Hungarian University of Agriculture and Life Science

Szent István Campus

Plant Biotechnology MSc Course

METABOLOMIC ANALYSIS AND SOME GENETIC TRAITS OF GRAFTINGS OF *SOLANUM TUBEROSUM* AND SOLANUM *LYCOPERSICUM PLANTS*

Primary Supervisor: Vanda Villányi

research fellow

Author: **Catherine Okaroni O.**

XAFZU5

Institute/Department: **Institute of Genetics and Biotechnology, Department of Plant Biotechnology**

Gödöllő

2023

Table of Contents

LIST OF ABBREVIATIONS

- RT-PCR- Reverse Transcriptase- Polymerase Chain Reaction
- PLS-DA- Partial least squares discriminant analysis
- qPCR-quantitative Polymerase Chain Reaction
- NCD- non communicable diseases
- PCA- Principal Component Analysis.
- VIP- Variable importance
- (bHLH)- basic-helix-loop-helix
- MBW - MYB-bHLH-WD40
- MYB- myeloblastosis
- WDR- WD-repeat
- LDL- Low density lipoprotein
- ROS- reactive oxygen species
- (ICAM-1- intercellular adhesion molecule-1
- VCAM-1, vascular cell adhesion molecule-1
- TNF- tumour necrotic factor
- ANT – anthocyanin
- CK- chemokines
- CRP- C-reactive protein
- IL – Interleukins
- CSC- cancer stem cells
- CRISPR- clustered regularly interspaced short palindromic repeats.
- UV- ultraviolet
- PAL- Phenylalanine
- ANS- anthocyanidin synthase
- DFR- dihydro flavonol-4-reductase
- bZIP-basic region/leucine zipper motif
- ERF- Ethylene responsive factor
- HSF- Heat shock factor
- ARF- Auxin responsive factor
- ZFC- Zinc factor cluster
- C2H2- Cys2His2 zinc finger motifs
- HD-Zip- homeodomain- leucine zipper
- NaAc- Sodium acetate
- EDTA- ethylenediaminetetraacetic acid
- SDS- Sodium Dodecyl Sulfate
- LiCl- Lithium chloride
- cDNA- Copy Deoxyribonucleic acid.
- TBE- Tris-borate-EDTA
- MEOX- Methoxamine
- MSTFA- Methyl-N-(trimethylsilyl)trifluoroacetamide

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is a perennial plant belonging to Solanaceae's family. It is native to Southern America near Lake Titicaca, probably on the borders of Bolivia and Peru. It is the most extensively consumed vegetable worldwide, producing approximately 376M tonnes annually. In yields per tonnes, China leads India and Russia with an annual estimate of more than 66M. Africa immediately follows in terms of production. Compared to other crops, potato is the fourth largest non-grain crop produced worldwide after wheat, maize, and rice. Currently, it is grown and consumed by over 150 countries, playing a pivotal role in food security (Enciso-Rodriguez et al., 2018).

In Europe, the potato, a requisite crop in Northern and Eastern parts, was brought by Spanish travelers by the end of the 16th century and further spread by the Britons and Portuguese. According to Tomlekova, et al. (2022), the European Union produces an estimated 112M tonnes of potatoes annually, whereas, in Hungary, production is estimated at 40285 tonnes.

In developing countries like Africa, where the population density is very high, the available land is usually small-scale. People depend majorly on these small farms to support their large families. As a result, the available food is usually limited. To salvage the situation, the focus is shifted to crops that have a shorter life cycle and can support either double cropping or intercropping systems.

Potato, a valuable crop that can provide a high-quality product and yield per unit input, forms a significant part of the diet worldwide. It has a pliable and short life cycle that ranges between 100 to 120 days compared to other crops, and it can thrive well in conditions that other vegetables cannot. Potato is an excellent source of carbohydrates, dietary fibers, vitamin C, and various health-promoting compounds, like antioxidants, caffeic acid, chlorogenic acid, and Patatin, a unique tuber storage protein. Starch in potatoes is used in paper, textiles, food, pharmaceuticals, and dye industries (Chen, et al., 2022).

To improve the quality of potatoes, human interventions must be implemented considering the poverty level in Africa. therefore cheap, easy, and simple techniques that don't require complicated equipment should be considered. Grafting technology has been applied in other crops to enhance crop resistance to biotic and abiotic stresses, improve the branching structure, and increase crop quality and yield. The technology is easy, simple, and cheap compared to molecular breeding and genetic engineering, which are expensive and require expertise and enclosed laboratories, and expensive equipment to execute it. This experiment requires conducive environment for tissue culture, expensive reagents equipment for gene expression and GC-MS analyses together with expertise. The equipment and reagents were limited in this case.

2. OBJECTIVES

Poverty in developing countries is a vital cause of malnutrition and unhealthy diets. This usually leads to changes in the lifestyle of the people. This results in risks of non-communicable diseases, including cardiovascular diseases, type 2 diabetes, cancer, and obesity, the primary cause of death (Mattoo et al., 2022a). However, these health conditions can be remedied using plant extract Phyto-molecules such as anthocyanin, which can be obtained from ready potatoes.

Anthocyanin, rich in antioxidants, can counteract the effects of chronic oxidative stress and inflammation caused by non-communicable chronic diseases (NCD) by donating hydrogen from hydroxyl groups located at the aromatic ring, thus eliminating free radical that causes oxidation of lipids and other biomolecules by neutralizing them.

The anthocyanin found in the potato is usually restricted to the epidermis of the tuber. However, the low anthocyanin concentration does not meet the recommended daily intake requirement. The pigmented potatoes with red and purple skin and flesh have elevated antioxidants. Therefore, to increase their concentration, grafting technology could be embraced.

In this study, grafting of tomato, a close relative of potato which belongs to the same nightshade family on potato rootstock, is anticipated to increase the concentration of anthocyanins in potatoes that need to be ascertained. The advantage is that the two plants remain genetically different while depending on each other for nourishment and growth. In this way, the land required by the two plants is significantly reduced because fruits and tubers are produced on the same plant.

In this experiment, non-grafted control, homo-grafted- and tomato (Mobile) grafted plants of three potato Cultivars; Désirée, Hópehely, and White Lady, were used. The goal was to establish the variation of anthocyanin content, the expression of some anthocyanin synthesis related genes in tuber skin, and metabolic composition of the tubers using Spectrophotometry, RT-PCR and RT-qPCR, and Gas Chromatography-Mass Spectrometry respectively.

Main objectives of these examinations were:

1. To assess the effects of tomato grafting on the potato skin color
2. To determine the effects of tomato grafting on potato tuber metabolites
3. To study the changes in gene expression of tuber skin because of heterografting

3. LITERATURE REVIEW

3.1. Anthocyanin biosynthesis

Anthocyanins are unique glycosylated flavonoids that are water-soluble vacuolar pigments of the phenylpropanoid pathway (Chaves-Silva, et al., 2018). Besides chlorophyll, anthocyanins are the most visible group of pigments, giving shades of pink, red, blue, and purple hues to leaves, flowers, fruits, seeds, stems, and roots (Ahn, et al., 2022). Anthocyanins are naturally occurring, restricted to the epidermis, provide quality to fruits, seeds, tubers, and vegetables, and are widely distributed in the plant kingdom (Jaakola, 2013).

Approximately 700 different Anthocyanins have been identified in nature their variation is based on the position of attachment of sugar, hydroxyl, acyl, and methyl, with the most common derivatives in plants being Malvidin(purple), Delphinidin(purple), Cyanidin(magenta), Petunidin(purple), Pelargonidin(red), and peonidin(magenta) **(see figure1)** (Oertel et al., 2017); (Xing et al., 2023).

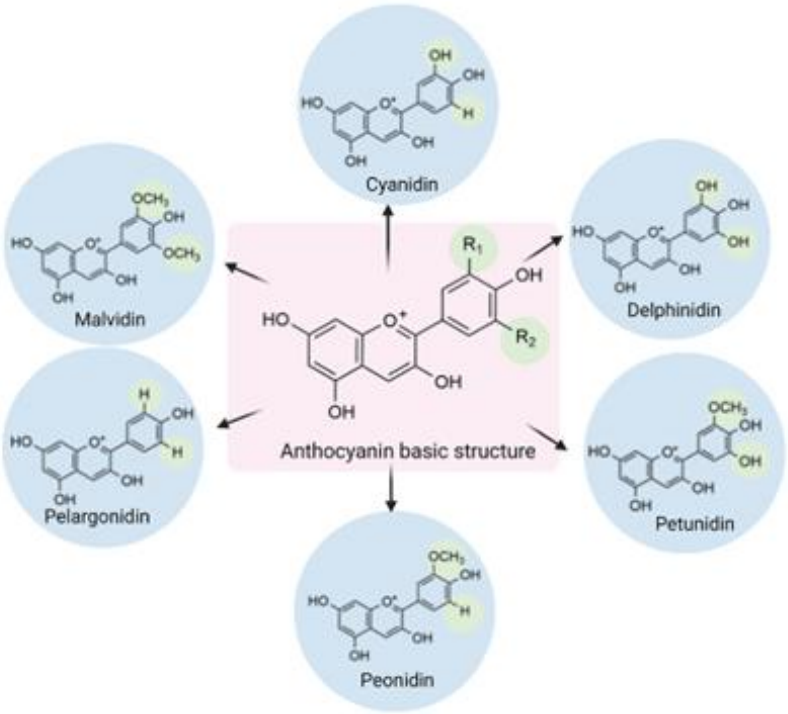

Fig. 1. The basic structure and variation of anthocyanin according to Xing, et al., (2023).

The Anthocyanin biosynthesis pathway has a complex regulated network, and it's well-explicated in all plants. It is a coordination between regulatory and structural genes that express and encode proteins (Staiti, et al., 2022). In summary, anthocyanin biosynthesis is carried out by the phenylpropanoid pathway; it occurs in the cytoplasm and is

stored in the plant vacuoles (Wang et al., 2023a). During its synthesis, the precursor, Phenylalanine is deaminated by phenylalanine ammonia-lyase to form trans-cinnamic acid, which is a substrate for cinnamic acid 4-hydroxylase that forms coumaric acid, Coenzyme A of Coumaric acid is produced by 4-coumaroyl Coenzyme A ligase.

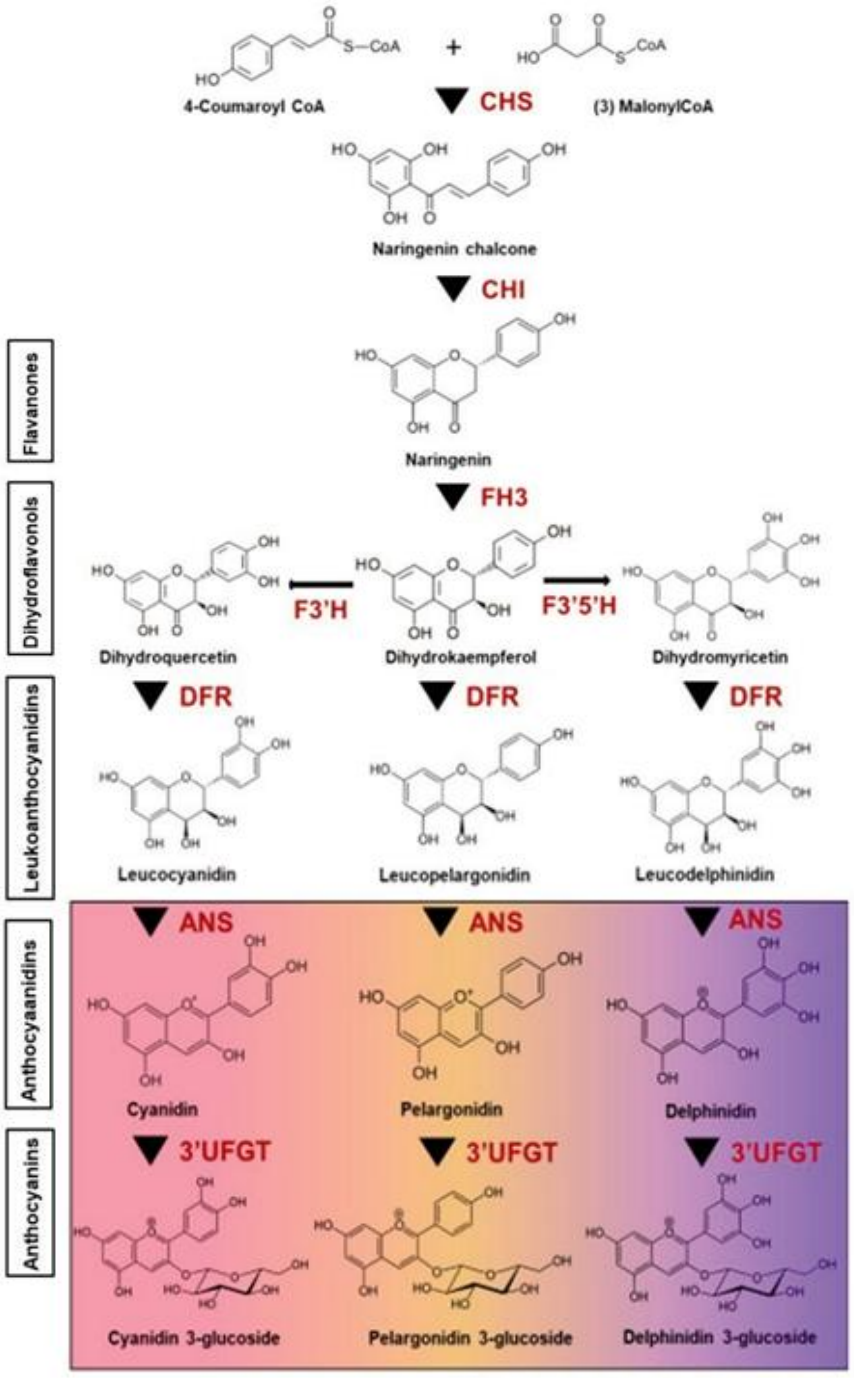

Fig.2. Schematic structure of anthocyanin biosynthesis according to (Staiti et al., 2022)

At this point, the branch for Lignin biosynthesis is formed. This is followed by synthesizing three molecules of malonyl-CoA and one molecule of coumaric acid to create a tetrahydroxy chalcone catalyzed by chalcone synthase. The chalcone isomerase catalyzes the flavanone naringenin and the flavanone 3-hydroxylase hydroxylates the naringenin to form dihydro flavanol**.** The dihydro flavonol-4-reductase produces the colorless anthocyanin, and the formation of colored anthocyanidin is catalyzed by anthocyanin synthase/leucocyanidin dioxygenase (S. Zhang et al., 2019)**;** (Yan et al., 2021)**.** The cinnamic acid 4-hydroxylase, 4-coumarate–CoA ligase, and phenylalanine ammonia-lyase are the upstream structural genes of the phenylpropanoid pathway.

The genes result in flavonoid biosynthesis as Early biosynthesis Genes which include: chalcone synthase, chalcone isomerase, flavanone 3-hydroxylase, flavonoid 3' 5 -hydroxylase, and flavonoid 3`-hydroxylase that are regulated by; MYB75/PAP1 and subsection of 7R2R3- MYB transcription factors which comprise of MYB111, MYB11, and MYB12 gene loci. Whereas the Late Biosynthetic Genes, which include dihydro flavanol 4-reductase, anthocyanidin synthase/leucoanthocyanidin dioxygenase, and UDP-glucose flavonoid glucosyl transferase are crucial in anthocyanin biosynthesis and are regulated by MBW Transcription factors: Myeloblastosis family, a basic helix-loop-helix, and the tryptophan-aspartic acid repeat.

