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Changes in the Activities of Sugars and Sugar Metabolic Enzymes in Grape Berries during Development

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Authors' contributions

This work was carried out in collaboration between both authors. Author SB performed the experiment, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NK and NKA designed and managed the analysis of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Accumulation of sugars is an important metabolic process that occurs during grape berry development. Sugars are transported into the berry mainly in the form of sucrose and are broken down into glucose and fructose by the enzymes invertase and sucrose synthase**.** Changes in activities of invertase and sucrose synthase in the juice of three table grape genotypes (Flame Seedless, Perlette and Muscat Hamburg) was studied during four developmental stages. The characteristic rapid increase in the rate of sugar accumulation in the juice throughout the development was preceded by the activity of invertase. At the same time the rate of sugar accumulation also increased the activity of sucrose synthase. There is a possibility that sugar accumulation in grape juice occurs by pathway involving changes in the activity of enzyme for this process to operate. The results of the present study suggests that in the grape genotypes studied, invertase and sucrose synthase activities are positively correlated with high berry sugar content.

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Keywords: Grape juice; table grapes; stages; enzymes; sugars; sucrose; glucose.

1. INTRODUCTION

Sugar accumulation is an important event in berry ripening physiology of grapevine and is a vital characteristic essential for superior enological characteristics and product value. Sugars are synthesized mainly in leaves as a result of photosynthesis and imported into the berry in the form of sucrose. Upon reaching the berry, sucrose is split into glucose and fructose by the enzymes invertase and sucrose synthase [1,2,3]. Any limitation or restriction in sugar transport and breakdown can adversely affect the sugar content and composition of grape berry as well as its quality. In grape berries, the major sugars - glucose and fructose are present almost in similar concentrations while sucrose is present in trace amounts [4]. According to some authors, glucose and fructose concentrations ranged between 45.9 to131.0 $mg.mL^{1}$ and sucrose generally accounts for less than 2.0% of total sugars [5]. Sucrose accumulation in grape berries depends on many factors such as metabolism in leaves, transport in phloem and metabolism in berry. The sucrose produced may be cleaved into glucose and fructose by acid invertase (AI, EC 3.2.1.26) and neutral invertase (NI, EC 3.2.1.26) or may produce UDP-glucose and fructose by sucrose synthase (SS,EC 2.4.1.13) [6]. Sugar accumulation and related enzymes have been widely studied in grape berries and it was found that generally the acid invertase activity was low at flowering but increased gradually during berry development and high in mature berries which results in low sucrose levels in mature berries [7]. Hence, the present investigation was undertaken to explore the mechanism regulating sugar accumulation in grape berries by understanding the function of enzymes acid invertase and sucrose synthase during development of berries.

2. MATERIALS AND METHODS

Grape berry samples were selected from the Fruit Research Farm, Department of Fruit Science, PAU Ludhiana at four different stages of berry maturation, during 2015 and 2016 .The three varieties selected for the present study were Flame Seedless, Perlette and Muscat Hamburg. First stage was when the berries were unripe and almost full size (fruit growth stage), second at maturity (beginning of verasion), third at maturing and fourth at post ripening. Three vines of each culivar were selected for berry sampling. The harvested samples were brought to the laboratory of Department of Botany, College of Basic Sciences, PAU, Ludhiana washed with distilled water and processed for various estimations.

The total sugar content in the grape juice was determined following the method of Dubois et al. [8] using sulphuric acid and phenol. The activity of invertase was determined by using acetate buffer (pH4.8) and 3,5-dinitrosalicylic acid reagent and the activity was expressed as µmoles sucrose hydrolysed min^{-1} mg^{-1} protein [9] and the sucrose synthase activity was determined by the method given by Morel and Copeland [10] using HEPES-NaOH buffer. Data was recorded for two successive years (2015 and 2016) and was statistically evaluated using Tukey's b range test. Differences were considered statistically significant at the levels, (p <0.05), using SPSS 16.0 for Windows.

