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Genetic diversity of total phenolic, flavonoid and antioxidant activity in pearl millet genotypes grown in semi-arid region of Rajasthan

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Abstract
Pearl millet (*Pennisetum glaucum*) is an important food staples in semi-arid tropics of Asia and Africa. Millet is cereal grains that have prospective to be used as substitute to wheat flour for celiac patients. It is considered as the good source of many important and essential fatty acids.

The present study was an effort to explore secondary metabolite composition of six millet varieties MPMH-17, HHB-67, RHB-177, RHB-173, GHB-558, and GHB-38 with special reference to Total Phenolic, Total Flavonoid and Antioxidant activity. These activities were analyzed using UV-VIS spectrophotometer. Total phenolics content were ranged from 152-202 mg GAE g^{-1} seed. Highest phenolics content were found in MPMH-17 variety (202.81 mg GAE g^{-1}) while lowest phenolics was recorded in RHB-177 (152.54 mg GAE g^{-1}). Total flavonoids content were ranged from 82-147 mg QE g^{-1} seed. MPMH-17 variety (147.20 mg QE g^{-1}) was superior in total flavonoid content and RHB-177 (82.56 mg QE g^{-1}) was found lowest flavonoid containing genotype. DPPH radical scavenging IC_{50} value of pearl millet genotypes were ranged from 8-12 mg ml^{-1}. HHB-67 variety recorded lowest IC_{50} value (8.16 mg ml^{-1}) while GHB-538 (12.25 mg ml^{-1}) contains highest IC_{50} value among six genotypes. Study result reveals that pearl millet contains adequate amount of secondary metabolites.

Keywords: Pearl millet; phenolics; flavonoids; antioxidant

Introduction
Millets are a group of cereal crops grown in semi-arid tropics of Asia and Africa for food and staples. The millets include species in several genera, mostly in the sub-family Panicoideae, of the grass family Poaceae. Pearl millet (*Pennisetum glaucum*) is one of the most important drought-resistant crops. Also, millet has resistance to pests and diseases, short growing season, and productivity under drought conditions, compared to major cereals [1]. Millets rank as the sixth most important cereal in the world especially in developed countries where they serve as staple foods for millions of people [2]. It is also known as 'bajra' and is a prominent food of western Rajasthan of India. It is one of the four most important cereals (rice, maize, sorghum and millets) grown in the tropics and is rich in iron and zinc, contains high amount of antioxidants and these nutrients may be beneficial for the overall health and wellbeing [3-4]. Millet is a primary sources of nutrients e.g. protein, mineral, vitamins and energy [5]. Pearl millet grain fractions and extracts were found to have antimicrobial activity [6]. Millets contain phenols, phytic acid and tannins which can contribute to antioxidant activity, important in health, ageing and metabolic diseases [7]. Secondary metabolites or phytochemicals, naturally occurring in plants are biologically active and play important role in defense system of plants [8]. While, extensive information is available on proximate composition and mineral accessibility, information on antioxidant activity and its influence on processing in pearl millet are scanty.

The methanolic seed extracts of pearl millet were analyzed for Total phenolics content, Total Flavonoid content and Antioxidant activity DPPH free radical scavenging IC_{50} value.

The Pearl Millet genotypes were collected from All India Coordinated Research Project on Pearl Millet, Project Coordinating Unit Mandor, Jodhpur and analyzed at Sanitary and Phytosanitary Laboratory of Agricultural Research Station, Mandor, (Agriculture University Jodhpur).

Materials and Methods
Total phenolics content (TPC)
The total phenolic content was determined using the Folin-Ciocalteu assay according to

procedure describe by Dewanto *et al.* [9] with slightly modification and the results were expressed as mg gallic acid g^{-1} seed. An aliquot of 1 ml of the crude seed methanol extract was mixed with 1 ml of the Folin-Ciocalteu reagent and 4 ml of a 20 % sodium carbonate solution. Distilled water was added to a final volume of 25 ml. Following incubation for 30 min, the absorbance of the reaction mixture was measured at 765 nm using Lab India make spectrophotometer against a blank. Gallic acid was used as the standard. The amount of total phenolic was calculated by using the standard curve of gallic acid (Figure-1) drawn within a concentration range of 8.0 x 10^{-4} to 4.0 x 10^{-3} mg ml^{-1} having R^2 value 0.994 and was expressed as mg Gallic acid equivalents g^{-1} (mg GAE g^{-1}) seed.

