Biochemical characterization of grape germplasm from Leh grown under tropical condition of India

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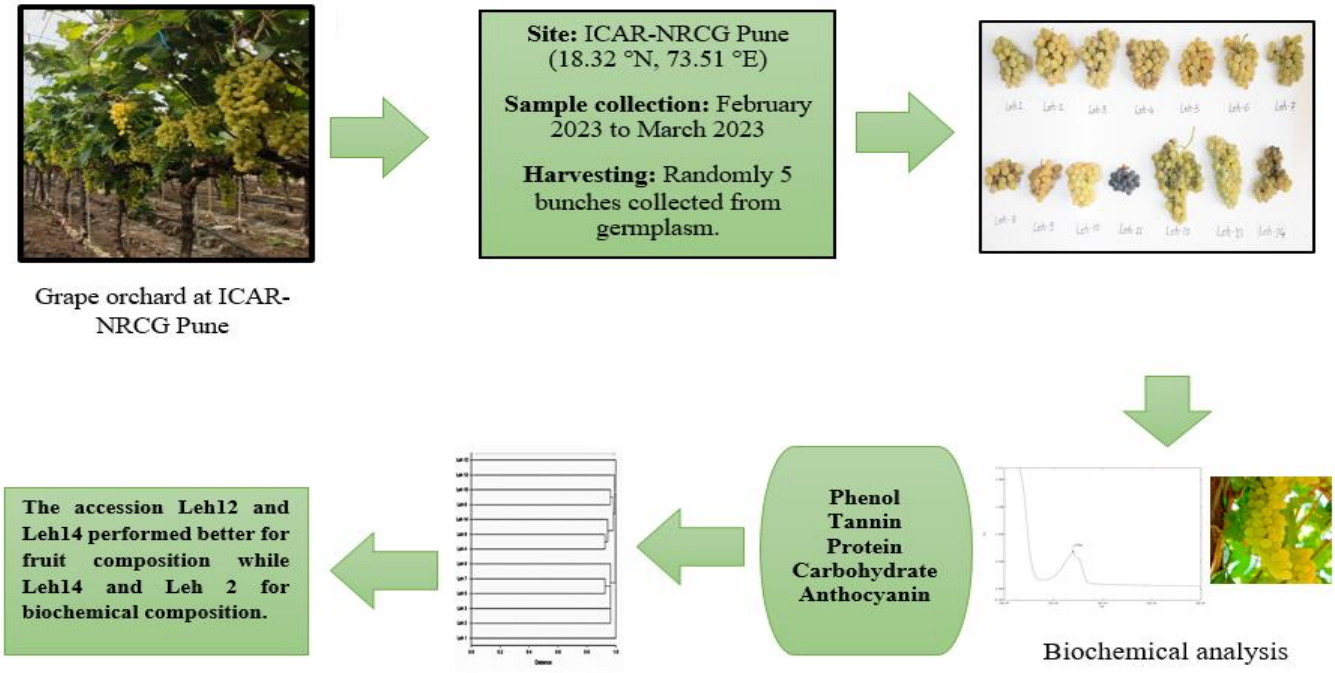
Abstract

Thirteen grape germplasm collected from local region of Leh district of Jammu and Kashmir were evaluated for physicochemical and biochemical characters for its suitability under tropical climate of India which has functional and nutritional value. Data on various vegetative, fruit physical and fruit chemical characters were recorded in the present investigation. Significant variations were observed for all the characters studied. Among the different accessions, Leh12 showed maximum average bunch weight (504.37g) and number of berries/bunch (126.30) followed by Leh14 (398.50g and 85.70 respectively), while highest 50 berry weight (192.53g), berry diameter (17.63 mm) and total soluble solid (22.63°Brix) was recorded in Leh14. The accession Leh12 had maximum acidity (0.83%) while maximum juice pH was recorded in Leh8. Maximum total phenol (7.44 mg/g), tannin content (9.01mg/g) and antioxidant activity (127.37 %) were observed in Leh14 while protein content (3.94mg/g), reducing sugar (266.25 mg/g) and carbohydrate (94.75mg/g) were highest in Leh2. Cluster analysis performed on the basis of yield parameters and biochemical attributes divided into two main groups. Group 1 was consisted of seven germplasm while six genotypes in group 2. All phenological, physical and biochemical studies showed useful attributes for future grapes improvement programs.