3. RESULTS AND DISCUSSION

3.1 Total Soluble Sugars

Total soluble sugars content varied among genotypes. Among the genotypes studied, Perlette had significantly high mean total soluble sugars (17.517g/100 ml) followed by Muscat Hamburg and Flame Seedless in 2015. During 2016, a similar trend was followed with maximum total soluble sugar content in Perlette and minimum in Flame Seedless at Post ripening (Table 1). The interaction of the pooled data showed that the total soluble sugars remained maximum at Post ripening in Perlette (24.761 g/100 ml) being statistically significant from the other two genotypes (Fig. 1). Earlier studies by Jain et al. [11] have shown varying levels of sugars in leaf and berry tissue among the grape genotypes, indicating possible differences in their sugar metabolizing enzymes. Artes-Hernandez et al. [12] investigated the total sugar content of autumn seedless grape varieties at harvest time in Spain for two consecutive years, and reported the similar results.

3.2 Enzyme Activity

The ability of invertase to cleave sucrose making them influential in plant growth and development. Invertases are involved in processes such as partitioning of carbon within the plant thus controlling the composition of stored sugars and

providing hexoses for metabolism, osmoregulation and gene regulation signalling [13]. It is also thought that the accumulation of hexoses resulting from cleavage of sucrose by invertases may also drive the cell expansion during berry ripening. Enzyme analysis revealed that acid invertase activity varied among grape genotypes during berry development. Peak invertase activity was observed between maturing and post ripened berries in all the genotypes evaluated. Among these, Perlette (7.988 µ molsucrose hydrolysed /min/mg protein) displayed higher invertase activity followed by Muscat Hamburg and Flame Seedless at post ripening stage during the year 2015. During the year 2016, the activity was maximum in Perlette and minimum in Flame Seedless (Table 2). The interaction of the pooled mean of data revealed that the activity of invertase enzyme was maximum at Post ripening in Perlette (9.903 µ mol sucrose hydrolysed /min/mg protein Fig. 2). Invertase activity was found to correlate positively with increasing sugar content. Deficiency in invertase activity has been shown to result in reduced glucose and fructose levels, and increased sucrose levels in the fruit [14,15,16]. The present studies are in agreement with Devaiah et al. [17] who studied the genetic variation in sucrose metabolizing enzymes among six Muscadine varieties and reported that invertase activity varied among cultivars during berry development. Peak invertase activity was observed in berries at 90 days from the full bloom and post ripened berries in all the cultivars tested. These data indicate existence of genetic differences in invertase activity among the genotypes evaluated.

In addition to invertase, sucrose synthase activity also varied among the genotypes studied during the course of berry development. Among these, Perlette (0.596 µ molsucrose hydrolysed /min/mg protein) displayed the highest sucrose synthase activity followed by Muscat Hamburg and Flame Seedless during the first year of investigation. During the second year of investigation a similar trend was followed with highest activity in Perlette followed by Muscat Hamburg and Flame Seedless (Table 3). The interaction values of overall mean of the data of both the years indicate that at Post ripening maximum activity of sucrose synthase was observed in Perlette (0.719 µ mol sucrose hydrolysed /min/mg protein, Fig. 3). The accumulation of roughly equal amounts of glucose and fructose suggests that cleavage of sucrose may be catalysed by sucrose metabolising enzymes such as invertase and sucrose. It was also found by some researchers that the activity of the invertase enzymes involved in sugar metabolism in grape berries was 200-300 times greater than that of sucrose synthase [18]. The present investigation on sucrose metabolising enzymes in grape berries showed that the enzyme activities varied with the berry development which is consistent with the studies of Takayanagi and Yokotsuka 1997 [19] who also reported that acid invertase involved in sucrose degradation has higher activity as compared to sucrose synthase.

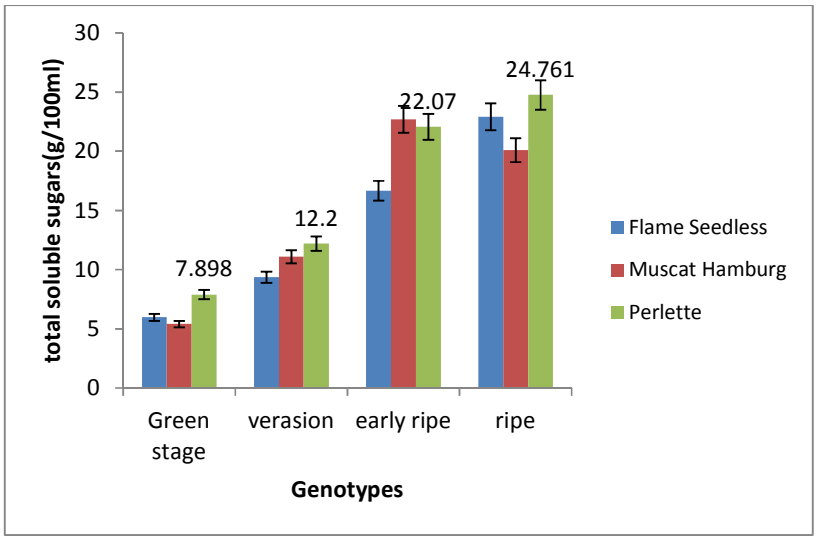

Fig. 1. Overall genotype × stage interaction of total soluble sugars in grape juice
(Value over the graphs is the interaction of pooled data and only maximum values are indicated)

