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Profiling of different grape (*Vitis vinifera* L.) genotypes for their nutraceutical attributes

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ABSTRACT

Experiments were conducted to evaluate twelve seeded grape genotypes, including three white (B-27-1, Italia and Sauvignon Blanc) and nine colored (Cardinal, Carolina Black Rose, Chasan-B, H-144, H-27, Kyoho, Muscat Hamburg, Punjab MACS Purple and Pusa Navrang). The seeds were separated manually to ascertain the potential of natural antioxidants and finding out the suitable genotype for cultivation. Among the evaluated coloured and white genotypes, the highest total phenols, flavonoids, anthocyanins (2.15 mg/100 g), hydroxyl radicals scavenging activity (57.29 %) and total antioxidants (76.21 %) were found in the seed extracts of Pusa Navrang. Further high nutraceutical properties of seeds were recorded with Pusa Navrang followed by H-27 due to the teinturier trait of berries, whereas other genotypes had pale juice color and lacked anthocyanins in the pulp and seeds. Pusa Navrang, Kyoho, and Sauvignon Blanc exhibited the highest enzymatic activities for phenylalanine ammonia lyase, peroxidase and polyphenol oxidase respectively. The high correlation coefficient (R^2) between phenolic content and antioxidant activity was also recorded. This study valuable insights into the nutraceutical potential of various grape genotypes, offering opportunities to repurpose juice and wine industry byproducts into nutraceutical products. Such initiatives could enhance the economic benefits for grape growers.

Key words: Anthocyanin, peroxidase, phenols, phenylalanine and scavenging activity.

INTRODUCTION

Nowadays interest has been increased for studying phenolic compounds and antioxidant activity in agro-industrial byproducts. Several researchers have revealed diverse antioxidant properties and phenolic profiles in food plant byproducts such as grape pomace, winery seeds, spent coffee grounds, pomegranate marc, and orange peel. Significant data suggests that these byproducts have the potential to be used in the production of innovative foods, as they may offer health benefits to humans. (Chellammal, 5). Among various fruits *Vitis vinifera* L. (Grape) is widely grown and consumed globally. Approximately 80% of the total harvest is used for winemaking, and the byproducts from this process account for about 20% of the grape's weight (Nurhan, 13). Black Queen is a grape variety commonly used for producing wine and juice, resulting in a large number of byproducts, including pomace (grape pulp, peels, and seeds). Several studies have shown that these byproducts are a valuable antioxidant source (Arif *et al.,* 1). Grape seeds contain sixty to seventy per cent phenolic compounds, with catechin, epicatechin, and procyanidin identified as the primary antioxidants (Ribéreau-Gayon *et al.,* 15) reported that. Grapes have high content of phenolic compounds, and their potential health benefits have been well known and studied, including the inhibition of oxidation in human low-density lipoproteins (LDL) and their anti-inflammatory effects (Frankel *et al.,* 7). This protective effect of polyphenols, is ascribed to their radical-scavenging capacity against the reactive free oxygen radicals (ROS) which damage the proteins, nucleic acids, lipids and carbohydrates in the human body. Therefore, for decades the antioxidant activity of polyphenols in grape and its products, particularly wine, which is included in the Mediterranean diet. The antioxidant character of these compounds is high and their quality depends on the genotype type and the agrochemical conditions of vine growing. Keeping in mind all these facts, research proposal was planned to quantify the polyphenol spectrum and to evaluate the antioxidant properties of grape seeds of grape genotypes suitable for cultivation under subtropical conditions of North India.

MATERIALS AND METHODS

Twelve grape genotypes, including three white and nine colored, were selected in the experimental block of Fruit Research Farm, Punjab Agricultural University, Ludhiana (India) (Table 1). Based on TSS (total soluble solids), acidity and colour traits fruits were harvested at maturity. The fresh grape samples after harvest were used for analysis studies and stored at 4°C during the analysis process. The seeds were separated manually from the mass

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Table 1: Grape genotypes evaluated and their place of origin.

