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# Assessment of biochemical basis of yield variation in small millets under rainfed condition

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## Abstract
Small millets are important climate-smart nutritious crops. Biochemical constituents play an important role in crop growth and development. Understanding the biochemical changes in crops plants at different stages helps to understand their adaptations to extreme climate changes and crop characteristics, their influence on grain yield. In this study, we assessed the biochemical constituents such as soluble protein, proline, nitrate reductase, catalase activity and peroxidase activity at five different growth stages (vegetative, flowering, grain development, grain maturity and harvest) and five small millets (foxtail millet, proso millet, kodo millet, little millet and barnyard millet). The proline accumulation was highest at grain development stage in all the small millets (1.43 to 2.02 mg/g) and was the maximum in barnyard millet. Soluble protein and nitrate reductase activity were the maximum in foxtail millet; both increased from flowering to grain development stages and decreased in later stages. Barnyard millet had the highest catalase activity in all five growth stages, followed by foxtail millet, and the lowest catalase activity was found in little millet across growth stages. Within each crop, a cultivar having high soluble protein, nitrate reductase, proline, and peroxidase activity had higher grain yield, while cultivar with high catalase activity had comparatively low yield, except little millet.

**Keywords:** Proline, Soluble protein, nitrate reductase, catalase activity, peroxidase activity, small millets

## Introduction
Small millets, such asfinger millet, foxtail millet, proso millet, kodo millet, little millet and barnyard millets are considered as important nutri-rich climate-smart crops (Vetriventhan and Upadhyaya 2015) [14]. The small millets are adapted to varied agro-climatic regions, and their use as food, feed and fodder make them important for food security (Upadhyaya *et al*. 2008) [13]. Small millets are suited to conditions of low and moderate rainfall area due to early maturity and their adaptation to water stress conditions. Besides India, small millets are also grown in China, Russia, Japan., USA and other African and East Asian countries. In India, the cultivation of small millets is confined to Andhra Pradesh, Karnataka, and Tamil Nadu.In India, small millets are cultivated in limited area of 2.32 m ha and occupy about 9.7 lakhs ha with a production of 4.67 lakhs tons, with a productivity of 480 kg/ha (averaged between 2006-2010). Potential yields of up to 3 tons in small millets were reported (http://www.aicrpsm.res.in/Reports.html), indicating a large yield gaps, and great opportunity to enhance productivity following improved crop management practices and cultivation high yielding cultivars.
Various biochemical constituents play important role in crop growth and development, and their concentration varies at different growth stages and growing conditions (stress and non-stress). Comparative investigation on small millets, with respect biochemical traits and grain yields were meager. Assessing the change in biochemical constituents in small millets, and at different growth stages and the relationship with yield would help in understanding the basis of crop and varietal differences in terms of productivity. This study aims to assess the biochemical constituents such as soluble protein, nitrate reductase, proline, catalase and peroxidaseat five different growth stages and their influence on grain yields.

## Materials and Methods
The experiment was conducted at Eastern Block Farm of Tamil Nadu Agricultural University, Coimbatore situated at 11Nº and 77Eº longitude with at an altitude of 426.7 m above mean sea level. This study included two cultivars each of barnyard millet (Co 1 and Co 2) and kodo millet (Co 3 and APK), three cultivars each of proso millet (Co 3, Co 4, and Co 5), little millet (Co2, Co 3 and Co4) and foxtail millet (Co 5, Co 6 and Co 7). Together, 13 cultivars of five small millets were planted

Following randomized complete block design with three replications. The experiment received NPK in the form of urea, single super phosphate and muriate of potash, respectively at the rate of 44: 22: 15 kg/ha. Full dose of P was applied as basal, whereas, N was applied in two splits, one as basal and another at 30 days after sowing (DAS). Potassium in the form of Muriate of potash was applied at $20^{th}$ and $40^{th}$ DAS.

