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# Geographical Variation in Phytochemical and Antioxidant Profile of Buckwheat (Fagopyrum Esculentum Moench)

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**ABSTRACT**

Buckwheat (Fagopyrum esculentum Moench) is an important pseudocereal crop known for its nutritional and phytochemical richness. The present study was conducted to evaluate the variation in phytochemical constituents and antioxidant enzyme activities among buckwheat seed samples collected from four locations in Haryana, namely Hisar (BWH), Bhiwani (BWB), Loharu (BWL) and Rohtak (BWR), during the Rabi season of 2023-2024. The collected seeds were grown under uniform pot conditions using a completely randomized design with three replications. Mature seeds were analysed for major phytochemical constituents, including flavonoids, alkaloids, carotenoids, tannins, terpenoids, steroids and saponins. Antioxidant defence responses were assessed through superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) activities, while lipid peroxidation was estimated through malondialdehyde (MDA) content. Significant variations were observed among the four locations for most biochemical parameters. BWL exhibited the highest flavonoid (7.54mg QE $g^{-1}$ DW), alkaloid (3.28mg $g^{-1}$ DW), tannin (2.74mg $g^{-1}$ DW), terpenoid (4.14mg $g^{-1}$ DW) and saponin (4.74mg $g^{-1}$ DW) contents, whereas steroid content showed non-significant variation. Similarly, BWL recorded maximum SOD (36.80U $mg^{-1}$ protein), CAT (18.40U $mg^{-1}$ protein), POX (13.90U $mg^{-1}$ protein) and APX (5.14U $mg^{-1}$ protein) activities. In contrast, the lowest MDA content (4.56nmol $g^{-1}$ FW) was recorded in BWL. Correlation analysis further revealed strong positive associations between antioxidant enzymes and several phytochemical constituents, while MDA showed strong negative correlations with SOD, CAT and POX. Findings from this study showed biochemical diversity among buckwheat where BWL exhibiting comparatively higher phytochemical accumulation and antioxidant activity coupled with lower lipid peroxidation.

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## 1. Introduction

The increasing global frequency of chronic non-communicable diseases concerns food and nutritional security, strengthening of environmental stresses and changing consumer preferences to gluten free foods and accelerated the exploration for nutrient rich and potential replacements of cereals (Arya & Bisht, 2022). Pseudocereals have emerged as promising alternative crops owing to their favorable nutritional composition, abundance of bioactive compounds and ability to perform under marginal and stressed environments (Ţopa et al. 2025; Sharma et al. 2026). Buckwheat (Fagopyrum esculentum) has acknowledged for the scientific and commercial attention because of its characteristic nutritional profile, naturally gluten free nature and significant antioxidant potential (Koç et al. 2025). Buckwheat not only contributes to human health through improved nutritional quality but also to support sustainable agricultural production under increasingly fluctuated environmental conditions (Chaudhari et al. 2026). These characteristics have established buckwheat as a valuable raw material for the development of functional foods and nutraceutical products and potential contribution to sustainable and nutritionally secure food systems (Lițoiu et al. 2025).

Buckwheat is an annual herbaceous pseudocereal belonging to Polygonaceae family and botanically classified as a dicotyledonous plant rather than a true cereal (Canbey et al. 2026). Grains are cultivated, processed and consumed like as conventional

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cereals such as wheat, rice and maize constitute an important source of dietary fibers (Kumari et al. 2026). Traditionally, buckwheat has been cultivated chiefly in moderate to temper region of the mountainous regions of Asia and Europe whereas, its cultivation and scientific importance have been extended significantly due to its short growth duration, efficient resources utilization and capacity to grow under relatively poor soil conditions and adverse environmental circumstances (Kreft et al. 2023; Żarczyński et al. 2026); these characteristics make buckwheat particularly significant under low input and climate resilient agricultural production (Rangappa et al. 2023). The fusion of promising agronomic features and nourishing cost has subsequently strengthened the position of buckwheat as a sustainable crop for the future food, nutritional and agricultural safety (Choudhury et al. 2026).

