Metabolic Cold Acclimation of 'Polka' and 'Honeoye' strawberries under Natural Field Conditions

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Abstract

The winter hardiness of strawberry varieties used in perennial production systems varies greatly. Still, little information is available on how plant metabolism adapts to cold and freezing temperatures under natural temperature and light conditions. In order to examine the hardening process of overwintering meristematic tissue in *Fragaria* × *ananassa*, crown samples of field-grown var. 'Polka' and 'Honeoye' were consecutively collected over a period of 15 weeks, i.e. from the end of the season (week 35/ end August) until midwinter (week 50/ December). Samples were subjected to qGC-MS metabolite profiling to assess the reconfiguration of central metabolism, and characterize the regulation of selected compatible solutes. Besides changes in amino acid patterns (glutamic acid, aspartic acid, and asparagine), monosaccharide levels (fructose) increased strongly in 'Honeoye' (180-fold compared to start control) towards the end of the acclimation period. In contrast, 'Polka' showed a concentration peak (36-fold) in week 47 and a decline towards week 50. Also sucrose levels were steadily increased throughout the cold hardening period with averagely 6-fold higher levels in 'Honeoye' compared to 'Polka', thus underscoring cultivar-dependent differences. However, both varieties showed a decline in sucrose levels after week 47. Particularly, the raffinose pathway was affected leading to strongly and transiently increased levels of the precursor galactinol (week 42/ mid-October) and the trisaccharide raffinose (weeks 43 to 47/ end October to mid-November). While galactinol biosynthesis was earlier induced in 'Polka' (week 38) compared to 'Honeoye' (week 39), subsequent raffinose production was delayed in 'Polka' (week 47) compared to 'Honeoye' (week 45). Major metabolic changes in both varieties coincided with a decrease in day length below 14 h in mid-September, and a consistent drop below 10°C average day temperature by the end of September.

INTRODUCTION

Cultivated strawberry (*Fragaria* × *ananassa* Duch.) is an important berry crop for the consumer market and food industry in Norway due to its pleasant taste, flavour, and health beneficial phytochemicals. A successful commercial production relies mainly on the plants acclimation, winter survival and rapid re-growth in spring time. The winter

hardiness of strawberry varieties used in perennial production systems in Northern Europe differs greatly, although strong similarities exists regarding transcriptional, protein and metabolic changes during cold acclimation (Koehler et al., 2012; Rohloff et al., 2012a) since this biological mechanism is highly conserved in plants (Davik et al., 2012; Rohloff et al., 2012b). Still, little information is available on how plant metabolism adapts to cold and freezing temperatures in the field. The present project addresses the following biological questions and varietal aspects: **(A)** Reconfiguration of primary metabolism in overwintering meristem (crown tissue), when strawberry plants are exposed to natural climatic conditions during cold acclimation, **(B)** Differences in metabolite profiles of freezing-tolerant *F.* × *ananassa* varieties regarding long-term metabolic regulation and plasticity, and **(C)** Specific impact of major abiotic factors such as temperature and light on the cold acclimation processes of field-grown strawberry plants.

MATERIALS AND METHODS

In order to study acclimation of overwintering meristems in *Fragaria* × *ananassa* Duch., crown tissue samples (fresh-frozen in liquid N_2) of field-grown var. 'Polka' and 'Honeoye' were collected 11 times over a period of 15 weeks - from the end of the growth season (week 35/ end August) until midwinter (week 50/ mid December). Sample processing followed a modified procedure described in Sissener et al. (2011), using 100 mg (f.w.) of crown tissue for extraction. Speedvac-dried extracts of crown samples containing polar compounds from central metabolism, were further derivatized and subjected to metabolite profiling by quadrupole gas chromatography/mass spectrometry (qGC/MS) (Davik et al., 2012). Compounds were identified using the Golm Metabolome Database (GMD) (Kopka et al., 2005). qGC/MS data was processed with AMDIS deconvolution software and normalized based on the internal standard (ribitol). The re-configuration of central metabolism was assessed based on the quantification of selected metabolites (sugars, polyols, Krebs compounds and amino acids). The following statistical analyses were carried out: (1) Variance analysis (ANOVA) to determine variety and time point differences of 10 selected metabolites, and (2) multivariate statistical analyses using principal component analysis (PCA) based on GC/MS-based chemical profiles including 31 metabolites (average concentration).

