International Journal of Chemical Studies 2019; 7(1): 1181-1184

International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 1181-1184

Received: 04-11-2018
Accepted: 08-12-2018

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Analysis of total flavonoids content in grape genotypes

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Abstract
Grape is an important fruit crop with tremendous nutritious and antioxidant properties. Its consumption helps to protect our human body system from external injuries by scavenging free radicals. We have evaluated the total flavonoids content of 11 grape hybrids along with their parents. The total flavonoids were estimated and expressed as quercetin equivalent (QE). We found that, the highest total flavonoids was in 'Hy.16/2A R_3P_{12}' followed by 'Hy.16/2A R_1P_8'. In conclusion, our studies identified some of the nutraceutical rich genotypes, which can be carried forward for further utilization at a commercial scale.

Keywords: antioxidant, flavonoids, hybrid, nutraceuticals

Introduction
Grape is one of the ancient fruit crops known to human civilization, which holds a unique position among the fruits owing to its diverse array of nutraceuticals and multifold uses. India ranks ninth in grape production, with an annual production of 2.43 million tons from an acreage of 0.12 million ha with very high (21.10 t/ha) productivity (NHB, 2015) [14]. Grapes processing industry especially for juice and wine produced an estimated amount of at least 10 million tonnes of press residues each year (Maier, Andreas & Dietmar, 2009) [13].

It is a well known fact that, the nutritional qualities of grape are affected by environmental, cultural, and post-harvesting conditions, but genotype is the determined factor leading to the variation. The consumption of grape berries play a noteworthy role in maintenance of health and in disease prevention, such as; inflammation, cardiovascular disease, cancer, and age-related disorders.

Though grape has origin in the temperate climate, but it can be cultivated in all the three i.e, temperate, tropical, and subtropical climatic conditions. The major commercial grape industry in India is located in tropical belts, but the subtropical plains of India also contributed a remarkable portion of grape production. Berry cracking associated with pre-monsoon shower is the main constraint of grape industry in Northern India. Therefore, we evaluated some of the early maturing grape hybrids developed in subtropical plains of Northern India for their antioxidant traits.

Flavonols are extensively studied compounds found in muscadine grapes. In humans, protection against carcinogenesis is a widely documented effect of flavonols (Williamson and Manach, 2005) [18]. Quercetin, a flavonol, has been extensively studied, and has been shown to protect against DNA mutations, colon cancer and heart disease (Hollman and Katan, 1999) [12]. Quercetin relaxes the blood vessel wall (Rendig *et al*., 2001) [15] and increases the production of enzymes that dissolve blood clots (Abou-Agag *et al*., 2001) [1]. The grape flavonoids comprising of anthocyanins, tannins, and flavonols play a major role in influencing colour and mouthfeel of red wine. As per the stages of berry development, these compounds are synthesized in different plant parts. Some environmental and viticultural practices such as; exposure to light can also influence the flavonoid composition of grapes.

To our knowledge, there is no comprehensive study regarding flavonoid profile of grape hybrids developed in India. The research on flavonoid composition of different fruit crops is well developed and vast in abroad, but the information regarding many Indian hybrids are completely lacking. In the present study, we have estimated the flavonoids content of 11 different grape hybrids developed at IARI, New Delhi along with comparison with their parents was done for the flavonoid composition.

Materials and Methods

Plant materials

A total of 11 hybrids along with their 9 parents were taken for this study. The details were given in Table 1.

Chemicals

For analysis the chemicals used are; sodium nitrite, sodium hydroxide and aluminium chloride. The chemicals used in our study were of the best quality available commercially from the suppliers.

Sample Preparation

Mature berries were collected from the grape germplasm block situated at IARI, New Delhi. Grape berries of uniform size; shape and colour, free from injuries were sorted out and used for this experiment. Five uniform bunches from the selected vines were used for taking morpho-physical parameters. Grape berries were removed from each bunch. Randomly selected 100 berries from each genotype were chosen for evaluating the phytochemical content. Four replicates for each cultivar were used for analytical work and 1 to 2 berries homogenized for analytical work. From this homogenate a 2 to 2.5 g of berry was accurately weighed and crushed with 80% ethanol and 10 ml sample volume was made with 80% ethanol and transferred to a 10 ml of sample volume. The mixture of all these were centrifuged at 10000 rpm for 10 minutes at 4 °C. For analytical work, the supernatant was collected and used for the estimation of total phenolics.

Quantitative determination of total flavonoids

The content of the total flavonoid content was measured through spectrophotometer. In this procedure, 1 ml sample of extract was drawn from each genotype. The each sample was added to a 10 ml volumetric flask containing 4 ml of distilled water and also added 0.3 ml of 5% $NaNO_2$ and allowed to stand for 5 min at ambient conditions. Another 0.3 ml portion of 10% $AlCl_3$ $6H_2O$ was also added to the mixture and allowed to stand for 6 min at ambient conditions. Finally 2 ml of 1N NaOH was added and the solution was diluted to the desired volume (10 ml) with distilled water. The absorbance of the solution was measured at 510 nm immediately by Spectrophotometer (UV 5704SS, ECIL, India). The quantity was calculated and expressed as quercetin equivalent (QE) using a standard curve (absorbance versus concentration) prepared from authentic quercetin.