Skin colour	Grape genotypes	Origin
White	B-27-1	India
	Italia	Italy
	Sauvignon Blanc	France
Coloured	Cardinal	USA
	Carolina Black Rose	USA
	Chasan-B	France
	H-144	India
	H-27*	India
	Kyoho	Japan
	Muscat Hamburg	UK
	Punjab MACS Purple	India
	Pusa Navrang*	India

*Teinturier hybrid having coloured skin and flesh

part. After washing the seeds with distilled water, Phenolic and enzymes were extracted with the different procedures. Total phenols were estimated with the Folin-Ciocalteu procedure as discussed by Swain and Hill, 18. Total flavonoids were estimated by the procedure suggested by Balababa *et al.* (2). The total antioxidant activity was measured by the 2, 4-dinitrophenylhydrazine (DPPH) reagent as suggested by Liyana and Shahidi, 12. The hydroxyl radicals in grape tissues were estimated by the Spectrophotometric method, as suggested by Barros *et al.* (3). Total monomeric anthocyanins were estimated by the method suggested by Zheng and Tian, (20). Tannins content was assayed by Folis-Denis reagent method suggested by Sadasivam and Manickam, (16). Polyphenol oxidases (PPO) were calculated by using Zauberman *et al.,* (19) method. Peroxidase (POD) was analyzed via exploring Shannon *et al.* (17) method. Phenylalanine ammonia lyase (PAL) was estimated by using Burrell and Rees, (4) method. The experiment was arranged using a Randomized Complete Block Design, with each treatment replicated three times. The data were analyzed for a variance by using Statistix 10 software. The treatments means were subjected to mean separation by Least Significant Difference (LSD, $p \leq 0.05$). To analyze the relationship among the attributes data were subjected to Pearson's correlation coefficient and regression analysis.

RESULTS AND DISCUSSION

The seeds of the grape genotypes under consideration had phenol content ranging from 8.69 to 18.76 mg/g equivalent to gallic acid and flavonoids from 6.29 to 13.03 mg/g FW (Table 2). The grape genotype Punjab MACS Purple (17.55, 13.03 mg/g) followed by H-27 (16.86, 12.60 mg/g) had the highest total phenol and flavonoids content, respectively. While, the lowest total phenol and flavonoid content was recorded in the seeds of the Cardinal (8.69, 6.45 mg/g). Most likely, the colour of the grape genotypes significantly influences the total phenolic content. The total phenol in coloured genotypes was recorded higher than that of the white genotypes in general, because of the inability

Table 2: Total phenols, flavonoids and tannins in grapes seeds of different genotypes.

Genotype	Pooled data		
	Total phenols (mg GAE/g FW)	Total flavonoids (mg/g FW)	Tannins (mg/g DW)
B-27-1	$9.27^{de} \pm 0.28$	$6.44^{c} \pm 0.20$	$2.36^{f} \pm 0.06$
Italia	$11.82^{cd} \pm 0.34$	$8.31^{bc} \pm 0.13$	$4.88^{a} \pm 0.17$
Sauvignon Blanc	$9.69^{de} \pm 0.26$	$6.70^{c} \pm 0.09$	$4.57^{ab} \pm 0.14$
Cardinal	$8.69^{e} \pm 0.34$	$6.28^{c} \pm 0.20$	$2.78^{ef} \pm 0.09$
Carolina Black Rose	$11.04^{cde} \pm 0.38$	$8.20^{bc} \pm 0.15$	$3.37^{cde} \pm 0.11$
Chasan-B	$12.95^{c} \pm 0.43$	$9.48^{b} \pm 0.16$	$3.29^{de} \pm 0.10$
H-144	$13.31^{c} \pm 0.30$	$9.74^{b} \pm 0.24$	$3.94^{bcd} \pm 0.13$
H-27	$16.86^{ab} \pm 0.28$	$12.60^{a} \pm 0.35$	$4.07^{bc} \pm 0.13$
Kyoho	$9.16^{de} \pm 0.20$	$6.53^{c} \pm 0.22$	$4.64^{ab} \pm 0.16$
Muscat Hamburg	$13.85^{bc} \pm 0.33$	$9.05^{b} \pm 0.19$	$3.66^{cd} \pm 0.14$
Punjab MACS Purple	$17.55^{a} \pm 0.44$	$12.55^{a} \pm 0.24$	$3.56^{cd} \pm 0.12$
Pusa Navrang	$18.76^{a} \pm 0.30$	$13.03^{a} \pm 0.32$	$3.24^{de} \pm 0.08$
CD at p<0.05	3.07	2.23	0.73

of the white genotypes to synthesize anthocyanins (Du *et al.,* 6).

