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Estimation of DPPH antioxidant activity in some of the grape genotypes

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Abstract

Today, there is a high demand for foods rich in antioxidants. 'Grape' is regarded as a versatile crop with wide range of bioactive compounds naturally present in it. These bioactive compounds attribute to the antioxidant properties. In the present study, the antioxidant activity in terms of DPPH was determined for 11 grape hybrids along with their 9 parents. The highest antioxidant activity was exhibited by the genotype 'Cardinal' followed by 'Banqui Abyad'. Henceforth, the potent antioxidant rich hybrids could be utilized to manufacture various nutraceutical products and also in improvement of grape germplasm.

Keywords: Antioxidant, DPPH, hybrid, bioactives

Introduction

Grape (*Vitis vinifera*) is one of the very old fruit crop species originated approximately 54 million years ago. In ancient times, it was commonly used as a fermented beverage namely 'wine' (McGovern, 2003) [9]. However, it was one of the established and successful fruit crop during that period mainly due to the delicious and nutritious fruits (Dos santos Lima *et al.*, 2014 and Toaldo *et al.*, 2015) [3, 11] and also being processed to different products like juices, raisins and wines (Granato *et al.*, 2016) [5]. About 23% of the total grapes harvested are table grapes for fresh consumption, while 86.6% of the crop is processed, especially for wine-making (Liu *et al.*, 2006) [8]. Commercial viticulture in India is around six-decade old and is currently being considered as one of the most remunerative horticulture enterprises.

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. In this study, we have evaluated some of the early maturing grape hybrids for their antioxidant traits, that are especially suited to the subtropical plains of Northern India.

Grape berries possess tremendous antioxidant activity due to numerous bioactives in it such as; phenolics, flavonoids, anthocyanins, resveratrol, catechins, epicatechins *etc.* These antioxidative compounds play a vital role in protecting human body from destructive effect of reactive oxygen species. The ROS generation greatly injures DNA and creates various imbalances in metabolism. Thus, the antioxidant rich fruits help in a way to tackle against them naturally and efficiently.

The research on antioxidant properties of grapes 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 antioxidative activity in terms of DPPH of 11 different grape hybrids developed at IARI, New Delhi along with comparison with their parents for the antioxidants level.

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.

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

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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 10,000 rpm for 20 minutes at 4 °C. For analytical work, the supernatant was collected and used for the estimation of DPPH antioxidant activity.

Free radical scavenging activity by DPPH assay

The antiradical capacity of the sample extract was measured as per the method suggested by Brand-Williams *et al.*, (1995) [2] and modified by Sanchez-Moreno *et al.*, (1998). DPPH is one of the stable and commercially available organic nitrogen radicals and has UV-VIS absorption maxima at 515 nm. On reduction of the colour solution fades and the reaction progress is monitored with a spectrophotometer at 515 nm. In methanolic solution (0.1 ml) of sample extract (15 mg/ml) added to 3.9 ml of DPPH (0.025g/ l) in methanol and absorbance measured at 515 nm (Double beam UV-VIS Spectrophotometer, UV 5704SS, ECIL, India). The absorbance was measured until the reaction reached a plateau (steady state). Results were expressed in terms of Trolox equivalents.

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 the DPPH antioxidant activity, which can increase the immune system of our body.

The DPPH scavenging activity was recorded and data are presented in Table 4.13. Among the genotypes, the highest DPPH scavenging activity was detected in the genotype 'Cardinal' (10.38) followed by 'Banqui Abyad' (9.74), 'Hy.ER-R₂P₃₆' (9.63), 'Hy.16/2A R₁P₁₉' (9.36), 'Madeline Angevine' (9.25) and 'Hy.16/2A R₁P₂' (8.82) $\mu\text{mol TE/g}$ were found significantly different as compared to other genotypes ($P \leq 0.05$). However, the minimum scavenging activity was detected in genotypes 'Hy.16/2A R₁P₈' (5.37) followed by 'A-5' (6.66) and 'Black Muscat' (6.80) $\mu\text{mol TE/g}$. The difference in antioxidant activities are mainly governed by the genotype irrespective of rest of the factors (Garrido and Borges, 2011) [4]. Bartolomé *et al.* (2004) [1] also suggested that the radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is influenced by grape variety and stage of berry ripening. Kedage *et al.* (2007) [7] and Katalinić *et al.* (2010) [6] also gave confirmation that coloured grape genotypes were rich in antioxidant activity as compared to white genotypes.

