Metabolomics of Oil Palm Sap: Extraction Methods & K rate effect

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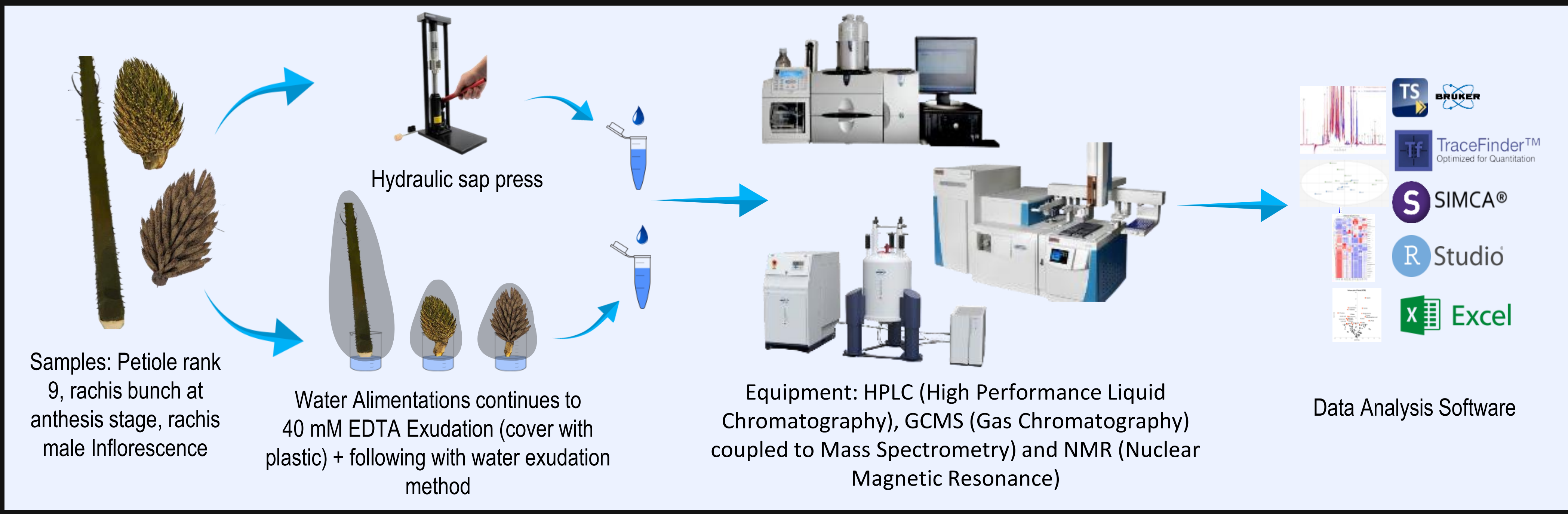
Introduction

