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Enzymatic antioxidant and antioxidants biochemical relation gives flavour in Nanjangud rasabale (*Rasthali* Musa sp. AAB silk) banana grown in different environment

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Abstract
Forty five NRB type cultivars of banana which were grown in Mysore and Hassan conditions and were analysed for different biochemical parameters. NRB collection 66 and 22 showed highest content total phenolics in both the locations. Total flavonoid content found highest in NRB 13, NRB 54, at Hassan region while NRB 53 found more in Mysore region. Total antioxidant analysis depicted more at both the location NRB type. The present study also adds to the current knowledge of nutritive values, antioxidant potential and antioxidant enzymes *viz*; Esterase, Superoxide dismutase, aspects of banana.

Keywords: NRB nanjangud rasabale, antioxidant, rasabale, super oxide dismutase, peroxidase

Introduction
Banana is one of the cheapest nutritious fruits available in India. India is the second largest producer of banana and plantains in the world after Brazil and produces about 4.8 million tons annually. Ripen banana is recommended and especially good for patients and children suffering from several diseases. Besides banana fruit, leaves, pseudo stems and inflorescences are used for various purposes in India. Banana (*Musa sps.*) is such a fruit yielding tropical plant that may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidants. The elevated level of oxidative stress present in banana minimises the incidence of the several diseases. The mechanism of the action these antioxidant compounds include suppression of reactive oxygen species (ROS) formation either by inhibition of enzymes involved in free radical production, scavenging of reactive species and up regulating and protecting antioxidant defence. Banana fruit contain various antioxidants compounds such as ascorbic acid, retinol, β-carotene and polyphenols. (Arora *et al.*, 2008) [4]. In banana antioxidant of fruit is mainly due to presence of flavonoid, phenolic and antioxidant enzyme activity. Further the antioxidant capacity of banana may also be attributed to the presence of gallocto-catechin and dopamine (N. Ummarat *et al*., 2011) [15]. These compounds play an important role in protecting neuronal cells from oxidative stress induced neurotoxicity. To deal with the free radicals or so called ROS, the human body is equipped with an effective defence system which includes various enzymes and high and low molecular weight antioxidants. As with the chemical antioxidants, cells are protected against oxidative stress by an interactive network of antioxidant enzymes and free radical scavengers may provide a defensive mechanism against the deleterious actions of ROS.
Some antioxidants enzymes are found to provide protection against the ROS are superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT). SOD is an enzyme that repairs cells and reduces the damages done to them by superoxide, the most common free radical in the body. Glutathione peroxidase, the body's primary antioxidant, which is in virtually every cell, is one of the most powerful free radical fighters that the body has in its arsenal. These enzyme transformed the verity of compounds including carcinogens, therapeutic drugs, and products of oxidative stress. These enzyme play a key to detoxification of such substances. Banana is the most popular food in the world and it is well known that it contain various antioxidant

compounds such as gallocto-catechin and dopamine. Banana to be considered a good source of antioxidant for food. Accumulating evidence has revealed that both banana pulp and peel contain various antioxidants, for instance vitamins (A, B, C and E), beta-carotene and phenolic compounds such as catechin, epicatechin, lignin, tannins, anthocyanins and flavonoid (Someya *et al*., 2002; Wall, 2006; Lim *et al.,* 2007) [14, 16, 12]. As oxidative damage of lipids, proteins, and nucleic acids is implicated in the pathology of many chronic diseases, a great interest was developed by many research groups in exploring the major phytochemicals with antioxidant properties in banana.

Though many groups have explored the phytochemical composition and the antioxidant properties of banana fruit, till date a comparative evaluation of various phytochemicals and its antioxidant properties in banana variety Nanjangud Rasabale (NRB) type Rasthali is not reported. This study is of paramount importance as the nutritional quality of banana NRB type fruit is highly variable with its varieties, climatic conditions, soil type, temperature, light intensity and many more factors. The enzymatic profiling and phytochemical profiling of NRB type of banana cultivar fruit is depend on maturity, cultivars, geographical, growing and post-harvest condition (N. Ummarat *et al*., 2011) [15]. The fact that banana is used in the preparation of many commercial dietary supplements and processed food products high lights the importance of studies on the phytochemicals and antioxidants studies of the most popular verity of banana NRB type. The presence of protective phytochemical in NRB type, there is a need for analysis of vital importance of these phytochemical and antioxidant enzymes activity.

