

# Are polyphenols viable targets for soft fruit breeding? A model study on blackcurrant.

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## Background

The increase of general and specific polyphenol level is a target for enhancement in crops, such as soft fruit, since they accruing evidence suggests they exhibit beneficial effects against a number of degenerative diseases. Blackcurrant (*Ribes nigrum*) represents a rich source of putatively beneficial polyphenols, such as anthocyanins, flavonols and phenolic acids, and is a widely consumed fruit, mainly in juice form. Here we report some advances in our research into the inheritance of nutritional and beneficial health traits on soft fruit.

## Methods

LC/MS: Analysis was performed on single-stage LTQ Orbitrap mass spectrometer, coupled to LC and diode array.

LC conditions: Eluent: A-water (0.1% formic acid) and B-acetonitrile (50%) + 0.1% formic acid, Injection volume of 8  $\mu$ l, and flow of 200  $\mu$ l.min<sup>-1</sup> at 30°C. Gradient: 5-50% B to 22 min, 100%B to 32 min, 100%B at 34 min.

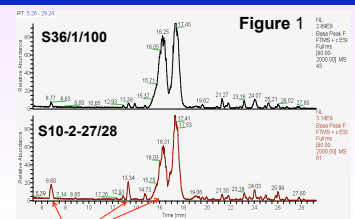
MS conditions- Analyzer: FTMS; Resolution: 100.000; MS range: 80 – 2000 m/z; Full scan in positive mode

Processing data- Sieve V 1.2<sup>TM</sup>  
RT- 2.2 min; frame m/z- 0.02, threshold - 500.000  
Statistical Analysis- Simca P 11<sup>TM</sup>.

## Results

As part of a broad study looking at polyphenol inheritance we have analysed the progeny (200 lines) from a well established segregating cross derived from the blackcurrant parental lines **S36/1/100** (high juice quality) and **S10-2-27/28** (gall mite resistant).

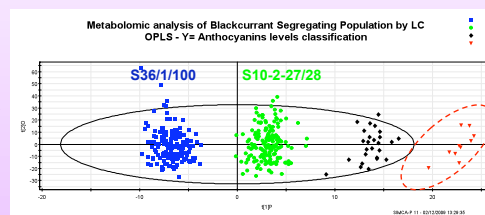
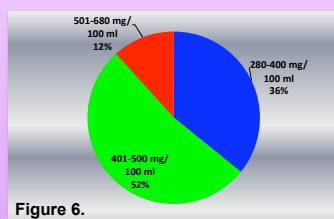
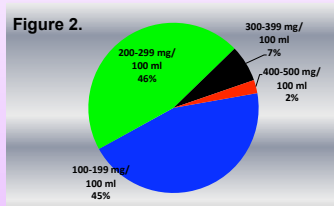
Metabolomic screening was undertaken for all progeny and the profiles of the parental lines is shown in Fig. 1.



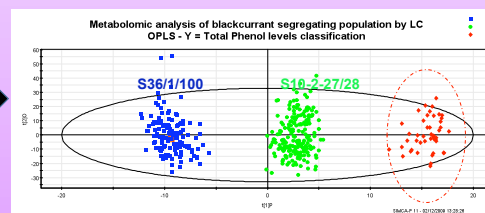
Polyphenolics driving segregation (see Fig. 4)

## General and specific polyphenols across the progeny

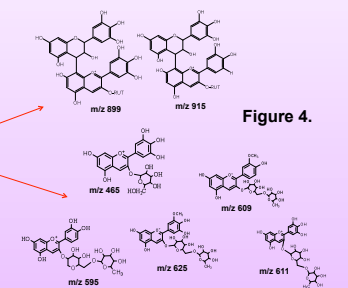
Total anthocyanin levels (mg/ 100 ml of juice) were used to classify the segregating population into 4 groups (Fig. 2). The same classification was used for OPLS analysis of the LC/MS data; a valid model to discriminate the genotypes is shown in Fig. 3. The loading plots (not shown) identified the outlier genotypes producing not only higher levels of anthocyanins but mainly conjugated anthocyanin-flavonols (Fig. 4). A similar strategy was used to analyse the general phenolics across the progeny, where OPLS analysis (Fig. 5) generated 3 groups (Fig.6), according to the total phenolics levels (mg/100 ml juice).



**Figure 3.** OPLS analysis (Y= 1, 2, 3 and 4)  
Observations (N) = 383, Variables (K) = 2561, A= 1+2+1, R2X= 0.72, R2Y= 0.97, Q2(cum)= 0.79



**Figure 5.** OPLS analysis (Y= 1, 2 and 3)  
Observations (N) = 383, Variables (K) = 2561, A= 1+2+0, R2X= 0.701, R2Y= 0.98, Q2(cum)= 0.88



Gallocatechin-Delphinidin (DP)-3  
Gallocatechin-cyanidin (CD)-3-glu  
Anthocyanins: m/z 611, 595, 625, 449, 609, 579  
Flavonols:  
Myr-3-Rut m/z 627  
Quercetin-3-O-glu m/z 465  
Isorhamnetin-3-Glu m/z 479  
Isorhamnetin-3-glu-malonate m/z 565

## Conclusion

The cross yielded progeny exhibiting a diversity of polyphenolic structure and quantity. The state-of-the-art analytical approaches ('omics) facilitated this screening in a timescale significantly quicker (~x10) than traditional analytical approaches. We are applying correlation analysis to this data along with sensorial scores to determine the impact of polyphenols on organolepsis and the interplay between the polyphenol biosynthetic pathways.