Keywords: Grape germplasm, Leh, biochemical, grapes.



Graphical abstract

Introduction

Grapevines (*Vitis vinifera* L.) is basically originated from the temperate regions. Varietal adaptability and technological interventions facilitated successful grape growing in all three distinct agro-climatic zones of mild humid tropical region, sub-tropical region, and dry temperate region. The area under grape cultivation in India is on 162.2 thousand hectares with production of 34.77 lakh MT. In the temperate region of Jammu and Kashmir, grapes are grown on an area of 318 hectare and produces 1734 MT (NHB 2023). Landraces, improved varieties, hybrids, and wild relatives comprises the wide range of grape variety that must be conserved to provide rich genetic variability for various breeding purposes (Dolkar *et al.* 2018). The current genetic pool must be evaluated for its diversity in order to be preserve and use effectively in the future. This can be done by identifying and differentiating grape accessions and determining the genetic relationships between local cultivars and wild relatives (Negrul, 1973). Early grape introduction occurred in the Leh district of Jammu and Kashmir, and small-scale plantings were established in the Leh area in the early century (Angcok *et al.* 2009). The maximum area under grape is in lower belt of Leh district. Traders, nomads, and invaders from Yarkand, Baltistan, Punjab, Kashmir, China, and Tibet used to pass through Ladakh in the past when it served as a transit station on the Central

Asian trade route (Jolden, 2012). During that period the traders might have disseminated or introduced the germplasm of grapes in Ladakh from where its cultivation had been started in lower belt of Leh district, which has a milder climate. In major parts of the grape growing regions of the country, grapes are harvested in February - May; however, in the Ladakh region, harvesting occurs in August -September, which is considered as off-season for the rest of the country and can result in higher prices even with low productivity (Dolkar *et al.* 2017).

Selection and domestication of superior genotypes of a particular fruit crop from existing genetic variability is essential to increase its adoption, production, and diversification for domestic consumption as well as for commercial purpose, which would lead to increase in farmer's income. For the effective use of genetic resources, information on genetic variation among plant species is essential. The grape variety grown in one region may not perform same in another region. Hence, evaluation for specific objectives in different regions becomes important. Keeping in view the importance of grape germplasm for improvement of grape, an attempt was made to evaluate the grape accessions collected from Leh under tropical condition in present investigation.

Materials and methods

The study was conducted during the year 2022-23 and 2023-24 at the experimental block of ICAR-National Research Centre for Grapes, Pune (India). Pune is located at 559 m above sea level in the Maharashtra State of India's Midwest (18.32°N and 73.51°E). Six-year-old vines of grape germplasm collected from Leh and grafted on Dogridge rootstock were selected for the study. These vines were spaced at 9 feet between rows and 4 feet between vines, drip irrigated as per the irrigation schedule developed for this region. The experiment was laid out in a randomized block design with five replications. Since the region falls under tropical condition, double pruning and single cropping is followed. The vines were pruned twice in year, once after the harvest of the previous crop during April to promote growth of canes and shoot popularly known as back pruning. Second pruning was done for fruit i.e. forward pruning after 5 to 6 months in October to encourage cluster development. The grapes were harvested when they attained total soluble solid (TSS) of more than 18 °Brix. Five randomly selected bunches were used to record average bunch weight. Fifty berries were collected from different part of bunches for recording data on 50 berry weight. A random berry sample were drawn from bunches for analysis for fruit quality parameters like TSS, acidity, juice pH and berry diameter. These sample were also used for analysis of biochemical

parameters like total phenol, tannin content, reducing sugar, total protein, proline content, flavonoid, anthocyanin, and antioxidants.

