

SWEET CHERRY BUDS OF TREES AND TWIGS AND THE RESPONSES OF POLYAMINES AND AMINO ACIDS TO TEMPERATURE CHANGES

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Abstract

The response of polyamines (PAs) and amino acids (AAs) to a sequence of environmental temperature changes {cold (orchard) - warm (controlled temperature of ~22 °C) - cold (orchard)}, of sweet cherry buds (cv. 'Summit') of twigs by gas chromatography-mass spectrometry (GC-MS) analyses was determined. Findings were compared with buds of twigs and buds of trees under natural conditions over six weeks between November and January in northeast Germany. Distinct changes of PA and AA content in the tree buds are evident. The level of PAs/AAs in buds of twigs in the orchard, compared to buds of trees, reveal differences after 21 days, which become much greater with increasing time (after 42 days). A temperature of ~22 °C led to increasing anabolism, and thus to a several hundred times increase of the PAs/AAs in buds of twigs compared to buds of twigs in the orchard, as well as to the buds of trees after 21 days. When these twigs after 21 days at ~22 °C were placed in the orchard, a clear decrease - well below the level in the buds of twigs under natural conditions - is apparent after 21 days, suggesting strong catabolic activity and consumption of PAs and most AAs within the buds. Compared to the buds of trees, in the buds of twigs kept for 21 days at ~22 °C, PA and AA levels increased considerably. After returning these twigs to the orchard for 21 days, some of the PAs, AAs exceeded the level in the buds of trees, whereas others were well below the level of the buds of trees. During winter rest, a phase characterized by no growth and development, and by environmental temperature changes for the buds, the turnover (anabolism/catabolism) of PAs and AAs in sweet cherry buds of twigs and trees is evident.

Keywords: *Prunus avium L., Buds, Twigs, Trees, Polyamines, Amino acids, Air temperature*

1. INTRODUCTION

Cold acclimation of deciduous trees is accompanied by complex physiological and biochemical reactions (Xin and Browse 2000, Ruttink et al. 2007). Low temperatures lead to changes of the content of a large number of metabolites, including AAs, PAs, carbohydrates and organic acids. Considerable evidence points to a correlation between stress tolerance and the capacity of plants to enhance the synthesis of PAs upon encountering the stress. The importance of PAs in plant responses to low temperature has also been shown by exogenous application of PAs or PA-biosynthetic inhibitors (Alcasar et al. 2011 and references therein). The review from Alcázar et al. (2011) integrate PA biosynthesis with transcriptional and metabolic changes that occur in response to low temperature, and discuss the potential of PA genetic engineering to develop plants with increase freezing tolerance. Certain amino acids, including proline and glycine, are belong to the array of naturally occurring low temperature protecting organic osmolytes (Bolen 2001). Other N-containing metabolites, like γ -aminobutyric acid (GABA), respond also to low temperatures (Bouché and Fromm 2004). Nevertheless, only a limited number of studies are focused on the involvement of PAs and AAs in chilling tolerance. Experimental research focused on mechanisms concerning whole tree physiology is generally possible (Chmielewski and Götz 2016), but may encounter methodological constraints since *in situ* manipulations for all issues are not practicable. Alternatively, twigs are used for phenological

observations like growth and development (bud burst, leaf unfolding) (Fu et al. 2013), especially in relation to the impact of abiotic factors (chilling/heat requirement, light quantity/quality (Gariglio et al. 2006, González-Rossia et al. 2008, Levizou and Manetas 2007). Here, the response of polyamines and amino acids to a sequence of environmental temperature changes {cold (orchard) - warm (controlled temperature of ~22 °C) - cold (orchard)}, of sweet cherry buds (cv. 'Summit') of twigs was determined. Findings were compared with buds of twigs and buds of trees under natural conditions over six weeks between November and January in northeast Germany. This study was intended to contribute to a better understanding of the behaviour of N-containing compounds, such as PAs and AAs, in sweet cherry buds.

2. MATERIAL AND METHODS

This study was conducted on trees and twigs of cultivar 'Summit', grafted on GiSelA 5 (GiessenerSelektionAhrensburg 5), a weakly- to medium-strongly growing stock, at the Experimental Sweet Cherry Orchard (area: 980 m²) at Humboldt-University in Berlin-Dahlem (52° 28' Northern latitude and 13° 18' Eastern longitude). The long-term (1981-2010) average annual air temperature and precipitation are 9.9 °C and 562 mm, respectively. Specific details for the Experimental Sweet Cherry Orchard are described in Götz et al. (2017).