The data on agronomic traits such as days to 50% flowering, plant height, panicle weight, 1000 grain weight, grain yield, straw yield and biological yield were recorded. Further, harvest index was estimated using grain yield divided by biological yield. Biochemical constituents such as soluble protein, nitrate reductase, proline, catalase activity and peroxidase activity were recorded at five different crop growth stages: vegetative, flowering, grain development, grain maturation and at harvest. Soluble protein content was estimated from the leaf samples following the method of Lowry *et al.* (1951) [7] and expressed as mg/g fresh weight. Nitrate reductase activity was estimated by following the method of Nicholas *et al*. (1976) [11] and the enzyme activity was expressed as µmol $NO_2$ $g^{-1}$ $h^{-1}$.Proline accumulation in the leaf was estimated by the method of Bates *et al.* (1973) [1] and expressed in mg/g fresh weight. Catalase activity was determined according to Teranishi *et al*. (1974) [12] and expressed as µg of $H_2O_2$ reduced $min^{-1}$ $g^{-1}$ fresh weight. Peroxidase activity was determined according to and expressed as µmol $min^{-1}$ $mg^{-1}$**.**

The data collected on the different parameters were statistically analysed by the 'F' test for significance as suggested by Gomez and Gomez (2010) [4]. The critical difference (CD) was computed at 5% probability. Biochemical traits at different crop growth stages were compared following Newman and Keul' stest (Newman 1939; Keuls 1952) [10, 6] using the GenStat 17th edition (http://www.genstat.co.uk).

## Results and Discussion

Small millets play an important role in food and nutritional security of the rural poor in dry land agriculture. However, from the past few decade small millets became a minor and continued under research and utilized, due to the cultivation of other highly yielding and commercial crops. Small millets are highly nutritious and have several health perspectives. Small millets also climate resilient crops, and they are less affected by insect pests and diseases and abiotic stress. Small millets cultivation and consumption has been a drastic declined in production mainly due to limited productivity, high drudgery involved in their processing and negative perceptions of small millets as a food for the poor.

In this study, we assessed the biochemical constituents such as soluble protein, proline, nitrate reductase and catalase at five different growth stages (vegetative, flowering, grain development, grain maturity and harvest) and five small millets (foxtail millet, proso millet, kodo millet, little millet and barnyard millet). Understanding the biochemical changes in crops plants at different stages helps to understand their adaptations and crop characteristics.

### Agronomic traits

Small millets cultivars used in this study included two each in barnyard and kodo millets, and three each in foxtail millet, proso, and little millets. Flowering duration of these cultivars varied from 40 to 65 DAS (Table 1.). Except kodo millet, remaining four crops' cultivars flowered within 52 DAS, and matured in less than 95 DAS. Barnyard millet cultivars were tallest (113 to 120 cm), while the Kodo millet cultivars were shortest (94 to 96 cm). The highest panicle weight was found in foxtail millet cultivar Co 7, and the least was in little millet cultivar Co 2. The 1000 grain weight of these small millets varied from 2.7 g to 4.5g, and the cultivars such as Co 7 of foxtail millet and Co 4 of little millet had maximum 1000 seed weight of 4.5 and 4.4 g. Grain yields of small millets cultivars varied from 1133 kg ha-1 (APK of kodo millet) to 3499 kg ha-1 (Co 7 of foxtail millet), and straw yield varied from 5083 kg $ha^{-1}$to7666 kg ha-1. Harvest index varied from 0.27to 4.10 among cultivars. Harvest index was highest in kodo millet (0.39 to 0.41, mean 0.40) and was lowest in foxtail millet (0.27 to 0.32, mean of 0.28).The foxtail millet cultivars yielded an average of 3033 kg/ha followed by proso millet (2877 kg/ha), and least was in kodo millet (1575 kg/ha).

