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Am Max-Planck-Institut für molekulare Pflanzenphysiologie
In Potsdam-Golm
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Betreuer: Dr. Saleh Alseekh

Thema: Metabolite profiling of different tomato accessions
and their F2 families

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1. Introduction

1.1 Personal introduction

I have been interested in biology since I can think because of my parents, who both work as ecologists. Being able to join my parents during their work, catching dragonflies, hamsters, birds and bats I learned a lot about this classic field of biology. With my interest in animals growing, I started to keep ants and breed butterflies at home and to attend to annual local bat conferences and camps organized by the *Arbeitskreis Fledermäuse Sachsen-Anhalt*. By that time, in class 9 my school demanded a 2 week practical experience in a company, so I applied at the Leibnitz Institute for plant biochemistry in Halle. Even though I have not had genetics in school and because my adviser explained everything very well and I was able to learn and do a lot of different experiments in the lab. Fascinated by this I applied at the same institute one year later to write my scientific practical article for school about the biosynthesis of carnosic acid. In 2016 I heard of the International Biology Olympiad and sadly was not able to reach the third round, being the 48th in the second round. In the next Olympiad 2017/18 I reached the third round, where I got this internship, and also the fourth round of the competition.

1.2 Institutional introduction

The Max-Planck-Institute of Molecular Plant Physiology (MPIMP) is located in the Max-Planck Campus in Potsdam Golm together with the MPI of research on colloids and interfaces (MPIKG) and the MPI of gravitational physics. The focus lies on *the development and implementation of phenotyping technologies and system approaches* [1]. It is divided in three departments: Metabolic Networks led by Prof. Dr. Stitt; Organelle Biology, Biotechnology and Molecular Ecophysiology led by the director of the Institute Prof. Dr. Bock and Molecular Physiology led by Prof. Dr. Willmitzer where my advisors' workgroup is located. The workgroup of Dr. Alisdair Fernie, central metabolism, mainly works on primary metabolites of tomato and *Arabidopsis thaliana* and especially on compounds involved in the Calvin- and Citric Acid Cycle [2].



Picture 1.2.1: The Max Planck Campus; from left to right: MPIMP, the central building and the MPIKG

1.3 Abstract

The main objective of this project was metabolite profiling of different tomato accessions. To achieve this, tomato fruits have been picked, the compounds extracted and derivatized for the following GC-MS analysis. In total 73 primary metabolites could be identified in the 42 lines using TagFinder software. It was found that some primary metabolites are found very concentrated in all fruits but the relative levels between the different lines vary strongly. This project is the first step to associate variable regions of the genome with the abundance of primary metabolites in the fruit and therefore the phenotypic trait of fruit quality.

2. Project referring information

2.1 Background information

This project belongs to the biological field of **metabolomics**, which is the analysis of all metabolites in a cell of an organism [3S.1017]. Metabolites can be primary or secondary, depending on the purpose either to be necessary for living or just being additional factors increasing the organism's fitness. Some primary metabolites are sugars, amino acids or nucleotides, whereas most antioxidants and compounds of essential oils are secondary metabolites.

To characterize an **organism's metabolome**, metabolic fingerprinting can be used to identify the phenotype. Because of the high variability of genotypes throughout animate beings and also within one species, the metabolic phenotype varies heavily.

The **tomato** (*Solanum lycopersicum*) originally comes from South America, where it was cultivated by the Maya more than 2500 years ago. Used a lot by the Aztecs, it was very uncommon for the arriving white people, so it took until the 1860s until tomatoes were widely grown and became popular [4]. It belongs to the nightshade family, of which many plants are poisonous, as some wild variations of the tomato still are. The fact that potato, eggplant and paprika also belong to this family favours the tomato's place as model plant. Over 50 % of the tomato's 34,727 protein coding genes are homologous to genes in potato [5 p.1 ff.] and therefore most research findings can be transferred similarly to other economically important members of the nightshade family.

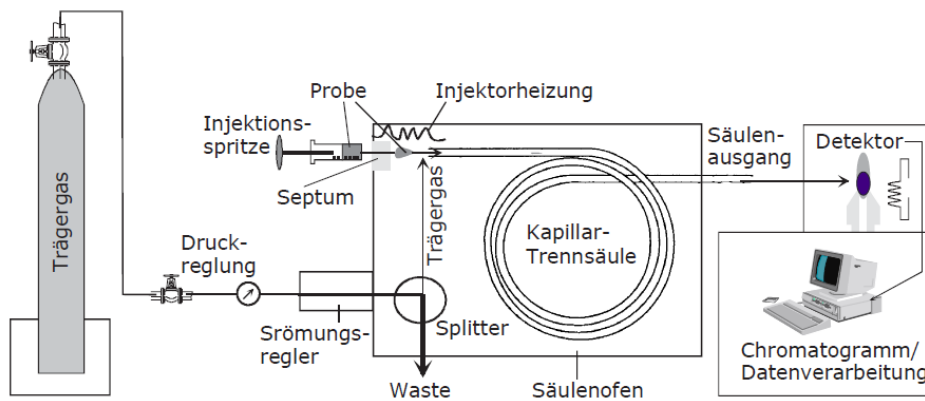
During **cultivation** some tomato lines were taken and bred resulting in our modern varieties, whereby many genes have been lost. It is believed that many compounds found in the wild tomatoes are good for our health [6] and therefore should be reintegrated into the economically grown tomatoes.

