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Front page photo: IBRA 1980, at buckwheat mill, Dolenjska, Slovenia. From right to left: Ludvik Rozman, Toshiko Matano, Takashi Nagatomo, Miroslav Bogdanović, Jožica Vodopivec, Taiji Adachi, miller's wife, guest, Marija Kraljević-Balalić, miller. Behind: guest, Björn O. Eggum, Marek Ruszkowski, guest, Ana Matičić, Slavko Borojević. Some participants and organizers are absent from the photo.

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Research paper

The Growth and Biomass Yield of Common Buckwheat (*Fagopyrum esculentum* (L.) Moench) Under Different Crop Management Systems

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ABSTRACT

Growing crops as cover or companion crops, as well as for green manure, forms the basis of sustainable and organic field crop production. This practice helps reduce soil degradation and supports sustainable soil management. The aim of this field study was to evaluate the effects of crop management systems on the growth and biomass yield of two varieties of common buckwheat. The crop management systems tested were: common buckwheat (Zoe and Harpe) grown alone (control), intercropped with sorghum (*Sorghum bicolor*), intercropped with a mixture of lacy phacelia (*Phacelia tanacetifolia*) and white mustard (*Sinapis alba*), and grown in postharvest wheat residues (straw). The experiment was laid out in a randomized complete block design with three replicates. Data were collected on plant height (cm), number of leaves/plant, number of branches/plant, total leaf area/plant (cm²), stem diameter (cm), and biomass yield (t/ha). Crop management systems had a significant effect on the number of branches/plant, stem diameter, and overall biomass yield of buckwheat. The highest biomass yield (1.13 t/ha dry weight) was obtained from Harpe variety intercropped with *Phacelia + Sinapis*, while the lowest value 0.71 t/ha was recorded in the control. Given the high biomass yields, intercropping common buckwheat with *Phacelia + Sinapis* mixtures is a promising option for green manure production. Although the buckwheat varieties differed in number of leaves, leaf area, and number of branches/plant, the variety used did not have a statistically significant effect on biomass yield.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Monch) is a fast-growing crop in the knotweed family Polygonaceae cultivated primarily for its achenes, but also as a cover crop or intercrop for sustainable crop production (Falquet et al. 2015). In soil, buckwheat enhances organic carbon, nutrient cycling and microbial activities, reduces erosion by mitigating raindrop impact and run off, and contributes to moisture conservation (Kato-Noguchi et al. 2007; Glaze-Corcoran et al., 2020). Additionally, buckwheat exhibits the ability to suppress weeds through root allelopathy and the specific leaf arrangement (Woźniak et al., 2025), provides effective soil protection and supports insect pollinators during flowering (Liszewski & Chorbiński, 2021).

A crop management system is a logical combination of agricultural practices orderly or operations applied to a field in order to obtain a desired level of crop production (Sun et al., 2018; Maitra et al., 2021). It also consists of a mixture of crops of different species grown in the same field, to achieve more sustainable and profitable crop cultivation (Maitra et al., 2019; Ren et al., 2019). A crop management system encompasses the strategies used by farmers to grow, maintain and harvest crops in a given agroecosystem (Gao et al., 2024). The system focuses on beneficial interactions, efficient resource use, and controlling pests, weeds, and diseases to maximize yield (Woźniak et al., 2025). Sustainable management strategies aim to improve soil fertility, water use, and plant protection by leveraging the synergistic effects between crops (Chen et al., 2019; Lin et al. 2019). Crop management strategies may differ in how they balance plant responses, competition, complementary, and functional diversity (Akhtar et al., 2018).

Intercropping utilizes complementary interactions between species, and crop mixtures promote increased vegetative growth and higher biomass yields (Qu et al., 2023; Groß et al., 2024). Intercropping and cover crops can significantly influence plant growth, therefore farmers should consider appropriate intercropping strategies, planting geometry, and plant protection measures to achieve desired yield (Maitra et al., 2019; Moreira et al., 2024). Mulching modifies the soil microenvironment and support plant growth, while crop mixtures use functional diversity to maintain biomass production and enhance ecosystem benefits (Zhang et al., 2011; Lin et al., 2019).

While monoculture provides a baseline, it often lacks resource efficiency and ecological benefits (Feng et

al., 2021; Gao et al., 2024) High-input systems, such as uncontrolled usage of mineral fertilizers and pesticides, often result in higher biomass production but can have negative environmental consequences (Sun et al., 2018; Basaran, et al., 2020). Crop management practices that enhance soil health and nutrient cycling, thereby increasing biomass production and resource use efficiency should be prioritized.

By comparing crop management systems in buckwheat production, their roles can be better understood — not only in terms of yield, but also in relation to green manuring and cover cropping for agricultural sustainability. Therefore, this study aims to evaluate the effects of different crop management systems on the vegetative growth and biomass yield of two varieties of common buckwheat (*F. esculentum*).

MATERIALS AND METHODS

Experimental location and design

The field study with common buckwheat was carried out at the Teaching and Research Farm, Faculty of Agriculture and Technology, University of South Bohemia, České Budějovice (48°58'43.15"N and 14°26'54.3"E, 380 m elevation, sandy-loam soil, pH 5.6, average annual temperature 9.7°C, average annual total precipitation 808 mm) during the 2024 cropping season.

The experiment was a 2×4 factorial scheme fitted into a Randomized Complete Block Design. The crop management systems tested were: two varieties of common buckwheat (Zoe and Harpe) grown alone (control), intercropped with sorghum (*Sorghum bicolor*, Ruzrok variety), intercropped with a mixture of lacy phacelia (*Phacelia tanacetifolia*, Fiona variety) and white mustard (*Sinapis alba*, Sněženka variety) and grown in postharvest wheat residues (straw) with the 8 treatment combinations replicated three times to give a total of 24 plots.