**Table 1:** Small millets cultivars and their agronomic performance.

| Crop | Days to 50% flowering | Plant height at maturity | Days to maturity (days) | Panicle weight (g plant-1) | 1000 grain weight (g plant-1) | Grain yield/plot Kg | Grain yield (Kg/ha) ha | Biological yield (g plant-1) | Harvest index |
|---|---|---|---|---|---|---|---|---|---|
| Barnyard millet | | | | | | | | | |
| CO 1 | 52 | 113 | 95 | 34 | 3.2 | 2.6 | 2197 | 77.25 | 0.35 |
| CO 2 | 48 | 120 | 92 | 38 | 3.9 | 3.7 | 3091 | 92.54 | 0.38 |
| Foxtail millet | | | | | | | | | |
| CO 5 | 43 | 106 | 89 | 25 | 3.1 | 3.2 | 2716 | 72.96 | 0.27 |
| CO 6 | 43 | 106 | 87 | 27 | 3.7 | 3.4 | 2883 | 76.38 | 0.28 |
| CO 7 | 40 | 111 | 83 | 39 | 4.5 | 4.2 | 3499 | 84.53 | 0.31 |
| Proso millet | | | | | | | | | |
| CO 3 | 47 | 105 | 94 | 25 | 3.2 | 3.4 | 2883 | 68.75 | 0.39 |
| CO 4 | 45 | 106 | 93 | 22 | 3.2 | 3.2 | 2666 | 73.79 | 0.39 |
| CO 5 | 44 | 113 | 90 | 29 | 3.6 | 3.7 | 3083 | 78.83 | 0.4 |
| Kodo millet | | | | | | | | | |
| CO 3 | 60 | 96 | 115 | 26 | 3.6 | 2.4 | 2016 | 56.37 | 0.41 |
| APK | 65 | 94 | 122 | 22 | 2.7 | 1.3 | 1133 | 48.85 | 0.39 |
| Little millet | | | | | | | | | |
| CO 2 | 48 | 103 | 86 | 20 | 3.2 | 2.9 | 2466 | 55.56 | 0.35 |
| CO 3 | 47 | 105 | 83 | 24 | 3.2 | 3.0 | 2499 | 60.19 | 0.36 |
| CO 4 | 43 | 106 | 80 | 24 | 4.4 | 3.3 | 2774 | 64.06 | 0.38 |
| Mean | 48 | 107 | 93 | 27 | 3.50 | 3.10 | 2608 | 70.00 | 0.36 |
| SED | | 0.113 | | 0.981 | 0.009 | 0.002 | 9.630 | 0.204 | 0.001 |
| CD(0.05) | | 0.234 | | 0.202 | 0.018 | 0.024 | 19.87 | 0.422 | 0.002 |

**Biochemical traits**

Biochemical traits such as soluble protein, nitrate reductase, proline and catalase activity were estimated at five different growth stages of small millets (Table 2 and 3). Soluble protein content was the highest in foxtail millet at vegetative (14.70), flowering (160.90), grain development (18.43) and grain maturation (12.90) stages and was second largest at harvest (7.40) stage harvest, and significantly different at all stages (Table 2). Among five crop growth stages, soluble protein content was the highest at grain development stage (14.43 in proso millet to 18.43 in foxtail millet) and the lowest at harvest (6.25 in barnyard millet and 8.05 in kodo millet) stage in all five small millets (Table 2 and Figure 1).