Tannins are a subclass of phenolic compounds and contribute to wine colour and mouth feel. The results obtained on the content of tannins in the seeds indicated a range between 2.36 to 4.88 mg/g DW (Table 2). White seeded genotype, Italia (4.88 mg/g DW) and coloured seeded genotype Kyoho (4.64 mg/g DW) had highest tannins content among the evaluated genotypes. Sauvignon Blanc had insignificant differences with Kyoho and Italia. Other coloured seeded genotypes such as Cardinal (2.78 mg/g DW), Carolina Black Rose (3.37 mg/g DW), Chasan-B (3.29 mg/g DW) and Muscat Hamburg (3.67 mg/g DW) had total tannins level below the average 3.93 mg/g DW, which were obtained from three white seeded genotypes. On average basis, white seeded grape (3.93 mg/g DW) was superior over the coloured seeded group (3.62 mg/g DW) for tannins content. Similar results were obtained by Ortega *et al.,* (14) they recorded the highest tannins in Merlot (2139.3 mg/kg) followed by Cabernet-Sauvignon (2031 mg/kg) grapes seed extract.

Total antioxidants and OH scavenging activity in the grape seeds had abroad range, depending on the grape genotype (Table 3). The data showed that the highest content of total antioxidants (76.21%) and OH scavenging activity (57.29 %) was observed in the seeds of Pusa Navrang genotype. Least antioxidants and OH scavenging activity were detected in seeds of genotype B-27-1 and Sauvignon Blanc. As per genotype studies, it was found that the coloured grapes had better capability for antioxidants and OH scavenging activities than white seeded grapes. Higher antioxidants and OH scavenging activity of coloured genotypes is associated with the higher phenolic contents as the phenols are known to have strong antioxidant potential and *detoxification of* hydroxyl radicals (ROS). Similar observations were recorded by Hassanpour and Khoshamad, (9) they recorded remarkable (DPPH) radical scavenging activity from 27.34 to 78.57 per cent in 20 wild grape accessions. Karasu *et al.,* (10) recorded antioxidant activity (DPPH radical-scavenging capacity) from seed extracts of five grape genotypes, which ranged from 86.25 to 94.47 per cent.

Anthocyanins function as the red-coloured phenols which are unstable in grapes and become stabilized when reacts with tannins. Among all the evaluated grape genotypes, significantly highest anthocyanins (2.15 mg/100 g FW) were recorded in the seed of Pusa Navrang (Table 3). Seed extracts of three white seeded genotypes (B-27-1, Italia, Sauvignon Blanc) and Kyoho, the coloured seeded genotype showed unavailability of anthocyanins in their seeds. The highest total anthocyanins recorded in Pusa Navrang and H-27 might be attributed to the teinturier character of these grape genotypes, whose pulp and juice are red due to anthocyanin accumulation. From the limited literature, similar results were found by Hao *et al.* (8) where seeds of grape berry consisting of 1.5-8.5 mg/100 g of total anthocyanins.

Table 3: Antioxidant activity, total monomeric anthocyanin and OH radicals scavenging activity in grape seeds of different genotypes.

Genotype	Pooled data		
	Total antioxidant activity (DPPH %)	Total monomeric anthocyanins (mg/100 g FW)	Hydroxyl radicals scavenging activity (%)
B-27-1	$63.89^{f} \pm 0.65$	$0.00^{d} \pm 0.00$	$34.85^{g} \pm 0.46$
Italia	$70.48^{bcde} \pm 0.30$	$0.00^{d} \pm 0.00$	$45.23^{de} \pm 0.56$
Sauvignon Blanc	$64.92^{ef} \pm 0.66$	$0.00^{d} \pm 0.00$	$35.82^{g} \pm 0.48$
Cardinal	$65.56^{def} \pm 0.67$	$0.44^{cd} \pm 0.04$	$41.45^{ef} \pm 0.62$
Carolina Black Rose	$67.58^{cdef} \pm 0.57$	$0.79^{c} \pm 0.03$	$42.12^{ef} \pm 0.55$
Chasan-B	$72.44^{abc} \pm 0.46$	$1.53^{b} \pm 0.04$	$48.03^{cd} \pm 0.35$
H-144	$73.06^{abc} \pm 0.61$	$1.55^{b} \pm 0.06$	$46.41^{d} \pm 0.48$
H-27	$75.05^{ab} \pm 0.48$	$1.82^{ab} \pm 0.08$	$51.71^{bc} \pm 0.32$
Kyoho	$68.13^{cdef} \pm 0.66$	$0.00^{d} \pm 0.00$	$40.38^{f} \pm 0.43$
Muscat Hamburg	$71.03^{abcd} \pm 0.55$	$1.42^{b} \pm 0.05$	$46.66^{d} \pm 0.29$
Punjab MACS Purple	$74.62^{ab} \pm 0.63$	$1.62^{ab} \pm 0.06$	$54.80^{ab} \pm 0.36$
Pusa Navrang	$76.21^{a} \pm 0.52$	$2.15^{a} \pm 0.07$	$57.29^{a} \pm 0.38$
CD at p<0.05	5.71	0.60	4.15