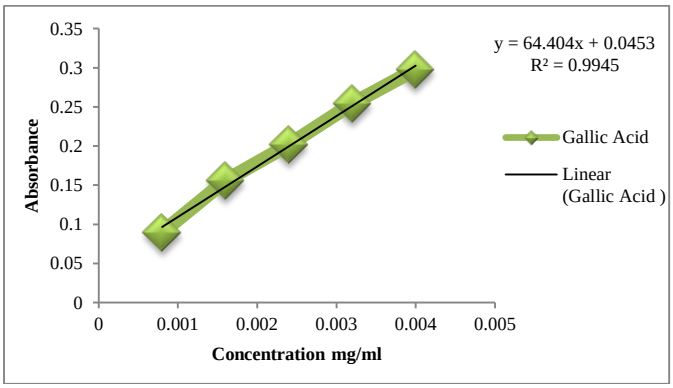


Fig 1: Gallic Acid Standard Linear Calibration Curve for Assessment of Total Phenolics

Total flavonoid content (TFC)

The total flavonoid content in methanol extract was determined using aluminium trichloride ($AlCl_3$) method, protocol described by Chang *et al.* [10] with slightly modification. Briefly, 2 ml of 2 % aluminium trichloride ($AlCl_3$) solution in methanol was mixed with the 2 ml of a diluted stock solution (0.01 or 0.02 mg ml^{-1}). Absorption readings were taken at 415 nm (Lab India spectrophotometer) after 10 min against a methanol blank, Quercetin was used as the standard. The total flavonoid content was determined using a standard curve (Figure-2) of Quercetin drawn within a concentration range of 4.0 x 10^{-3} to 2.0 x 10^{-2} mg ml^{-1} having R^2 value 0.996 and was expressed as mg Quercetin equivalents g^{-1} seed (mg QE g^{-1} seed)

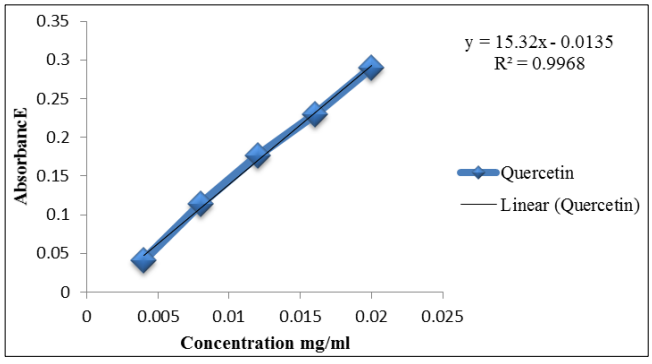


Fig 2: Quercetin Standard Linear Calibration Curve for Assessment of Total Flavonoids

Antioxidant activity DPPH assay

There are several methods commonly used to determine the antioxidant activity of natural products, we have choose the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical reagent because it is an easy, precise, and accurate method. 2,2-diphenyl-1-picryl-hydrazyl DPPH is a free radical, and produces a violet solution in alcohol. It is reduced in the presence of an antioxidant molecule [11]. Antioxidant activity of the methanol extract of pearl millet seed and standard were assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH). The diluted working solutions of the test samples were prepared in methanol. Gallic acid was used as the standard in solutions ranging from 5 x 10^{-4} to 4 x 10^{-3} mg ml^{-1}. 0.135 mM DPPH solution was prepared in methanol. Then 2 ml of this DPPH solution was mixed with 2 ml of sample solutions (ranging from 5 mg ml^{-1} to 15 mg ml^{-1}) and the standard solution were tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using Lab India make spectrophotometer against methanol. Two ml of methanol with 2 ml of DPPH solution was used as control [12-13]. The optical density (O.D.) was recorded and percentage of inhibition was calculated using the formula given below:

$$\% \text{ of inhibition of DPPH activity} = \frac{\text{O.D. of the control- O.D. of the sample x 100}}{\text{O.D. of the control}}$$

The IC_{50} values were calculated using linear regression of plots where the abscissa represented the concentration of the test solution and the ordinate was the percent of antioxidant activity.

