Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
www.phytojournal.com
JPP 2024; 13(4): 97-106
Received: 12-04-2024
Accepted: 10-05-2024

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# Comparative study of Indian wheat (*Triticum aestivum* L.) varieties with respect to phytochemical profiling and antioxidant activity

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**DOI:** https://doi.org/10.22271/phyto.2024.v13.i4b.14999

**Abstract**
Wheat is one of the major grains in human diet. Differences in phytochemical profiles and antioxidant activities of different wheat varieties influences the nutritional and health benefits of this cereal. Germinated wheat grains are more packed with health promoting bioactive compounds. Therefore, the objectives of this study were to determine the phytochemical, enzyme and antioxidant status of 10 different commercial Indian wheat varieties during germination. The experiments included estimation of total phenolic, protein, carbohydrate, flavonoids and carotenoids and enzyme activity of catalase, peroxidase and superoxide dismutase. Antioxidant activity was determined by DPPH and FRAP assay. The results showed a multifold increase in most of the tested parameters, with varietal differences. Heat map and PCA were performed and categorized on the basis of their correlation.

**Keywords:** PCA, phytochemical, phenolic compound, antioxidant, enzyme activity, heat map

## Introduction
The focus is turning to examining better ways to enhance the functionality of meals as people become more aware of the connection between nutrition and health. Edible seeds that have been sprouted are becoming more and more prevalent in modern human diets [1]. Today, there is a growing body of research about the therapeutic benefits of sprouted foods, and with the recent coronavirus outbreak, there is also a growing desire for functional foods that boost body immunity [2]. Sprouts' rising popularity is mostly attributable to their advantageous health effects. Sprouts have been linked to a number of biologically active components that may have positive health effects. Seeds are soaked in water and kept under appropriated conditions for germination. According to reports, germination also raises the amounts of a number of phytochemicals, minerals, and vitamins, improving the plant's nutritional value [3]. Complex macromolecules like protein, carbohydrate, and fat are hydrolyzed into simpler forms of amino acids and sugars that are easier for the body to absorb and digest due to a rapid rise in different enzyme activity [4]. In germinated seeds, there are fewer antinutritional substances such trypsin inhibitor, phytic acid, tannin, etc. [5]. Cereals are major source of antioxidants and it has been found that their antioxidant activity increases during germination [6]. The quantities of amino acids, sugars, folate, aminobutyric acid, phenolic compounds, flavonoids, vitamins, and minerals in the seeds of many different plant species, such as wheat, sorghum, rice, oat, barley, buck wheat, and moong, have been found to increase during germination [7, 8].
One of the most significant cereal crops is wheat (*Triticum aestivum* L.). For the preparation of various cereal foods, including baked bread, steaming bread, biscuits, pancakes, cakes, etc., several wheat kinds are utilized [9]. Varieties of wheat are used for the production of different food products, which provide calories for human activity. Wheat grains are a great source of fiber, vitamins, minerals, enzymes, and phytochemicals in addition to carbs. These nutrients have been linked to a number of health advantages, including a lower risk of cancer, type II diabetes, obesity, cardiovascular disease, and other chronic diseases [10]. Studies have reported enhancement in the nutritional content of wheat grains after germination [11]. The nutritional content varies in terms of phytochemical composition, enzyme activity and antioxidant activity between grain varieties [12, 13, 14]. Therefore, the objective of this study was to investigate the effect of germination on the nutritional and functional properties of some commercial Indian wheat varieties.

## Materials and Methods

### Seed collection and germination

Ten different varieties of wheat seeds (*Triticum aestivum* L.) were purchased from agriculture farm of Banaras Hindu University (HVW-243, HVW-468, HVW-510, HVW-669), Varanasi and Narendra Dev University, Faizabad (SR-303, DBW-187, PBW-343, PBW-502), Uttar Pradesh, India, two varieties (HD-3086, HI-6759) were purchased from local market. The seeds of each wheat variety were cleaned with distilled water and surface sterilized with 2% sodium hypochlorite solution than immersed in distilled water for 8 hours at room temperature. Fifteen seeds were germinated on two layers of filter paper that had been soaked with distilled water in petri dishes. Germination was conducted at $25^0$ C in complete darkness. Morphological and biochemical characteristics were tested on 0, 2nd day, 5th day and 7th day of germination. Equation GP=GS/TSx100 was used to compute the percentage of germination. GS stands for germinated seed, TS for total seed in the petri dish, and GP represents germination percentage. For each germination circumstance, each cultivar underwent three replications.

### Phytochemical assays

### Estimation of total protein

Total protein in wheat seed was quantified by Lowry method as described by Singh S., 2016 with slight modification [15]. Two-gram seeds of all wheat varieties were crushed in 2 ml of 0.1 M phosphate buffer (pH 7.0). This mixture was centrifuged at 5000 rpm for 5 minutes and supernatant was used as protein source. About 0.5 ml extract was taken and diluted by 0.5 ml of distilled water, 4 ml of reagent C was added in sample mixture and incubated in dark for 10 minutes at room temperature. Reagent C was prepared by adding reagent A (2g $Na_2CO_3$ was added in 0.1% aqueous NaOH) and reagent B (0.1gm $CuSO_4$ was added in 1% aqueous Na-K tartrate in 50:1 ratio). Later 400µl of dilute Folin-Ciocalteau's reagent was added in sample mixture and kept in dark for 30 minutes at room temperature. Absorbance was read at 650 nm and concentration was calculated by comparing with standard curve prepared by using bovine serum albumin.

### Estimation of total carbohydrate

Total carbohydrate in all wheat varieties were estimated by phenol-sulphuric method as described by Rakesh *et al*., 2021 with some modification [16]. Two-gram seeds were grinded well in 2 ml of 1N HCl, this mixture was incubated in a boiling water bath for 3 hours. After cooling at room temperature, sodium carbonate was added till effervescence ceased. Mixture was centrifuged at 7000 rpm for 8 minutes, after that 0.5 ml supernatant was taken and diluted by adding 0.5 ml distilled water. Then 5% phenol was added followed by 1 ml 98% sulphuric acid and mixed properly. Absorbance was read at 490 nm after 30 minutes incubation at room temperature. Glucose was used as standard to prepare curve and unknown concentration was calculated using standard graph.

