5th International Conference of the International Commission of Agricultural and Biosystems Engineering (CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Québec City, Canada - May 11-14, 2021

APPLICATION OF INTEGRATED OMICS: TOWARDS UNDERSTANDING THE INCIDENCE OF HUSK SCALD OF POMEGRANATE FRUIT DURING POSTHARVEST HANDLING

ZINASH ASSEFA BELAY[12*], ALVERA VORSTER[3], CAREL VAN HEERDEN[3], LUCKY MOKWENA[4], UMEZURUIKE LINUS OPARA[1], OLUWAFEMI JAMES CALEB[2]

[1]Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Horticultural Science, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa
[2]Agri-Food Systems and Omics Laboratory, Post-Harvest and Agro-Processing Technologies (PHATs), Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch 7599, South Africa
[3]The Central Analytical Facilities (CAF), the DNA-sequencing unit, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa
[4]The Central Analytical Facilities (CAF), the DNA-sequencing unit, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa
*Corresponding author: E-mail:BelayZ@agric.co.za

CSBE20XXXX – Presented at World Congress on Computers in Agriculture 2020 (WCCA); *CSBE20*

ABSTRACT

Pomegranate fruit loses quality during long term storage due to continued metabolic responses and physiological disorders, which usually coincides with commercial shipping period. Physiological disorder such as husk scald affects the fruit appearance and limits marketability. However, the molecular mechanism underpinning husk scald incidence on pomegranate fruit peel remain unclear. Therefore, the aim of this study was to identify the molecular responses of pomegranate fruit peel to husk scald incidence using RNA-seq (Ion Torrent™ Proton™ Next Generation Sequencing) and gas chromatograph mass spectroscopy (GC-MS). The RNA-seq analysis generated 98,441,278 raw reads and genes were annotated using UniPort Consortium. Approximately 652 differentially expressed genes (DEGs) with a fold change of > |2|, a *p* value ≤ 0.05 and a false discovery rate (FDR) of < 0.05 were identified between healthy and husk scald fruit peels. Various enzyme classes and pathways which are linked to structural metabolism of pectin, amino acid biosynthesis and protein modification as well as transferase and hydrolase activities were identified. Metabolites analysis also indicated higher concentration of amino acids, sugars and volatile organic compounds in the scald fruit. Higher concentration of Lysine_1 (8,17 to 10,13 mg/L), Threonine_1 (8,38 to 10,40 mg/L), α-Bergamotene (0,331 g/L) and γ-Terpinene (0,224 g/L) were observed in scald fruit. The data obtained will provide valuable information for unravelling the molecular mechanism of scald incidence and lay a foundation for future studies towards understanding and eliminating husk scald development in pomegranate fruit.

Keywords: cv. 'Wonderful', Enzyme classes, gene expression, metabolites, *Punica granatum*, physiological disorder, RNA-Seq,

INTRODUCTION

Pomegranate fruit has become an emerging profitable fruit and has been considered as a functional food with high content of health promoting metabolites in its peel and arils (Yuan et al., 2017). The colour of pomegranate peel tissue is of special interest since it produces simultaneous metabolites of anthocyanin and hydrolysable tannins pathways that directly influences the sensitivity of the fruit to biotic and abiotic stresses (Harel-Beja et al., 2019). Moreover, the red colour of pomegranate fruit free from any defects drive its consumer acceptance and marketability. Mechanical damage, microbial decay and/or development of physiological disorder are the main factors affecting the appearance and colour of pomegranate fruit. Therefore, proper handling, transportation, pre-treatments and appropriate storage conditions are crucial during postharvest handling of pomegranate fruit to ensure superior quality and longer self-life.

Recommended storage temperature (> 5°C) for pomegranate fruit to avoid chilling injury, however, this could result in husk scald development on the peel during long-term storage (Defilippi et al., 2006). Husk scald is a major physiological disorder that is characterised by the superficial fruit skin/peel browning. Various studies suggested that the mechanism leading husk scald development in pomegranate fruit peel could be related to: oxidative stress, PPO activities, total tannins (Arendse et al., 2018), and enzymatic oxidation of phenolic compounds and carotenoids (Zhang & Zhang, 2008). The hypothesis behind scald development mostly linked to the oxidation of α-farnesene sesquiterpene and its subsequent oxidation into conjugate trionls (CTols). Furthermore, the physiological causes of scald in fruit has also been suggested to be due to PPO activity via the oxidation of chlorogenic acid that leads to the accumulation of quinones and melanin (Giné-Bordonaba et al., 2020).

