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Study on variation in photosynthetic area and pigments during different phenological stages of small millets

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Abstract
Despite the high yield potential and wider adaptability, the productivity of small millets is low compared to major millets and cereals. This is mainly due to the lack of research on yield and its contributing traits such as photosynthesis on these marginalised crops. To accomplish this, a comprehensive study was taken up to in a pot culture experiment with six cultivated small millets, *viz.,* finger millet (CO15), little millet (CO4), barnyard millet (CO2), proso millet (CO5), foxtail millet (CO7) and kodo millet (CO3) during *Rabi,* 2018 to explore the photosynthesis and its related traits involved in yield production. The photosynthetic area and pigments were analysed in this study which are considered as vital traits for photosynthesis process. In this study, it is observed that that there was a considerable variation in photosynthetic area and pigments concentration in leaves among the six small millets studied. But there was no positive correlation observed between leaf area and the concentration of photosynthetic pigments. The highest leaf area was measured in finger millet and the highest contents of photosynthetic pigments, chlorophyll a, b, total and carotenoid recorded in proso millet at flowering stage.

Keywords: Small millets, leaf area, chlorophyll pigments, carotenoids

Introduction

At present, the increase in demand for primary food grains is outstripping increase in yields, an expanding gap that indicates large potential food shortages by mid of this century. This comes at a time when yield improvements are slowing or stagnating as the approaches of the Green Revolution reach their biological limits. This compels to opt for alternate crop species such as millets which are nutritionally equal or superior over other cereals but still underutilized. Millet is a collective term commonly referred to a number of small seeded annual grass grain crops. Millets such as maize, sorghum and pearl millet are considered as coarse millets while the others are called small millets or minor millets comprising of six species *viz.,* finger millet (*Eleusine coracana*), little millet (*Panicum sumatrense*), foxtail millet (*Setaria italica*), barnyard millet (*Echinochloa frumentaceae*), proso millet (*Panicum miliaceum*) and kodo millet (*Paspalum scrobiculatum*) (Gupta, 2006). Small millets would be more viable and offer great benefits in terms of lower water requirements, adaptation to climate change and marginal soils, along with social impact in terms of the greater self-reliance of local populations as well as more resilient and accessible food systems (Padulosi *et al.,* 2015) [6].

Despite the high yield potential and wider adaptability, the productivity of small millets is low compared to major millets and cereals. This is mainly due to the lack of scientific research on these underutilized crops. Under such scenario, the scientists must broaden the focus of research to include less common food crops i.e. minor millets so as to supplement the demand for food staples and to close the gap in yield increase in modern varieties. However, limited information describing basic aspects of their genetics, physiology and agronomic performance across environments remains a hindrance in exploitation and further crop improvement. Any improvement in minor millets could play a role in the "New Green Revolution" a term coined to reflect novel strategies which will be required to deal with complex challenges in developing nations including increasing population and ever-diminishing arable land (Herder *et al*., 2010) [10]. To accomplish this, a comprehensive study on these underutilized crop plants is an immediate need to fully understand the underpinning physiology and biochemistry linked to various quantitative and qualitative traits of these crops to improve the yield. Photosynthesis is one of the key physiological processes exhibits greater variability among plant species.

Understanding morphological and physiological traits which modulate photosynthesis is one of the reasonable approaches for improving crop productivity. Though there is a clear discrepancy in photosynthetic performance of C3 and C4 species, evidences are much less regarding the variations in physiological, biochemical and structural traits related to photosynthesis among C4 plant species especially these minor millets. This requires a basic understanding of the photosynthetic area (leaf) architecture and photosynthesis related physiological parameters in minor millets.

Improving photosynthesis is a challenging task, but will become increasingly essential if the necessary yield increases are to be achieved especially in crops such as minor millets to supplement the demand for food grains. Investigations into morpho-physiological variations in photosynthesis will provide insights into genetic regulation of this complex trait. Such information could be used to comprehend the processes that affect primary production, allow larger understanding of the genetic regulation of photosynthesis and eventually increase the productivity of crops. Photosynthetic traits of leaves are one of the most important physiological factors responsible for plant productivity. The improvement of photosynthetic traits promises further increases in plant productivity (Evans, 1989; Zhu *et al*., 2010) [3, 10].

The nature and significance of variation in photosynthesis is dependent on the unit of measurement ie. quantified as photosynthetic rate per unit ground area, the rate per individual or the rate per unit area of the leaf. The standard way of describing photosynthesis is as a rate of CO2 fixation per unit leaf area. When considering variation in the properties of photosynthesis, focus must be on those expressed on a unit leaf area basis. Variation in photosynthesis is often associated with chlorophyll content, leaf size and specific leaf mass. Hence, it is planned to study the leaf architecture and pigments composition to determine their contribution towards the variation in photosynthetic efficiency among the minor millets.

