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The Biochemistry of Grape Berry Development

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A B S T R A C T

The grape berry is considered a sink for primary key metabolites and relies on the use of available carbohydrates produced by photosynthesis to support its growth and development. The berry size and quality at the time of harvest mainly depends on water, sugars such as glucose, fructose and sucrose, organic acids (mainly malic and tartaric acids), amino acids (arginine, proline and glutamic acid), phenolic compounds (anthocyanins, flavonoids and tannins) and aroma precursor. The physiological ripening is reached when the grapes achieve sufficiently high levels of sugar without losing too much acidity. The understanding of how and when specific metabolites accumulate and how the metabolism of the fruit of each cultivar responds to the environment is crucial for the viticulturists for improving the grape growing strategies.

Introduction

Grape (*Vitis vinifera* L.) is a temperate fruit crop, but has over the years adapted well in different agroclimatic regions of the world viz; temperate, tropical and subtropical regions**.** About7.6 million hectares area of the world is under grape cultivation (FAO, 2014). The area allocated to vine yards is increasing by about 2% per year. Mughal invaders introduced the grape in India in 1300AD from Iran and Afghanistan and now India is placed as the thirteenth largest grape producing country in the world with a production of 1,546300 (tones) that accounts for a share of 2.24 percent of total grape production in world. The grape vines are grown under two different climatic conditions in India: the subtropical and tropical climatic conditions. Maharashtra, Karnataka, Andhra Pradesh and Tamilnadu are the major grape growing states in India. In Punjab, grapes are grown on an area of 297 ha with an annual production of 8493 thousand MT with productivity of 28.6 metrictonnes per hectare (Anonymous 2016). The grape vines are truly propagated by hard wood stem cuttings and frequently through grafts on standard root stocks. The fruit of grape is a berry. Grape is a non-climacteric fleshy fruit of great economic importance (Coombe and Hale 1973). Berry blooming follows a typical double sigmoid growth curve having the three definite phases, the first two growth phases are separated by a lag phase when the seeds mature and expansion is slow (Coonde *et al.*, 2007). The first phase is signalized with the upsurge of organic acids in the vacuoles and various precursors of phenolic compounds such as tannins and hydroxycinnamates. At the end of the lag phase, a small growth phase known as verasion is signalized by the upsurge of sugars and anthocyanin pigments in coloured genotypes. After the verasion, high concentration of glucose and fructose accumulation occurs however the concentration of organic acids decreases and the berry softens (zoccatelli *et al.,* 2013).One of the most important constituents of grapes besides sugars and organic acids are the phenolic compounds, the structurally diverse substances that are present in various amounts. They have a major role in the color and flavor of the wine. Anthocyanins and tannins the two main substances included in this group of compounds. The pigments that are responsible for the red and purple color of the grapes are anthocyanins that begin to accumulate during verasion and continue all through ripening. Besides the phenolic compounds, the aroma compounds in the grapes exist as non- volatile odourless bound forms that may be released by chemical /enzymatic reaction during wine making.

An insight into the distribution pattern of various metabolites is of importance for manipulation of grape growing practices in order to enhance its production potential as a table fruit and wine quality.

Berry development

A grape inflorescence arises in the flower cluster from individual flowers and consists of a peduncle, pedicels, rachis and berries. A single berry is made up of skin, pulp and seeds. On the outside of skin, a waxy layer or bloom is present that helps to prevent water loss. As the berry forms, the vital nutrients are delivered through the vascular system, viz; the phloem and xylem. From the root system, the xylem transports water, growth regulators, sugars, mineral and other nutrients. During berry development, the xylem predominantly plays an important role until verasion. The phloem is primarily involved in carbohydrate transport from the leaves to the vine and consequently to the berry and becomes the primary source of nutrition after verasion. After verasion, with the increase in sugar content the berry size surges and this varies by cultivar. The grape berry is an independent biochemical machinery that contains water, sugar, amino acids, minerals, and micronutrients (Kennedy 2002). The berry development has been divided into three main stages:

Stage I: Berry formation to lag phase

The first stage associated with berry formation starts at bloom upto approximately for 60 days. During this phase, rapid cell division occurs and the berry starts to accumulate solutes such as tartaric and malic acids and expands in volume. The tartaric acid accumulates during the early stages of berry development with the highest accumulation in the skin of berry providing acidity for winemaking. However, malic acid has the highest accumulation in the flesh. At this stage, the other important acids, such as hydroxycinnamic acid starts accumulating. Hydroxycinnamic acid is the precursor of volatile phenols (eg. tannins) that are responsible for bitterness and astringency.