Current investigations regarding husk scald development in various fruit indicated that this physiological disorder is linked to α-farnesene synthase gene (AFS). The recent attempt to understand the transcriptomic dynamics for pomegranate (cv. Wonderful) scald incidence was reported by Belay et al. (2020). The authors showed a significantly up regulation of protein modification associated with cell membranes and biosynthesis of tannins related to scald incidence. Recently, Baghel et al. (2021) related the metabolic changes in pomegranate fruit peel to chilling injury. The authors suggested that development of chilling injury on the pomegranate fruit peel could be associated with the total phenolic content and PPO activities. However, the integrated analysis of primarily metabolites and gene expression to understand the incidence of scald during storage of pomegranates has not been fully understood. Therefore, the aim of this study is to investigate the incidence of husk scald in pomegranate fruit peel by using primary metabolite studies, volatile organic compounds emission and gene expression analysis.

MATERIAL AND METHODS

Fruit sampling and storage condition

Pomegranate fruit (cv. Wonderful) were obtained at the commercially ripened stage with characteristic deep-red skin and arils with mature from Sonlia Pack House, Wellington, Western Cape, South Africa. Fruit were transported in well-ventilated boxes to the Postharvest Technology Research Laboratory, Stellenbosch University, South Africa. On arrival, fruit were stored in a cold storage room at 5 °C for sorting. After sorting, fruit

were stored at 7 °C with relative humidity (RH) of 91 ± 4% for 3 months, followed by 2 weeks shelf life at ambient storage to simulate marketing conditions before final quality evaluations. On each sampling day, pomegranate peels (from fruit with disorder (SP) and without disorder (HP)) were manually separated from fruit. The peels were pooled separately into liquid nitrogen. All samples were stored at −80 °C until further analysis. RNA-Seq and metabolite analysis was done at the Stellenbosch University, Central Analytical Facility (CAF) DNA Sequencing Unit and GC-MS unit, respectively.

Primary metabolite analysis

Primary metabolite (sugars, and amino acids) analysis of pomegranate peel were determined by gas chromatography-mass spectrometry (GC-MS) approach as described by Wu et al. (2018). Pomegranate fruit peel was extracted in methanol. Volatile compounds were analysed by HS-SPME-GC-MS. The relative content (%) of each volatile compound was calculated by dividing the peak area of each component by the total peak area of all the compounds identified.

RNA extraction and Sequence

Whole transcriptome sequencing was performed on the Ion Torrent™ Proton™ next generation sequencing platform (ThermoFisher Scientific, Waltham, MA, USA). The detail RNA-Seq steps and protocols are presented in Belay et al. (2020). Differential gene expression (DGEs) analysis was done based on gene expression using Partek flow package. Punica granatum ASM765513v2 (https://www.ncbi.nlm.nih.gov/genome/ 13946) was used as the reference genome and genes were annotated using Swiss-Port (http://www.uniport.org). A p value ≤ 0.05, false discovery rate (FDR) of < 0.05 was used as a selection criterion, and a two-fold change were used to determine significant differences in gene expression. The GO, enzyme class and pathway analysis was performed using UniProt Consortium.

Statistical analysis

The results presented a mean value of three replicates using one-way analysis of variance (ANOVA). The analysis was performed using Statistica software (version 13, StatSoft Inc. TIBCO Software Inc., USA). The mean differences were determined by Ducan's multiple range test at ($p \leq 0.05$) significant difference.

RESULTS

Primary metabolites

Sugars and sugar alcohols

Sugars (D-Fructose, D-Glucose and Sucrose) and sugar alcohols (Mannitol and Myo-Inositol) were found on both HP and SP of pomegranate (Figure 1). Compared to the heathy peel, the scald pomegranate peel had higher concentration of D-Fructose (1005 mg/L), and Mannitol (181.98 mg/L). In contrast, concentration of sucrose and Myo-Inositol was similar in both HP and SP. Similarly, Belay et al. (2018) and Palma et al. (2006) found out that fructose was the predominant sugar in pomegranate 'Wonderful' and 'Primosole' fruit followed by glucose and succors. On the contrary, Hasnaoui et al. (2018) reported xylose and arabinose (60%) followed by galactose (14%), and glucose (10%) as main sugars in 12 Tunisian pomegranate varieties. Similarly, Gavlighi et al. (2018) found out the main sugar in Iranian pomegranate peel are glucose (44-68%) followed by

galactose (14-19%). The variation of sugars and sugar alcohols on the above studies clearly distinguish the effects of different cultivars towards the concentration of these metabolites on pomegranate fruit peel.

