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Original Research Article

Changes in the activities of sugars and sugar metabolic enzymes in grape berries during development

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.

Key words: *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 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

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investigation was undertaken toexplore 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 (green stage), second at maturity (verasion), third at early ripe stage and fourth at ripe stage. 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/100ml) 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 ripened stage (Table 1). The interaction of the pooled data showed that the total soluble sugars remained maximum at ripened stage in Perlette (24.761g/100ml) 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, obtaining the similar results.

3.2 Enzyme Activity: The abilityof invertase to cleave sucrose makes them powerful players 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 early ripe and ripened

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berries in all the genotypes evaluated. Among these, Perlette (7.988µ molsucrose hydrolysed /min/mg protein) displayed higher invertase activity followed by Muscat Hamburg and Flame Seedless at ripened 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 maximumat ripened stage inPerlette (9.903 µ molsucrose 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 between 90-day-old and 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 Seedlessduring 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 (Table3). The interaction values of overall mean of the data of both the years indicate that at ripened stage maximum activity of sucrose synthase was observed in Perlette (0.719 µ molsucrose 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 workers 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 degredation has highest activity as compared to sucrose synthase.

4.CONCLUSION

Among the genotypes studied, Perlette displayed the highest total soluble sugars, invertase and sucrose synthase activities while Flame Seedless showed the total soluble sugars, lowest invertase and sucrose synthase activities. These results suggest that genotypes that are sweeter contain higher levels of sucrose synthase and invertase activities than the less sweeter genotypes. Flame Seedless exhibiting 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.

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

Stages / Varieties	2015					2016				
	Green stage	Verasion stage	Early ripe	Ripened stage	Mean	Green stage	Verasion stage	Early ripe	Ripened stage	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

Table2: Invertase activity (µ mol sucrose hydrolysed /min/mg protein) in grape berries at different stages of development

Stages / Varieties	2015					2016				
	Green stage	Verasion stage	Early ripe	Ripened stage	Mean	Green stage	Verasion stage	Early ripe	Ripened stage	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

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

Stages / Varieties	2015					2016				
	Green stage	Verasion stage	Early ripe	Ripened stage	Mean	Green stage	Verasion stage	Early ripe	Ripened stage	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

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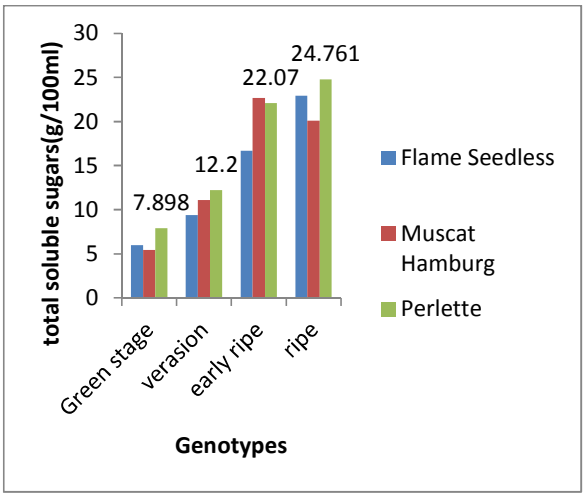

Fig.1 **Overall genotype ×stage interaction of total soluble sugars in grape juice**

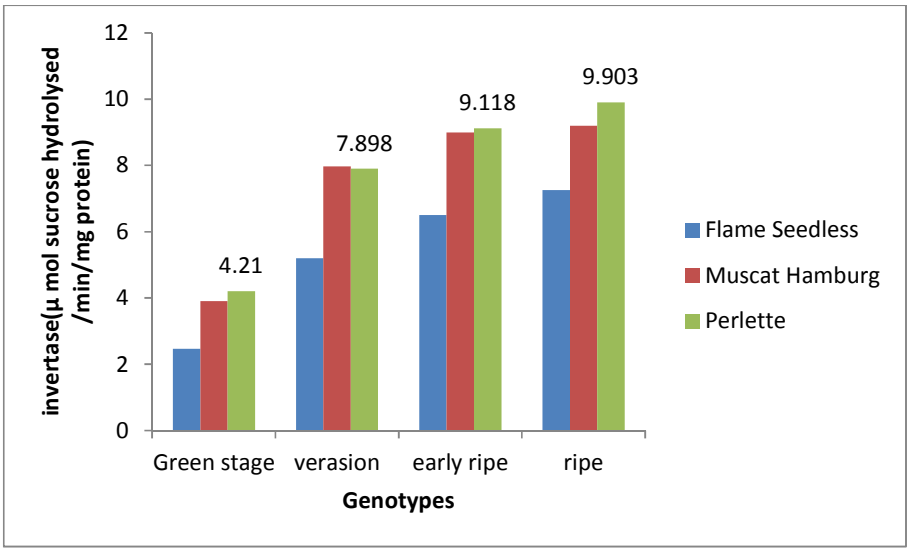

Fig.2 **Overall genotype ×stage interaction of invertase activity in grape juice**

