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Original Research Paper

Antioxidant profiling in bael (*Aegle marmelos* Correa.) genotypes

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ABSTRACT

The present investigation was undertaken to characterize the non-enzymatic and enzymatic antioxidant activities among 18 bael genotypes. Amongst the bael genotypes, highest marmelosin was observed in 'Pant Bael-1', at par with 'Pant Bael-15' and 'Pant Bael-14'. The maximum enzymatic antioxidant activity of SOD and CAT was obtained in 'Pant Shivani', while, maximum POD enzyme activity was recorded in 'Pant Bael-1'. The maximum DPPH radical scavenging activity was observed from 'Pant Shivani'. The correlation study showed positive correlation of non-enzymatic antioxidant content and enzymatic antioxidant activity with DPPH free radical scavenging activity (%). Principal component analysis revealed that out of nine principal components axis, four had eigen values greater than one and all together account for 77.22 of the total variability.

Keywords: Antioxidants, bael, marmelosin

INTRODUCTION

Bael (*Aegle marmelos* Correa.) belongs to monotypic genus *Aegle* and family Rutaceae. All parts of the tree are used ethnomedicine for the cure of various human diseases. The fruits are good source of vitamins, minerals, dietary fibers, proteins and carbohydrates. Besides the nutritional importance, the fruits are highly reputed for their use in the preparation of a number of indigenous traditional ayurvedic medicine. The importance of bael as medicinal plant has also been emphasized by World Health Organization. A number of therapeutic products like squash, candy, powder, marmalade, jelly etc. are made from bael fruit pulp after its processing. The nutritive and medicinal properties together with its hardy nature make bael an ideal fruit for future.

Among the important pharmaceutical properties of bael fruits its antioxidant properties hold a prominent place. The antioxidants are the substances that protect our cells from oxidative stress and inflammation. In our body, free radicals are generated during normal biochemical reactions, increased exposure to the environment, and due to higher levels of dietary xenobiotics (Lobo et al., 2010). Free radicals adversely alter lipids, proteins, and DNA and trigger a number of human diseases. The reported chemical evidences suggest that dietary antioxidants immensely help in disease prevention. These natural antioxidants exhibit a wide range of biological effects and health benefits including anticancer, anti-aging, and protective action against cardiovascular diseases, diabetes mellitus, obesity and neurodegenerative diseases (Zhang et al., 2015). Studies on antioxidant activities of fruits have increased remarkably due to increased interest in their potential of being used as a rich and natural source of antioxidants**.** In view of the above, an attempt was made to investigate the antioxidant potential in fruits of diverse genotypes of bael. Proper assessment of antioxidants from fruits of various bael genotypes will help in exploring potential antioxidant rich genotype which can be further utilized commercially in the manufacturing of functional foods, pharmaceuticals and food additives.

MATERIAL AND METHODS

Fruits of 18 bael genotypes were collected from Horticulture Research Centre, Pattharchatta, G. B. Pant University of Agriculture and Technology, Pantnagar, District, U. S. Nagar, Uttarakhand during the year 2016-17. The experimental site was located at an altitude of 29º N latitude and 79.3º E longitudes and at 243.84 meters above mean sea level. The climatic condition of experimental site is humid

