Comparisons of metabolomes of *Quercus serrata* and *Quercus mongolica* by altitude along Hwaeumsa valley in Mt.Jiri

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ABSTRACT

There are many environmental stresses in nature, and plants produce metabolomes under various stimuli from these stresses. Since the environmental conditions differ depending on the altitude, we would like to identify metabolomes produced by plants at various altitudes and analyze the difference of amounts. Metabolomes were extracted from leaves of *Q. serrata* and *Q. mongolica* and were analized using GC-MS. PCA and OPLS-DA were used to analyze metabolomes differences in each plant group. Through the analysis results, it was possible to determine which metabolites were produced and difference in amounts according to the species and altitude. Especially, by analyzing the function of metabolomes, it was found that *Q. mongolica* growing at high altitude is subject to a large amount of oxidative stress, *and Q. mongolica* growing at low altitude is subject to a large amount of disease stress.

INTRODUCTION

- Plants adapt to environments and face various environmental stresses(dry, temperature, salinity, etc.) while living. When exposed to these stresses, tissues can be damaged. So, plants produce metabolomes to overcome the stress. Metabolomes perform functions such as signal transport, protein translation and maintenance of homeostasis depending on the type. Examples of typical metabolites include vitamins, amino acids, nucleotides, etc.

METHODS & MATERIALS

Table1. Sample list of *Q. serrata* and *Q. mongolica*. Groups by altitude were divided into data from the Ministry of the Environment (김광임, 1996). All of samples were collected on 30 August 2021

Species	Altitude(m)	Sample size
Q. serrata	0~600	13
Q. serrata	600~1000	4
Q. mongolica	0~600	5
Q. mongolica	600~1000	5
Q. mongolica	1000~1300	9

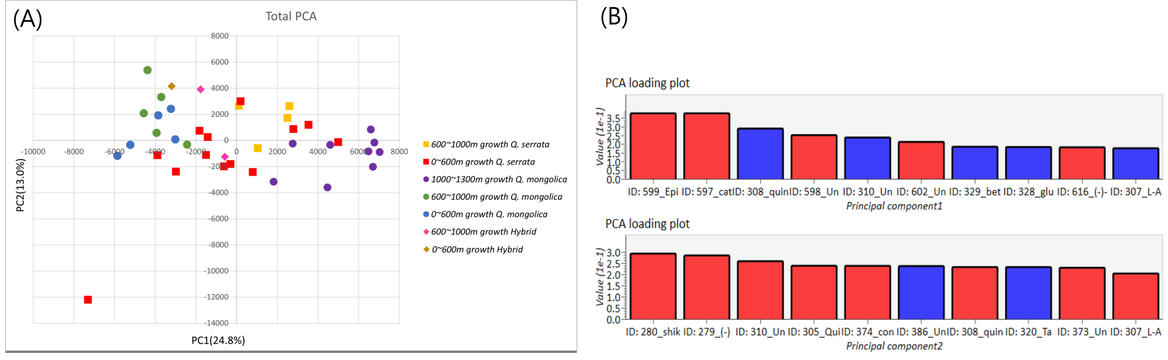

Fig.1. Workflow of this study.

1. Plant metabolites extraction (modified with Lisec *et al.*, 2006)
① Extraction of metabolites with methanol after comminution the samples.
② Separation using non-polar chloroform and polar water.
③ Addition of pyridinic methoxyamine to stabilise carbonyl moieties.
④ Derivatisation by MSTFA.

2. Metabolomes detection using

3. Data alignment using MS-DIAL (Tsugawa *et al.*, 2015)
Alignment of multiple samples, normalization based on internal standard D-sorbitol-1-^{13}C- and statistical analysis Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA).

4. Data processing using R program
Using packages.dplyr (Sorting), ggplot2 (Visualization)

RESULTS

- PCA was run on all samples to confirm associations between the data.

Fig. 2. (A) Scatterplot of all samples analyzed with MS-DIAL. (B) Loading plot of Principal component1 and Principal component2.

- Since no association was observed, PCA was performed for each species.

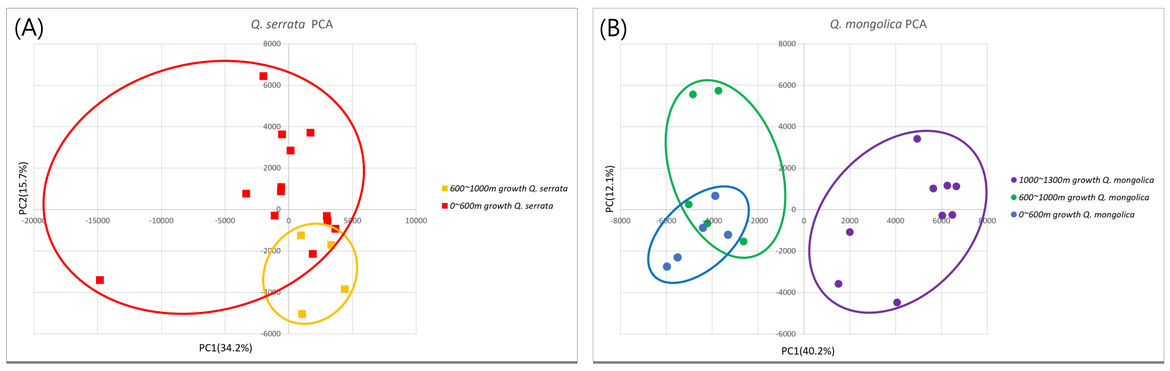

Fig. 3. (A) Scatterplot of *Q. serrata* analyzed with MS-DIAL. (B) Scatterplot of *Q. mongolica* analyzed with MS-DIAL.

