



**PEARL MILLET (*Pennisetum glaucum*) GROWTH AND  
PRODUCTIVITY UNDER DIFFERENT SOWING METHODS, MULCHING  
AND FOLIAR FEEDING OF NUTRIENTS**

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**DOI - 10.5281/zenodo.14523337**

**ABSTRACT:**

*Pearl millet (***Pennisetum glaucum***), also known as Bajra, is one of the four most important cereals (rice, maize, sorghum and millets) grown in tropical semi-arid regions of the world primarily in Africa and Asia. Our aim is to review the potential health benefits of pearl millet. Desk reviews from Gujarat Agricultural Universities, libraries, PubMed and other web sources, key informant interviews of farmers (n=30), local leaders (sarpanch) (n=30) and women (n=960) from pearl millet belt of Bhojpur, Buxar, Rohtas and Kaimur district of Bihar. Pearl millet is rich in several nutrients as well as non-nutrients such as phenols. It has high energy, has less starch, high fiber (1.2g/100g, most of which is insoluble), 8-15 times greater  $\alpha$ -amylase activity as compared to wheat, has low glycemic index (55) and is gluten free. The protein content ranges from 8 to 19% and it is low in lysine, tryptophan, threonine and the sulfur-containing amino acids. The energy of millet is greater than sorghum and nearly equal to that of brown rice because the lipid content is generally higher (3 to 6%). Pearl millet can be recommended in the treatment of celiac diseases, constipation and several non-communicable diseases. Nutritional studies on the population living in the pearl millet belts of the world and clinical trials on the impact of pearl millet in specific disease conditions are needed.*

**Keywords:** *Estimation of phenolic and flavonoid content of raw and fermented pearl millet.*

**INTRODUCTION:**

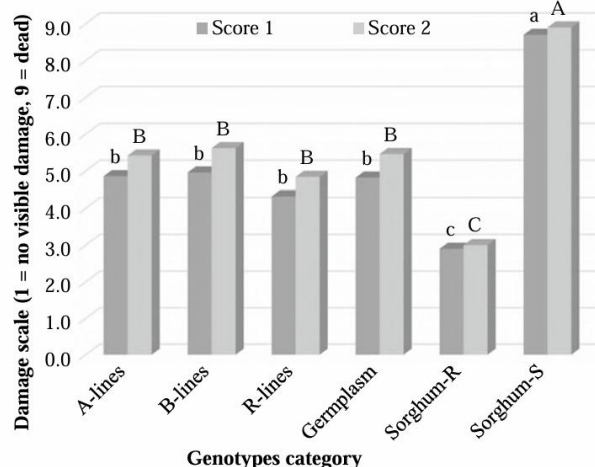
Pearl Millet (*Pennisetum glaucum*), also known as Bajra, is a cereal crop grown in tropical semi-arid regions of the world primarily in Africa and Asia Bajra is well adapted to production systems characterized by low rainfall (200-600 mm), low soil fertility, and high temperature, and thus can be grown in areas where other

cereal crops, such as wheat or maize, would not survive. In its traditional growing areas, pearl millet is the basic staple for households in the poorest countries and among the poorest people. It is also one of the most drought resistant crops among cereals and millets. Pearl millet is generally used as a temporary summer pasture crop or in some areas as a food crop. Pearl millet 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 along with the antioxidants may be beneficial for the overall health and wellbeing.

**MATERIALS AND METHODS:**

The genetic improvement program progressed effectively initiating from the selection of local and traditional germplasm to the development of high-yielding hybrids possessing inbuilt tolerance and resistance to climatic stresses such as drought and heat along with different diseases. These hybrids were grown on 70% of the total pearl millet area, resulting in a 124% enhancement in productivity since 1986–1990. ICAR-All India Coordinated Research Project on Pearl Millet has developed several precise production and protection technologies for different agroecological regions of different states since its beginning in 1965. Till now, a total of 180 hybrids and 62 varieties have been identified and released for growing in different agroecological regions of India through ICAR-All India Coordinated Research Project on Pearl millet (Satyavathi et al., 2020).