Reagents and chemicals

The standard reference chemicals viz., (+) catechol (98% purity), gallic acid (98% purity), L- Proline, bovine serum (fraction v) albumin, glucose, ascorbic acid was obtained from HI media Laboratories Pvt. Ltd. (Mumbai,India). All other solvents and chemicals used in this study were of HPLC grade and purchased from Merck Ltd. (Mumbai, India).

Fruit quality parameter

Fresh berry samples were used for the analysis of fruit quality parameters such as berry diameter, total soluble solid (°Brix), titratable acidity, juice pH, etc. Berry diameter was measured by using vernier caliper (ACCUPLUS, 3M-300). Total soluble solids were measured using hand refractometer (ERMA ehb-32 Manual Handheld Refractometer, 0-32% Brix Refractometer), while titratable acidity was measured by titrating a known volume of juice with 0.1N NaOH using phenolphthalein as indicator.

Sample preparation and analysis

A 0.5 g of crushed berry sample was lyophilized in three replications and each were extracted by overnight shaking at room temperature on a mechanical shaker (Scigenics Orbitek, RPM 250) for uniform mixing of sample. To extract polyphenols more effectively, 80% aqueous methanol was utilized as the solvent (Bonilla *et al*. 2003). The extract was centrifuged at 5000 rpm for 5 minutes and supernatant was collected. The residue was re-extracted twice under similar conditions. Finally, supernatant was combined and stored at 0°C until further analysis.

Estimation of biochemical

Total phenolic content in the grape sample was determined by Folin- Ciocalteu method (Singletonand Rossi, 1965) using gallic acid as standard. The concentration of total phenolics was expressed as gallic acid equivalent (GAE mg g- ^{1}) of crushed sample. The tannins were determined by Folin - Ciocalteu method using tannic acid as the standard and was expressed in mg/g of extract. Protein content was estimated according to method described by Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard and total protein concentration was

expressed as mg/g. Proline was determined using method given by Bates et al. (1973). The concentration of proline was determined using the L- proline standard curve and expressed as mg/g. Dinitrosalicyclic acid (DNS) method using glucose as a standard was used to estimate the reducing sugar content and expressed as mg/g fresh weight. Total carbohydrate was determined using Anthrone method with D glucose (Sadasivam and Manikam, 1996) and was expressed as mg/g fresh berry weight.

Statistical analysis

The results were analyzed using statistical software SAS (Version 9.1.3, service pack 3, SAS Institute, Cary, NC, USA) and the comparison of treatment means was done using least square difference at $p < 0.005$. Cluster analysis was recorded using PAST software.

Result and Discussion

The data on yield and fruit composition parameters of grape germplasm collected from Leh grafted on Dogridge rootstocks are presented in Table 1. All the germplasm grown under Pune condition showed significant variations in bunch and berry characters (Fig 1 and 2). Significant differences were recorded for average bunch weight, number of berries per bunch, 50 berry weight, berry diameter, TSS, acidity and juice pH in all studied germplasm. Among the different accessions studied, average bunch weight ranged from 103.53 to 504.37 g. Highest bunch weight was recorded in Leh12 (504.37 g) followed by Leh14 (398.50g) while it was lowest in Leh1 (103.53g). Bunch weight is an important yield parameter. The grape yield per vine increased with the increase in bunch number and bunch weight. Differences in bunch weight in different varieties can be attributed to the inherent genetic characteristics of the variety, differences in the number of canes, number of berries per bunch and berry size, as well as differences in the canopy size of the vine (Walker *et al.* 2000; Havinal *et al.* 2008). Earlier reports suggested high variation for bunch weight ranging from 66.84 g to 233.2 g in different cultivars (Joshi *et al.* 2015). In Thompson Seedless, Jogaiah *et al.* (2014) measured bunch weights ranging from 175 to 229 g. High variation was observed among Leh germplasm for number of berries per bunch. The accession Leh12 had highest number of berries per bunch (126.30) while lowest in Leh1 (35.70). The difference in the number of berries per bunch can be attributed to differences in the size and diameter of the berry (Ratnacharyulu, 2010). The mean 50 berry weight was maximum (192.53 g) in Leh14 while minimum in Leh 1 (66.17 gm). Wide range of hundred berry weight was reported by several

