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# Estimation of CUPRAC Activity in some Grape Genotypes

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**A B S T R A C T**

The potential of antioxidants in our diet has attained the major focus now-a-days. Grape often termed as 'Super food of the future' has been a crop of choice to analyse for various its nutraceuticals. In the present study, the antioxidant activity in terms of CUPRAC was determined for 11 grape hybrids along with their 9 parents. The highest antioxidant activity was exhibited by the genotype 'Hy.16/2A $R_1P_{19}$' and among parents, it was found maximum in 'Beauty Seedless'. In conclusion, the potent antioxidant rich hybrids could be utilized to manufacture various nutraceutical products and also in improvement of various genotypes.

## Introduction

Grape (*Vitis vinifera* L.) is one of the most nutritious fruit crop having beneficial effects on human health and also gives higher returns per unit area when grown on commercial scale are widely grown and eaten around the world. Now-a-days, there are three main species of grapes: European grapes (*Vitis vinifera*), North American grapes (*Vitis labrusca* and *Vitisrotundifolia*) and French hybrids. During the ancient time, grapes were esteemed for their use in winemaking. These are classified as table grapes, wine grapes (used in viniculture), raisin grapes, and seedless grapes. People often enjoy the various grape products, such as fruit, raisins, juice and wine.

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). 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. Therefore, we evaluated some of the early maturing grape hybrids developed in subtropical plains of Northern India for their antioxidant traits.

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.

To our knowledge, there is no comprehensive study regarding antioxidant activity of grape hybrids developed in India. 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 CUPRAC of 11 different grape hybrids developed at IARI, New Delhi along with comparison with their parents was also done.

## 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 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 10ml 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 CUPRAC antioxidant activity.

### Cupric reducing antioxidant capacity (CUPRAC)

The CUPRAC is an assay used to measure antioxidant capacity of grapes. This method measures the copper (II) or cupric ion reducing ability of polyphenols, vitamin C and vitamin E (Apak *et al.,* 2004). It uses copper (II)-neocuproine [Cu (II)-NC] reagent as the chromogenic oxidizing agent.

In this method there is mixing of the antioxidant solution with a copper (II)

chloride solution, a neocuproine alcoholic solution and an ammonium aqueous buffer at pH 7 and subsequent measurement of the developed absorbance at 450 nm after 30 min. In the grape samples it was measured as below:

Added 1 ml each of copper (II) chloride solution ($10^{-2}$ M), neocuproine solution (Nc) of 7.5 x $10^{-3}$ M, and ammonium acetate ($NH_4Ac$) buffer (pH 7) solutions in a test tube. Antioxidant sample (or standard) solution (x ml) and $H_2O$ (1.1- x ml) were added to the initial mixture so as to make the final volume 4.1 ml. The tubes were plugged and after one hour, the absorbance at 450 nm was recorded against a reagent blank. The standard calibration curve of each antioxidant compound was constructed in this manner as absorbance versus concentration. The molar absorptivity of the CUPRAC method for each antioxidant was found from the slope of the calibration line concerned and the antioxidant activity was expressed as 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 CUPRAC antioxidant activity, which can increase the immune system of our body.

The cupric reducing power activity indicates about the antioxidant compounds which are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process. In the present study, CUPRAC assay was used based on the reaction in which reduction of Cu(II) to Cu(I) by antioxidants occurs. The activity was recorded among genotypes was found significantly variable and data are presented in Table 2.

**Table.1** Grape hybrids with their parentage

| Hybrid | Cross (indicating parentage) |
|---|---|
| **16/2A $R_1P_2$** | Madeleine Angevine X Ruby Red |
| **16/2A $R_1P_7$** | Madeleine Angevine X Ruby Red |
| **16/2A $R_1P_{18}$** | BanquiAbyad XBeauty seedless |
| **16/2A $R_1P_{19}$** | BanquiAbyad XBeauty seedless |
| **16/2A $R_4P_{13}$** | BanquiAbyad XBeauty seedless |
| **16/2A $R_3P_{12}$** | Black Muscat XBeauty seedless |
| **ER-$R_1P_{19}$** | Pearl of csaba XBeauty seedless |
| **ER-$R_2P_{36}$** | Pearl of csaba XBeauty seedless |
| **ER-$R_2P_{19}$** | Pearl of csaba XBeauty seedless |
| **16/2A $R_1P_{14}$** | CardinalBeauty Xseedless |
| **16/2A-$R_1P_8$** | Hur XA-5 |