These lost wild genes could also improve **fruit quality**, which largely depends on the composition of primary metabolites in the plants [6]. Systematic gene based breeding or genetic modifying of plants, to achieve better traits, is an important objective for the industry but very little of the genes involved in primary metabolism are yet characterized. To find candidate genes, likely being responsible for a high or low level of a specific metabolite a Genome Wide Association Study (GWAS) approach can be used.

The concept of a **GWAS** is used to associate genetic polymorphisms to phenotypes levels to find the genomic region involved in interesting phenotypic traits, like the abundance of different metabolites. For example, differences in the abundance of a metabolite M found associated with nucleotide variance (for example allele A) in a certain genomic region, indicates that this nucleotide variance in the genome is a region that affects the level of metabolite M.

The nucleotide variances are called **Single Nucleotide Polymorphisms (SNPs)**. SNPs are variations of a specific nucleotide throughout the alleles of a locus, where each variation is present in an appreciable amount in a population. An SNP can affect the phenotype directly when the nucleotide sequence of an exon and therefore the primary structure of the expressed protein is changed. If the SNP is located in an intron, a different phenotype can be caused by differences in the gene regulation, for example because of alternative splicing or the RNA's stability.

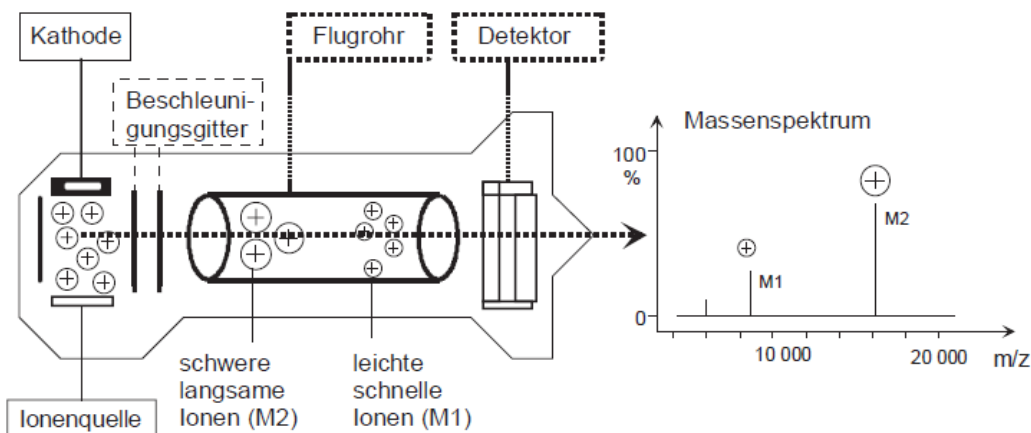
To perform a GWAS, also **phenotyping** is required, which is realized by taking samples of tomato fruits, extracting the compounds, separating them and measuring the concentration levels of each one. The last two steps are done with chromatography and mass spectrometry. Chromatography in general is a process of separating chemicals depending on some of their physical or chemical characteristics and uses a stationary and a mobile phase. Depending on the affinity of the chemicals to the stationary phase, the mobile phase can take them more or less with it. In a fixed amount of time different compounds will move different distance with the mobile phase, while a fixed distance will be covered in different amount of time. Thus, separation of the chemicals is achieved. In gas chromatography (GC), the mobile phase is a gas like nitrogen, helium or hydrogen, whereas the stationary phase is a fused silica capillary. The capillary has a fixed length and can be up to 100 m long which is why the separation is expressed in the time (retention time), the chemicals need to run through. In the GC only volatile substances and those, who do not decompose when heated can be separated. When the sample gets injected the molecules adsorb to the stationary phase differently depending on their interaction. After that, the temperature of the column slowly increases and the different compounds reach their boiling point one after another, evaporate, stop adsorbing to the column and instead float with the carrier gas [7 p.186 ff.]. This way the molecules get separated by their boiling temperature and their affinity to the column material.



Picture 2.1.1: Schematic structure of a gas chromatograph [9]

In the following “time of flight mass spectrometry” (MS-TOF) the separated molecules get ionized and often fragmented. The resulting ions are accelerated in an electric field and fly through a pipe, where the ions get separated by mass. Lighter particles fly faster and reach the detector earlier than heavier ones. All ions resulting from these fragmentation events are detected and form a fragmentation pattern, characteristic for every molecule. [7 p.327]

The MS-TOF is coupled with the GC so the separated molecules, leaving the column after their specific retention time are ionized and fragmented, which then is detected. This ensures that only one metabolite’s fragmentation is detected at a time.



Picture 2.1.2: Principle of a time of flight mass spectrometer [10]

2.2 Project information

In recent research, a GWAS of tomato crossings between wild and cultivated variations (parents) was made. Many candidate genes for primary metabolites were found, but the results need to be validated. To do this, the F1 Plants were then self-pollinated, to obtain homozygote plants in the F2 generation. The fruits of these plants were harvested and prepared for a GC-MS analysis, while a collaborating institute identified the genotypes. If this GWAS shows the same results and candidate genes as the previous, effort can be put in the further characterization of those genes.