Material and Method

Enzyme protein extraction

NRB type banana leaf samples were collected from collage of agriculture Hassan location and collage of horticulture Mysore location at different growth stages for protein extraction. The collected of leaves samples were kept under ice box (4 ˚C) after every collection. For the extraction of SOD leaf samples were cut into small pieces and it was homogenised under ice cold condition in a prechilled pestle and mortar. The buffer used for extraction is 100mM sodium phosphate buffer (pH 7.4). The enzyme POX was extracted from leaf tissue with 100mM sodium phosphate buffer, pH 6.0. The homogenate were centrifuged at 12,000 rpm for 10 min. and supernatant was obtained and used as an enzyme source. The protein content was determined by modified method of Lowry's and was read at 660nm with bovine serum albumin as a standard (Zor and Selinger 1996) [17].

Native Profiling for Iso-enzyme analysis

The equal amount of protein isolated from leaves were subjected to discontinuous polyacrylamide gel electrophoresis under non reducing and non-denaturing condition as described by Laemmli (1970) [17]. The resolving gel constitute 12 percent acrylamide and staking gel concentration was 5 percent gel. The enzyme extraction was mixed with tracking dye (62.5mM Tris HCL pH 6.8, 10% Glycerol, 1mM PMSF) were loaded on to well-made on stacking gel. The electrophoretic separation was performed at 4 °C under an electric current of 20-30 mA. The electrophoresis were carried out for about 4-5 hour for enzymes.

Enzyme specific activity staining of the gel

Super Oxide Dismutase (SOD), EC 1.15.1.1

The enzyme specific staining for SOD was performed as described by Rao *et al*. 1997 [21], with a miner modification. Solution 1 containing 20mg NBT (Nitro blue tetrazolium) dissolved in 20ml water DDW (preferably autoclave), 500µl (0.2M) EDTA, 50ml 100mM pH 7.5 phosphate buffer, 330µl TEMED, solution 2 containing 30 ml (25Mm) Riboflavin. Both the solution 1 and 2 were mixed and the electrophoresis was done at 65-75mV, electrophoresed gel was incubated for 15 minute under dark condition and immediately it was kept under the sun or high beam fluorescent light for 15-20 min. till coloured bands are seen. The enzyme reaction was stopped using 10% acetic acid and glycerol and gel was preserved in 4 ˚C.

Peroxidase (POX), E.C 1.11.17

Activity staining for POX was performed using the method of Simons (1986) [13]. Gels were incubated for 10-15minute in 100mM phosphate buffer at pH 6.1 containing 90mM guaicol and 0.25% H_2O_2. After the appearance of bands gel were incubated in a stop solution of 5% acetic acid.

Esterase (EST), E.C.3.11

Esterase isoforms were visualized by incubating the gel for 30 minutes at 4 ˚C in a solution containing α-napthyl acetate 100 mg/ml, 50 mg/ml β-napthyl acetate dissolved in 1ml acetone, 100ml of 0.1M sodium phosphate buffer with pH 6.5 and 50 mg fast garnet GBC salt (Solis and Soltis 1989) [31] was used as colour developer for visualization.

Visualization of Isozymes

Following electrophoresis, isoforms of enzymes were detected using the specific activity stains. General principles of enzyme activity staining and the chemistry of enzyme localization in gels have been reviewed by Gabriel (1971) [11], Ostrowski (1983) [21] and Vallejos (1983) [29]. Following immersion of the gel into the staining solution, the substrates and the other required reagents diffuse into the gel where they are acted upon by the enzyme. Detection is primarily based upon precipitation of soluble indicator dyes, which become insoluble and coloured in zones of enzyme activity. Redox dye guaicol was used in detection of peroxidases, which undergo changes in colour and/or solubility upon oxidation.

Evaluation and Documentation

The relative mobility (Rm) of relative front Rf value of each stained enzyme band was calculated by following formula

$$\text{Relative mobility (Rm)} = \frac{\textit{The distance travelled bt specific band}}{\textit{Distance travelled by the tracking dye}}$$

Table 1: Gel recipe for native poly acrylamide gel electrophoresis for *in-gel* enzyme assay

Stock Solution	Resolving Gel Tris buffer 1.50M, pH 8.8	Stacking Gel Tris buffer 1.00 M, pH 6.8	Reservoir buffer Tris glycine 0.025M, 0.192M pH 8.3
Acrylamide: 2, 2-Methyl Bisacrylamide (29:1)	12.480ml	0.750ml	-
1.5M Tris buffer pH 8.8	3.750ml	-	-
1.0M Tris buffer pH 6.8	-	1.5ml	-
0.25M Tris,1.92M Glycine, pH 8.3	-	-	200ml

10% (w/v) Ammonium per sulphate	0.255ml	0.045ml	-
Distilled water	13.530ml	3.669ml	1800ml
TEMED	0.015ml	0.006ml	-
Total (ml)	30.00	06.00	2000

w/v – Weight/volume

Data analysis for Isozymes in gel assay

The electrophoretic analysis of ISO enzymes POX, SOD and EST were analysed on 10% polyacrylamide slab gels. Detection of POX was carried out by the method described by Larsen and Benson (1970) [18], SOD was performed by the method of Siciliano and Shaw (1976). ISO enzymes banding pattern were recorded according to their relative mobility (Rm) values. Interpretation of banding pattern followed standard principles (Wendel and Weeden 1989) [31].