Results and Discussion

The evolution of reactive oxygen species cause a great injury to the DNA inside the human body system. The examples of ROS are, $^1O_2$, H_2O_2, O_2^- and OH^-. These affect normal cellular functioning and lead to chronic human diseases such as; cancer, heart diseases, and cerebrovascular diseases. The developing countries like India suffered a lot from these chronic diseases due to change in lifestyle and diet pattern. In the present context the food basket comprising of ample fruits and vegetables can serve the purpose of overcoming these health curses as they are rich in phytochemicals. Hence, we have evaluated some of the Indian grape hybrids and their parents for their flavonoids content, which can increase the immune system of our body.

Determination of Total flavonoids by biochemical analysis

The extracts from different grape genotypes were used for determination of their total flavonoids are presented in Figure 1 and 2. The grape extracts derived from berries of different genotypes differed significantly in total flavonoid content ranged from 41.02 ('A-5') to 175.29 ('Hy.16/2A R_3P_{12}') mg, QE/100g. The maximum total flavonoid content was measured in genotype 'Hy.16/2A R_3P_{12}' (175.29) followed by 'Hy.16/2A R_1P_8' (135.17), 'Hy.ER-R_2P_{36}' (133.09), 'Hy.16/2A R_1P_{14}' (129.71), 'Hy.16/2A R_1P_2' (128.03), and 'Hy.ER-R_1P_{19}' (126.64) mg QE/100g were found rich in total flavonoids. However, the minimum total flavonoids were recorded in genotype 'A-5' (41.02) followed by 'Banqui Abyad' (41.47), 'Black Muscat' (44.49), 'Pearl-of-Csaba' (47.05), 'Cardinal'(66.50) and 'Ruby Red' (68.66) mg QE/100g.

The flavonoids are the group of polyphenolic compounds with wide distribution in various plant parts reported different forms of flavonoids like flavones, flavanones, falvan-3-ols, flavonols, anthocyanins, flavonones and isoflavones in fruit crops. They act as anti-bacterial, anti-viral, anti-inflammatory, antiallergic and vasodilatory actions in the human body. Therefore, they have received special attention from researchers for the use of human health. Flavonoids inhibit certain enzymes through their antioxidant activity and help in treating the diseases. Therefore, it is very essential to study the grape genotypes for total flavonoid content. In the present study, the amount of total flavonoids was observed in the range of 41.02 (A-5) to 175.29 (16/2A R_3P_{12}) mg QE/100 g. The fruits of hybrids 16/2A R_3P_{12}, 16/2A R_1P_8, ER-R_2P_{36} were found most rich in terms of total flavonoids under study. These three hybrids are good for nutritional point of view to fight against deadly chronic diseases and good for human consumption. Similar findings also reported by Yang *et al*. (2009) who assessed 14 grapes genotypes for total flavonoid content and found 3- times higher total flavonoids in 'Pinot Noir' (301.8 mg/100 g) as compared to 'Baco Noir'. Hogan *et al*. (2009) also evaluated 'Cabernet Franc', 'Clone1' and 'Cabernet Franc clone313' for total flavonoids content and the maximum content was recorded in 'Cabernet Franc' then 'Clone1', and 'Cabernet Franc clone 313'. Recently in Spain, Montoro *et al*. (2015) also analyzed total flavonoids in 20 commercial grape juice and 10 typical Spanish wine varieties. The higher content was recorded in red grape juice varieties as compared to white grape juice varieties (98 vs. 63 mg catechin/l).

Table 1: Grape hybrids with their parentage

Hybrid	Female parent♀	Male parent♂
16/2A R_1P_2	Madeleine Angevine	Ruby Red
16/2A R_1P_7	Madeleine Angevine	Ruby Red
16/2A R_1P_{18}	Banqui Abyad	Beauty seedless
16/2A R_1P_{19}	Banqui Abyad	Beauty seedless
16/2A R_4P_{13}	Banqui Abyad	Beauty seedless
16/2A R_3P_{12}	Black Muscat	Beauty seedless
ER-R_1P_{19}	Pearl of csaba	Beauty seedless
ER-R_2P_{36}	Pearl of csaba	Beauty seedless
ER-R_2P_{19}	Pearl of csaba	Beauty seedless
16/2A R_1P_{14}	Cardinal	Beauty seedless
16/2A-R_1P_8	Hur	A-5

