



E-ISSN: 2278-4136

P-ISSN: 2349-8234

www.phytojournal.com

JPP 2021; 10(1): 703-706

Received: 25-11-2020

Accepted: 27-12-2020

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Biochemical changes during Seed development and maturation in little millet cv.CO (Samai) 4

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DOI: <https://doi.org/10.22271/phyto.2021.v10.i1j.13406>

Abstract

Study on seed development and maturation studies in little millet revealed that optimum stage of physiological maturity was attained at 40 days after anthesis (DAA). The individual panicles of bulk crop raised in the field and were tagged at the time of flower opening. The panicles were collected at 5 days intervals up to 45 DAA and subjected to the biochemical changes *viz.*, electrical conductivity, leachate amino acid content, leachate sugars, protein content and dehydrogenase activity in seeds. Biochemical parameters were superior in the seeds collected at 40 DAA.

Keywords: Little millet, physiological maturity, days after anthesis, dehydrogenase activity

Introduction

Good quality seeds implies high vigour, uniformity and its genetic and physical purity. Seed crops should be harvest at its maximum seed quality obtained during physiological maturity. Successful agriculture depends on the quality of seeds used for sowing. Production of quality seeds and its maintenance till next sowing is very important activity in crop production. Stage of harvest is one of the most important factors that can influence the quality of seeds. For seed crop, physiological maturity is the right stage to harvest, found to induce several physiological and biochemical changes during seed growth and development. Present study was planned to study the biochemical changes associated with seed development and maturation in little millet cv. CO (Samai) 4

Materials and Methods

The study was conducted with little millet seeds obtained from the Department of millets, Tamil Nadu Agricultural University, Coimbatore formed the base material for this study. The experiment was conducted at the department of seed science and technology, AC&RI, Madurai to determine the physiological maturity status of the seed. The bulk little millet crop seed was raised in the field. Individual flower heads were tagged at the time of flower opening. The panicles were collected at 5 days intervals and subjected to the following seed quality assessment. Enzyme activities and nutrient accumulation such as electrical conductivity, leachate amino, leachate sugars, protein content and dehydrogenase enzyme activity observations recorded.

Electrical conductivity

Little millet seeds (25 no) were soaked for 12 h with deionized water (50 ml) and the electrical conductivity was measured in replicates with little modification of Presley (1958) ^[12] using Electrical conductivity meter and expressed in dS m⁻¹.

Leachate amino acids

The amount of amino acid in little millet seed leachate was estimated by the method described by Moore and Stein (1948) ^[10] with minor modifications. To 3 ml of leachate, 0.5ml of 0.1M acetic acid sodium acetate buffer (pH 5.3) and 1 ml of 0.5 % Ninhydrin solution were added and the reaction mixture was heated in a boiling water bath for 15 min for colour development. It was then cooled in running water. The colour intensity was measured in Cary UV spectrophotometer at 550 nm. The amount of amino acid was expressed as µg g⁻¹ of seed against a leucine standard.

Leachate sugars

The seed leachates of little millet after the electrical conductivity test were used for determining the amount of sugar following the method described by Somogyi (1952) with minor modifications. Duplicate analyses were made using 3 ml of leachate with 1 ml of copper reagent and boiled for 15 min. After cooling this mixture in running water, 1 ml of Nelson's arsenomolybdate reagent was added for colour development and the final volume was made up to 5 ml with distilled water. The intensity of colour was measured in carry UV spectrophotometer at 580 nm. The amount of leachate sugars was expressed as $\mu\text{g g}^{-1}$ of seed.

Protein content

Protein content was estimated by the colorimetric method as described by Ali-khan and Young (1973) [2]. One hundred milligram of ground seed meal was taken in a 50 ml polyethylene screw cap bottle and 25 ml of 1N sodium hydroxide was added. The mixture was shaken for 15 minutes in a wrist shaker to disperse the protein. Then 10 ml of the suspension was poured into a graduated test tube and used as a blank to compensate for the difference in the amount of natural pigments extracted. To the remaining suspension in bottle, 0.25 ml of 10 per cent copper sulphate solution was added and the bottle was retaken for five minutes to develop the colour complex. The sample solution was then poured in to a separate test tube and kept overnight along with its blank to allow the dispersed material to settle down. The optical density of the clean supernatant solution obtained after centrifugation at 3000 rpm for 10 minutes was measured in