Result and Discussion

Total phenolics content (TPC)

The colorimetric method using the Folin-Ciocalteau reagent is frequently used for total phenolic content estimation. A blue colour complex forms due to the reaction of Folin-Ciocalteau reagent and phenols that allow quantification. The total phenolic content in peal millet genotypes were ranged from 152-202 mg GAE g^{-1} seed (Figure-3). MPMH-17 variety contain highest 202.81 mg GAE g^{-1} seed followed by GHB-558 (198.96 mg GAE g^{-1}), GHB-538 (175.60 mg GAE g^{-1}), HHB-67 (162.32 mg GAE g^{-1}) and RHB-173 (156.73 mg GAE g^{-1}) while RHB-177 contains lowest (152.54 mg GAE g^{-1}) total phenolics content among six genotypes (Table-1). According to Zhang *et al.* [14], the TPC of free phenolics extracts of three different varieties of proso millet ranged from 27.48 to 151.14 mg gallic acid equiv (GAE)/100 g.

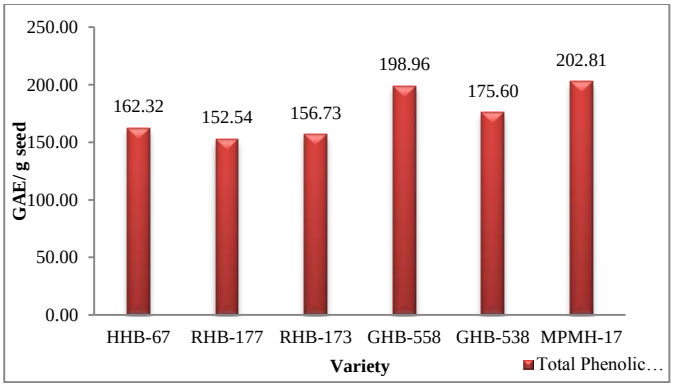


Fig 3: Total Phenolics Content GAE g^{-1} Seed in Pearl Millet genotypes

Total flavonoid content (TFC)

Total flavanoids content was estimated by aluminium chloride method. Quercetin dihydrate was taken as standard flavonoid and results were calculated as means of triplicate and represented as mg Quercetin equivalent g^{-1} seed (mg QE g^{-1})

with standard deviation. The total flavonoid content was ranged from 82-147 mg QE g^{-1} seed (Figure-4). In MPMH-17 variety highest Flavonoid content (147.20 mg QE g^{-1}) was found among six genotypes followed by GHB-538 (142.08 mg QE g^{-1}), HHB-67 (87.39 mg QE g^{-1}), RHB-173 (85.71 mg QE g^{-1}) and RHB-177 (82.56 mg QE g^{-1}). Pushparaj and Urooj [15] analyzed flavonoid content in Karnataka, Tamilnadu and Maharashtra growing pearl millet varieties Kalukombu and Maharashtra Rabi Bajra. The flavonoid content was found 27 mg g^{-1} in Kalukombu followed by 21 mg g^{-1} in Maharashtra Rabi Bajra. Ju-Sung *et al.* [16] also showed that the TFC of five cultivars of whole grains of proso millet ranged from 3.4 to 11.5 mg quercertin equivalents (QE) /g of sample. Current study results reveal that western Rajasthan growing pearl millet genotypes contain adequate amount of total flavonoids.