**Table 2:** Mean performance of small millets for different physiological traits at different growth stages.

| Crop and trait | Crop Stages# | | | | |
|---|---|---|---|---|---|
| | Vegetative | Flowering | Grain development | Grain maturation | Harvest |
| **Soluble protein(mg $g^{-1}$)** | | | | | |
| Barnyard millet | 8.90ab | 11.40bc | 14.75c | 11.95bc | 6.25a |
| Foxtail millet | 14.70bc | 16.90cd | 18.43d | 12.90b | 7.40a |
| Proso millet | 9.26b | 12.90c | 14.43c | 10.57b | 6.53a |
| Kodo millet | 9.15a | 12.80ab | 15.65b | 11.40ab | 8.05 |
| Little millet | 10.57b | 13.03c | 16.07d | 12.37c | 5.77a |
| **Nitrate reductase(µg $NO_2$ $g^{-1}$ $hr^{-1}$)** | | | | | |
| Barnyard millet | 36.35a | 45.84a | 59.00a | 60.98a | 39.61a |
| Foxtail millet | 34.56a | 47.49b | 64.46c | 72.35d | 34.35 |
| Proso millet | 38.26a | 46.21a | 56.93b | 65.84b | 35.58a |
| Kodo millet | 38.08a | 44.79a | 61.55b | 64.63b | 38.61a |
| Little millet | 31.00a | 43.17b | 60.21c | 61.76c | 36.86ab |
| **Proline(mg/g)** | | | | | |
| Barnyard millet | 1.23b | 1.29bc | 2.02d | 1.40c | 1.07a |
| Foxtail millet | 1.24b | 1.31b | 1.64c | 1.36b | 1.02a |
| Proso millet | 1.21b | 1.34c | 1.43c | 1.31c | 1.04a |
| Kodo millet | 1.24a | 1.32a | 1.65b | 1.33a | 1.04a |
| Little millet | 1.22ab | 1.29b | 1.85c | 1.35b | 1.07a |
| **Catalase activity** | | | | | |
| Barnyard millet | 6.95a | 9.77b | 9.11b | 8.94b | 10.94c |
| Foxtail millet | 5.67a | 9.17a | 8.14a | 6.91a | 8.11a |
| Proso millet | 3.20a | 7.74c | 6.21bc | 4.97ab | 5.83bc |
| Kodo millet | 2.60a | 7.54b | 4.47ab | 2.77a | 4.53abc |
| Little millet | 2.00a | 5.83c | 3.56b | 2.05a | 4.08b |
| **Peroxidase activity** | | | | | |
| Barnyard millet | 24.48a | 43.92c | 46.12c | 75.55d | 34.52b |
| Foxtail millet | 16.89a | 38.83b | 43.19b | 75.17c | 27.47ab |
| Proso millet | 16.67a | 25.43b | 34.22c | 45.72d | 14.68a |
| Kodo millet | 20.53a | 38.80b | 41.15b | 82.53c | 39.40b |
| Little millet | 17.47 | 36.28 | 44.30b | 58.33c | 18.80 |

#Growth Stages: Mean values of a trait at different growth stages were compared using Neman and Kuel's test (Newman 1939; Keuls 1952) [10, 6]. The means followed by different letter for a given trait and crop at different stages indicating significant difference at 5% probability.

**Table 3:** Soluble protein (mg $g^{-1}$) content of small millets at different growth stages.

| Crop | Stages | | | | |
|---|---|---|---|---|---|
| | Vegetative | Flowering | Grain development | Grain maturation | Harvest |
| Foxtail millet | | | | | |
| CO 5 | 13.4 | 17.5 | 19.9 | 13.6 | 6.5 |
| CO 6 | 14.2 | 15.7 | 16.6 | 12.3 | 6.9 |
| CO 7 | 16.5 | 17.5 | 18.8 | 12.8 | 8.8 |
| Kodo millet | | | | | |
| CO 3 | 9.8 | 11.7 | 16.8 | 12.5 | 7.7 |
| APK | 8.5 | 13.9 | 14. 5 | 10.3 | 8.4 |
| Little millet | | | | | |
| CO 2 | 10.1 | 13.0 | 15.7 | 12.6 | 5.3 |
| CO 3 | 10.5 | 12.5 | 14.8 | 11.8 | 5.7 |
| CO 4 | 11.1 | 13.6 | 17.7 | 12.7 | 6.3 |
| Barnyard millet | | | | | |
| CO 1 | 8.3 | 10.2 | 13.1 | 11.5 | 5.7 |
| CO 2 | 9.5 | 12.6 | 16.4 | 12.4 | 6.8 |
| Proso millet | | | | | |
| CO 3 | 8.72 | 11.5 | 13.1 | 10.1 | 6.2 |
| CO 4 | 9.52 | 12.3 | 14.0 | 10.4 | 5.6 |
| CO 5 | 9.53 | 14.9 | 16.2 | 11.2 | 7.8 |
| Mean | 10.74 | 13.61 | 15.97 | 11.86 | 6.75 |
| SED | 0.0403 | 0.0367 | 0.0339 | 0.0178 | 0.0189 |
| CD (0.05) | 0.0833 | 0.0756 | 0.0700 | 0.0368 | 0.0379 |