2.1 Bud sampling

Buds of trees (treatment A) were compared with buds of cut twigs (placed in plastic flasks), kept for 42 days (six weeks) under the same natural conditions as for the orchard (treatment B). Also, twigs (C) were kept first for 21 days (3 weeks) under controlled conditions at an air temperature of ~22 °C (day/night), and subsequently, the twigs treated in this way then were placed for 21 days in the orchard under natural conditions (twigs B, C: in Styrofoam containers and isolation film, and avoiding effective freezing of the water in the plastic flask). Bud clusters (n=3/sampling) were taken weekly (2017: 22 November - 3 January 2018), each cluster from 3 trees (A) and from 3 twigs each for B and C, respectively. After cutting, the flower buds were selected (exception: the smaller leaf bud in the middle), immediately frozen in liquid nitrogen, stored at -80 °C, and later freeze-dried. All buds were ground in a ball mill (Retsch M1, Haan, Germany) before analysis.

2.2 Metabolite analysis

To identify metabolites in the sweet cherry buds, GC-MS analyses were performed, as described elsewhere (Lisec et al., 2006; Alseekh et al., 2020) and recording of the mass scans were determined, using a fragmentation pattern ('fingerprint') after gas chromatographic separation. These fingerprints were then compared to those of the relevant database to identify the respective substances. For these analyses, selected bud samples of treatments A, B, and C at DOY 326 (start of the experiment), DOY 347 (3 weeks later) and DOY 3 (6 weeks after the start of the experiment) of the year 2017/18 were analysed.

3. RESULTS AND DISCUSSION

3.1 Temperature in 2017/2018

The mean air temperature (Tab. 1) experienced during week 1 through week 3 in the orchard, for the trees (A) and twigs in water (B), was 6.1, 3.3, and 2.8 °C at DOY (day of year) 333, 340, and 347, respectively. During week 4 through week 6 (A, B, and C) the mean air temperature was 2.0, 6.4, and 5.0 °C at DOY 354, 361, and 3, respectively. The coldest temperatures ($T_{\min}$) during the six-week period was in a range between 0.2 (DOY 354) and 4.4 °C (DOY 361), whereas the range for the warmest temperatures ($T_{\max}$) varied between 3.7 (DOY 354) and 8.5 °C (DOY 333).

Table 1. Mean weekly air temperature (T_{mean}), minimum (T_{min}), and maximum (T_{max}) temperature ($^{\circ}\text{C}$) in the orchard under natural conditions during the experiment from DOY (day of year) 326 – DOY 3 (2017/18) (treatments A,B,C). Data in bold: sampling.

DOY	$T_{\text{mean}} (^{\circ}\text{C})$	$T_{\text{min}} (^{\circ}\text{C})$	$T_{\text{max}} (^{\circ}\text{C})$
326	5.5	7.5	3.2
333	6.1	3.3	8.5
340	3.3	1.4	7.4
347	2.8	0.5	5.3
354	2.0	0.2	3.7
361	6.4	4.4	5.1
3	5.0	1.5	7.8

3.2 Metabolite profiling by GC-MS

Polyamines are small aliphatic amines. The compounds putrescine (Put), spermidine (Spd), spermine (Spm), cadaverine (Cad) and thermospermine (t-Spm), a Spm isomer, also occur in plants. Polyamine homeostasis is determined by PA metabolism, conjugation, interconversion, chemical alteration, and transport (Wang et al. 2019). PAs correlate with vital biochemical functions, including protein regulation, regulation of chemiosmosis and photo-protection in chloroplasts, ATP synthesis, membrane fluidity, and control of N/C balance. Exogenous PA application has been demonstrated to increase plant tolerance to abiotic stress conditions, such as salinity, drought, osmotic stress, and extreme temperatures (Wang et al. 2019, and references therein, Majumdar et al. 2013). Seventy-one metabolites were identified in buds according to the Golm Metabolome Database (GMB; <http://gmd.mpimp-golm.mpg.de/>; Hummel et al. 2010).