Phenol and flavonoids isolation

About 0.1 gram NRB type Banana leaf and fruit samples were taken for phenol and flavonoids isolation and homogenised using 1:10 sample to extraction medium of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 15 minutes. Again residue was re-extracted with 1:5 residue to extraction medium (80% ethanol) ratio and it was centrifuged for 10,000rpm and 15 minutes, supernatant were pooled in to a small beaker. The collected supernatant was allowed to evaporate in water bath at the temperature 60-70 ˚C. After evaporation of ethanol the dried powder was dissolved in a 2ml distilled water. The dissolved solution were stored at 4 ˚C until further analysis.

Spectroscopic assay of phenols and flavonoids

Total Phenolics

The total phenolics were determined using the Folin ciocalteu reagent (FCR) methods with the slight modifications, for spectroscopic analysis of phenol content, 0.2ml aliquot was taken for the analysis and volume was made up to 3ml using double distilled water. 0.5ml of 1N FCR was added in to the reaction mixture, after the 3 minute of incubation 20% of 2ml sodium carbonate was added into the each tubes. The reaction mixtures were mixed thoroughly and tubes were incubated into a boiling water for one minutes, the tubes were allowed to cool at normal room temperature and absorbance was measured at 650nm (Anyasi *et al*., 2015) [3]. Ethanol was used as an extraction solvent, while gallic acid was used as the standard phenolic compound. Final results of total phenolic were expressed as Gallic acid equivalent mg per 100g of dry weight of sample (GAE/100 g d.w).

Total Flavonoids

The total flavonoids content was determined using a spectrophotometric method (Meyers *et al*., 2003). The sample were read at 510nm using spectrophotometer. The reaction mixture for flavonoid assay was containing 0.2ml of sample, 2ml of 95% methanol, 0.2ml 1M sodium acetate, 10% Aluminium chloride of 0.1ml and the volume was made up to 3ml with distilled water. All the values were expressed in grams of rutin equivalents g^{-1}. The data were reported as a means, critical difference (CD), co-variance (CV) and range for all replications.

Antioxidant capacity of banana

In determining the ability of phenolic compounds extracts from banana samples to scavenge the unstable free radicals 1, 1-diphenyl-2-picrylhydrazol the method proposed by Anyasi, Jideani, and Machau (2015) [3]. The dilution of different concentration of 10, 20, 30, 40 and 50 mg/ml of the sample with final values of IC50 obtained by plotting the percentage. The DPPH does not dimerize as happens with most free radicals. The reaction mixtures were incubated in the dark at room temperature for 30 minutes. The delocalisation on the DPPH molecule determines the occurrence of a purple colour with an absorption band with a maximum around 520nm. The percentage of DPPH free radical quenching activity determined using the following equation

$$\text{DPPH \% scavenging activity} = \frac{(\text{DPPH Absorbance}) - (\text{Extracted sample Absorbence})}{\text{DPPH Absorbence}} X100$$

Where a DPPH and an extraction absorbance values at 520nm for the methanolic solution of DPPH and the sample extract, respectively.

Results and discussion

Analysis of isozymes profiles by Native PAGE

Native gel banding pattern analysis of each NRB collection representative sample was used for antioxidants and variability study. The each lane represent respective NRB collection *viz*., Lane1 (NRB 5), Lane 2 (NRB 3), Lane 3 (NRB 9), Lane 4 (NRB 7), Lane 5 (NRB 2), Lane 6 (NRB 4), Lane 7 (NRB 6), Lane 8 (NRB 1) and Lane 9 (NRB 8).

After visualization of gel, data analysis were done for all the three enzymes *viz*., Peroxidase (E.C.1.11.1.7), Superoxide dismutase (E.C 1.15.1.1) and Esterase (E.C.3.1.1.6) enzymes. Reproducibility of ISO enzyme banding patterns was tested three times. Zymograms obtained from three enzyme system showed good resolution staining and banding pattern were reproducible for each enzyme system among all the NRB type cultivars. For ISO enzyme analysis, the relative mobility (Rm) of each band was calculated presented in Fig1 and Fig2.