Table 1. Total soluble sugars (g/100 ml) in the juice of grape varieties /genotypes at different stages of development

Stages / Varieties	2015					2016				
	Fruit growth	Verasion stage	Maturing	Post ripening	Mean	Fruit growth	Verasion stage	Maturing	Post ripening	Mean
Flame seedless	6.013^{cd}	10.013^{cc}	18.070^{cb}	23.607^{da}	14.426^{c}	5.940^{cd}	8.723^{cc}	15.270^{cb}	22.240^{ca}	13.043^{c}
Muscat hamburg	5.917^{bd}	12.143^{bc}	24.207^{bb}	21.260^{ba}	15.882^{b}	4.907^{bd}	10.043^{bc}	21.187^{bb}	18.947^{ba}	13.771^{b}
Perlette	8.903^{ad}	12.867^{ac}	22.760^{ab}	25.540^{aa}	17.517^{a}	6.893^{ad}	11.533^{ac}	21.380^{ab}	23.983^{aa}	15.894^{a}
Mean	6.944d	11.674c	21.679b	23.407a		5.913d	10.100c	19.279b	21.652a	

Values with different superscripts are significantly different at p<0.05 by Tukey's test for interaction between varieties and stages for each year within the columns and rows

Table 2. Invertase activity (µ mol sucrose hydrolysed /min/mg protein) in grape berries at different stages of development

Stages / Varieties	2015					2016				
	Fruit growth	Verasion stage	Maturing	Post ripening	Mean	Fruit growth	Verasion stage	Maturing	Post ripening	Mean
Flame seedless	2.870^{cd}	5.543^{cc}	7.060^{cb}	7.763^{ca}	5.809^{c}	2.063^{cd}	4.847^{cc}	5.947^{cb}	6.747^{ca}	4.901^{c}
Muscat hamburg	4.047^{bd}	8.043^{bc}	9.240^{bb}	9.453^{ba}	7.696^{b}	3.747^{bd}	7.883^{bc}	8.743^{bb}	8.947^{ba}	7.330^{b}
Perlette	4.467^{ad}	8.047^{ac}	9.370^{ab}	10.067^{aa}	7.988^{a}	3.953^{ad}	7.750^{ac}	8.867^{ab}	9.740^{aa}	7.578^{a}
Mean	3.794^{d}	7.211^{c}	8.557^{b}	9.094^{a}		3.254^{d}	6.827^{c}	7.852^{b}	8.478^{a}	

Values with different superscripts are significantly different at p<0.05 by Tukey's test for interaction between varieties and stages for each year within the columns and rows

Table 3. Sucrose synthase activity (µ mol sucrose hydrolysed /min/mg protein) in grape berries at different stages of development

Stages / Varieties	2015					2016				
	Fruit growth	Verasion stage	Maturing	Post ripening	Mean	Fruit growth	Verasion stage	Maturing	Post ripening	Mean
Flame seedless	0.302^{cd}	0.333^{cc}	0.342^{cb}	0.374^{ca}	0.338^{c}	0.284^{cd}	0.325^{cc}	0.338^{cb}	0.354^{ca}	0.325^{c}
Muscat hamburg	0.405^{bd}	0.543^{bc}	0.603^{bb}	0.734^{ba}	0.571^{b}	0.364^{bd}	0.441^{bc}	0.526^{bb}	0.647^{ba}	0.495^{b}
Perlette	0.457^{ad}	0.532^{ac}	0.663^{ab}	0.734^{aa}	0.596^{a}	0.431^{ad}	0.505^{ac}	0.631^{ab}	0.704^{aa}	0.568^{a}
Mean	0.388^{d}	0.470^{c}	0.536^{b}	0.614^{a}		0.360^{d}	0.423^{c}	0.498^{b}	0.568^{a}	