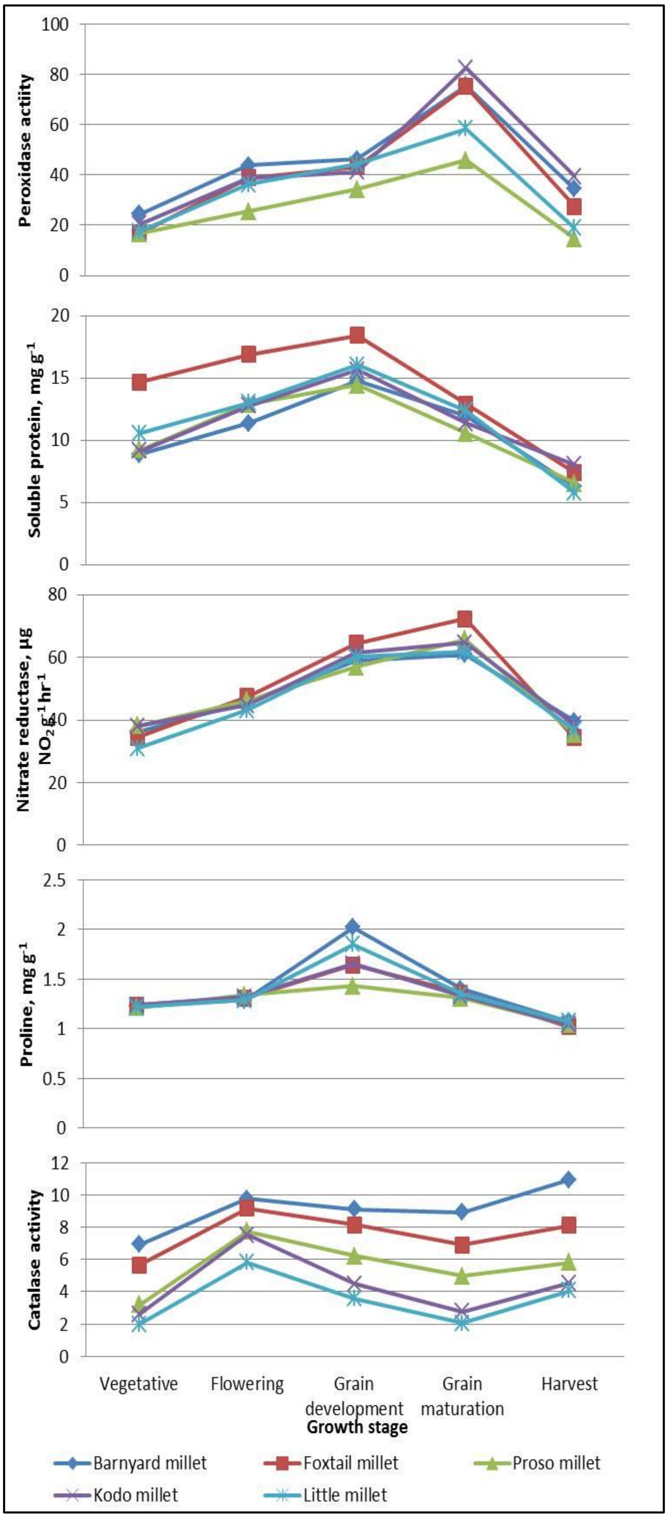


**Fig 1:** Biochemical traits at different growth stages in small millets

In this study, soluble protein was the maximum in foxtail millet in first four growth stages, and was the second highest at harvest stage, as compared to other small millets. It reached the maximum at grain developmental stage and reduced at maturity. The soluble protein content of the leaf, being a measure of RuBP carboxylase activity was considered as an index for photosynthetic efficiency. There were reports that RuBP-case enzyme forms nearly 80% of the soluble proteins in leaves of many plants (Joseph *et al*., 1981) [5]. Diethelm and Shibles (1989) [3] opined that the RUBISCO content per unit leaf area was positively correlated with that of soluble protein content of the leaf. Soluble protein, world's most abundant protein containing the enzyme RUBISCO, is involved in $CO_2$ assimilation; therefore, the reduction in soluble protein might have a direct adverse effect on photosynthesis.

Nitrate reductase was the highest at grain maturation stage (61.76 in little millet to 72.35 in foxtail millet) and lowest during vegetative (31.0 in little millet to 38.26 in proso millet) and harvest (34.35 in foxtail millet to 39.61 in barnyard millet) stages. Nitrate reductase was the maximum at flowering, grain development and grain maturation stages in foxtail millet, while in proso millet at flowering and barnyard millet at harvest stage. We have observed the maximum nitrate reductase (NR) activity at flowering, grain development and grain maturity stages in foxtail millet, while it was the maximum at harvest stage in barnyard millet, it reached the maximum at grain development stage in all five small millets. Nitrate reductase (NR) is a key enzyme for nitrogen acquisition by plants, algae, yeasts, and fungi (Chamizo-Ampudia *et al*. 