The bHLH and MYB usually bind directly to structural genes' promoters while the WD40 coordinates the interaction. Together with other modifying and structural genes, the modification and transportation of anthocyanin are achieved **see figure 3** (H. Liu et al., 2021)**.**

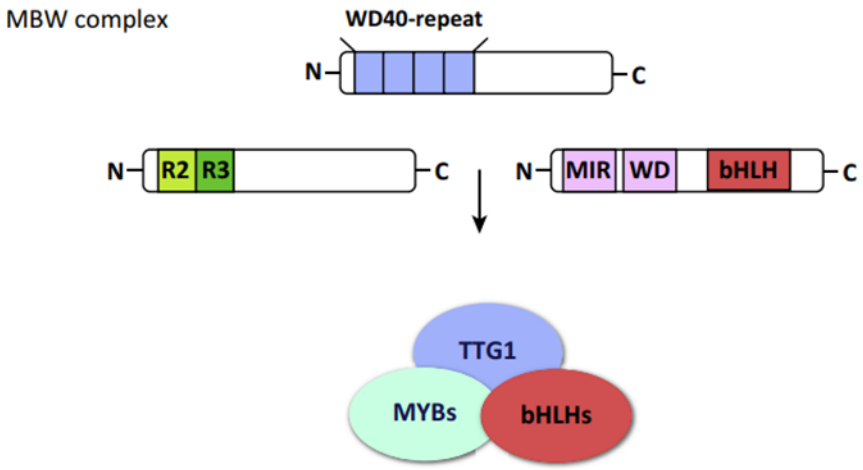

Fig 3. Schematic structure of WDR complex. According to Xu, Dubos and Lepiniec, (2015)

Anthocyanin biosynthesis is mainly regulated by the R2R3-MYB, basic-helix-loop-helix, and WD-repeat proteins which are involved in hormone signal transduction and abiotic stress tolerance **(See figure 3).** The process is initiated on the onset of stress, activating the R2R3-MYB genes. The activated R2R3 forms an MBW complex with Bhlh1 and WDR proteins, this complex in turn activates the Bhlh2. The Bhlh2 forms a complex with R2R3-MYB and

WDR proteins that eventually activates the genes that encode for R2R3-MYB repressor, DFR, and R3-MYB repressor (J. Liu et al., 2015).

These TFs can work either dependent or independently of the MYB-bHLH-WD40 complex and can activate or repress the expression of genes responsible for anthocyanin synthesis. It's documented that, the activation of SlMYB12 in tomatoes, VvMYBA2, and VvMYBA1 in grapevine, AtMYB75, AtMYB113, AtMYB 114, and AtMYB90, in Arabidopsis Thaliana, MdMYBA, MdMYB110a, MdMYB1, and MdMYB10 in Apples enhanced the biosynthesis of anthocyanin positively (Y. Liu et al., 2019).

The negative expression effect is usually brought by the action of R3-MYB and R2R3-MYB repressors, which possess one or two repeats of the MYB domain region. The R2R3 MYB repressors have a characteristic C-terminus that carries the C2/EAR motif (LxLxL or DLNxxP, and the C1(LlsrGIDPxTN HR as reported by (Li et al., 2022). Similarly, repression of AtMYB3/4/6 in Arabidopsis, PhMYB17, and PhMYB4 in petunia, FaMYB1 in strawberry, and MdMYB16/17/111 in Apples showed negative expression of anthocyanin in the plants' tissues(Chen, Zhang, et al., 2022). In addition to the MYB-Bhlh-wd40 complex, other families involved in the regulation include MADS-box, WRKY, HD-Zip, C2H2 ZFC, Bzip, ERF, HSF, and ARF(Naik et al., 2022).

Besides regulatory and structural genes, other factors such as light, nutrient availability, temperature, and biotic stress affect the action of transcription factors by either up-regulating or down-regulating the synthesis and accumulation of anthocyanins. Plants subjected to low temperatures showed upregulated anthocyanin, while on the other hand, high temperatures repressed the accumulation. In addition, high light intensity upregulated anthocyanin biosynthesis; however, nutrient deficiency upregulated anthocyanin accumulation in case of phosphorous deficiency. Pests and diseases also induced the expression of anthocyanin (S. Li et al., 2022).

In apples, light intensity downregulates the biosynthesis of anthocyanin by repressing the activity of the MdBBX3 transcription factor. However, in red pears, light intensity enhanced the accumulation of anthocyanins by upregulating the expression of PpBBX16 (H. Liu et al., 2021). According to Yan, et al. (2021), high sucrose and light intensity stimulated anthocyanin biosynthesis in Arabidopsis Thaliana by regulating the AtPAP1 transcription factor, whereas in kiwifruit low temperatures that regulate AcMYBA1-1 and AcMYB5-1 transcription factors, induced anthocyanin biosynthesis **see figure 4 below.**

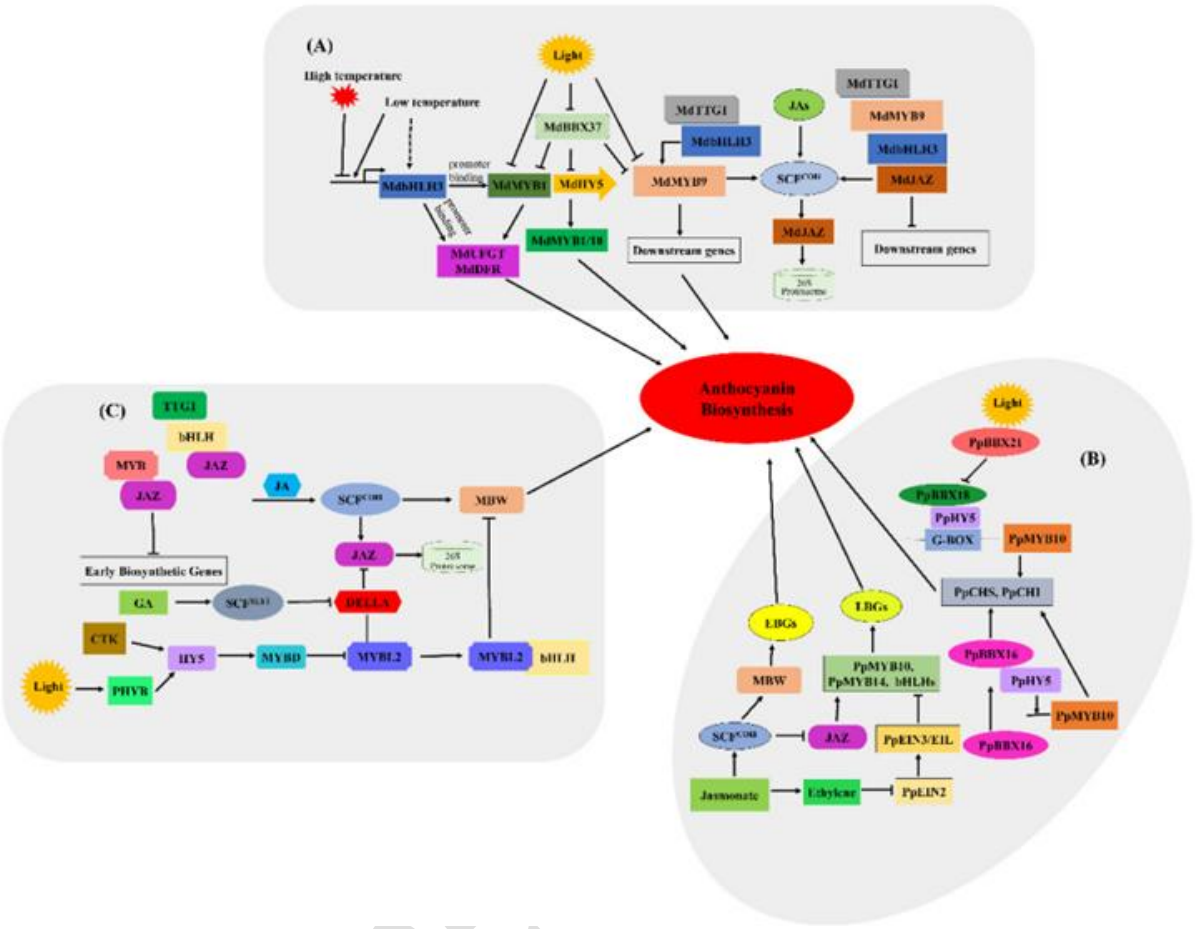

Fig.4 schematic figure detailing the effects of temperature and light on anthocyanin biosynthesis in red-fleshed Apples(A), phytohormones involved in anthocyanin biosynthesis in Arabidopsis, and Pears B and C respectively according to Liu, et al. (2021).

3.2. Significance of anthocyanins

The agglomeration and appearance of anthocyanin usually depend on species, variety, cultivar, stage of plant development, and atmospheric conditions. In plants, anthocyanin is brightly colored and is crucial in pollination and seed dispersal by attracting pollinators. In addition, it protects the plant against distress from cold, salt, drought, UV radiation, nutrient deficiency, pests, and diseases (ZHANG et al., 2020).

The above-mentioned stresses usually alter plants' biochemical and physiological state; in this way, plants respond by heightening the anthocyanin synthesis. According to Kaur, et al., (2023a), Hao, et al., (2022), (Wang et al., 2023b), the studies showed that in potatoes, Arabidopsis, pigeon peas, barley, grapevine, and tobacco, the accumulation of anthocyanin helped to alleviate drought strain.

In addition, Arabidopsis, retained a high dry mass in salt stress, plants were protected from osmotic potential in salt trauma, Arabidopsis and grapevine were protected from nutrient stress, and UV rays were photo filtered by the accumulation of anthocyanin. As indicated in **(figure 5).**

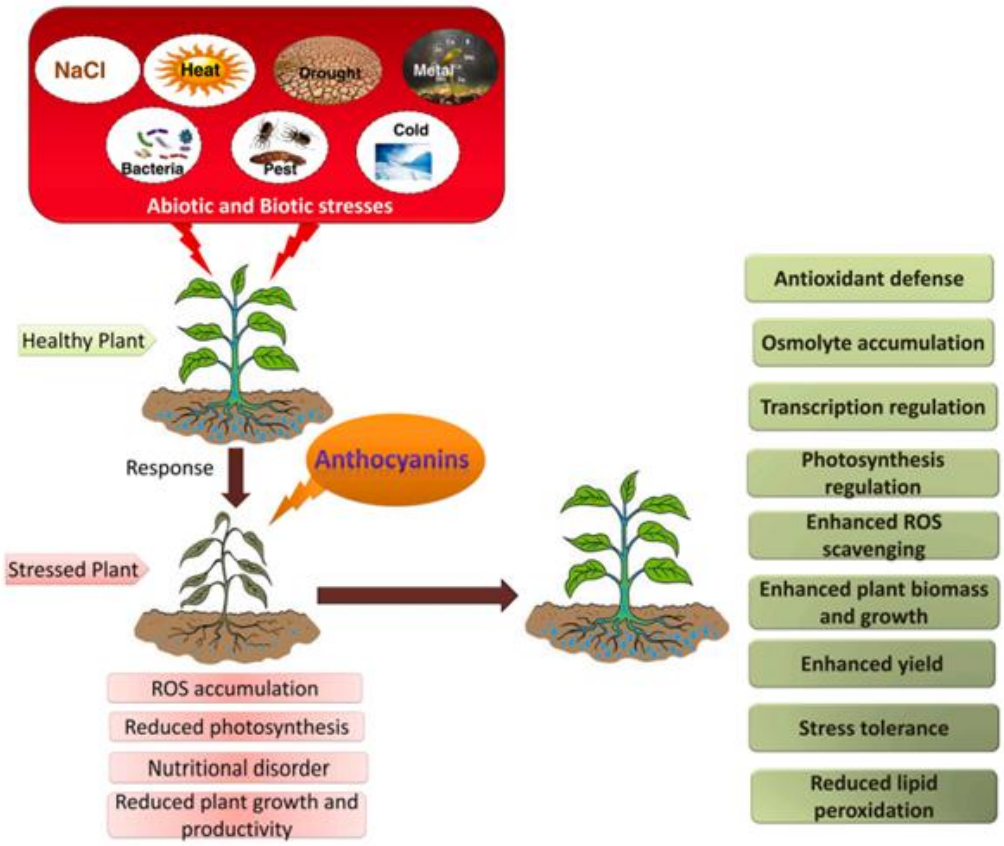

Fig. 5. Plants respond to various stress by the heightened accumulation of anthocyanin, according to Kaur, et al. (2023b).

In the human body, anthocyanin plays a crucial role in alleviating the occurrence of non-communicable diseases that are on the rise worldwide. The primary cause of Non-Communicable Diseases is malnutrition, which is brought about by unhealthy diets, and lack of physical activity (Bigna et al., 2017).

Globally, over forty million people die annually, accounting for 71% of total deaths because of non-communicable diseases, namely Cancer, cardiovascular diseases, respiratory diseases, and type 2 diabetes. In developing nations like Africa, where poverty is on the rise and the population growth rate is rapidly increasing, resulting in small, fragmented parcels of land that cannot sustain large families, impacting their nutrition and lifestyle, NCD is on the double. It is estimated that by 2030 NCD will surpass communicable diseases (Chikowore et al., 2021), (Owino, 2019), (Hunter-Adams et al., 2019).

In the endothelium lining, NCD commences and advances as chronic inflammation, leading to atherosclerosis development caused by the deposition of low-density lipoproteins. Anthocyanin enhances the synthesis of Nitric Oxide, which protects the endothelial cells that produce inflammatory mediators vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, that convey attachment molecules for the white blood cells to anchor and burn the deposited low-density lipoprotein in the walls of arteries Mattioli et al. (2020) as elaborated in **figure 6.**

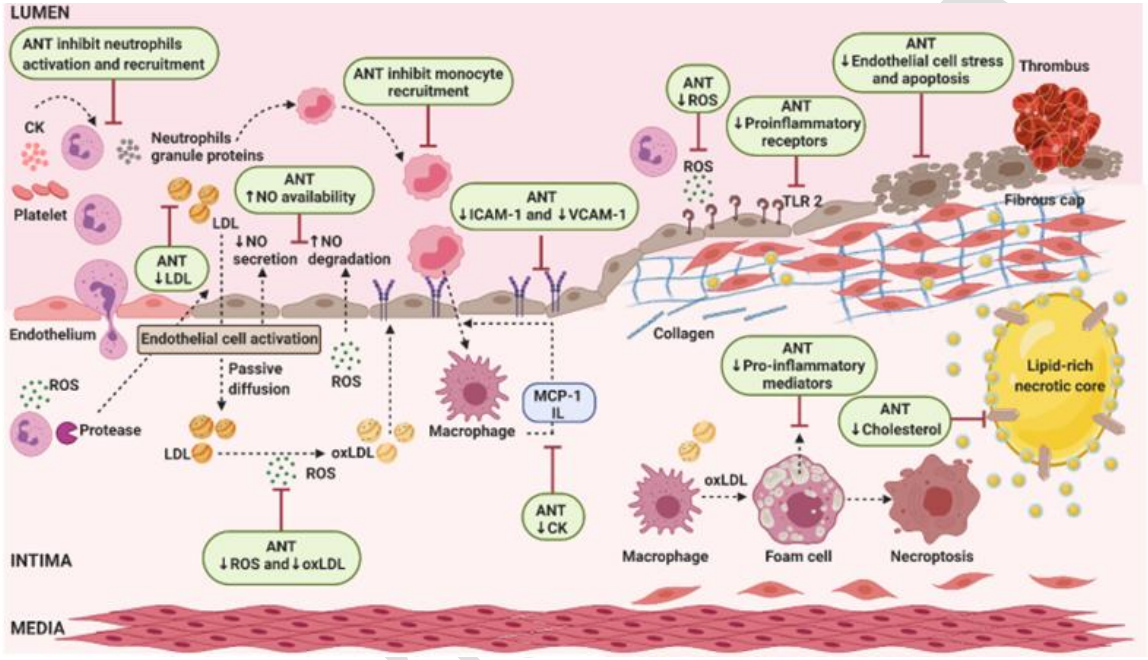

Fig 6. A detailed account of antioxidant activity of anthocyanin in human physiology (Mattioli et al., 2020)

Anthocyanins are abundant in Fruits and vegetables and are natural sources. Fruits like blueberries, pomegranates, elderberry, chokeberry, grapes, bilberry, and raspberry are rich in anthocyanins. In addition, vegetables like purple cabbage, purple tomatoes, and colored potatoes also contain anthocyanin restricted to the epidermis and the flesh. (Sunil & Shetty, 2022), (K. V. Strygina & Khlestkina, 2017).