Seeds of the 2 varieties of buckwheat were sown in rows at a spacing of 25cm while sorghum and *Phacelia* + *Sinapis* were sown between rows of buckwheat at a spacing of 12.5cm by a precision seed drill. A total population of 200 plants/m² was involved for buckwheat planted alone (control) and 100 plants/m² each for both buckwheat in intercrop with sorghum treatment as well as in mixtures with *Phacelia* + *Sinapis* treatment. The individual testing plot size of 1.25 × 4m was measured with 1m within plots and between replicates. Planting took place

on 23rd June, 2024. At 4 weeks after planting (at flowering stage) data were collected.

Vegetative growth parameters

In each plot, a total of ten plants from two middle rows per plot were randomly tagged for data collection. The parameters measured were:

Plant height (cm) was taken with a measuring tape from the soil surface to the apex of the crop where the youngest leaf branches.

Number of leaves per plant and **number of branches per plant** were visually counted.

Total leaf area per plant (cm²) was measured from leaves at the middle canopy (fifth fully expanded leaf) using Petiole Pro plant leaf area meter app (Breskinaa & Chuyana, 2021) and the value was multiplied by total number of leaves/plant.

Stem diameter (cm) was obtained using a digital vernier caliper (at 2 cm) above the ground level.

Biomass yield (t/ha) was determined by harvesting the whole plant at 2 cm above the ground level and weighed. The forage yield was calculated using the formula described by Nwajei et al. (2019), as stated below:

$$\text{Forage yield (t/ha)} = \frac{\text{Fresh weight (g)}}{\text{Harvested plot area (m}^2\text{)}} \times \frac{10000 \text{ (m}^2\text{)}}{1000} \times \frac{1}{1000}$$

The dry matter weight of the harvested ten plants per plot was determined by oven-drying the plants at 70°C to a constant weight according to Saifullah et al. (2011) and the values were calculated to t/ha using the same formula as used for forage yield.

The dry matter % was calculated using the formula described by Saifullah et al. (2011) as shown below:

$$\% \text{ Dry Matter} = \frac{\text{dry weight}}{\text{fresh weight}} \times \frac{100}{1}$$

Statistical analysis

All data obtained were analyzed using analysis of Variance (ANOVA) with GenStat 12th edition software program (GenStat, 2009). Means were compared using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS

Plant height

The tallest plants were recorded by the buckwheat grown with straw residues while the sole buckwheat plants were the shortest (Table 1). Zoe variety was taller than Harpe. However, the variety as well as the different crop management systems did not significantly influence the plant height of buckwheat. The variety and crop management system interaction affected the height of buckwheat significantly. Plants of Zoe variety mulched with straw had the highest plant height (49.24cm), while the plants of sole Harpe variety had the lowest height (32.85cm).

Number of leaves

The mean number of leaves per plant varied from 8.17–8.60 in Zoe and 7.17 to 8.57 in Harpe variety (Table 1). Zoe mulched with straw had the highest number of leaves per plant (8.60), while Harpe in monoculture had the lowest (7.17). The varieties sowed and their interaction with the crop management system significantly affected the number of leaves per plant of buckwheat. Although Zoe had generally a higher number of leaves/plants than Harpe, both varieties had similar values, approximately 8 leaves per plant. Similarly the plants in-cropped with *Phacelia + Sinapis*, which had the highest number of leaves/plant, showed values close to 8 leaves, comparable to other treatments.

Number of branches

The number of branches per plant of two common buckwheat varieties was significantly influenced by the varieties, crop management systems and variety × crop management system interaction (Table 1). The number of branches per plant ranged from 4.23 – 4.87 and 2.93 – 4.40 in Zoe and Harpe. Overall, Zoe + sorghum intercrop had the highest number of branches/plant (4.87) while sole Harpe had the lowest (2.93). Generally, the plants intercropped with sorghum, which had the highest values, produced a similar number of branches per plant, approximately 5, to those grown in the mixture with *Phacelia + Sinapis*. Zoe had the higher (4.51) average number of branches/plant than Harpe (3.71).

Table 1. Effect of crop management system on the growth of two varieties of common buckwheat

| Treatment | Plant height (cm) | Number of leaves/plant | Number of branches/plant | Total leaf area (cm ²) | Stem diameter (cm) |
|--|-------------------|------------------------|--------------------------|------------------------------------|--------------------|
| Variety (V) | | | | | |
| ZOE | 45.86 | 8.32a | 4.51a | 7490.70a | 0.61 |
| HARPE | 37.64 | 7.83b | 3.71b | 6141.99b | 0.58 |
| Crop management system (CMS) | | | | | |
| Sole | 39.53 | 7.75 | 3.58b | 7175.91a | 0.54b |
| Intercrop with Sorghum | 42.14 | 8.03 | 4.63a | 7496.81a | 0.64a |
| Mulched with straws | 43.57 | 8.15 | 3.75b | 7508.69a | 0.56b |
| Mixture with <i>Phacelia + Sinapis</i> | 41.77 | 8.37 | 4.47a | 5083.96b | 0.64a |
| Interaction (V x CMS) | | | | | |
| Sole Zoe | 46.20ab | 8.33a | 4.23a | 8399.87a | 0.56c |
| Zoe + Sorghum | 43.87ac | 8.17ab | 4.87a | 7457.47ab | 0.60ac |
| Zoe + Straw | 49.24a | 8.60a | 4.23a | 8455.62a | 0.57bc |
| Zoe + <i>Phacelia + Sinapis</i> | 44.13ac | 8.17ab | 4.70a | 5649.83bc | 0.60ac |
| Sole Harpe | 32.85d | 7.17b | 2.93b | 5951.95bc | 0.52c |
| Harpe + Sorghum | 40.42bc | 7.90ab | 4.40a | 7536.16ab | 0.68a |
| Harpe + Straw | 37.90cd | 7.70ab | 3.27b | 6561.77ab | 0.56c |
| Harpe + <i>Phacelia + Sinapis</i> | 39.41bd | 8.57a | 4.23a | 4518.09 c | 0.67ab |
| SL | | | | | |
| V | 1.52ns | 0.23* | 0.44* | 426.95* | 0.05ns |
| CMS | 2.16ns | 0.32ns | 0.62* | 603.60* | 0.07* |
| V x CMS | 3.05* | 0.46* | 0.87* | 853.90* | 0.09* |

Values with same letter(s) in columns for: V. Variety, CMS. Crop management system and VxCMS. Interaction, are not significantly different using Duncans' multiple range test at 5% level of probability. SL: Significant level; ns: not significant.

