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Review Article

Introgression and Environmental Effects on Gene Expression and Aroma Volatiles As Biomarkers in a Melon Near-Isogenic Line

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Abstract

This review paper discusses a study aimed at determining the aroma profile and global quality of a non-climacteric near-isogenic line (NIL) SC10-2 of melon and identifying the most discriminant flesh aroma volatiles at harvest as potential biomarkers of textural differences. The introgression in the linkage group X allows the NIL SC10-2 to behave differently, changing the non-climacteric behavior by producing aroma volatiles characteristic of non-climacteric lines or inhibiting the production of some compounds important for ripening. The study identifies thirty-two volatiles organic compounds (VOCs) that show correlation with textural traits in season 1, while in season 2, forty-one VOCs were identified. This paper also discusses the impact of environmental effects and introgression effects on VOCs and textural traits. Transcriptomic analysis from RNA-Seq revealed 2954 differentially expressed genes (DEGs) having introgression or introgression x ripening time effects and 2068 DEGs postharvest ripening time effect. The study also identifies at least thirty-four genes affecting direct and/or indirectly in the delay of ripening of SC10-2 versus PS in general and particularly to respiration rate, ethylene production, textural traits and volatile production and probably differential non-climacteric response.

Keywords: *Cucumis melo* L.; Near-isogenic line (NIL); Volatile organic compounds (VOCs); Quantitative trait loci (QTLs), RNA-Seq; Transcriptomic analysis; Differentially expressed genes (DEGs); Fruit quality traits; Fruit senescence; Fruit texture

Introduction

Melon is an annual diploid plant that has a high intra-specific genetic variation and a small genome size, making it technologically exploitable for flavor development and textural changes that occur during fruit ripening. The biochemical, physiological, and organoleptic changes that occur during the development and ripening process of melons are well known, but non-climacteric melon fruit ripening is still poorly understood. One of the main evident changes during melon fruit softening is flesh softening, which occurs in non-climacteric types and consumers prefer a fruit of medium firmness, crunchy, and outstanding firmness [1]. The aroma profile and the textural traits discriminate climacteric from non-climacteric near-isogenic lines at harvest or after postharvest, but little information is still available about aroma formation in non-climacteric melons, particularly during postharvest ripening. New genetic and genomic tools are available for studying fruit ripening, including near-isogenic lines, tilling platforms, saturated genetic maps, and the genome sequence, and many QTLs and eQTLs positioned, gene expression, etc [1]. However, non-climacteric melon fruit ripening and quality have been little studied compared to climacteric melons, and this review aims to address this gap in knowledge by summarizing the current understanding of the ripening process and postharvest quality of non-climacteric melons. This review provides an overview of the current knowledge regarding the ripening process and postharvest quality of non-climacteric melons.

Methodology to Remove Significant Outliers in Aroma Analysis of a Non-Climacteric Melon Fruit Obtained by Using HS-SPME GC-MS at Harvest

The analysis of volatile aroma compounds (VOCs) in non-climacteric melons is complicated due to variability in fruit maturity, genotype, and environment. Outliers in data can be problematic for identifying QTLs, so statistical methods are used to detect and remove them. In previous studies [2], multivariate and univariate statistical methods were tested, and PCA and PLS-DA were applied for dimension reduction. The box-whisker plot is a useful tool for detecting univariate outliers. The objective of this part of work is to develop a methodology for systematically observing strong outliers in VOC analysis, particularly in cases where the exact degree of fruit maturity is difficult to assess. The study is based on the same parental line studied in two different seasons. During a previous examination of the data using PCA, several mistakes were identified due to a malfunction in our alignment program, affecting two samples, and a sample with many acetate esters and sulfur-derived compounds that were unique in fruit VOC of both seasons. The Grubbs test only allowed the detection of a few outliers as the normality assumption was rejected for most of the variables. The data on three groups of variables based on total areas were analyzed visually and the outliers identified were fruits 2B and 6A (S1), and 3B (S2). Using PLS-DA, the same outliers were identified as well as 16B (S1) and 2A (S2) and 5B or 6D (S2) to a lesser extent. The main reasons for identifying outliers were the excess of acetate esters and aldehydes, ketones, and others in certain fruits. One outlier was detected in the VOCs of fruit 15A (S1), and no outliers were detected in S2 when analyzing four groups of variables representing percentages of different compound classes based on different total areas of the chromatograms. Certain differences between seasons were detected by PCA or the PLS-DA of percentages. The fruit classified as outliers in the previous section by reference to the area in absolute values were not classified as outliers using PCA but using variables of compound classes based on percentages of the sum of the areas. In the last of the percentage variables, fruit 5A (S1) was considered as an outlier due to a slightly higher proportion of acids, acetate esters, and/or alkanes but not finally considered as a strong outlier [2].

