International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 601-604

Received: 01-09-2017
Accepted: 03-10-2017

R Samundeswari
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

D Durga Devi
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

P Jeyakumar
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
R Samundeswari
Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Assessment of biochemical basis of yield variation in proso millet and little millet

R Samundeswari, D Durga Devi and P Jeyakumar

Abstract
Proso millet and little millet is a highly nutritious cereal grain used for human consumption, bird seed, and/or ethanol production. Unique characteristics, such as drought and heat tolerance, make proso and little millet a promising alternative cash crop for future climate scenario. These smallmillets are act as important climate-smart nutritious crops. Understanding the biochemical changes in proso millet and little millet 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 two small millets cultivars (prosomillet and little millet). The proline accumulation was highest at grain development stage in these two small millets (1.54 to 2.05 mg/g) and was the maximum in liitle millet. Soluble protein and nitrate reductase activity were the maximum in little millet; both increased from flowering to grain development stages and decreased in later stages. Proso millet had the highest catalase activity in all five growth stages and the lowest catalase activity was found in little millet across growth stages. Within each crop, a cultivar having high proline, soluble protein, nitrate reductase, 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, growth stages

Introduction
Small millets of proso millet and little millets are considered as important nutri-rich climate-smart crops (Vetriventhan and Upadhyaya 2015) [13]. These 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) [12]. Small millets of little and proso millets are suited to conditions of low and moderate rainfall area due to early maturity and their adaptation to drought 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, these small millets are cultivated in limited area of 0.82 m ha and occupy about 3.7 lakhs ha with a production of 2.67 lakhs tons, with a productivity of 310 kg/ha (averaged between 2006-2010). Potential yields of up to 2 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 little and proso 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 proline, soluble protein, nitrate reductase, catalase and peroxidase 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 three cultivars each of little millet (Co 2, Co 3 and Co 4) and three cultivars each of proso millet (Co 3, Co 4, and Co 5). Together, 6 cultivars of two small millets were planted following randomized complete block design with three replications. The experiment received NPKin the form of urea, single super phosphate and muriate of potash, respectively 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 20th and 40thDAS.

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 proline, soluble protein, nitrate reductase, catalase activity and peroxidase activity were recorded at five different crop growth stages: vegetative, flowering, grain development, grain maturation and atharvest. Proline accumulation in the leaf was estimated by the method of Bates *et al.* (1973) [1] and expressed in mg/g fresh weight. Soluble protein content was estimated from the leaf samples following the method of Lowry *et al.* (1951) [6] and expressed as mg/g fresh weight. Nitrate reductase activity was estimated by following the method of Nicholas *et al*. (1976) [10] and the enzyme activity was expressed as µmol NO_2 g^{-1} h^{-1}.Catalase activity was determined according to Teranishi *et al*. (1974) [11] and expressed as µg of H_2O_2 reduced min^{-1} g^{-1} fresh weight. Peroxidase activity was determined according to Addy and Goodman (1972) 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). 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) [9, 5] using the GenStat 17th edition (http://www.genstat.co.uk).

Results and Discussion

Biochemical Traits

Biochemical traits such as proline, soluble protein, nitrate reductase, catalase and peroxidase activity were estimated at five different growth stages of little and proso millets cultivars (Table 1 and 2). Proline content was the highest at grain developmental stage and reduced significantly afterwards in two of these small millets (Table 2). A maximum proline content of 1.85 was found at grain development stage in little 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 (Mattioli *et al.* 2008). In this study, the proline accumulation was highest at grain development stage in all small millets cultivars (1.43 to 1.85 mg/g) and was the maximum in little millet and was low in proso millet at grain development stage. This shows that the little millet cultivars able to tolerate under stress compared to proso millet cultivars, and proline accumulation is the highest at grain developmental stage these crops.