Total leaf area

The crop management system, variety, and their interaction significantly affected the total leaf area produced by the buckwheat (Table 1). The total leaf area varied from 5649.83–8455.62 cm² in Zoe and 4518.09–7536.16 cm² in Harpe. In total, the highest leaf area of buckwheat was recorded in Zoe grown with straw residues (8455.62 cm²), while the lowest was observed in the Harpe + mixture (4518.09 cm²). Plants mulched with straw and those in mixture with *Phacelia + Sinapis* showed the highest and lowest total leaf area/plant. Zoe had a higher total leaf area than Harpe.

Stem diameter

The crop management system had a significant effect on the stem diameter of both common buckwheat varieties (Table 1). The effect of variety on the stem diameter

was not significant. There was also a significant (P≤ 0.05) interaction between variety and crop management system.

The highest stem diameter was recorded in the Harpe + sorghum treatment (0.68 cm), while the lowest was observed in sole Harpe (0.52 cm) (Table 1). On average, Zoe had a larger stem diameter than Harpe. Across the different crop management systems, the stem diameter of the plants was approximately 1 cm.

Yield

Plants harvested from the mixtures with *Phacelia + Sinapis* produced the highest forage yield of buckwheat, while those mulched with straw had the lowest. It was also observed that Zoe produced a higher forage yield (4.42 t/ha) than Harpe (4.20 t/ha). The highest (5.52 t/ha) was obtained from Harpe intercropped with *Phace-*

lia + Sinapis, while the lowest (2.75 t/ha) was recorded in sole-cropped Harpe.

The total dry matter yield varied significantly from 0.86 to 1.05 t/ha in Zoe and 0.79–1.13 t/ha in Harpe. However, Zoe had a slightly higher (0.97 t/ha) average dry matter yield compared to Harpe (0.94 t/ha). The highest and lowest dry matter yields were recorded in Harpe intercropped with *Phacelia + Sinapis* (1.13 t/ha) and in the sole-cropped Harpe control (0.71 t/ha), respectively.

The results also showed that crops mulched with straw had a significantly higher dry matter percentage, while those intercropped with *Phacelia + Sinapis* had the lowest. The dry matter percentage ranged from 20.38% to 25.58% in Zoe and from 20.49% to 27.65% in Harpe. Although Harpe had a higher dry matter percentage overall, the difference between the two varieties was not statistically significant. The highest dry matter percentage (27.65%) was observed in sole-cropped Harpe, while the

lowest (20.38%) was recorded in Zoe intercropped with *Phacelia + Sinapis*.

DISCUSSION

Effect of crop management system on the plant height of two varieties of common buckwheat

Plant height is an important component of vegetative parameter which serves as a key indicator of a plant's growth status, health, and genetic potential. It is a crucial parameter in agriculture for predicting crop yield, biomass, and susceptibility to lodging. In this study, crop management systems as mulching promoted the growth of taller plants in both common buckwheat varieties compared to monoculture. This may be due the fact that crop management system influence buckwheat growth through effect on resource availability by improving water and nutrient accessibility. Virili et al. (2024) report-

Table 2. Effect of crop management system on the forage and dry matter yield of two varieties of buckwheat

| Treatment | Forage yield (t/ha) | Dry matter yield (t/ha) | Dry matter % |
|--|---------------------|-------------------------|--------------|
| Variety (V) | | | |
| ZOE | 4.42 | 0.97 | 22.69 |
| HARPE | 4.20 | 0.94 | 23.72 |
| Crop management system (CMS) | | | |
| Sole | 3.85bc | 0.83c | 24.46ab |
| Intercrop with Sorghum | 4.73ab | 0.88bc | 22.14ab |
| Mulched with straws | 3.29c | 1.02ab | 25.78a |
| Mixture with <i>Phacelia + Sinapis</i> | 5.37a | 1.09a | 20.43b |
| Interaction (V x CMS) | | | |
| Sole Zoe | 4.96ab | 1.05ab | 21.27ab |
| Zoe + Sorghum | 4.05ac | 0.92ac | 23.52ab |
| Zoe + Straw | 3.46bc | 0.86bc | 25.58ab |
| Zoe + <i>Phacelia + Sinapis</i> | 5.22a | 1.05ab | 20.38b |
| Sole Harpe | 2.75c | 0.71c | 27.65a |
| Harpe + Sorghum | 5.40a | 1.12a | 20.77b |
| Harpe + Straw | 3.12c | 0.79c | 25.99ab |
| Harpe + <i>Phacelia + Sinapis</i> | 5.52a | 1.13a | 20.49b |
| SL | | | |
| V | 0.37ns | 0.05ns | 1.43ns |
| CMS | 0.53* | 0.07* | 2.02* |
| V x CMS | 0.74* | 0.11* | 2.86* |

Values with same letter(s) in columns for: V. Variety, CMS. Crop management system and V x CMS. Interaction, are not significantly different using Duncans' multiple range test at 5% level of probability. SL: Significant level; ns: not significant

ed that buckwheat in mixtures produced highest plant heights and differed significantly from those in monocultures which agreed with the result of the present study.