In a highly populated and developing nation like Africa, there is a need to adopt a crop that is used as food, cheap to maintain, has a high yield, and has a short life cycle. In addition, it should be able to survive in adverse conditions and can be grown at least twice annually. Potato is considered the most valuable food crop as it forms a significant part of the diets of over a billion consumers worldwide. Its ability to provide a high yield of high-quality product per unit input with a shorter crop cycle (mostly < 120 days) than significant cereal crops such as maize, is considered to have a high potential for food security (Ulas et al., 2021).

In addition, Potatoes usually grow at an altitude ranging from sea level to 4,700 meters and perform best in a temperate climate. They are cheap, readily available, and have anthocyanins restricted to their peel and flesh.

Anthocyanin has antioxidant properties that benefit human health and could help protect the body against inflammation, reducing the instances and occurrence of non-communicable diseases (H. Zhang, Hassan, et al., 2017).

According to Tsang, et al. (2018), potatoes rich in anthocyanin were utilized on I individuals suffering arterial stiffness. It concluded that anthocyanin lowered the pulse wave velocity, which affects the vascular tone of the arteries, a risk factor for cardiovascular diseases. Charepalli et al., (2015), used purple potatoes rich in anthocyanin to determine the effects on colon cancer. The anthocyanin subdued the proliferation of cancer cells and increased cell death in a p53-independent mode. The crucial controller of CSC proliferation and its subsequent proteins were downregulated while Bax and cytochrome c, which conciliate mitochondrial cell death, increased.

While Jiang, et al. (2016), used purple potato anthocyanin to enhance antioxidant activity against alcohol-induced hepatic injury. The anthocyanin protected against increased alcohol-induced levels and activity of cytochrome P450 2E1 (CYP2E1 by inhibiting its action, thus protecting the liver against alcohol-induced injuries.

Ngamsamer, Sirivarasai and Sutjarit, (2022), assessed The Benefits of Anthocyanins against Obesity-Induced Inflammation and concluded that anthocyanin could reduce markers of CRP, IL-6, and TNF-α that promote obesity Mattioli, et al. (2020), reviewed Comprehensively anthocyanin and its Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases and concluded that anthocyanin counteracts ROS in both the luminal and intimal side, reducing LDL oxidation in the vessel wall. Fallah et al. (2020) analyzed the Impact of dietary anthocyanins on systemic and vascular inflammation. The findings indicated that dietary anthocyanins decreased the effects of CRP, IL-6, TNF-α, and VCAM-1, responsible for systemic inflammation of the blood vessels.

Based on the yield production of potatoes globally, it passes as one of the tuber plants that could be explored for anthocyanin production. However, its anthocyanin concentration is low and needs to be elevated. For this reason, grafting with tomato plants, which equally have a moderate anthocyanin production, is hypothesized to increase anthocyanin concentration in potatoes, hence this study.

3.3. Grafting technique

Grafting is the technique of joining a scion and a rootstock of two distinct species of plants to allow growth as a new single plant. It's a vegetative hybrid technique dating back to 424 BC. Usually, branches or buds are used as scions, whereas the root stem is a rootstock. The scion is inserted into the rootstock via an incision, and the two are allowed to heal, forming a grafted plant. The healing process is brought by the growth of parenchyma cells around the point of joining, forming a vascular connection.

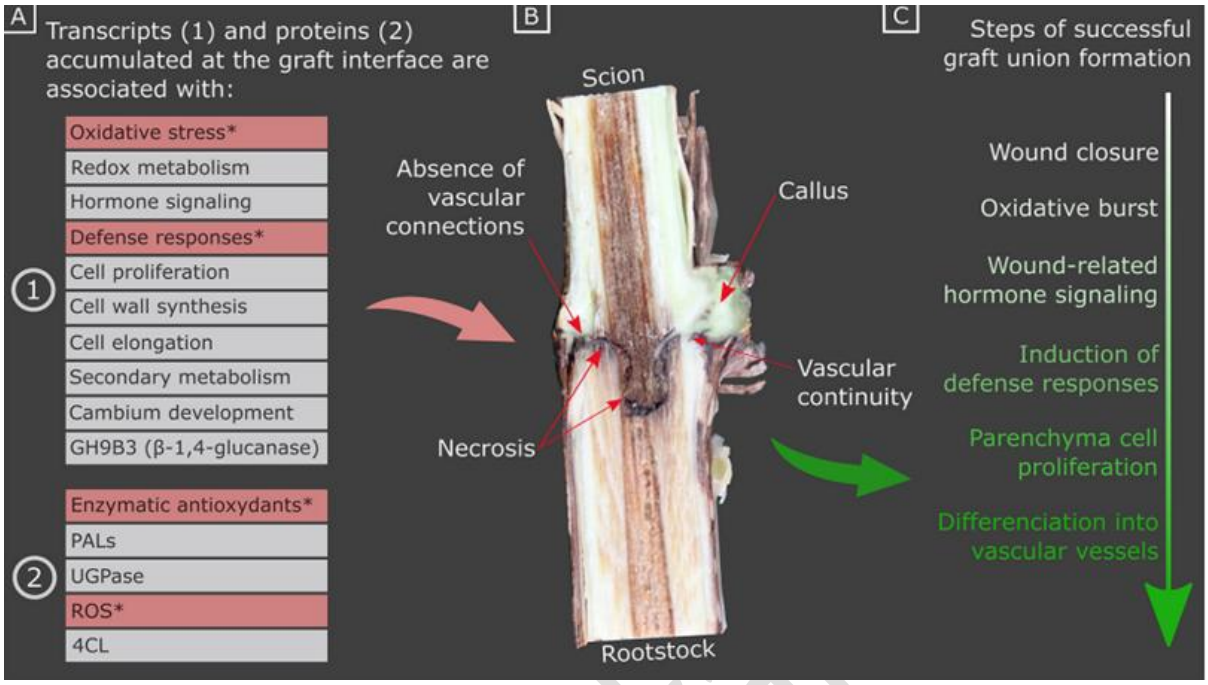

Fig 7. Detailed representation of graft union formation according to Loupit and Cookson, (2020).

For the formation of a successful graft, various stages of development occur. The first initial stage is the onset of mechanical injury that requires a quick response to close the wound protecting it against pathogen invasion and water loss. This calls for gathering lignin and suberin, acting as barriers around the wound. This activates oxidative stress rapture, switch in metabolism, production of pathogenesis-related proteins, and signal hormones associated with wounding. Callus forms a connection between the scion and the rootstock, and cambial cells transform into vascular vessels allowing them to reunite (Loupit & Cookson, 2020) see **figure 7** below.

Hand grafting can be done by farmers at their nursery units using different methods of grafting, such as tube grafting, hole insertion, cleft grafting, tongue grafting, pin grafting, and slant grafting. The slant or wedge method is preferable for the Solanaceae family, forming a tapered wedge to be joined to the rootstock using a clip or tape. In addition, micrografting and single and double tomato grafts are recent innovations. The technique is considered horticultural though it started with perennial fruit crops such as apples, citrus, grapevine, pears, avocados, pears, figs, and quince. In addition, the technique has been applied to vegetable crops like cucumbers, eggplants, watermelons, potatoes, pepper, and tomatoes (Tsaballa et al., 2021). In horticulture, the technique was applied in the agricultural sector to curb the effects of nematodes, soil-related pathogens, PH, and salinity on cucurbitaceous crops, to increase yield and quality (Noor et al., 2019). as shown in **Figure 8** below.

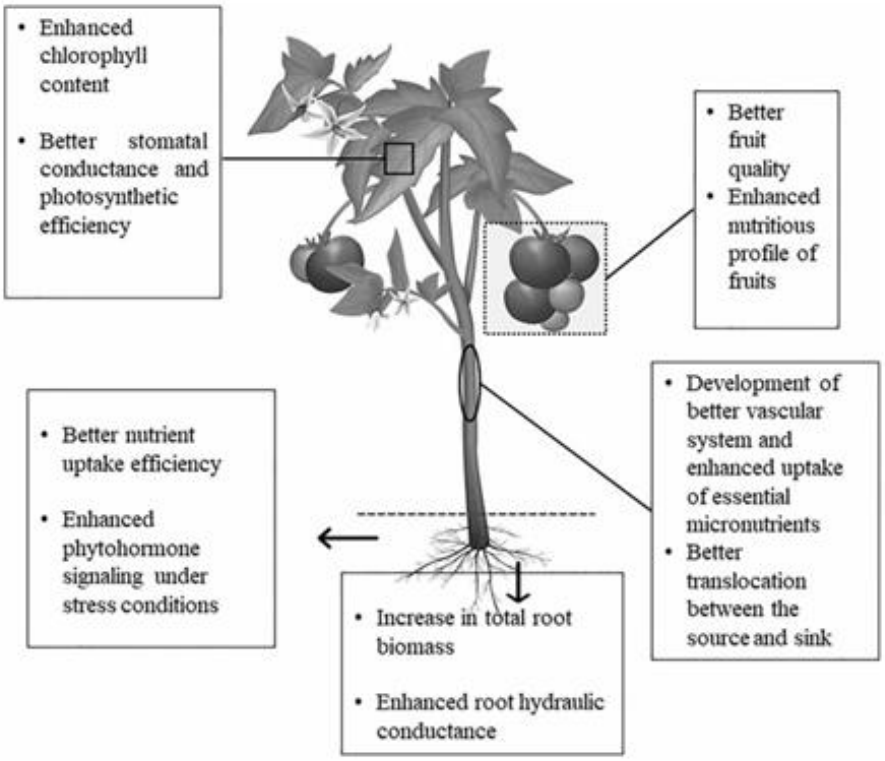

Fig 8 is a schematic illustration of grafting benefits on the morphology and physiology of vegetables according to (Noor et al., 2019)

Parthasarathi, Ephrath and Lazarovitch, (2021), grafted tomato on the potato to improve salinity and concluded that potato rootstock could improve tomato resistance to salinity through the introduction of mineral compartmentalization and allocation of dry mass Spanò et al. (2020), assessed the effects of grafting of commercial tomato varieties and hybrids on the tomato ecotype Manduria and concluded that high levels of tolerance to the infection of Sw5 resistance-breaking strains of tomato spotted wilt virus and severe cucumber mosaic virus emanated because of grafting.

Yuan, et al. (2019), grafted plants using Solanum torvum as rootstock to investigate the effects of grafting on Cd accumulation in shoots. The results showed that grafting on S. torvum could efficiently reduce Cd accumulation in leaves of eggplant and tomato. Abdeldym, et al. (2020), determined grafting's efficiency in improving tomato growth and production under salinity stress conditions, and the results revealed that the four genotypes of tomatoes exhibited better performance under salinity stress conditions in addition, grafting could be a low-cost alternative method to improve salt tolerance in sensitive tomato genotypes.

Noor, et al., (2019). investigated the interactive effect of different grafting techniques of hybrid scion on local rootstocks on plant survival, plant phenological growth, fruit yield, and fruit quality under a controlled environment. The results indicated that grafting hybrid cucumber onto four local cucurbitaceous rootstocks positively influenced

growth, yield, and fruit quality. Singh et al., (2020), a reviewed vegetable grafting research results and concluded that grafting will help growers deal with climate change and overcome the unsustainable vegetable production practices that result in soil degradation and the rapid depletion of natural resources.

Darré, et al. (2022), grafted a purple eggplant scion cv. Monarca onto a cold-tolerant hybrid Solanum rootstock ('Java') and evaluated the changes in growth, quality, postharvest chilling tolerance, and antioxidant stability and concluded that grafting modulated fruit growth, quality at harvest, and increased fruit chilling injury tolerance during storage Shehata, et al. (2022) ; Coşkun, (2023), grafted a novel rootstock on cucumbers to enhance tolerance to drought and suggested that grafting with local varieties of novel drought-tolerant rootstock genotypes could improve drought tolerance in drought-sensitive cucumber genotypes.

Smith and Saravanakumar, (2022), grafted a tomato on Solanum torvum to develop resistant tomato against R. solanacearum. Results showed a high level of wilt resistance in plants grafted onto S. torvum rootstock. They proposed this as an effective management tool for controlling tomato bacterial wilt.

In addition to the grafting technology used to protect the plant against abiotic and abiotic stresses, the technique has been used in several studies to upregulate the concentration of anthocyanin in plants Zhong, et al. (2022), performed metabolomic and transcriptomic analysis on homo and hetero-grafted grapevine to show the effects. The results revealed that rootstock grafting enhanced anthocyanin biosynthesis by upregulating the expression of PAL, ANS, and DFR genes when heterografted and homograft. According to Chen, et al. (2022), the effects of three commonly used rootstocks on the blood orange fruit quality were determined. The analysis revealed that the rootstock affected the anthocyanin accumulation in the blood.

3.4. Other attempts to boost anthocyanin in plants.

Besides grafting technology, other attempts have been made to increase anthocyanin concentration in plants, such as molecular breeding and gene technology (C. Liu et al., 2022). New F30H and Pur7.1-K1 molecular markers were used to design non-anthocyanin green broccoli and anthocyanin-rich purple broccoli varieties. They obtained two green broccoli lines without anthocyanin and three anthocyanin-rich purple lines with the best yield/quality characteristics.

Mattoo, et al. (2022b), reviewed the use of biotechnology and crossbreeding in obtaining fruits and vegetables rich in anthocyanin and concluded that the production of anthocyanin-rich potatoes and tomatoes was achieved through genetic engineering techniques. D'Amelia et al. 2022), knocked out potato Inducer Silencing of

Anthocyanins in Cell culture using CRISPR-Cas9 editing, and the results indicated that mutant cell lines doubled the accumulation level of anthocyanins biosynthesized.

However, these technologies are expensive and need qualified expertise, a laboratory, and expensive equipment. In addition, it has yet to be embraced by many people. On the other hand, grafting technology is a safer, cheaper technique that farmers can do by hand in nursery units. It doesn't require special equipment, expertise, personnel, or laboratories to carry it out. In this regard, grafting technology is proposed to enhance anthocyanin levels in potatoes.

In this experiment, grafting tomato on potato rootstock is expected to raise the level of anthocyanin in the tuber by elevating the expression of the genes directly involved in the flavonoid biosynthesis pathway. Evaluation of the effect of grafting technology on the expression level of the ANS genes, on three potato cultivars Désirée, Hópehely, and White Lady when self and hetero grafted with tomato using the RT-PCR and qPCR techniques would justify the reason why grafting technology should be embraced as the cheapest and safest method to improve the quality of potatoes.

4. MATERIALS AND METHODS

4.1. Plant Material and growth conditions

Three potato cultivars cv. Désirée, Hópehely , White Lady , and tomato (Mobil) were obtained from the Center of Potato Research, Keszthely, Hungary. The plant materials were propagated in vitro in Murashige and Skoog media with vitamins excluded in exchange for 0.8% agar and 2% sucrose. The cultured tissues were stored under 75 µmol m-2 s -1 light intensity, 24°C at a 16 h/8h photoperiod cycle for four weeks. The 3-4 cm long plantlets were acclimatized in Tabaksubstrat A200 sterile soil in polythene pots in a greenhouse under 80% humidity, a 12h daylenght, 18-24°C, and the ambient light was provided by sodium lamps (Odgerel & Bánfalvi, 2021).

4.2. Grafting and growth conditions

After successful acclimatization, homo, and heterografting were done with each cultivar's scions from potatoes and tomatoes. The slant technique was used for grafting the plants with an incision approximately 2 cm above the soil. The scions were attached to the rootstocks using rubber clips, and the grafted plants were kept in a shaded healing chamber under 24°C at high humidity for 7 days to allow callus formation. Meanwhile, the controls were left in the greenhouse.

The plants were transferred to 14 cm by 14 cm pots, watered regularly under 20-26°C, at 12-15h/ 12-9h cycle under ambient light for 6 weeks, and the plants were carefully uprooted to confirm tuber formation and re-planted. The experiments consisted of 12-15 grafted plants for each treatment and were performed for the three cultivars. The tubers were harvested at the end of the growing season of 3 months, and the shape and color of the freshly harvested tubers were assessed visually and recorded (Villányi, Gondor and Bánfalvi, 2022).