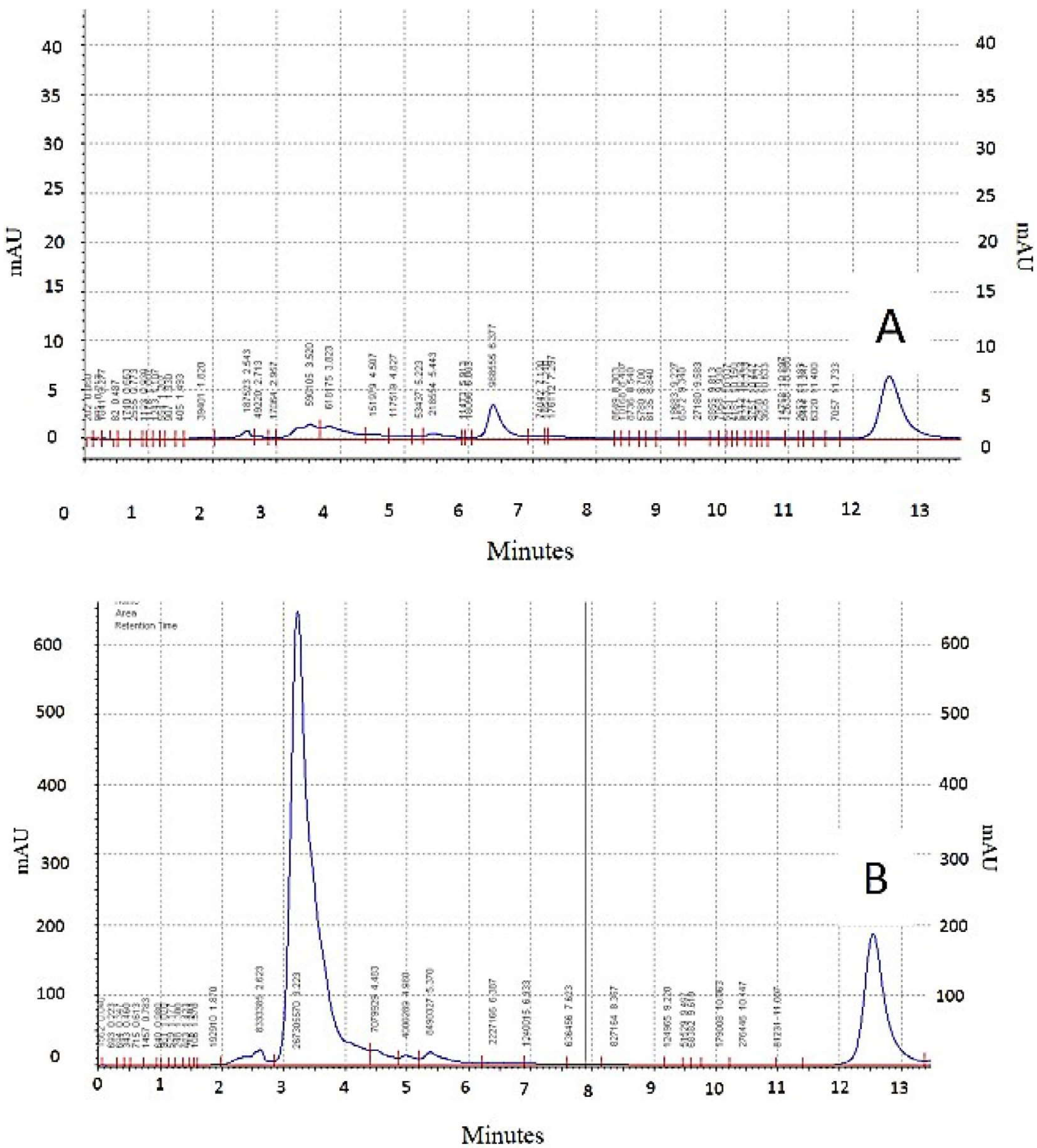

Fig 1 : Chromatogram obtained by HPLC, A: Marmelosin peak in standard mixture, B: Marmelosin peak in bael fruit extract mixture

subtropical. The plants with uniform growth and vigour were selected for the experiment. Uniform cultural practices were adopted for all the selected plant of different genotypes. The bael fruits of different genotypes were harvested during first forth night of March 2016-17. The fresh mature unripe bael fruits were thoroughly washed in clean water to remove unwanted material and dirt and were stored at -20^{o}C. Quantification of non-enzymatic antioxidant marmelosin was estimated through reverse phase-high performance liquid chromatography (RP-HPLC) method given by Shinde & Laddha (2014), and chromatograms obtained by HPLC presented in Fig.1.

The riboflavin was estimated as per method of AOAC (1980). Total phenol and carotenoids were estimated as per method of Sagar & Samuel (2008). Total flavonoids were measured by procedure given by Dewanto et al. (2002). Quantification of enzymatic antioxidants i.e. the activity of superoxidase dismutase (SOD) and peroxidase (POD) in bael fruit pulp was assayed spectrophotometrically (Abassi et al., 1998). Catalase (CAT) activity in bael fruit pulp was assayed (Luck, 1965). To measure the antioxidant activity DPPH (2,2-diphenyl-1-picrylhydrazyl) assayed by method as suggested by Yamaguchi (1998).