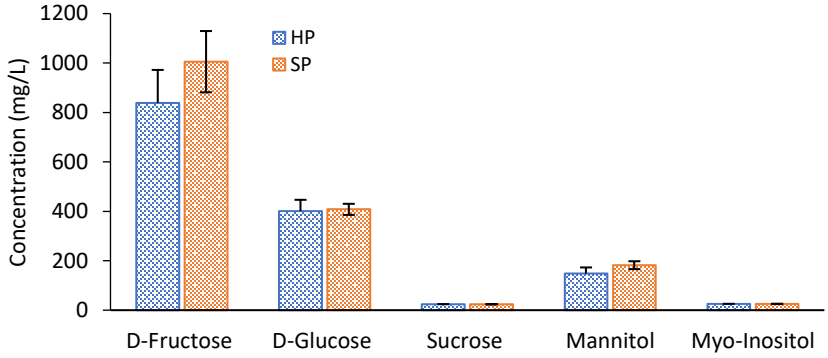

Figure 1. Individual sugar and Sugar alcohols presented in healthy (HP) and scald pomegranate (SP) fruit peel

Sugars and sugar alcohols are significant sources of energy, contribute to the quality of the fruit and affects fruit stress resistance (Der Agopain et al., 2011). The increase in fructose, D-Glucose and mannitol during scald incidence could be explained by the hydrolysis of sucrose. Consistently, the sucrose content on both HP and SP was the lowest than other sugars or sugar alcohols. Wang et al. (2013) reported that high sucrose content in peaches played an important role to alleviate chilling injury than fructose and glucose. Similarly Jiang et al. (2013) suggested sucrose is more important than hexose to protect grape branches from cold injury. Sugars are involved in signal transduction and gene expression throughout all stages of the plants (Xu et al., 2016). Sugars that accumulate in the peel tissue of banana was reported to be the major factor that regulates chlorophyll degradation (Yan et al., 2009). In addition, sucrose regulates osmotic pressure, stabilize cell membrane structure and regulate metabolic pathways (Yu et al., 2016). The lowest concentration of sucrose obtained on the peel therefore could be linked to the susceptibility of pomegranate to physiological disorder.

Amino acids

Results of the study showed that, in both HP and SP a total of 27 amino acids (AA) were identified (Figure 2). Overall, concentration of all the AAs were higher in scald fruit than HP. According to previous studies by Rhodes et al. (1998, these amino acids plays an important role in fruit response to stress. The accumulation or biosynthesis of lysine under stress is considered a general response to environmental stresses that affects the cellular energy status, and leads to an energy limitation (Ali et al., 2019). On the other hand, the accumulation of glutamate is linked to the increase in stress tolerance as it can serve as a source of nitrogen and carbon.