The outliers found in the total area and those detected by percentages did not match due to variations in analysis and aroma intensity among fruits. It is not advisable to convert percentages into logarithms for anything other than classification or prediction purposes. PLS-DA detected more outliers in the total area than PCA and the box-whisker plots detected more outliers than the multivariate statistical analysis because the variables could not be analyzed assuming normality. The Grubbs test could not be applied because it can only be used if the individual variables follow a normal distribution, losing the perspective of a multivariate analysis. Anomalies detected by the box-whisker plot should be in the same range when two seasons are compared. The outliers detected by correlation analysis were more than those considered as strong outliers. Variability in melon fruit VOCs could be associated with differences in physiological maturity and fruit sampling. Previous publications have applied PCA and PLS-DA for the classification and comparison of VOCs of melon near-isogenic lines. From a physiological point of view, the higher the relative acetate ester concentration in flesh tissue, the more senescent the PS melon flesh. Some outliers can be detected by specific compounds like isobutyl acetate, phenylmethyl acetate and isopropyl acetate, which are typical of climacteric cultivars.

Exploring the impact of seasonal variations on flesh volatile concentrations and texture at harvest in a melon near-isogenic line with introgression in Lg X

The production and composition of volatiles in melons can be influenced by various factors, such as the fruit's genetics, maturity, postharvest handling, storage, and environmental conditions during production [3]. There is also intrinsic variability in melon flesh during sample extraction and analysis [4-6]. Researchers have shown interest in the melon NIL collection developed from the cross between the Korean accession 'Songwhan Charmi' and the 'Piel de Sapo' (PS) strain [7] to map quantitative trait loci associated with textural properties and VOCs [8-10]. This part of the study aims to examine the fixed effect of introgression and seasonal effects and identify the most discriminant flesh aroma volatiles at harvest as potential biomarkers of textural differences, particularly flesh firmness. The hypothesis is that the genetic factor (introgression in melon chromosome X present in the NIL SC10-2, but not in PS) and the environment (seasons) differentially contribute to the relative effects on the traits analyzed. Additionally, the study aimed to explore three methods for analyzing the effect of the presence of null values within consistent VOC variables. The results of an experiment studying the effect of introgression on the aroma, texture, and ripening behavior of melons grown in two different seasons. The two seasons had similar temperatures and radiation, but season 1 had significant precipitation, resulting in delayed harvest. The fruit from season 1 had higher juiciness than season 2, and the introgression had a significant effect on the texture of the fruit. The study identified and analyzed 311 volatile organic compounds (VOCs) in the melon juice and found that 60 VOCs were consistent across both seasons, while others were season specific. The introgression was found to significantly affect 28 VOCs. The study concludes that introgression can significantly modify the aroma, texture, and ripening behavior of melons.