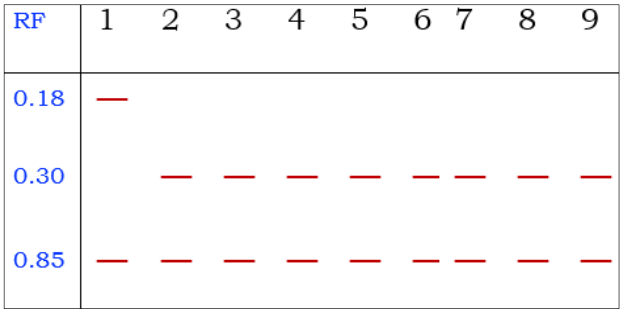

Fig 1: Zymogram of POX at vegetative (HSN).

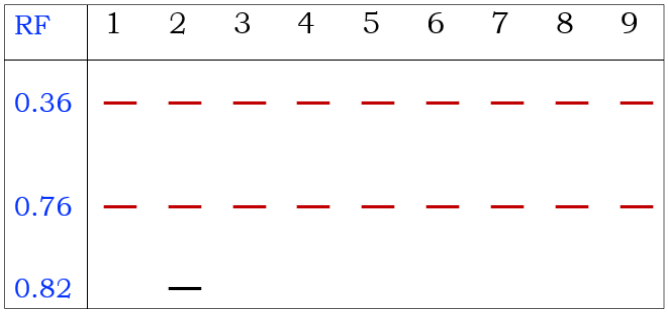

Fig 2: Zymogram of POX at flowering stage (HSN).

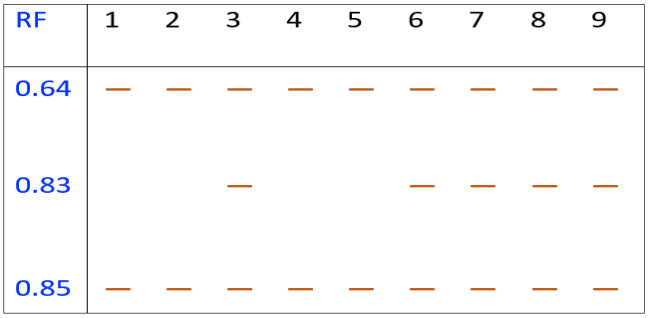

Fig 3: Zymogram of Esterase flowering stage

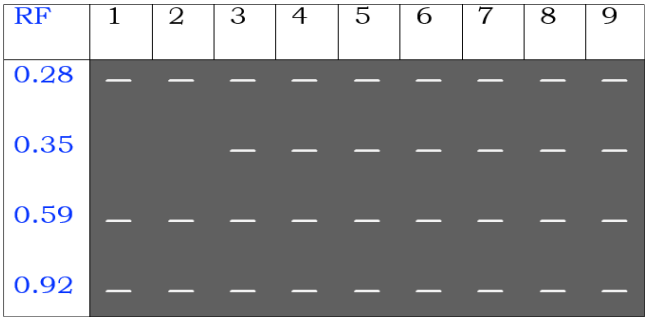

Fig 4: Zymogram of SOD flowering (Mysore).

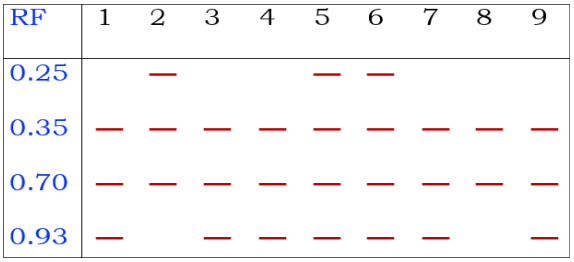

Fig 5: Zymogram of POX (Mysore)

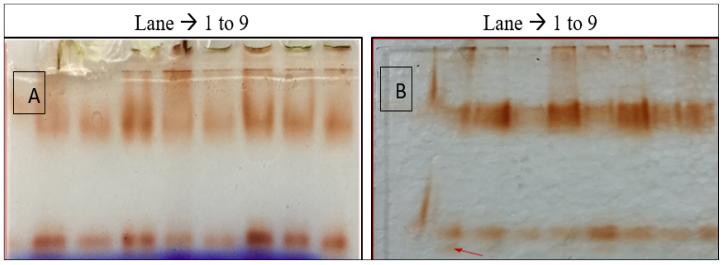

Fig 6: *In gel* assay of Peroxidase (POX) at different stage in Hassan location. A: Vegetative stage B; Reproductive stage of banana