### Estimation of total phenolic content

Total phenolic content of all 10 wheat varieties were computed by Folin-Ciocalteau's method, as described by Lawag *et al*., 2023 [17]. Briefly, 2g seeds were homogenized in 2 ml distilled water by using mortar pestle. Mixture was centrifuged at 5000 rpm for 5 minutes and supernatant was collected. About 0.5 ml extract was taken in a test tube and 0.5 ml distilled water was added and mixed properly, followed by addition of diluted 300µl of Folin-Ciocalteau's phenol reagent and left for 10 minutes in complete darkness at room temperature. 1 ml of 20% sodium carbonate solution was mixed in reaction mixture than add distilled water to ensured that the final volume remained at 3 ml and left for 60 min at room temperature in darkness before their absorbance was measured at 650 nm. Different concentrations of Gallic acid were used for preparing Standard curve.

### Estimation of total flavonoid

Total flavonoid content in wheat seeds were quantified by using aluminum chloride colorimetric method as described by Ordonez *et al*., 2006. with slight modification [18]. Two-gram seeds of each wheat variety were dried and grinded well by using mortar and pestle. Powder was taken and in it 2 ml methanol was added and mixed properly. This was kept for 2 hours at room temperature. Mixture was centrifuge at 5000 rpm for 5 minutes. Supernatant was collected, 1 ml extract was taken and diluted with 1 ml methanol, and then 1 ml of 2% AlCl3 was mixed well. This combination was incubated at room temperature for 60 minutes. At 420nm, absorbance was measured. Various concentrations of quercetin were utilized to prepare standard graph.

### Estimation of total carotenoid

Total carbohydrate in all wheat varieties were tested as described by Cong *et al*., 2009 with some modification in our laboratory [19]. Two-gram seeds of all wheat varieties were taken and dried crushed well and power was transferred in Eppendorf tube, then 1.5 ml hexane was added and mixed well. Mixture was kept for 30 minutes at room temperature and centrifuged at 5000 rpm for 5 minutes. One ml supernatant was diluted by 2ml hexane and absorbance was read at 450 nm. Quantification was done by using beta carotene as standard.

### Antioxidant activity

### 2, 2-Diphenyl-1-picrythydrazyl (DPPH) radical studies

The effect of methanolic extract of wheat on scavenging DPPH radical was determined by using modified DPPH method explained by Aryal *et al*., 2019 [20]. Briefly, 2g seed was crushed in 2 ml methanol and centrifuged at 5000 rpm for 5 minutes. 2.5 ml methanol was added in 0.5 ml methanolic extract than 1 ml of 0.004% DPPH solution was added in solution. Absorbance was recorded at 517nm after 30 min dark incubation. Antioxidant activity was calculated by using following formula:

$$\text{Radical scavenging activity \%} = \frac{\text{Absorbance of Control- Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

Where sample was methanolic wheat extract and control was 1 ml DPPH with 3 ml methanol.

### Ferric-reducing antioxidant power (FRAP) assay

The Guo *et al*., 2003 procedure was followed while performing the Frap assay [21]. After diluting around 0.5 ml of methanolic extract with 0.5 ml of methanol, 2 ml of frap reagent was added, and the mixture was left for 15 minutes at 37 °C. By comparing with the ascorbic acid standard solutions, antioxidant activity was determined. Frap reagent was prepared freshly by addition of 2.5 ml of 10mmol/L TPTZ (2,4,6-Tripyridy-s-triazine, sigma) in 40 mmol/L HCl

and 2.5 ml of 20 mmol/L FeCl3 in 25 ml of 0.3mol/L acetate buffer, pH 3.6, maintained at $37^0$ C.

**Estimation of antioxidant enzyme activity**

**Catalase**

Catalase activity of all wheat variety was tested according to the method described by Herzog, V. & Fahimi, H. D. (1974) with slight modification [22]. About 2 g seeds were homogenized in 2ml 0.1M cold phosphate buffer (7 pH) with chilled mortar and pestle, centrifuged at 7000 rpm for 2 minutes. Supernatant was collected and used for calculation of CAT and POD activity. To measure CAT activity, 1 ml extract was taken in cuvette and diluted by 2 ml 0.1M cold phosphate buffer (7 pH), 50 µl of 10% $H_2O_2$ was added in reaction mixture and mixed well. Cuvette was placed in spectrophotometer and absorbance was read till 60 seconds at 240 nm. These values were used for further calculation.

**Peroxidase**

Peroxidase activity of all wheat variety was tested by checked according to the method described by Putter *et al*., 1974 [23]. A cuvette was filled with 3 ml of 0.1 M phosphate buffer (pH 7.0), 0.05 ml of 4% guaiacol, and 0.1 ml of extract. The mixture was then thoroughly mixed. Added 0.03 ml of 30% $H_2O_2$ and set the cuvette in the spectrophotometer at 436 nm. recorded the amount of time, in minutes, needed to raise the absorbance by 0.1.

**Superoxide dismutase**

Superoxide dismutase activity was performed by the method described by Beauchamp & Fridovich 1971 with some modification [24]. Two grams of seeds were crushed in 2 ml of 250 mM cold phosphate buffer (7.8 pH) by pre-chilled mortar and pestle. Mixture was centrifuge at 7000 rpm for 2 minutes and supernatant was used as extract. Reaction mixture was prepared in duplicate by adding 0.3 ml of 250 mM cold phosphate buffer (7.8 pH), 0.3 ml of 13 mM methionine, 0.3 ml of 2 uM riboflavin, 0.3 ml of 1 mM EDTA, 0.3 ml of 750 uM NBT and 50 ul of enzyme extract, added 1.45 ml of distilled water to make final volume 3 ml. Blank was prepared without enzyme and NBT to calibrate the spectrophotometer and control was prepared by adding only NBT and no enzyme. One set of the reaction mixture were exposed in 200 W bulb and another set of reaction mixture in dark for 15 minutes. Absorbance was read at 560 nm immediately.

**Results and Discussion**

**Percentage germination and Seedling length**

It is crucial to define the term 'germination' because it is frequently and occasionally misused in the scientific literature. The process of germination starts with the seed absorbing water (known as imbibition), and it concludes with the embryonic axis typically the radicle emerging through its surrounding components. The seed has finished germination (or germinated) at this latter stage, which is frequently referred to as "visible germination." [25]. Germination potential as well as its rate varies between different plant types and even within varieties of same species [26, 27]. Our results demonstrate a high germination potential of all the wheat varieties, showing sprouting within 24 to 30 hours of germination. Variation was observed between varieties. Highest germination of hundred percent was observed in seeds of HI-6759, DBW-187 and PBW-343 whereas lowest in HVW-243, HVW-468 and HVW-669 around 80% (Figure 1A, Supplementary Material-Table 1). Length of the seedling also varied between different wheat varieties. After seven days of germination, highest seedling growth was observed in PBW-343, 13.28±0.08 cm followed by DBW-187, 12.34±0.32 cm and HI-6759, 10.56±0.28 cm. Lowest Seedling length was found in HVW-669, 5.16±0.55 cm followed by HVW-468, 5.64±0.11 cm and HD-3086, 6.04±0.30 cm (Figure 1B, Supplementary Material-Table 1).