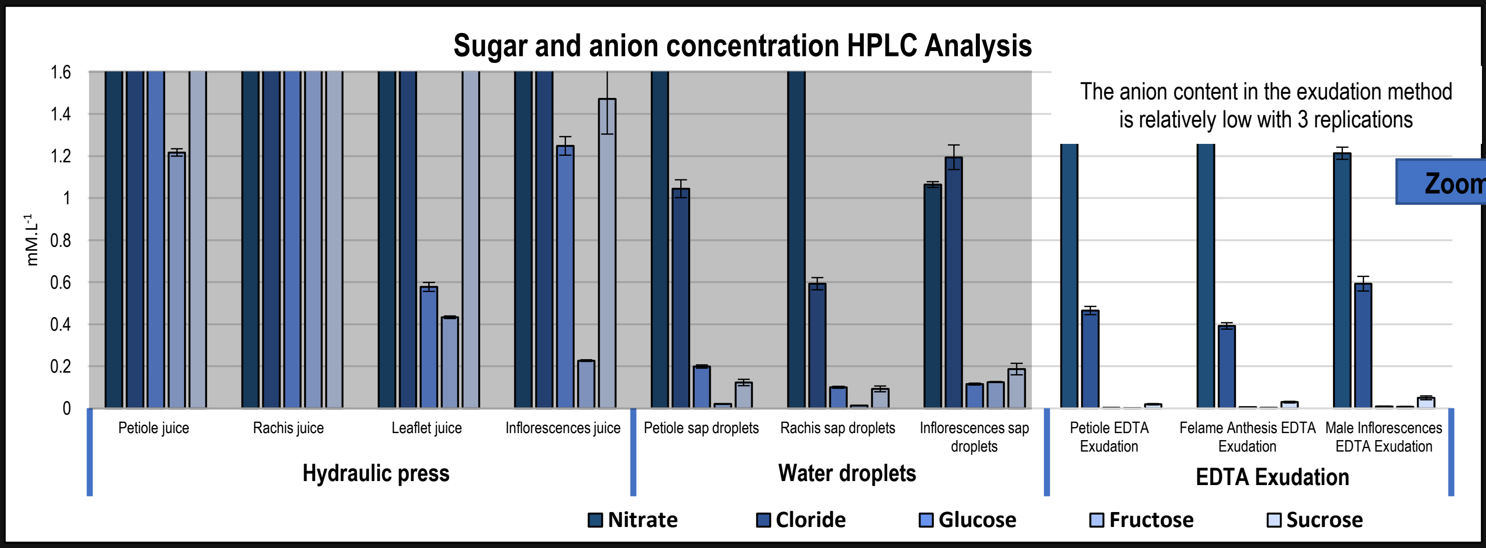
Oil palm sap has been less studied compared to other species like *Ricinus*, *Lupinus*, and various crops due to its unique monocot xylem and phloem structure and the small size of its vessels. However, understanding its mineral and metabolic composition is important for investigating nutrient and metabolite transport within the tree. Potassium plays a key role in sucrose translocation from leaves to fruits and in glucose mobilization from starch storage in different organs. Recent metabolomic studies have demonstrated that potential of advanced biotechnology in oil palm research, particularly in analysing potassium impact on leaf and fruit metabolism. This study aims to determine the best method for detecting metabolite content in pure phloem sap.