Soluble protein content was the highest in little millet at vegetative (10.5), flowering (13.03), grain development (16.07), grain maturation (12.37) and harvest stage (5.77) and significantly different at all stages (Table 1 and 3). Among five crop growth stages, soluble protein content was the highest at grain development stage (16.07 in little millet to 14.43 in foxtail millet) and the lowest at harvest (5.77 in little millet and 6.53 in proso millet) stage in two of these small millets. In this study, soluble protein was the maximum in little millet in first four growth stages (16.07 to 14.43 mg/g), and was the second lowest at harvest stage, as compared to proso millets cultivars. 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) [4]. 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 flowering stage (61.76 in little millet to 65.84 in proso millet) and lowest during vegetative (31.0 in little millet to 38.26 in proso millet) and harvest (35.58 in proso millet to 36.86 in little millet) stages (Table 1 and 4). Nitrate reductase was the maximum at flowering and grain development stages in little millet and in proso millet at flowering stage was only found maximum Nrase activity. We have observed the maximum nitrate reductase (NR) activity at flowering and grain development stages in little and proso millets, while it was the maximum at flowering stage in prosomillet, it reached the maximum at grain development stage in these small millet cultivars. 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.

Catalase activity was highest in proso millet at all five growth stages (Table 2), while lest was in little millet at five crop growth stages (Table 1 and 5). Proso millet had the highest catalase activity in all five growth stages 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.

Peroxidase activity was highest in proso millet at all five growth stages (Table 1 and 6), while lest was in little millet at five crop growth stages. Within each crop, a cultivar having high proline, soluble protein, nitrate reductase, catalase and peroxidase activity had higher grain yield, while cultivars with high catalase activity had comparatively low yield, except little millet

Table 1: Mean performance of little and proso millet cultivars 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})					
Little millet	10.57b	13.03c	16.07d	12.37c	5.77a
Proso millet	9.26b	12.90c	14.43c	10.57b	6.53a
Nitrate reductase (µg NO_2 g^{-1} hr^{-1})					
Little millet	31.00a	61.76c	60.21c	43.17b	36.86ab
Proso millet	38.26a	65.84b	56.93b	46.21a	35.58a
Proline (mg/g)					
Little millet	1.22ab	1.29b	1.85c	1.35b	1.07a
Proso millet	1.21b	1.34c	1.43c	1.31c	1.04a
Catalase activity					
Little millet	2.00a	5.83c	3.56b	2.05a	4.08b
Proso millet	3.20a	7.74c	6.21bc	4.97ab	5.83bc
Peroxidase activity					
Little millet	17.47	36.28	44.30b	58.33c	18.80
Proso millet	16.67a	25.43b	34.22c	45.72d	14.68a

Table 2: Proline Content (mg g^{-1}) of little millet and proso millet cultivars at different growth stages

Crop	Stages				
	Vegetative	Flowering	Grain development	Grain maturation	Harvest
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
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	1.21	1.31	1.64	1.33	1.06
SED	0.008	0.186	0.006	0.015	0.013
CD (0.05)	0.017	0.039	0.014	0.033	0.027

Table 3: Soluble protein (mg g^{-1}) content of little and prosomillet cultivars at different growth stages

Crop	Stages				
	Vegetative	Flowering	Grain development	Grain maturation	Harvest
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
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	9.91	12.96	14.65	11.46	6.15
SED	0.101	0.190	2.46	0.219	0.077
CD (0.05)	0.216	0.405	5.259	0.466	0.165

Table 4: Nitrate reductase activity (µg NO_2 g^{-1} hr^{-1}) of little and proso millet cultivars at different growth stages

Crop	Stages				
	Vegetative	Flowering	Grain development	Grain maturation	Harvest
Little millet					
Co 2	31.42	58.11	56.58	41.00	33.11
Co 3	30.26	59.95	58.21	40.26	34.47
Co 4	31.32	67.21	65.84	48.26	43.00
Proso millet					
Co 3	38.74	61.79	51.00	45.11	29.63
Co 4	38.58	64.79	55.11	46.26	30.42
Co 5	37.47	70.95	64.68	47.26	46.68
Mean	34.63	63.80	58.57	44.57	36.21
SED	0.574	1.289	0.849	0.329	0.304
CD (0.05)	1.223	2.747	1.809	0.701	0.648