The enzymatic browning reaction, which involves the oxidation of phenolic compounds, is driven by polyphenol oxidase. This reaction plays a crucial role in fruit processing, as it leads to the formation of undesirable color, flavor changes, and nutrient loss. Meanwhile, peroxidase facilitates the breakdown of hydrogen peroxide by utilizing electron acceptors and reduces the ROS load. All the evaluated seed extracts showed that PPO activity differed significantly depending on the genotype and grape colour (Table 4). The comparatives value of PPO in the seeds was evaluated. Sauvignon Blanc grapes had the highest activity (2.02 U/min/g FW), followed by Carolina Black Rose (1.97 U/min/g FW). In contrast minimum polyphenol oxidase activity were recorded in Pusa Navrang (0.64 U/min/g FW) and H-27 (0.87 U/min/g FW). Similarly, the highest peroxidase activity was recorded in genotype Kyoho (2.20 U/min/g FW) as compared to other genotypes, however, Kyoho was statistically at par with B-27-1, Italia, Cardinal, Carolina Black Rose, Chasan-B and H-144 genotypes. The minimum peroxidase activity was observed in Muscat Hamburg (1.39 U/min/g FW), which had insignificant, results with Punjab MACS Purple (1.58 U/min/g FW), Pusa Navrang (1.62 U/min/g FW) and Sauvignon Blanc (1.71 U/min/g FW). The white peel grapes had relatively high PPO and POD activity than coloured genotypes. The polyphenol oxidase and peroxidase activity in grape peel and pulp extract were searched from literature, but noticeable literature on grape seed enzymatic activity could not be found.

PAL is a key plant enzyme that removes ammonia from phenylalanine, converting it into trans-cinnamic acid, a precursor for the synthesis of lignins, flavonoids, and coumarins. The PAL values of the samples varied from 3.50 to 7.51 U/g FW and as expected, the highest PAL value 7.51 U/g FW was obtained in Pusa Navrang, followed by H-27 with 6.47 U/g FW (Table 4). Similarly, the lowest PAL value was recorded in B-27-1 (3.50 U/g FW), followed by Cardinal (3.84 U/g FW). Its activity was prominently higher in the coloured than white peel grapes. PAL values of the grape genotypes differed significantly. Kataoka *et al.,* (11) recorded phenylalanine ammonia lyase activity within the range of 0.05-0.20 moles of t-cin-namic acid/g/FW/hour from veraison to harvesting phase in seeds of three grape genotypes. They also found higher phenylalanine ammonia lyase activity in the peel than seed.

There was a significant correlation among all of the assessed components and the antioxidant activity (Table 5). A positive correlation was recorded of total phenols with flavonoids (0.941), anthocyanins (0.863), hydroxyl radicals scavenging activity (0.815) and total antioxidant activity (0.863), while polyphenol oxidase (-0.632) and peroxidase activity (-0.490) was negatively correlated. Phenylalanine ammonia lyase activity had an indirect correlation with antioxidants and hydroxyl radical's activity as they are positively correlated with total phenols. Correlation studies among the enzymatic activity indicated a positive correlation between polyphenol oxidase

Table 4: Enzyme activity in grape seeds of different genotypes.

Genotype	Pooled data		
	Polyphenol oxidase (U/min/g FW)	Peroxidase (U/min/g FW)	Phenylalanine ammonia lyase (U/g FW)
B-27-1	$1.66^{abc} \pm 0.09$	$2.03^{a} \pm 0.12$	$3.50^{e} \pm 0.28$
Italia	$1.83^{a} \pm 0.09$	$2.10^{a} \pm 0.08$	$4.76^{de} \pm 0.26$
Sauvignon Blanc	$2.02^{a} \pm 0.06$	$1.71^{ab} \pm 0.11$	$4.25^{de} \pm 0.29$
Cardinal	$1.71^{ab} \pm 0.12$	$2.05^{a} \pm 0.09$	$3.84^{e} \pm 0.19$
Carolina Black Rose	$1.98^{a} \pm 0.10$	$2.01^{ab} \pm 0.10$	$4.80^{cde} \pm 0.25$
Chasan-B	$1.33^{bcd} \pm 0.11$	$2.13^{a} \pm 0.13$	$6.33^{ab} \pm 0.20$
H-144	$1.65^{abc} \pm 0.09$	$1.94^{ab} \pm 0.10$	$4.21^{de} \pm 0.23$
H-27	$0.87^{de} \pm 0.09$	$1.81^{ab} \pm 0.14$	$6.47^{ab} \pm 0.22$
Kyoho	$1.61^{abc} \pm 0.07$	$2.20^{a} \pm 0.12$	$5.30^{bcd} \pm 0.15$
Muscat Hamburg	$1.58^{abc} \pm 0.11$	$1.39^{b} \pm 0.12$	$4.07^{de} \pm 0.15$
Punjab MACS Purple	$1.21^{cd} \pm 0.07$	$1.58^{ab} \pm 0.07$	$6.24^{abc} \pm 0.26$
Pusa Navrang	$0.64^{e} \pm 0.07$	$1.62^{ab} \pm 0.09$	$7.51^{a} \pm 0.16$
CD at p<0.05	0.46	0.63	1.45