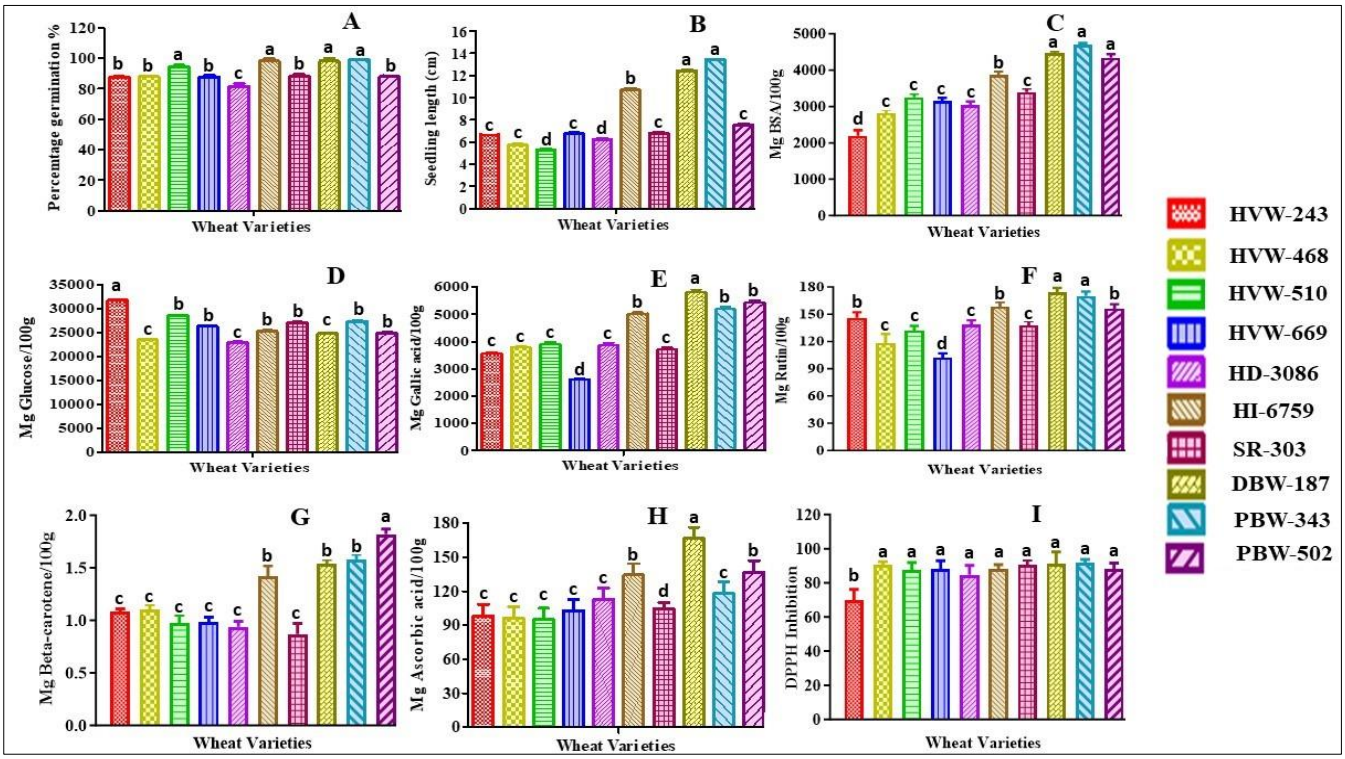


**Fig 1:** Morphological and biochemical parameters of different wheat sprouts (A) Percentage germination, (B) Seedling length, (C) Protein, (D) Carbohydrate, (E) Total phenolic, (F) Total flavonoids, (G) Carotenoids, (H) FRAP and (I) DPPH. Values are the means ± standard deviation, at significance difference p< 0.05 (different superscripts, a, b, c, d e, bc, cd shows significantly different values).

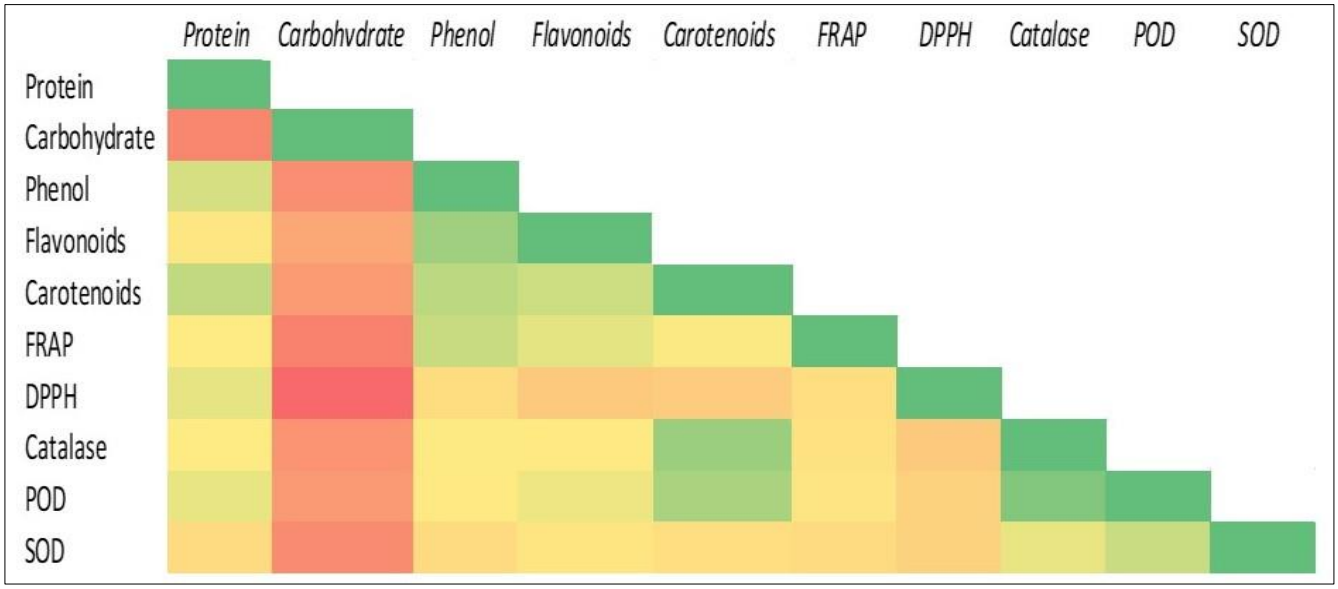


**Fig 2:** The variable correlations are explained by a heat map and hierarchical clustering.

**Table 1:** Percent germination calculated for entire period of observation and seedling length of ten wheat varieties estimated on 0, 2nd, 5th and 7th day of germination, mean ± standard deviation, N=3