Table 5: Catalase activity (µg of H_2O_2min^{-1} g^{-1}) of little and proso millet cultivars at different growth stages

Treatments	Stages				
	Vegetative	Flowering	Grain development	Grain maturation	Harvest
Little millet					
Co 2	2.2	6.05	3.91	2.31	1.30
Co 3	2.0	5.84	3.56	2.14	1.21
Co 4	1.8	5.62	3.22	1.71	1.31
Proso millet					
Co 3	3.7	8.76	7.15	6.75	5.21
Co 4	3.2	7.12	5.76	4.12	4.02
Co 5	2.7	7.34	5.72	4.04	3.25
Mean	2.6	6.79	4.89	3.51	2.71
SED	0.048	0.072	0.068	0.039	0.026
CD (0.05)	0.102	0.154	0.149	0.081	0.055

Table 6: Peroxidase activity (µmol min^{-1} mg $^{-1}$) of little and proso millet cultivars at different growth stages

Crop	Stages				
	Vegetative	Flowering	Grain development	Grain maturation	Harvest
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
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	17.07	30.85	39.25	52.02	16.74
SED	0.179	0.551	0.687	0.043	0.205
CD (0.05%)	0.383	1.174	1.466	0.911	0.438

Conclusion

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

References

1. Bates LS, Waldren RD, Teare ID. Rapid determination of free Proline in water stress studies. Plant and Soil. 1973; 39:205-208.
2. Chamizo-Ampudia A, Sanz-Luque E, Llamas A, Galvan A, Fernandez E. Nitrate reductase regulates plant nitric oxide homeostasis. Trends in Plant Science. 2017; 22:163-174.
3. Diethelm R, Shibles R. Relationship of enhanced sink demand with photosynthesis and amount and activity of Ribulose-1, 5 biphosphate carboxylase in soybean leaves. Journal of Plant Physiology. 1989; 134:70-74.
4. Joseph MC, Randall DD, Nelson CJ. Photosynthesisin polyploid tall fescue. II. Photosynthesis and RUBP case of polyploid tall fescue. Plant Physiology. 1981; 68:894-898.
5. Keuls M. The use of the “Studentized range” in connection with an analysis of variance. Euphytica. 1952; 1:112-122.
6. Lowry OH, Rose Brought NT, Farr LA, Randall RJ. Protein measurement with folin phenol reagent. Journal of Biology and Chemistry. 1951; 192:265-275.
7. Mattioli R, Costantino P, Trovato M. Proline accumulation in plants: not only stress. Plant Signal Behaviour. 2009; 4(11):1016-1018.
8. Mujtaba SM, Khan MA, KBKMUSKMA, Nitrate SMK. reductase activity, nitrate and relative water contents in wheat as fluctuated during 24 h cycle under water stress. Sky Journal of Agricultural Research. 2016; 5(8):161-167.
9. Newman D. The distribution of range in samples from a normal population expressed in terms of an independent estimate of standard deviation. Biometrika. 1939; 31:20-30.
10. Nicholas JC, Harper JS, Hageman RH. Nitrate reductase activity in soybean. Effect of light and temperature. Plant Physiology. 1976; 58:731-735.
11. Teranishi Y, Tanaka A, Osumi M, Fukui S. Catalase activity of hydrocarbon utilizing candida yeast. Agricultural biology and Chemistry. 1974; 38:1213-1216.
12. Upadhyaya HD, Gowda CLL, Reddy VG, Singh S. Diversity of small millets germplasm in genebank at ICRISAT. In: 5th International Symposium on New Crops and Uses: their role in a rapidly changing world, 3-4 September, 2007, University of Southampton, Southampton, UK, 2008.
13. Vetriventhan M, Upadhyaya HD, Dwived SL, Pattanashetti SK, Singh SK. Finger and foxtail millets. In Singh, M., Upadhyaya, H.D. (eds.), Genetic and Genomic Resources for Grain Cereals Improvement. Oxford: Academic Press, 2015, 291-319.