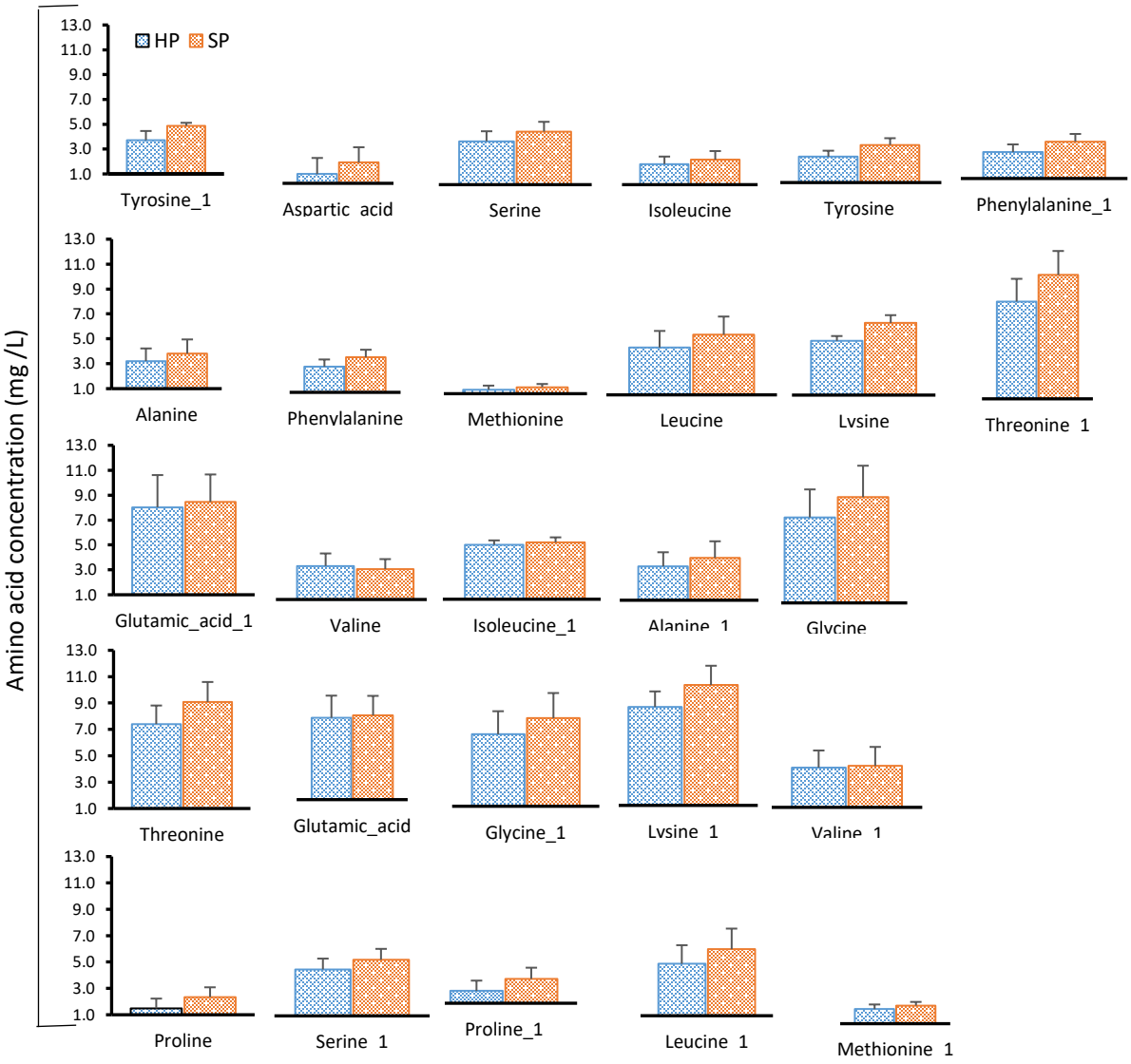

Figure 2. List of Amino acids presented in healthy and scald pomegranate fruit peel

Amino acid content affected to a large extend by postharvest storage, as these compounds are involved in several pathways induced during postharvest handling. Luengwilai et al. (2017) reported that amino acid increased during chilling stress of different varieties of pineapple. According to Ali et al. (2019), accumulation of AAs is the most common response of plant to abiotic stresses with multifarious functional roles by acting as compatible osmolytes, involved in pH regulation and detoxification of reactive oxygen species (ROS). However, in some cases the accumulation of AAs concentration could indicates cell functionality damage than an adoptive response to environmental stress (Munns, 2002).

In the current study, the concentration of Aspartic acid (1.71, 2.58 mg/L), Methionine (1.31, 1.51 mg/L), Proline_1 (1.96, 2.90 mg/L) and Proline (1.49, 2.34 mg/L) was the lowest in both HP and SP, respectively. From various amino acids, proline is a well-documented stress related AA that accumulated during stress, that plays important role on membrane protection and ROS scavenging activates (Vazquez-Hernandez et al., 2018). However, in

the current study the proline content was very low comparing to other AA. Halilova and Yildiz (2009) reported that the effects of change in environmental condition on the Proline continent of Turkish cultivar pomegranate. Furthermore, according to Bustamante et al. (2016), the role of AA in counteracting stress could be species dependant. Therefore, the lower ratio of proline observed in the current could be due to the storage condition or the difference in cultivar of pomegranate fruit than the above-mentioned studies. The study done by Rowayshed et al (2013) on Egyptian pomegranate fruit peel powder demonstrated that the concentration of glutamine (0.52 g/100g), glycine (0.41g/100g and aspartate (0.3g/100g) was the most abundant amino acids. According to Ya'akov et al. (2019) amino acid content in pomegranate fruit peel can be influenced by the environmental condition particularly temperature. Amino acids plays an important role in protein biosynthesis, secondary metabolites synthesis as well as signaling processes in plant metabolism regulation and plant stress response (Ya'akov et al., 2019).