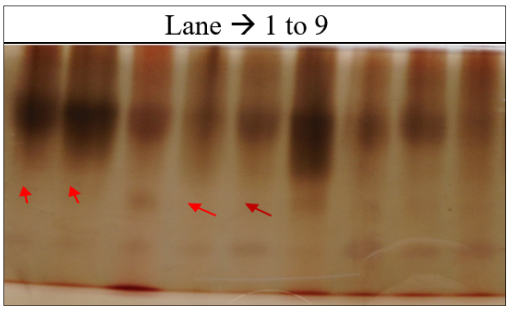

Fig 7: In gel assay of Esterase at reproductive Stage grown in Hassan location.

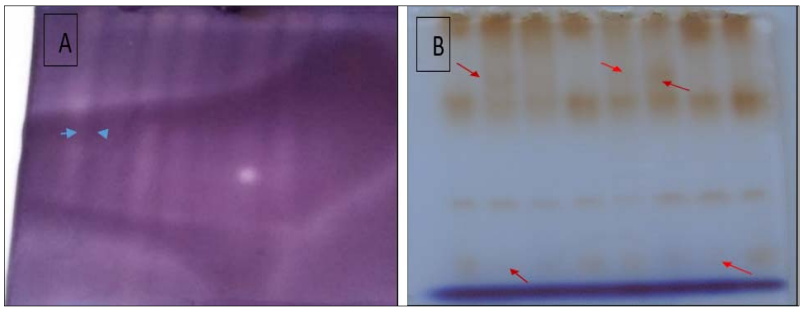

Fig 8: In gel assay of different anti-oxidant enzymes at Reproductive stage of banana A. Super oxide dismutase (SOD) and B. Peroxidases (POX).

The peroxidase profile at vegetative stage, at Hassan grown region only two bands were observed shown in (Fig: 1) with less variability among the banana cultivars. The Rf value ranged from 0.18-0.85. Only one band observed in Lane-1 which represented NRB 5 at Rf 0.18. At the Rf value of 0.30 showed dissimilarity between the NRB 5 and other 8 cultivars were showing similar banding pattern. At reproductive stage (Fig: 2) was observed in lane 2 represented NRB 9 with Rf 0.82. However, a band with Rf 0.82 was absent in other cultivars. A bands with Rf 0.36 and 0.76 were present all nine NRB type cultivars. At vegetative stage monomorphic bands were found. Reproductive stage of Mysore location the peroxidase banding pattern lane 2, 5 and 6 represented collection NRB 3., NRB 2 and NRB 4 with two bands (Rf 0.25,0.35) showed total dissimilarity with NRB type NRB 5., NRB 1., NRB 4., NRB 7., NRB 6 and 8 (Figure 21) However a band Rf 0.93 was absent in NRB 3 and NRB 1 in represented lane 2 and 8. While found in other NRB type cultivars (Plate 13 B).

At the reproductive stage of Mysore grown region samples banding pattern lane 2, 5 and 6 represented collection NRB 3., NRB 2 and NRB 4 with two bands (Rf 0.25, 0.35) showed total dissimilarity with NRB type NRB 5., NRB 1., NRB 4., NRB 7., NRB 6 and 8 (Fig: 3). However a band Rf 0.93 was absent in NRB 3 and NRB 1 in represented lane 2 and 8 while found in other NRB type cultivars (fig.3). At vegetative stage in Mysore location very slight polymorphism was observed.

Esterase isozymes in samples of leaf extracts of cultivars of Mysore field at reproductive stage showed in Fig: 4. five major bands in faster zone of zymograms with the Rf 0.83.Absence of four bands with Rf 0.83 may be useful for identification of variability. In this study, Esterase Zymograms showed different banding pattern in the lane-1, lane-2, lane-4 and lane -5 showed unique double band pattern with the Rf 0.64 and 0.85 *viz*. lane-1(NRB 5.), lane-4 (NRB 7.) and lane-5 (NRB 2.) while with the Rf 0.83 bands were present lane-3 (NRB 9.), lane-6 (NRB 4.), lane-7 (NRB 6.), lane-8 (NRB 1.), lane-9 (NRB 8.) showing similarity among the cultivars. In Superoxide dismutase both of the locations monomorphic bands has been observed.

Table 1: Mean performance of different NRB lines for phenolics, flavonoids and percent anti-oxidants at two location.