3. Material and methods

3.1 Aliquotation

After harvesting, fruits were immediately frozen in liquid nitrogen to stop all enzymatic activity and then ground to a powder. To make all samples comparable and to prevent overloading in the chromatography $120\text{mg} \pm 5\text{mg}$ of the tomato powder have to be weighed and put into a labelled Eppendorf Tube (Eppi) and the exact weight must be written down. It is very important that the powder never melts, because the enzymes would become active and alter the metabolite composition.

3.2 Extraction

At first a stock of the extraction buffer with around 3.5 vol% ribitol in methanol is mixed, to ensure that every sample is extracted with exactly the same ribitol concentration. Ribitol is used later as internal standard to correct errors during pipetting or the chromatography. The estimated number of samples was smaller than 500, so the total volume of the stock requires $500 * 1,2\text{ml} = 600\text{ml}$ of methanol. To create the right concentration of ribitol $600\text{ml} * 0,035 = 21\text{ml}$ are added and the final concentration of $\frac{21\text{ml}}{621\text{ml}} \approx 3,38\%$ is acceptable as internal standard.

Adding 1200 μl of the extraction buffer deactivates all enzymes and extracts the compounds out of the powdered fruit. To support both processes, the samples are heated up to 70 °C and left for 15 minutes in the thermocycler. After taking out of the machine and cooling down 600 μl of water are added to dissolve also the more hydrophilic substances during the following vortexing of around 20 seconds duration. To separate the liquid phase with the solved metabolites from the solid phase consisting of dead cell parts, the tubes are then centrifuged at 12000 rpm for 10 minutes. 120 μl and 400 μl of the supernatant are each transferred into an Eppi and then dried in a Speedvac. The Speedvac is a machine which centrifuges under vacuum, so the liquid like methanol and water evaporate relatively quickly. The first sample (120 μl) will be used for the derivatization and the larger one (400 μl) is filled with argon gas to prevent oxidations and then stored at -80 °C as a backup.

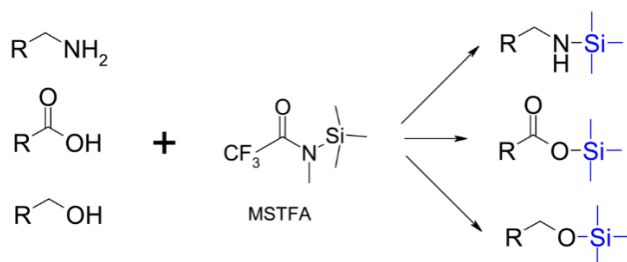
3.2.1. Quality Control

Because of the high number of samples each step was performed with a set of 60 samples at once, requiring some methods to obtain comparability. After the extraction of the first set some extraction leftovers were mixed, aliquoted to 120 μl and put in the Speedvac. These are Quality Control (QC) samples that all have the same composition. During later GC-MS analysis one QC is measured after

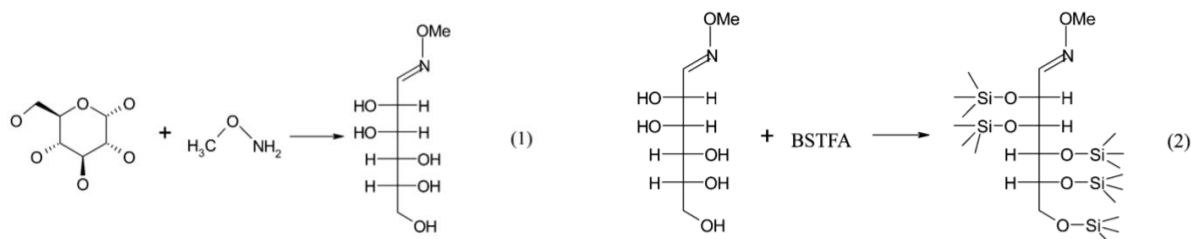
every 20 Samples, to identify errors or differences between the sets. In every set, all these steps are equally performed on an empty Eppi as a blank.

3.3 Derivatization

Because many primary metabolites like sugars and amino acids can have different forms they have to be fixed in one or two of these configurations to make the chromatography easier. This is done by adding 40 μl of 20 $\frac{\text{mg}}{\text{ml}}$ of Methoxyaminhydrochlorid (MeOX) dissolved in pyridine to the dried samples. This alters the structure of sugars by replacing aldehyde- and keto-groups with a methoxyimino group (see Picture 3.3.2). To complete this reaction the Eppis are shaken for 2 hours at 37 °C. After a short centrifugation to spin all drops to the ground, 70 μl N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) mix with 20 $\frac{\mu\text{l}}{\text{ml}}$ of fatty acid methyl ester mix (FAME) are added to the tubes. While now shaking for 30 minutes at 37 °C all hydrogens of amino- and hydroxyl-groups are trimethylsilyliated (see Picture 3.3.1) to make the metabolites more volatile for the chromatography. After this last step 120 μl of the samples are pipetted to the sample vials.