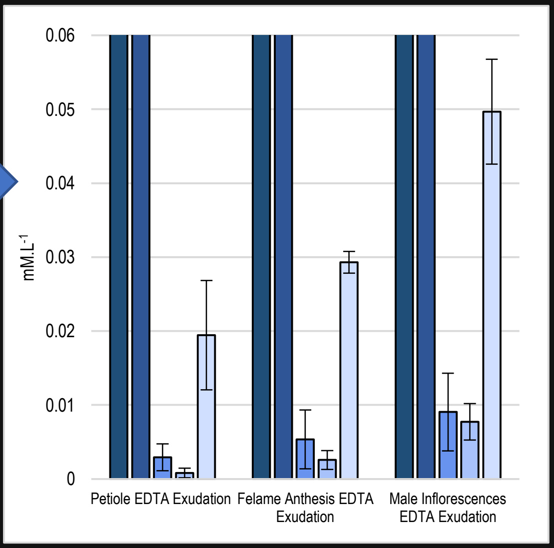
Methods

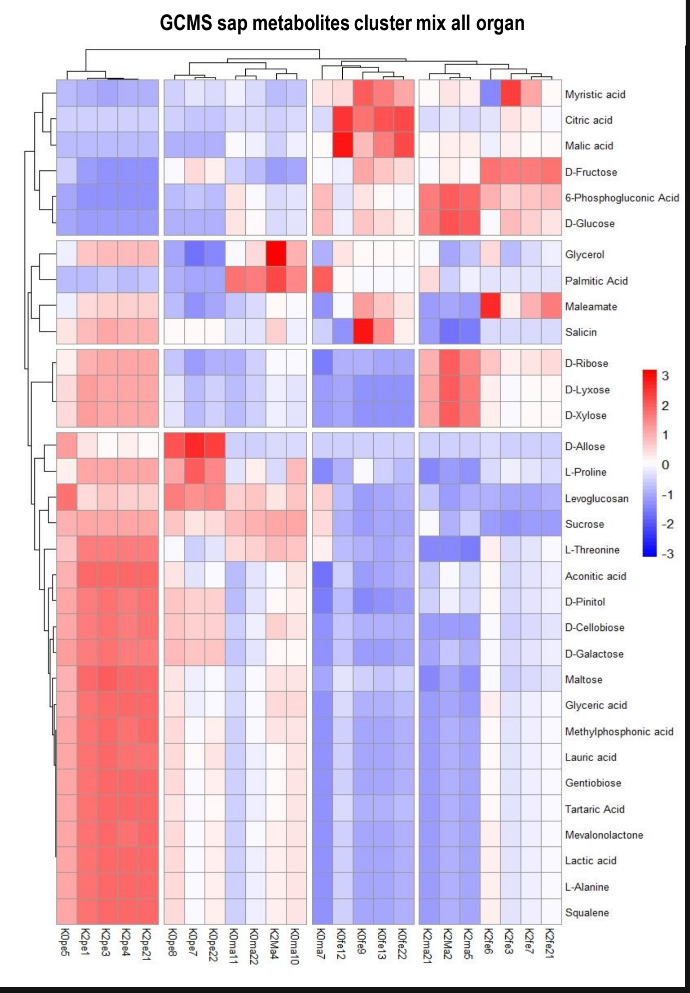
This study examined the effects of KCl (Muriate of potash) fertilizer on mature Dura x Pisifera palm tree (planting year 2016). Two potassium levels were tested: no K fertilizer and 100% standard K rate. Three sap extraction methods were tested: water, EDTA, and hydraulic press from petiole, stalk of female anthesis and male inflorescence. Metabolite analysis was conducted using HPLC, GC-MS, and NMR, with five replicates.

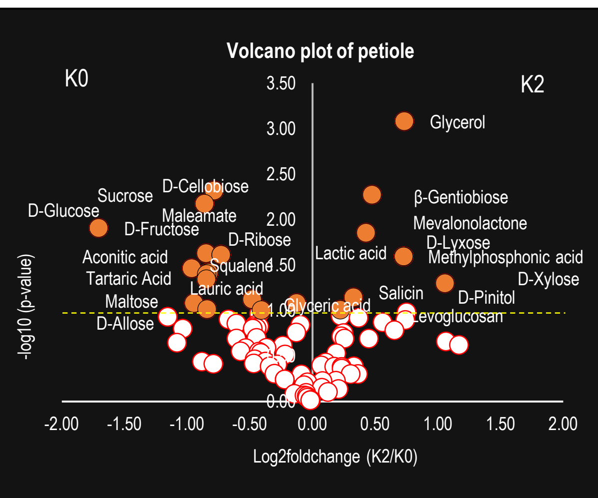


Results









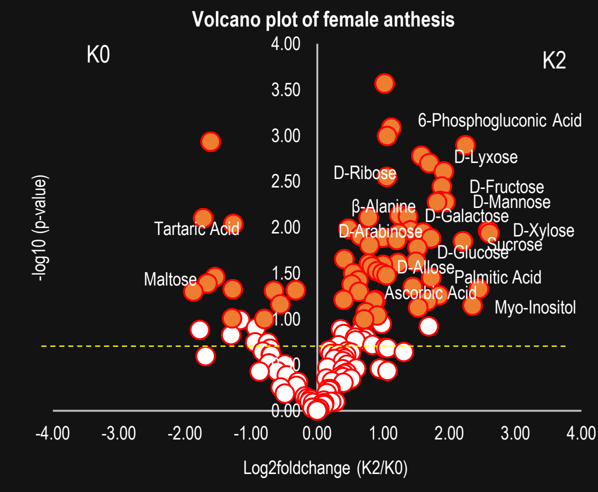
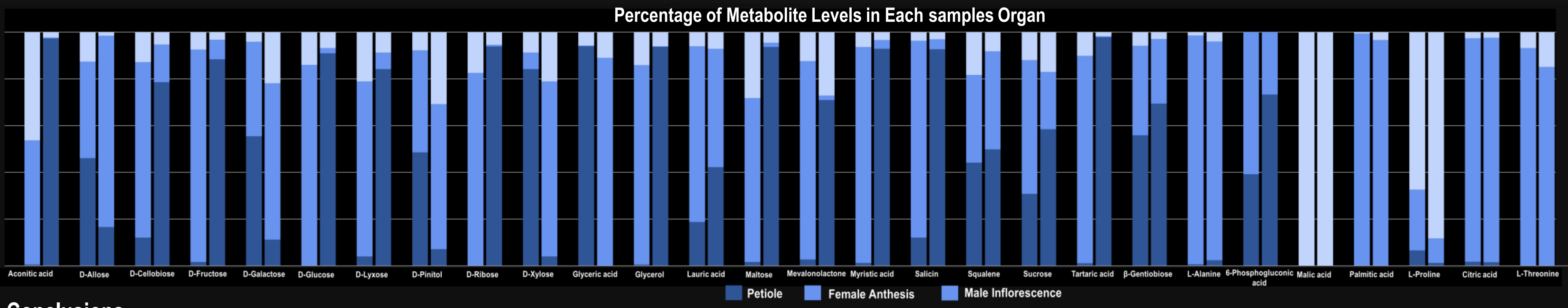