Effect of crop management system on the number of leaves/plant of common buckwheat

The number of fully expanded leaves produced by a single plant reflects the plant's developmental stage and their ability to capture light for photosynthesis and production of assimilates. In this study, crop management systems interacted significantly with the varieties. However, the mixture of Harpe with *Phacelia + Sinapis* encouraged more leaves per plant than other treatments. This may be due to interspecific competition within the mixture, which could have stimulated leaf development as a response to shading. Similarly, Heuermann et al. (2019); Groß et al. (2024) reported that crop mixtures interactions among species can promote plant growth.

Effect of crop management system on the number of branches/plant of common buckwheat

Branches are stem-like structures that grow from the main stem of a plant and contribute to canopy expansion and biomass production. Intercropping systems appeared to promote branching in buckwheat, potentially as a strategy to fill canopy gaps and compensate for shading, especially under taller intercrop partners like sorghum. Overall, intercropping buckwheat with sorghum or with *Phacelia + Sinapis* promoted a higher number of branches per plant compared to monoculture. This suggests that intercropping may stimulate lateral growth in response to light competition. Wortman et al. (2012); Couëdel et al. (2018) reported similar findings, suggesting that competitive species in intercrops may benefit from complementary interactions. Gao et al. (2024) also observed increased vegetative branching in buckwheat intercropped with alfalfa compared to monoculture.

Effect of crop management system on the total leaf area/plant of common buckwheat

Total leaf area is a critical determinant of photosynthetic capacity and biomass accumulation (Chen et al., 2019; Nwajei et al., 2019). In this study, buckwheat mulched with straw had the highest total leaf area, likely due to enhanced soil moisture retention, temperature

regulation, and nutrient availability. These findings are consistent with those of Qu and Feng (2022), who reported that straw mulching increased leaf area in cereals and pseudocereals by conserving soil moisture and stabilizing soil temperature.

Effect of crop management system on the stem diameter of common buckwheat

Stem diameter is a measure of stem thickness which indicate plants strength mechanism, ability to absorb water and nutrients and allocation of assimilate to their structural tissues. Buckwheat plants intercropped with sorghum or with *Phacelia + Sinapis* developed thicker stems compared to those grown in monoculture or under straw mulch. This may be due to the fact, that thicker stems are associated with the ability to withstand or resist lodging conditions, higher nutrient uptake and greater support for plant development. These results align with the findings of Woźniak et al. (2025), who reported increased stem diameter in intercropped buckwheat compared to monoculture. Similar results were also observed by Basaran et al. (2020) in alfalfa–intercrop with an annual companion crop, supporting the findings of the present study.

Effect of crop management system on the biomass yield of common buckwheat

The forage yield is the weight of the above ground plant part taken at a specific stage of growth (Mariotti et al., 2016). It also includes water content, structural tissues and assimilates which are needed for animal feeding, soil cover, and short-term biomass supply. The dry biomass yield on the other hand is the oven dried weight of the above ground plant parts representing the structural tissues and biomass accumulated by crops after water have been removed (Omoregie et al., 2020).

In this study, intercropping systems involving sorghum, *Phacelia + Sinapis*, and straw mulch significantly improved forage and dry matter yields compared to monoculture. The mixture of buckwheat with *Phacelia + Sinapis* in particular provided canopy closure, which is beneficial for weed suppression and pollinator habitat provision. These results are in agreement with those of Virili et al. (2024), who reported significantly higher buckwheat biomass yields in crop mixtures compared to monocultures.

CONCLUSION

Crop management systems had a statistically significant effect on the number of branches/plant, total leaf area, stem diameter, and biomass yield of buckwheat with the intercrops and the mixtures being more favourable than other treatment and the control.

The effect of the crop management system—particularly the mixture with *Phacelia* + *Sinapis* and intercropping with sorghum—resulted in higher growth and biomass yield of buckwheat compared to monoculture and treatments mulched with wheat straw.

The mixture of Harpe + *Phacelia* + *Sinapis* produced the highest fresh (5.52 t/ha) and dry matter (1.13 t/ha) yields, while the control (monoculture) recorded the lowest values - 2.75 t/ha and 0.71 t/ha, respectively. Given these high biomass yields, intercropping common buckwheat with lacy phacelia and white mustard is a prom-

ising option for green manure production. Although the buckwheat varieties differed in vegetative traits such as number of leaves, total leaf area, and number of branches per plant, the variety used did not have a statistically significant effect on biomass yield.

Sorghum as a companion crop synergistically improved the overall biomass yield of buckwheat. This underscores the value of diversified crop management systems—particularly intercropping—for enhancing buckwheat's potential use in organic manuring and cover cropping.

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IZVLEČEK

Rast in pridelek biomase navadne ajde (*Fagopyrum esculentum* (L.) Moench) pri različnih sistemih pridelovanja