The impact of introgression, or the transfer of genetic material from one species or subspecies to another, on the volatile organic compound (VOC) profile and fruit texture of melon plants. Specifically, the we compared the VOC profiles and texture of a non-climacteric melon line (NIL) that had been intro-Grassed with a quantitative trait locus (QTL) for firmer fruit texture, called SC10-2, with those of its parental line, called PS. We found clear differences in VOC production between SC10-2 and PS, indicating that VOC production may be affected by environmental and seasonal factors [11]. Moreover, the aroma volatile variability observed in the study is a typical problem for cultivars grown for the fresh-cut industry that do not reach adequate maturity. The differences in textural

traits observed between SC10-2 and PS might be associated with differences in the cell wall composition between the two lines. The textural effect associated with the QTL ff10.2 is located in SC10-2 and is very consistent across different studies. However, the lack of complete agreement between the VOCs identified or associated with the introgression in previous studies was partially due to the strong seasonal effect, and the difficulties in growing many replicates of SC10-2. The study identified 28 VOCs that were implicated in differences in aroma production due to the introgression in both seasons. Seven VOCs exerted a positive effect on the VOC content when NIL SC10-2 was compared with PS, and the others exerted negative effects compared with PS. The compounds associated with the introgression effect on the aroma profile were VOCs typical of other types of non-climacteric melons and were consistent with the VOCs identified in previous studies [11]. This part of the study highlights the importance of considering environmental and seasonal factors when studying the VOC profile and texture of melons, especially when introgression is involved. The findings could inform breeding programs aimed at improving the aroma and texture of melons for different applications.

Gene expression profile analysis of a melon near-isogenic line with increased fruit flesh firmness during ripening using transcriptomic analysis

Melon fruit ripening involves a complex process of physiological, biochemical and sensory changes that are influenced by a genetic program. This program is characterized by a coordinated cascade of responses that begin in the placental tissue of the fruit and end in the rest of the mesocarp. Non-climacteric melon fruit ripening is not well understood, which makes it challenging to apply postharvest techniques to delay ethylene-independent processes such as flesh softening or the production of certain aromas. However, recent advancements in genetic and genomic tools have made it possible to study fruit ripening in melon. These tools include collections of near-isogenic lines, tilling platforms, genetic maps, the genome sequence, quantitative trait loci (QTLs) and gene expression. RNA-Seq technology has also been used to analyze the transcriptomic changes during melon fruit ripening and to identify candidate genes related to fruit quality. This part of the study aims to compare the transcriptomes of a near-isogenic line of melon with its parental line during ripening to reveal the genes associated with the introgression during melon postharvest ripening, particularly those related to textural traits and volatile organic compounds (VOCs) and to identify potential pathways and regulators. Two lines of melon, PS and SC10-2 were examined, to compare their postharvest ripening characteristics and VOCs production [12]. The SC10-2 line had higher flesh firmness and whole fruit hardness than PS, which was slower to ripen postharvest, resulting in reduced juiciness [12]. SC10-2 could be stored for over 40 days, while PS could only be stored for about 30 days due to flesh sugar loss and fungal decay [12]. During storage, both lines showed different patterns of VOC production, with SC10-2 generally having higher levels of aldehydes, alcohols, acids, and terpenes [12]. The study identified 13 individual VOCs that showed significant effects during storage, most of them belonging to four compound classes (aldehydes, ketones, alcohols, and sulfur-derived compounds) [12]. Also, 2954 differentially expressed genes (DEGs) were identified between the two lines, with 909 of them showing a significant effect for introgression but not ripening time [12]. Overall, the study provides insights into the postharvest ripening characteristics and VOC production of different melon lines, which could have implications for improving storage and quality of melon fruits. This part of the review discusses the differences in gene expression and metabolic changes in two melon lines with different fruit ripening characteristics. The study found that the respiration rate was lower in one line than the other, which could be due to differences in the expression of genes involved in mitochondrial enzymatic activities and transporters. The expression of some genes such as NADH: Quinone oxidoreductase and isocitrate dehydrogenase was higher in one line than the other [12]. However, the expression of succinate dehydrogenase was not significantly different between the two lines.

