## Characterization of the tuber metabolic differentiation between two homologically related Cyperaceae species

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Abstract: **[Background]** Yellow nutsedge (*Cyperus esculentus*, YN) and purple nutsedge (*Cyperus* rotundus, PN) are homologically related Cyperaceae species which both rely on the underground tubers for energy reservation and propagation. However, the tuber components between YN and PN show great distinction. PN is rich in secondary metabolites such as monoterpenes, sesquiterpenes and their oxides, whereas YN mainly contains lipids and carbohydrates. Such a dramatic metabolic differentiation therefore indicates it might be meaningful to characterize the underlying mechanisms within, which may provide critical insights for understanding the biogenesis of the sedge plant family, a traditional weed but with outstanding application values in the agricultural production. **(Objective)** (1) Dynamic analysis of the major carbon anabolic products in YN and PN tubers over the whole growth duration. (2) Identifying the most obvious physiological node at which the metabolite flux starts to differentiate. (3) Establishment of the metabolic network which exclusively reflects the differences in gene expression and metabolic flux distribution in the tuber carbon metabolism between the two Cyperaceae species. [ Method ] The joint omics analysis including transcriptomics and metabolomics were utilized as the dominant tool to investigate the metabolic difference between YN and PN tubers. Several tuber-specific compounds like lipid, phenols, esters, flavonoids, amino acids etc. have been qualified and quantified via the chromatography dependent technologies. Measurement of regular carbohydrates (e.g. starch, amylose, soluble sugars), as well as the gene expression verification were carried out through the kits. **[Results]** (1) Three developmental stages have been divided to reveal the tuber component variation in YN and PN, of which the tuber developing stage was found to be the most active phase for metabolites accumulation and differentiation. (2) Both the total starch and total lipid contents tended to decrease first then increased later in YN and PN over the three divided developmental stages, with the total lipid displaying almost 10-fold higher level in YN. In comparison, the total sugar content showed a continuously reducing trend from the tuber initiation stage. Amylose and resistant starch were analyzed in the mature tubers, PN had a higher ratio than YN in both but did not reflect significant changes in the pasting property and starch morphology. (3) Joint omics analysis was implemented at the tuber developing stage. By using the Arabidopsis genome as the reference template for gene functional annotation, a total of 22,349 differentially expressed genes were identified, and there were 102 differentially accumulated metabolites detected in the metabolomics. (4) Multiple transcriptional factors involved in the central carbon metabolic network, such as WRI1 (WRINKLED1) and FUS3 (FUSCA3), which displayed significantly differentiated expression levels between YN and PN, as well as

some important rate-limiting genes including AGPase (ADP-glucose pyrophosphorylase), DGAT (diacylglycerol acyltransferase), PDAT (phosphatidiotylglycerol acyltransferase), LDAP (lipid-droplet associated proteins) and ALDHs (aldehyde dehydrogenases) etc. were found to be the key regulators that may determine the metabolite flow redistribution in tubers according to the metabolic model established based on the omics results. **[Conclusions]** Our study has revealed that the metabolic difference between YN and PN in terms of the tuber components, to a large extent, could be attributed to the highly differentiated expression levels of the key transcriptional factors and structural genes identified in the tuber developing stage. As a result, the carbon repartitioning formed afterwards could profoundly impact the discrepant tuber phenotype. These findings may help the future research to better elucidate the genetics in sedge plants on the molecular level, which could also give applicable information for the genetic engineering improvement in other crops.

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