Twenty-two biogenic polyamines, amino acids were selected, and the changes within the samplings at DOY 326, 347, and DOY 3 for buds of trees (treatment A) and buds of twigs (treatment B and C) compared (Fig. 1A-H). The other 49 metabolites identified in the buds represent different catechins, which are polyphenolic compounds assigned to flavonoids, organic acids like different quinic acids, malic acid, glycerol and the phosphoric ester of glycerol, and sugar acids (data not shown).

Regarding the buds of trees, marked content changes are evident for PAs and amino acids (AAs) between DOY 326 and DOY 347, and between DOY 347 and DOY 3, respectively (Fig. 1A,B). The amino acids glutamate (Glu), glutamine (Gln), ornithine (Orn), arginine (Arg) and histidine (His) especially demonstrated increases of 166-, 111-, 145-, 236-, and 167-fold at DOY 347 (Fig. 1A). And even more pronounced (at DOY 3) were increases of Orn, Arg, and His {increases of 556-, 863-, and 311-fold (Fig. 1B), respectively}. Orn is an intermediate in the Glu- to Arg pathway in plants, and both Glu and Arg are often present in large quantities, whereas in most plants the cellular concentration of Orn is much lower, compared to levels of Arg or Glu (Majumdar et al. 2013). While aspartate (Asp), asparagine (Asn), threonine (Thr), and isoleucine (Ile) were clearly reduced below 100% at DOY 347, these amino acids showed a marked (410-, 357-, 148-, 160-fold, respectively) increase at DOY 3. This finding was also true for phenylalanine (Phe) and tyrosine (Tyr), (194-, 243-fold, respectively). The non-protein amino acid GABA increased at both sampling dates, demonstrating similar values (DOY 347, DOY 3, 229-, 226-fold, respectively). The diamine Put, synthesized either by direct decarboxylation of Orn or indirectly of Arg in most plants (Majumdar et al. 2013), increased from 119- to 181-fold at DOY 347 and DOY 3, respectively.

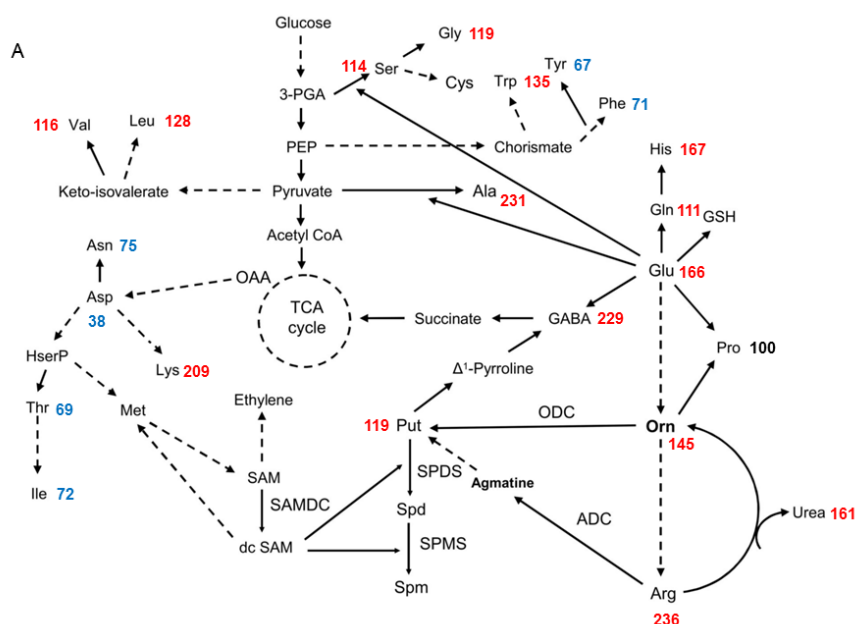


Figure 1A. Changes in the abbreviated pathway for the biosynthesis of polyamines and amino acids in sweet cherry buds in 2017/18. A: buds of trees (treatment A) at DOY 347 (Values are given as % of DOY 326=100%). Data in blue: decreasing value; data in red: increasing value.

Abbreviations (PA enzymes): ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase (modified from Majumdar et al. 2013).