RESULTS AND DISCUSSION

Statistical significant differences between the varieties 'Polka' and 'Honeoye' could be found for several metabolites including the amino acids aspartate, glutamate, and asparagine, and sucrose (Table 1). When comparing sample time points, significant differences were detected for fructose, galactinol and raffinose. PCA analysis depicting variety and time point segregation patterns (Fig. 1), further emphasized a genotype-dependent metabolic regulation during cold acclimation. Besides changes in amino acid patterns (glutamic acid, aspartic acid, and asparagine), monosaccharide levels (fructose) were strongly enhanced until the end of the acclimation period in 'Honeoye' (180-fold compared to start control) (Fig. 2). In contrast, 'Polka' showed a concentration peak (36-fold) in week 47 and a decline towards week 50. Also sucrose levels were steadily enhanced throughout the cold hardening period with averagely 6-fold higher levels in 'Honeoye' compared to 'Polka', thus emphasizing cultivar-dependent differences. However, both varieties showed a decline in sucrose levels after week 47.

Particularly, the cold-inducible raffinose pathway (Fig. 3) was affected leading to strongly and transiently increased levels of the precursor galactinol (week 42/ mid October) and

the trisaccharide raffinose (weeks 43 to 47/ end October to mid November). While galactinol biosynthesis was earlier induced in 'Polka' (week 38) compared to 'Honeoye' (week 39), the subsequent raffinose production and concentration peaks occurred later in 'Polka' (week 47) compared to 'Honeoye' (week 45). Major concentration changes in the raffinose pathway of both varieties coincided with a decrease in day length below 14 h after week 37 (mid September), and a consistent drop below 10°C average day temperature by the end of September (week 39).

CONCLUSIONS

Our results have revealed how central metabolites, which function as compatible solutes, are regulated in two *Fragaria* × *ananassa* varieties during cold acclimation under field conditions. Genotype-dependent differences in long-term metabolic adaptations over a period of 15 weeks (105 days) were observed in 'Polka' and 'Honeoye'. However, both varieties showed similar temporal changes in metabolite patterns, thus indicating the time points and significance of environmental factors (temperature, day length) necessary to induce cold and freezing tolerance in cultivated strawberry.

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Tables

Table 1. Determination of variance (ANOVA) between varieties and time points based on 10 selected metabolites ($p \leq 0.05$).

ANOVA testing	COMPOUND	Malic Acid	Aspartic Acid	Glutamic Acid	Asparagine	Citric Acid
VARIETY differences		ns	1.02E-03	4.14E-03	4.31E-02	ns
TIME POINT differences		ns	ns	ns	ns	ns

ANOVA testing	COMPOUND	Fructose	Glucose	Sucrose	Galactinol	Raffinose
VARIETY differences		ns	ns	0.000106	ns	ns
TIME POINT differences		4.23E-02	ns		2.00E-04	5.40E-04