4.3. Spectrophotometric investigation of anthocyanins

Wash 12 freshly harvested tubers from each treatment for the three Cultivars with clean water, weigh, and peel carefully with a knife and grin in liquid nitrogen using a mortar and a pestle, transfer to Eppendorf and store at -70 °C prior to analysis. Weigh 100mg of each sample into a 1,5 ml Eppendorf tube, add 1ml of solution (1ml of 1% HCl mixed with 36ml of methanol), vortex, and keep at 4 °C overnight. vortex the stored sample for 1minute, centrifuged for 10 minutes at 4°C and 13000 rpm. Transfer supernatant into cuvettes and measure optical density at 540nm using a spectrophotometer: Jenway Genova UV-Visible spectrophotometer (Jenway, Essex, England).

4.4. RNA extraction

Use the previously prepared skin samples described in **section 4.3** above. weigh 2.5g of frozen skin samples using a weighing scale in a cooled mortar. Grind in 500µl of extraction buffer (39.6 µl of 3M NaAc PH 5.2, 12 µl of 0.5 EDTA, and 60 µl of10% SDS) and 300 µl of chloroform, transfer mixture into labeled Eppendorf vortexed for 1 minute, allow to stand at room temperature for 10 minutes. Centrifuge samples at 13000 rmp at 4 ℃ for ten minutes. Transfer supernatant to a new tube containing 500 µl of phenol and 500µl of chloroform, vortexed for 60 seconds, centrifuge, and repeat the step to purify the samples. Take 500 µl of the supernatant into a new Eppendorf, wash with 170 µl of 10M LiCl, gently shake by hand, and keep the samples in the ice in the cold room overnight. Centrifuge the stored samples at 13000 rmp for 10 minutes at 4℃. Discard supernatant, and wash pellets with 1ml of 2.5M OF LiCl centrifuge; discard supernatant, and rinse pellets twice with 1ml of 70% Ethanol centrifuging and discarding the supernatant, and finally air dry the pellets and dilute with 30 µl of Milli Q water and keep at -70℃ until analysis (Stiekema et al., 1988).

4.5. Nanodrop measurements

To determine RNA concentration, vortex samples shortly and place on ice. With an open Nanodrop program, clean the pedestal using tissue paper, calibrate with 2 µl of MQ water, and take a blank measurement. select the nucleic acid on the program and measure 2 µl of the samples and record the result. Dilute samples to 200 ng/ul RNA concentration and store at -70℃ until use. Nanodrop ND-1000 V3.5 Spectrophotometer, (Nanodrop Technologies, Inc; Wilmington, USA) was used.

4.6. CDNA synthesis

The first strand cDNA was synthesized using MAXIMA H MINUS FIRST STRAND CDNA SYNTHESIS KIT from THERMOSCIENTIFIC (Waltham, MA, USA) protocol. add 13 µl nuclease-free water, 4 µl of 5x reaction buffer, 1 µl of RNA and 2 µl maxima enzyme mix, into Eppendorf tube, vortex for 30 seconds, and set at 10min at 25℃ ,15min at 50℃, 5min at 85℃ and store at -70℃. Test the quality cDNA using the EF1α primer pairs GACAAGCGTGTTATTGAGAGG and CACAGTGCAGTAGTACTTAGTG for the EF1 housekeeping genes to serve as an RT-PCR control using a thermal cycler- Light Cycler-96 from Roche.

4.7. RT-PCR

Into a new Eppendorf pipette 17.25 µl of MilliQ water, 3 µl of MgCl2, 1 µl of dNTP 2.5 µl of GRS buffer, 0.5 µl of forward primer,0.5 µl of the reverse primer. 1 µl of DNA and 0.25 µl of GRS taq polymerase were mixed in PCR tubes, centrifuge shortly, and place in a thermocycler set 10-15min at 45°C(cDNA synthesis), 3 min at 95°C (hot start),30-40 cycles of 10 seconds at 95°C, 10 seconds at Ta*(56°C for AN1 gene and 40cycles), 15seconds/kb at 72°C, and 1 min at 72°C(final extension) then put on hold at 4°C and store at -20°C. Visualize products on 1.2% agarose gel.

For Gel electrophoresis, measure1.2% agarose in a conical flask on a weighing scale, add 100ml of 1x TBE, and boil in a microwave until clear. Prepare the tray and the comb. Add 10 µl of ethidium bromide when cooled to hand temperature, shake gently, pour on the tray, and allow to set down. Pipette 2.5 µl of 2X RNA Loading Dye from Thermo Scientific and add to the samples, mix well. Load 5µl of the gene ruler from Thermo Scientific into the first well and the samples into the following wells. Run the gel for 30- 40 mins at a voltage of 85mv. Visualize the gel using Gel Doc EZ Imager from BIO-RAD, and the results were recorded. For RT- qPCR for AN1 gene, Xpert Fast SYBR qPCR Master Mix kit (grisp) without Rox was used. Pipette 3.2 µl DNA nuclease free waster, 5 µl of master mix, 0.4 µl of 10 uM Forward primer, 0.4 µl of 10uM reverse primer, 1 µl 200ng of cDNA into qPCR plate, seal and shake on a shaker and measure the amplification using Light Cycler-96 thermal cycler (Roche Diagnostics GmbH, Mannheim, Germany) set at UDE pretreatment at 50°C at 2min, initial denaturation at 95°C for 10min, Denaturation at 95°C for 15second, Annealing at 58°C for 50 seconds, Extension at 72°C for 30 seconds and cooling at 37°C for 30 seconds. 40 cycles were set and water was used for negative controls. EF1α served as a reference gene.

N	Gene name	Primer name	Forward (5' – 3')	Reverse (5' – 3')	NCBI ref.seq	PGSC ref.seq
1	PHE- NYLALANINE AMMONIA LYASE	StPAL	TGGTGCTCCTCTTCCAATCTG 56.8 ºC	ATACATCCTTCCAGAATTGTTGCT 54.6 ºC	XM_0063674 72.2	Soltu.DM.03G011 480.1
2	CINNAMATE 4-HYDROXYLASE (C4H)	StC4H	ACCAAGAGCATGGACAGCAA 57.1 ºC	CTAGTTCCGCGATACCCCAC 57.4 ºC	XM_0063508 25.1	Soltu.DM.06G032 860.1
3	4-COUMARYOL COA LIGASE (4CL)	St4CL	GCTAGACTGGCTGCTGGTATT 56.9 ºC	TTTCGTCCGTTGCCAAACTG 56.3 ºC	XM_0063413 75.2	Soltu.DM.03G032 090.1
4	CHALCONE SYNTHASE (CHS)	StCHS	GGCTTGAATGGGGTGTCCT 57.6 ºC	GCCCACAATATAAGCCCAACC 56.3 ºC	NM_0012884 23.1	Soltu.DM.09G028 .1
5	CHALCONE ISOMER-ASE (CHI)	StCHI	TCCGAAAAGGTGGCAGGAAA 57 ºC	GCCAGTCTCTCTGCATCACT 56.8 ºC	XM_0063485 48.2	Soltu.DM.05G001 960.1
6	FLAVANONE 3-HYDROXYLASE (F3H)	StF3H	TCGAATTGCCTCCTGACGAA 56.4 ºC	ACGCCAGTCTTGAACCACTT 56.9 ºC	NM_0012880 01.1	Soltu.DM.02G023 850.1
7	DIHYDROFLAVONOL 4-REDUCTASE (DFR)	StDFR	AGCACTGCAGACAATGGAAG 55.5 ºC	CAGATATCAAGAACCAAGAAGAACA 52.9 ºC	NM_0012884 80.2	Soltu.DM.02G024 900.2
8	ANTHOCYANIDIN SYNTHASE (ANS)	StANS	GACGAGCAGGATGCAGTTG 56.3 ºC	AGGGATAGGGGGATCACAAA 55.2 ºC	NM_0012879 30.1	Soltu.DM.08G026 700.1
9	UDP-GLUCOSE:FLAVONOID 3-O-GLUCOSYL TRANSFERASE (UFGT)	StUFGT	ACAAGGTCCCCTACCATCCA 57.8 ºC	CCTGGCTCCCAAAACAGAGT 57.4 ºC	XM_0063536 67.2	Soltu.DM.07G013 870.1

Table 1 Table of the primer pairs used for testing.

4.8. GC-MS metabolites extraction

Sample extraction, derivatization and chromatography was according to (Villányi et al., 2022). Measurements were made in three biological repetitions of the 9 different combinations (3 cultivars in 3 grafting combinations). Each sample consisted of the middle sections of three freshly harvested potato tubers of average size. Tubers were sporadically picked and thoroughly cleaned using ionized water and dried using tissue paper, grind the samples in liquid nitrogen using a mortar and a pestle and stored at -70°C until analysis. Weigh 100mg of frozen samples, Pipette 700 µl of GC-grade methanol, 30 µl of 0.2 mg $ml^{-1}$of Ribitol as internal standard. Vortex the mixture and shake at 1000 rpm for 15 minutes, at 70 °C. Subsequently, Pipette and add 375 µl of GC- grade chloroform and 730 µl of MQ water, vortex, and centrifuge at 13000 rpm for 15 minutes. Finally, pipette 150 µl of the supernatant of the water phase and vacuum dry and store at – 70 °C until further processing.

4.8.1. Derivatization

In the 1.5-ml Eppendorf tubes of dried samples, add 40 µl MEOX, shake at 300 rpm and 37 °C for 90 minutes; add 60 µl of MSTFA, shake at 300 rpm and 37 °C for 30 minutes.

4.8.2. Gas Chromatography

The quadrupole GC-MS system from Thermo Electron Corp., Austin, TX, USA was used for chromatographic analysis. Column type was TG-5MS, with the following dimensions: 30 m×0.25 mm×0.25 µm, from Thermo Scientific, Waltham, MA, make injections using AS 3000 injector, with 1 µl of the sample. Carrier gas was helium, with a flow of 1 ml min^{-1}. A gradient temperature program was used: after initial 90 °C for 2 min, then ramp to 165 °C for 15min, held for another 15min, ramp for 6min at 320°C followed by cooling down to 90 °C.

4.8.3. Mass Spectrometry

The full scanning was done using a quadrupole mass spectrometer -Trace DSQ. The spectrometer uses EI electron source. The scan was carried in TIC mode, in a mass range between 50 and 650 m/z.

4.8.4. Data Transformation

Data transformed using Xcalibur Data System Software 1.4.1 SP3 from Thermo Finnigan, San Jose, CA, USA. The relative peak area derived from the peak area of the formerly added Ribitol as internal standard, which was referred to as one unit. Relative peak areas used for further analysis. The metabolites identified using NIST MS Search 2.0 software based on their relative retention time and mass spectra.

4.9. Data analysis

For all statistical analysis, the online MetaboAnalyst 5.0 (www.metaboanalyst.ca) statistical software used. Data normalized by median, and log transformed for reaching the normal distribution. The following analyse; dendrograms, Principal Component Analysis (PCA), heat maps, and one-way Analysis of Variance (ANOVA). The post hoc Tukey's HSD test adjusted to 0.05, the Partial Least Squares Discriminance Analysis's VIP values cut put to 1,5. *Student's* t-test used to compare two treatments of each cultivar ($P \leq 0.05$)

5. RESULTS

5.1 Grafting experiment

To investigate the effects of grafting on potato skin and metabolites, potato and tomato seedlings were grafted four weeks after *in vitro* culture, as shown in **Fig 9 B.** Grafted plants, homo (self-grafted) and hetero (tomato grafted on potatoes), were monitored together with non-grafted control plants grown under the same greenhouse conditions for 12 weeks. Grafting was conducted with a success of 70-85%. The plants grow under greenhouse conditions. The potted plants were grafted four weeks after in vitro culture. Tubers harvested 12 weeks after grafting.

A B

Figure 9. The cover of one of the cultivars Hópehely two weeks after potting. Non-grafted (A) and self-grafted (B) control plants. The arrow indicates the point of grafting with the rubber clip.

5.2. Effects of grafting on morphology

To investigate the effects of grafting on tuber morphology, freshly harvested sets of tubers from the three non-grafted, homo-grafted, and hetero-grafted potato plants per each of the three cultivars (Desiree, Hópehely, and White Lady) were examined. Tubers were weighed and examined visually for shape, size, and skin color and photographed as shown in **Figure 10.**

Desiree's non-grafted (A) shape was elongated, whereas the hetero-grafted tubers (B) seemed oval. This confirms the tomato grafting induced change in shape from cylindrical to ovoid. The size of the hetero-grafted tubers **(B)** was reduced compared to non-grafted ones. There was a considerable color change from red to darker red

because of hetero grafting. In the Hópehely cultivar, there was a change in the shapes from round to oval, the non-grafted **(C)** appeared round shaped while the hetero grafted **(D**) looked oval. Change in size was also observed with non-grafted**(C)** looking bigger than the hetero-grafted tubers **(D**). The skin color improved in the hetero-grafted tubers as it appeared more yellow than that of the non-grafted ones **(10 C, D).** In White Lady, there was a change in shape from oval to round. The non-grafted **(E)** appeared oval, whereas the hetero grafted **(F)** looked round. The size also changed; the non-grafted **(E)** tubers appeared smaller than that of hetero grafted **(F)** plants. There was no grafting induced color change observed in White Lady.

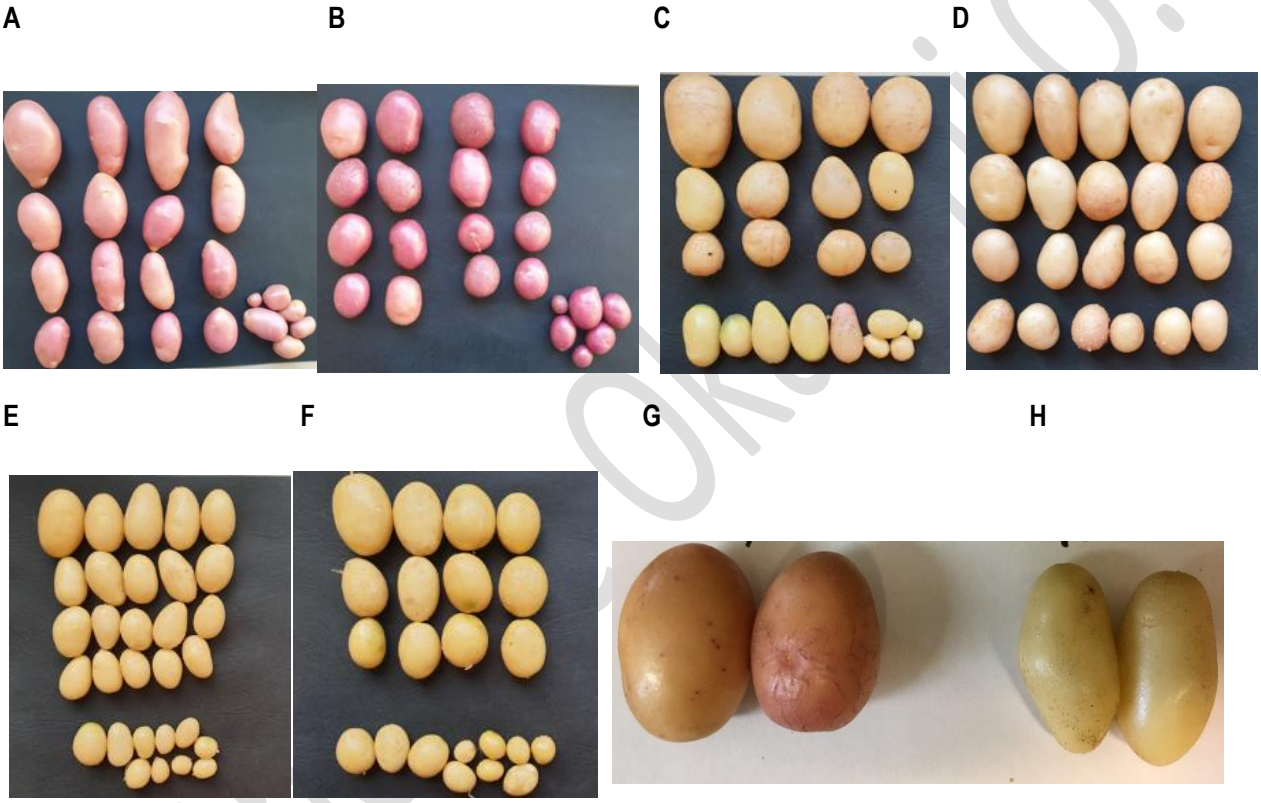

Figure 10. shows the morphology of freshly harvested tubers after 120 days in greenhouse conditions. **A** and **B** show nongrafted and tomato grafted on potatoes of the Désirée cultivar. **C** and **D** show the non-grafted, and tomato grafted Hópehely cultivar; **E** and **F** are non-grafted, and tomato grafted White Lady; **G** and **H** show self-grafted Hópehely and White Lady respectively.