| Wheat varieties | Percentage germination (%) | Germination stages (Days) | Seedling length (cm) |
|---|---|---|---|
| HVW-243 | 86.66±11.54 | G0 | 0.00±0.00 |
| | | G2 | 1.34±0.16 |
| | | G5 | 3.42±0.08 |
| | | G7 | 6.6±0.1 |
| HVW-468 | 86.66±11.54 | G0 | 0.00±0.00 |
| | | G2 | 1.40±0.15 |
| | | G5 | 3.46±0.16 |
| | | G7 | 5.64±0.11 |
| HVW-510 | 93.33±11.54 | G0 | 0.00±0.00 |
| | | G2 | 1.06±0.20 |
| | | G5 | 3.14±0.15 |
| | | G7 | 5.16±0.55 |
| HVW-669 | 86.66±11.54 | G0 | 0.00±0.00 |
| | | G2 | 2.22±0.13 |
| | | G5 | 4.92±0.17 |
| | | G7 | 6.84±0.16 |
| HD-3086 | 80.00±20 | G0 | 0.00±0.00 |
| | | G2 | 1.34±0.11 |
| | | G5 | 3.72±0.14 |
| | | G7 | 6.04±0.30 |
| HI-6759 | 100.00±00 | G0 | 0.00±0.00 |
| | | G2 | 3.46±0.13 |
| | | G5 | 6.04±0.38 |
| | | G7 | 10.56±0.28 |
| SR-303 | 86.66±11.54 | G0 | 0.00±0.00 |
| | | G2 | 1.16±0.11 |
| | | G5 | 4.14±0.34 |
| | | G7 | 6.70±0.20 |
| DBW-187 | 100.00±00 | G0 | 0.00 |
| | | G2 | 3.10±0.1 |
| | | G5 | 7.84±0.11 |
| | | G7 | 12.34±0.32 |
| PBW-343 | 100.00±00 | G0 | 0.00 |
| | | G2 | 3.06±0.05 |
| | | G5 | 5.76±0.33 |
| | | G7 | 13.28±0.08 |
| PBW-502 | 86.66±11.54 | G0 | 0.00 |
| | | G2 | 1.68±0.08 |
| | | G5 | 4.68±0.08 |
| | | G7 | 7.44±0.37 |

**Protein content**

Protein content of all wheat variety was estimated by Lowry method using phosphate buffer (pH 7) and expressed in mg BSA equivalent per 100g of seeds (Supplementary material, Table 2). Proteins were found to be higher in seeds with sprouts when compared with non-sprouted seeds. Total protein was observed in range from 84.89 mg BSA/100 g (HVW-243) to 197.04 mg BSA/100 g (PBW-343) in non-sprouted seeds and 2046.47 mg BSA/100 g (HVW-243) to 4643.07 mg BSA/100 g (PBW-343) in seven days germinated seeds. Protein content of all wheat varieties increased rapidly after germination with a multifold increase ranging from 9.4 (PBW-343) to 12.79 (HVW-243) observed after 2 days. Enhancement in the protein content followed till seventh day, with highest increment shown by HVW-510, 35.28-folds and PBW-187, 31.89-folds. Varieties HVW-243 and HVW-468 showed lowest increment, 24.10-folds and 26.80-folds respectively (Figure 1C, Supplementary Material-Table 2). Our findings are in consistent with the studies reported in literature, showing high protein concentration in sprouted cereals [28]. It was also observed that the rate of increase was very high during early times of germination, but later increment was slowed. This is due to rapid increase in metabolic activity during start of germination, thereby increasing gene expression levels and overall protein profile of the plant [29].