Volatile organic compounds

A total of 22 volatile organic compounds (VOCs) were tentatively identified in the healthy fruit peel (HP) and scald fruit peel (SP). These VOCs categorized under five functional group as presented in Table 1. Aldehyde and aromatic hydrocarbons represented the most abundant group in the HP, while monoterpene, sesquiterpene and sesquiterpenoids were predominant in SP (Table 1). Similarly, Melgarejo et al. (2010) and Belay et al. (2019) reported that aldehyde and monoterpene as a predominate functional groups in 9 Spanish pomegranate cultivars and 'wonderful' pomegranate, respectively. VOCs such as, Trans-2-Hexenal and Xylene only presented in HP. Comparing the concentration of VOCs, which are presented in both HP and SP, a significant increase of Ethylbenzene, β-Pinene, p-Cymene in the SP was observed compared to the HP. On the other hand, VOCs such as, Hexanal and l-Limonene emitted higher concentration in both HP and SP. From the identified 22 VOCs 13 of the VOCs only found in scald fruit. These include p-cymene, α-Cedrene, α-Bergamotene, β-Bisabolene β-Farnesene, Trans.β- Farnesene and α-Curcumene, which had a high ratio that indicates terpenes were the prevalent class of VOCs in the scald fruit peel.

Table 1. Volatile organic compounds (VOCs) in healthy and scald peel pomegranate fruit

			Value (ratio)	
Functional group	**VOCs**	**RT**	**Healthy fruit peel (HP)**	**Scald fruit peel (SP)**
Aldehyde	Hexanal	12.60 – 12.63	0.067	0.053
	Trans-2-Hexenal	19.37	0.026	–
Aromatic hydrocarbons	Ethylbenzene	14.20 – 16.78	0.004	0.022
Monoterpene	β-Pinene	13,04 – 13.07	0.010	0.030
	m-Xylene	14.55 – 14.60	0.003	0.007
	p-Xylene	14.81 – 14.85	0.006	0.011
	Xylene	16.67	0.008	–
	l-Limonene	16.85 – 17.05	0.095	0.109
	p-Cymene	21.34 – 21.59	0.004	0.063
	l-Phellandrene	15.51	–	0.006
	Myrcene	15.67	–	0.015

	α-Terpinene	16.14	–	0.016
	γ-Terpinene	19.85	–	0.224
	p-cymene	21.59	–	0.063
	α-terpinolene	22.05	–	0.013
	α-Cedrene	37.34	–	0.099
Sesquiterpene	α-Bergamotene	38.12	–	0.331
	β-Bisabolene	42.34	–	0.100
Sesquiterpenoids	β-Farnesene	40.80	–	0.069
	Trans.β- Farnesene	41.24	–	0.090
	γ-curcumene	41.58	–	0.042
	α-Curcumene	43.11	–	0.149

Mean (*n* = 3), RT = Retention time

The high emission of VOCs under sesquiterpene functional group suggested that the similarity of apple and pomegranate scald. According to Whitaker (2004) scald in apple occurred due to the oxidation of conjugated triene products of α-Farnesene*,* which is a *s*esquiterpene. There are sufficient studies done for scald control in apple fruit, therefore, the identified cause similarities would be used to strategize the control of scald development in pomegranate fruit. α-Cedrene is a tricyclic sesquiterpene, the biological role of α-Cedrene is a membrane stabilizer that support the formation and maintenance of a structurally sound and functioning cell or organelle membrane, which further strengthen the membrane and decrease its permeability (Phospholipids). Tietel et al. (2012) demonstrated the high emission of terpenoid in chilling sensitive mandarin fruit, which deceased fruit palatability, as terpenes can, contributes to musty, sticky and oily notes. According Pott et al. (2019) the accumulation of monoterpene, (limonene) and sesquiterpene (α-farnesen) over the storage duration could be related to temperature stress response.

Gene expression in pomegranate healthy and scald fruit peels

Differential gene expression was done to match the metabolite data, giving emphasis to the key gene in sugar, amino acid and volatile organic compounds biosynthesis. Gene expression was done before and after the development of scald on pomegranate fruit peel during storage. Results indicated that 652 differentially expressed genes (DEG) with a fold change of >2, a p value < 0.05 and a false discovery rate (FDR) < 0.05. The differentially expressed genes categorized under four enzymatic classes: transferase (45%), hydrolase (30%), oxidoreductase (18%) and lyases (95%). The only enzyme class down regulated in scald fruit peel was the transferase. The suppression of hexosyltransferase, which encode enzyme responsible for the biosynthesis of pectin, results in losing cell wall. On the other hand, husk scald development in the peel further resulted in up regulation of glucose-6-phosphate isomerase (*Pgr021428*), 1,2-dihydroxy-3-keto5-methylthiope (Pgr025648), lipoyl synthase, and mitochondrial (*LIP1*), which play an important role in cellular amino acid and carboxylic acid biosynthesis. Moreover, these up-regulated genes in the scald fruit peel activated amino-acid biosynthesis. Amino acid metabolism is tightly linked to ammonium (absorbed and synthesized from and demand for protein synthesis as well as secondary metabolism (Pratelli & Pilot, 2014). This could suggest cellular response towards mending the damaged scald tissues.