Picture 3.3.1: general scheme of chemical derivatization of metabolites by silylation [11]



Picture 3.3.2: reaction of glucose with MeOX (1) and its trimethylsilylation by BSTFA (similar to MSTFA) [12]

3.4 Gas chromatography – mass spectrometry (GC-MS)

The used carrier gas of the gas chromatography is helium at a constant flow rate of $2 \frac{ml}{s}$; the used column 30 m long and of the type DB-35. The injection temperature was 230°C and the transfer line and ion source were set to 250 °C. The initial temperature of the oven (85 °C) increased at a rate of $15 \frac{K}{min}$ up to a final temperature of 360 °C. After a solvent delay of 180 sec mass spectra were recorded at 20 scans per second with a m/z 70-600 scanning range.

The samples were placed in the GC-MS machine, and the program for the cleaning of the needle and the order of the QC samples is written into the software. In each set one QC should be tested every regular 20 samples.

3.5 Data processing

Chromatograms and mass spectra were analysed by using Chroma TOF 4.5 (Leco) and TagFinder 4.2 software. In Chroma TOF 4.5 the spectra of the blank and the QC samples could be evaluated. To connect the peak data of the mass spectrometry to a molecule and to save the data as a table TagFinder was used:

The first step using the program TagFinder is the time index calculation. The program has to find the different FAMES peaks in all samples and calculate the relative retention time index from the absolute retention time. To do this, different chromatograms are visualized in the Pegasus software and the range where each FAME appears is written into TagFinder.

Secondly, TagFinder is ran and finds the similar chromatography peaks of all samples depending on the retention time index and the specific fragmentation pattern. Lastly the chromatography peaks have to be annotated by hand. The criteria for correct annotating are: a similar retention time with only a small time deviation, a similar spectrum to the standard compound, a sufficient detection of major fragments and a linear correlation of most masses' intensities to each other [8]. A linear correlation between the masses' intensities has to be found throughout the whole chromatography peak, centre and sides. If the ratio between intensities changes, another compound must have eluted from the column at this time, interfering with the first compound's spectrum For every chromatography peak, this has to be checked and it must be decided if this is a real compound and if yes, which molecule it is. After exporting of the data from each sample and each compound the values must be normalized by the weight of the aliquotation and the measured ribitol concentration. This is necessary because a different weight of extracted material and imprecise pipetting results in different detected concentrations. To make the data reliable many replicate samples have been harvested and tested, so now the average concentrations of each replicate can be taken and used for further analysis. Some metabolites were detected too sparse to make statements about them, so they have been excluded

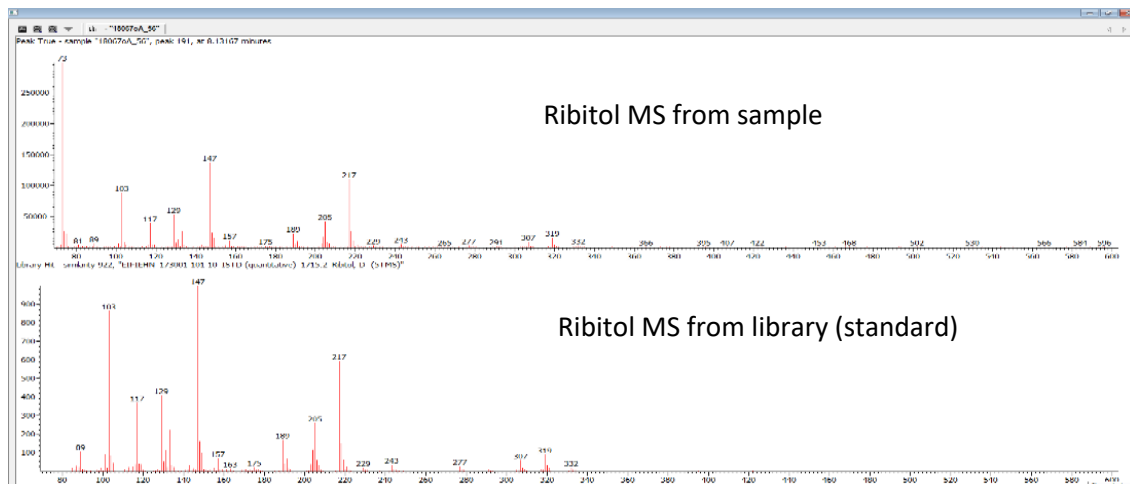
from further analysis. To create a heatmap the average concentrations (μ) and the standard deviation (σ) for each substance are calculated. Standardized values are received by applying the following formula to each measurement: $c_N = \frac{c - \mu}{\sigma}$.

4. Results

4.1 Quality Control

By visual examination of the quality controls was no major deviation visible.