Gojenje rastlin kot pokrovnih ali spremiševalnih kultur ter za zeleno gnojenje je osnova trajnostne in ekološke pridelave poljščin. Tak način pomaga zmanjševati degradacijo tal in podpira trajnostno upravljanje s tlemi. Namenski tega poskusa pridelovanja je bil oceniti vpliv sistemov pridelovanja rastlin na rast in pridelek biomase dveh kultivarjev navadne ajde. Testirani sistemi upravljanja s pridelkom so bili: navadna ajda (kultivarja Zoe in Harpe) kot samostojen posevek (kontrola), v vmesnem posevku s sirkom (*Sorghum bicolor*), v vmesnem posevku z mešanico facelije (*Phacelia tanacetifolia*) in bele gorčice (*Sinapis alba*), ter gojena z ostanki pšenice po žetvi (slama). Poskus je bil zasnovan v popolnoma randomiziranem bloku s tremi ponovitvami. Podatki so bili zbrani o višini rastlin (cm), številu listov na rastlino, številu vej na rastlino, skupni površini listov na rastlino (cm^2), premeru stebla (cm) in skupni pridelek biomase (t/ha). Načini pridelovanja so imeli pomemben vpliv na število vej na rastlino, premer stebla in skupni pridelek biomase ajde. Najvišji pridelek biomase (1,13 t/ha sušine) je bil dosežen pri sorti Harpe, posejani z vmesnim posevkom facelijo in belo gorčico, medtem ko je bila najnižja vrednost 0,71 t/ha ugotovljena v kontrolni skupini. Glede na visoke pridelke biomase je skupna setev ajde z mešanico facelije in bele gorčice obetavna možnost za pridelavo zelene mase za podor. Čeprav sta se sorte ajde razlikovali po številu listov, površini listov in številu vej na rastlini, uporabljeni sorte nista imeli značilnega vpliva na pridelek biomase.

Research paper

The Influence of Different Cultivation Technologies on the Changes in Quantitative and Qualitative Parameters of Buckwheat

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ABSTRACT

Changes of the quantitative and qualitative parameters of buckwheat were observed on gleyic Fluvisols (locality Milhostov, Slovak Republic) at different tillage between 2013 and 2015. The experiment was conducted using two soil tillage treatments: conventional tillage and reduced tillage, and three conditioner application treatments: soil conditioner PRP SOL, a combination of soil conditioner PRP SOL and plant auxiliary substance PRP SOL+EBV, and control. In buckwheat crops, basic physical properties were also monitored. The statistically significantly higher yields of buckwheat were achieved with reduced tillage. Significant differences were found in buckwheat yield between years. The lowest yields of buckwheat were recorded in the dry and extremely hot year of 2015. In the variant with conventional tillage, better values of basic soil physical properties were recorded compared with the reduced tillage. Significantly higher yields of buckwheat were found with applications of conditioners than in the control. The application of plant auxiliary substance PRP SOL+EBV on the variant with PRP SOL did not substantially increase the yields of buckwheat. The content of nitrogen substances in the grain of buckwheat was dependent on the fertilization options. Higher content of nitrogen substances in the grain of buckwheat was found in the control than with the application of conditioners. A negative correlation was found between the yield and nitrogen substances in the grain of buckwheat ($r = -0.74$).

INTRODUCTION

Climate change poses a serious challenge to soil in ensuring optimal food production. Intensive agricultural practices and the use of monocultures have led to the loss of biodiversity. Changes in agricultural routines are needed to address biodiversity. There is a need to grow crops that are more resilient to climate change. Such crops include buckwheat, which can be grown in different climatic and soil conditions

Buckwheat (*Fagopyrum esculentum* Moench) is a cereal of growing agricultural and nutritional importance. It is valued for its short vegetation period, adaptability to marginal soils, and high content of protein and minerals, which makes it a valuable raw material for food production.

Quantitative and qualitative parameters of buckwheat are strongly influenced by cultivation practices and climatic conditions (Popovic et al., 2014). Buckwheat grain yields depend on the agro-ecological conditions of its cultivation and sowing times (Ikanović et al. 2013; Mariotti et al., 2016; Mikami et al., 2018; Nikolic et al., 2019; Jukić et al., 2021; Hassona et al., 2024).

The content of nitrogenous substances in buckwheat grain varies differs considerably, not only depending on soil and climatic conditions, but also on the variety and sowing time (Guo et al., 2007; Jukić et al., 2021).

At extremely high temperatures and consequently dried soil, buckwheat could be exposed to water stress because of the thin root system (Zamaratskaia et al., 2024). It should be noted that buckwheat is highly susceptible to dryness, particularly in early growth stages, during rooting, flowering, and the yielding period. However, moisture excess during the later stages of growth also has strong detrimental effects on buckwheat development (Nikolic et al., 2019).

Buckwheat can be cultivated under a reduced tillage system (Chrungoo and Chetry, 2021). Reduced tillage can boost buckwheat crop germination and establishment by creating a seedbed that facilitates optimal seed-to-soil contact. Nevertheless, buckwheat can be drilled without tillage, which is a viable choice especially for mid-summer planting. This strategy can reduce soil erosion and help preserve soil moisture (Vieites-Álvarez et al., 2024).

The nutrient requirements of buckwheat are low, and intensive fertilization is not required because buckwheat can easily absorb macro- and microelements from the soil. Some studies have highlighted the importance of nitrogen fertilization and water management. For instance,

Ciftci et al. (2025) demonstrated that the combined application of irrigation and nitrogen fertilization significantly increased grain yield and protein content.

Despite these findings, relatively little is known about how cultivation technologies interact with soil physical properties (e.g., bulk density, total porosity). Soil conditions can influence quantitative and qualitative parameters of buckwheat. To address this gap, the present study investigates the impact of different tillage practices and conditioner applications on buckwheat yield, grain quality, and soil physical parameters. The aim was to identify cultivation practices that maximize yield and quality while maintaining soil fertility.

MATERIAL AND METHODS

Experimental site and design

The field experiment with different tillage technologies and conditioner applications for buckwheat grown was conducted at the locality Milhostov (National Agriculture and Food Centre – Research Institute of Plant Production – Institute of Agroecology in Michalovce, Slovak Republic) during the 2013-2015 growing seasons. The site is located at (48°40'02.3"N. 21°43'51.2"E), situated in the central part of the East-Slovak Lowland at an altitude of 101 m. The monitored location is included in the climatic region T 03 (Linkeš et al. 1996), which is characterized as warm, very dry, and lowland. The long-term normal (1981 – 2010) for the annual air temperature in Milhostov is 9.4 °C (16.6 °C during the growing season), and the long-term normal for precipitation is 567 mm (374 mm during the growing season) (Mikulová et al. 2020).

