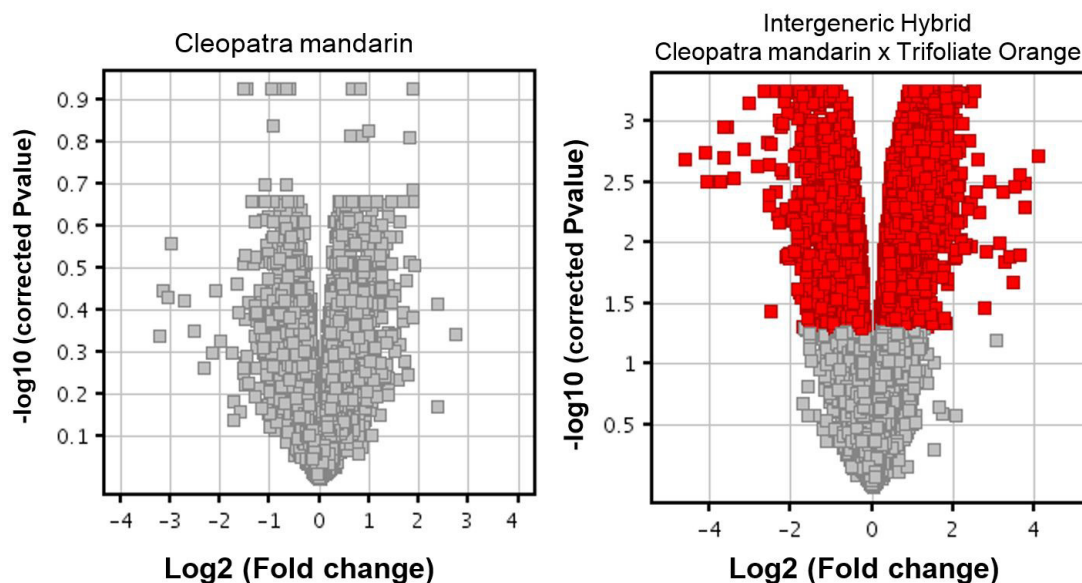


Figure 1. Comparison of gene expression in 2x versus 4x genotypes. Analysis was performed using Genspring software. A few genes presented a significant differential expression between 2x and 4x Trifoliolate Orange (data not showed). In Cleopatra, mandarin very limited number of genes ($< 0.1\%$) presented a gene expression change > 2 fold. Large changes of expression were observed when comparing the 2x intergeneric hybrid Cleopatra mandarin \times Trifoliolate orange and its.

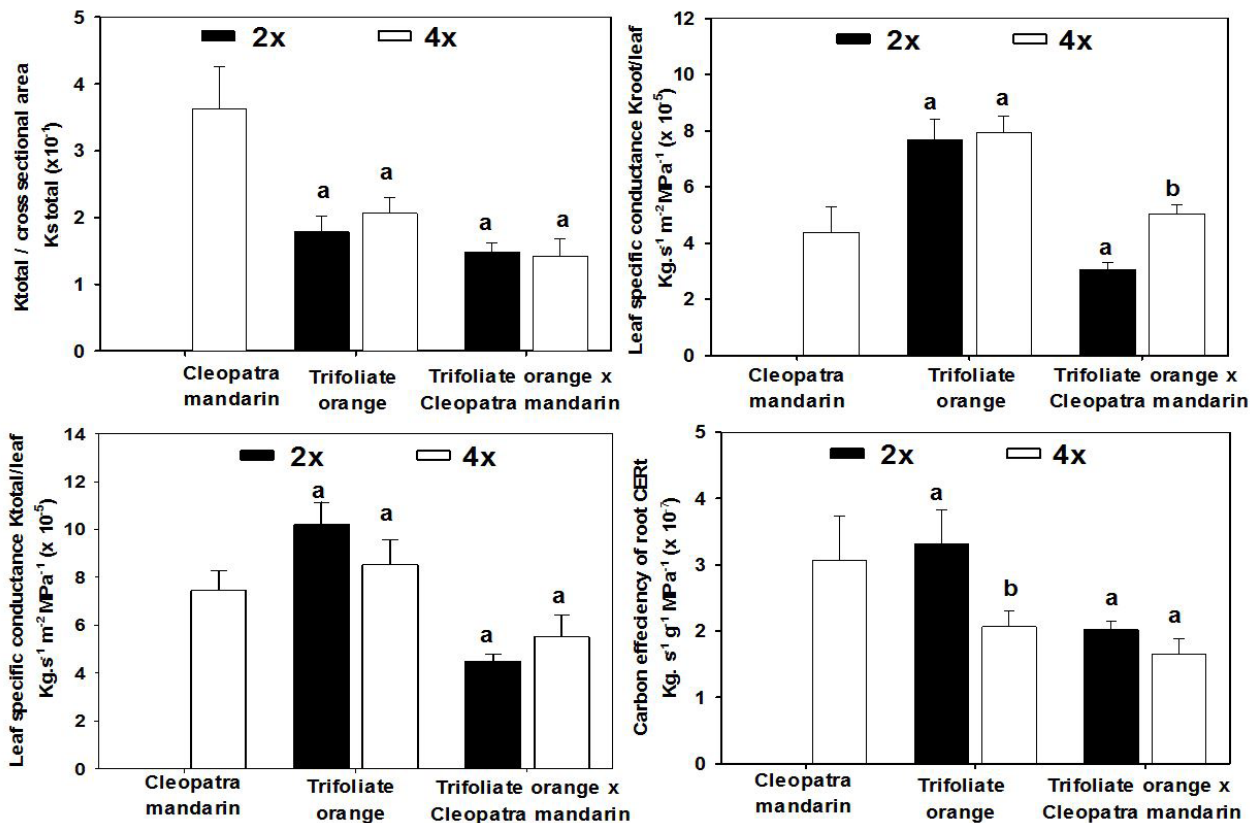


Figure 2. Specific conductances (K_{total} cross sectional⁻¹, Leaf specific conductance (K_{root} leaf⁻¹, K_{total} leaf⁻¹) and $CERT_r$ were measured in the different genotypes. Different letters indicate a statistical difference between 2x and 4x belonging to the same genotype (t-test, $P \leq 0.05$).

of subfunctionalization (Adams et al., 2003). Thus, changes in gene expression observed between the 2x intergeneric hybrid Cleopatra mandarin \times Trifoliolate orange and its respective 4x may be the result of genome hybridization rather than changes in genome ploidy (Chen, 2010).

Hydraulic architecture

Different hydraulic parameters of plant architecture were investigated such as the carbon efficiency of root ($CERT_r$) and specific conductances (K_{total} leaf⁻¹, K_{total} cross sectional⁻¹ are, K_{root} leaf⁻¹) were investigated. Unfortunately 2x Cleopatra mandarin were not available in the experimental design, and comparisons between the three 2x and 4x couples were not possible. Results did not reveal large changes between 2x and 4x genotypes, except $CERT_r$ between 2x and 4x Trifoliolate orange and K_{root} leaf⁻¹ between 2x and 4x hybrid Cleopatra mandarin \times Trifoliolate orange (Figure 2). These results suggest that tetraploidy do

not induces large changes in hydraulic architecture in absence of stress. Since doubled diploid were showed to present greater traits of adaption to stress, it would be necessary to do such physiological measurement under stress conditions to decipher the impact of polyploidy.

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