Buckwheat holds a distinctive nutritional profile characterized by high proteins content, essential amino acid, complex carbohydrates, dietary fibre, fatty acids, vitamins and essential minerals (Sonawane et al. 2024) and important source of various phytochemicals such as flavonoids, phenolic acids, carotenoids, tocopherols, tannins, alkaloids, saponins, terpenoids and phytosterols (Chandla et al. 2024); whereas rutin and quercetin are the characteristic flavonoids of buckwheat because of their relatively high abundance in overall phenolic composition and antioxidant activity (Dziedzic et al. 2025). Their capacity for free radical scavenging, metal ion chelation and inhibition of lipid peroxidation is considered to contribute substantially to the biological and health promoting properties associated with buckwheat consumption (Rajabboyevna et al. 2025). The antioxidant capacity of buckwheat is dominated not only by the accumulation of phytochemical constituents but also by the synchronized functioning of its endogenous antioxidant defense system (Song et al. 2025) comprises a series of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX), which act sequentially and cooperatively to regulate reactive oxygen species (ROS) and maintain cellular redox homeostasis (Rajabboyevna & Jomurodovich, 2025). Malondialdehyde (MDA) and proline are widely used as biochemical indicators associated with oxidative membrane damage and physiological responses to environmental stress (Luca et al. 2026) and any changes in these enzymatic and non-enzymatic constituents provide direct information of the oxidative status and antioxidant defence capacity of plant tissues (Dash et al. 2025).

Despite increasing scientific interest in the antioxidant potential of buckwheat the inter relationships between phytochemical accumulation, oxidative stress related indicators and enzymatic antioxidant defense mechanisms remain uncertain. Furthermore, comparatively limited information is available to the extent to which genotype-dependent variation affects the integrated antioxidant defence system and consequently contributes to differences in the nutritional and functional quality of buckwheat. The present study was undertaken to compare the phytochemical composition and antioxidant properties of four buckwheat (Fagopyrum esculentum) genotypes collected from different regions of Haryana, India. In contrast to studies primarily focused on quantitative assessment of diverse phytochemicals and biochemical characteristics of buckwheat.

## 2. Materials and Methods

Selection and Collection of Seed Material: The seeds was collected during the Rabi season of 2023-2024 from four distinct agro-ecological locations representing different geographical regions of the state, namely Mayar, Hisar (29.106976°N, 75.860966°E); Bamla, Bhiwani (28.798250°N, 76.191926°E); Loharu, Bhiwani (28.433846°N, 75.812192°E); and Samchana, Rohtak (28.856437°N, 76.787912°E). The collected seed samples were marked as BWH, BWB, BWL and BWR corresponding to Hisar, Bhiwani, Loharu and Rohtak, respectively. After collection of the seeds properly labelled, packed and transported to the laboratory at Hindu Girls College, Sonipat, Haryana, for further biochemical and another physiological analysis.

Raising of Crop and Experimental Layout

The collected seeds were germinated under uniform agronomic conditions with regular irrigation and pot maintenance. The experiment followed a Completely Randomized Design (CRD) with three replications. After 30 days, healthy seedlings were transplanted into earthen pots containing 8kg sandy loam soil and grown until physiological maturity. Harvested seeds were labelled and used for biochemical analyses.

### Preparation of Seed Extract and biochemical analysis

Mature buckwheat seeds were manually cleaned to remove impurities, dried at room temperature and ground into a fine powder using a laboratory grinder (WSG60E). The powdered samples were stored in airtight containers. A known quantity was extracted with 80% methanol under continuous shaking, filtered through Whatman No. 1 filter paper, and the filtrate was stored in sealed 5mL Eppendorf tubes for subsequent phytochemical and antioxidant analyses.