**RESULTS:**

[*Pennisetum glaucum* (L.) R. Br.] In arid circumstances. Experiment consisted of 3 sowing methods, viz. direct seed sowing (45 cm × 15 cm); 2-week-old seedling (60 cm × 22.5 cm); and 4-week-old seedling (60 cm × 22.5 cm) transplanting; and 3 mulches, viz. no mulch; dust mulch; and straw mulch assigned to main-plot and 2 levels of NPK foliar fertilization [control and NPK @1% foliar spray at 35 and 50 DAS (days after sowing)] assigned to sub-plot. Results showed that transplanting 4-week-old seedlings led to significantly higher plant height (170.1 cm), dry-matter accumulation (133.6 g), total and effective tillers (3.41 and 3.14), ear head length (24 cm), ear head girth (2.28 cm), weight of ear head (31.5 g), grains/ear (1964), weight of grains/ear (18.9 g), comparable to 2-week seedling transplanting. The grain yield (2396 kg/ha) and straw yield (6640 kg/ha) were significantly superior with 4-week-

old seedlings over 2-week seedling by 8.2, 5.4% on pooled basis. Straw mulch exhibited superior most for growth and yield parameters, followed by dust mulch. Additionally, NPK @1% foliar spray at 35 and 50 DAS resulted in significantly higher plant height (170.5 cm), dry-matter accumulation (131.6 g), ear head length (23.7 cm), ear head girth (2.27 cm), weight of ear head (31.1 g), grains/ear (1921), weight of grains/ear (18.5 g), compared to the control. The grain yield (2303 kg/ha) and straw yield (6548 kg/ha) were significantly superior with NPK @1% by 9.5% and 11% over control. In conclusion, the combination of transplanting 4-week-old seedlings, straw mulch and NPK @1% foliar spray offering a potential solution for enhancing agricultural productivity in the region.

#### **DISCUSSION:**

Bajra and Allergies Pearl millet is a gluten free grain and is the only grain that retains its alkaline properties after being cooked which is ideal for people with wheat allergies. Pearl millet grains are all very high in calories—precisely the reason they do wonders for growing children and pregnant women). Gluten intolerance persons (Celica) allergic to gliadin, aprolmine specific to wheat and some other common grains, comprise approximately 500,000 persons in the United States or 1 in every 541 people (based on US census bureau resident

population estimate, 1998). More complete characterization of sorghum and pearl millet proteins and their functionality would provide useful information for marketing celiac foods. The total phenol content of various treatments was found to be in a range of 32.74-46.43 µg GAE/mg. The maximum phenolic content was found to be highest in treatment 11 i.e. 46.43 µg GAE/mg. the change in phenolic content in various treatment was observed with the change in yeast concentration, fermentation temperature and fermentation time. The maximum phenolic content was observed at yeast concentration 2%, fermentation temperature 45°C and fermentation time of 18h. The total flavonoid content was observed in range from 2.24-9.46 µg CAT/mg .This change could be due to alteration in pH values of various treatments. The decrease in pH content of different treatment during fermentation could be the main reason affecting the disintegration of native structure of the grain. Apart from this, the secretion of various enzymes during fermentation could possibly help in releasing the phytochemicals, especially those phenolic compounds found attached to insoluble cell wall matrix (Ilowefah et al., 2015; Adebo and Medina-Meza, 2020, Salar et al., 2016).

### Effect of Fermentation Conditions on Phenolic Compounds:

Phytochemicals, such as phenolic acids and flavonoids, scavenge free radicals and aid in the prevention of a variety of chronic syndromes by reducing oxidative stress (Guo et al., 2017; Dadwal and Gupta, 2021). These phenolic acids are present in both soluble and bound form, belonging to both hydroxyl-benzoic and hydroxyl-cinnamic acids groups in variety of millet species (Shahidi & Chandrasekara, 2013). The primary phenolics (ferulic acid, cinnamic acid, vanillic acid, and catechin) in raw millet (*Pennisetum glaucum*) were quantified using chromatographic gradient method using UHPLC-DAD and fermented samples were compared to understand the chemical behavior of phenolics during fermentation. Results showed that millet sample treated with a 2% yeast concentration and at 45°C for an interval of 18h, yields maximum amount of ferulic acid (33.66 µg/mg), catechin (20.10 µg/mg) and vanillic acid (5.67 µg/mg). The plausible reason for increased ferulic acid concentration could be explained by the presence of feruloyl esterase activity of *Saccharomyces cerevisiae* (Coghe et al., 2004). Apart from this, results signify no substantial increase in the phenolic acid after 18min of the time interval. Similar trend of decline in phenolics, flavonoid content and antioxidant activity above