Genotypes	Phenol (mg/g)		Flavonoid (mg/g)		%Antioxidant	
	Hassan	Mysore	Hassan	Mysore	Hassan	Mysore
NRB 11	15.05	13.73	12.73	2.80	87.73	85.70
NRB 12	14.77	14.25	13.27	3.47	89.08	92.55
NRB 13	14.22	14.32	14.46	3.71	92.41	92.56
NRB 14	14.64	14.22	13.54	3.34	89.91	92.55
NRB 15	14.61	14.26	13.66	3.50	90.26	92.55
NRB 21	33.00	35.33	8.89	3.06	90.58	88.35
NRB 22	34.17	35.67	8.90	3.29	90.40	91.84
NRB 23	33.79	34.33	8.88	3.31	90.62	91.66
NRB 24	33.47	34.83	8.89	3.30	90.59	91.73
NRB 25	33.25	35.42	8.88	3.29	90.72	91.81
NRB 31	14.67	24.30	2.35	6.65	99.90	105.30
NRB 32	17.00	24.56	2.88	6.70	89.35	97.80
NRB 33	18.75	25.06	2.85	5.98	89.45	98.00
NRB 34	18.75	24.92	2.85	6.18	89.45	97.94
NRB 35	18.31	24.80	2.85	6.35	89.43	97.90
NRB 41	21.91	14.25	8.78	8.92	130.77	85.85
NRB 42	21.11	14.85	9.02	9.22	88.65	86.29
NRB 43	20.76	14.56	9.41	8.97	88.38	86.18
NRB 44	20.42	13.87	9.80	8.26	88.10	86.03
NRB 45	20.94	13.60	9.21	8.64	88.51	85.33
NRB 51	6.43	15.34	26.21	26.30	78.47	91.80
NRB 52	6.30	15.97	26.66	26.66	91.68	91.82
NRB 53	6.34	15.40	26.62	26.75	91.69	91.77
NRB 54	6.28	16.17	26.68	26.67	91.68	91.83
NRB 55	6.29	15.93	26.67	26.75	91.68	91.81
NRB 61	55.44	49.71	3.68	4.68	100.03	96.31
NRB 62	55.73	48.99	3.74	4.97	88.59	98.38
NRB 63	55.00	50.42	3.65	4.89	89.70	96.71
NRB 64	55.54	50.56	3.71	4.73	88.62	95.78
NRB 65	55.35	49.32	3.69	4.67	88.65	96.61
NRB 71	8.75	7.14	6.99	13.95	85.63	86.91
NRB 72	9.73	7.14	6.84	13.95	91.60	86.91
NRB 73	9.59	7.24	6.82	13.91	91.24	84.73
NRB 74	9.70	7.02	6.84	13.99	91.52	89.64
NRB 75	9.68	7.05	6.83	13.68	91.46	85.51
NRB 81	14.53	14.53	5.61	13.61	105.60	93.77
NRB 82	13.08	13.15	5.82	11.00	97.35	91.87
NRB 83	13.07	14.40	5.82	12.95	97.35	93.63
NRB 84	13.40	13.97	8.05	12.10	96.28	93.05
NRB 85	13.38	13.40	7.87	11.49	96.37	92.23
NRB 91	21.76	33.49	18.03	41.68	94.37	84.27
NRB 92	21.88	33.49	17.39	41.69	91.60	84.27
NRB 93	21.99	33.51	17.85	41.34	91.53	83.80
NRB 94	21.74	33.45	16.82	41.26	91.67	84.39
NRB 95	21.83	33.58	17.19	40.68	91.62	82.57
Mean	21.48	23.14	10.63	13.41	92.21	91.07
CV%	7.49	4.96	9.46	8.17	13.55	5.17
S.E	0.93	0.66	0.58	0.63	7.21	2.72
C.D %	3.46	2.47	2.16	2.35	-	10.12
Lowest	6.28	7.02	2.35	2.80	78.47	82.57
Highest	55.73	50.56	26.68	41.69	130.77	105.30

Phenol (mg/g), Flavonoid (mg/g) and total antioxidant (percentage)

A range 6.30 to 55.40 recorded in case of phenol content with an average 21.48 at Hassan location. Maximum value (55.40 mg/g) recorded in NRB 53 followed by NRB 51 followed NRB 52 and NRB 53 minimum value (6.30 mg/g) recorded in NRB 52 collection (Table 1).

Phenol content at Mysore location fruit ranged was from 7.02 to 50.56 with an average 23.14. Maximum value (50.56 mg/g) recorded in NRB74 and minimum value (7.02 mg/g) recorded in NRB 64 collection (Table 1).