2017) [2]. Nitrate reductase plays a pivotal role in the plant nitrogen supplement for growth, development and productivity, in the initiation of nitrogen metabolism and the level of protein synthesis, and NR mediates conversion of nitrate to nitrite.

Proline content was the highest at grain developmental stage and reduced significantly afterwards in all five small millets (Table 2). A maximum proline content of 2.02 was found at grain development stage in barnyard millet. It is well described that under stress conditions many plant species accumulate proline as an adaptive response to adverse conditions and plays important role in plant growth and development. In this study, the proline accumulation was highest at grain development stage in all the small millets (1.43 to 2.02 mg/g) and was the maximum in barnyard millet, followed by little millet, kodo millet and foxtail millet and was low in proso millet at grain development stage. This shows that the barnyard millet cultivars able to tolerate under stress compared to other four millets studies, and proline accumulation is the highest at grain developmental stage in all crops.

Catalase activity was highest in barnyard millet at all five growth stages (Table 2), followed by foxtail millet, while lest was in little millet at five crop growth stages (Figure 1). Within each crop, a cultivar having high soluble protein, nitrate reductase, proline,and peroxidase activity had higher grain yield (Table 3, 4, 5, 6, 7), while cultivars with high catalase activity had comparatively low yield, except little millet (Table 8).