The size increased in self-grafted tubers for Hópehely **(G)** and White Lady **(F)**. The shapes looked like non-grafted tubers, round for Hópehely **(C)** and oval for White Lady**(E)**. In Hópehely the color was more yellow, while no change occurred in the White Lady, as indicated by **G** and **H**. The total anthocyanin concentrations confirmed the visual observations as shown on **Figure 10**.

5.3. Relative anthocyanin concentration

To determine the effects of grafting on relative anthocyanin concentration, optical densities of tuber skin samples were measured **(Figure 11)**. The results clearly showed that the relative absorbance in the Désirée cultivar was the highest, followed by Hópehely and the White Lady gave the lowest absorbance value. Among the non-grafted samples, Désirée tubers contained the highest amount of anthocyanin with a mean of OD 0.288, followed by Hópehely with OD 0.111, and White Lady had the lowest (OD 0.062). Among the self-grafted treatments, also Désirée had the highest anthocyanin with a mean of 0.310, followed by homo grafted Hópehely with 0.146 and the lowest in White Lady with 0.060. Hetero grafting increased anthocyanin content in Desiree at the highest level with a

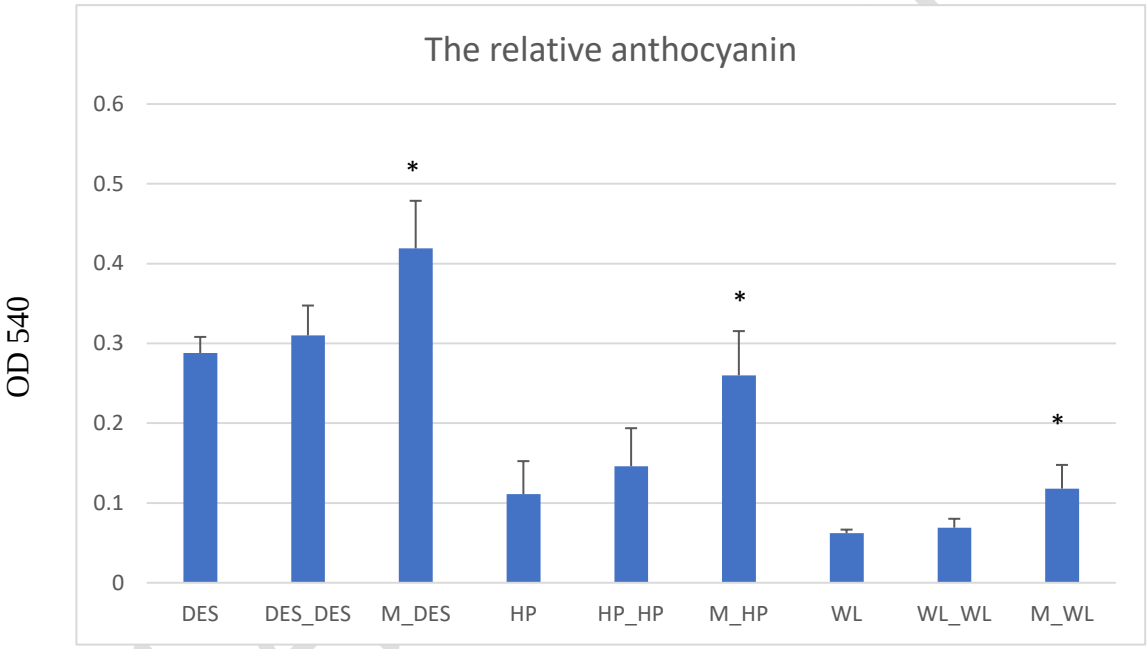

Figure 11. The relative anthocyanin content of tuber skin samples measured 540 using a spectrophotometer. Arrow bars indicate the standard deviation.

mean of 0.419, followed in Hópehely (0.26) and lastly in White Lady with a mean of 0.118. The heterografting gave the highest level of anthocyanin in all cultivars compared to non-grafted ones.

5.4. Gene expression

To determine the gene responsible for the skin color change in grafted potato tubers, the RNA extracted from the potato skin was amplified by RT-PCR. The quality of the cDNA was tested using the housekeeping gene EF1 and

the result was visualized by Gel electrophoresis as shown in **(A)**. The cDNA was used to test the expression of the nine genes involved in the Phenylpropanoid biosynthetic pathway. out of the nine, AN1 showed a promising result visualized by Gel electrophoresis, as shown in **Figure 12 C**. qPCR done to capture the real-time amplification of the products as the fluorescence passed through. the relative ratios were used to generate the bar graph shown in (B). The result showed that the quality of the cDNA was good in all the samples and the band's strength was also similar, as shown in **A**.

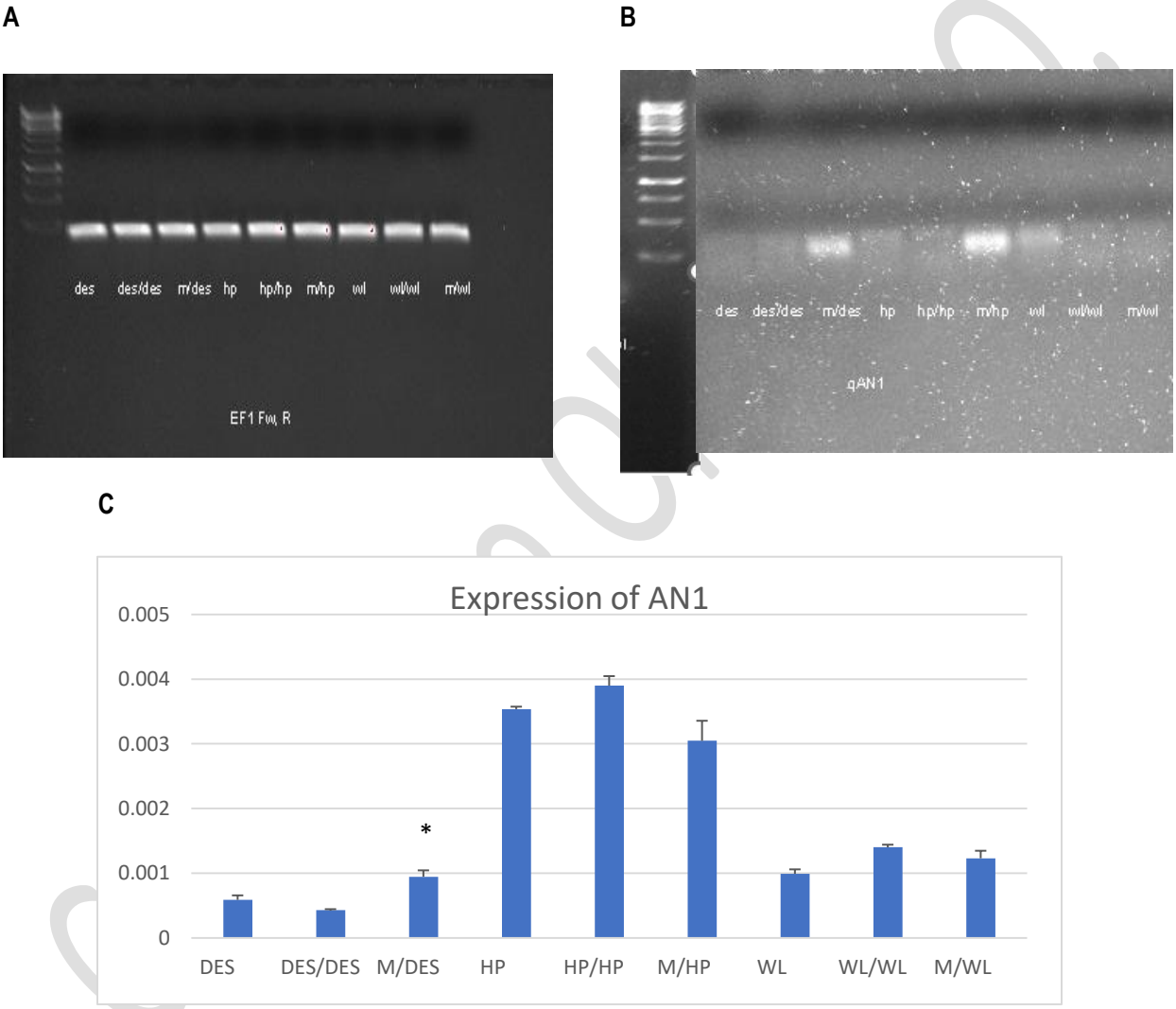

Figure 12. RT-PCR of EF1 housekeeping gene **(A)** and AN1 **(B)** and a bar graph showing relative expression from RT-qPCR for AN1.error bars indicate standard deviation.

On the other hand, the presence of anthocyanidin Synthase was significantly reduced in non- and homo-grafted samples of Désirée and Hópehely, as compared to White Lady where the decrease was observed in homo and hetero-grafting. Still, the band's strength was highest in the hetero-grafted Désirée and Hópehely, whereas the strength in the non-grafted White Lady looked faint, as shown by **Figure 12 B**.

The bar graph depicts that the expression of AN1 is reduced by homo grafting but increased by hetero grafting in Desiree. In contrast, it increased by homo grafting in Hópehely and White Lady and decreased by hetero grafting in Hópehely. This contradicts the results obtained from RT-PCR, which indicates that hetero grafting in Desiree and White Lady increased the expression.

5.5. Metabolomic Analysis

5.5.1. Effects of grafting on tuber metabolites.

37 metabolites from the chromatograms of freshly harvested tubers of the nine different treatments were determined. PCA Scores Plot **Figure 13**, illustrates the hierarchy of clusters based on metabolites composition. the first Principal component separated the hetero-grafted samples from all the non- and homo-grafted groups. Hetero grafted Hópehely was also separated from the other two hetero-grafted treatments by the second principal

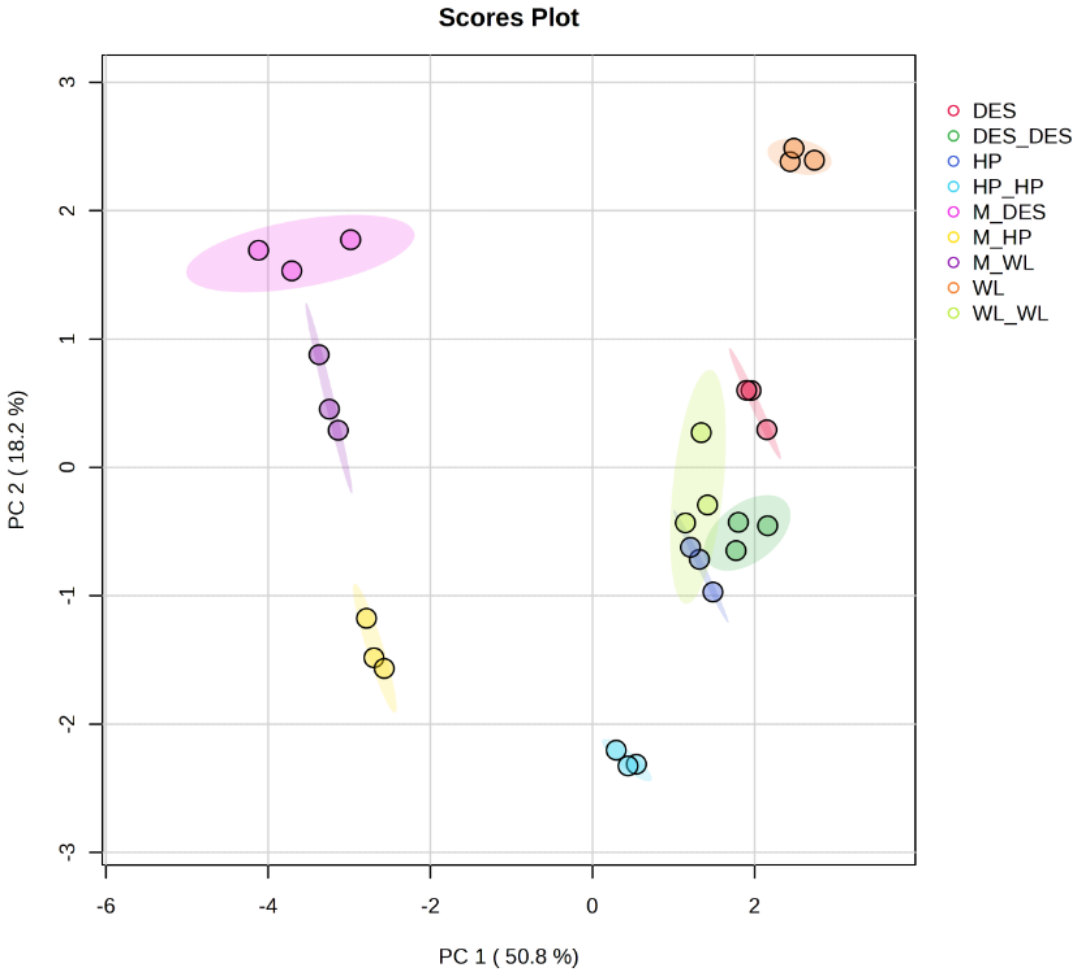

Figure 13. PCA scores plot showing hierarchical clustering of the non-grafted, homo, and hetero grafted treatments of three potato cultivars based on tuber metabolites.

component. Similarly, the groups of non-grafted White Lady and homo grafted Hópehely are separated from all the other non-grafted and self-grafted groups along with the second principal component.