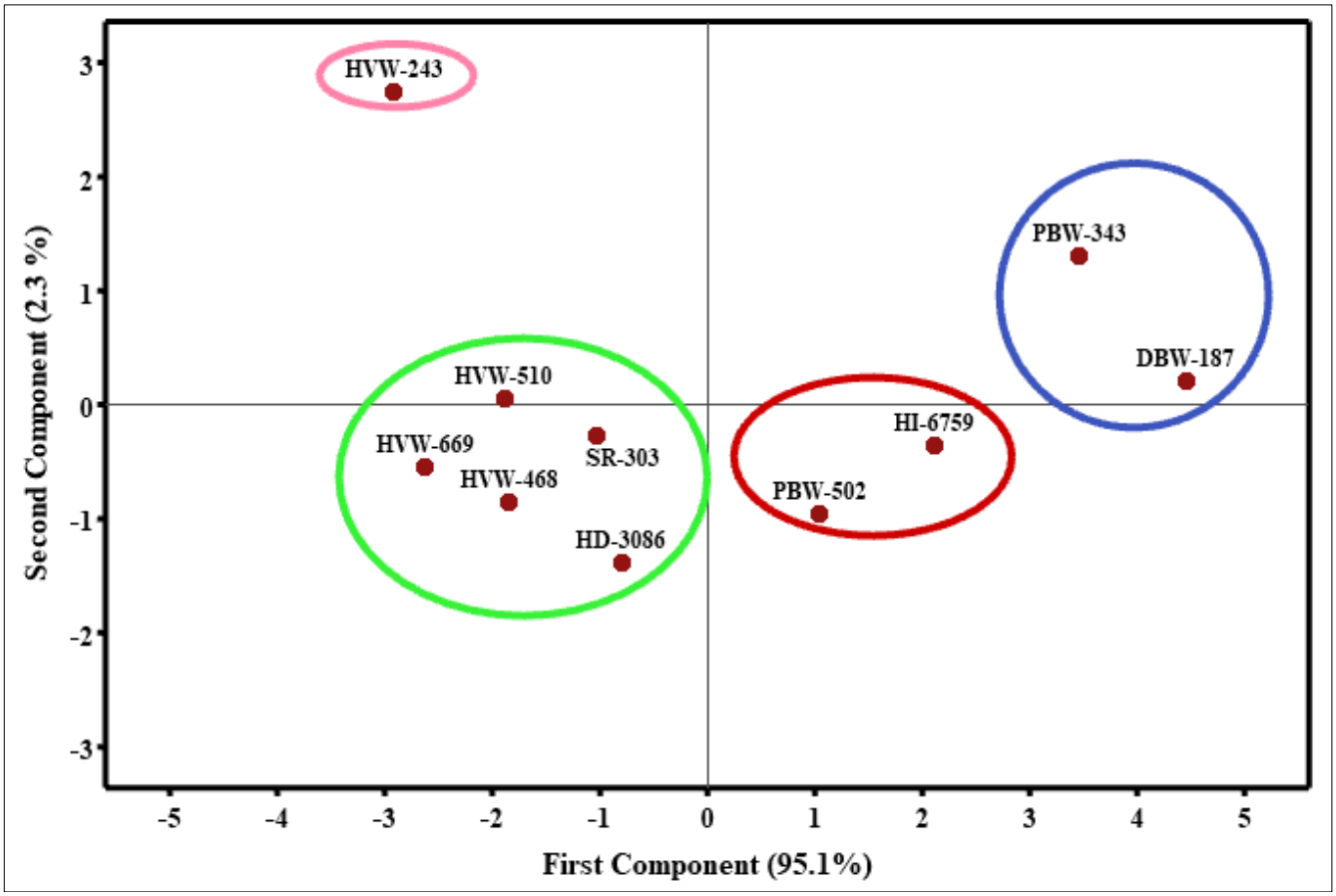


**Fig 3:** Principal component analysis: Score plot categorizing wheat varieties on the basis of their correlation.

**Carbohydrates**

The total carbohydrates present in all wheat variety were estimated using the phenol-sulphuric acid method, quantified spectrophotometrically and tabulated in Table 2. Sugar is treated in acidic medium forming hydroxymethyl furfural after dehydration, which forms green colour after reaction with phenol. Wheat is a rich source of carbohydrate [30]. Our results also demonstrate that carbohydrates were present in high concentration in non-sprouted seeds, ranging between 4.190 g Glucose/100 g of seeds (HI-6759) to 12.783 g Glucose/100 g of seeds (HVW-243). It had been reported that that sugar content increases during germination, thereby providing energy to the growing embryo (Megat *et al*., 2016). Our findings also report an increase in the total carbohydrate content during germination, with estimated values ranging between 22.672 g Glucose/100 g (HD-3086) to 31.714 g Glucose/100 g (HVW-243) after seven days.

Carbohydrate concentration in seeds increased after germination, but when we compare it with phenol and protein, increment was slow. After two days of germination highest increment was shown in HI-6759, 2.51-folds and PBW-187, 1.94-folds and lowest in HVW-669, 1.23 folds and HVW-510, 1.43-fold. It was shown to increase continuously after 5$^{th}$ day of germination but with less variation. On seventh day of germination highest increase was found in HVW-243, 5.2-folds and HVW-510, 4.64-folds and lowest increment in HVW-669, 2.57-folds and HI-6759, 2.72-folds (Figure 1D, Supplementary Material-Table 2).

**Table 2:** Phytochemical composition and antioxidant activity of ten wheat varieties estimated on 0, 2nd, 5th and 7th day of germination. Values expressed as mg/100 g of seed; mean ± standard deviation, N=3