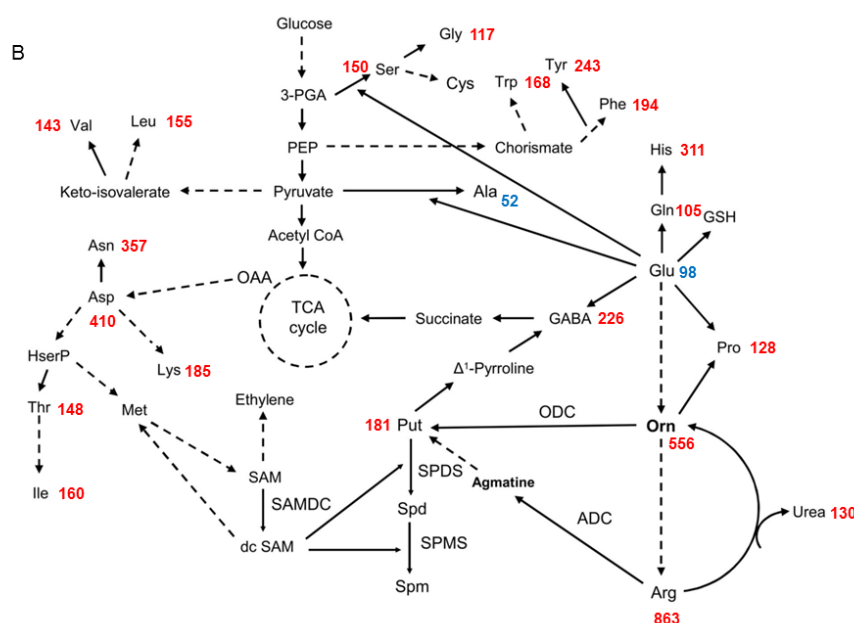


Figure 1B. Changes in the abbreviated pathway for the biosynthesis of polyamines and amino acids in sweet cherry buds in 2017/18. B: buds of trees (treatment A): at DOY 3 (Values are given as % of DOY 347=100%). Data in blue: decreasing value; data in red: increasing value.

Abbreviations (PA enzymes): ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase (modified from Majumdar et al. 2013).

The level of PAs, AAs in buds of twigs under natural conditions (Fig. 1C,D, treatment B) in the orchard, compared to values for buds of trees, reveal differences at DOY 347, which became much stronger with increasing time following 42 days at DOY 3. Glu, His, Asn, Thr, Ile, Tyr, Val, Leu (Fig. 1C) increased 140-, 132-, 136-, 137-, 174-, 357-, 144-, and 156-fold, and much stronger (387-, 216-, 354-, 311-, 486-, 805-, 588-, 433-fold), at DOY 347 and DOY 3, respectively (Fig. 1D). Put initially demonstrated a 136-fold increase at DOY 347, and was markedly reduced to 51% at DOY 3. Also remarkable are the changes of alanine (Ala) and GABA, which increased from 118-, 147-fold at DOY 347 to 1985-, 631-fold at DOY 3, respectively. GABA probably plays a dual role as both a signalling molecule and a metabolite. GABA is involved in general nitrogen metabolism and possibly in the storage and/or transport of nitrogen. The GABA shunt is also a way to assimilate carbons from glutamate and also to generate C:N fluxes that enter the tricarboxylic acid (TCA) cycle. It is hypothesized that the degradation of GABA could limit the accumulation of reactive oxygen intermediates under oxidative stress conditions, resulting in an inhibition of certain enzymes of the TCA cycle. In addition, Proline-GABA transporters can transport organic osmolytes, also called compatible solutes, which serve several protective roles (Bouché and Fromm 2004).