Phytochemical constituents of buckwheat seeds were estimated using standard spectrophotometric and gravimetric methods. Total flavonoids were determined according to Zhishen et al. (1999) using quercetin as the reference standard and expressed as mg QE $g^{-1}$ dry weight (DW). Alkaloids were estimated following Harborne (1973) and expressed as mg $g^{-1}$ DW. Carotenoid content was

measured using chilled 80% acetone following Lichtenthaler (1987), with absorbance recorded at 470, 646.8 and 663.2nm, and expressed as mg $g^{-1}$ fresh weight (FW). Tannins were quantified using the Folin–Ciocalteu colorimetric method and expressed as mg tannic acid equivalents (TAE) $g^{-1}$ DW. Terpenoids were estimated through petroleum ether extraction according to Ferguson (1956), while steroids were determined using a chloroform–sulphuric acid colorimetric reaction with cholesterol as the reference standard; both were expressed as mg $g^{-1}$ DW. Total saponins were quantified using the gravimetric method of Obadoni and Ochuko (2001), involving aqueous ethanol extraction and n-butanol partitioning, and expressed as mg $g^{-1}$ DW.

Antioxidant enzyme activities were determined using established spectrophotometric methods. Superoxide dismutase (SOD) activity was estimated according to Dhindsa et al. (1981) by measuring the inhibition of nitro blue tetrazolium (NBT) photoreduction at 560nm. Catalase (CAT) activity was assayed following Aebi (1984) by monitoring the decomposition of hydrogen peroxide ($H_2O_2$) at 240nm. Peroxidase (POX) activity was determined using the method of Kar and Mishra (1976), based on guaiacol oxidation, with absorbance measured at 470nm. Ascorbate peroxidase (APX) activity was estimated according to Nakano and Asada (1981) by measuring ascorbate oxidation at 290nm. Lipid peroxidation was assessed as malondialdehyde (MDA) content following Heath and Packer (1968). Plant tissue was homogenized in trichloroacetic acid (TCA), reacted with thiobarbituric acid (TBA), and absorbance was recorded at 532 and 600nm. Enzyme activities were expressed per milligram of protein, while MDA content was expressed as nmol $g^{-1}$ fresh weight.

Statistical Analysis: The experimental data were analysed for one way analysis of variance (ANOVA) using OPSTAT statistical software developed by CCS HAU, Hisar at the 5% probability level ($p \leq 0.05$).

## 3. Results and Discussion

The biochemical analysis showed considerable variation in buckwheat genotype collected from Hisar (BWH), Bhiwani (BWB), Loharu (BWL) and Rohtak (BWR) in phytochemical and antioxidant activities in table- 1. BWL recorded the highest values for most parameters, indicating a comparatively stronger biochemical and antioxidant profile. Flavonoid content (mg quercetin equivalents [QE] $g^{-1}$ dry weight [DW]) was highest in BWL (7.54) followed by BWB (6.86), BWR (6.80) and BWH (6.43) with differences significant at the 1% level. Alkaloid content (mg $g^{-1}$ DW) ranged from 2.82 in BWH to 3.28 in BWL, while carotenoid content (mg $g^{-1}$ DW) increased from 1.47 to 1.68. Tannin content (mg $g^{-1}$ DW) was highest in BWL (2.74), compared with 2.37 in BWH. Terpenoid content (mg $g^{-1}$ DW) also reached its maximum in BWL (4.14), followed by BWB (3.85), BWR (3.79) and BWH (3.54). Saponin content (mg $g^{-1}$ DW) followed the same trend, with values ranging from 4.10 in BWH to 4.74 in BWL. These differences were significant at the 5% level. In contrast, steroid content (mg $g^{-1}$ DW) varied from 1.29 in BWH to 1.53 in BWL but the differences were found non-significant. Superoxide dismutase (SOD; U $mg^{-1}$ protein) activity was significantly higher in BWL (36.80) than in the other groups, followed by BWB (34.10), BWR (33.20) and BWH (31.40). Catalase (CAT; U $mg^{-1}$ protein) activity was highest in BWL (18.40) and lowest in BWH (15.60), with differences significant at the 0.01 level. Peroxidase (POX; U $mg^{-1}$ protein) activity also peaked in BWL (13.90), while ascorbate peroxidase (APX; U $mg^{-1}$ protein) reached 5.14 in BWL compared with 4.53 in BWH; both showed significant differences. In contrast, malondialdehyde (MDA) content, an indicator of lipid peroxidation (nmol $g^{-1}$ fresh weight [FW]), was lowest in BWL (4.56) and highest in BWH (5.21) with differences significant at the 1% level as indicated in the table-1.