| Wheat variety | Germination stages | Protein (mg BSA/100g seeds) | Carbohydrate (mg Glucose/100g seeds) | Phenol (mg Galic acid/100g seeds) | Flavonoids (mg quercetin/100g seeds) | Carotenoids (mg beta-carotene/100g seeds) | FRAP (mg ascorbic acid/ 100g seeds) | DPPH (% inhibition) |
|---|---|---|---|---|---|---|---|---|
| HVW-243 | G0 | 84.89 ±7.55 | 7769.47±49.26 | 572.30±15.38 | 26.95±0.75 | 0.17±0.19 | 22.55±0.31 | 5.58±0.03 |
| | G2 | 1075.12±23.54 | 11594.35±46.52 | 965.63±20.88 | 54.27±1.36 | 0.37±0.18 | 37.52±0.35 | 29.72±0.06 |
| | G5 | 1419.74±16.93 | 27453.84±42.33 | 3038.97±23.09 | 77.61±1.21 | 0.82±0.25 | 54.44±0.24 | 39.50±0.10 |
| | G7 | 2046.47±26.85 | 31714.70±48.16 | 3519.99±21.53 | 140.14±2.82 | 1.04±0.18 | 88.38±0.31 | 53.27±0.08 |
| HVW-468 | G0 | 103.40±13.25 | 6584.35±48.10 | 689.22±26.29 | 39.38±0.80 | 0.24±0.15 | 21.43±0.45 | 4.08±0.02 |
| | G2 | 1271.79±26.52 | 10707.33±59.34 | 1169.22±23.22 | 63.07±1.46 | 0.42±0.18 | 36.47±0.27 | 27.47±0.07 |
| | G5 | 1623.67±93.01 | 20573.30±68.52 | 2128.20±18.19 | 84.44±1.07 | 0.80±0.33 | 51.85±0.29 | 37.00±0.11 |
| | G7 | 2719.99±18.46 | 23556.41±62.12 | 3746.69±29.52 | 108.26±1.09 | 1.05±0.32 | 86.35±0.29 | 72.40±0.14 |
| HVW-510 | G0 | 89.35±6.92 | 10558.97±62.72 | 579.48±25.80 | 27.38±0.88 | 0.14±0.18 | 19.57±0.40 | 5.07±0.08 |
| | G2 | 1373.33±21.82 | 15125.12±59.36 | 1929.22±17.13 | 58.95±1.14 | 0.42±0.22 | 35.41±0.31 | 28.82±0.08 |
| | G5 | 2236.14±23.22 | 23573.33±71.44 | 2789.08±17.62 | 78.03±1.20 | 0.67±0.37 | 50.45±0.14 | 49.5±0.09 |
| | G7 | 3152.30±24.61 | 28542.56±63.38 | 3833.00±18.46 | 127.13±1.43 | 0.90±0.27 | 84.97±0.59 | 78.96±0.016 |
| HVW-669 | G0 | 116.61±6.45 | 12783.59±58.57 | 671.79±18.55 | 37.68±0.80 | 0.23±0.15 | 20.51±0.48 | 6.15±0.01 |
| | G2 | 1669.19±56.17 | 15743.94±57.04 | 879.99±24.61 | 48.82±1.01 | 0.38±0.24 | 38.56±0.27 | 26.12±0.09 |
| | G5 | 1948.70±26.17 | 20155.9±46.29 | 1787.68±19.21 | 68.37±1.27 | 0.78±0.30 | 56.77±0.21 | 37.50±0.14 |
| | G7 | 3044.10±15.18 | 26121.02±34.03 | 2551.79±15.38 | 97.08±1.12 | 0.93±0.34 | 92.65±0.38 | 72.95±0.13 |
| HD-3086 | G0 | 110.53±7.27 | 6557.43±69.35 | 742.05±24.07 | 47.52±0.84 | 0.17±0.24 | 26.14±0.26 | 4.08±0.01 |
| | G2 | 1429.78±14.54 | 11515.89±40.15 | 2117.92±29.55 | 64.69±1.13 | 0.37±0.26 | 38.51±0.30 | 35.38±0.05 |
| | G5 | 1983.07±25.41 | 18336.92±57.42 | 3304.61±30.77 | 79.41±3.23 | 0.77±0.27 | 57.48±0.31 | 49.00±0.013 |
| | G7 | 2938.46±20.17 | 22672.82±77.37 | 3801.02±15.48 | 133.34±1.09 | 0.89±0.41 | 102.61±0.64 | 79.23±0.014 |
| HI-6759 | G0 | 130.05±6.69 | 4190.76±48.06 | 861.79±35.96 | 44.51±0.85 | 0.24±0.25 | 26.54±0.33 | 7.61±0.04 |
| | G2 | 1832.66±58.63 | 10554.87±56.92 | 2564.10±50.02 | 73.39±2.59 | 0.52±0.28 | 40.24±0.26 | 36.32±0.07 |
| | G5 | 2457.43±16.94 | 17664.46±70.73 | 4373.32±23.09 | 96.87±1.25 | 1.03±0.48 | 78.56±0.35 | 61.50±0.14 |
| | G7 | 3768.18±26.91 | 25026.15±50.01 | 4989.75±16.82 | 153.48±1.09 | 1.32±0.43 | 124.34±0.28 | 85.73±0.17 |
| SR-303 | G0 | 105.17±5.52 | 8733.33±64.63 | 614.35±21.82 | 48.65±0.89 | 0.19±0.25 | 25.84±0.45 | 6.59±0.02 |
| | G2 | 1308.20±16.94 | 14722.82±46.87 | 1596.92±20.17 | 69.29±0.98 | 0.47±0.30 | 37.88±0.78 | 31.03±0.6 |
| | G5 | 2056.40±27.92 | 20172.82±74.49 | 2356.92±61.46 | 83.04±1.38 | 0.82±0.24 | 56.10±0.32 | 56.00±0.14 |
| | G7 | 3296.92±81.13 | 26841.74±65.24 | 3640.50±45.29 | 132.49±1.17 | 0.97±0.34 | 99.09±0.40 | 78.41±0.13 |
| DBW-187 | G0 | 138.51±12.79 | 5468.21±40.83 | 852.81±43.52 | 50.54±0.84 | 0.23±0.17 | 29.61±0.40 | 6.09±0.01 |
| | G2 | 1996.32±41.66 | 10642.05±63.38 | 2494.22±23.08 | 74.66±2.55 | 0.50±0.17 | 42.54±0.46 | 36.92±0.07 |
| | G5 | 2747.69±15.98 | 17405.12±54.02 | 5337.43±16.94 | 98.97±6.11 | 1.29±0.34 | 81.99±0.18 | 59.50±0.18 |
| | G7 | 4417.43±30.82 | 24641.53±52.91 | 5757.94±18.19 | 179.02±1.32 | 1.90±0.49 | 156.37±0.39 | 84.15±0.12 |
| PBW-343 | G0 | 197.04±6.91 | 9535.38±43.07 | 919.48±24.87 | 59.78±0.84 | 0.24±0.21 | 26.50±0.43 | 7.10±0.02 |
| | G2 | 1852.81±27.19 | 15470.25±48.90 | 2576.40±18.54 | 74.66±2.55 | 0.64±0.34 | 37.83±0.18 | 34.68±0.15 |
| | G5 | 2894.86±26.60 | 23430.48±71.38 | 4583.58±20.48 | 88.51±1.04 | 1.34±0.21 | 60.96±0.11 | 57.00±0.19 |
| | G7 | 4643.07±20.17 | 27151.32±53.16 | 5130.25±15.48 | 165.03±1.54 | 1.93±0.43 | 108.02±0.39 | 79.78±0.24 |
| PBW-502 | G0 | 146.82±6.91 | 8489.22±64.61 | 685.38±24.62 | 49.91±0.66 | 0.21±0.19 | 27.36±0.36 | 8.62±0.02 |
| | G2 | 1739.74±33.27 | 16338.97±79.70 | 2349.74±38.43 | 68.40±1.03 | 0.50±0.27 | 40.60±0.35 | 37.83±0.10 |
| | G5 | 2732.30±23.22 | 21693.82±60.78 | 4051.15±34.23 | 89.46±1.64 | 0.93±0.35 | 78.51±0.13 | 68.00±0.21 |
| | G7 | 4239.99±26.29 | 24701.21±50.18 | 5365.05±6.68 | 151.08±4.39 | 1.47±0.21 | 126.92±0.51 | 84.69±0.16 |

**Phenolic content**

Research on phenols has increased due to their positive impacts on human health. They are known to possess several biological activities like antioxidants, anti-inflammatory, anti-carcinogenic, anti-ulcer, anti-thrombotic, analgesic, vasodilatory etc. [31]. Eating a diet rich in phenolic compounds is very much in demand. It is generally reported that the total phenolic content in cereals increases during germination [32]. In our experiment also, we observed that the phenolic content of all wheat varieties increased after germination. The total phenolic content estimated for all germinated and non-germinated seeds are presented in Supplementary material-able 2, expressed as mg of Gallic acid (GA) equivalent per 100 g seeds. The results also demonstrate that the amount of total phenolic content varied between different wheat varieties and also with time, showing an increasing value with the increase in time period. Total phenolic in wheat varieties ranged from 572.30 mg GA/100 g seeds (HVW-243) to 919.48 mg GA/100 g (PBW-343) in non-germinated seeds and 2551.79 mg GA/100 g seeds (HVW-669) to 5757.94 mg GA/100 g seeds (DBW-187) in seven days germinated seeds (Figure 1E, Supplementary material, Table 2).