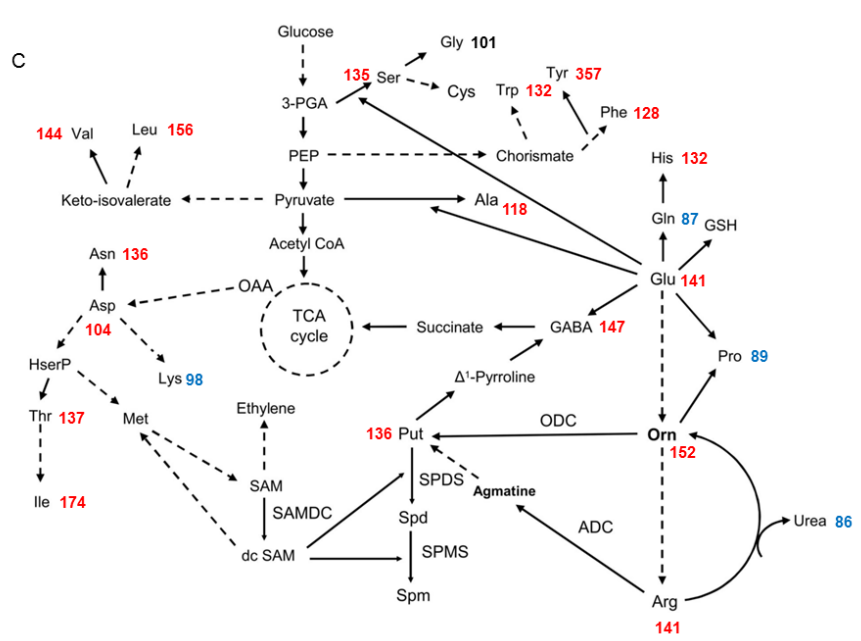


Figure 1C. Changes in the abbreviated pathway for the biosynthesis of polyamines and amino acids in sweet cherry buds in 2017/18. C: buds of twigs under natural conditions in the orchard (treatment B) at DOY 347 (Values are given as % of DOY 326=100%). Data in blue: decreasing value; data in red: increasing value.

Abbreviations (PA enzymes): ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase (modified from Majumdar et al. 2013).

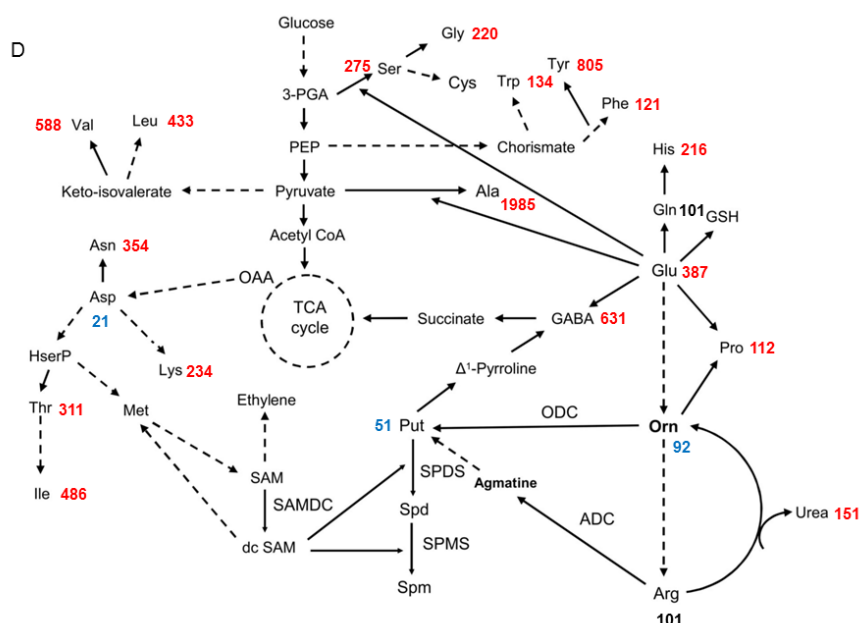


Figure 1D. Changes in the abbreviated pathway for the biosynthesis of polyamines and amino acids in sweet cherry buds in 2017/18. D: buds of twigs under natural conditions in the orchard (treatment B) at DOY 3 (Values are given as % of DOY 347=100%). Data in blue: decreasing value; data in red: increasing value.

Abbreviations (PA enzymes): ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase (modified from Majumdar et al. 2013).

Temperatures of ~22 °C lead to rising anabolism, and thus to a several hundred times increase of the PAs, AAs in buds of twigs (treatment C), compared to buds of twigs in the orchard (treatment B) after 21 days at DOY 347 (Fig. 1E), with the exception of Put (decrease) and Ala (similar). This is particularly obvious for Orn, Arg, Asp, Thr, Ile, and GABA (637-, 971-, 737-, 493-, 219-, 304-fold), but more distinctly for Asn and His (2354-, 1303-fold, respectively). When these twigs after 21 days at ~22 °C were placed in the orchard under natural conditions, a clear decrease, well below the level for the buds under natural conditions (treatment B), is evident (Fig. 1F), suggesting strong catabolic activity and consumption within the buds of PAs and most AAs measured at DOY 3. Exceptions are Asp and Asn, whose levels decreased between DOY 347 and DOY 3 (from 737-, 2354-fold to 309-, 153-fold, respectively), and yet are higher than in the buds kept 42 days in the orchard (treatment B). Aspartate derives from transamination of oxaloacetate, and is a precursor of the amino acids lysine (Lys), Thr, and Ile (Gaufichon et al. 2010, Lea and Azevedo 2007), which are strongly reduced (Fig. 1F).