Table 1: Newly developed grape hybrids and parents used for the study.

Parental combination	Hybrids	No. of Hybrids
Madeleine Angevine x Ruby Red	16/2A R ₁ P ₂ , 16/2A R ₁ P ₇	2
Banqui Abyad x Beauty seedless	16/2A R ₁ P ₁₈ , 16/2A R ₁ P ₁₉ , 16/2A R ₄ P ₁₃	3
Black Muscat x Beauty seedless	16/2A R ₃ P ₁₂	1
Pearl of Csaba x Beauty seedless	ER-R ₁ P ₁₉ , ER-R ₂ P ₃₆ , ER-R ₂ P ₁₉	3
Cardinal x Beauty seedless	16/2A R ₁ P ₁₄	1
Hur x A-5	16/2A-R ₁ P ₈	1

Table 2: DPPH radical scavenging activity in grape genotypes

S. No.	Genotype	DPPH (TE, $\mu\text{mol/g}$)
Hybrids		
1	Hy.16/2A R ₁ P ₂	8.82 \pm 0.07 ^{cd}
2	Hy.16/2A R ₁ P ₇	7.24 \pm 0.03 ^{gh}
3	Hy.16/2A R ₁ P ₈	5.37 \pm 0.01 ^h
4	Hy.16/2A R ₁ P ₁₄	6.47 \pm 0.08 ^{bc}
5	Hy.16/2A R ₁ P ₁₈	8.21 \pm 0.02 ^{gh}
6	Hy.16/2A R ₁ P ₁₉	9.36 \pm 0.05 ^{de}
7	Hy.16/2A R ₃ P ₁₂	7.86 \pm 0.04 ^{ef}
8	Hy.16/2A R ₄ P ₁₃	8.31 \pm 0.08 ^{bc}
9	Hy.ER-R ₁ P ₁₉	7.54 \pm 0.08 ^{bc}
10	Hy.ER-R ₂ P ₁₉	7.37 \pm 0.03 ^{gh}
11	Hy.ER-R ₂ P ₃₆	9.63 \pm 0.09 ^b
Parent		
12	Madeline Angevine	9.25 \pm 0.06 ^{cd}
13	Banqui Abyad	9.74 \pm 0.04 ^{ef}
14	Beauty Seedless	8.46 \pm 0.04 ^{ef}
15	Pearl-of-Csaba	7.75 \pm 0.04 ^{ef}
16	Hur	7.54 \pm 0.02 ^{gh}
17	Cardinal	10.38 \pm 0.11 ^a
18	Ruby Red	8.62 \pm 0.09 ^b
19	A-5	6.66 \pm 0.06 ^{cd}
20	Black Muscat	6.80 \pm 0.06 ^{cd}
	Mean	8.07
	LSD (p \leq 0.05)	0.177

Values represent the mean \pm standard error of four replicates. Means with same superscript within a column are not significantly different at 5% level of significance when compared with LSD value. Different letters in the same column represent statistically different results ($p < 0.05$).

Conclusion

From the present study, it was inferred that, among the different varieties assessed for their DPPH antioxidant activity, 'Cardinal' showed the maximum followed by 'Banqui Abyad'. So, the antioxidant rich grape genotypes can be exploited commercially to manufacture potent nutraceutical products, which confer tremendous health benefits and fight against age related and life style diseases. These can also be utilized in further development of new grape varieties through breeding programme.

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