Figures

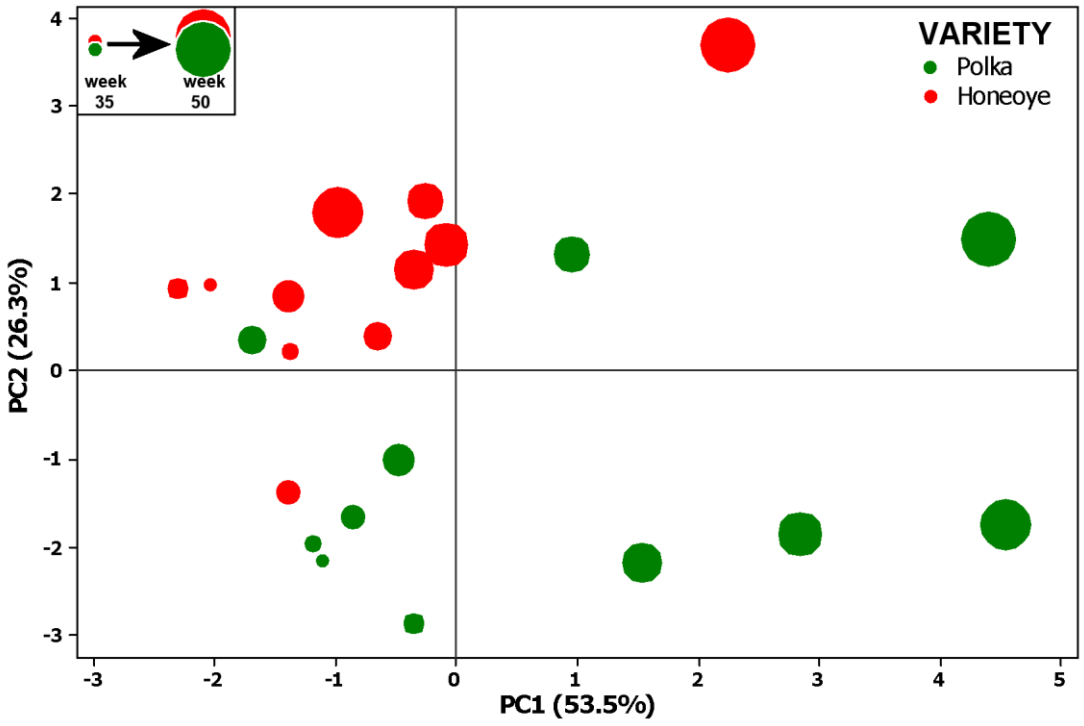

Fig. 1. Principal component analysis (PCA) depicting components PC1 and PC2 based on crown GC/MS profiles (31 metabolites). Segregation patterns of *F.* × *ananassa* samples (var. 'Polka' and 'Honeoye') harvested at different time points during cold acclimation (11 time points from week 35 to week 50) are shown.

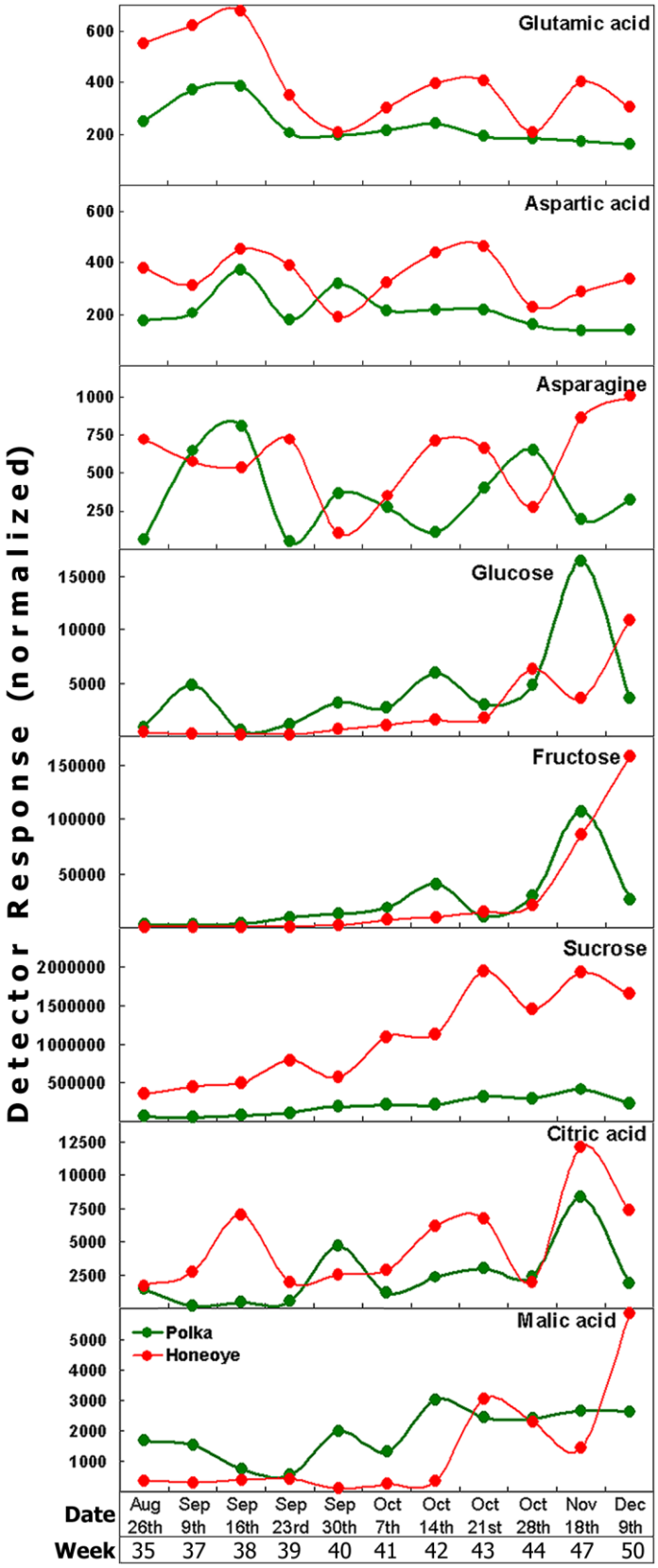

Fig. 2. Concentration changes (normalized detector response) of selected amino acids, sugars, and Krebs cycle metabolites.