Table of metabolites content in potassium fertilizer and without potassium fertilizer treatment

Metabolites	Potassium Fertilizer (ppm.kg⁻¹)	Without potassium Fertilizer (ppm.kg⁻¹)	p Value	Group
Aconitic acid	1.8796	1.0340	9.12	OA
D-Allose	9.7501	13.7604	7.72	Carb
D-Cellobiose	2.3686	2.4554	10.16	Carb
D-Fructose	1521.1176	703.3105	5.56	Carb
D-Galactose	0.9907	1.9371	12.22	Carb
D-Glucose	795.8777	260.6382	8.69	Carb
D-Lyxose	18.2929	2.7116	13.71	Carb
D-Pinitol	0.8274	3.3682	9.88	Carb
D-Ribose	17.4183	4.6422	11.25	Carb
D-Xylose	2.7116	18.2929	14.10	Carb
Glyceric acid	1.6111	1.5827	3.34	OA
Glycerol	13.0381	9.4851	5.54	Flav
Lauric acid	0.4366	0.1689	12.55	FA
Maltose	1.2386	3.4104	10.21	Carb
Mevalonolactone	0.1207	0.0681	2.21	FA
Myristic acid	229.0334	109.0670	7.89	FA
Salicin	14.2258	19.0261	8.81	Flav
Squalene	0.0045	0.0128	6.89	FA
Sucrose	930.2498	441.2315	7.88	Carb
Tartaric Acid	0.2638	0.5776	5.56	OA
β-Gentiobiose	0.1011	0.1355	5.49	Carb
L-Alanine	470.0368	130.5592	14.11	AA
6-Phosphogluconic Acid	6.3209	8.6567	3.38	Carb
Malic acid	63.3790	11.0068	5.54	OA
Palmitic Acid	558.7590	408.9869	2.27	FA
L-Proline	15.3775	45.8373	5.58	AA
Citric acid	409.2729	408.0364	9.12	OA
L-Threonine	4.1194	2.7091	5.56	AA

The content of metabolites was obtained from metabolite accumulation in the following: petiole, female anthesis, and male inflorescence, for each treatment calibration with ribitol.



Conclusions

1. **Sap Extraction:** The EDTA method demonstrated the highest efficacy in extracting phloem sap with minimal xylem contamination, evidenced by low nitrate levels.
2. **Sugar Distribution:**
   High sugar concentrations, particularly sucrose, were observed in stalk bunches (Female anthesis), indicating their vital role in fruit filling.
   Under potassium (K) deficiency, sugar accumulation was observed in petioles.
3. **K Role in Sugar Transport:** These findings suggest that K plays a crucial role in facilitating sugar transport from source tissues (leaves) to important sinks (fruits).
4. **K Deficiency Implications:** When K is deficient, glucose may accumulate in petioles or even trunks, potentially inhibiting their further remobilization for fruit development and ultimately impacting yield
5. **Metabolite Groups:** The impact of K fertilizer treatment indicates that the metabolite groups that demonstrate involvement consist of carbohydrates, which include 13 metabolites with the highest concentration, such as sucrose, glucose, fructose, followed by lipids (5 metabolites), organic acids (5 metabolites), amino acids (3 metabolites), and phenolic groups (2 metabolites).