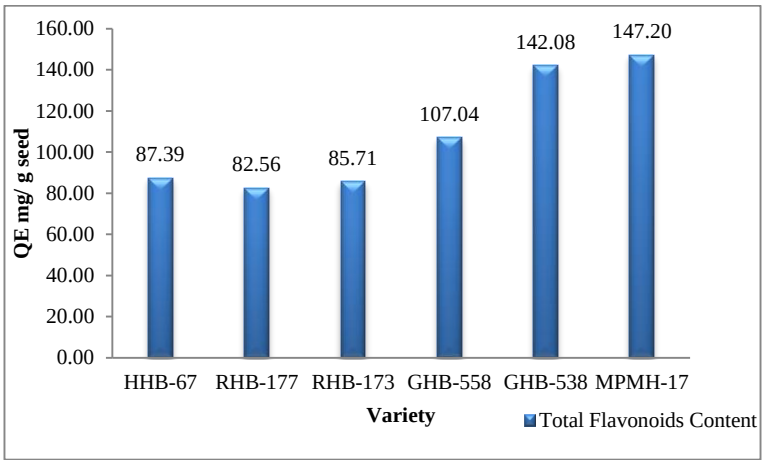


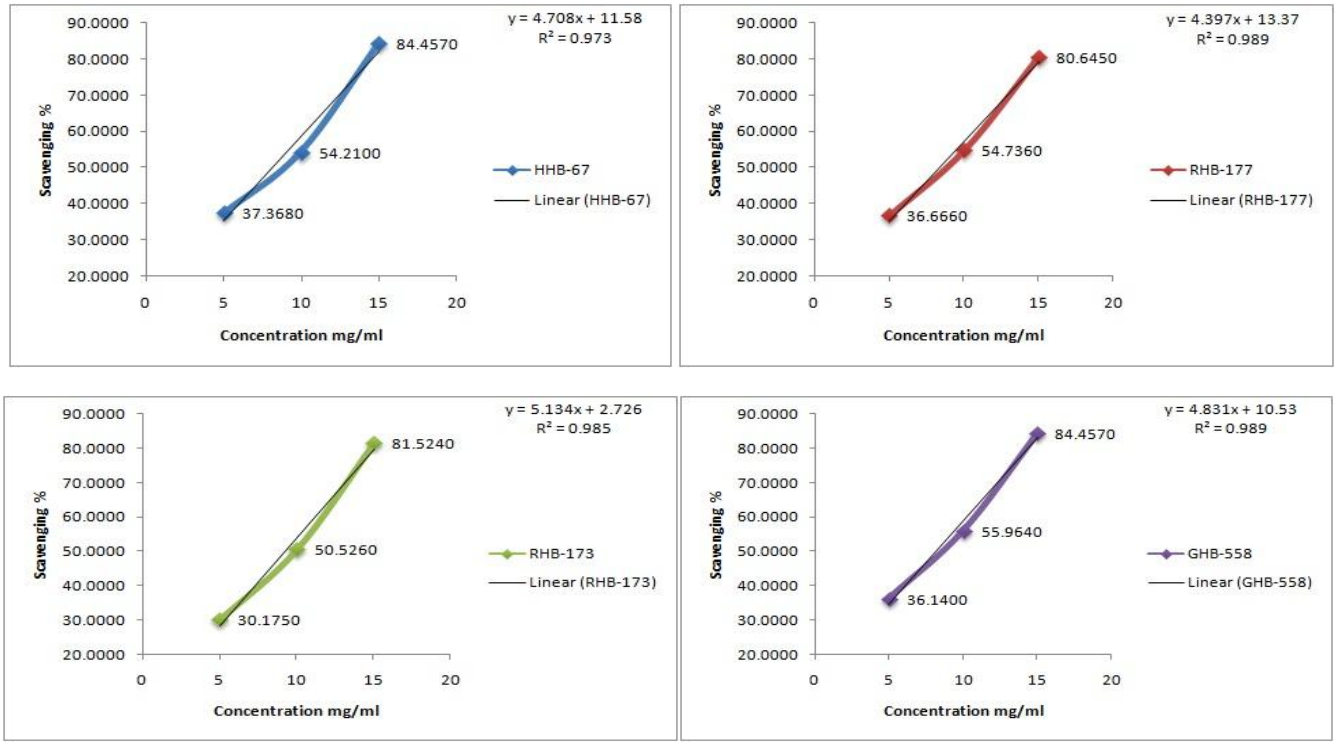
Fig 4: Total Flavonoid Content QE g^{-1} Seed in Pearl Millet genotypes

Antioxidant activity DPPH assay

The determination of the antioxidant activity of pearl millet seed extract was based on the DPPH radical scavenging activity through the IC_{50} parameter, which represents the concentration of the material necessary to inhibit 50% of free radicals. Thus, a lower IC_{50} value shows a superior ability to neutralize free radicals and potential antioxidant content.

The scavenging ability of methanol seed extract of pearl millet genotypes represented as IC_{50} of DPPH radical are shown in Table-1 while DPPH radical scavenging % is showed in Table-2.