Amount of precipitation [mm] and air temperature [°C] in 2013 – 2015 and during vegetation in these years, and their qualitative evaluation are shown in Table 1. The growing season of 2013 and 2014 was warm, and 2015 was very warm. In terms of precipitation, the growing season of 2013 and 2014 was normal, and 2015 was very dry.

The soil was classified as Gleyic Fluvisols, with an initial organic matter content of 2.9 % and a pH in KCl of 6.4. According to the Novak classificatory scale (Zaujec et al. 2009), this soil subtype belongs to heavy soils. The soil particle size distribution before the establishment of experiments with buckwheat is shown in Table 2. The average content of clay particles was 53.2 %.

Table 1. Amount of precipitation [mm] and air temperature [°C] in 2013 – 2015 and their qualitative evaluation

| Evaluated parameter | | DN | 2013 | 2014 | 2015 |
|---------------------------------|------------------------|-------|--------|--------------------|--------------------|
| Amount of precipitation I.-XII. | [mm] | 567 | 530 | 613 | 447 |
| | Percentage to DN [%] | 100.0 | 93.5 | 108.1 | 78.8 |
| | Evaluation | - | normal | normal | very dry |
| Amount of precipitation IV.-IX. | [mm] | 374 | 298 | 425 | 227 |
| | Percentage to DN [%] | 100.0 | 79.7 | 113.6 | 60.7 |
| | Evaluation | - | normal | normal | very dry |
| Air temperature I.-XII. | [°C] | 9.4 | 10.3 | 11.1 | 11.0 |
| | Deviation from DN [°C] | 0.0 | +0.9 | +1.7 | +1.6 |
| | Evaluation | - | warm | extraordinary warm | extraordinary warm |
| Air temperature IV.-IX. | [°C] | 16.6 | 17.4 | 17.2 | 18.0 |
| | Deviation from DN [°C] | 0.0 | +0.8 | +0.6 | +1.4 |
| | Evaluation | - | warm | warm | very warm |

where: DN – long-term normal

Table 2. Soil particle size distribution before experiment establishment

| Fraction | Values [%] |
|--|-----------------------------|
| 1 st fraction, clay (< 0.001 mm) | 30.3 |
| 2 nd fraction, soft and middle silt (0.001 – 0.01 mm) | 22.9 |
| 3 rd fraction, crude silt (0.01 – 0.05 mm) | 27.9 |
| 4 th fraction, soft sand (0.05 – 0.25 mm) | 16.3 |
| 5 th fraction, middle sand (0.25 – 2 mm) | 2.6 |
| Content of particle I. category (< 0.01 mm) | 53.2 |
| Soil evaluation | heavy soil, clay-loamy soil |

The experiment was arranged in a randomized complete block design with three replications. Treatments consisted of different tillage technologies, including variants in conditioner applications. Plot size with buckwheat was 60 × 45 m, the variant size 15 × 10 m (150 m²).

Crop management

Sowing common buckwheat (*Fagopyrum esculentum* Moench) variety Hajnalka was carried out in May (3 May 2013, 2 May 2014, 11 May 2015). The experiment was conducted using two soil tillage technologies: conventional tillage and reduced tillage, and three conditioner application treatments: soil conditioner PRP SOL, a combination of soil conditioner PRP SOL and plant auxiliary substance PRP SOL+EBV, and control.

The trial was established with two types of tillage:

CT – conventional tillage – after harvesting of the forecrop, stubble breaking was performed, autumn

medium-deep ploughing, spring pre-sowing soil treatment was done using a share cultivator, and sowing.

RT – reduced tillage – after harvesting of the forecrop, stubble breaking was performed, spring pre-sowing soil treatment was done using a share cultivator, and sowing.

The trial was established with three conditioner applications:

PRP – soil conditioner PRP SOL,

PRP+EBV – a combination of soil conditioner PRP SOL and plant auxiliary substance PRP EBV,

C – control.

The soil conditioner PRP SOL was applied for pre-sowing soil preparation at a dose of 200 kg ha⁻¹. The plant auxiliary substance PRP EBV was applied in the 3-leaf phase at a dose of 1.5 l ha⁻¹.

Table 3. Buckwheat phenology in 2013 – 2015

| Phenology | Year | | |
|------------------------|--------|--------|--------|
| | 2013 | 2014 | 2015 |
| Sowing | 03.05. | 02.05. | 11.05. |
| Emergence | 17.05. | 19.05. | 26.05. |
| Spike formation | 06.06. | 05.06. | 21.06. |
| Flowering | 12.06. | 14.06. | 29.06. |
| Technological maturity | 20.09. | 10.10. | 05.10. |
| Harvesting | 24.09. | 13.10. | 07.10. |

The beginning of the basic phenological phases of buckwheat growth in 2013 – 2015 is shown in Table 3.

Standard buckwheat management practices (weed control, pest protection) were applied uniformly across all treatments.

All interventions in establishing and maintaining the experiments were carried out in one day, strictly respecting the principles of experimental equality.

Yield assessment

Buckwheat was harvested after reaching harvest maturity with a small-plot combined harvester. Grain yield was determined by weighing harvested seeds. During harvest, grain samples were taken to determine the harvest moisture content. Buckwheat yields were converted to 13 % moisture content and were expressed in $t\ ha^{-1}$.

Grain quality analysis

Quality parameters were determined from grain representative samples collected at harvest. The content of nitrogenous compounds in buckwheat grains was determined using the Kjeldahl method according to ISO 1871 (2009). The concentration of nitrogenous substances in buckwheat grain was converted to dry matter and expressed in $g\ kg^{-1}$.