The amount of phenolic content increased drastically within 2 days of germination. Highest increment was shown by PBW-502, with a 3.42-fold increase, followed by HVW-510, 3.32-folds and HI 6759, 2.97-folds. Lower values were observed for varieties HVW-669 with 1.30-fold, HVW-243 with 1.68-folds and HVW-468 with 1.69-folds. Phenolic content increased with further increase in germination time. On fifth day of germination highest increment was shown by DBW-187, 6.25-folds followed by PBW-502, 5.91-fold and HVW-243, 5.31-folds. When we compared the presence of phenolic content of non-germinated seeds with seven days germinated seeds, we found that it increased further, with highest increment shown by PBW-502, 7.82-folds and DBW-187, 6.75-folds. The effect of germination on the total phenolic content varied between varieties, for example highest phenolic content in non-germinated seed was found in PBW-343 (919.48 mg GA/100 g seeds), and however the increase after seventh day was only 5.58 as compared to HVW-502 showing 7.82-folds increases (Figure 1E. Supplementary material, Table 2). It was also observed that the rate of increase was maximum during early times of germination, till 5th day, after which it decreased.

**Flavonoids**

Total flavonoid content was quantified by calorimetric method and the results obtained are shown in Table 2, expressed as mg of quercetin/100 g seeds. Results reveal that total flavonoid content was highest in PBW-343 (59.78 mg Quercetin/100 g seeds) and lowest in PBW-502 (19.91 mg Quercetin/100 g seeds) in non-sprouted seeds, whereas

highest in DBW-187 (179.02 mg Quercetin/100 g seeds) and lowest in HVW-669 (97.086 mg Quercetin/100 g seeds) in seven day sprouted seeds.

The study shows that flavonoid content also increases with germination, with highest increment shown by HVW-510 (2.15-folds) followed by HVW-243 (2.01-folds) and lowest increment shown by PBW-343 (1.24-folds) followed by HVW-669 (1.29-folds) after two days. The increment was observed up to seventh day ranging between 2.57 to 5.20-fold (Figure 1F. Supplementary material, Table 2), however like phenol highest increment was observed on fifth day of germination in most of the wheat varieties. The results are in consistent with the previous report, showing increase in flavonoid content during germination [6].

**Carotenoids**

Carotenoid Content of all Wheat Varieties was quantified and expressed in Table 2 as mg of Beta-Carotene/100 g seeds. It was observed that Carotenoid concentration in all tested wheat variety was very low ranging from 0.147 mg Beta-carotene/100 g of seeds (HVW-510) to 0.247 mg Beta-carotene/100 g of seeds (PBW-343) in non-sprouted seeds. The increase during germination was also very low, ranging from 0.871 mg Beta-carotene/100 g of seeds (PBW-502) to 1.937 mg Beta-carotene/100 g of seeds (PBW-243) after 7$^{th}$ day. No significant change in carotenoid concentration was observed in varieties HVW-243, HD-3086, HI-6759 and PBW-187 (Figure 1G. Supplementary material, Table 2). Previous studies have also reported similar results, where carotenoid concentration was not affected by germination [12].

**Antioxidant activity**

**Frap assay**

In the FRAP method electron-donating substances reduced yellow $Fe^{3+}$ into blue $Fe^{2+}$ under acidic conditions [33]. The absorbance of all samples was compared with standard curve made from ascorbic acid and expressed in mg ascorbic acid/100g seeds. In the present study antioxidant activity by frap assay was observed in range of 19.57 mg ascorbic acid/100 g seeds (HVW-510) to 29.61 ascorbic acid /100 g seeds (DBW-187) in non-sprouted seeds and 84.97 mg ascorbic acid /100 g seeds (HVW-510) to 156.37 mg ascorbic acid /100 g seeds (DBW-187) in seven-day germinated seeds. Antioxidant activity increased after germination, with highest increment shown by HVW-669, 1.88-fold, followed by HVW-510, 1.80-folds and HVW-468, 1.70-folds. After seventh day antioxidant activity increased up to 5.30-folds in DBW-187, followed by HI-6759, 4.68-fold (Figure 1H. Supplementary material, Table 2). These results show a positive co-relation between phenolic and flavonoids, indicating that high levels of these compound in germinated wheat may act as a good source of natural antioxidant [31].

**DPPH assay**

DPPH is a stable and free radical compound which is solubilized in methanol and gives purple-colour solution. DPPH after getting reduced by the electrons donated by antioxidant molecules of the extract, becomes colorless [34]. The reduction in color is measured spectrophotometrically; lower the absorbance higher is the antioxidant activity. The antioxidant activities of all wheat variety were estimated by this method and the results are tabulated in Table 2. In non-germinated seeds, PBW-502 showed 8.62% inhibition of DPPH as compared with control, followed by HI-6759 (7.61%) and then PBW-343 (7.10%). After two days of germination percentage inhibition of DPPH was increased by 4.77-fold (HI-6759-187) to 8.67-fold (PBW-343). On seventh day of germination highest increment was shown by HD-3069, 19.41-folds followed by HVW-468, 17.74-folds and HVW-510, 15.57-folds and lowest by HVW-243, 9.54-folds followed by PBW-502-folds, 9.82 and PBW-343, 11.23-folds (Figure 1I. Supplementary material, Table 2). Antioxidant activity calculated by DPPH method also exhibits a positive co-relation between phenolic and flavonoids, demonstrating them as a source of free radical scavengers [35].

**Enzyme activities**

Seed germination is associated with generation of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals. Rapid detoxification of ROS is essential to prevent oxidative damage. Plant cells have well developed defence system consisting of antioxidant enzymes (e.g., catalase, peroxidase and superoxide) and small molecules that provide protection against oxidative stress by scavenging free radicals and reducing $H_2O_2$ to water [6]. It is observed that antioxidant enzyme activity increases during germination [9]. Our experiments also confirmed an increase in enzyme activity during germination and the results obtained are shown in Table 3. Catalase (CAT) activity started as soon as the seeds were hydrated and a significant increase was observed within 24 hours. Highest CAT activity after 24 hours was shown by PBW-343, 56.66±0.45 unit/ml followed by DBW-187, 46.00±0.78 unit/ml and HI-6759, 42.00±0.78 unit/ml and lowest in HVW-510,16.01±0.57 followed by SR-303, 17.33±0.56 and HD-3086, 24.00±0.89. After 78 hours an increase of 6.56 (HVW-243) to 16.8 (DBW-187) fold was observed. Peroxidase (POD) activity was negligible in non-sprouted seeds but increased with germination. A significant increase was shown in 48 hours, with highest values observed by HI-6759, 6.62±0.05 unit/ml followed by PBW-343, 6.21±0.04 unit/ml and DBW-187, 6.02±0.03 unit/ml. After 78 hours POD was shown to increase multifold, around 7.8 (HVW-468) to 13.6 (DBW-187) times higher than sprouted seeds. Superoxide dismutase (SOD) activity showed an increase within 24 hours of germination. Highest activity was observed in HI-6759,13.39±0.02 unit/ml followed by DBW-187,16.85±0.51 unit/ml and PBW-343, 20.00±0.56 unit/ml and lowest in SR-303, 5.41±0.02 followed by HVW-669, 5.44±0.07 and HVW-243, 5.55±0.06. After 78 hours sprouted, seeds reached its highest level, around 8.34 (HD-3086) to 16.89 (DBW-187) times.