**Table- 1.** Phytochemical and antioxidant responses of buckwheat collected form the Hisar, Bhiwani, Loharu and Rohtak

| Parameter | BWH | BWB | BWL | BWR | Pooled SE(m) | CD (5%) | Sign. |
|---|---|---|---|---|---|---|---|
| Flavonoids | 6.43 ± 0.18 | 6.86 ± 0.10 | 7.54 ± 0.14 | 6.80 ± 0.06 | 0.13 | 0.42 | ** |
| Alkaloids | 2.82 ± 0.15 | 3.12 ± 0.26 | 3.28 ± 0.11 | 2.92 ± 0.10 | 0.17 | 0.54 | * |
| Carotenoids | 1.47 ± 0.10 | 1.55 ± 0.04 | 1.68 ± 0.17 | 1.57 ± 0.12 | 0.12 | 0.38 | * |
| Tannins | 2.37 ± 0.04 | 2.48 ± 0.17 | 2.74 ± 0.08 | 2.43 ± 0.03 | 0.10 | 0.31 | * |
| Terpenoids | 3.54 ± 0.23 | 3.85 ± 0.08 | 4.14 ± 0.07 | 3.79 ± 0.10 | 0.14 | 0.45 | * |
| Steroids | 1.29 ± 0.12 | 1.46 ± 0.10 | 1.53 ± 0.17 | 1.36 ± 0.09 | 0.12 | 0.40 | NS |
| Saponins | 4.10 ± 0.21 | 4.39 ± 0.15 | 4.74 ± 0.10 | 4.24 ± 0.12 | 0.15 | 0.49 | * |
| SOD | 31.40 ± 0.17 | 34.10 ± 0.08 | 36.80 ± 0.04 | 33.20 ± 0.08 | 0.10 | 0.34 | ** |
| CAT | 15.60 ± 0.09 | 17.10 ± 0.06 | 18.40 ± 0.15 | 16.50 ± 0.25 | 0.16 | 0.51 | ** |
| Peroxidase | 11.40 ± 0.19 | 12.60 ± 0.10 | 13.90 ± 0.09 | 12.10 ± 0.26 | 0.17 | 0.57 | ** |
| APX | 4.53 ± 0.12 | 4.81 ± 0.11 | 5.14 ± 0.07 | 4.69 ± 0.17 | 0.12 | 0.40 | * |
| MDA | 5.21 ± 0.11 | 4.89 ± 0.11 | 4.56 ± 0.05 | 5.03 ± 0.07 | 0.09 | 0.29 | * |

BWH- Buckwheat genotype collected from Hisar, BWB- from Bhiwani, BWL- from Loharu, BWR- from Rohtak, SOD- Superoxide dismutase, CAT- Catalase, APX- Ascorbate peroxidase, MDA- Malondialdehyde and * Significant at the 0.05 level and ** Significant at the 0.01 level