Table 1: Evaluation of different bael genotypes for non-enzymatic antioxidants

Genotype	Marmelosin (mg/g)	Total phenol (mg GAE/g)	Riboflavin (mg/100 g)	Total carotenoids (mg/100 g)	Total flavonoid (mg catechin/g DW)
Pant Bael-1	1.29a	77.60h	0.80bc	5.11a	42.88fg
Pant Bael-2	0.90b	90.40e	0.45c	3.86ab	41.00hi
Pant Bael-3	0.60c	95.20d	0.30d	2.50c	44.70de
Pant Bael-4	0.62c	98.80c	0.38d	1.25d	44.55de
Pant Shivani	0.61c	84.50f	1.01b	2.12c	52.28c
Pant Urvashi	0.28d	77.00h	0.93b	1.54d	43.75ef
Haldi Nurmohamd	0.08e	76.65h	0.19d	1.25d	36.30j
Patharchatta-1	0.23de	62.45i	0.34d	1.25d	32.28k
Faizabad No.9	0.58c	78.80h	0.65c	1.74c	42.45fgh
Faizabad Local	0.58c	77.50h	0.67c	2.81bc	45.05de
Pant Aparna	0.30d	101.50b	1.05b	1.60cd	75.08a
Pant Bael-10	0.44d	83.75fg	0.57c	0.96d	45.85d
Pant Sujata	0.67c	105.00a	1.21	2.31c	63.65b
Pant Bael-13	1.13a	78.35h	0.59c	1.63c	40.03i
Pant Bael-14	0.50cd	97.70c	0.55c	1.54d	41.88gh
Pant Bael- 15	1.25a	82.05g	0.32d	1.74c	42.88fg
Pant Bael -16	0.59gh	51.50j	0.44cd	1.06c	45.98d
Gonda No.2	0.65c	89.45e	0.73c	1.16c	44.90de
S.E.m.±	0.06	0.58	0.08	0.34	0.63
C.D. at 5%	0.21	2.13	0.28	1.25	2.33

The experiment was laid out in completely randomized design (CRD) with 18 genotypes of bael replicated thrice. The quantitative data were analyzed in a one-way ANOVA to determine differences between genotype through WASP 2.0 (Web Based Agricultural Statistics Software Package, ICAR-CCARI, India). The correlation analysis was done using standard statistical software (OPSTAT, HAU, India). Pearson's correlation coefficients were calculated at 5%. The principal components analysis (PCA) of all genotypes using 9 quantitative traits was performed by XLSTAT 2019.1.3.58328. The principal component score with eigen values >1 was used as new variable for cluster analysis.

RESULTS AND DISCUSSION

A wide range of variation with respect to different bioactive compounds was observed among the eighteen bael genotypes evaluated. The recovery of various non enzymatic antioxidants like marmelosin, total phenols, riboflavin, total carotenoids and total flavonoids is presented in Table 1.

Marmelosin is considered to be the main therapeutically active compound of bael fruit pulp and have been associated with profound beneficial effects on human health, due to their antioxidant properties. The value of marmelosin ranged between 0.08 to 1.29 mg/g. The maximum value of marmelosin was obtained in 'Pant Bael-1' (1.29 mg/g), and was found at par with Pant Bael -15/g (1.25 mg/g) and Pant Bael-13 (1.13 mg/g). 'Haldi Nurmohamd-2' the marmelosin content recovered was minimum (0.08 mg/g). Shailajan et al. (2012) reported a marmelosin concentration of 1.251±0.0069 mg/g in bael fruit pulp, whereas Shinde and Laddha (2014) observed a marmelosin content ranging from 5 to 6.5% in bael fruit.

The quantitative profiling of total phenols in the eighteen genotypes suggested that there was significant effect of genotypes on total phenol of bael fruits. The value of total phenol ranged between 51.50 to 105.00 mg GAE/g. 'Pant Sujata' was found to have most abundant total phenol (105.00 mg GAE /g), which was significantly superior than all other genotypes. The minimum total phenol content was recovered in 'Pant Bael-16' (51.50 GAE mg/g). Charoensiddhi & Anprung (2008) reported 87.34 mg GAE/g in Thai Bael. Similar effect of genotype on phenol content has been observed in other fruit crops like banana (Deshmukh et al., 2009) and pineapple (Lu et al., 2014).