ELICO SL 150 spectrophotometer using a red filter (620nm) against their respective blank. From the optical density values, the protein content was calculated using the following formula and the mean value of five replications was expressed in per cent.

$$\text{Protein content (\%)} = 3.78 + (61.6 \times \text{Optical Density value})$$

Dehydrogenase enzyme activity

The Dehydrogenase enzyme activity of the seeds was estimated in duplicate following the method of Kittock and Law (1968) [9] with minor modifications. 20 seeds from each treatment were preconditioned for 12h between fold of moistened filter papers and five embryonic axes were excised and incubated in darkness with 5ml of 0.2 percent tetrazolium chloride solution in glass vials for one hour. After one hour incubation the tetrazolium chloride solution was decanted and the embryos were thoroughly washed with distilled water and surface dried with blotters. The formazon was eluted by soaking the stained seeds in five ml of methyl cellosolve (2 methoxy ethanol) for one hour and the optical density was measured using Cary UV spectrophotometer at 470 nm.

Statistical analysis

The data obtained from experiments were analyzed by the 'F' test for significance by following Completely Randomized Design. Wherever necessary, the percent values were transformed to angular (Arc-sine) values before analysis. The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance. (Gomez and Gomez, 1984) [5]

Table 1: Changes in biochemical seedling quality characteristics during seed development and maturation in little millet cv.CO (Samai) 4

Days after anthesis (DAA)	Electrical conductivity (dSm^{-1})	Leachate amino acids ($\mu\text{g g}^{-1}$)	Leachate sugars ($\mu\text{g g}^{-1}$)	Protein content (%)	Dehydrogenase enzyme activity (OD value)
5 DAA	0.0	0.0	0.0	0.0	0.0
10 DAA	0.0	0.0	0.0	0.0	0.0
15 DAA	0.0	0.0	0.0	0.0	0.0
20 DAA	0.018	0.023	0.039	18.23	0.08
25 DAA	0.023	0.031	0.044	19.85	0.11
30 DAA	0.030	0.035	0.049	20.24	0.16
35 DAA	0.034	0.038	0.054	20.98	0.18
40 DAA	0.038	0.041	0.060	21.15	0.20
45 DAA	0.035	0.040	0.057	21.10	0.18
SEd	0.0014	0.0005	0.0008	0.242	0.0021
CD (P=0.05)	0.0031**	0.0011**	0.0016**	0.520**	0.0045**