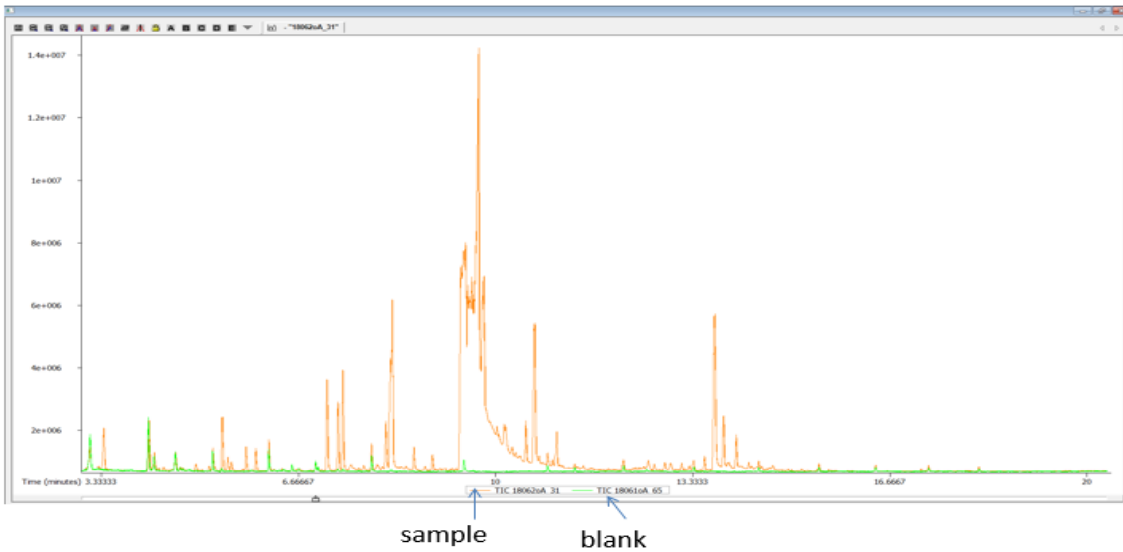
4.2 Ribitol



Picture 4.1.1: Fragmentation pattern of ribitol from a sample and a standard

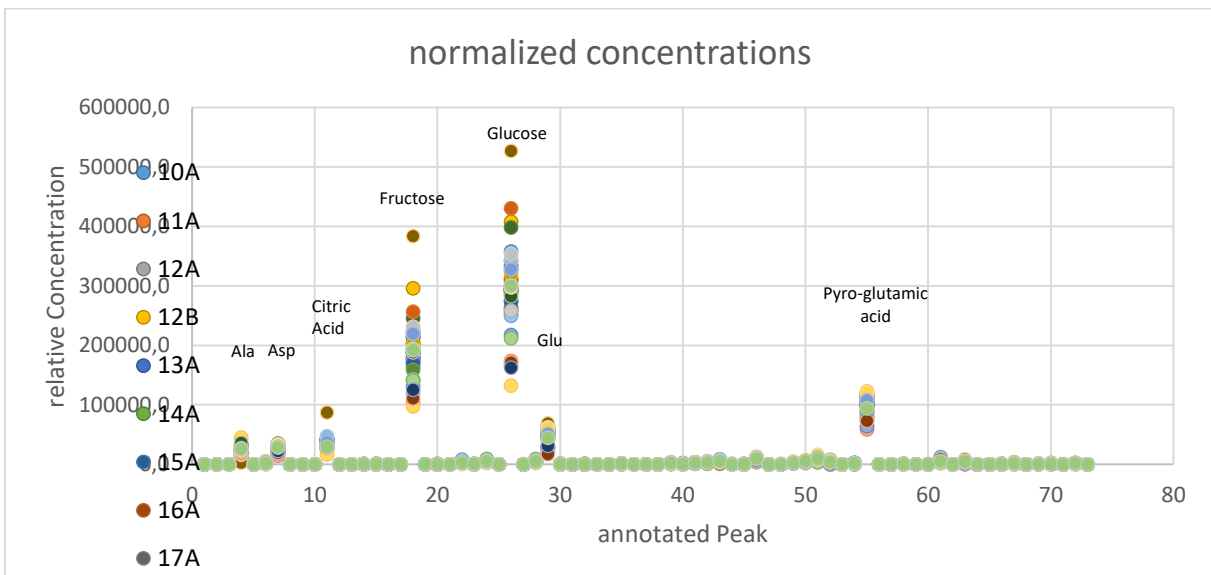
The picture shows a fragmentation pattern, which means every peak stands for a fragment with exactly that mass on the x-axis. The height of the peak shows the abundance of that fragment on the y-axis. Because every molecule is fragmented differently, many fragments are detected, but because for every molecule a most likely fragmentation exists, these fragments form the main peaks, like 103, 205 and 217 from ribitol. The real pattern differs from the standard, because of background signals and sometimes low detection levels.

4.3 Samples



Picture 4.3.1: Example chromatogram (all masses) of a blank and a sample compared

This chromatogram shows the sum of all detected masses (y-axis) based on the time (x-axis). The sample has high detection levels in the middle, which is caused by the presence of large amounts of sugars like glucose and fructose. The blanks have been looked through and all looked like in Picture 4.3.1. All blanks showed low concentrations, only a few and always the same peaks. Some of the blanks' peaks are the FAMES, which spread across the whole chromatogram.



Picture 4.3.2: Diagram showing the normalized concentrations of the compounds

This diagram is similar to the chromatogram, but only shows annotated peaks. The x-axis represents the peak's number, whereas the y-axis is the normalized concentration of the compound. The numeric integral of the chromatogram peak is the concentration, which was then divided by ribitol levels and the weight to get the normalized values drawn here.

