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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DOI: https://doi.org/10.22271/chemi.2020.v8.i1t.8457

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 spp.) 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, anthocyansins 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 profiling of NRB type of 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].

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 minor modification. Solution 1 containing 20mg NBT (Nitro blue tetroxidum) 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. 1.11.1.7
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% H2O2. After the appearance of bands gel were incubated in a stop solution of 5% acetic acid.

Esterase (EST), E.C.3.11
Esterase isofoms were visualized by incubating the gel for 30 minutes at 4 °C in a solution containing α-naphyl acetate 100 mg/ml, 50 mg/ml β-naphyl acetate dissolved in 1ml acetone, 100ml of 0.1M sodium phosphate buffer with pH 6.5 and 50 fast garnet GBC salt (Solis and Soltis 1989) [31] was used as colour developer for visualization.

Visualization of Isozymes
Following electrophoresis, isofoms 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

\[
Rm = \frac{\alpha}{\alpha + \beta}
\]

\[
\alpha = \text{Distance travelled by specific band}
\]

\[
\beta = \text{Distance travelled by the tracking dye}
\]

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

| Stock Solution | Resolving Gel Tris buffer | Stacking Gel Tris buffer | Reservoir buffer Tris glycine |
|----------------|--------------------------|--------------------------|-----------------------------|
| 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 |
**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 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, Judeani, 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

\[
\text{DPPH} \% \text{ scavenging activity} = \left( \frac{\text{Extracted sample absorbance}}{\text{DPPH absorbance}} \right) \times 100
\]

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.

| RF     | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 0.18   |     |     |     |     |     |     |     |     |     |
| 0.30   |     |     |     |     |     |     |     |     |     |
| 0.85   |     |     |     |     |     |     |     |     |     |

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

**Fig 2:** Zymogram of POX at flowering stage (HSN).
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 (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.
| Genotypes | Phenol (mg/g) | Flavonoid (mg/g) | %Antioxidant |
|-----------|--------------|-----------------|--------------|
| Hassn Mysore | Hassn Mysore | Hassn Mysore | Hassn 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 | 3.29 | 90.40 | 91.84 |
| NRB 31 | 14.67 | 24.30 | 2.35 | 6.65 | 99.90 | 105.30 |
| NRB 32 | 17.00 | 24.56 | 2.88 | 6.70 | 91.24 | 84.73 |
| NRB 33 | 18.75 | 25.56 | 8.78 | 8.92 | 130.77 | 85.70 |
| NRB 34 | 18.75 | 24.92 | 2.85 | 6.35 | 89.45 | 97.90 |
| NRB 35 | 18.31 | 24.80 | 2.85 | 6.35 | 89.45 | 97.90 |
| NRB 41 | 19.01 | 14.25 | 8.78 | 8.92 | 130.77 | 85.85 |
| NRB 42 | 20.94 | 13.60 | 9.21 | 8.64 | 88.51 | 95.78 |
| NRB 51 | 6.30 | 15.97 | 26.66 | 26.66 | 91.68 | 91.82 |
| NRB 52 | 6.34 | 15.40 | 26.62 | 26.75 | 91.69 | 91.77 |
| NRB 53 | 6.28 | 16.17 | 26.68 | 26.75 | 91.68 | 91.83 |
| NRB 54 | 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 57 | 6.30 | 15.97 | 26.66 | 26.66 | 91.68 | 91.82 |
| NRB 58 | 6.34 | 15.40 | 26.62 | 26.75 | 91.69 | 91.77 |
| NRB 59 | 6.28 | 16.17 | 26.68 | 26.75 | 91.68 | 91.83 |
| NRB 60 | 6.29 | 15.93 | 26.67 | 26.75 | 91.68 | 91.81 |
| NRB 71 | 8.75 | 7.14 | 6.99 | 13.95 | 85.63 | 83.38 |
| NRB 72 | 7.93 | 7.14 | 6.84 | 13.95 | 91.60 | 86.91 |
| NRB 75 | 9.68 | 7.02 | 6.84 | 13.99 | 91.52 | 89.64 |
| NRB 81 | 14.53 | 14.53 | 5.61 | 13.68 | 91.46 | 85.51 |
| 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 | 92.73 |
| 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 of 10.63 at Hassan location. Highest value (26.68 mg/g) recorded in NRB 41 collection and minimum value (2.80 mg/g) recorded for NRB 11 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 2.80 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.
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 (Jahnke et al., 1991) [13]. Elevated level of SOD and POX have been correlated 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) and potato (Giovanni et al., 1993). Since ample variability in peroxidase profile was observed, this isozone 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 monomorphistic 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).

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 marker 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.

Acknowledgment
The authors acknowledge thanks to Dean College of Agriculture, Hassan, Dean (Hort) College of Horticulture for providing the place for conducting the afore said experiment. Author also acknowledge the University Grant Commission for providing the funds for doing the research.

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