**Table 4:** Nitrate reductase activity (μg $NO_2$ $g^{-1}$ $hr^{-1}$) of small millets at different growth stages.

| Crop | Stages | | | | |
|---|---|---|---|---|---|
| | Vegetative | Flowering | Grain development | Grain maturation | Harvest |
| Foxtail millet | | | | | |
| Co 5 | 31.26 | 46.79 | 61.11 | 67.21 | 35.32 |
| Co 6 | 35.95 | 47.68 | 64.11 | 71.74 | 31.00 |
| Co 7 | 36.47 | 48.00 | 68.16 | 78.11 | 36.74 |
| Kodo millet | | | | | |
| Co 3 | 37.84 | 47.74 | 64.95 | 70.11 | 39.74 |
| APK | 38.32 | 41.84 | 58.16 | 59.16 | 37.47 |
| Little millet | | | | | |
| Co 2 | 31.42 | 41.00 | 56.58 | 58.11 | 33.11 |
| Co 3 | 30.26 | 40.26 | 58.21 | 59.95 | 34.47 |
| Co 4 | 31.32 | 48.26 | 65.84 | 67.21 | 43.00 |
| Barnyard millet | | | | | |
| Co 1 | 34.05 | 43.37 | 51.47 | 54.53 | 38.11 |
| Co 2 | 38.65 | 48.32 | 66.53 | 67.42 | 41.11 |
| Proso millet | | | | | |
| Co 3 | 38.74 | 45.11 | 51.00 | 61.79 | 29.63 |
| Co 4 | 38.58 | 46.26 | 55.11 | 64.79 | 30.42 |
| Co 5 | 37.47 | 47.26 | 64.68 | 70.95 | 46.68 |
| Mean | 35.41 | 45.53 | 60.45 | 65.47 | 36.68 |
| SED | 0.053 | 0.047 | 0.094 | 0.107 | 0.083 |
| CD (0.05) | 0.110 | 0.098 | 0.194 | 0.221 | 0.171 |

**Table 5:** Proline Content (mg $g^{-1}$) of small millets at different growth stages.

| Crop | Stages | | | | |
|---|---|---|---|---|---|
| | Vegetative | Flowering | Grain development | Grain maturation | Harvest |
| Foxtail millet | | | | | |
| Co 5 | 1.22 | 1.30 | 1.59 | 1.32 | 1.01 |
| Co 6 | 1.25 | 1.31 | 1.78 | 1.35 | 1.02 |
| Co 7 | 1.26 | 1.32 | 1.56 | 1.42 | 1.03 |
| Little millet | | | | | |
| Co 2 | 1.20 | 1.27 | 1.71 | 1.29 | 1.09 |
| Co 3 | 1.21 | 1.28 | 1.78 | 1.30 | 1.08 |
| Co 4 | 1.24 | 1.31 | 2.05 | 1.47 | 1.04 |
| Kodo millet | | | | | |
| Co 3 | 1.25 | 1.35 | 1.78 | 1.38 | 1.01 |
| APK | 1.22 | 1.29 | 1.51 | 1.27 | 1.06 |
| Barnyard millet | | | | | |
| Co 1 | 1.21 | 1.28 | 1.96 | 1.36 | 1.05 |
| Co 2 | 1.24 | 1.29 | 2.08 | 1.45 | 1.09 |
| Proso millet | | | | | |
| Co 3 | 1.18 | 1.29 | 1.33 | 1.29 | 1.04 |
| Co 4 | 1.20 | 1.36 | 1.43 | 1.30 | 1.02 |
| Co 5 | 1.25 | 1.36 | 1.54 | 1.35 | 1.07 |
| Mean | 1.22 | 1.31 | 1.70 | 1.35 | 1.05 |
| SED | 0.0004 | 0.0005 | 0.0038 | 0.0011 | 0.0005 |
| CD (0.05) | 0.0008 | 0.0010 | 0.0079 | 0.0022 | 0.0010 |