It is seen that from left to right alanine, aspartic acid, citric acid, fructose, glucose, glutamic acid and pyroglutamic acid have a significantly high concentration throughout the samples.

Picture 4.3.3: Heatmap showing abundance of compounds in the different plants

A heatmap is a good way to visualize small amounts of data. Different numbers of a table are coloured differently depending on their value. In this heatmap positive values are red and negative ones are

blue whereas 0 is black. The data was hierarchically clustered, making it easier to find connections between the plants and metabolites.

5. Discussion

5.1 Errors

During the preparation and testing of the samples multiple errors could appear and affect the results. To prevent this, multiple techniques have been applied, like the use of blanks or the QC. If the pipets take a different volume than pre-set, every sample gets extracted and derivatized slightly different. Errors in pipetting can be filtered out using ribitol, as internal standard. Ribitol is not produced in tomatoes and gets added to every sample in the same concentration. Therefore, if the ribitol level is lower in the end, there must have happened some error, which will probably have influenced ribitol and all other substances equally. To adjust the ratio of all concentrations, they must be divided through the measured ribitol, which gives normalized values.

The blank samples had all very similar chromatograms and only a few peaks of the fames, so there was no contamination of the MeOX or the FAMES. The quality controls did not show anomalies either, which means that there is no notable difference between the sets.

In the GC-MS, the samples could run a little bit faster or slower through the column every time testing. To be able to adjust the retention time, the FAMES have been added to the samples. FAMES consist of fatty acid methyl esters of different length. Because they are added to every sample equally, their respective retention times can be compared and the retention time index can be calculated for each run. When annotating the peaks by hand in TagFinder many criteria must be evaluated, which requires experience and careful working. This may cause that not all possible compounds have been annotated.

5.2 Outcome

All the replicates and different samples are taken from the F2 generation of one set of parents. These parents differed strongly in the expression of some metabolites in fruit. Even though primary metabolites are important for all organisms to keep the cells alive, the concentrations in the fruit can differ strongly. This is because the primary metabolites are important for the flavour and some get overexpressed in the fruit.

Alanine, aspartic acid, citric acid, glutamic acid and pyroglutamic acid show the highest absolute concentrations, which is reasoned in their importance for the fruit. During ripening, the concentrations of free amino acids increases heavily. Especially glutamic acid is important for the umami flavour of

the tomato fruits [13]. Organic acids and sugars heavily influence the fruit's flavour [14], which explains the high values of glucose, fructose and citric acid. This is supported by the fact that cultivated tomatoes were analysed and therefore a good flavour and high levels of these metabolites have been achieved during cultivation. The results of glucose and fructose are not quantitatively reliable because they are both overloaded in the samples, which is visible in the chromatograms. To get exact values of the sugars, a split in the GC must be done.

As visible in the heatmap, the samples A31 to A40 are very similar to each other and completely, which could be reasoned in a similar genotype. B3 is completely different from these samples and shows very high relative metabolite concentration levels.

5.3 Outlook

To conclude this project the plants of which the samples have been taken will be genotyped and the SNPs will be identified. In a GWAS analysis, differences in metabolites can then be mapped to the different SNPs.

6. Conclusion

For me, working in the lab was very interesting. Even though there were so many samples and some tasks like labelling all the tubes or pipetting 120 µl of all samples in a new tube were a little boring, all in total I liked it very much because that is what scientists have to do (or let do) to get new knowledge. I could see the greenhouse and hear from the postdocs and PhD Students about their work. Before doing this internship, I wanted to work in the lab, and now I am even assured in this. In the few days where my adviser was not around I had to organize my work all by myself. It was a good experience when I had to switch all my plans as I realized someone else booked the machines because I forgot to.

I want to thank the whole workgroup for supporting me and making me feel like being part of the group. Most thank goes to Saleh Alseekh for supervising me and explaining the whole project in an understandable way and to Regina Wendenburg for the direct help with the experiments and also giving me a little insight in her other projects. All this could not have been possible without the *Förderverein der BiologieOlympiade e.V.*, who organized and payed this internship for me.

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- [10] Picture 2.1.2: Gey (2015) Instrumentelle Analytik und Bioanalytik, 3.Auflage, Berlin Heidelberg: Springer Verlag(S.328)
- [11] Picture 3.3.1: Silas G. Villas-Bôas , Kathleen F. Smart, Subathira Sivakumaran and Geoffrey A. Lane (2010) Alkylolation or Silylation for Analysis of Amino and Non-Amino Organic Acids by GC-MS?
- [12] Picture 3.3.2: Lunzhao Yi, Shuting Shi, yi Zhibiao, Yizeng Liang (2014), MeOx-TMS derivatization for GC-MS metabolic profiling of urine and application in the discrimination between normal C57BL/6J and type 2 diabetic KK-Ay mice