workers, 106 to 403g (Ratnacharyulu 2010); 130 to 480 g (Thakur et al. 2008); 150 to 300 g (Ghosh *et al.* 2008). Significant variation was recorded in berry diameter. Highest berry diameter was recorded in Leh14 (17.63 mm), while lowest in Leh2 (13.80 mm). The high berry diameter might be due to a lower number of berries in a bunch, whereas a higher number of berries may lead to a lower diameter of the grape berries in the bunch. Total soluble solids (TSS) varied from 17.0 to 22.63 °Brix while the acidity from 0.64 to 0.83%. In most cases, less acidic varieties are preferred in grapes for table purpose. The acidity of grapes varies according to climatic conditions, genotypes and cultural practices. Under tropical condition during ripening stage, temperature increases that helps to increase the sugar and reducing the acids. Yinshan *et al.,* (2017) reported that sugars and acids of grapes are greatly influenced by cultural practices, soil topography, climatic as well as by environmental conditions. There is great variation occur in different regions which is called the interaction of genotype and environment. So, it is desirable to find out the variation and changes in sugars and organic acids of varieties grown in the same region. De Orduna, (2010) found large difference between the grapes harvested from cool regions compared to warm regions. Under warm climate, grapes mature earlier and acid reserves convert to sugars earlier compared to temperate regions so the growers have to wait less to get early crop. The grape juice pH is also considered an important factor for flavor and resistance to spoilage (Amerine, 1965). In our finding, grape juice pH ranged between 3.25 to 3.78. These results are harmony with scientists Soltekin *et al.,* (2015) and Somkuwar et al., (2020) who evaluated different grape cultivars and found pH of most varieties above 4. The pH of a fresh grape is always acidic because the water content of the grapes evaporates during the drying process, leading to increased acidity (Somkuwar *et al.*, 2020).

Table 1. Fruit composition and quality parameters of Leh germplasm grafted on Dogridge rootstocks.

Leh accession	Average bunch weight (g)	No. of berry per bunch	50 berry weight (g)	Berry diameter (mm)	TSS (°Brix)	Acidity (%)	Juice pH
Leh 1	103.53	35.70	66.17	15.40	22.50	0.77	3.43
Leh 2	132.60	42.00	148.10	13.80	18.20	0.81	3.32
Leh 3	157.60	64.30	127.87	16.47	20.53	0.66	3.46

Leh 4	255.77	68.20	183.43	16.10	22.47	0.69	3.51
Leh 5	196.77	51.30	170.90	17.17	20.03	0.72	3.52
Leh 6	189.87	46.70	128.17	16.53	21.13	0.73	3.52
Leh 7	136.80	62.00	144.83	17.33	20.07	0.72	3.44
Leh 8	255.80	40.00	137.63	17.10	22.40	0.64	3.74
Leh 9	109.43	55.00	129.27	16.53	20.17	0.69	3.68
Leh 10	223.27	57.00	107.63	16.20	18.80	0.73	3.30
Leh 12	504.37	126.30	183.93	16.77	17.97	0.83	3.25
Leh 13	237.60	68.30	157.03	15.33	19.90	0.70	3.47
Leh 14	398.50	85.70	192.53	17.63	22.63	0.73	3.42
LSD(p=0.05)	8.15	6.03	8.64	1.16	1.11	0.03	0.08