With view on changes of the PAs and AAs, also notable is the fact that water uptake for the twigs/bud clusters were equal, 0.039 ± 0.005 g H₂O/g twig and 0.047 ± 0.018 g H₂O/g twig, during the low temperature period in the orchard (treatment B) and at ~22 °C (treatment C), respectively.

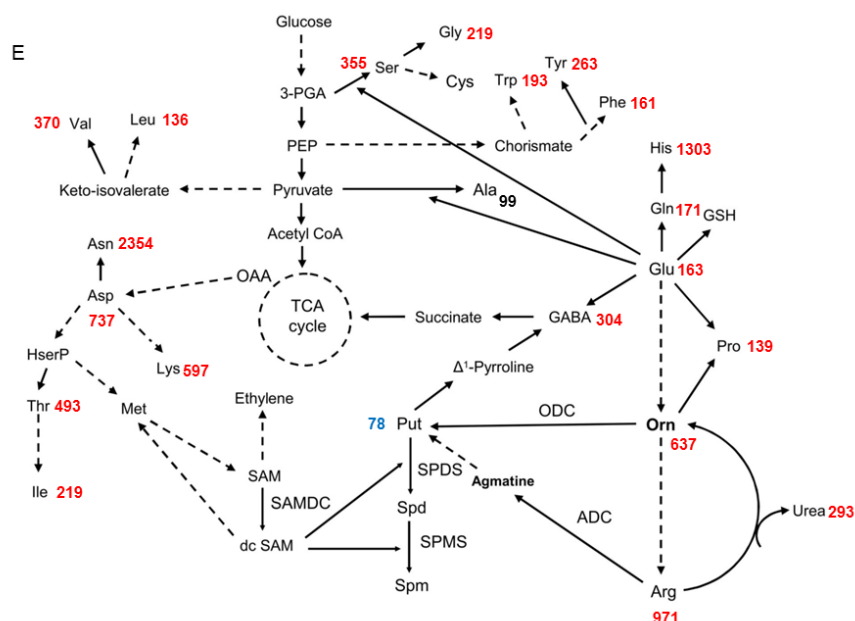


Figure 1E. Changes in the abbreviated pathway for the biosynthesis of polyamines and amino acids in sweet cherry buds in 2017/18. E: buds of twigs after 21 days at ~22 °C at DOY 347 (treatment C) compared to buds of twigs under natural conditions in the orchard (treatment B). Values are given as % of DOY 347=100%). Data in blue: decreasing value; data in red: increasing value.

Abbreviations (PA enzymes): ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase (modified from Majumdar et al. 2013).