5.5.2. Important metabolites

To determine the most essential metabolites differentiated by treatments, VIP scores of the top 6 metabolites out of the 37 analyzed metabolites, with a score of more than 1.5 were identified by PLS-DA and the effect of grafting on the three cultivars illustrated. Glutamate was the highest metabolite followed by sorbitol, proline, glucose, leucine, and L-threonic acid. The relative amounts and differences of the metabolites in each tuber sample are indicated by **Figure 14.**

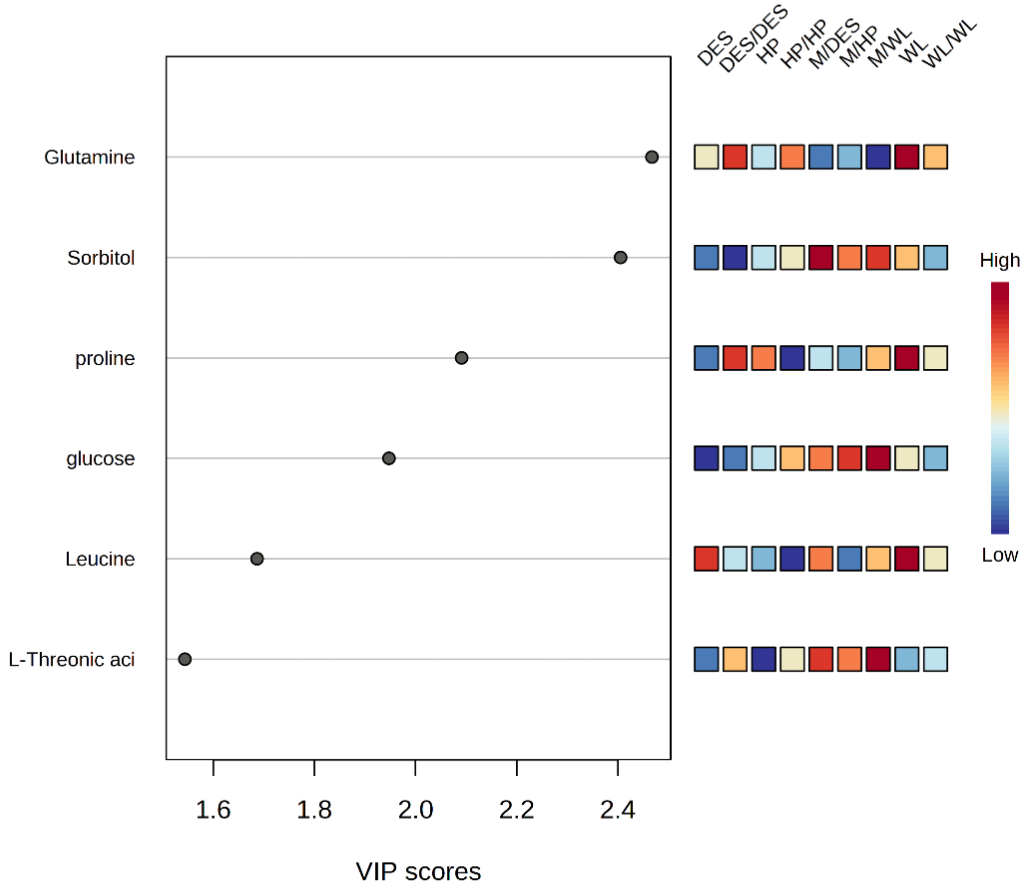

Figure 14. The top 6 important metabolites in potato tuber. VIP scores plot from PLS-DA indicating the 6 top important metabolites.

In **Figure 15,** the dendrogram clearly illustrates that all the hetero-grafted tuber samples of the three cultivars are distinct from the non, and homo-grafted combinations as they distinctively composed on their own branch. The

non-grafted samples were also separated into different sets based on cultivars, just like the homo-grafted ones but were differentiated from each other.

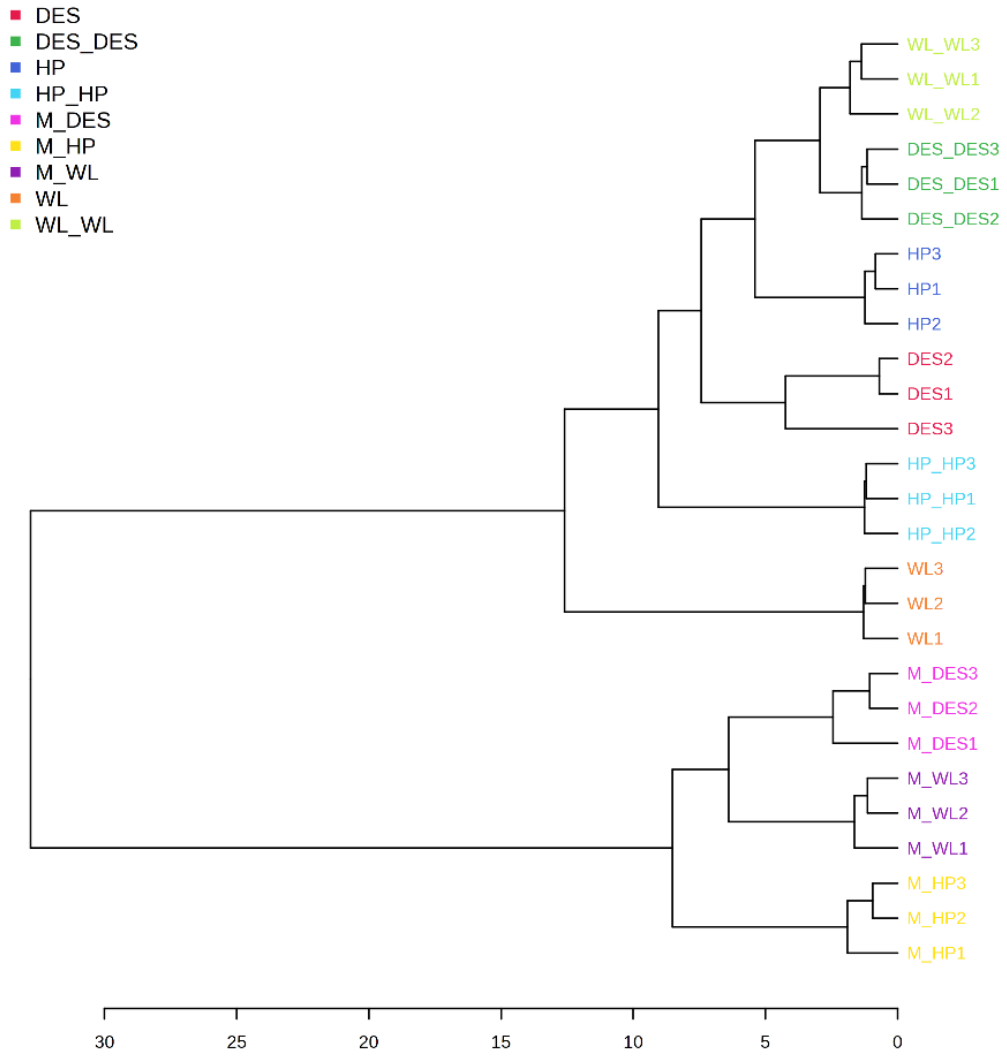

Figure 15. Dendrogram showing hierarchical clustering of three potato cultivars based on the constitution of metabolites.

5.5.3. The effects of treatments on metabolite composition

To examine the impact of tomato grafting on the relative abundance of tuber metabolites of different cultivars, one-way ANOVA with Tukey's post hoc HSD test was done. The boxplots of different metabolites **Figure 16** showed that heterografting reduced the amount of glutamine of all three cultivar's tubers, whereas homo grafting had no impact on glutamine concentration. On the other hand, hetero grafting increased the amount of myo-inositol in all three cultivars, whereas homo grafting reduced the amount. The amount of citric acid was increased by hetero grafting, whereas homo grafting decreased the amount. Conversely, sucrose was increased by both hetero and homo grafting.

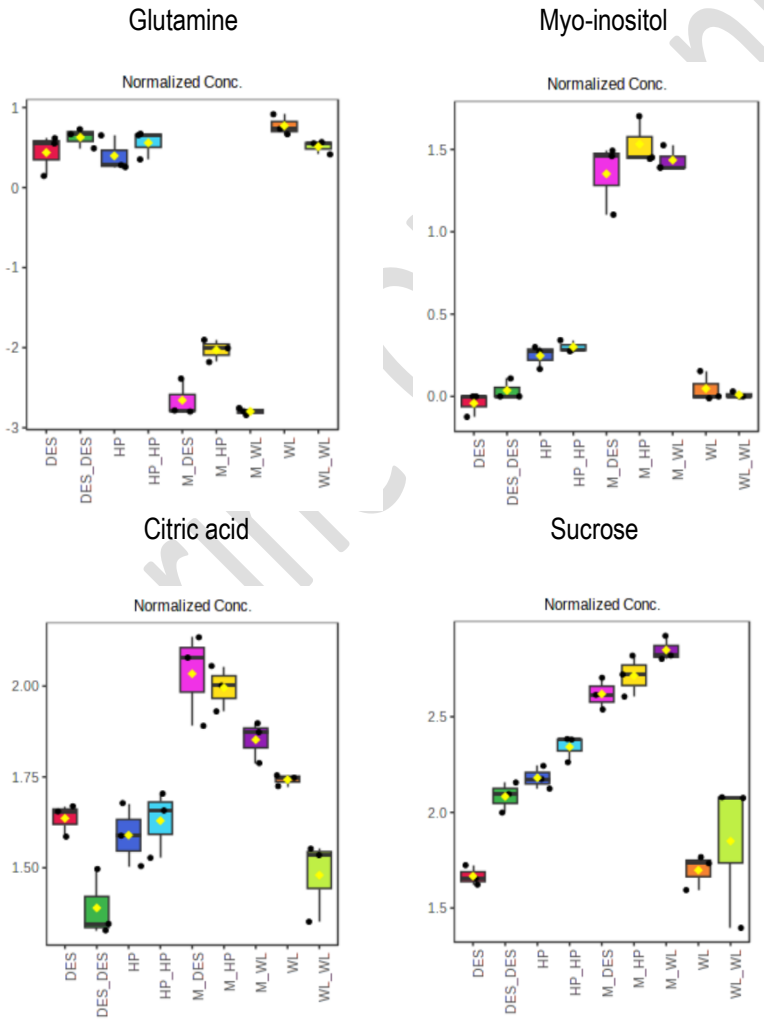

Figure 16. One-way ANOVA analysis of the effects of grafting on different metabolites. The analysis was based on Tukey's post hoc HSD test showing significance difference.

5.5.4. Metabolites proportion

To differentiate tuber samples based on metabolite proportion, cultivars, and grafting category, a heat map of 37 analyzed metabolites was prepared. This analysis, as shown in **Figure 17** showed a clear cut between the heterografted models from the non- and homo-grafted ones. 19 metabolites were higher, and 18 metabolites were lower in the hetero-grafted samples of all three cultivars as compared to control treatments.

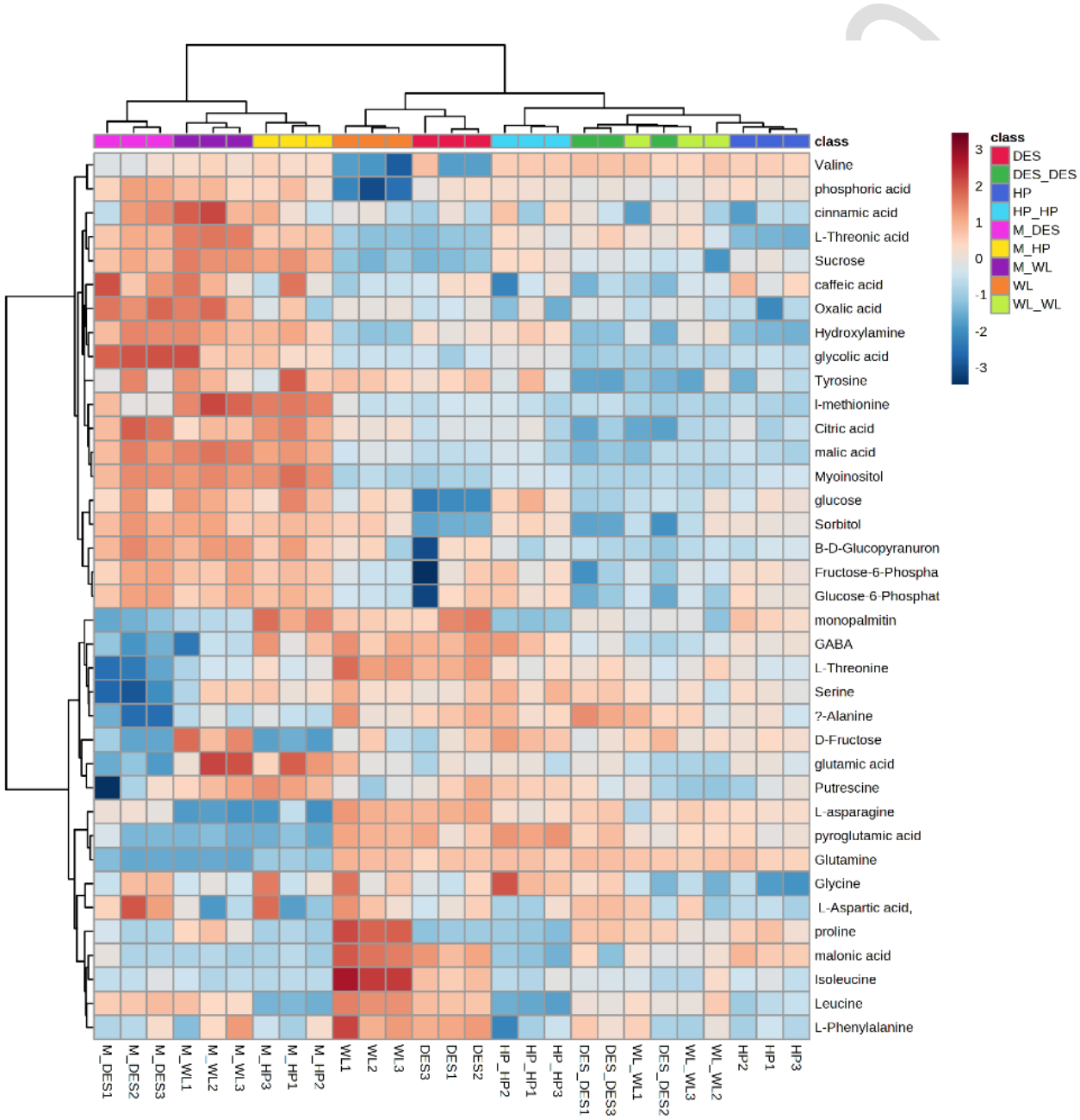

Figure 17. Heat map differentiating the nine treatments based on metabolite composition.

6. CONCLUSION

Several studies have been conducted to improve the quality of potatoes using crossbreeding and transgenic techniques. However, the grafting method has yet to be explored intensively to enhance the quality of potatoes. in this study, the effects of grafting on the skin color of potato tubers were established in an experiment using a spectrophotometer.

Three potato cultivars, Désirée, Hópehely, and White Lady, were self and grafted with tomatoes juxtaposed to their control, non-grafted ones. The effects of hetero grafting on Desiréé improved the tuber skin color from a light red tint to deep red compared to the non-grafted one, as shown in **Figures 11A** and **B**. This confirms that grafting elevated potatoes' synthesis of the red color from 0. 288 to 0.419, as indicated by the relative absorbance ratios shown in **fig 12** measured using a spectrophotometer at 540 nm.

This corresponds with Li, et al., (2014), who transformed Désirée by cloning potatoes using StAN11 in Agrobacterium and found that the color deepened, like (Jung et al., 2009). who transformed two potato cultivars, Désirée and Bintje, using the Stans 2 gene and found that the transgenic potato tuber lines of Désirée had a deep red color compared to the non-transformed ones. The slight color improvement in Hópehely as shown in **Fig 11 C, D**, could be attributed to the less grafting effect on the yellow color synthesis in this cultivar.

However, the absorbance doubled from 0.111 to 0.26, as indicated by the spectrophotometer reading in **Fig 12.** No color change observed in White Lady could be that grafting does not affect white color synthesis though the absorbance also doubled from 0.062 to 0.118. This is confirmed by Zhang, et al., (2017), Who examined 8 potato genotypes and discovered that the Redsen genotype had the highest anthocyanin content. But Bonar, et al., (2018), analyzed four potato cultivars for anthocyanin content using liquid chromatography–mass spectrometry and found out the DB22670 potato cultivar with yellow skin and white flesh had no anthocyanin detected.

The results do not correspond with the result from this experiment. on the other hand, the results from the post hoc Tukey HSD Test Calculator from ASTATSA online software using the absorbance values of this experiment showed a significant difference of P-value<0.05 when the control Désirée was compared to its heterografts, whereas its homo graft and heterograft of Hópehely showed no significant difference. but it showed a significant difference of P-value<0.1 when compared to non-grafted Hópehely and both non, homo, and hetero grafts of the White Lady.

Other studies have been conducted to establish genetic control of pigmentation in potatoes. However, information on the genetic control of grafted potatoes has yet to be intensively studied. Strygina and Khlestkina, (2017), reviewed anthocyanin biosynthesis using genetic markers for innovative breeding and found StAN1 as the leading cause of pigment variation in potatoes.