The data on biochemical constituents of fresh grape sample is presented in Table 2. Among the different germplasm studied, total phenol, tannin and anthocyanin content ranged from 2.71 to 7.44 mg/g fresh weight; 3.72 to 9.01 mg/g fresh weight; 3.52 to 24.66 mg/l, respectively. The accession Leh 14 recorded highest phenol content of 7.44 mg/ g, while Leh 2 recorded the lowest concentration of 2.71 mg/g. Similar trend was also recoded for tannins where maximum tannins were recorded in Leh 14 (9.01 mg/g) while minimum in Leh 2 (3.72 mg/g). Grapes are rich in polyphenols, anthocyanins, phenolic acids, and ascorbic acid (Prakash *et al.,* 2020). A large extent of biochemical diversity was also observed in the genotypes collected from Leh region. Total phenolics are the main constituents of fruits and have antioxidant potential and are beneficial to human health. The production of secondary metabolites depends on genotypes and environmental effects. Therefore, the total phenolic content may vary according to the geographical location (Prakash *et al.,* 2020). The total phenol content of grapes also varies according to the year and variety and decreases during the ripening period (Polat *et al.,* 2022). The accumulation of more phenol in the vine parts might also contribute to the development of resistance against pests and diseases (Somkuwar et al., 2013). The formation of anthocyanins in the berry skin at the beginning of verasion, especially in red grape cultivars, is one of the most important characteristics of berry growth and development (Balik *et al.,* 2013). The total anthocyanin content ranged from 3.53 to 24.66 mg/ L in all grape accessions studied. The results obtained in the present study for matured fruits are within the range of earlier studies in grape cultivars reported by Polat *et al.,* (2022). Grape

varieties with higher amounts of phenolics, flavonoids, and anthocyanin compounds exhibit potent antioxidative activities at full berry maturation under biotic and abiotic stress conditions (Prakash *et al.,* 2020). A positive correlation observed between total phenolics, anthocyanin content, and antioxidant activities. Anthocyanin plays an antioxidant role against reactive oxygen species (ROS), leading to the formation of melanin-like pigments during ripening in white grapes (Rustioni *et al.,* 2015). The grape varieties showed a variation in DPPH activity ranged from 18.24 to 124.37 % at full maturity stage. However, there was difference in antioxidant activity observed in all grape varieties which may be due to the high accumulation of phenolic acids and various flavonoids at different developmental stages of grape berries (Ignat *et al.*, 2011).

Proline is one of the major important amino acid that plays a crucial role in the physiological activities, including normal growth, flower bud differentiation and stress responses. In the present study, maximum proline content was reported in Leh 10 (12.74 mg/g) while minimum in Leh 3 (2.79 mg/g). Proline content in grapevine improves abiotic stress tolerance for plants (Wie *et al.,* 2022). Significant differences were recorded for protein content in fresh grape of Leh accession. Higher amount of protein in fresh grape was estimated in Leh2 (3.94 mg/g) followed by Leh 4 (3.54 mg/g) and Leh 14 (3.45 mg/g) whereas minimum protein was estimated in Leh 8 (1.24 mg/g). The present results are in accordance with the result obtained by Somkuwar *et al.,* 2013) who reported variation in berry protein of Thompson Seedless due to genetical variation. Significant differences were recorded for carbohydrate content in grape berries. Higher amount of carbohydrate was recorded in Leh 2 (94.75 mg/g) followed by Leh 14 (86. 32 mg /g). However, the lowest carbohydrate was recorded in Leh 9 (21.42 mg /g). Similar trend was observed for reducing sugar in fresh grape of Leh accessions. At harvesting time, reducing sugar content in grape berries increased due to carbohydrate get converted in reducing sugar (Somkuwar *et al.,* 2014). Sugar accumulation can vary with genotype and environment (Kuhn *et al.,* 2013).

Table 2. Biochemical parameters of fresh grape of different Leh accession grafted on Dogridge rootstock.