A range of flavonoid was from 2.85 to 26.68 with an average 10.63 of Hassan location. Highest value (26.68 mg/g) recorded in NRB 54 collection and minimum value (2.85 mg/g) recorded in NRB 31 collection. Genotypic and phenotypic coefficient variation was (67.54) and (68.20) shown in Table 1.

A range of flavonoid in fruit was from 2.80 to 41.69 with an average of 13.41 at Hassan location. Highest value (41.69 mg/g) recorded in NRB 92 collection and minimum value (2.80 mg/g) recorded for NRB 11 collection (Table 1).

A range of 78.47 to 130.77 was recorded in case of total antioxidant activity at Hassan location (Table 1). Maximum value was recorded in NRB 41 (130.77%) whereas, NRB 51 recorded the minimum value (78.47%). A range of (Table 1) 84 to 105% recorded in case of total antioxidant activity at Mysore location. Maximum value (105%) observed in NRB 31

collection while minimum value (84%) was observed in NRB 91 followed NRB 73 collection.

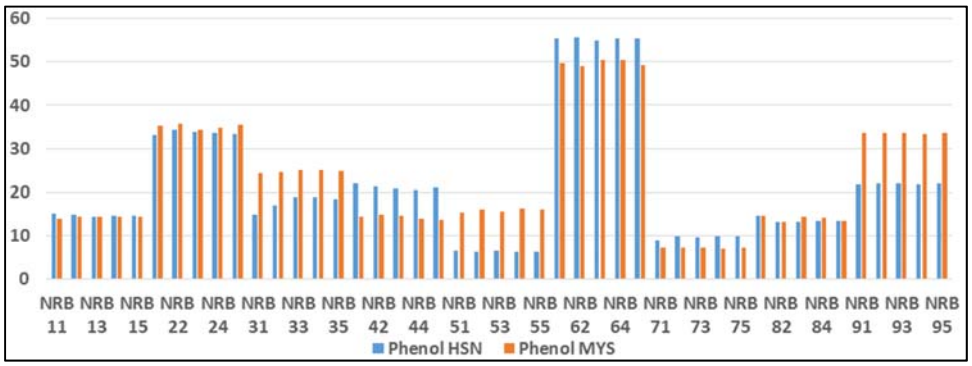

Fig 9: Total phenolic studies from NRB collection at different location

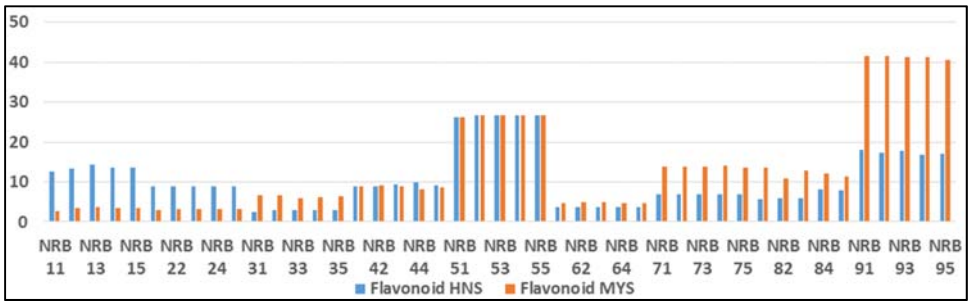

Fig 10: Total flavonoids studies from NRB collection at different location

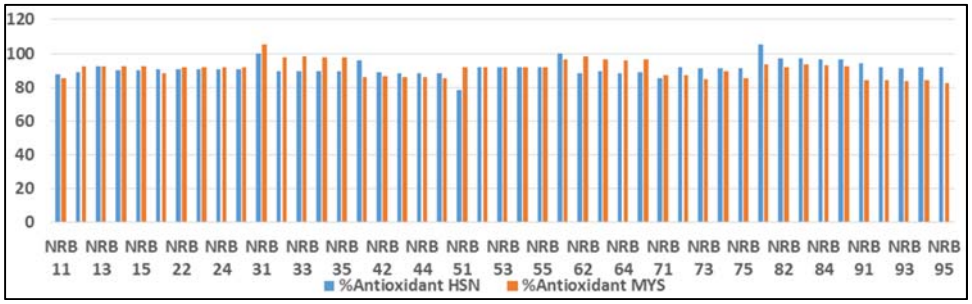

Fig 11: Total percent anti-oxidants studies from NRB collection at different location

Based on the phenolics, flavonoids and total antioxidant activity of Mysore location has been taken as a reference value for the comparison with the Hassan location. Sensory and nutritional qualities of foods are closely associated with phenolics, contributing directly or indirectly to desirable or undesirable aroma and taste. The present investigation data supported with the result of Mahesh Deshmukh *et al*., (2009) [8] who reported phenolic and flavonoid content in different banana cultivars of pulp extract of Jawari and Rasbale 40 mg/100g and 86 mg/100g respectively and flavonoid content measured in Rasbale 98 mg/100g and Jawari 46mg/100g (Mahesh Deshmukh *et al*., 2009) [8]. There is very less difference between regions of phenol content whereas flavonoid is found more in Mysore region cultivars, which belong to Devarasanahalli region NRB4 and NRB5.