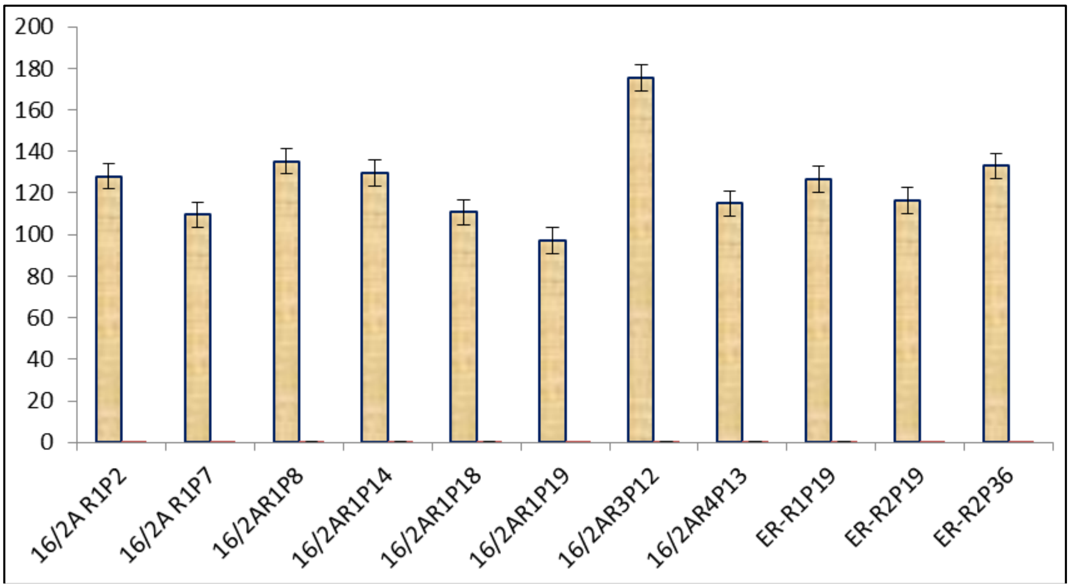

Fig 1: Total flavonoids content of eleven grape hybrids

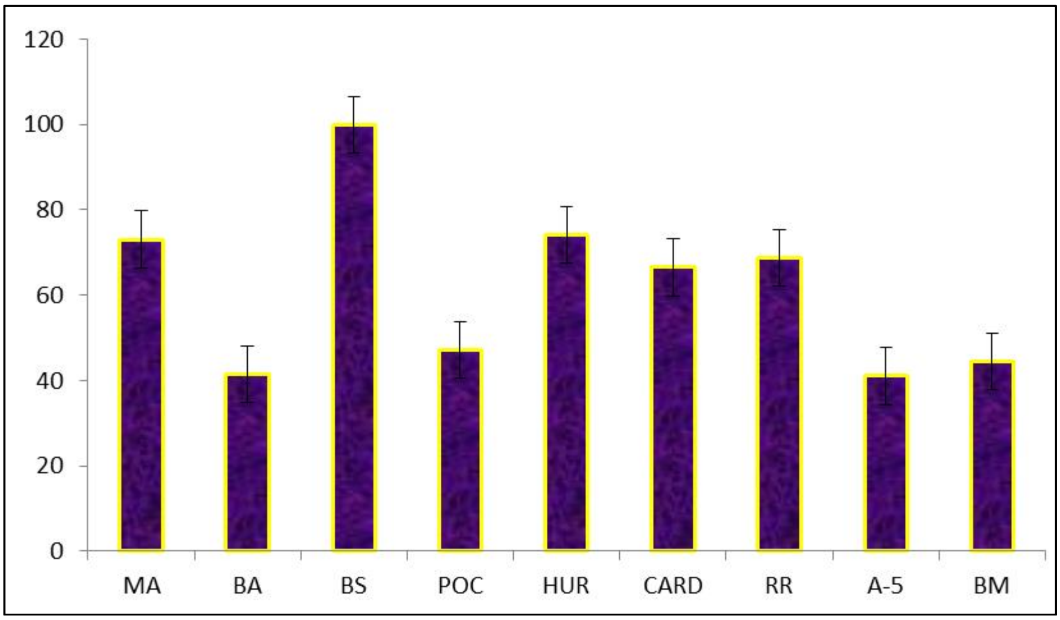

Fig 2: Total flavonoids content of nine parental grape genotypes

Conclusion

From the present study, it was inferred that, among the different varieties assessed for their total flavonoids content, 'Hy.16/2A R_3P_{12}' and 'Hy.16/2A R_1P_8' have the maximum content. There is positive correlation exists between the flavonoids content and antioxidant activities. So, these grape hybrids can be exploited commercially to manufacture potent nutraceutical products. The flavonoids are very much helpful to stabilize the immune system of body, hence the consumption of flavonoids rich grape berries can lessen the vulnerability to chronic disorders. There is also scope for further development of new grape hybrids by exploiting the promising genotypes in future breeding programme.

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