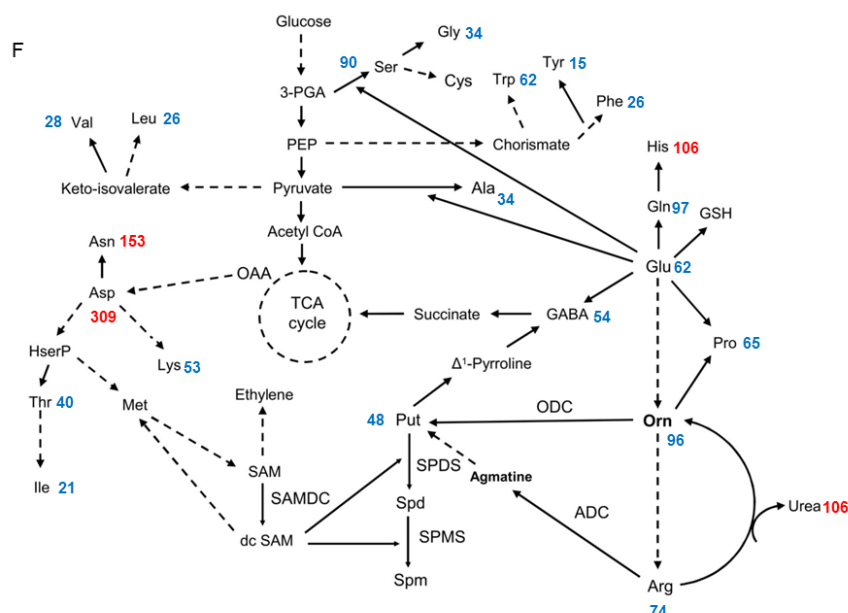


Figure 1F. Changes in the abbreviated pathway for the biosynthesis of polyamines and amino acids in sweet cherry buds in 2017/18. F: buds of twigs placed 21 days to the orchard (treatment C) at DOY 3 compared to buds of twigs under natural conditions in the orchard (treatment B). Values are given as % of DOY 3=100%). Data in blue: decreasing value; data in red: increasing value.

Abbreviations (PA enzymes): ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase (modified from Majumdar et al. 2013).

Compared to the buds of trees, the buds of twigs kept for 21 days at ~22 °C, demonstrated {for levels of the PAs and AAs at DOY 347 (Fig. 1G)} considerable increases for Orn, Arg, Glu, Gln, His, Asp, Thr, Ile, Asn, Tyr, Val, and GABA, for 966-, 1367-, 227-, 149-, 1724-, 678-, 381-, 764-, 3211-, 940-, 533-, and 447-fold, respectively. Changes of AAs in sweet cherry buds (Götz et al. 2017, and references therein) are intimately related to the carbon and nitrogen metabolism of arginine. Concerning further metabolism, if required, ammonium can be re-assimilated by glutamine synthase and incorporated into amino acids, with resulting high N content. In particular, Asn, Gln, and Arg are used as nitrogen storage and transport compounds. In sink tissues, the amide groups of asparagine and glutamine, and also the guanidine group of arginine, are hydrolysed by asparaginase, glutaminase, and arginase, respectively, to remobilize nitrogen. After placing these twigs for 21 days in the orchard environment (treatment C), there was a notable decrease in the level of the PAs and AAs at DOY 3, compared with the buds of trees at DOY 347. Glu, His, Asn, Thr, Lys, Ala, Val, Leu, Tyr, and GABA exceeded the level of the buds of trees (240-, 229-, 543-, 124-, 124-, 683-, 165-, 122-, and 344-fold, respectively), whereas the levels of Orn, Arg, Put, and Asp (88-, 75-, 24-, and 65-fold, respectively), were well below the levels for the buds of trees (Fig. 1H). Results from Majumdar et al. (2013) reveal that ornithine, even though present in relatively small quantities compared with other amino acids of the glutamate–arginine–proline pathway, may not only be the key regulator of polyamine biosynthesis in *Arabidopsis thaliana*, but it may also regulate the entire subset of pathways for metabolism of Glu to Arg and to Pro. Indirectly, it could also regulate Put catabolism, therefore contributing to the GABA content of the cells.