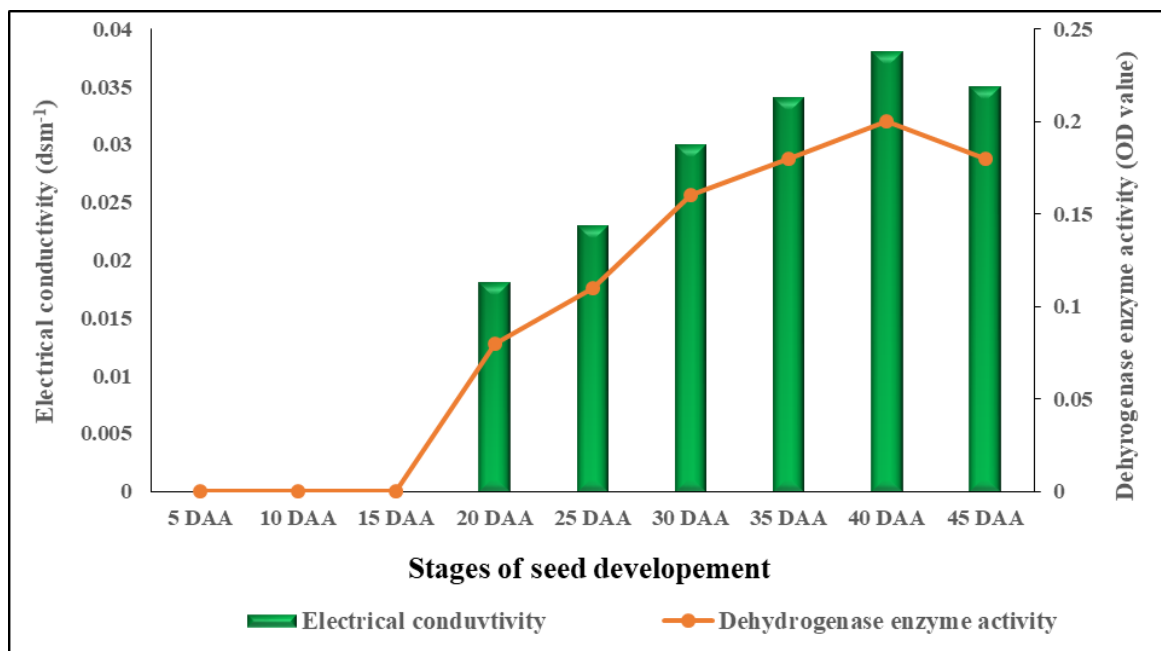


Fig 1: Changes in electrical conductivity and dehydrogenase enzyme activity during seed development and maturation in little millet cv.CO (Samai) 4



Plate 1: Panicle developmental stages in little millet cv. CO (Samai) 4

Results and Discussion

Electrical conductivity was significantly influenced by stages of development with maximum at 40 DAA (0.038 dS m⁻¹) followed by 45 DAA (0.035 dS m⁻¹). The minimum electrical conductivity was recorded at 20 DAA (0.018 dS m⁻¹); whereas, the seeds from first two stages of development (5 and 10 DAA) and did not produce any seeds. Leachate amino acid was significantly influenced by stages of development with maximum at 40 DAA (0.041 μg g⁻¹) followed by 45 DAA (0.040 μg g⁻¹). The minimum leachate amino acids were recorded at 20 DAA (0.023 μg g⁻¹); whereas, the seeds from first two stages of development (5 and 10 DAA) did not produce any seeds. Leachate sugars were significantly differed by recording maximum at 40 DAA (0.060 μg g⁻¹) followed by 45 DAA (0.057 μg g⁻¹). The minimum leachate sugars were recorded at 20 DAA (0.039 μg g⁻¹); whereas, the seeds from first two stages of development (5 and 10 DAA) did not produce any seeds. Protein content (%) was maximum at 40 DAA (21.15 %) followed by (21.10 %). The minimum

Protein content was recorded at 20 DAA (18.23 %); whereas, the seeds from first two stages of development (5 and 10 DAA) did not produce any seeds. Dehydrogenase enzyme activity (OD value) was significantly influenced by developmental stages with maximum at 40 DAA (0.20) followed by 35 and 45 DAA (0.18). The Dehydrogenase enzyme activity (OD value) recorded at 20 DAA was minimum with 0.08 OD value; whereas, the seeds from first two stages of development (5 and 10 DAA) did not produce any seeds. As the maturity of seeds advanced, the electrical conductivity, leachate amino acid content, leachate sugars, protein content and dehydrogenase activity also increased. They reached the maximum at 40 DAA and there after a marginal decrease in this enzyme activity was noticed. The present findings are in agreement with the results of Sridevi, *et al.*, 2019 [14]; Kalavathi *et al.*, 2000 [8]; Noggle and Fritz, 1991 [11]; Dahatonde and Adhao, 1978 [4]; Adib sultana *et al.*, 1994 [1]; Javaregowda, 1986 [7]. According to Harrington (1972) [6], physiological maturity is the stage at which the

seed reaches its maximum dry weight and nutrient flow into the seed from mother plant is ceased due to breakage of vascular connection by the formation of abscission layer. Seed germination, seedling vigour and other biochemical parameters were at their peak by physiological maturity at 48, 36, 52 and 44 DAA respectively in ragi, panivaragu, kuthiraivalli, thenai (Angamuthu and Karivaratharaju, 1974) and at 50 DAA in Italian millet (Kalavathi *et al.*, 2000) ^[8]. The results of the study thus suggested that the physiological maturity of little millet (*Panicum sumatrense* Roth ex Roem. & Schult.) cv. CO (Samai) 4, occurred 40 days after anthesis with accumulation of maximum biochemical parameters of seed accompanied with decrease in seed moisture content, higher germination and vigour parameters.

Conclusion

The bulk little millet (*Panicum sumatrense* Roth ex Roem. & Schult.) cv. CO (Samai) 4 crop was raised in the field and the panicles were collected at 5 days intervals up to 45 DAA and subjected to the biochemical changes *viz.*, electrical conductivity, leachate amino acid content, leachate sugars, protein content and dehydrogenase activity in seeds. Biochemical parameters were superior in the seeds collected at 40 DAA. Thus physiological maturity was found to be attained at 40 days after anthesis.

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