Bael fruit is considered to be one of the richest sources of riboflavin. The value of riboflavin was ranged between 0.19 to 1.21 mg/100g. The highest riboflavin was found in 'Pant Sujata' (1.21mg/100 g), which was at par with 'Pant Aparna' (1.05 mg/100 g) and 'Pant Shivani' (1.01mg/100 g), whereas, lowest was recorded in 'Haldi Nurmohamd' (0.19 mg/100 g). Variation in riboflavin content of fruits as affected by different genotypes has been reported in banana (Englberger et al., 2010). The values of riboflavin in bael signify potential as a good natural source of this important water-soluble vitamin. The antioxidant effect of riboflavin reveals that it protects the body against oxidative stress, especially lipid peroxidation and reperfusion oxidative injury (Ashoori & Saedisomeolia, 2014). Beside this, the prescribed recommended daily intake (RDI) of riboflavin is 1.3 mg daily for men and 1.1 mg for women.

There was remarkable genotypic diversity with regard to total carotenoid content being estimated in bael fruit pulp. The value of total carotenoids ranged between 0.96 mg to 5.11 mg/100 g, maximum being recorded in 'Pant Bael-1' (5.11 mg/100 g) which was however, at par with 'Pant Bael-2' (3.36 mg/100 g) while, minimum was observed in 'Pant Bael-10' (0.96 mg/ 100 g). Similar findings on effect of genotypes on total carotenoids have been reported in banana (Englberger et al., 2010). The carotenoids are the derivatives of tetraterpenes and render yellow, orange, or red colour to the fruits. Some of these pigments show provitamin A activity and play an important role in the alleviation of vitamin A deficiency. They exhibit excellent antioxidant potential and help in protecting the skin against photooxidative damage, also play role in Singlet oxygen quenching and peroxyl radical scavenging (Stahl & Sies, 2003).

The analysis of total flavonoids content in bael genotypes reflected great variation. The value of total flavonoid content of the investigated genotypes ranged from 32.28 to 75.08 mg catechin/g dry weight, highest was observed in 'Pant Aparna' (75.08 mg catechin/g Dw) which was found significantly superior over other genotype. However, the lowest total flavonoid content was noted in 'Patharchatta -1' (32.28 mg catechin/g Dw). Lu et al. (2014) in pineapple also reported significant variation among different genotypes with regard to total flavonoids.

The superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are the key enzymatic antioxidants by which the free radicals that are produced during oxidative stresses are removed. These oxidative stresses lead to many serious human diseases. Superoxide dismutase catalyzes the breakdown of the superoxide anion into oxygen and hydrogen peroxide. Catalase and peroxidase enzyme catalyze the decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide is harmful by-product of many normal metabolic processes. The enzymatic antioxidants SOD, POD and catalase activity studied in different genotype of bael and significant variation was observed (Table 2). The maximum superoxidase dismutase enzyme activity was noted in 'Pant Shivani' (31.06 U/g fresh weight), while, the lowest superoxidase dismutase enzyme activity was noted in 'Faizabad Local' (11.50 U/g fresh weight). The data on peroxidase activity revealed that there was significant difference among diverse genotypes of bael. The highest peroxidase enzyme activity was observed in 'Pant Bael -1' (6.55 △O.D./min/g fresh weight), whereas, it was lowest in 'Pant Bael-2' (0.44 △O.D./ min/g fresh weight). The catalase activity was also found to be significantly affected by the genotypic diversity. The highest catalase enzyme activity was reported in 'Pant Shivani' (5.47 △O.D./min/g fresh weight) and was at par with 'Pant Bael-10' (5.20 △O.D./min/g fresh weight) and 'Pant Bael-4' (4.72 △O.D./min/g fresh weight). However, the lowest catalase enzyme activity was recorded in 'Patharchatta-1' (2.21 △O.D./min/g fresh weight). The similar effect of genotypes on different enzymatic antioxidants has also been reported by Desouza et al. (2014) in acerola.