And reports that the long introgression covering the entire LG X delayed fruit ripening in general, as seen from physiological traits such as respiration rate, ethylene production, and volatile production [12]. The introgression also affected textural traits, and the effects on aroma volatiles and textural traits could be due to independent genes or pleiotropic effects due to a unique gene. Several potential candidate genes have been proposed as being associated with melon textural traits during ripening, including firmness, whole fruit hardness, and flesh juiciness [12]. Overall, the study provides insights into the genetic and metabolic changes that occur during melon fruit ripening, which could be useful for improving melon fruit quality and shelf life. The metabolic pathways associated with the differences in aroma production between two lines of melon fruit : PS and SC10-2. Specifically, the differences in gene expression and enzyme activity lead to differences in the production of aldehydes, alcohols, terpenes, and other volatile organic compounds (VOCs) that contribute to the fruit's aroma [12]. One metabolic pathway that contributes to aroma production involves the free L-amino acid metabolism [12]. Four amino acids in particular, phenylalanine, valine, isoleucine, and leucine, are considered aroma precursors. We suggested that differences in the expression of certain genes, such as GDPDL4 (MELO3C013101), may explain why SC10-2 has a higher abundance of alcohols than PS. GDPDL4 is involved in the hydrolysis of glycerophosphodiesters, which can lead to the production of alcohols. Also, some differences were noted in the expression of *Cm*LOX18 (MELO3C024348), which is involved in the generation of fruit aroma in melon, may explain why SC10-2 has higher levels of aldehydes, terpenes, and other VOCs than PS.

The enzymatic reaction of LOX causes polyunsaturated fatty acids to generate hydroperoxide, which is then catalyzed by hydroperoxide lyase (HPL) to produce aldehydes. These aldehydes can then be converted to alcohols by alcohol dehydrogenases (ADH) and esters by alcohol acyl-transferase (AAT). The expression of *Cm*ADH1 (MELO3C019548) were higher in PS than in SC10-2, which may explain the differences in aroma production between the two lines. The expression of *Cm*OPR2 (MELO3C008075)

may increase the activity of the β-oxidation pathway, leading to increased production of aldehydes in SC10-2. Overall, this part provides insight into the complex metabolic pathways involved in the production of fruit aroma and how differences in gene expression and enzyme activity can lead to differences in aroma production between different lines of fruit.

The introgressions delayed the non-climacteric fruit ripening, resulting in reduced ethylene production and respiration rates, delayed softening, and increased flesh juiciness [12]. The delay in ripening also affected volatile production, as evidenced by the levels of acetate esters and non-acetate esters in the experiment. The study analyzed the transcriptome of the melon NIL SC10-2 and its parental PS and identified differentially expressed genes (DEGs) associated with postharvest ripening time [12]. The analysis showed that most genes are upregulated over time, but SC10-2 had more upregulated genes compared to PS at harvest or after 8 days of ripening, and 29% of the DEGs were downregulated. Specific genes involved in ripening pathways that were dynamically regulated during fruit ripening were identified. Some genes were activated during ripening time, such as *Cm*LOX18, *Cm*AOS, *Cm*DFR4, *Cm*OPR2, *Cm*GATA5, *Cm*TCP15, and *Cm*GDSL esterase/lipase process genes [12]. On the other hand, other genes related to cellular components such as *Cm*NAC18, *Cm*NADH1, *Cm*CAD1, *Cm*ACO2, and *Cm*ADH1 were delayed in their expression during fruit ripening [12]. The study suggested that the identified genes located in different linkage groups might be controlled by an expression QTL (eQTL) located in LG X, contributing to crosstalk with other QTLs associated with differential textural traits and aroma volatile production in NIL SC10-2 and PS during postharvest ripening. Finally, the study concluded that all the identified genes in the experiment were affected by the introgressions in LG X.