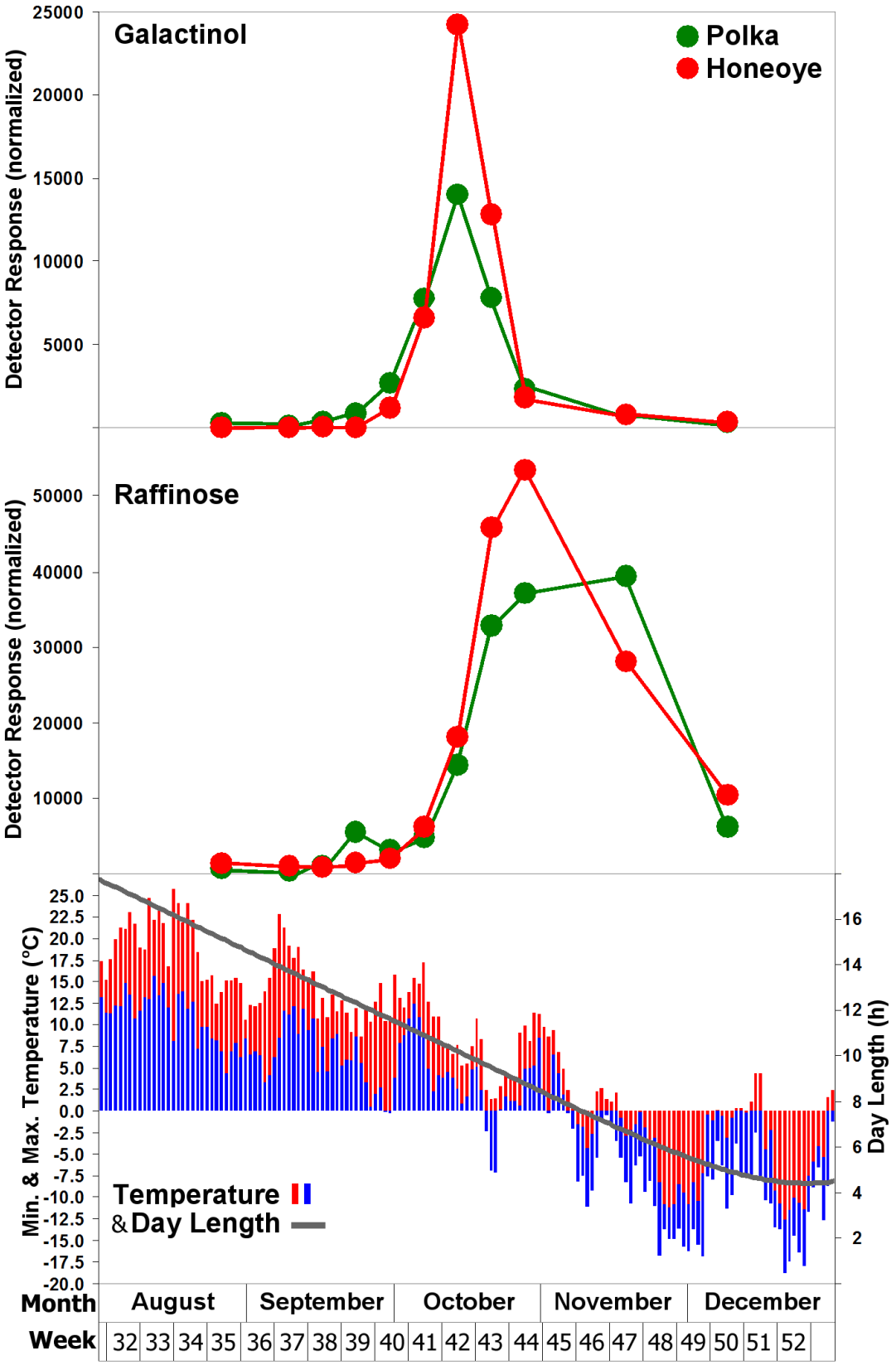

Fig. 3. Relationship between maximum/minimum temperature (°C) and day length (h), and concentration levels of galactinol and raffinose, the central metabolites of the cold-inducible raffinose pathway.