Strygina, Kochetov and Khlestkina, (2019), Investigated potatoes' regulatory genes responsible for anthocyanin biosynthesis by developing diagnostic markers for their dominant and recessive alleles. They found that StAN1 is the significant regulatory gene controlling anthocyanin biosynthesis. This result matches the outcome of this experiment where the homo and hetero grafting samples of three potato cultivars compared to their non-grafted ones were tested using nine genes. Only AN1 showed the expression with the hetero grafted samples of Désirée and Hópehely cultivars when visualized by the Agarose gel electrophoresis **Fig 12 B**., but the RT- qPCR results **Fig. 12 C** were not consistent with the gel results of Hópehely, and White Lady. The RT- qPCR results showed that homo grafting elevated the expression of qAN1, whereas hetero grafting reduced its expression.

This result differs from that of Zhang, et al., (2020). who analyzed the potato Anthocyanin synthase gene and found that the expression in colored potatoes was higher than in the yellow ones. This could result from cultivar variation to grafting and other background factors in play that elevate the expression of AN1 in the two cultivars. This can be justified by (Payyavula et al., 2013), who investigated the role of sucrose and its metabolic genes together with Transcription factors in regulating the phenylpropanoid pathway and found that there is a positive interaction between sucrose its genes and sucrose and the pathway. Also Laimbeer, et al., (2020), who highlighted that the dynamic of the F locus of StFIAN2 in potatoes might alter the expression.

Numerous research has been conducted to investigate the effects of grafting on potato metabolites. However, the information on the changes in metabolite levels when interspecies are grafted has yet to be fully explored. In this experiment, three potato cultivars, Désirée, Hópehely, and White Lady, were homo and heterografted with tomatoes. The effects on untargeted tuber metabolites were assessed compared to the non-grafted ones.

Odgerel and Bánfalvi, (2021), analyzed the effects of grafting on two homo and hetero-grafted potato cultivars Hópehely and White Lady. 31 metabolites were analyzed, and found that Hópehely differed from White Lady significantly, and the difference was based on sucrose, and grafting had no effect on it. In this experiment, 37 metabolites were analyzed, the three cultivars were different, and Désirée and White Lady were separated from Hópehely by the second PCA **Fig 13**. Though Désirée and White Lady were clustered together, they were different from each other. This could be attributed to differences in metabolite composition.

The first PCA separated the heterografts from the controls and homograft's; however, all three cultivars differed, and this could be the effects of tomato scion on the tuber that altered the composition of the metabolites as shown by the heat map **Fig 17.** Odgerel and Bánfalvi, (2021), found a significant difference in sucrose higher in Hópehely than in White Lady, and heterografting had no effect; similarly, in this experiment, sucrose was high in Hópehely compared to the White Lady. However, hetero grafting elevated the sucrose considerably. this could result from altering enzymes involved in the starch metabolism by the tomato scion that raises levels (White et al., 2016).

A significant difference was observed with homo and hetero grafting in this experiment in sucrose level, which is also contrary to (Odgerel and Bánfalvi,2021). This is because they used to inter cultivar as a hetero graft while in this experiment, interspecies was used. This coincides with Villányi, et al., (2022), who analyzed the effects of grafting on early and maturing potato lines and found that heterografting had a significant alteration in the metabolites. This could also be due to the earliness and lateness effect. (Inostroza-Blancheteau et al., 2018). found the lowest level of sucrose in Désirée, which concurs with the results of this experiment. However, hetero elevated the sucrose level significantly compared to homo grafting.

Grafting technology could be the cheapest method to improve the quality of potatoes in developing countries where poverty is on the rise and usually populated. It can be done without expertise right at the nurseries. This way, farmers can maximize the use of their small farms as they can produce two vegetables simultaneously, saving time and money. Therefore, future experiments are recommended to elucidate the variations brought by grafting.

7. SUMMARY

Hungarian University of Agriculture and Life Sciences

MSc. Plant Biotechnology

METABOLOMIC ANALYSIS AND GENETIC TRAITS OF GRAFTINGS OF *SOLANUM TUBEROSUM* AND *SOLANUM LYCOPERSICUM* PLANTS

Author: **Catherine Okaroni Ong'iro**

XAFZU5

Primary Supervisor: Vanda Villányi

Institute/Department: **Institute of Genetics and Biotechnology, Department of Plant Biotechnology**

Gödöllő

2023.

Potato (*Solanum tuberosum* L.), native to the Andes in South America, is the most consumed vegetable and fourth most nongrain crop in the world after wheat, rice, and maize, with an annual production of 376M tonnes. In yields per tonne, China leads India, and Russia, whereas Europe and Africa are upcoming in production.

Potatoes are a source of starch, proteins, minerals, crude fiber, and antioxidants. Starch is used in textiles, pharmaceuticals, food, dye, and paper. It has a pliable and short life cycle of 100 to 120 days compared to other crops. It's cheap, readily, and can thrive in conditions other vegetables cannot.

Poverty in developing countries causes Malnutrition, unhealthy diets, and lifestyle changes that are risk factors for noncommunicable diseases, which are the primary cause of death worldwide. Anthocyanins rich in antioxidants found in potatoes can help to alleviate conditions like Cancer, type 2 diabetes, and heart disease, which are a result of inflammation.

However, their low concentration in the epidermis of potatoes needs to meet the daily intake. Therefore, to increase their concentration, grafting technology has been upheld. Currently, grafting of tomato on potato rootstock is anticipated to increase the concentration of anthocyanins in potatoes which needs to be ascertained.

In this experiment, three potato cultivars cv. Désirée, Hópehely, and White Lady, were homo and hetero grafted using tomatoes, their close relative. The effects of grafting in greenhouse conditions were investigated using a spectrophotometer for relative anthocyanin absorbance for tuber skin color change; RT-PCR and RT- qPCR for gene expression in tuber skin and GC-MS analysis for untargeted metabolites changes in comparison to the non-grafted as the control.

Objectives

1. To assess the effects of tomato grafting on the potato skin color
2. To determine the effects of tomato grafting on potato tuber metabolites
3. To study the changes in gene expression of tuber skin because of heterografting

Results

1. Spectrophotometry results showed that hetero grafting in Désirée had a double fold upregulation of relative anthocyanin absorbance of 0.419 compared to 0.288 of the non-grafted control, followed by hetero grafted Hópehely with relative absorbance of 0.26 compared to 0.11 non grafted control and lastly hetero grafted White Lady had the lowest relative absorbance of 0.118 compared to 0.062 non grafted control. This indicates that heterografting elevated the synthesis of anthocyanin, which varies with the cultivar.
2. The RT-PCR and RT- qPCR results showed that the expression of AN1 was elevated by hetero grafting in Désirée and reduced by homo grafting, contrary to Hópehely and White Lady, which showed elevation with homo grafts and decreased with hetero grafts. This variation could be brought by the cultivars and other secondary factors that are in play in the background.
3. The GC-MS analysis showed that hetero grafting significantly altered the tuber metabolites and impacted sucrose by elevating it.

8. ACKNOWLEDGEMENT

First, I thank God Almighty for the gift of life, Grace, and Favor that enabled me to undertake this study successfully. I thank my supervisor, Dr. Vanda Villányi, and Dr. Zsófia Bánfalvi, for allowing me to conduct the research in their department. I thank Kongorzul Odgerel for being so patient with me. I also thank Monika Tekla Kiss and Flóra Karsai-Rektenwald for their support, and finally, I thank Dr. Onyango, who advised me on the topic, and my family for their moral support.

9. BIBLIOGRAPHY

1. Abdeldym, E. A., El-Mogy, M. M., Abdellateaf, H. R. L., & Atia, M. A. M. (2020). Genetic Characterization, Agro-Morphological and Physiological Evaluation of Grafted Tomato under Salinity Stress Conditions. *Agronomy*, *10*(12), p.1948. https://doi.org/10.3390/agronomy10121948
2. Ahn, J. Y., Kim, J., Yang, J. Y., Lee, H. J., Kim, S., Cho, K.-S., Lee, S.-H., Kim, J.-H., Lee, T.-H., Hur, Y., & Shim, D. (2022). Comparative Transcriptome Analysis between Two Potato Cultivars in Tuber Induction to Reveal Associated Genes with Anthocyanin Accumulation. *International Journal of Molecular Sciences*, *23*(7), p. 3681. https://doi.org/10.3390/ijms23073681
3. Bigna, J. J., Noubiap, J. J., Nansseu, J. R., & Aminde, L. N. (2017). Prevalence and etiologies of pulmonary hypertension in Africa: a systematic review and meta-analysis. *BMC Pulmonary Medicine*, *17*(1), p.183. https://doi.org/10.1186/s12890-017-0549-5
4. Bonar, N., Liney, M., Zhang, R., Austin, C., Dessoly, J., Davidson, D., Stephens, J., McDougall, G., Taylor, M., Bryan, G. J., & Hornyik, C. (2018). Potato miR828 Is Associated With Purple Tuber Skin and Flesh Color. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01742
5. Charepalli, V., Reddivari, L., Radhakrishnan, S., Vadde, R., Agarwal, R., & Vanamala, J. K. P. (2015). Anthocyanin-containing purple-fleshed potatoes suppress colon tumorigenesis via elimination of colon cancer stem cells. *The Journal of Nutritional Biochemistry*, *26*(12), pp.1641–1649. https://doi.org/10.1016/j.jnutbio.2015.08.005
6. Chaves-Silva, S., Santos, A. L. dos, Chalfun-Júnior, A., Zhao, J., Peres, L. E. P., & Benedito, V. A. (2018). Understanding the genetic regulation of anthocyanin biosynthesis in plants – Tools for breeding purple varieties of fruits and vegetables. *Phytochemistry*, *153*, pp.11–27. https://doi.org/10.1016/j.phytochem.2018.05.013
7. Chen, Q., Li, Y., Han, H., Ma, Y., Xie, X., Feng, Y., Zhao, Q., Dong, Y., Zhao, X., Gu, H., Huang, J., & Zhang, Y. (2022). Transcriptome and metabolite profiling analyses reveal the molecular mechanism underlying the characteristic accumulation of anthocyanins and flavonols in Fritillaria unibracteata and F. delavayi. *Industrial Crops and Products*, *186*, p.115183. https://doi.org/10.1016/j.indcrop.2022.115183
8. Chen, Q., Min, A., Luo, S., He, J., Wu, R., Lin, X., Wang, Y., He, W., Zhang, Y., Lin, Y., Li, M., Zhang, Y., Luo, Y., Tang, H., & Wang, X. (2022). Metabolomic Analysis Revealed Distinct Physiological Responses of Leaves and Roots to Huanglongbing in a Citrus Rootstock. *International Journal of Molecular Sciences*, *23*(16), p. 9242. https://doi.org/10.3390/ijms23169242
9. Chen, Q., Zhang, X., Fang, Y., Wang, B., Xu, S., Zhao, K., Zhang, J., & Fang, J. (2022). Genome-Wide Identification and Expression Analysis of the R2R3-MYB Transcription Factor Family Revealed Their

Potential Roles in the Flowering Process in Longan (Dimocarpus longan). *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.820439

10. Chikowore, T., Kamiza, A. B., Oduaran, O. H., Machipisa, T., & Fatumo, S. (2021). Non-communicable diseases pandemic and precision medicine: Is Africa ready? *EBioMedicine*, *65*, p. 103260. https://doi.org/10.1016/j.ebiom.2021.103260
11. Coşkun, Ö. F. (2023). The Effect of Grafting on Morphological, Physiological and Molecular Changes Induced by Drought Stress in Cucumber. *Sustainability*, *15*(1), p. 875. https://doi.org/10.3390/su15010875
12. D'Amelia, V., Staiti, A., D'Orso, F., Maisto, M., Piccolo, V., Aversano, R., & Carputo, D. (2022). Targeted mutagenesis of *StISAC* stabilizes the production of anthocyanins in potato cell culture. *Plant Direct*, *6*(8). https://doi.org/10.1002/pld3.433
13. Darré, M., Valerga, L., Zaro, M. J., Lemoine, M. L., Concellón, A., & Vicente, A. R. (2022). Eggplant grafting on a cold-tolerant rootstock reduces fruit chilling susceptibility and improves antioxidant stability during storage. *Journal of the Science of Food and Agriculture*, *102*(8), pp. 3350–3358. https://doi.org/10.1002/jsfa.11682
14. Enciso-Rodriguez, F., Douches, D., Lopez-Cruz, M., Coombs, J., & de los Campos, G. (2018). Genomic Selection for Late Blight and Common Scab Resistance in Tetraploid Potato (*Solanum tuberosum*). *G3 Genes|Genomes|Genetics*, *8*(7), pp.2471–2481. https://doi.org/10.1534/g3.118.200273
15. Fallah, A. A., Sarmast, E., Fatehi, P., & Jafari, T. (2020). Impact of dietary anthocyanins on systemic and vascular inflammation: Systematic review and meta-analysis on randomised clinical trials. *Food and Chemical Toxicology*, *135*, p.110922. https://doi.org/10.1016/j.fct.2019.110922
16. Hao, Y., Wang, J., Hu, C., Zhou, Q., Mubeen, H. M., & Hou, X. (2022). Regulation of BcMYB44 on Anthocyanin Synthesis and Drought Tolerance in Non-Heading Chinese Cabbage (Brassica campestris ssp. chinensis Makino). *Horticulturae*, *8*(5), p.351. https://doi.org/10.3390/horticulturae8050351
17. Hunter-Adams, J., Battersby, J., & Oni, T. (2019). Food insecurity in relation to obesity in peri-urban Cape Town, South Africa: Implications for diet-related non-communicable disease. *Appetite*, *137*, pp. 244–249. https://doi.org/10.1016/j.appet.2019.03.012
18. Inostroza-Blancheteau, C., de Oliveira Silva, F. M., Durán, F., Solano, J., Obata, T., Machado, M., Fernie, A. R., Reyes-Díaz, M., & Nunes-Nesi, A. (2018). Metabolic diversity in tuber tissues of native Chiloé potatoes and commercial cultivars of Solanum tuberosum ssp. tuberosum L. *Metabolomics*, *14*(10), p. 138. https://doi.org/10.1007/s11306-018-1428-7
19. Jaakola, L. (2013). New insights into the regulation of anthocyanin biosynthesis in fruits. *Trends in Plant Science*, *18*(9), pp. 477–483. https://doi.org/10.1016/j.tplants.2013.06.003

20. Jiang, Z., Chen, C., Wang, J., Xie, W., Wang, M., Li, X., & Zhang, X. (2016). Purple potato (Solanum tuberosum L.) anthocyanins attenuate alcohol-induced hepatic injury by enhancing antioxidant defense. *Journal of Natural Medicines*, *70*(1), pp. 45–53. https://doi.org/10.1007/s11418-015-0935-3
21. Jung, C. S., Griffiths, H. M., De Jong, D. M., Cheng, S., Bodis, M., Kim, T. S., & De Jong, W. S. (2009). The potato developer (D) locus encodes an R2R3 MYB transcription factor that regulates expression of multiple anthocyanin structural genes in tuber skin. *Theoretical and Applied Genetics*, *120*(1), pp.45–57. https://doi.org/10.1007/s00122-009-1158-3
22. Kaur, S., Tiwari, V., Kumari, A., Chaudhary, E., Sharma, A., Ali, U., & Garg, M. (2023a). Protective and defensive role of anthocyanins under plant abiotic and biotic stresses: An emerging application in sustainable agriculture. *Journal of Biotechnology*, *361*, pp. 12–29. https://doi.org/10.1016/j.jbiotec.2022.11.009
23. Kaur, S., Tiwari, V., Kumari, A., Chaudhary, E., Sharma, A., Ali, U., & Garg, M. (2023b). Protective and defensive role of anthocyanins under plant abiotic and biotic stresses: An emerging application in sustainable agriculture. *Journal of Biotechnology*, *361*, pp. 12–29. https://doi.org/10.1016/j.jbiotec.2022.11.009
24. Laimbeer, F. P. E., Bargmann, B. O., Holt, S. H., Pratt, T., Peterson, B., Doulis, A. G., Buell, C. R., & Veilleux, R. E. (2020). Characterization of the *F* Locus Responsible for Floral Anthocyanin Production in Potato. *G3 Genes|Genomes|Genetics*, *10*(10), pp. 3871–3879. https://doi.org/10.1534/g3.120.401684
25. Li, S., He, Y., Li, L., Li, D., & Chen, H. (2022). New insights on the regulation of anthocyanin biosynthesis in purple Solanaceous fruit vegetables. *Scientia Horticulturae*, *297*, 110917. https://doi.org/10.1016/j.scienta.2022.110917
26. Li, W., Wang, B., Wang, M., Chen, M., Yin, J., Kaleri, G. M., Zhang, R., Zuo, T., You, X., & Yang, Q. (2014). Cloning and characterization of a potato *StAN11* gene involved in anthocyanin biosynthesis regulation. *Journal of Integrative Plant Biology*, *56*(4), pp. 364–372. https://doi.org/10.1111/jipb.12136
27. Liu, C., Yao, X., Li, G., Huang, L., Liu, C., & Xie, Z. (2022). Development of Novel Markers and Creation of Non-Anthocyanin and Anthocyanin-Rich Broccoli (Brassica oleracea var. italica) Cultivars. *Applied Sciences*, *12*(12), p. 6267. https://doi.org/10.3390/app12126267
28. Liu, H., Liu, Z., Wu, Y., Zheng, L., & Zhang, G. (2021). Regulatory Mechanisms of Anthocyanin Biosynthesis in Apple and Pear. *International Journal of Molecular Sciences*, *22*(16), p. 8441. https://doi.org/10.3390/ijms22168441
29. Liu, J., Osbourn, A., & Ma, P. (2015). MYB Transcription Factors as Regulators of Phenylpropanoid Metabolism in Plants. *Molecular Plant*, *8*(5), pp.689–708. https://doi.org/10.1016/j.molp.2015.03.012