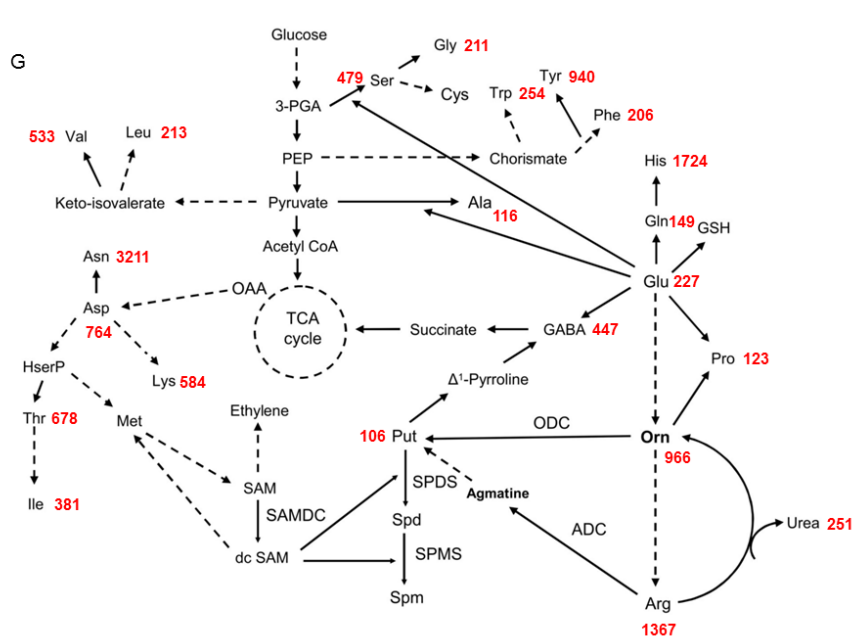


Figure 1G. Changes in the abbreviated pathway for the biosynthesis of polyamines and amino acids in sweet cherry buds in 2017/18. G: buds of twigs after 21 days at ~22 °C at DOY 347 (treatment C) compared to buds of trees (treatment A). Values are given as % of DOY 347=100%). Data in blue: decreasing value; data in red: increasing value.

Abbreviations (PA enzymes): ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase (modified from Majumdar et al. 2013).

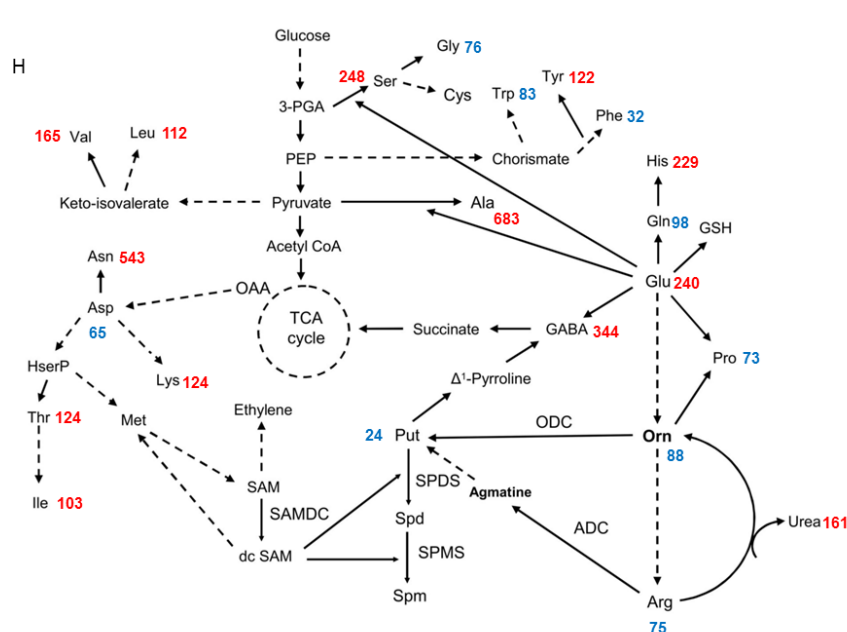


Figure 1H. Changes in the abbreviated pathway for the biosynthesis of polyamines and amino acids in sweet cherry buds in 2017/18. H: buds of twigs placed 21 days to the orchard (treatment C) at DOY 3 compared to buds of trees (treatment A). Values are given as % of DOY 3=100%. Data in blue: decreasing value; data in red: increasing value.

Abbreviations (PA enzymes): ODC, ornithine decarboxylase; ADC, arginine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase (modified from Majumdar et al. 2013).

4. CONCLUSIONS

The level of PAs and AAs in buds of twigs in the orchard, compared to buds of trees, reveal differences (after 3 weeks), which become much greater with increasing time (after 6 weeks), indicating metabolic activity during winter rest. Temperature of ~22 °C led to increasing anabolism, and thus to a several hundred times increase of the PAs, AAs in buds of twigs compared to buds of twigs in the orchard (after 3 weeks). When these twigs after 3 weeks at ~22 °C were placed in the orchard, a clear decrease - well below the level in the buds of twigs under natural conditions - is apparent (after 3 weeks), suggesting strong catabolic activity and consumption of PAs and most AAs within the buds. Compared to the buds of trees, in the buds of twigs kept for 3 weeks at ~22 °C, PA and AA levels increased considerably. After returning these twigs to the orchard (for 3 weeks), some of the PAs, AAs exceeded the level in the buds of trees, whereas others were well below the level of the buds of trees. During winter rest, a phase characterized by no growth and development, and by environmental temperature changes, the turnover (anabolism/catabolism) of PAs and AAs in sweet cherry buds is evident. Currently, an insight into the proteomic analysis of PA-responses to low temperature might be required for a better integration of PAs with transcriptome and metabolome data.

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