Stage II: Lag phase to verasion

At this stage berries reach half of their ultimate size and cells continue to accumulate acids and tannins, which reach their maximum threshold at verasion. Seed embryos start to grow and seed reach their final size about 10-15 days before verasion.

Stage III: Post-verasion berry ripening

The third stage (verasion) includes accumulation of sugars, reduction in acids, berry softening and coloring. The berry doubles in size. The malic acid and aroma compounds decline. However, there is an increase in compounds like glucose and fructose from sucrose after verasion.

Sugar composition of grape berries at different developmental stages

Sugar accumulation in grape berries is an important phenomenon which has a direct impact on the amount of alcohol in wine in case of wine grapes. In case of table grapes, total sugar is also an important fruit quality factor. Sugars are produced by the green berries at the beginning of berry development while afterwards the berry becomes a typical "sink" and uses carbohydrates produced by photosynthesis of leaves. Sucrose produced in the leaves is then transported for long distance to berries into the phloem. From verasion, xylem flow is almostobstructed, hence water may reach berries via phloem with sugars. Photosynthesis generates glucose, which is spontaneously inter-converted with fructose. One each of these molecules is pooled into sucrose before their journey through the phloem and then on to the sink (growing point, fruit etc.) where it is transformed back into glucose and fructose.

The optimization of crop efficiency depends on the proficient assimilation and use of nutrients by plants. Grape berries support their growth and development by the accessible carbohydrate resources produced by photosynthesis. The assimilate partitioning between the photosynthetic "source tissues" and the heterotrophic "sink tissues" is a key determinant of plant growth and productivity (Kingston 2001).

The levels of sugars are important factors in determining fruit quality. Glucose and fructose which are present in almost comparable concentrations are the main sugars in the grape berries. Sucrose is however, present only in trace amounts (Kliewer 1965). According to some reports, glucose and fructose concentrations ranged from 45.9 to 131.0mg.ml^{-1}, and sucrose generally accounts for less than 2.0 % of total sugars (Shiraishi 1993).

The sugar concentration of berry during the early stages of fruit development is quite low, typically about 2 per cent of fresh weight of the fruit. Sugar concentration however increases rapidly at the onset of verasion and by the time of harvest it can reach twenty five per cent or more of fresh weight of the berry. By the time of harvest, glucose and fructose present almost in equal amounts counts for 8 to 12 percent of fresh weight of the berry.

There is remarkable increase in compounds like glucose and fructose during the later stages of development as an outcome of total biochemical shift into fruit ripening mode. Sugar influx into the berry begins at the verasion. During fruit ripening, sucrose formed during photosynthesis is imported into the grape berry. Once transported into the berries, the sucrose is hydrolyzed into its constituent sugars, i.e., glucose and fructose by cell wall invertase maintaining the sucrose gradient by precluding its retrieval by the phloem (Robinson and Davies 2000).

The main role of sugars in grape berries is to provide the carbon skeleton of many compounds viz., amino acids and organic acids and to be energy sources for cells. By now it is scientifically accepted that sugars play also an important role as signalling molecules in control of growth and development (Rolland *et al.,* 2006).

Liu *et al.,* (2006) while studying the sugar concentration of 98 different grape cultivars concluded that glucose and fructose are the principal sugars in grape berries and ranged from 45.86 to 122.89 mg/mL and from 47.64 to \131.04 mg/mL, respectively. In addition in most of the cultivars studied sucrose was also present at trace amounts except for two cultivars of hybrids between *Vitis labrusca* and *Vitis vinifera*, which contained large amounts of sucrose.

One of the main features of the ripening process in grape berries is the accumulation of sugar mainly in the form of glucose and fructose within the cellular medium, specifically in the vacuoles and is a major commercial consideration for the grape grower, winemaker, and dried grape producer. Thus, sugar content is an indicator often used to assess ripeness and to mark the harvest. Moreover, as most of the sugar is fermented to alcohol during the winemaking process, the measurement of sugar content, the so-called "must weight", allows the control of alcohol content in the wine.