Table 5: Correlation analysis of different enzymes and nutraceuticals parameters.

	POD	PAL	SN***	SH****	TP	TF	TAN	TMA	OH	TAA
PAL	0.044									
SN	0.267*	-0.204								
SH	-0.128	-0.057	-0.030							
TP	-0.490**	0.538**	-0.122	0.029						
TF	-0.331**	0.557**	-0.022	0.029	0.941**					
TAN	0.159	0.167	-0.096	-0.427**	-0.104	-0.119				
TMA	-0.366**	0.583**	0.010	0.124	0.774**	0.801**	-0.090			
OH	-0.258*	0.676**	-0.165	0.067	0.815**	0.835**	-0.008	0.772**		
TAA	-0.342**	0.533**	-0.038	-0.018	0.863**	0.836**	0.071	0.743**	0.792**	
PPO	0.331**	-0.375**	0.415**	-0.060	-0.632**	-0.640**	0.445**	-0.443**	-0.606**	-0.470**

*Significant at p<0.05, **Significant at p<0.01, ***Seed Number per berry, ****Seed Hardness (kg/cm^{2})s

and peroxidase (0.331). While polyphenol oxidase and phenylalanine ammonia-lyase enzymes had a negative correlation (-0.375) possibly as phenylalanine ammonia-lyase enzyme enhances the biosynthesis of phenols and phenols restrict the polyphenol oxidase activity. Correlation studies revealed that the total antioxidant activity was positively correlated with all the parameters except of polyphenol oxidase (-0.470), peroxidase (-0.342), seed number (r = -0.038, p < 0.05) and seed hardness (r = -0.018, p < 0.05). Hydroxyl radicals scavenging activity was found to have a maximum significant positive correlation n (0.835) with flavonoids and strongest negative association (-0.640) with polyphenol oxidase activity.

The principal component analysis (PCA) effectively distinguished the variables in the factorial space. Each variable within a given principal component (PC) had an associated Eigenvector weight or factor loading value (Table 6). The most heavily weighted variables were those with the highest factor loading values within a specific PC. The first five principal components (PC_1-PC_1) accounted for 90.67% of the variability in the dataset. The bold-face values for PPO, PAL, Total Phenols, Flavonoids, Anthocyanin, OH radical scavenging activity and AO activity were the highly weighted Eigenvectors in PC_1, seed hardness and tannins were highly weighted Eigenvectors in PC_2. The high

Table 6: Factor loading and Eigenvector of principal component analysis (PCA) explaining variance in data-set.

Principal Components (PCs)	PC_1	PC_2	PC_3	PC_4	PC_5
Eigen value	5.333	1.673	1.236	0.948	0.784
% of Variance	48.481	15.205	11.239	8.614	7.129
Cumulative %	48.481	63.686	74.925	83.539	90.668
Factor loading/Eigenvector variables					
POD	-0.442	0.398	0.285	0.683	-0.164
PPO	-0.712	0.367	0.307	-0.216	0.391
PAL	0.666	0.391	-0.064	0.469	0.130
SN	-0.197	0.133	0.918	-0.184	-0.173
SH	0.075	-0.665	0.245	0.262	0.629
TP	0.950	-0.003	0.040	-0.163	-0.045
TF	0.940	0.039	0.159	-0.039	-0.107
TAN	-0.120	0.843	-0.241	-0.191	0.347
TMA	0.860	0.045	0.231	-0.050	0.149
OH	0.912	0.124	0.027	0.142	0.059
TAA	0.878	0.192	0.123	-0.157	0.048

weighted Eigenvectors in all PCs were considered in the MDSs. PC1, which explained the greatest variance, had a positive factor loading of 0.950 for total phenols and a negative factor loading of -0.712 for PPO. PC2, which accounted for the second-largest variance, showed the highest positive loading value of 0.843 for tannins and a negative loading of -0.665 for seed hardness.

AUTHOR'S CONTRIBUTION

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DECLARATION

Regarding this article there are no conflicts of interest.

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