24h of the time interval has been reported earlier (Adetuyi & Ibrahim, 2014). The possible reason for decreased activity could be assigned to the presence of micro-organisms utilizing the available compounds for growth (Karimi et al., 2010; Adetuyi & Ibrahim, 2014). Other than this, activation of polyphenol oxidase after germination might have resulted in loss of phytochemicals (Sharma & Kapoor, 1996). Increases in total phenolics and flavonoids were found in fermented cereals due to higher enzyme activity, and there was a positive correlation between total phenolics and antioxidant activity was also observed in previous studies (Saharan et al., 2017). Similarly, in a recent reports fermentation of *Murraya koenigii* leaves for beverage development resulted in a considerable rise in phenolics which also results higher antioxidant activity (Bhatt et al., 2021). Considering all these reported factors, a significant increase in the phenolic compounds in present study were can be co-related. Whereas, it is critical to optimize the fermentation time to maximize the amount of liberated of phenolic acids. Presently researchers explored room temperature (20-25°C) as a suitable temperature condition for fermentation. But in present study, it was observed that temperature treatments between 37.5-45°C yield the maximum amount of all major phenolic acids.

**FUTURE PERSPECTIVES:****Determination of Chemical Composition:**

The samples were analysed for moisture, crude protein, total fat, total ash and crude fiber content in accordance with official method of Association of Official Analytical Chemists (AOAC,2010).

**Moisture Content:**

Briefly, sample moisture determined using hot air dry method for which samples were ground, weight and taken in previously weighed moisture dishes then the samples along with moisture dish were kept in a hot air oven at 105 °C for 1 h. Moisture dish was taken out after 1 h and placed in a desiccator for cooling. Weight of moisture dish was recorded thereafter; this process repeated until a constant weight was attained. The loss in weight represents the moisture content of sample:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \times 100$$

**Fat Content:**

Fat estimation was performed using the soxhlet extraction method in which moisture free samples were weighed and placed in thimbles; for fat extraction these thimbles were kept in soxhlet apparatus with petroleum ether for 16-18h. The fat extract was filtered through a sintered funnel in a pre-weighed beaker. Petroleum ether were

evaporated and beakers were weighed to get the fat content using formulae: Fat (%)

$$= \frac{\text{Amount of ether extract (g)}}{\text{Weight of sample (g)}} \times 100$$

**Ash Content:**

Ash was analysed using incineration in a muffle furnace; samples were weighed and placed in pre-weighed silica crucibles to burn organic matter. After that, crucibles were placed in muffle-furnace at 600°C for 4h. Crucibles were kept for cooling in the desiccator followed by weighing. Per cent ash content was calculated as: Ash (%) =  $\frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$

**Protein Content:**

For protein content determination micro-Kjeldhal apparatus and a multiplication factor of 6.25 was used for converting nitrogen content into crude protein. In Kjeldahl digestion flask samples were digested by mixing them with 2 g digestion mixture (10 parts of potassium sulphate and 1 part of copper sulphate) and 25 mL of sulphuric acid. Digestion flask was kept on hot plate for boiling for complete digestion. The contents were cooled and filtered then transferred to 100 mL volumetric flask to make up volume with distilled water. This was followed by distillation, in a distillation flask 5 mL sample and 40% sodium hydroxide were taken and liberated ammonium borate was trapped in 4% boric acid

solution containing mixed indicator (methyl red and Bromo cresol green).

The obtained distillate was titrated with 0.1 N HCL.  $\text{Nitrogen(\%)} = \text{Titrevalue} \times \text{Normality of HCL} \times 14 \times 100 / \text{Weight of sample (g)} \times 100$   
 $\text{Crude protein(\%)} = \text{Nitrogen(\%)} \times 6.25$ .

#### **Crude Fiber Content:**

Crude fiber was determined in fat free samples by acid-alkali digestion method, ground fat free samples were weighed and subjected to acidic digestion by boiling them with 200 mL of 1.25% sulphuric acid for half an hour followed by alkali digestion with 200 mL of 1.25% sodium hydroxide for half an hour. Residue was obtained and dried in hot air oven. After drying initial weight of the crucible with sample was recorded and placed in the muffle furnace at  $600 \pm 5^\circ\text{C}$  for 4h. Final weight of the sample was recorded after cooling the crucible. Crude fiber content was calculated as follows:  
 $\text{Crude fiber(\%)} = \frac{\text{Wt. of sample (before ignition)} - \text{Wt. of sample (after ignition)}}{\text{Weight of sample (g)}} \times 100$

#### **Total Carbohydrate Content:**

Total carbohydrate content was determined by the using phenol sulphuric method described by (Masuko et al., 2005). Digested sample was taken in a test tube and using distilled water final volume was made up to 1 mL, 1 mL of 5% phenol was added to test tube followed by 5 mL of 96% sulphuric acid. Samples were

incubated by placing the tubes in water bath along with blank at  $25-30^\circ\text{C}$  for 20 min. O.D of each sample was taken at 490 nm.

