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Estimation of total phenolics in some of the grape genotypes

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Abstract

Grape is a versatile crop being the reservoir of diverse array of nutraceuticals. Grape products can serve as a potential weapon to fight against many deadly chronic diseases owing to its tremendous antioxidant activity. We have evaluated the phenolics content of 11 grape hybrids along with their parents. The total phenolics were estimated by using Folin-Ciocalteu reagent. We found that, the highest total phenolics was in 'Hy. 16/2A R₁P₂' followed by 'Hy.16/2A R₁P₈'. In conclusion, our studies identified some of the potential genotypes, which can be used in future breeding programme to produce a variety with enhanced level of nutraceuticals.

Keywords: Antioxidant, phenolics, hybrid, nutraceuticals

Introduction

Good health is one of the greatest blessings of life. It is evident that, regular dietary intake of fruits and vegetables is associated with reduced risk of cardio-vascular diseases (Bazzano *et al.*, 2003) [1]. 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. According to FAO (2013) [6] estimates 67.2 million tonnes of grapes being produced from an area of around 6.9 million ha in the world. 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) [12]. Among dietary antioxidants, phenolic compounds are abundant present in several fruits and grape in particular (Hollman and Arts, 2000; Crozier *et al.*, 2006) [10, 2]. The higher amount of phenolic compounds (gallic acid, catechin, anthocyanins, resveratrol, procyanidins) in grapes is generally associated with the richness of the fruits for consumption. These phenolic compound acts as antioxidants, anti-carcinogenic, anti-atherogenic, anti-inflammatory and antimicrobial (Falcao *et al.*, 2010; Darra *et al.*, 2012) [7, 4].

Though grape originated in the temperate zones, but it can be cultivated in all the three i.e., temperate, tropical, and subtropical climatic conditions. The major commercial grape growing region 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.

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) [11]. The phenolic compounds in grape are mainly concentrated in berry skin and seeds. The by-products emerged from the processing industry as waste can be converted in powerful nutraceutical products for overcoming various chronic diseases. Phenolics of grape seeds may help to inhibit enzyme systems that are responsible for the production of free radicals causing great injury to human system (Shi *et al.*, 2005). Grape seeds contains mainly phenols such as proanthocyanidins (oligomeric proanthocyanidins) [14]. Scientific studies have shown that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C (Shi *et al.*, 2005) [14].

To our knowledge, there is no comprehensive study about nutraceutical properties of grape hybrids developed in India. Though, these kind of study were well-known in many of the wine varieties and table varieties at the global level, the information regarding many Indian hybrids are completely lacking. In the present study, we have determined the phenolics content of 11 different grape hybrids developed at IARI, New Delhi. We also compared these with their parents.

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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.

Table 1: Grape hybrids with their parentage

Hybrid	Female parent ♀	Male parent ♂
16/2A R ₁ P ₂	Madeleine Angevine	Ruby Red
16/2A R ₁ P ₇	Madeleine Angevine	Ruby Red
16/2A R ₁ P ₁₈	Banqui Abyad	Beauty seedless
16/2A R ₁ P ₁₉	Banqui Abyad	Beauty seedless
16/2A R ₄ P ₁₃	Banqui Abyad	Beauty seedless
16/2A R ₃ P ₁₂	Black Muscat	Beauty seedless
ER-R ₁ P ₁₉	Pearl of csaba	Beauty seedless
ER-R ₂ P ₃₆	Pearl of csaba	Beauty seedless
ER-R ₂ P ₁₉	Pearl of csaba	Beauty seedless
16/2A R ₁ P ₁₄	Cardinal	Beauty seedless
16/2A-R ₁ P ₈	Hur	A-5

Chemicals

Folin and Ciocalteu's phenol reagent (1N), ethanol (80%), sodium carbonate (20%). 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 phenolics

The total phenolic content was measured through use of the spectrophotometer (Double beam UV-VIS Spectrophotometer, UV 5704SS, ECIL, India) and using Folin-Ciocalteu reagent and gallic acid as a standard (Singleton *et al.*, 1999) [13]. To the 100 µl of the sample extract, 2.9 ml of deionized water, 0.5 ml of Folin-Ciocalteu reagent and 2.0 ml of 20% Na₂CO₃ solutions were added. The absorbance of the mixture was then measured at 760 nm. The amount of total phenolic content was expressed as mg of gallic acid equivalents (GAE)/100g fresh weight.

Results and Discussion

ROS generation occurs constantly at basal level under favourable conditions, but they are unable to cause damage as being scavenged by different antioxidant mechanism (Foyer and Nocter, 2005) [8]. The ROS mainly comprise of ¹O₂, H₂O₂, O₂⁻ and OH⁻. These are very lethal and causes extensive

damage to protein, DNA and lipids and thereby affects normal cellular functioning. These ROS may ultimately leads to degenerative 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 antioxidant traits, that can become a potential tool to fight against the chronic diseases.