**Table.2** Cupric reducing antioxidant capacity (CUPRAC) ingrape genotypes

| Sl. No. | Genotype | CUPRAC (µmol TE/g) |
|---|---|---|
| | **Hybrids** | |
| **1** | Hy.16/2A $R_1P_2$ | $15.92 \pm 0.10^{gh}$ |
| **2** | Hy.16/2A $R_1P_7$ | $27.26 \pm 0.24^{bc}$ |
| **3** | Hy.16/2A $R_1P_8$ | $24.58 \pm 0.12^{gh}$ |
| **4** | Hy.16/2A $R_1P_{14}$ | $27.72 \pm 0.17^{cb}$ |
| **5** | Hy.16/2A $R_1P_{18}$ | $17.05 \pm 0.19^{de}$ |
| **6** | Hy.16/2A $R_1P_{19}$ | $31.94 \pm 0.25^{b}$ |
| **7** | Hy.16/2A $R_3P_{12}$ | $25.84 \pm 0.20^{cd}$ |
| **8** | Hy.16/2A $R_4P_{13}$ | $31.78 \pm 0.36^{a}$ |
| **9** | Hy.ER-$R_1P_{19}$ | $5.41 \pm 0.02^{l}$ |
| **10** | Hy.ER-$R_2P_{19}$ | $5.18 \pm 0.07^{ij}$ |
| **11** | Hy.ER-$R_2P_{36}$ | $13.26 \pm 0.12^{gh}$ |
| | **Parents** | |
| **12** | Madeline Angevine | $14.67 \pm 0.07^{ij}$ |
| **13** | BanquiAbyad | $6.58 \pm 0.06^{k}$ |
| **14** | Beauty Seedless | $18.90 \pm 0.15^{ef}$ |
| **15** | Pearl-of-Csaba | $6.34 \pm 0.08^{ij}$ |
| **16** | Hur | $6.21 \pm 0.21^{cd}$ |
| **17** | Cardinal | $12.47 \pm 0.03^{l}$ |
| **18** | Ruby Red | $10.38 \pm 0.06^{k}$ |
| **19** | A-5 | $5.60 \pm 0.08^{ij}$ |
| **20** | Black Muscat | $11.58 \pm 0.07^{ij}$ |
| | Mean | 15.93 |
| | LSD ($p \leq 0.05$) | 0.453 |

Values represent the mean ± 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 ($p < 0.05$).

Among the 20 genotypes it was ranged from 5.18 ('Hy.ER-$R_2P_{19}$') to 31.94 µmol TE/g ('Hy.16/2A $R_1P_{19}$'). However, among all the genotypes, the maximum CUPRAC reducing power was detected in genotype 'Hy.16/2A $R_1P_{19}$' (31.94) followed by 'Hy.16/2A $R_4P_{13}$' (31.78), 'Hy.16/2A $R_1P_{14}$' (27.72), 'Hy.16/2A $R_1P_7$' (27.26), 'Hy.16/2A $R_3P_{12}$' (25.84) and 'Hy.16/2A $R_1P_8$' (24.58) µmol TE/g. The minimum CUPRAC reducing power was recorded genotype in 'Hy.ER-$R_2P_{19}$' (5.18), 'Hy.ER-$R_1P_{19}$' (5.41), 'A-5' (5.60), 'Hur' (6.21), and 'Pearl-of-Csaba' (6.34) µmol TE/g.

These findings are also comparable with the antioxidant activities reported by (Jiang and Zhang, 2011; Rockenbach *et al.*, 2011; Meng *et al.*, 2012). Rockenbach *et al.*, (2011) studied four wine grape varieties Cabernet Sauvignon, Merlot, Bordeaux and Isabel, which Cabernet Sauvignon grape pomace was found to have the highest content of antioxidant activity of cupric reducing capacity and hydroxyl radical-scavenging activity.

In conclusion, from the present study, it was inferred that, among the different varieties

assessed for their CUPRAC antioxidant activity, 'Hy.16/2A $R_1P_{19}$' showed the maximum followed by 'Hy.16/2A $R_4P_{13}$'. In general the new grape hybrids showed better antioxidant activity over their parents.So, these grape hybrids can be exploited commercially to manufacture potent nutraceutical products and also in further development of new grape varieties by exploiting the promising genotypes in future breeding programme.

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