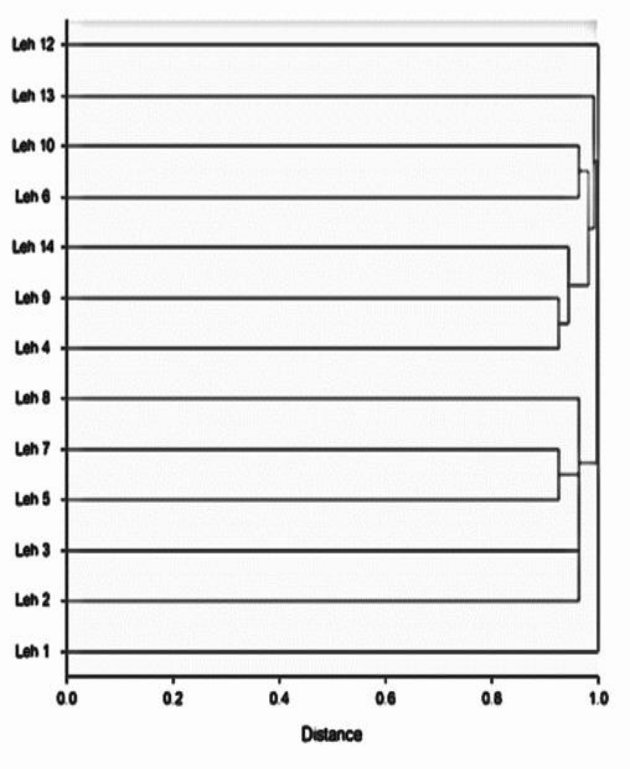
Leh accession	Phenol mg/g	Tannin mg/g	Proline mg/g	Protein mg/g	Reducing sugar mg/g	Carbohydrate mg/g	Anthocyanin mg/L	DPPH Antioxidant activity (%)
Leh 1	4.63	5.77	5.68	2.03	245.50	60.32	17.62	82.35
Leh 2	2.71	3.72	10.33	3.94	266.25	94.75	14.09	107.31

Leh 3	3.48	4.17	2.79	1.69	215.67	83.58	14.09	18.24
Leh 4	4.84	5.56	7.86	3.54	251.83	75.03	7.05	60.13
Leh 5	3.79	4.69	12.19	1.89	208.75	80.73	14.09	106.93
Leh 6	4.57	4.97	11.56	2.28	193.67	75.07	3.52	56.53
Leh 7	4.45	5.49	11.24	2.11	212.58	78.78	14.09	36.64
Leh 8	4.40	5.29	5.38	1.24	195.67	80.60	14.09	93.01
Leh 9	4.05	5.06	11.53	2.27	190.00	21.42	7.05	75.23
Leh 10	5.74	6.96	12.74	1.74	197.67	75.91	21.14	66.11
Leh 12	3.40	4.00	3.12	2.17	204.25	53.95	24.66	106.16
Leh 13	5.43	6.56	6.25	1.75	220.97	86.27	21.14	49.87
Leh 14	7.44	9.01	7.72	3.45	205.58	86.32	7.05	124.37
LSD (p=0.05)	0.67	0.55	1.33	0.62	22.70	17.59	13.94	19.40

Fig 1. Variation for bunch characteristics in Leh germplasm.

Fig 2. Variation for berry characteristics in Leh germplasm

Cluster analysis



The Dendrogram was developed on the basis of yield parameters and biochemical attributes recorded in the present study (Fig. 1). Based on yield parameters and biochemical attributes, it divided in germplasm in two main groups. In group A, there were seven accessions (Leh12, Leh13, Leh14, Leh10, Leh6, Leh9, Leh4) which have maximum resemblance within group on the basis of biochemical characteristics and have least resemblance or with other group B.

Fig.3. Dendrogram on the base of physicochemical and biochemical attributes showing relation between germplasm grown under tropical climate of India

Conclusion

The grape germplasm collected from Leh region possesses many potentially informative and useful fruit quality parameters. In addition, regional groups of Leh accessions will have practical applications in selection of parental stocks for breeding and in establishing a core germplasm collection. Concise and informative collection of accessions with minimal complexity is the goal of the core collection, which aims to incorporate the genetic diversity of the cultivated species. In the present study, the accession Leh12 and Leh14 performed better for fruit composition while Leh14 and Leh 2 for biochemical composition. The germplasm investigated in the present study may constitute a useful core collection of table grapes for future breeding and evaluative work.

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