#### **Phenolics Quantification UHPLC-DAD Gradient Approach:**

Previously reported phenolic acids including ferulic, cinnamic and vanillic acids and flavanol compound including catechin exhibited in millet crops were used as reference standards and compared with samples. For quantitative analysis a gradient approach using ultra-high performance liquid chromatography diode array detector (UHPLC-DAD) (Agilent Technologies, USA) (Dadwal, Joshi, & Gupta, 2021).

#### **Determination of Total Phenolic and Flavonoid Content:**

Total phenolic and flavonoid content (TPC, TFC) was determined using spectrophotometric method as previously described by Bhatt et al., 2020. In brief, for TPC, 1 mg/mL of fermented millet extract was mixed with 1 N Folin-Ciocalteu (FC) reagent (500  $\mu\text{L}$ ) and then 7% of saturated sodium bicarbonate (100  $\mu\text{L}$ ) was added. Further, the reaction solution was made up with distilled water to 25 mL followed by incubation at  $25 \pm 2^\circ\text{C}$  for 30 minutes. Thereby, the absorbance was observed at 730 nm. For TFC, 1 mg/mL of fermented millet extract was mixed 5% of sodium nitrite (60  $\mu\text{L}$ ) along with 10% of aluminum chloride (60  $\mu\text{L}$ ) and

was then incubated for about 5 min at room temperature ( $25 \pm 2^\circ\text{C}$ ). Further, there was addition of 1 N sodium hydroxide (400  $\mu\text{L}$ ) with final volume make up to 10 mL with distilled water. Afterwards, absorbance was measured at 730 nm.

#### **Physicochemical Analysis:**

##### **pH Determination:**

The samples were thoroughly stirred to homogenize the mixture and achieve uniformity. The pH electrode was dipped into the sample and measurement was taken using a Eutech pH meter.

##### **Total Titratable Acidity (TTA):**

The total titratable acidity was estimated using method described by Tamani et al. (2013). Aliquot (10 mL) of the fermented samples of pearl millet flour were pipette into an Erlenmeyer flask, and then 2drops of phenolphthalein were added. It was titrated using 0.1 N NaOH until a faint pink colour appeared. The titre volume was noted and used to calculate TTA. TTA was expressed as mL of 0.1M NaOH/10g of flour suspension.

##### **Total Soluble Solids (TSS):**

The determination of TSS was carried out using hand refractometer (Erma, Japan). Briefly, 10 g sample was thoroughly mixed with 100 mL of distilled water. Few drops of sample were positioned on refractometer and analyzed.

#### **Determination of Free Amino Acid Composition:**

Amino acid composition was determined by using Waters Acquity UPLC-H class system with binary solvent manager, an auto sampler, e photodiode array detector (PDA), 600 controller pump with online degasser, column heater (Agrawal et al., 2020). Separations was made using Water BEH C18 column ( $2.1 \times 100$  mm, 1.7  $\mu\text{m}$  particle size) integrated with a suitable guard column. Mobile phase A was prepared using Sodium acetate (0.14 mol/L) and tri-ethylamine adjusted to pH 6.7 using glacial acetic acid and methanol (90:10). Acetonitrile was used as mobile phase B. All Samples and standard mix were derivatized with O-phthalaldehyde and passed through a 0.45  $\mu\text{m}$  Millipore membrane syringe filter before injecting into system.

#### **Principle Component Analysis:**

PAST software was used for principal component analysis to examine the variation patterns of amino acid content from UPLC-H analysis to identify the main groups among the samples.

