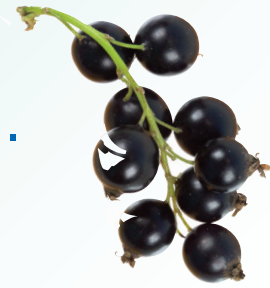# Are polyphenols viable targets for soft fruit breeding?
A model study with blackcurrant.

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Increases in general and specific polyphenol level is a target for enhancement in crops, such as soft fruit, since evidence is accruing that 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 nutritionally important polyphenols in blackcurrant.

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 ml, and flow of 200 ml.min-1 at 30ºC. Gradient: 5-50% B to 22 min, 100%B to 32 min, 100%B at 34 min.
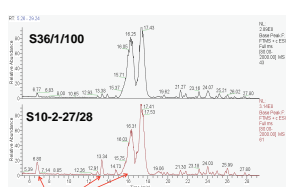
MS conditions-
Resolution: 100.000;
MS range: 80 – 2000 m/z;
Full scan in positive mode

Processing data-
Sieve V 1.2™
RT- 2.2 min; frame m/z- 0.02, threshold - 500.000

Statistical Analysis - Simca P 11™.

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 are shown in Fig. 1.

Polyphenol inheritance across the progeny
Total anthocyanin levels (mg/ 100 ml of juice) were used to separate the population into 4 groups (Fig. 2). The same classification was used for OPLS analysis of the LC/MS data and 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 also anthocyanin-flavanol conjugates (Fig. 4). A similar strategy was used to generate an OPLS analysis (Fig. 5) with 3 groups (Fig.6) classified according to the total phenol levels (mg/100 ml juice) which drew out other interactions between polyphenol classes.
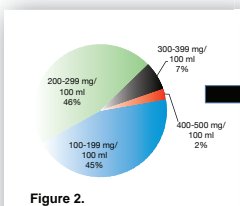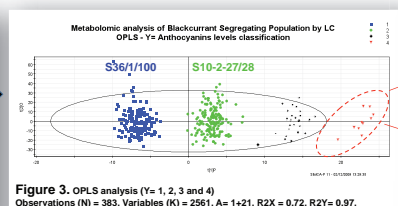

Figure 1
S36/1/100
S10-2-27/28
Polyphenolics driving segregation (see Fig. 4)

Figure 2.
300-399 mg/ 100 ml 7%
400-500 mg/ 100 ml 2%
100-199 mg/ 100 ml 45%
200-299 mg/ 100 ml 46%

Metabolomic analysis of Blackcurrant Segregating Population by LC OPLS - Y= Anthocyanins levels classification
S36/1/100   S10-2-27/28

Figure 3. OPLS analysis (Y= 1, 2, 3 and 4)
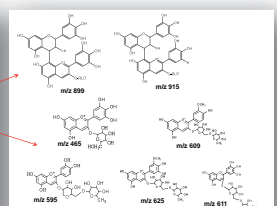Observations (N) = 383, Variables (K) = 2561, A= 1+21, R2X = 0.72, R2Y= 0.97, Q2(cum)= 0.79

m/z 899   m/z 915
m/z 465   m/z 609
m/z 625   m/z 611
Figure 4.

Figure 6.
501-680 mg/ 100 ml 12%
280-400 mg/ 100 ml 36%
401-500 mg/ 100 ml 52%

Metabolomic analysis of blackcurrant segregating population by LC OPLS - Y = Total Phenol levels classification
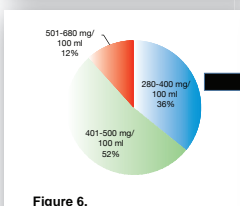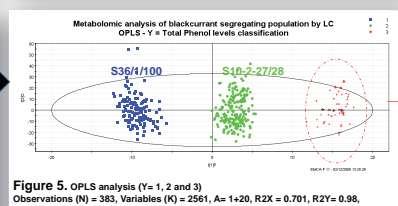S36/1/100   S10-2-27/28

Figure 5. OPLS analysis (Y= 1, 2 and 3)
Observations (N) = 383, Variables (K) = 2561, A= 1+20, 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 with a diversity of polyphenolic structures and total phenol content. The state-of-the-art analytical "omic" approach facilitated screening of polyphenolic composition on a significantly quicker timescale (~x10) than traditional analytical approaches.

We are correlating polyphenolic composition against genetic maps to assess inheritance of specific polyphenols. By also applying correlation analysis of this data with sensorial scores, we hope to determine the impact of polyphenols on organolepsis.