**Table 3:** Enzyme activity of ten wheat varieties estimated on 24, 48 and 72 hours of germination. Values expressed as unit/ml, mean ± standard deviation, N=3

| Wheat variety | Germination time in hours | Catalase activity (unit/ml) | Super oxide dismutase activity (unit/ml) | Peroxidase activity (unit/ml) |
|---|---|---|---|---|
| HVW-243 | 24 | 34.66±1.03 | 5.55±0.06 | 0.00 |
| | 48 | 103.98±2.32 | 12.00±0.09 | 3.20±0.02 |
| | 72 | 287.67±2.43 | 49.87±1.67 | 25.28±0.93 |
| HVW-468 | 24 | 30.66±0.98 | 7.05±0.07 | 0.00 |
| | 48 | 107.31±2.25 | 13.71±0.09 | 4.16±0.03 |

| | | | | |
|---|---|---|---|---|
| | 72 | 370.98±2.76 | 61.94±0.26 | 32.44±0.86 |
| HVW-510 | 24 | 16.00±0.56 | 8.07±0.04 | 0.00 |
| | 48 | 57.60±1.85 | 17.09±0.09 | 3.22±0.03 |
| | 72 | 157.92±2.05 | 82.43±1.54 | 25.76±0.95 |
| HVW-669 | 24 | 24.00±0.98 | 5.44±0.07 | 0.00 |
| | 48 | 93.6±1.02 | 12.85±0.38 | 2.68±0.04 |
| | 72 | 285.36±3.78 | 65.33±0.85 | 21.70±0.88 |
| HD-3086 | 24 | 25.33±0.98 | 9.00±0.04 | 0.00 |
| | 48 | 97.52±1.93 | 19.00±0.79 | 5.50±0.02 |
| | 72 | 303.96±3.21 | 75.08±1.03 | 59.50±1.03 |
| HI-6759 | 24 | 42.00±0.78 | 13.39±0.02 | 0.00 |
| | 48 | 169.60±1.83 | 61.14±0.06 | 6.62±0.03 |
| | 72 | 663.64±3.21 | 202.17±1.02 | 75.46±1.16 |
| SR-303 | 24 | 17.33±0.56 | 5.41±0.02 | 0.00 |
| | 48 | 99.79±1.67 | 15.36±0.69 | 5.81±0.04 |
| | 72 | 241.57±2.45 | 72.93±1.08 | 58.97±1.08 |
| DBW-187 | 24 | 46.00±0.78 | 16.85±0.51 | 0.00 |
| | 48 | 270.94±2.56 | 60.57±0.87 | 6.02±0.03 |
| | 72 | 772.80±5.46 | 284.64±1.84 | 81.87±1.02 |
| PBW-343 | 24 | 56.66±0.45 | 20.00±0.56 | 0.00 |
| | 48 | 330.32±2.89 | 64.94±1.39 | 6.21±0.04 |
| | 72 | 912.22±7.53 | 299.25±1.95 | 81.28±1.86 |
| PBW-502 | 24 | 35.66±0.16 | 6.00±0.03 | 0.00 |
| | 48 | 142.64±1.28 | 24.13±1.03 | 2.13±0.03 |
| | 72 | 370.15±2.64 | 72.41±1.85 | 19.71±0.88 |

**Heat Map**

A heat map is a graphical display of numerical data in which the colors correspond to the individual values. The display is combined with a particular sequence in heat map figure. The features that are most closely connected to the target feature can be identified by automatically rearranging the matrix data using various clustering techniques. The squares represent the connections between sets of rows and columns (Singh *et al* 2023). The susceptibility indices of various morphological features were aggregated and normalized. The intensity of the several parameters normalized mean values is shown, we can infer information about the correlation between attributes from the colors. Larger values are often represented by green squares, and lesser values by red squares. Three unique clusters were observed among all the investigated factors in wheat cultivars (Figure 2). According to the result as shown in Figure, the maximum intensity was seen in phenolic content and also positive correlation with other biochemical, antioxidant and enzyme activities. It was showing strong correlation with flavonoid, carotenoid and FRAP, less correlation with SOD activities but it was negatively correlated with carbohydrate. Protein content was also showing strong correlation with carotenoid, phenol and flavonoid, it was weakly correlated with SOD activity. Carbohydrate was negatively correlated with almost all tested parameter except flavonoid (less correlation). Carotenoids were also depicting strong correlation with Catalase and POD activity while catalase were showed highest correlation with POD. All tested wheat varieties varied from one another, still showing a favorable association.

**Principal component analysis**

A mathematical process known as principal component analysis (PCA) splits a large number of positively correlated variables into a smaller number of uncorrelated variables known as principal components. The variability in the data is mostly explained by the first principal component to the greatest extent feasible, with the remaining variability being explained by each subsequent component (Figure 3). The score plot in this report was generated using the first two principal components, which together accounted for 97.4% of the variation (Figure 3). PCA finds new, significant underlying variables and reduces the complexity of the data set. With PCA we can categorize wheat varieties into four groups based on their correlations with one another. HVW-510, HVW-669, HVW-468, SR-303 and HD-3086 are grouped in green circle, showing strongest correlation to each other than other varieties. Similarly, HI-6759 and PBW-502 are grouped in red circle, showing positive correlation and PBW-343 and DBW-187 are in blue circle, correlated to each other. HVW-243 enclose in pink circle have no correlation. We also find that these groups are less correlated to each other.

**Conclusion**

Germination studies on wheat seeds clearly demonstrated varietal differences in phytochemical content, enzyme activity and antioxidant activity. Although a similar pattern was observed in biochemical changes, their amount varied between varieties. A multifold increase in total phenolics, proteins, carbohydrates, enzyme and antioxidant activity were observed after germination in all the wheat varieties, however each variety responded differentially depending on their genotypes. The study is useful in selecting varieties with higher nutritional values for development of functional foods.

**Acknowledgements**

The authors acknowledge Banaras Hindu University (BHU), Varanasi, India, for providing laboratory facilities and fund support.

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