#### **Determination of Functional Properties:**

##### **Water and Oil Absorption Index (WAI and OAI):**

WAI and OAI were determined as described by Bhatt et al., 2021. In brief, 1 g sample was mixed with distilled water (10 mL) mixed, incubated ( $85 \pm 2^\circ\text{C}$  for

10 min) and centrifuged at 3,000 rpm for 10 min. The supernatant was discarded and sample was weighed. For OAI, 1 g sample was mixed with vegetable oil (10 mL) vortex (1 min) and kept at room temperature for 30 min. Further sample was centrifuged and surplus oil was decanted while the residues were weighed. The WAI and OAI were expressed as:  $WAI/OAI(g/g) = \frac{\text{Weight of water/oil absorbed}(g)}{\text{Weight of sample}(g)}$

**Water Solubility Index (WSI) and Water Activity:**

The water solubility index was obtained as describe by kumari et al. 2018. The presence of dry matter present in supernatant from WAI. The supernatant was dried for 1-2h in pre weighed petriplates at  $100 \pm 2^\circ\text{C}$  cooled and weighed. The WSI was expressed as:  $WSI(\%) = \frac{\text{Weight of dry matter in supernatant}}{\text{Weight of sample}} \times 100$

The water activity of the samples was analyzed by kumari et al. (2021) with the help of water activity meter (Aqua lab 4TE dew point water). All the experiments were carried out in triplicate.

**Statistical Analysis:**

Response variables (TPC, TFC, total protein, total CHO, TTA, TSS and pH) obtained from yeast fermented pearl millet flour were subjected to regression analysis and analysis of variance (ANOVA) to determine

regression coefficients and statistical significance of model terms; and to fit the mathematical models to the experimental data, aiming at an overall optimal region for the response variable. Multiple regression coefficients were determined by employing the least-squares technique to predict linear and quadratic polynomial models for the response variable studied. The behavior of the response surface was investigated for the response function ( $Y_i$ , the predicted response) using the regression polynomial equation. The generalized polynomial model proposed for predicting the response variable is given

$$Y_i = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3$$

Where  $\beta_0$  is the intercept,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  as coefficient. The significance of the equation parameters for each response variable was also assessed by F ratio at a probability (P) of 0.05. The adequacy of the model was determined using model analysis, lack-of-fit test and coefficient of determination ( $R^2$ ) analysis as described by (Lee, Ye, Landen, & Eitenmiller, 2000). For a good fit of a model,  $R^2$  should be at least 0.80 (Joglekar & May, 1987). The experimental design matrix, data and analysis, and optimization procedure were performed using the Design-Expert Version 13, trial (Stat-Ease, Inc, Minneapolis, MN, USA).



**RESULTS AND DISCUSSION:****Fitting the Models:**

The study used RSM to develop a prediction model for optimizing the yeast fermentation conditions of PM flour. RSM always been used a prominent statistical tool for determining accurate variables for food processing with desirable results. In respect to millet processing, previously, Chakraborty, Singh, Kumbhar, & Chakraborty (2011) used RSM as statistical approach in extrusion processing with characteristic properties of extruded millet legumes, which demonstrated it as an effective statistical implementation for processing millet edibles to understand physiochemical behavior.

**Estimation of Phenolic and Flavonoid Content of Raw and Fermented Pearl Millet:**

The total phenol content of various treatments was found to be in a range of 32.74-46.43  $\mu\text{g GAE/mg}$ . The maximum phenolic content was found to be highest in treatment 11 i.e. 46.43  $\mu\text{g GAE/mg}$ . the change in phenolic content in various treatment was observed with the change in yeast concentration, fermentation temperature and fermentation time. The maximum phenolic content was observed at yeast concentration 2%, fermentation temperature 45°C and fermentation time of 18h. The total flavonoid content

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#### CONCLUSION:

Fermentation is a traditional approach developing widespread of food products although scientific validations help to optimize the perfect conditions to acquire effective results. Present study successfully demonstrated the optimized fermentation conditions for pearl millet flour for the enhancement of nutritional and phytochemical constituents. Whereas, the optimal fermentation temperature for pearl millet flour was 30°C for 18h with 2% baker's yeast. Fermentation improved the protein content was co-related with the higher levels of essential amino acids after fermentation. Phytochemicals were also significantly increased with higher phenolic and flavonoid content. Whereas, major phenolics acids were also detected in elevated amounts. Thus,

fermentation of pearl millet flour to improve nutrition and phytochemical contents is uncomplicated and can be more efficient in improving pearl millet flour quality for future product development. Potential health benefits and its possible nutraceutical properties of pearl millet have been highlighted in this paper. Pearl millet serves as a major staple food for many populations around the globe, however, it is still considered poor man's food and does not find place in the food purchase lists of the elite. Millets, which are currently consumed in the rural and tribal areas of the world, need to be popularized. Unique health foods as well as traditional foods made from pearl millet need to be promoted.

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