Values with different superscripts are significantly different at p<0.05 by Tukey's test for interaction between varieties and stages for each year within the columns and rows

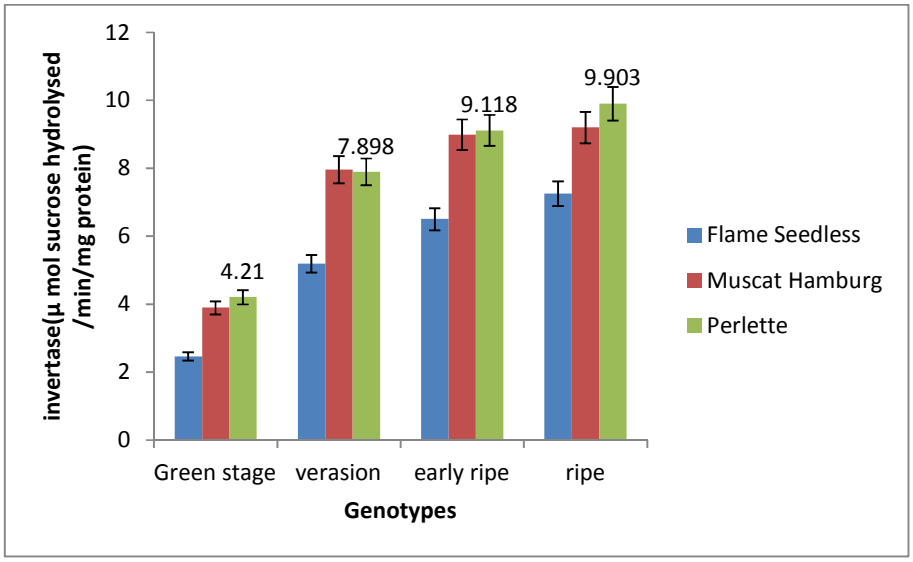

Fig. 2. Overall genotype × stage interaction of invertase activity in grape juice
(Value over the graphs is the interaction of pooled data and only maximum values are indicated)

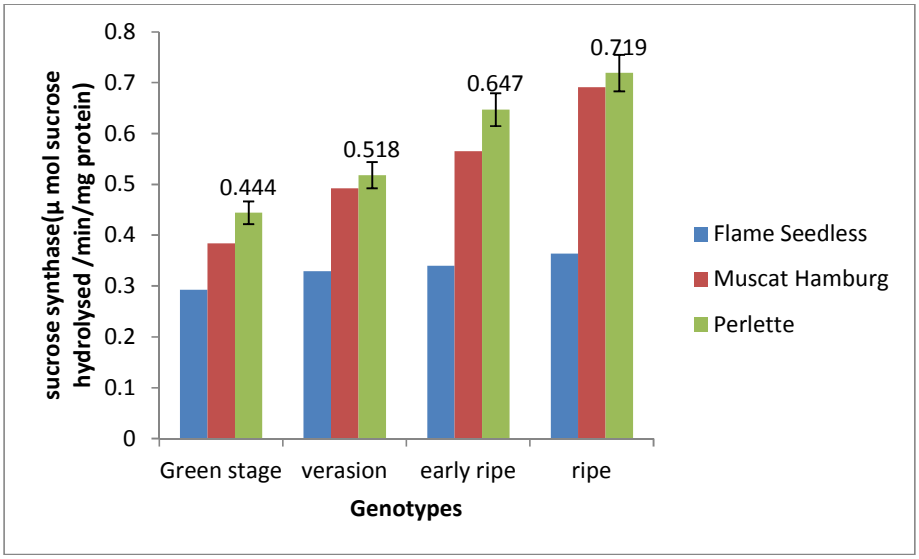

Fig. 3. Overall genotype × stage interaction of sucrose synthase activity in grape juice
(Value over the graphs is the interaction of pooled data and only maximum values are indicated)

4. CONCLUSION

Among the genotypes studied, Perlette displayed the highest total soluble sugars, invertase and sucrose synthase activities while Flame Seedless showed the lowest total soluble sugars, invertase and sucrose synthase activities. These results suggest that genotypes that have higher levels of total soluble sugars contain higher levels of sucrose synthase and invertase activities than the genotypes with less total soluble sugar content. Flame Seedless exhibiting lower invertase and sucrose synthase activities, may be lower in hexose (glucose and fructose) content due to the lower breakdown of transported sucrose in the berries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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