30. Liu, Y., Lin-Wang, K., Espley, R. V, Wang, L., Li, Y., Liu, Z., Zhou, P., Zeng, L., Zhang, X., Zhang, J., & Allan, A. C. (2019). StMYB44 negatively regulates anthocyanin biosynthesis at high temperatures in tuber flesh of potato. *Journal of Experimental Botany*, *70*(15), pp. 3809–3824. https://doi.org/10.1093/jxb/erz194
31. Loupit, G., & Cookson, S. J. (2020). Identifying Molecular Markers of Successful Graft Union Formation and Compatibility. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.610352
32. Mattioli, R., Francioso, A., Mosca, L., & Silva, P. (2020). Anthocyanins: A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases. *Molecules*, *25*(17), p. 3809. https://doi.org/10.3390/molecules25173809
33. Mattoo, A. K., Dwivedi, S. L., Dutt, S., Singh, B., Garg, M., & Ortiz, R. (2022a). Anthocyanin-Rich Vegetables for Human Consumption—Focus on Potato, Sweetpotato and Tomato. *International Journal of Molecular Sciences*, *23*(5), p. 2634. https://doi.org/10.3390/ijms23052634
34. Mattoo, A. K., Dwivedi, S. L., Dutt, S., Singh, B., Garg, M., & Ortiz, R. (2022b). Anthocyanin-Rich Vegetables for Human Consumption—Focus on Potato, Sweetpotato and Tomato. *International Journal of Molecular Sciences*, *23*(5), p. 2634. https://doi.org/10.3390/ijms23052634
35. Naik, J., Misra, P., Trivedi, P. K., & Pandey, A. (2022). Molecular components associated with the regulation of flavonoid biosynthesis. *Plant Science*, *317*, p. 111196. https://doi.org/10.1016/j.plantsci.2022.111196
36. Ngamsamer, C., Sirivarasai, J., & Sutjarit, N. (2022). The Benefits of Anthocyanins against Obesity-Induced Inflammation. *Biomolecules*, *12*(6), p. 852. https://doi.org/10.3390/biom12060852
37. Noor, R. S., Wang, Z., Umair, M., Yaseen, M., Ameen, M., Rehman, S.-U., Khan, M. U., Imran, M., Ahmed, W., & Sun, Y. (2019). Interactive Effects of Grafting Techniques and Scion-Rootstocks Combinations on Vegetative Growth, Yield and Quality of Cucumber (Cucumis sativus L.). *Agronomy*, *9*(6), p. 288. https://doi.org/10.3390/agronomy9060288
38. Odgerel, K., & Bánfalvi, Z. (2021). Metabolite analysis of tubers and leaves of two potato cultivars and their grafts. *PLOS ONE*, *16*(5), p. 0250858. https://doi.org/10.1371/journal.pone.0250858
39. Oertel, A., Matros, A., Hartmann, A., Arapitsas, P., Dehmer, K. J., Martens, S., & Mock, H.-P. (2017). Metabolite profiling of red and blue potatoes revealed cultivar and tissue specific patterns for anthocyanins and other polyphenols. *Planta*, *246*(2), pp. 281–297. https://doi.org/10.1007/s00425-017-2718-4
40. Owino, V. O. (2019). Challenges and opportunities to tackle the rising prevalence of diet-related non-communicable diseases in Africa. *Proceedings of the Nutrition Society*, *78*(4), pp. 506–512. https://doi.org/10.1017/S0029665118002823
41. Parthasarathi, T., Ephrath, J. E., & Lazarovitch, N. (2021). Grafting of tomato (Solanum lycopersicum L.) onto potato (Solanum tuberosum L.) to improve salinity tolerance. *Scientia Horticulturae*, *282*, p. 110050. https://doi.org/10.1016/j.scienta.2021.110050

42. Payyavula, R. S., Singh, R. K., & Navarre, D. A. (2013). Transcription factors, sucrose, and sucrose metabolic genes interact to regulate potato phenylpropanoid metabolism. *Journal of Experimental Botany*, *64*(16), pp. 5115–5131. https://doi.org/10.1093/jxb/ert303
43. Shehata, S. A., Omar, H. S., Elfaidy, A. G. S., EL-Sayed, S. S. F., Abuarab, M. E., & Abdeldaym, E. A. (2022). Grafting enhances drought tolerance by regulating stress-responsive gene expression and antioxidant enzyme activities in cucumbers. *BMC Plant Biology*, *22*(1), p. 408. https://doi.org/10.1186/s12870-022-03791-7
44. Singh, H., Sethi, S., Kaushik, P., & Fulford, A. (2020). Grafting vegetables for mitigating environmental stresses under climate change: a review. *Journal of Water and Climate Change*, *11*(4), pp. 1784–1797. https://doi.org/10.2166/wcc.2019.177
45. Smith, J., & Saravanakumar, D. (2022). Development of resistance in tomato plants grafted onto Solanum torvum against bacterial wilt disease. *Journal of Plant Diseases and Protection*, *129*(6), pp. 1389–1399. https://doi.org/10.1007/s41348-022-00650-3
46. Spanò, R., Ferrara, M., Gallitelli, D., & Mascia, T. (2020). The Role of Grafting in the Resistance of Tomato to Viruses. *Plants*, *9*(8), p. 1042. https://doi.org/10.3390/plants9081042
47. Staiti, A., D'Amelia, V., & Carputo, D. (2022). Exploring and exploiting anthocyanins for human needs: potato as a research case study. *Italus Hortus*, *29*(2), pp. 18–34. https://doi.org/10.26353/j.itahort/2022.2.1834
48. Stiekema, W. J., Heidekamp, F., Dirkse, W. G., van Beckum, J., de Haan, P., Bosch, C. ten, & Louwerse, J. D. (1988). Molecular cloning and analysis of four potato tuber mRNAs. *Plant Molecular Biology*, *11*(3), pp. 255–269. https://doi.org/10.1007/BF00027383
49. Strygina, K. V., & Khlestkina, E. K. (2017). ANTHOCYANINS SYNTHESIS IN POTATO (Solanum tuberosum L.): GENETIC MARKERS FOR SMART BREEDING (review). *Sel'skokhozyaistvennaya Biologiya*, *52*(1), pp. 37–49. https://doi.org/10.15389/agrobiology.2017.1.37eng
50. Strygina, K. V., Kochetov, A. V., & Khlestkina, E. K. (2019). Genetic control of anthocyanin pigmentation of potato tissues. *BMC Genetics*, *20*(S1), p. 27. https://doi.org/10.1186/s12863-019-0728-x
51. Sunil, L., & Shetty, N. P. (2022). Biosynthesis and regulation of anthocyanin pathway genes. *Applied Microbiology and Biotechnology*, *106*(5–6), pp. 1783–1798. https://doi.org/10.1007/s00253-022-11835-z
52. Tomlekova, N., Mladenov, P., Dincheva, I., & Nacheva, E. (2022). Metabolic Profiling of Bulgarian Potato Cultivars. *Foods*, *11*(13), p. 1981. https://doi.org/10.3390/foods11131981
53. Tsaballa, A., Xanthopoulou, A., Madesis, P., Tsaftaris, A., & Nianiou-Obeidat, I. (2021). Vegetable Grafting From a Molecular Point of View: The Involvement of Epigenetics in Rootstock-Scion Interactions. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.621999

54. Tsang, C., Smail, N. F., Almoosawi, S., McDougall, G. J. M., & Al-Dujaili, E. A. S. (2018). Antioxidant Rich Potato Improves Arterial Stiffness in Healthy Adults. *Plant Foods for Human Nutrition*, *73*(3), pp. 203–208. https://doi.org/10.1007/s11130-018-0673-2
55. Ulas, F., Yetisir, H., & Ulas, A. (2021). Root-growth Characteristics Contributing to Nitrogen Efficiency of Reciprocally Grafted Potatoes (Solanum tuberosum L.) Under Hyroponic Conditions. *Gesunde Pflanzen*, *73*(4), pp. 417–425. https://doi.org/10.1007/s10343-021-00560-8
56. Villányi, V., Gondor, O. K., & Bánfalvi, Z. (2022). Metabolite profiling of tubers of an early- and a late-maturing potato line and their grafts. *Metabolomics*, *18*(11), p. 88. https://doi.org/10.1007/s11306-022-01950-3
57. Wang, L., Yang, S., Ni, J., Teng, Y., & Bai, S. (2023a). Advances of anthocyanin synthesis regulated by plant growth regulators in fruit trees. *Scientia Horticulturae*, *307*, p. 111476. https://doi.org/10.1016/j.scienta.2022.111476
58. Wang, L., Yang, S., Ni, J., Teng, Y., & Bai, S. (2023b). Advances of anthocyanin synthesis regulated by plant growth regulators in fruit trees. *Scientia Horticulturae*, *307*, p. 111476. https://doi.org/10.1016/j.scienta.2022.111476
59. White, A. C., Rogers, A., Rees, M., & Osborne, C. P. (2016). How can we make plants grow faster? A source–sink perspective on growth rate. *Journal of Experimental Botany*, *67*(1), pp. 31–45. https://doi.org/10.1093/jxb/erv447
60. Xing, C., Chen, P., & Zhang, L. (2023). Computational insight into stability-enhanced systems of anthocyanin with protein/peptide. *Food Chemistry: Molecular Sciences*, *6*, p. 100168. https://doi.org/10.1016/j.fochms.2023.100168
61. Xu, W., Dubos, C., & Lepiniec, L. (2015). Transcriptional control of flavonoid biosynthesis by MYB–bHLH–WDR complexes. *Trends in Plant Science*, *20*(3), pp. 176–185. https://doi.org/10.1016/j.tplants.2014.12.001
62. Yan, H., Pei, X., Zhang, H., Li, X., Zhang, X., Zhao, M., Chiang, V. L., Sederoff, R. R., & Zhao, X. (2021). MYB-Mediated Regulation of Anthocyanin Biosynthesis. *International Journal of Molecular Sciences*, *22*(6), p. 3103. https://doi.org/10.3390/ijms22063103
63. Yuan, H., Sun, L., Tai, P., Liu, W., Li, X., & Hao, L. (2019). Effects of grafting on root-to-shoot cadmium translocation in plants of eggplant (Solanum melongena) and tomato (Solanum lycopersicum). *Science of The Total Environment*, *652*, pp. 989–995. https://doi.org/10.1016/j.scitotenv.2018.10.129
64. ZHANG, D., LIU, L., ZHOU, D., LIU, X., LIU, Z., & YAN, M. (2020). Genome-wide identification and expression analysis of anthocyanin biosynthetic genes in Brassica juncea. *Journal of Integrative Agriculture*, *19*(5), pp. 1250–1260. https://doi.org/10.1016/S2095-3119(20)63172-0
65. Zhang, H., Hassan, Y. I., Renaud, J., Liu, R., Yang, C., Sun, Y., & Tsao, R. (2017). Bioaccessibility, bioavailability, and anti-inflammatory effects of anthocyanins from purple root vegetables using mono- and

co-culture cell models. *Molecular Nutrition & Food Research*, *61*(10), p. 1600928. https://doi.org/10.1002/mnfr.201600928

66. Zhang, H., Yang, B., Liu, J., Guo, D., Hou, J., Chen, S., Song, B., & Xie, C. (2017). Analysis of structural genes and key transcription factors related to anthocyanin biosynthesis in potato tubers. *Scientia Horticulturae*, *225*, pp. 310–316. https://doi.org/10.1016/j.scienta.2017.07.018
67. Zhang, H., Zhao, X., Zhang, J., Yang, B., Yu, Y., Liu, T., Nie, B., & Song, B. (2020). Functional analysis of an anthocyanin synthase gene StANS in potato. *Scientia Horticulturae*, 272, p. 109569. https://doi.org/10.1016/j.scienta.2020.109569
68. Zhang, S., Zhang, A., Wu, X., Zhu, Z., Yang, Z., Zhu, Y., & Zha, D. (2019). Transcriptome analysis revealed expression of genes related to anthocyanin biosynthesis in eggplant (Solanum melongena L.) under high-temperature stress. *BMC Plant Biology*, *19*(1), p. 387. https://doi.org/10.1186/s12870-019-1960-2
69. Zhong, H., Liu, Z., Zhang, F., Zhou, X., Sun, X., Li, Y., Liu, W., Xiao, H., Wang, N., Lu, H., Pan, M., Wu, X., & Zhou, Y. (2022). Metabolomic and transcriptomic analyses reveal the effects of self- and hetero-grafting on anthocyanin biosynthesis in grapevine. *Horticulture Research*, *9*. https://doi.org/10.1093/hr/uhac103

DECLARATION

on authenticity and public assess of final essay/thesis/master's thesis/portfolio[1]

Student's name: OKARONI CATHERINE ONG'IRO

Student's Neptun ID: XAFZU5

Title of the document: **METABOLOMIC ANALYSIS AND SOME GENETIC TRAITS OF GRAFTINGS OF *SOLANUM TUBEROSUM* AND *SOLANUM LYCOPERSICUM* PLANTS**

Year of publication: ____2023__

Department: **Institute of Genetics and Biotechnology, Department of Plant Biotechnology**

I declare that the submitted final essay/thesis/master's thesis/portfolio[2] is my original individual creation. Any parts taken from another author's work are clearly marked and listed in the table of contents.

If the statements above are not true, I acknowledge that the Final examination board excludes me from participation in the final exam, and I am only allowed to take the final exam if I submit another final essay/thesis/master's thesis/portfolio.

Viewing and printing my submitted work in a PDF format is permitted. However, the modification of my submitted work shall not be permitted.

I acknowledge that the rules on Intellectual Property Management of Hungarian University of Agriculture and Life Sciences shall apply to my work as an intellectual property.

I acknowledge that the electric version of my work is uploaded to the repository system of the Hungarian University of Agriculture and Life Sciences.

Place and date: __Budapest_____ year ____2023__month ___03____day

_____ _____
Student's signature

[1]Please select the one that applies, and delete the other types.
[2]Please select the one that applies, and delete the other types.

STATEMENT ON CONSULTATION PRACTICES

As a supervisor of Catherine Okonji (Student's name) XAFZU5 (Student's NEPTUN ID), I here declare that the final essay/thesis/master's thesis/portfolio[1] has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

I recommend/don't recommend[2] the final essay/thesis/master's thesis/portfolio to be defended in a final exam.

The document contains state secrets or professional secrets: yes no*[3]

Place and date: Gödöllő, 2023 year May month 2 day

Internal supervisor

[1] Please select applicable and delete non-applicable.
[2] Please underline applicable.
[3] Please underline applicable.