IC_{50} values were ranged from 8-12 mg ml^{-1} among six genotypes. Figure 5 represents the DPPH radical scavenging activity of pearl millet genotypes. HHB-67 (8.16 mg ml^{-1}) contains lowest IC_{50} value followed by GHB-558 (8.17 mg ml^{-1}), RHB-177 (8.33 mg ml^{-1}), RHB-173 (9.29 mg ml^{-1}), MPMH-17 (9.74 mg ml^{-1}) and GHB-538 (12.25 mg ml^{-1}).



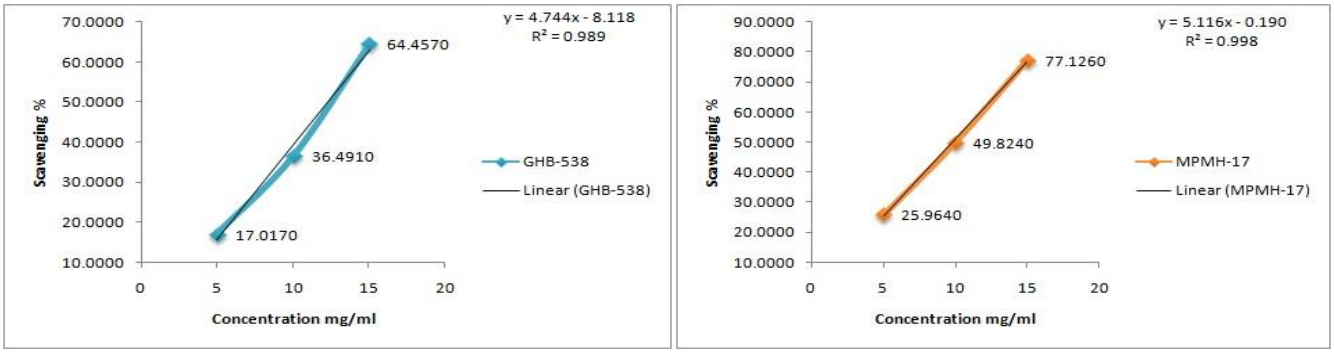


Fig 5: DPPH radical scavenging activity of Pearl Millet genotypes

Table 1: Total Phenolics, flavonoids and Antioxidant activity of Pearl Millet genotypes

S. No.	Variety	TPC mg GAE g^{-1} Seed	TFC mg QE g^{-1} Seed	IC_{50} Value mg ml^{-1}
1.	HHB-67	162.32 ± 4.11	87.39 ± 1.28	8.16
2.	RHB-177	152.54 ± 3.24	82.56 ± 1.27	8.33
3.	RHB-173	156.73 ± 2.48	85.71 ± 0.38	9.29
4.	GHB-558	198.96 ± 2.29	107.04 ± 1.08	8.17
5.	GHB-538	175.60 ± 4.74	142.08 ± 0.78	12.25
6.	MPMH-17	202.81 ± 4.70	147.20 ± 0.92	9.74

Mean ± SD

Table 2: DPPH radical scavenging % in crude extract of Pearl Millet genotypes

S. No.	Variety	DPPH Radical Scavenging % at Different Concentration of Crude Seed Methanol Extract		
		5 mg ml^{-1}	10 mg ml^{-1}	15 mg ml^{-1}
1.	HHB-67	37.3680	54.2100	84.4570
2.	RHB-177	36.6660	54.7360	80.6450
3.	RHB-173	30.1750	50.5260	81.5240
4.	GHB-558	36.1400	55.9640	84.4570
5.	GHB-538	17.0170	36.4910	64.4570
6.	MPMH-17	25.9640	49.8240	77.1260

Conclusion

Pearl millet is one of the most important crops produced prominently from western Rajasthan of India and occupies significant place in Indian agriculture. Current study conducted to evaluate the total phenolics, Flavonoid and antioxidant activity in methanolic extract of pearl millet genotypes which will lead to characterization of these genotypes with reference to secondary metabolites constituents. Based on the results of studies carried out, we can observe that millet grains contain many health-promoting components. Research is also needed to determine the bioavailability, metabolism, and health contribution of millet grains and their different fractions in humans. Experimental results reveal that pearl millet genotypes growing in Western Rajasthan were contains adequate amount of secondary metabolites and an immense genetic variability among six genotypes were observed. Highest total phenolics and flavonoids were recorded in MPMH-17. The information provided by this research will help breeders in future breeding programmes to develop improved varieties of pearl millet.

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