**Table 7:** Peroxidase activity of small millets.

| Crop | Stages | | | | |
|---|---|---|---|---|---|
| | Tillering | Vegetative | Panicle initiation | Grain development | Grain maturation |
| Foxtail millet | | | | | |
| Co5 | 18.11 | 45.50 | 40.35 | 62.60 | 20.10 |
| Co6 | 14.74 | 34.05 | 49.68 | 73.65 | 25.30 |
| Co7 | 17.82 | 36.93 | 39.55 | 89.25 | 37.00 |
| Little millet | | | | | |
| Co2 | 19.50 | 31.78 | 43.05 | 49.95 | 16.90 |
| Co3 | 18.91 | 37.45 | 47.05 | 50.35 | 18.40 |
| Co4 | 14.00 | 39.60 | 42.80 | 74.70 | 21.10 |
| Kodo millet | | | | | |
| Co3 | 22.03 | 41.80 | 43.65 | 85.05 | 39.40 |
| APK | 19.03 | 35.80 | 38.65 | 80.01 | 39.40 |
| Barnyard millet | | | | | |
| Co1 | 23.62 | 42.41 | 44.11 | 72.44 | 33.16 |
| Co2 | 25.34 | 45.44 | 48.13 | 78.65 | 35.88 |
| Proso millet | | | | | |
| Co3 | 15.03 | 18.60 | 28.10 | 43.60 | 12.10 |
| Co4 | 16.31 | 29.05 | 39.40 | 45.20 | 15.35 |
| Co5 | 18.68 | 28.63 | 35.15 | 48.35 | 16.60 |
| Mean | 18.70 | 35.93 | 41.51 | 65.67 | 25.44 |
| SED | 0.055 | 0.124 | 0.094 | 0.266 | 0.164 |
| CD(0.05%) | 0.114 | 0.256 | 0.193 | 0.549 | 0.339 |

**Table 8:** Catalase activity of small millets at different growth stages.

| Treatments | Stages | | | | |
|---|---|---|---|---|---|
| | **Tillering Stage** | **Vegetative stage** | **Panicle initiation stage** | **Grain development stage** | **Grain maturation stage** |
| Foxtail millet | | | | | |
| Co 5 | 6.5 | 9.67 | 8.97 | 8.73 | 9.22 |
| Co 6 | 5.8 | 8.60 | 8.21 | 7.78 | 8.87 |
| Co 7 | 4.7 | 9.23 | 7.24 | 4.21 | 6.25 |
| Little millet | | | | | |
| Co 2 | 2.2 | 6.05 | 3.91 | 2.31 | 4.30 |
| Co 3 | 2.0 | 5.84 | 3.56 | 2.14 | 4.21 |
| Co 4 | 1.8 | 5.62 | 3.22 | 1.71 | 3.72 |
| Kodo millet | | | | | |
| Co 3 | 2.8 | 8.96 | 4.92 | 3.09 | 4.65 |
| APK | 2.4 | 6.12 | 4.02 | 2.46 | 4.42 |
| Barnyard millet | | | | | |
| Co 1 | 7.1 | 10.2 | 8.89 | 9.24 | 11.01 |
| Co 2 | 6.8 | 9.34 | 9.32 | 8.64 | 10.87 |
| Proso millet | | | | | |
| Co 3 | 3.7 | 8.76 | 7.15 | 6.75 | 7.21 |
| Co 4 | 3.2 | 7.12 | 5.76 | 4.12 | 5.02 |
| Co 5 | 2.7 | 7.34 | 5.72 | 4.04 | 5.25 |
| Mean | 3.97 | 7.91 | 6.22 | 5.02 | 6.54 |
| SED | 0.032 | 0.027 | 0.036 | 0.045 | 0.043 |
| CD (0.05) | 0.066 | 0.055 | 0.075 | 0.095 | 0.088 |

Barnyard millet had the highest catalase activity in all five growth stages, followed by foxtail millet, and the lowest catalase activity was found in little millet across growth stages. Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide to water and oxygen. The enzyme is found in all aerobic eukaryotes and is important in the removal of hydrogen peroxide generated in peroxisomes by oxidases involved β-oxidation of fatty acids, the glyoxylate cycle (photorespiration) and purine catabolism.

In conclusion, the biochemical contents follows similar pattern at different growth stages in all five small millets investigated. However concentration varies with crops, suggesting differential response of crops with respect to their adaptation. Within each crop, a cultivar having high soluble protein, nitrate reductase, proline, and peroxidase activity had higher grain yield, while cultivar with high catalase activity had comparatively low yield, except little millet.

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