300	7027-5A	30,0	120,8	19,5	30311,4	140,5	3362,7	26993,6	87,2	16,9	95,0	45707,8	110,9	8,1	673,8	545,6	85,5	43,9	262838,3	41,1	328,1	25,0	2705,7
301	7031-22A	47,1	132,0	85,0	6696,9	192,9	1930,2	38068,6	13,6	13,7	42,6	24303,8	117,2	4,0	564,1	461,5	112,9	60,7	281139,3	37,9	431,3	92,0	1507,6
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311	7008-11A	21,4	141,7	10,9	5991,1	63,6	1515,8	17209,4	20,6	3,4	26,2	33047,7	88,3	5,9	0,8	268,1	53,8	28,7	182122,4	29,6	268,4	25,2	314,1
312	7025-5A	229,6	156,1	91,8	28161,4	222,4	1873,2	32178,1	48,4	66,5	113,2	76749,4	83,9	9,9	571,3	517,3	120,0	59,3	373422,0	36,1	307,8	87,8	1786,4
313	7041-20A	61,6	158,6	32,5	43123,5	104,1	2380,7	27044,2	215,2	18,6	85,0	28092,2	55,6	15,5	0,9	191,9	100,9	47,0	266912,8	57,4	335,6	24,8	7016,4
314	7043-1A	110,1	172,8	142,4	13737,6	282,6	1352,2	41569,6	44,6	78,8	181,3	21076,5	60,1	15,6	492,0	440,1	112,5	60,8	522274,7	45,7	382,5	195,3	2589,8
315	7043-24A	19,3	150,5	41,3	4973,6	166,3	1116,4	26406,8	14,1	49,5	112,9	23725,4	113,4	8,3	726,7	609,8	80,8	41,8	372385,7	53,6	377,2	23,5	859,2
316	7035-27A	35,1	171,2	74,7	11538,9	188,0	3279,7	35235,9	59,8	51,6	114,4	55925,8	146,0	13,8	497,2	431,0	128,9	66,9	434999,1	54,9	383,8	71,6	3270,3
317	7012-12B	57,9	144,8	30,8	7503,4	116,7	801,4	31117,9	44,4	55,7	139,7	24916,2	46,4	11,5	265,8	197,6	94,0	47,9	296343,4	55,8	333,2	30,9	6552,6
318	7035-1 or	418,8	159,5	67,3	2610,4	125,1	1462,1	27492,9	17,4	13,0	93,5	#WERT!	40,4	7,0	363,1	314,4	79,7	41,1	#WERT!	28,4	372,3	34,5	1586,9
319	7031-6A	29,8	156,3	38,5	12694,0	98,3	5361,2	29577,1	83,4	24,3	98,5	39737,8	124,4	13,3	360,0	289,3	56,1	26,8	238942,6	42,8	283,2	30,8	4068,2
320	7029-8A	43,4	164,2	85,2	29776,2	216,1	2466,0	39641,9	128,4	85,6	277,0	28114,2	107,1	15,9	551,0	516,4	101,5	52,2	527918,8	36,8	376,7	21,2	10882,2
321	7043-25A	121,8	169,2	23,3	52762,6	199,7	2304,0	37572,0	159,4	104,7	226,2	28465,5	116,0	20,5	472,7	421,4	104,1	52,9	464637,7	64,7	412,0	58,6	6974,3