Organic acids and phenols in grape berries

Compared to sugars, organic acids are present in lesser quantity and do not surpass more than one percent of the total weight of the juice. However, they significantly contribute to the overall taste (Nelson 1985). The principal acids in the grape berry are tartaric and malic acids (approximately 90%), followed by citric acid, succinic acid and others. The amount of these organic acids varies in different grape genotypes. Differences in the acidity of the table grapes at the time of harvest can also be due to environmental conditions, duration of storage and other factors (Diakou *et al.,* 1997). Malic and tartaric acids generally accumulate before verasion, followed by a strong decline in malic acid content. However, there is a little change in tartaric acid content until harvest. The ratio of tartaric to malic acid depends on the genetic background and is usually cultivar-specific. Grapes among the exceptional fruits that contain tartaric acid present as free acid and a salt, such as potassium bitartrate. Regardless of the importance of tartric acid to the overall flavor, TSS (sweetness) is mainly used as an indicator of ripeness by the cultivators and most of the commercial genotypes are considered mature when TSS ranges from 15 to 18 percent. A vital factor controlling berry acid content is temperature. The ideal temperature for acid synthesis during the early stages of fruit development ranges between (20° and 25°C).

During grape berry development metabolic changes along with the environmental factors such light and temperature, are the main parameters that control the malate synthesis or degradation rate (Taureilles-Saurel *et al.*, 1995). Malic acid concentration at the time of harvest is negatively correlated with temperature during the ripening period. The ideal temperature for acids synthesis in grape berries is between 20°C and 25°C and with increasing temperature, synthesis of malic acid decrease and catabolic rate hastens (Kliewer *et al.,* 1967).

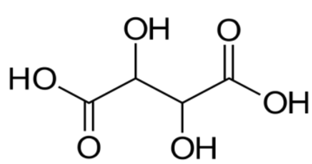
Even the potassium amount in grape berries could avoid tartrate and malate salts formation and therefore it could cause a reduction of the total acid concentration (Iland and Coombe 1988).

On the whole, fruits have greater total acidity at low temperature (particularly due to malic acid) as compared to the fruits ripened at high temperatures. Fruit acidity hence varies among regions and years, with higher levels of acidity reported in cooler regions.

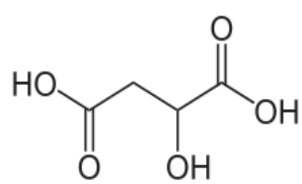
Phenolic compounds are the most abundant constituents present in grapes next to sugars and organic acids. Phenolic compounds are widely distributed in the plant kingdom. In grape berries, they are predominantly located in the skin and seeds of the berry with a very little amount (3 to 5% of total phenols) present in the grape juice. They are the important quality parameters of grapes as they contribute to the colour as well as organoleptic traits, viz; astringency, bitterness and flavor of the fruit. Phenolic compounds not only have an important role in the development of grape products, but also as effective antioxidants contributing to the nutritive value of the fruit. In grape juice, the flavonoids such as catechin, epicatechin, quercetin and anthocyanins have antioxidant effects and are also able to reduce LDL oxidation and oxidative damage to DNA, both in vitro and in animal studies (Frankel *et al.,* 1998, Singletary *et al.,* 2003).

Yang *et al.,* (2009) proposed that grapes are rich in phenolics, flavonoids, and anthocyanins, which have been suggested to be responsible for their health benefits. Grape phenolics, especially high in the grape peel, are classified into two groups: the flavonoids and non-flavonoids. The flavonoids include flavan-3-ols (catechin), flavonols (quercetin) and anthocyanins. The non-flavonoids include hydroxybenzoates (gallic acid), hydroxycinnamates and stilbenes (resveratrol) Colour along with the other variable is the most important indicator of the quality of grape juice which is directly reliant on the phenolic composition of the juice and the anthocyanins present in the grape skin. The colour of grape products is promoted by the anthocyanins, mainly through co-pigmentation and formation of polymeric pigments (Wrolstad *et al.,* 2005).

The final phenolic composition and anthocyanins of the grape genotypes vary significantly among different species and also depends on the weather, time of maturity, region where grapes are grown and the viti-cultural practices adopted (Mazza 1995, Bautista 2007). Different methods adopted during the grape juice production also effect the phenolic composition, for instance, the high temperature used during grape juice extraction leads to the degradation of anthocyanins which consequently effects the total phenolic content and colour of the juice (Morris *et al.,* 1986).