DPPH radical scavenging activity significantly varied among the various genotypes (Table 2). The maximum DPPH radical scavenging activity was observed in 'Pant Shivani' (81.10%) followed by 'Haldi Nurmohamd' (80.84%) and 'Faizabad Local' (80.62%), while, it was recorded minimum in 'Pant Bael-14' (40.32%). These results are in conformity with the findings of Deshmukh et al. (2009) in banana, Lu et al. (2014) in pineapple and Hua et al. (2018) in citrus.

Table 2 : Evaluation of different bael genotypes for enzymatic antioxidants and DPPH radical scavenging activity (%)

Genotype	SOD (U/g Fw)	CAT (“O.D./min/g Fw)	POD (“O.D /min/g Fw)	DPPH radical scavenging activity (%)
Pant Bael-1	13.91q	4.08b	6.55a	72.11 (58.12)[d]
Pant Bael-2	27.89d	3.58b	0.44g	78.21 (62.17)[b]
Pant Bael-3	28.24b	2.77c	1.03h	51.07 (45.61)[hi]
Pant Bael-4	22.04m	4.72a	0.66ig	68.47 (55.84)[e]
Pant Shivani	31.06a	5.47a	4.35de	81.10 (64.23)[a]
Pant Urvashi	27.19g	4.46a	5.17c	75.27 (60.18)[c]
Haldi Nurmohamd	26.49i	3.54b	5.83b	80.84 (64.04)[a]
Patharchatta-1	23.70l	2.21c	0.96i	58.94 (50.15)[g]
Faizabad No.9	27.41f	4.11b	1.55g	53.75 (47.15)[h]
Faizabad Local	11.58r	3.61b	4.72d	80.62 (63.88)[ab]
Pant Aparna	26.98h	3.61b	3.69e	76.12 (60.75)[bc]
Pant Bael-10	24.19k	5.20a	4.50d	64.67 (53.53)[f]
Pant Sujata	28.80c	3.20bc	5.09c	78.27 (62.21)[b]
Pant Bael-13	16.19p	4.55a	1.92f	72.27 (58.22)[cd]
Pant Bael-14	18.93n	4.57a	4.72d	40.32 (39.42)[k]
Pant Bael- 15	18.38o	4.40ab	5.39c	46.25 (42.85)[j]
Pant Bael -16	25.19j	2.70c	1.85g	57.39 (49.25)[g]
Gonda No.2	27.51e	3.60b	4.06e	58.67 (49.99)[g]
S.E.m. ±	0.01	0.37	0.13	(0.52)
C.D. at 5%	0.03	1.08	0.38	(1.93)

*Figure shown in parenthesis are arc sign transformed values

The correlation study revealed that there was high positive correlation between DPPH radical scavenging activity (%) and non-enzymatic antioxidants *i.e.* marmelosin, riboflavin, total phenol, and total carotenoids (Table 3). Positive correlation was noted with total flavonoids present in the fruits of evaluated bael genotypes. High positive correlation was also found between DPPH radical scavenging activity (%) and enzymatic antioxidants (SOD, CAT, POD) activity present in the fruits of different Bael genotypes. Similar positive correlation between DPPH radical scavenging activity (%) and non-enzymatic antioxidant has also been reported by Gil et al. (2002) in nectarine, peach, and plum cultivars and Yan et al. (2013) in underutilized fruit.

Table 3 : Correlation Coefficient (r) of different non- enzymatic and enzymatic antioxidants with between DPPH radical scavenging activity (%)

Non-enzymatic and enzymatic antioxidants	DPPH radical scavenging activity (%)
Marmelosin	0.827**
Total phenol	0.655**
Riboflavin	0.829**
Total carotenoids	0.848**
Total Flavonoid	0.507*
SOD	0.709**
CAT	0.769**
POD	0.742**

Table 4 : Principal component analysis among eighteen genotypes of bael

PCA	Character	Eigen value	Variance (%)	Cumulative variance (%)
PCA 1	Marmelosin	2.60	28.94	28.94
PCA2	Total phenol	2.02	22.45	51.39
PCA3	Riboflavin	1.22	13.57	64.96
PCA4	Total carotenoids	1.10	12.26	77.23
PCA 5	Total Flavonoid	0.73	8.14	85.37
PCA6	SOD	0.53	5.85	91.22
PCA7	CAT	0.46	5.09	96.31
PCA8	POD	0.21	2.29	98.60
PCA9	DPPH radical scavenging activity	0.13	1.40	100.00