Materials and Methods

The pot culture experiment was conducted with six cultivated small millets, *viz.,* finger millet (CO15), little millet (CO4), barnyard millet (CO2), proso millet (CO5), foxtail millet (CO7) and kodo millet (CO3) during *Rabi*, 2018 in Completely Randomized Design (CRD) with four replications. Pot mixture was prepared by using red soil, sand and vermicompost in the ratio of 3:1:1 and the pots (42×30 cm size) were filled with 12 kg of soil. The seeds were directly sown in the pot since minor millets used for the study are direct seeded. After the establishment of seedlings, thinning was done to maintain three seedlings per pot uniformly across the replications. Crop was applied with recommended dose of fertilizers. Fertilizer dosage for pot culture was calculated using per hectare recommendations of small millets and other cultivation operations including plant protection measures were carried out as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore.

Observations on leaf length, leaf width and photosynthetic pigments were recorded at seedling, vegetative, flowering and maturity stages. Since the test crops have different maturity duration, the observations were made separately in individual crops according to their phenological phases after establishment of the crop. Leaf length was measured from the tip to the base of the fully opened third leaf on the primary tiller at different growth stages from the randomly selected plants in each replication and the mean leaf length was expressed in cm. Leaf width was measured in centimeters at the widest portion of the leaf blade at different growth stages from the randomly selected plants in each replication and the mean leaf length was expressed in cm.

To estimate the photosynthetic pigments, leaf samples were collected from randomly selected plants from each replication. Photosynthetic pigments such as Chlorophyll a, Chlorophyll b, total Chlorophylls and Carotenoids content in leaves were estimated by using the method described by Hiscox and Israelstam (1979) [5] and expressed in mg g^{-1} fresh weight. 100 mg of fully expanded young leaf tissue was placed in vial containing 7 ml of Dimethyl sulphoxide (DMSO) and chlorophyll was extracted without grinding at 65°C by incubating overnight. The extract was transferred to graduated tube and made up to 10 ml with DMSO and assayed immediately. Pigments content were calculated following the equations used by Arnon (1949) [1].

Results and Discussion

The length and breadth of leaf are the basis for total leaf area which ultimately act as floor of photosynthesis in a plant. From the data on leaf length and width measured at different phenological stages, it was observed that the leaf length and width increased linearly from seedling to flowering stage in all the millets taken for this study but thereafter the increase in leaf length and width was non significant and a slight decrease in leaf length was observed in barnyard, proso and foxtail millets (Table 1). The rate of increase in leaf length during seedling to flowering was found to be maximum in foxtail millet and the minimum in barnyard millet. The maximum leaf length of 47.43cm was observed in little millet at its flowering stage followed by foxtail millet (45.68cm). Barnyard millet recorded the maximum leaf width (2.63cm) followed by foxtail millet (2.38cm) at reproductive stage. The observations on length and width of leaf of small millets revealed a positive correlation with the leaf area measured in all the small millets taken in this study.

Leaf area or assimilatory surface area gives a fairly good idea of photosynthetic capacity of the plant. The problem of increasing agriculturally yield is fundamentally the problem of manipulation of increase in the total annual photosynthesis per unit area of the crop. It has been demonstrated that yield is always closely correlated with variation in mean leaf area. Large leaf area development aids in the effective interception of light thus leading to higher dry matter production. In the present study, leaf area increased from seedling to flowering stage and showed significant decline towards maturity in all the millets taken for observation (Table 1). The decline in leaf area at maturity is due to progressive senescence of leaves. Shibles and Weber (1966) [7] reported that leaf area increased with the crop age attaining their peak at flowering and decreased thereafter with crop age. Thereby it indicated a decline in the capacity of the plant to produce assimilates. The results of the present study showed that the peak value for leaf area was recorded at flowering stage and the highest leaf area was found in finger millet (2878.7 cm^2) followed by barnyard millet (1837.8 cm^2) at flowering stage and the minimum leaf area was observed in little millet (789.32 cm^2). This was in confirmity with Thandapani (1985) [8] who pointed out that high leaf area at peak vegetative and peak flowering contributes to the better yielding ability of millets. The decline in leaf area after reproductive stage towards maturity might be due to transport of assimilates from the lower leaves to the developing sinks which later caused senescence of lower leaves.

The content of photosynthetic pigments represents the photosynthetic capacity of the plants.