Tartaric acid



Malic acid

Antioxidative status of grape berries

Antioxidants are agents that delay or prevent the damage to lipids, proteins, enzymes, carbohydrates and DNA by scavenging the free radicals present in the cell before they can attack. The antioxidants may be either natural or the synthetic ones. Some of natural substances with a high degree of antioxidant activity include polyhydroxy flavones, flavanones, flavanols, isoflavones and chalcones. They play an important role in various fields such as medical field, food industries and others. Antioxidants are used in drug formulations for the prevention and treatment of diseases like diabetes, stroke, atherosclerosis, Alzheimer's disease and cancer. The antioxidant potential of grape phenolic compounds has been comprehensively examined in *vitro* and in *vivo*. The extracts of grape skin, seed and pomace possess potent free radical scavenging activities. The dietary intake of grape antioxidants prevents lipid oxidation by scavenging of reactive oxygen species. Grape seeds have enhanced antioxidative potential than the skin or wine byproducts. In grape seeds the functional constituents with phenolic nature include several flavonoids such as monomeric flavanols, dimeric, trimeric and polymeric procyanidins and phenolic acids. The antioxidant activity of grape seed phenolic compounds is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders (Yilmaz *et al.,* 2004). The enzyme system responsible for production of free radicals associated with inflammatory reactions is inhibited by the grape seed phenolics.

Aroma compounds

An important quality aspect in grapes and wines is aroma. Among several compounds that are responsible for aroma of grapes, terpens are known to contribute to floral or fruity characters. Aroma compounds are usually present as free and bound glycosides in both pulp and skin of grape genotypes. The accumulation of aromatic compounds in grapes is termed as "engusting" and is not correlated with the sugar concentration. In colored genotypes, the volatile compound content reaches to maximum at maturity and then remains constant (Salinas *et al.,* 2004, Coelho *et al.,* 2006). However in white genotypes, there are changes in the concentration of volatile compounds during ripening, thus making it more difficult to determine maturity on the basis of volatiles content (Garcıa *et al.,* 2003).

Potassium, calcium and nitrogen in grape berries

At least 17 elements are considered essential for the plant growth and development of plants, with some having structural role while others having role in enzyme activation, or as a charge carrier and osmoregulator (Marschner 1995).Grape berry development is associated with an influx of water, carbon and a number of mineral elements. The mineral nutrient concentration in grapes changes throughout the growing season. The grape vine takes up the minerals from the soil each year and redistribute throughout the plant from the root tissues that function as storage organs. The nutrient status of grape berries is not only of interest to the viticulturists but also to oenologists because of their impact on juice nutritional value. The quality of grapes and organoleptic properties of wine are influenced by the mineral composition of soil. During the early stages, when the growth rate is high potassium accumulation is highest in leaves. There is a sharp increase in berry potassium after verasion, as a result of redistribution potassium from leaves to berries (Blouin and Cruege, 2003). Davies *et al.,* (2006) reported that excess levels of potassium in berries regardless of its importance may reduce the fruit quality which consequently affects the wine quality. A critical determining factor of wine quality in grape juice pH. In grape berries, anthocyanins are located in the skin where potassium concentration is generally higher than in the pulp. The levels of potassium in the berries are often more important to red than to white wines, as during red wine fermentation after crushing, the skin is left for some period a for the extraction of anthocyanins. During this period, even more potassium leaches out of the skins into the juice (Mpelasoka *et al.,* 2003). Several studies have shown that maximum potassium uptake by the vines occurs between bloom and verasion. However, phosphorus uptake occurred predominantly before bloom (Conradie 1981, Schreiner *et al.,* 2006, Williams and Biscay 1991). Depending on the developmental stage, potassium may play different roles during grape berry development. For instance, during early stages of berry growth, when the rate of cell division and expansion is high, potassium may play a significant role as an osmoregulator. After verasion, when the berries enlarge in size due to increase in sugars in the cell vacuole potassium plays an secondary role in the sugar accumulation.

In grape berries, minerals, such as magnesium and calcium, together with negligible amounts of sodium and iron are also present. The concentration of calcium reaches its maximum during verasion, and afterwards either remains stable or decreases. Calcium is mainly restrained to the tissues with large number of cells and, similarly, also with a high proportion of cell walls i.e to the inner and outer most part of the berry (Swift *et al.,* 1973). In contrast to that potassium is present at high concentration in almost all parts of the berries. In grape berries nitrogen is either present as mineral compound such as ammonia, nitrate or nitrite or in organic form such as free amino acids, proteins, urea and nucleic acids. Nitrogen in the form of minerals such as ammonia represents 80% of total nitrogen before verasion but after maturation it decreases to 5-10%. The nitrate and nitrite levels are negligible about 0.5-2mg/l and 5-40µg/l respectively (Blouin and Cruege, 2003).

The grape berries function as sophisticated biochemical factories that import and accumulate water, minerals, sugars, aminoacids, organic acids and synthesize aroma and flavor compounds. Thus an understanding in how these metabolites accumulate and influence the grape berry quality is of prime importance to the grape growers which can help in better economic returns.

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