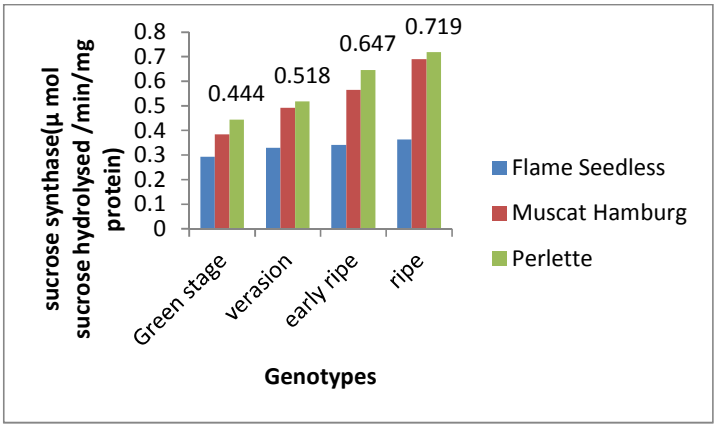

Fig. 3. **Overall genotype ×stage interaction of sucrose synthase activity in grape juice**

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Refrences:

1. Dantas BF, Ribeiro LDS, Da Silva AP, De Souza Luz SR. Foliar carbohydrates content and invertase activity in vines at Sao Francisco River Valley–Brazil. *RevistaBrasileira de Fruticultura*. 2005; 27:198–202.

2. Patrick J W, Zhang W, Tyerman SD,Offler CE, Walker NA. Role of membrane transport in phloem translocation of assimilates and water. *Austral. J. Plant Physiol*. 2001; 28:695–707.

3. Quick WP. Sucrose metabolism in sources and sinks, In: E. Zamski and A.A. Schaffer (eds.). Photoassimilate distribution in plants. *Marcel Decker*, New York.1996; 115–156
4. Kliewer, WM. Changes of concentration of glucose, fructose and total soluble solids in flowers and berries of *VitisVinifera*. *Am. J. Enol. Vitic*.1965; 16: 101-110.
5. Liu HF, Wu BH , Fan PG Li SH.Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. *J. Sci. Food Agric*.2006; 86: 1526-1536.
6. Fernie AR, Willmiter L.Sucrose to starch: a transition in molecular plant physiology. *Trends Plant Sci*.2002; 7: 35-41
7. Xie ZS , Li B ,Forney C H ,Xu W P , Wang S P.Changes in sugar content and relative enzyme activity in grape berry in response to root restriction.*Sci. Hort*.2009; 123: 39-45
8. Dubois M, Gilles KA,Hamilton JK, Rebers PA, Smith F.Colorimetric methods for determination of sugars and related substances.*Ann Chem.*1956;28: 350-56
9. Singh MB, Malik CP, Thapar H. changes in the activities of some enzymes of carbohydrate metabolism in *Amaryllis vitata* pollen suspension culture.*Plant CellPhysiol*.1978;19:677-84
10. Morell and Copeland.sucrose synthase of soyabean nodules. *Plant Physiol*.1985;78:149-54
11. Ashok K Jain,Basha SM,Alferdo B,Lorenz J LO,Stephen Leong.Variation in sugar accumulation pattern of Muscadine grape genotypes.*Proc.Fla.State Hort.Soc*.2002;115:329-336
12. Artes-hernandez F, Artes F Allende A. Sugar composition changes in 'Autumn Seedless' table grape during long term cold storage. *ActaHort* (ISHS) 2003;628: 363-66.
13. Sturm A.Invertases.Primary structure,functions and roles in plant developmentand sucrose partitioning.*Plant Physiol*.1999;121:1-7
14. Chetelate RT, DevernaJW,Bennett AB. Effects of the *Lycopersiconchmielewskii*sucrose accumulator gene (*sucr*) on fruit yield and quality parameters following introgression into tomato. *Theor. Appl. Genet*.1995; 91:334–339
15. Stommel JR. Enzymatic components of sucrose accumulation in the wild tomato species *Lycopersiconperuvianum.Plant Physiol*. 199299:324–328.
16. Yelle S,Chetelat RT,DoraisM, Deverna JW, Bennet A. Sink metabolism in tomato fruit: IV.

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Genetic and biochemical analysis of sucrose accumulation. *Plant Physiol*.1991 95:1026-1035.

17. Devaiah K,Hemanth KN,Vasanthaiah, Sheikh. M ,Basha.Genetic Variation in Sucrose Metabolizing Enzymes among Six Muscadine Varieties*Proc. Fla. State Hort. Soc.*2010;123:32–34.
18. Hawker J S.Changes in the activities of enzymes concerned with sugar metabolism during the development of grape berries. *Phytochem*1969;8: 9–17.
19. Takayanagi T, Yokotsuka K. Relationship between sucrose accumulation and sucrose-metabolizing enzymes in developing grapes. *Amer. J. Enol. Vitic*.1997;48, 403-407.