The Pearson correlation analysis revealed significant associations among phytochemical constituents, antioxidant enzyme activities and lipid peroxidation in buckwheat (Table 2). Flavonoids exhibited strong positive correlations with terpenoids (0.839), tannins (0.741), superoxide dismutase (SOD; r=0.918), catalase (CAT; r=0.893) and peroxidase (0.861). Significant positive relationships were also observed between flavonoids and carotenoids, saponins and ascorbate peroxidase (APX). Alkaloids were positively correlated with tannins (0.720), SOD (0.579), CAT (0.581) and peroxidase (0.666). Carotenoids showed a positive association with steroids (0.577). Similarly, tannins were positively associated with terpenoids, SOD, CAT, peroxidase and APX. Terpenoids also showed positive correlations with SOD (0.761), CAT (0.681) and peroxidase (0.694). Saponins exhibited positive associations with SOD (0.730), CAT (0.813), peroxidase and APX. Among antioxidant enzymes, strong positive correlations were observed between SOD and CAT (0.972), SOD and peroxidase (0.961), and CAT and peroxidase (0.963). APX was also positively correlated with SOD (0.763), CAT (0.753) and peroxidase (0.788), indicating coordinated activity among antioxidant enzymes. In contrast, malondialdehyde (MDA), an indicator of lipid peroxidation, showed negative correlations with flavonoids (−0.806), terpenoids (−0.696), SOD (−0.904), CAT (−0.841), peroxidase (−0.890) and APX (−0.620). Findings indicate that higher phytochemical accumulation and antioxidant enzyme activities were generally associated with lower lipid peroxidation, suggesting a relationship between antioxidant defence and membrane protection in buckwheat.

**Table-2.** Pearson Correlation Matrix of Phytochemicals, Antioxidant Activities and Lipid Peroxidation of buckwheat collected form the Hisar, Bhiwani, Loharu and Rohtak

| Variables | Flv | Alk | Car | Tan | ter | Ste | Sap | SOD | Cat | Per | APX | MDA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Flv** | **1** | 0.524 | 0.618* | 0.741** | 0.839** | 0.568 | 0.688* | 0.918** | 0.893** | 0.861** | 0.662* | -0.806** |
| **Alk** | 0.524 | **1** | 0.098 | 0.720** | 0.502 | 0.097 | 0.455 | 0.579* | 0.581* | 0.666* | 0.512 | -0.465 |
| **Car** | 0.618* | 0.098 | **1** | 0.139 | 0.434 | 0.577* | 0.527 | 0.435 | 0.468 | 0.38 | 0.315 | -0.449 |
| **Tan** | 0.741** | 0.720** | 0.139 | **1** | 0.623* | 0.028 | 0.467 | 0.681* | 0.656* | 0.705* | 0.709** | -0.505 |
| **ter** | 0.839** | 0.502 | 0.434 | 0.623* | **1** | 0.341 | 0.472 | 0.761** | 0.681* | 0.694* | 0.546 | -0.696* |
| **Ste** | 0.568 | 0.097 | 0.577* | 0.028 | 0.341 | **1** | 0.341 | 0.493 | 0.495 | 0.392 | -0.072 | -0.515 |
| **Sap** | 0.688* | 0.455 | 0.527 | 0.467 | 0.472 | 0.341 | **1** | 0.730** | 0.813** | 0.681* | 0.628* | -0.521 |
| **SOD** | 0.918** | 0.579* | 0.435 | 0.681* | 0.761** | 0.493 | 0.730** | **1** | 0.972** | 0.961** | 0.763** | -0.904** |
| **Cat** | 0.893** | 0.581* | 0.468 | 0.656* | 0.681* | 0.495 | 0.813** | 0.972** | **1** | 0.963** | 0.753** | -0.841** |
| **Per** | 0.861** | 0.666* | 0.38 | 0.705* | 0.694* | 0.392 | 0.681* | 0.961** | 0.963** | **1** | 0.788** | -0.890** |
| **APX** | 0.662* | 0.512 | 0.315 | 0.709** | 0.546 | -0.072 | 0.628* | 0.763** | 0.753** | 0.788** | **1** | -0.620* |
| **MDA** | -0.806** | -0.465 | -0.449 | -0.505 | -0.696* | -0.515 | -0.521 | -0.904** | -0.841** | -0.890** | -0.620* | **1** |

Flv - Flavonoids, Alk - Alkaloids, Car- Carotenoids, Tan- Tannins, Ter- Terpenoids, Ste- Steroids, Sap- Saponins, SOD- Superoxide Dismutase; CAT- Catalase, Per – Peroxidase, APX - Ascorbate Peroxidase; MDA- Malondialdehyde, *Significant at the 5% level ($p < 0.05$) and **significant at the 1% level ($p < 0.01$).