298	413,3	5962,0	11,6	316860,9	219,6	5962,0	23773,9	125,5	4,1	272,5	12,8	19,1	334,1	53,9	12,8	46,1	672,5	536,8	827,2	2020,8	7460,7	21,6	231,2
299	586,5	2027,5	24,9	454721,3	422,1	2027,5	55704,9	191,3	11,3	204,8	42,8	110,3	1092,3	190,4	31,5	105,1	2475,7	377,6	2729,5	2231,4	7873,0	44,8	377,6
300	394,9	5561,1	14,4	343425,2	365,1	5561,1	33169,9	494,4	8,2	143,8	38,5	27,0	978,1	131,4	31,8	80,6	3724,6	304,4	2670,6	3094,0	3130,1	8,6	349,1
301	420,9	4314,9	19,6	402063,9	310,1	4314,9	70539,2	183,1	8,4	243,4	24,4	72,1	381,7	299,2	10,0	103,0	1186,8	525,9	2696,6	2489,4	8473,1	118,8	286,2
302	372,9	5028,2	17,6	304794,1	322,4	5028,2	46889,1	351,4	8,4	253,7	1,6	53,0	965,7	122,2	26,3	58,6	1068,5	1146,6	1248,3	1852,0	2744,8	29,4	150,5
303	392,5	3834,7	17,4	272128,4	382,2	3834,7	60008,8	555,7	5,5	172,4	7,4	51,0	880,2	303,2	47,4	47,9	2200,1	555,7	2553,6	3913,0	4160,5	40,1	359,9
304	584,8	3263,7	15,4	500591,9	333,7	3263,7	76811,7	360,8	14,9	183,2	59,4	67,8	1003,3	257,4	31,2	87,3	3099,4	633,5	4436,7	2942,1	5944,5	216,0	932,6
305	363,8	2992,6	12,7	242660,2	305,5	2992,6	32323,2	454,5	5,2	224,5	5,6	23,7	1143,9	124,1	45,4	50,3	1298,0	2943,8	1610,9	3141,0	4583,3	36,8	404,6
306	462,2	1831,7	37,6	768281,0	415,2	1831,7	88150,2	316,9	15,8	268,7	8,4	52,4	458,6	226,7	14,9	139,8	1703,2	1647,6	3275,9	3198,3	919,9	196,1	773,2
307	258,3	3889,7	8,5	173449,9	314,9	3889,7	37646,1	980,4	6,3	177,1	7,3	53,2	1818,8	187,4	95,0	47,4	2091,5	1617,1	2882,1	4739,3	5880,6	30,5	743,3
308	362,0	2573,1	14,6	326168,9	221,9	2573,1	26376,3	328,4	6,4	169,1	1,1	19,9	927,0	68,2	11,6	57,0	1861,8	1374,9	1884,6	3028,5	2599,7	28,9	348,2
309	272,6	2637,0	13,3	296827,2	433,1	2637,0	54428,7	742,3	6,3	195,0	59,6	43,3	1255,7	230,5	46,7	43,4	2543,9	755,4	3782,8	4206,2	1722,9	58,0	520,3
310	414,6	1124,6	12,8	184267,9	446,4	1124,6	85902,3	1156,8	6,3	248,2	45,9	36,7	886,0	488,5	63,2	64,9	1538,3	2201,0	1824,0	5516,6	10884,6	65,2	625,7
311	219,3	4766,4	16,0	276024,5	271,7	4766,4	17870,7	220,7	5,4	209,6	1,0	20,7	333,8	59,8	28,4	65,4	367,2	1105,8	536,1	1266,5	4938,5	7,3	249,0
312	743,9	3871,0	23,4	521888,9	368,1	3871,0	57689,9	226,0	8,5	239,3	5,7	38,0	1018,2	238,2	11,7	53,5	1165,8	662,4	1879,1	1637,8	16392,4	47,9	169,0
313	328,0	1098,7	11,3	376463,9	285,7	1098,7	42376,5	541,8	9,0	241,9	32,9	73,2	1166,7	146,2	64,2	88,6	1599,2	748,0	2749,7	3424,2	1938,7	159,5	420,6
314	525,4	5807,5	24,6	789043,6	315,6	5807,5	70477,4	132,5	17,1	292,5	35,2	64,1	1175,5	162,4	16,9	98,9	3447,9	467,7	3312,9	1876,7	5782,0	157,5	602,0
315	349,8	4734,6	19,9	526739,6	363,5	4734,6	36118,6	144,2	9,6	236,2	13,3	89,1	398,0	116,9	18,7	114,8	945,0	934,5	1254,8	1288,9	2260,5	18,1	327,2
316	384,2	5016,2	21,6	602556,5	399,8	5016,2	68670,3	403,7	9,9	202,0	44,2	108,5	656,9	281,6	35,4	0,5	1001,8	940,9	1680,4	3041,8	10706,3	111,1	888,0
317	422,0	3743,5	12,3	408219,3	288,6	3743,5	56566,1	170,6	8,2	239,2	17,9	62,6	955,1	86,2	22,1	123,2	909,1	768,2	1521,7	1289,4	1394,6	129,7	128,5
318	743,4	2848,5	21,1	#WERT!	301,1	2848,5	64480,2	278,9	5,0	247,7	19,6	41,7	271,1	259,7	22,6	0,7	567,7	1192,8	1649,8	3279,5	3313,9	48,2	288,1
319	375,6	3017,2	14,9	354608,4	258,2	3017,2	49715,7	1384,7	5,9	166,6	1,0	44,2	514,9	235,5	44,0	49,1	1027,2	561,3	2619,3	4271,7	4630,0	42,3	1003,1
320	408,5	3444,6	29,4	792436,9	375,0	3444,6	90418,3	531,0	8,4	248,5	51,5	89,0	1980,3	273,6	23,1	118,5	2579,1	811,8	3540,5	4360,3	813,7	289,0	440,2
321	465,8	3470,0	22,0	673883,2	386,7	3470,0	60246,0	493,0	15,6	232,8	12,8	76,0	2733,0	204,4	64,1	110,1	4490,8	1388,1	7142,3	2804,3	6269,7	73,9	1035,1

The column headings from left to right:

2-oxo, glutaric acid; adenine; adenosine-5-monophosphate; alanine; ascorbic acid; asparagine; aspartic acid; beta, alanine; calystegine A; calystegine B2; citric acid; cysteine, S-methyl; cysteine; dehydroascorbic acid; dehydroascorbic acid dimer; erythritol; erythrose; fructose; fructose-6-phosphate; fucose; fumaric acid; GABA; galactinol; galactonic acid; galacturonic acid; glucose; glucose-1-phosphate; glucuronic acid, lactone; glutamic acid; glutamine; glyceric acid; glycerol; glycerol-2-phosphate; glycerol-3-phosphate; glycine; histidine; homoserine; inositol-1-phosphate; isoleucine; lactic acid; leucine; lysine; malic acid; maltose; methionine; myo, inositol; nicotinic acid; ornithine; phenylalanine; phosphoric acid; proglutamate; proline; proline-4-hydroxy; putrescine; pyroglutamic acid; pyruvic acid; quinic acid, 3-caffeoyl; quinic acid; raffinose; rhamnose; serine; succinic acid; sucrose; sugar similar to raffinose; talose alpha; threonic acid; threonine; tri-sugar; tryptophan; tyrosine; urea; valine; xylose