Soil physical properties

Selected physical properties of Gleyic Fluvisol were determined from undisturbed soil samples taken in the spring period. Soil samples were collected from each tillage in cylinders of $100\ cm^3$ at a depth of 0–0.3 m with three replications. Soil bulk density ($kg\ m^{-3}$) and total po-

rosity (%) were determined by methods as published by Hrivňáková, Makovníková et al. (2011).

Statistical analysis

Differences between treatment means were assessed by the least significant difference (LSD) test at $p < 0.05$. All statistical analyses were performed using the Statgraphics software package. Interrelationships between monitored parameters were evaluated using regression analysis.

RESULTS AND DISCUSSION

Buckwheat grain yield

The buckwheat grain yield was significantly influenced by the applied cultivation tillage (Table 4). The statistically significantly highest yield was obtained under reduced tillage (average $1.40\ t\ ha^{-1}$), while the lower yield (average $1.29\ t\ ha^{-1}$) was recorded in conventional tillage.

In none of the monitored years did the buckwheat yield exceed $2\ t\ ha^{-1}$ on heavy soils. Similar low yields were obtained in Sweden, where, however, buckwheat yield varied in a wide range depending on the type of buckwheat (Knicky et al., 2024).

Without the application of conditioners, the yield was $1.27\ t\ ha^{-1}$ with conventional tillage and $1.31\ t\ ha^{-1}$ with reduced tillage in 2013. The application of soil conditioner, as well as in combination with the plant auxiliary substance EBV, increased the yield by approximately $0.5\ t\ ha^{-1}$ (Table 5).

In 2014, yields were below $1.50\ t\ ha^{-1}$, with a tendency to increase with the application of conditioners. In the year of extreme dry in 2015, buckwheat yields were the

Table 4. Statistical evaluation of the observed parameters

| Source variability | d.f. | Factor | Yield | | Nitrogenous substances | |
|-------------------------|------|---------|-----------------------|---------|------------------------|---------|
| | | | [t ha ⁻¹] | F-ratio | [g kg ⁻¹] | F-ratio |
| Tillage | 1 | CT | 1.29 a | 26.15 | 117.0 a | 1.25 |
| | | RT | 1.40 b | | 118.1 a | |
| Conditioner application | 2 | PRP | 1.46 b | 79.39 | 117.9 ab | 2.40 |
| | | PRP+EBV | 1.42 b | | 116.1 a | |
| | | C | 1.17 a | | 118.6 b | |
| Year | 2 | 2013 | 1.62 c | 214.59 | 97.6 a | 409.3 |
| | | 2014 | 1.34 b | | 125.4 b | |
| | | 2015 | 1.09 a | | 129.6 c | |
| Residual | 63 | | | | | |
| Total | 71 | | | | | |

where: d.f. – degrees of freedom, F-ratio – calculated F-ratio, letters (a, b, c) between factors refer to statistically significant differences ($\alpha = 0.05$) – LSD test

Table 5. Buckwheat yield [t ha⁻¹] in 2013 – 2015 at 13 % moisture

| Tillage | Conditioner application | Year | | |
|----------------------|-------------------------|------|------|------|
| | | 2013 | 2014 | 2015 |
| Conventional tillage | PRP | 1.74 | 1.24 | 1.07 |
| | PRP+EBV | 1.76 | 1.36 | 1.16 |
| | C | 1.27 | 1.17 | 0.88 |
| Reduced tillage | PRP | 1.75 | 1.46 | 1.24 |
| | PRP+EBV | 1.86 | 1.44 | 1.18 |
| | C | 1.31 | 1.34 | 1.02 |

where: PRP – soil conditioner PRP SOL, PRP+EBV – a combination of soil conditioner PRP SOL and plant auxiliary substance PRP EBV, C – control.

lowest, reaching only 0.88 t ha⁻¹ in the control variant of conventional tillage (Table 5). Weather conditions significantly influenced the yield quantity during the researched period, which was also found by Popović et al. (2014) and Kolarić et al. (2021).

These findings are in agreement with studies reporting that fertilization combined with suitable tillage systems increases buckwheat yield and improves its qualitative parameters (Zhou et al., 2023; Vieites-Álvarez et al., 2024).

Grain quality parameters

Qualitative parameters were also influenced by the use of conditioners (Table 4). Higher content of nitrogen substances in the grain of buckwheat was found in the control than with the application of conditioners.

In terms of the year, statistically significantly, the lowest concentrations of nitrogenous substances in dry matter were measured in 2013, and the highest in 2015 (Table 4). In 2013, the content of nitrogenous substances in grain was only up to 105.0 g kg⁻¹ dry matter. In 2014, higher concentrations of nitrogenous substances were measured, and the difference between the variants was minimal in the interval from 123.1 to 127.5 g kg⁻¹ dry matter. In 2015, the concentration of nitrogenous substances ranged in a wider interval (Table 6), from 121.3 to 136.3 g kg⁻¹ dry matter. Similarly, Knicky et al. (2024) found in buckwheat grain from 10.8 % to 11.4 % protein content, and Domingos and Bilsborrow (2021) found 12 % protein.

No statistically significant differences were found in the concentration of nitrogenous substances between tillage treatments (Table 4).

Table 6. Nitrogenous substances [g kg⁻¹] in buckwheat grain in 2013 – 2015

| Tillage | Conditioner application | Year | | |
|----------------------|-------------------------|-------|-------|-------|
| | | 2013 | 2014 | 2015 |
| Conventional tillage | PRP | 89.3 | 123.1 | 131.3 |
| | PRP+EBV | 93.6 | 127.5 | 130.6 |
| | C | 98.0 | 123.1 | 136.3 |
| Reduced tillage | PRP | 105.0 | 126.9 | 131.9 |
| | PRP+EBV | 98.9 | 124.4 | 121.3 |
| | C | 100.6 | 127.5 | 126.3 |

where: PRP – soil conditioner PRP SOL, PRP+EBV – a combination of soil conditioner PRP SOL and plant auxiliary substance PRP EBV, C – control.