The estimation of photosynthetic pigments such as chlorophylls and carotenoids in minor millets showed a progressive increase over phenological phases upto flowering and towards maturity, a significant decrease in pigments observed between stages and among the crops as well (Table 2). In general, a significant difference in pigments content observed among the small millets in all the stages of crop growth. The variation might be attributed to the ability of these millets to effectively utilize the available resources for building macro and micro molecules such as chlorophylls, enzymes etc., that are directly involved in metabolic activities. Among the small millets, proso millet recorded the highest contents of all the photosynthetic pigments such as chlorophyll a, b, total chlorophyll and carotenoid at flowering stage, followed by little millet which was on par with each other. The rate of increase in chlorophyll ‘a’ content of proso and little millets was high between vegetative to flowering stage compared to other millets. The lowest total chlorophyll and carotenoid contents were observed in kodo millet followed by foxtail millet. But, the chlorophyll ‘b’ was found to be high in barnyard millet followed by finger millet which was on par with proso millet. The rate of increase in total chlorophylls and carotenoids was maximum in proso millet between vegetative and flowering stages followed by little millet. The close relationship between leaf chlorophyll content and photosynthetic rate was observed by Yoshida (1972) [9] and stated that higher chlorophyll is one of the important factors responsible for better yield.

The photosynthetic surface and pigments analysed in this study are the vital traits for photosynthesis process and are having positive correlation with photosynthetic efficiency of crops. In this study, it is observed that that there was a considerable variation in photosynthetic area and pigments concentration in leaves among the six small millets studied. But there was no positive relationship observed between leaf area and the concentration of photosynthetic pigments. The highest leaf area was measured in finger millet and the highest contents of photosynthetic pigments, chlorophyll a, b, total and carotenoid recorded in proso millet at flowering stage.

Table 1: Leaf morphological characters of small millets at different growth stages

Crop	Leaf Length				Leaf Width				Leaf Area			
	SS	VS	FS	MS	SS	VS	FS	MS	SS	VS	FS	MS
Finger millet	16.23	36.14	40.35	41.48	0.63	0.90	1.50	1.88	247.01	749.42	2878.7	2107.2
Barnyard millet	19.43	27.33	38.55	37.58	0.78	1.28	2.60	2.63	354.21	697.71	1837.8	1391.3
Kodo millet	15.10	29.60	41.18	42.18	0.63	0.80	1.18	1.60	182.08	408.95	1608.2	1189.9
Proso millet	15.75	43.55	44.45	43.05	0.95	1.60	1.68	1.83	216.10	336.47	958.08	680.5
Little millet	15.20	41.88	47.43	48.33	0.50	0.98	0.98	1.13	183.42	345.56	789.32	455.8
Foxtail millet	13.15	43.33	45.68	45.23	0.73	1.65	2.33	2.38	253.34	447.14	1596.0	1067.2
Mean	15.81	36.99	42.77	44.48	0.70	1.20	1.71	1.83	239.36	464.13	1611.3	1248.6
SEd	0.29	0.36	0.36	0.39	0.03	0.12	0.19	0.24	6.12	4.86	9.46	7.16
CD (p=0.05)	0.62	0.75	0.75	0.82	0.06	0.25	0.41	0.49	12.87	10.08	19.22	15.08

SS: Seedling stage; VS: Vegetative stage; FS: Flowering stage; MS: Maturity stage

Table 2: Photosynthetic pigments content of small millets at different growth stages

Crop	Chlorophyll ‘a’				Chlorophyll ‘b’				Total Chlorophyll				Carotenoids			
	SS	VS	FS	MS	SS	VS	FS	MS	SS	VS	FS	MS	SS	VS	FS	MS
Finger millet	0.98	1.41	1.80	0.97	0.24	0.33	0.70	0.31	1.23	1.73	2.50	1.28	0.33	0.34	0.48	0.46
Barnyard millet	0.99	1.29	1.41	0.75	0.27	0.38	0.96	0.57	1.27	1.68	2.37	1.32	0.30	0.44	0.52	0.44
Kodo millet	0.94	1.21	1.63	0.60	0.25	0.35	0.57	0.18	1.19	1.56	2.20	0.78	0.36	0.43	0.34	0.26
Proso millet	0.92	1.02	2.18	0.98	0.25	0.28	0.69	0.47	1.17	1.30	2.87	1.45	0.34	0.27	0.72	0.46
Little millet	0.88	1.38	2.22	1.02	0.24	0.38	0.64	0.52	1.12	1.76	2.86	1.54	0.33	0.43	0.60	0.44
Foxtail millet	1.09	1.72	1.84	1.00	0.30	0.44	0.42	0.54	1.40	2.16	2.26	1.55	0.40	0.60	0.62	0.55
Mean	0.97	1.34	1.81	0.89	0.26	0.36	0.66	0.43	1.23	1.70	2.48	1.32	0.34	0.42	0.49	0.45
SEd	0.01	0.02	0.03	0.02	0.03	0.004	0.03	0.02	0.02	0.02	0.03	0.06	0.02	0.04	0.05	0.006
CD (p=0.05)	0.03	0.05	0.06	0.05	0.06	0.010	0.07	0.05	0.05	0.05	0.06	0.12	0.04	0.08	0.11	0.012

SS: Seedling stage; VS: Vegetative stage; FS: Flowering stage; MS: Maturity stage

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