Three most significantly up regulated genes include *Pgr02508* (uncharacterized protein, 50% similarity to Glycine rich protein (GRP))*, Pgr007593* (Glycosyltransferase)*,*

and *Pgr016200* (*WAT1-related protein*). WAT1-related protein is a tonoplast-localized auxin transporter that encode a plant specific protein of 398 amino acids (Ranocha et al., 2013), and it is associated with integrating auxin signaling and secondary cell wall formation (Ranocha et al., 2010). Jaquinod et al. (2007) reported localization of *WAT1* in both plasma membrane and tonoplast fraction of Arabidopsis. The expression of amino acid biosynthesis pathways and secondary metabolites genes can be related to the higher emission of all amino acids in scald peel compared to healthy peel (Figure 3).

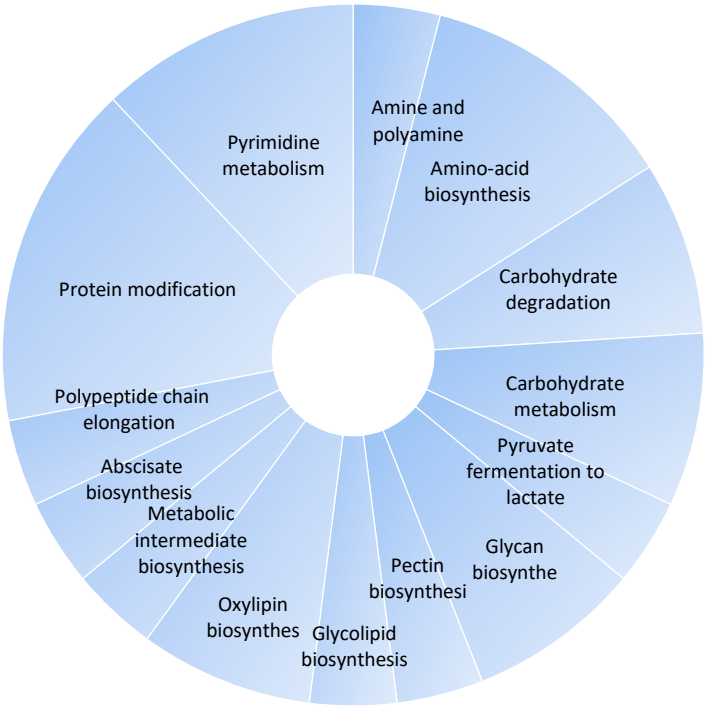

Figure 3. Up regulated pathways in pomegranate fruit peel

As can be seen in Table 2, from the isomerase enzyme activities group the expression of two gene (*CDL15_Pgr015913 and CDL15_Pgr020868*) encoding the terpene cyclase/mutase family member (EC 5.4.99) was observed during scald development. Terpene cyclase/mutase family member (EC 5.4.99) is involved in the triterpenoid biosynthetic process that result in the formation of triterpenoid compounds, terpenoids with six isoprene units. This could be associated with the higher emission of terpenes in scald fruit peel. The biosynthesis of sesquiterpenes is known to occur mainly through the mevalonic acid pathway (MVA), in the cytosol. However, recent studies have found evidence of pathway crosstalk with the methyl-eritritol-phosphate (MEP) pathway in the plastid. Terpenoids are derived from either the cytosolic mevalonic acid (MVA) pathway or the plastidic methylerythritol phosphate (Mep) pathway (Sallaude et al., 2009). Most of the monotropenes synthesised by downstream terpene synthases after the formation of geranyl diphosphate (GPP) (monoterpenes precursor) from the two-C5-isoprene building units, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) that could be regulated by transcription factors, including *AP2/ERF*(Li et al., 2017).