## 4. Discussion

Fagopyrum esculentum is valued as a pseudocereal rich in the phytochemicals and antioxidant compounds as well as nutraceutical properties. In this study, the four seed lots differed in antioxidant enzyme activity, malondialdehyde and phytochemical composition. Collected seed samples from the Hisar (BWH), Bhiwani (BWB), Loharu (BWL) and Rohtak (BWR) showed the strongest antioxidant and phytochemical profile among all traits. BWL had the highest SOD, catalase (CAT), peroxidase and ascorbate peroxidase (APX) activities, whereas BWH generally had the lowest. Genotype-dependent variation in buckwheat antioxidant enzymes has also been reported under drought and salinity treatments (Hossain et al. 2024; Ma et al. 2019). MDA an indicator of lipid peroxidation, ranged from 4.56 to 5.21 nmol $g^{-1}$ FW; BWL had the lowest value and BWH the highest. The negative associations observed between MDA and several antioxidant traits are consistent with coordinated antioxidant responses. However, these correlations do not demonstrate causation, and stress studies are not directly comparable with the mature seed measurements in this experiment (Zhou et al. 2015; Janovská et al. 2021). Flavonoids ranged from 6.43 to 7.54 mg QE $g^{-1}$ DW with BWL highest and BWH had lowest. This variation agrees with studies reporting differences in buckwheat flavonoids and phenolic profiles among cultivars and growing locations (Oomah & Mazza, 1996; Kiprovski et al. 2015; Janovská et al. 2021). Carotenoid values ranged from 1.47 to 1.68 again with BWL highest. Previous work has

reported variation in buckwheat carotenoids across developmental stages and growth conditions (Zhou et al. 2015; Ma et al. 2019). Tannin values ranged from 2.37 to 2.74 mg $g^{-1}$ DW, terpenoids from 3.54 to 4.14 mg $g^{-1}$ DW, and saponins from 4.10 to 4.74 mg $g^{-1}$ DW. BWL had the highest value for each trait. Buckwheat studies have documented tannins and other bioactive compounds in seeds and seed fractions and have quantified saponins in buckwheat seed material (Steadman et al. 2001; Huda et al. 2021; Jing et al. 2016; Aloo et al. 2021, 2023). Steroids ranged from 1.29 to 1.53 mg $g^{-1}$ DW but differences among the four lots were not statistically significant.

## 5. Conclusion

The present study revealed the seed collected from BWL (Loharu) exhibited superior phytochemical accumulation, with higher concentrations of flavonoids, alkaloids, carotenoids, tannins, terpenoids and saponins. It also recorded the highest activities of antioxidant enzymes, including SOD, CAT, POX and APX, accompanied by the lowest MDA content. Correlation analysis further supported the close association between phytochemical constituents and antioxidant defence activities, while the negative relationship between antioxidant enzymes and MDA indicated reduced lipid peroxidation. These findings provide a basis for further evaluation of buckwheat diversity, nutritional quality and selection of promising genetic resources for future crop improvement programmes.

Competing of interest- We declare that we have no competing interests or personal relationships that influenced in the reported work in this paper.

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**Data Availability-** The data supporting the findings of present study are available from the corresponding author.

**Ethics Approval**- Not applicable, as the study involved plant material and did not involve animals participation.

**Consent to Participate and Consent to Publish**- Not applicable.

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