Esterase enzyme also imparts role in flavour of fruits (Janhnke *et al*., 1991) [13]. Elevated level of SOD and POX have been co-related with increased levels of oxidative stress resistance in several cases (Jansen *et al*., 1989; Jahnke *et al*., 1991) [14, 13]. Flavonoids of banana stimulate the activity of SOD and POX which might be responsible for reduced level of peroxidation (Vijayakumar *et al*., 2008) [30]. Peroxidase activity was more in Mysore region at the flowering stage than in Hassan region whereas SOD activity was more in Hassan region at vegetative and flowering stage This suggest both of the trait are influenced more by environment.

The percentage of antioxidants radical scavenging activity towards DPPH free radical assay allows comparison of relativities of powerful oxidants such as BHT with those present in extract obtained from fruit residue. Free radical scavenging ability by hydrogen donation is a known mechanism for antioxidation. All the methanolic extract did not show much difference in antioxidant activity or scavenging activity. The correlation between total phenolic concentration and antioxidant activity has been widely studied in different foodstuffs, such as fruits and vegetables (Jayaprakasha *et al*., 2008 [15], Klimczak *et al*., 2007) [16]. Maximum antioxidant activity recorded at Mysore region which have been collected from Devarasanahalli region which has been identified as genuine NRB type and it is advantageous.

Isozyme banding pattern

In the present study, three and two active peroxidase zones were identified, and in Mysore location grown cultivars at

reproductive stage exhibited a high degree of polymorphism and Hassan grown cultivars showed moderate level of polymorphism at reproductive stage. Similar findings were also observed for 44 cultivars of Indian banana and plantain (Bhat *et al*., 1992) [5] and potato (Giovanni *et al.,* 1993) [10]. Since ample variability in peroxidase profile was observed, this isozyme may be useful for identification of NRB type cultivars of NRB 5 collection and NRB 9 collection which was grown in Hassan location and the cultivars which were grown in Mysore location NRB collection cultivars NRB 9, NRB 6 and NRB 1 collection. Thus identified genuine NRB type from Devarasanahalli could be easily distinguished from mixture.

In Esterase five bands with Rf 0.83 were discriminable in the esterase profiles cultivars grown in Hassan at reproductive stage. The esterase zone II was found to be highly polymorphic while zone I and III were monomorphic represented in zymogram (Plate). There were no visible bands for esterase profile for NRB collection cultivars of 5, 7 and 2 with Rf 0.83. Three bands with Rf 0.64, 0.83 and 0.85 were found in NRB collection NRB 9, NRB 4, NRB 6, NRB 1 and NRB 8. Based on the above analyses, polypeptide profile seems to be inappropriate for cultivars identification due to minimal variability determined by the present study with 9 NRB type cultivars. Isozymes such as POX, esterase and SOD could be used as biochemical markers for effective cultivars identification in banana taxonomy. The variation in polypeptide and isozyme pattern may reflect true genetic diversity rather than the variation in diverse horticultural traits, which are generally influenced by the surrounding environment (Vijay Kumar *et al*., 2008) [30].

Conclusion

A positive correlation was obtained between the phenolics, flavonoids, total antioxidant activity and antioxidant enzyme activity of NRB type Rasabale may also be considered to be one of the best sources of antioxidants. The present study also stressed the identification of NRB type of locally grown banana cultivars from Nanjangud in and around Mysore region that are likely to be nutritionally rich. It is reflected from the present study and the literature cited that bananas have an enormous potential as a nutritional fruit crop. The NRB 13, 54, 53, 66 and 22 are known to the falling in a same group. This may be concluded from above results. Biochemical analysis with biochemical maker in gel analysis is useful in understanding diversity and distribution of the cultivars. Cultivars of NRB type Rasabale banana that are rich in antioxidant activity could be realistically and nutritionally reasonably promoted to contribute to improved nutritional status. Selection of particular NRB type cultivars for its health significance may be an important consideration. Cultivars of NRB 13, 54, 53, 66 and 22 are nutritious promotion of such local cultivars for their wider use in recommended.

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