Table 2. Up regulated enzyme classes and protein names in scald pomegranate fruit peel

Enzyme classes	Protein name	Gene name
Oxidoreductases	Lipoxygenase	*CDL15_Pgr008562 CDL15_Pgr018980*
	Glyceraldehyde-3-phosphate dehydrogenase	*CDL15_Pgr019814, CDL15_Pgr014238*
	Proline dehydrogenase	*CDL15_Pgr027475*
	Peroxidase	*CDL15_Pgr002290 CDL15_Pgr012014*
Transferases	Glycosyltransferase	*CDL15_Pgr007593CDL15_Pgr003386 CDL15_Pgr023012CDL15_Pgr023013 CDL15_Pgr005743CDL15_Pgr002032 CDL15_Pgr022565 CDL15_Pgr004941 CRG98_009377 CDL15_Pgr003390 CRG98_046102 UGT84A23*
	Phosphotransferase	*CDL15_Pgr013361*
	Hexosyltransferase	*CDL15_Pgr020123CDL15_Pgr001425 CDL15_Pgr024399 CRG98_03695 CDL15_Pgr020178 CDL15_Pgr024012*
Hydrolases	Beta-galactosidase	*CDL15_Pgr015546 CDL15_Pgr005523 CDL15_Pgr001207 CDL15_Pgr025109*
	Carboxypeptidase	*CDL15_Pgr018192 CDL15_Pgr004597*
	Phosphoinositide phospholipase C	*CDL15_Pgr010348*
	Methionine aminopeptidase	*CDL15_Pgr019031*
	Beta-amylase	*CDL15_Pgr023254*
Lyases	Fructose-bisphosphate aldolase	*CDL15_Pgr004336*
	Threonine dehydratase (Threonine deaminase)	*CDL15_Pgr025511*
	Fructose-bisphosphate aldolase	*CDL15_Pgr012152 CRG98_015457*
Isomerases		
	Terpene cyclase/mutase family member	*CDL15_Pgr013941 CRG98_041458*

Glycosylation is significant for the structural modification of compounds with biological activities that allows the conversion of lipophilic compounds into hydrophilic once (Rivas et al., 2013). Glycosyltransferases (GTs) is the second highly expressed genes in the current study, which is one of the alternative glycosylation tool that can be used in biotransformation strategies that employ nucleotide activated sugars as glycosyl donor. From the up regulated transferase enzyme classes the highest number of genes encoding UDP-glycosyltransferase were observed, of which 13 of them were duplicated. UDP-glycosyltransferase catalysis the transfer of glycosyl group from a UDP-sugar to a small hydrophobic molecule (Lugue et al., 2002). In pomegranate genome, Yuan et al. (2018) reported expression of UDP-glycosyltransferase with the potential role of ellagitannin biosynthesis. Therefore, the up regulation GTs and biosynthesis of tannin could lead to the change in peel colour of pomegranate fruit.

Conclusion

In the present study, pomegranate fruit with healthy peel and scald peel were used to analyse and differentiate the metabolic changed. The results showed that scald incidence on the fruit peel enhanced the activation of metabolic and transcriptional pathways and

profile. The scald fruit promotes high concertation of sugars, sugar alcohols and amino acids profile. Furthermore, the differentially expressed genes (DEGs) indicated the up regulation of gene similar to Glycine rich protein (GRP)*),* Glycosyltransferase*,* and *WAT1-related protein* that are associated with amino acid, secondary metabolites biosynthesis, and membrane fractions. Scald incidence also influenced the up regulation of genes encoding the Terpene cyclase/mutase family member, which is responsible for biosynthesis of terpenoids. The DEGs identified in this study can be used as an indicator gene to reflect the physiological state of pomegranate fruit peel to incidence of scald. These DEGs could also be useful as a candidate gene for pomegranate breeders to improve the fruit tolerance to scald incidence.

Acknowledgements

This work is based on research supported wholly/in part by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) and the Agricultural Research Council (ARC). Dr O Caleb and Dr Z Belay acknowledges the award of NRF research support grant (116272), and Free standing Post-Doctoral Fellowship (120729), respectively.

REFERENCES

Ali, Q., Haider, M. Z., Shahid, S., Aslam, N., Shehzad, F., Naseem, J., & Hussain, S. M. (2019). Role of amino acids in improving abiotic stress tolerance to plants. In *Plant Tolerance to Environmental Stress* (pp. 175-204). CRC Press.

Arendse, E., Fawole, O. A., Magwaza, L. S., Nieuwoudt, H., & Opara, U. L. (2018). Evaluation of biochemical markers associated with the development of husk scald and the use of diffuse reflectance NIR spectroscopy to predict husk scald in pomegranate fruit. *Scientia Horticulturae*, *232*, 240-249.

Bar-Ya'akov, I., Tian, L., Amir, R., & Holland, D. (2019). Primary metabolites, anthocyanins, and hydrolyzable tannins in the pomegranate fruit. *Frontiers in Plant Science*, *10*, 620.