Determination of Total Phenolics by biochemical analysis

The extracts from different grape genotypes were used for determination of their total phenolics are presented in Figure 1. The range of the total phenol content ranged from 101.36 ('A-5') to 304.22 ('16/2A R₁P₂') mg GAE/100 g fw. There were significant differences were noticed for total phenolics in genotype '16/2A R₁P₂' (304.22) followed by 'Hy.16/2A R₁P₈' (276.05), 'Black Muscat' (268.47), 'Hy.ER-R₁P₁₉' (246.19) and 'Hy.16/2A R₁P₇' (227.15) mg/ GAE/100g as compared to other genotypes. However, the minimum total phenols were observed in genotypes 'A-5' (101.36) followed by 'Banqui Abyad' (128.81), 'Pearl of Csaba' (140.54) and 'Hy.16/2A R₄P₁₃' (172.34) mg GAE/100g. Grapes are chiefly appreciated for their higher content of phenolic contents in terms of gallic acid, catechin, resveratrol and wide range of procyanidins. In the present investigation, the amount of total phenol content was measured as gallic acid equivalent which was ranged from 101.36 ('A-5') and 304.22 ('16/2A R₁P₂') mg GAE/100 g fw. The similar findings was also reported by Du *et al.* (2012) [5] in 'Cabernet Sauvignon', 'Cabernet Franc', 'Merlot', 'Cabernet Gernischt', 'Muscat', 'Red Globe', '*Vitis labrusca* Kyoho' and 'Milk grape' and they reported the maximum content in 'Cabernet Gernischt' (257.0 mg/100 g). In grapes, majority of phenols are present in pulp, skin, and seeds. These are also considered to play major role in imparting the quality parameters of grapes which contribute to their colour and organoleptic characteristics like flavour, bitterness, and astringency (Dangles *et al.*, 1992; Gómez-Cordovés and González-Sanjósé, 1995) [3,9].

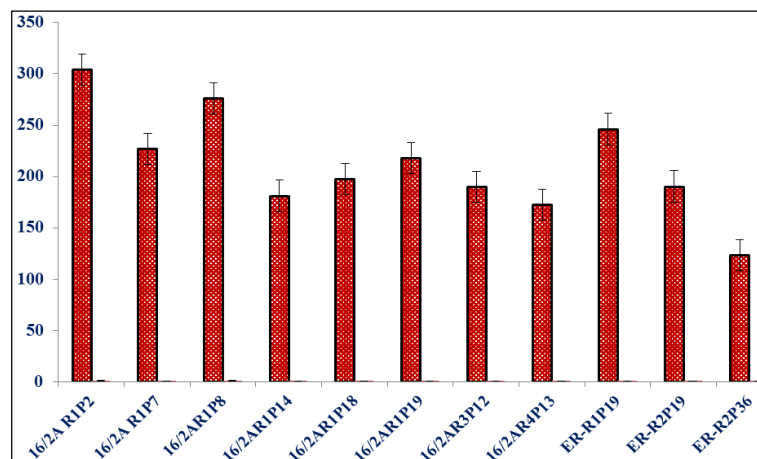


Fig 1: Total phenolics content of eleven grape hybrids

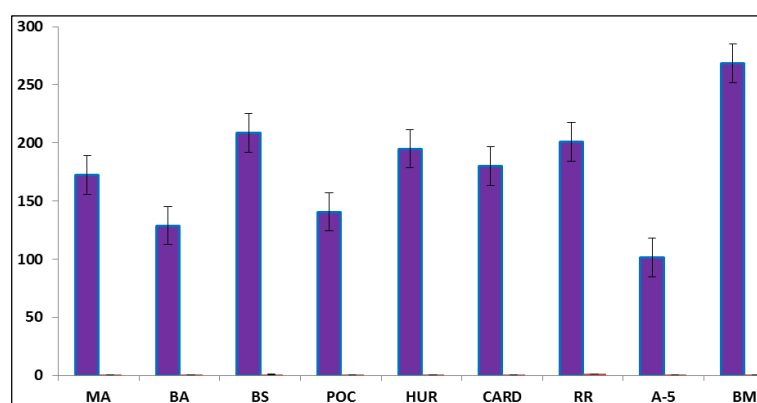


Fig 2: Total phenolics content of 9 parentage grape varieties

Conclusion

We got to know from our studies that among the different varieties assessed for their phenolics content, 'Hy.16/2A R₁P₂', 'Hy.16/2A R₁P₈' and Cv. Black Muscat have the maximum content. There is positive correlation exists between the phenolic contents and antioxidant activities. So, these grape hybrids can be exploited commercially to manufacture potent nutraceutical products, which if consumed in ample amount, may confer health benefits. The potential parents can be utilized in further breeding programme to evolve new potential hybrids. So our studies will certainly helpful for the countries with higher incidence of CVD.

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