Fig. 4. (A) *Q. serrata* analyzied by OPLS-DA. (B) *Q. serrata* analyzied by S-plot. The material with the lowest P correlation and P loading values is beta-D-(+)-glucose and the material with the highest values is unknown (retention time: 9.811min). (C) Beta-(+)-glucose's amounts of *Q. serrata*(*Q. serrata*_1's altitude is 0~600m and *Q. serrata*_2's altitude is 600~1000m). (D) Unknown's amounts of *Q. serrata*.

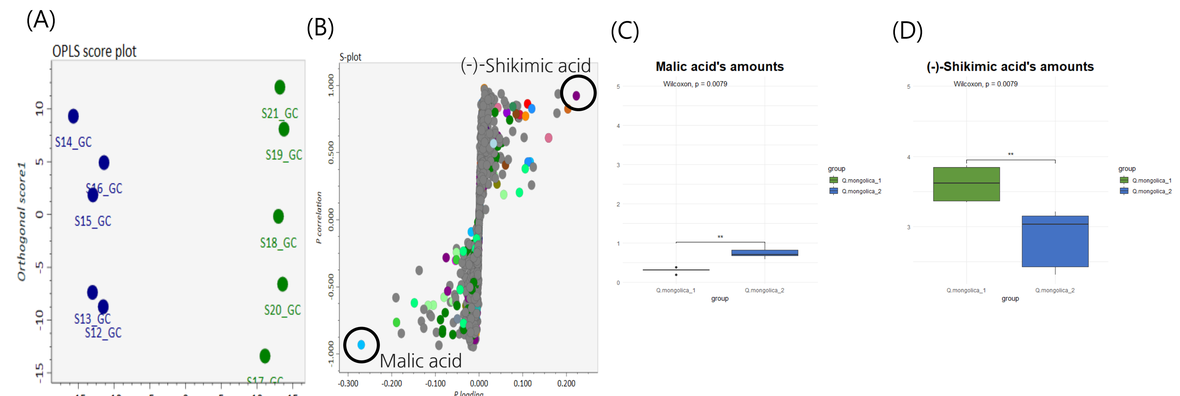

Fig. 5. (A) *Q. mongolica*(altitude 0~600m and 600~1000m) analyzied by OPLS-DA. (B) *Q. mongolica* analyzied by S-plot. The material with the lowest P correlation and P loading values is Malic acid and the material with the highest values is (-)-Shikimic acid. (C) Malic acid's amounts of *Q. mongolica*(*Q. mongolica*_1's altitude is 0~600m and *Q. mongolica*_2's altitude is 600~1000m). (D) (-)-Shikimic acid's amounts of *Q. mongolica*.

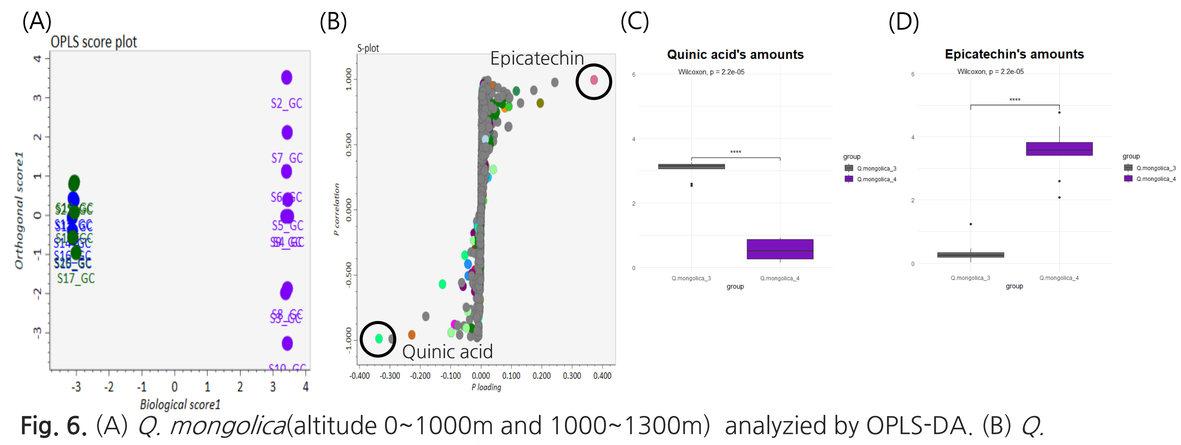

Fig. 6. (A) *Q. mongolica*(altitude 0~1000m and 1000~1300m) analyzied by OPLS-DA. (B) *Q. mongolica* analyzied by S-plot. The material with the lowest P correlation and P loading values is Quinic acid and the material with the highest values is Epicatechin. (C) Quinic acid's amounts of *Q. mongolica*(*Q. mongolica*_3's altitude is 0~1000m and *Q. mongolica*_4's altitude is 1000~1300m). (D) Epicatechin's amounts of *Q. mongolica*.

DISCUSSION & CONCLUSIONS

- *Q. mongolica* growing at the hightest altitude produce a lot of epicatechin that antioxidants (MartÍn *et al.*, 2013). So, *Q. mongolica* growing at high altitude is thought to be under a lot of oxidative stress.
- Previous study has shown that healthy tissues have high expression of malic acid and diseased tissues have high expression of shikimic acid (Kesari, 1975). Therefore, through *Q. mongolica* analysis, stress at low altitude is thought to be mainly caused by diseases.

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