General Discussion

The texture of melon fruits is dependent on both environmental conditions and genotype. Previous research has shown that different textural traits, such as flesh firmness, hardness, and juiciness, are associated with aroma volatile organic compounds (VOCs) that are ripening-dependent, even in non-climacteric fruit. However, seasonal effects have been found to have a greater impact on VOCs than on textural traits, potentially due to changes in biochemical pathways affecting fruit ripening. Gene expression differences have also been observed between postharvest ripening of non-climacteric melon lines PS and SC10-2, as well as in climacteric melons. This research has shown that introgressions in LG X do not affect melon climacteric behavior but may influence flesh firmness, whole fruit hardness and juice textural properties. However, it has been found that environmental and seasonal effects may also impact VOCs production. The same NIL can behave differently in different seasons depending on environmental growth conditions.

The introgression in LG X, which covers almost the entire LG X, can affect not only textural traits at harvest or aroma volatiles but also during postharvest ripening. This is in contrast with other QTLs mapped in tomato or melon. This could be due to its effect on respiration rate and ethylene production during fruit ripening. A QTL identified for texture in the introgression in LG X is linked to aroma pathways, with benzaldehyde potentially acting as a precursor of some aromatic volatiles. In a previous study, higher flesh firmness of the NIL SC10-2 was mainly associated with volatiles linked to pathways whose putative precursors are L-amino acids and fatty acids [13]. In addition to these findings, the study revealed discriminant compounds associated with textural traits, such as aldehydes, alcohols, and ketones, which are generally derived from L-amino acids phenylalanine, isoleucine, valine and leucine pathways. Isoleucine is one of the putative precursors of some discriminant compounds, including 2-methylbutanal and nonanal, and feeding of exogenous L-Ile into strawberry peduncles or in apples resulted in enhanced levels of the corresponding volatile derivatives in fruits (Obando et al., 2009). Aliphatic compounds and alcohols are derived from free fatty acids, and by β-oxidation, fatty acids can also produce shorter chains compounds. Both pathways can be used to reduce aldehydes to alcohols by alcohol dehydrogenase catalysis. Overall, this research highlights the complex interplay between environmental factors, genetic factors, and biochemical pathways that influence the texture and aroma of melon fruits.

Conclusion

The objective of this review was to summarize the relationship between aroma volatiles and other characteristics in near-isogenic line SC10-2 with firm flesh texture compared to the parental PS. Additionally, to investigate the effects of seasonality on QTLs located on LG X, specifically on texture and aroma volatiles, using multivariate statistics to differentiate aroma volatiles and map texture and aroma QTLs and transcriptomic analysis during postharvest ripening. Our findings revealed that the introgression in the LG X in the NIL SC10-2 delayed fruit ripening compared to the non-climacteric parental PS, as evidenced by reduced ethylene production and respiration rate, higher whole fruit hardness and firmness, reduced flesh juiciness, and delayed presence of some aroma volatiles (esters). Seasonal effects on introgression were less important in textural traits but more relevant in volatile organic compound production. A season with better environmental conditions produced more volatile compounds than a season with worse growing conditions, irrespective of the presence or absence of introgression in LG X. VOCs of different classes, such as alcohols and aldehydes, showed a significant introgression effect, with lower levels in PS than in SC10-2 during ripening.

The transcriptome analysis of the melon NIL SC10-2 and its parental PS revealed that the genes involved in the ripening pathways are regulated dynamically to activate the expression of some genes (i.e biosynthesis *Cm*LOX18, *Cm*AOS, *Cm*DFR4, *Cm*OPR2, *Cm*GATA5, *Cm*TCP15 and *Cm*GDSL esterase/lipase process genes) and to delay the expression of others (i.e genes related to cellular component such as *Cm*NAC18, *Cm*NADH1, *Cm*CAD1, *Cm*ACO2 and *Cm*ADH1) during fruit ripening. Additionally, the identified genes differentially expressed located in different linkage group (others than LG X) might be controlled by an expression QTL (eQTL) located in LG X, which would contribute to differential textural traits and

aroma volatile production between the NIL SC10-2 and PS during ripening.

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