Belay, Z. A., Caleb, O. J., Mahajan, P. V., & Opara, U. L. (2018). Design of active modified atmosphere and humidity packaging (MAHP) for 'wonderful'pomegranate arils. *Food and Bioprocess Technology*, *11*(8), 1478-1494.

Belay, Z. A., Caleb, O. J., Vorster, A., van Heerden, C., & Opara, U. L. (2020). Transcriptomic changes associated with husk scald incidence on pomegranate fruit peel during cold storage. *Food Research International*, *135*, 109285.

Gavlighi, H. A., Tabarsa, M., You, S., Surayot, U., & Ghaderi-Ghahfarokhi, M. (2018). Extraction, characterization and immunomodulatory property of pectic polysaccharide from pomegranate peels: Enzymatic vs conventional approach. International journal of biological macromolecules, 116, 698-706.

Hasnaoui, N., Wathelet, B., & Jiménez-Araujo, A. (2014). Valorization of pomegranate peel from 12 cultivars: Dietary fibre composition, antioxidant capacity and functional properties. *Food Chemistry*, *160*, 196-203.

Jiang, H., Li, W., He, B., Gao, Y., Lu, J., 2013. Sucrose metabolism in grape (Vitis vinifera L.) branches under low temperature during overwintering covered with soil. Plant Growth Regul. 72, 229–238

Li, X., Xu, Y., Shen, S., Yin, X., Klee, H., Zhang, B., & Chen, K. (2017). Transcription factor CitERF71 activates the terpene synthase gene CitTPS16 involved in the synthesis of E-geraniol in sweet orange fruit. *Journal of Experimental Botany*, *68*(17), 4929-4938.

Luengwilai, K., Beckles, D. M., Roessner, U., Dias, D. A., Lui, V., & Siriphanich, J. (2018). Identification of physiological changes and key metabolites coincident with postharvest internal browning of pineapple (*Ananas comosus* L.) fruit. Postharvest Biology and Technology, 137, 56-65.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell & Environment*, *25*(2), 239-250.

Palma, A., Schirra, M., D'Aquino, S., La Malfa, S., & Continella, G. (2006, October). Chemical properties changes in pomegranate seeds packaged in polypropylene trays. In I International Symposium on Pomegranate and Minor Mediterranean Fruits 818 (pp. 323-330).

Rivas, F., Parra, A., Martinez, A., & Garcia-Granados, A. (2013). Enzymatic glycosylation of terpenoids. *Phytochemistry Reviews*, *12*(2), 327-339.

Rhodes, D., Verslues, P. E., & Sharp, R. E. (1998). Role or Amino Acids in Abiotic Stress Resistance. In *Plant Amino Acids* (pp. 333-370). CRC Press.

Rowayshed G, Salama A, Abul-Fadl M, Akila-Hamza S, Mohamed EA. 2013. Nutritional and chemical evaluation for pomegranate (*Punica granatum* L.) fruit peel and seeds powders by products. Middle East J. Appl. Sci., 3(4): 169- 179.

Vazquez-Hernandez, M., Navarro, S., Sanchez-Ballesta, M. T., Merodio, C., & Escribano, M. I. (2018). Short-term high CO_2 treatment reduces water loss and decay by modulating defense proteins and organic osmolytes in Cardinal table grape after cold storage and shelf-life. *Scientia Horticulturae*, *234*, 27-35.

Wang, K., Shao, X., Gong, Y., Zhu, Y., Wang, H., Zhang, X., Cao, J., & Lu, H. (2013). The metabolism of soluble carbohydrates related to chilling injury in peach fruit exposed to cold stress. *Postharvest Biology and Technology*, *86*, 53-61.

Xu, F., Wang, H., Tang, Y., Dong, S., Qiao, X., Chen, X., & Zheng, Y. (2016). Effect of 1-methylcyclopropene on senescence and sugar metabolism in harvested broccoli florets. *Postharvest Biology and Technology*, *116*, 45-49.

Yu, L., Liu, H., Shao, X., Yu, F., Wei, Y., Ni, Z., & Wang, H. (2016). Effects of hot air and methyl jasmonate treatment on the metabolism of soluble sugars in peach fruit during cold storage. *Postharvest Biology and Technology*, *113*, 8-16.

Yuan, Z., Fang, Y., Zhang, T., Fei, Z., Han, F., Liu, C., & Zheng, H. (2018). The pomegranate (*Punica granatum* L.) genome provides insights into fruit quality and ovule developmental biology. *Plant Biotechnology Journal*, *16*(7), 1363-1374.