Principal component analysis is an important technique which is used to examine associations between characters and measure genotype genetic diversity. The PCA was used to determine the extent of the variation and percentage similarity within genotypes. Out of 9 principal components axis, four had eigen values greater than one and all together account for 77.22 of the total variability (Table 4). The PCA 1 accounted for 28.93 per cent of total variation, while PCA 2 accounted for 22.45 per cent of total variation. The cumulative per cent of variation varied from 28.93 to 77.22 per cent for the PCAs which had eigen value more than 1. The two-dimensional graphical representation of component patterns based on PCA2 and PCA3 is shown in Fig. 2. The genotypes were classified into four distinct

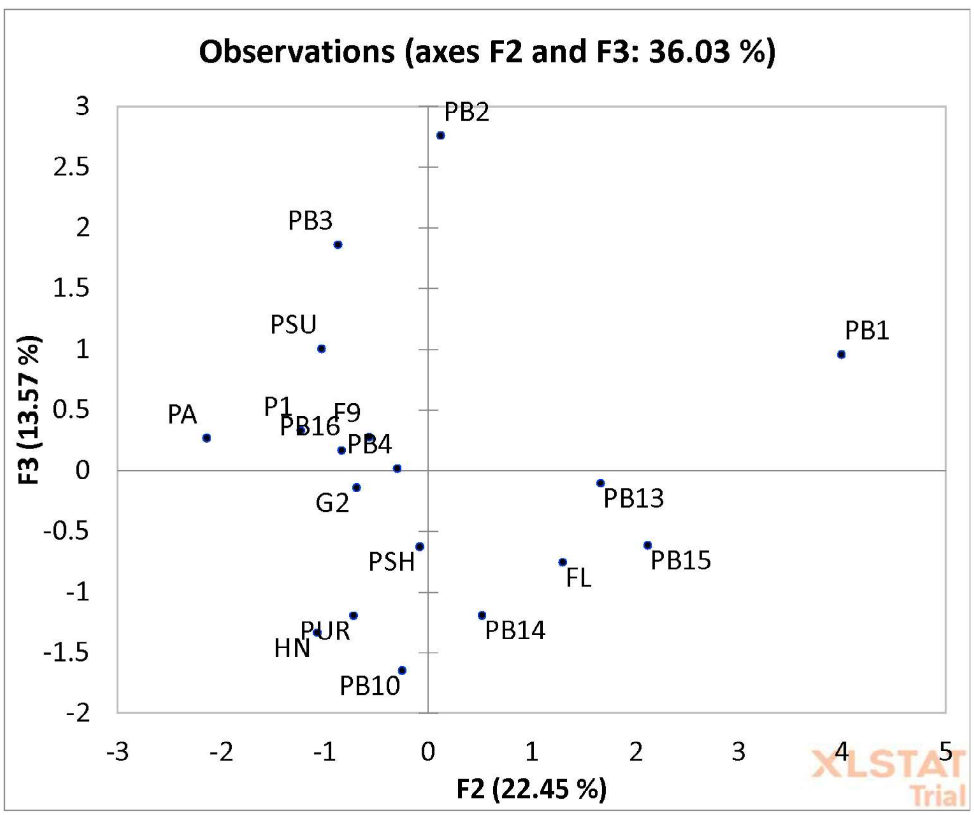

Fig 2 : A principal components analysis (PCA) scatterplot of 18 bael genotypes using non enzymatic and enzymatic antioxidants related parameters cluster group suggesting that these traits had significant contribution in bael diversity. Similar findings have also been reported in peach (Nowicka et al., 2019).

CONCLUSION

Thus, characterization of antioxidant content of bael fruits of different genotypes will help in choosing a suitable genotype for a particular requirement. Potential genotypes can be used as a rich source of antioxidants by the consumers and the pharmaceutical and processing industries. This study will also help in selection of superior parent in the breeding programmes.

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