The content of nitrogenous substances in buckwheat grain is closely related to the achieved grain yield. With higher grain yields, the content of storage substances decreases, including proteins. Therefore, even among the yield and nitrogen substances in the grain of buckwheat was found a negative correlation ($r = -0.74$).

Based on the determined grain yields and the determined nitrogenous substances content, at the monitored variants of soil tillage and conditioner applications, the nitrogenous substances yield was calculated and expressed in kg ha⁻¹ (Table 7).

In terms of tillage, higher nitrogenous substances yield was found with reduced tillage (163.4 kg ha⁻¹) compared to conventional tillage (147.4 kg ha⁻¹). The applications of conditioners had a positive impact on the nitrogenous substances yield. Average nitrogenous substances yield using a combination of soil conditioner PRP SOL

and plant auxiliary substance PRP EBV was 166.0 kg ha⁻¹, at using soil conditioner PRP SOL 163.5 kg ha⁻¹, and only 136.6 kg ha⁻¹ at the control (Table 7).

Soil physical properties

Research into the basic physical properties of soil in buckwheat crops was also monitored. Table 8 shows the average physical characteristics of the soil determined during different tillage in the monitored period (2013 – 2015).

The average values of bulk density at conventional tillage were from 1229 kg m⁻³ to 1 455 kg m⁻³, and at reduced tillage in the range 1301 – 1511 kg m⁻³. In 2015, bulk density values higher than 1400 kg m⁻³ were found, which is the limit value for clay-loam soil according to Act on the Protection and Use of Agricultural Land No.

Table 7. Nitrogenous substances yield [kg ha⁻¹] of buckwheat in 2013 – 2015

| Tillage | Conditioner application | Year | | | |
|----------------------|-------------------------|--------------|--------------|--------------|--------------|
| | | 2013 | 2014 | 2015 | Average |
| Conventional tillage | PRP | 155.4 | 152.6 | 140.5 | 149.5 |
| | PRP+EBV | 164.7 | 173.4 | 151.5 | 163.2 |
| | C | 124.5 | 144.0 | 119.9 | 129.5 |
| Reduced tillage | PRP | 183.8 | 185.3 | 163.6 | 177.5 |
| | PRP+EBV | 184.0 | 179.1 | 143.1 | 168.7 |
| | C | 131.8 | 170.9 | 128.8 | 143.8 |
| Average tillage | PRP | 169.6 | 169.0 | 152.0 | 163.5 |
| | PRP+EBV | 174.3 | 176.3 | 147.3 | 166.0 |
| | C | 128.1 | 157.4 | 124.4 | 136.6 |

where: PRP – soil conditioner PRP SOL, PRP+EBV – a combination of soil conditioner PRP SOL and plant auxiliary substance PRP EBV, C – control.

Table 8. *Soil physical parameters under different tillage in 2013 – 2015*

| Evaluated parameter | Tillage | Year | | | |
|------------------------------------|----------------|--------------|--------------|--------------|--------------|
| | | 2013 | 2014 | 2015 | Average |
| Bulk density [kg m ⁻³] | CT | 1372 | 1229 | 1455 | 1352 |
| | RT | 1301 | 1315 | 1511 | 1376 |
| | Average | 1337 | 1272 | 1483 | 1364 |
| Porosity [%] | CT | 46.53 | 52.09 | 43.29 | 47.30 |
| | RT | 49.26 | 48.72 | 41.08 | 46.35 |
| | Average | 47.90 | 50.41 | 42.19 | 46.83 |

where: CT – conventional tillage, RT – reduced tillage.

220/2004 Coll. (2004). With a higher bulk density of the soil, soil compaction and adverse changes in the water and air regime of the soil may occur.

The variant with conventional tillage, better values of basic physical properties of the soil were recorded (Table 8), i.e. lower values of soil bulk density (average 1352 kg m⁻³) and higher values of total soil porosity (average 47.30 %) were found in comparison with the reduced tillage (average 1376 kg m⁻³, respectively 46.35 %).

Swelling and shrinkage processes are typical for heavy soils with a high content of clay particles and affect soil porosity and its changes. Total porosity is a function of bulk density, therefore, its values are lower at higher bulk density. The optimal total porosity for clay-loam soils should be higher than 47% (Act 220/2004 Coll., 2004). Average porosity values for different tillage in 2015 (43.29 % at conventional tillage, 41.08 % at reduced tillage) and the average value of 46.53 % at conventional tillage in 2013 indicate compaction of the soil profile (Table 8).

The claim that soil physical properties subsequently affect buckwheat yields was confirmed by regression analysis. A significant negative correlation was found between soil bulk density and buckwheat yield ($r = -0.68$, and a significant positive correlation was found between soil total porosity and yield ($r = 0.68$).

CONCLUSION

This study demonstrated that different cultivation technologies significantly influenced both the quanti-

tative and qualitative parameters of buckwheat, as well as soil physical properties. The statistically significantly higher yields of buckwheat were achieved with reduced tillage (average 1.40 t ha⁻¹) in comparison to conventional tillage (average 1.29 t ha⁻¹).

Optimized application of conditioners produced a higher grain yield, and also a higher nitrogenous substances yield. Average grain yield and nitrogenous substances yield using a combination of soil conditioner PRP SOL and plant auxiliary substance PRP EBV was 1.42 t ha⁻¹, respectively 166 kg ha⁻¹, at using soil conditioner PRP SOL 1.46 t ha⁻¹, respectively 163.5 kg ha⁻¹, and only 1.17 t ha⁻¹ grain yield and 136.6 kg ha⁻¹ nitrogenous substances yield at control.

The results suggest that integrated buckwheat management approaches, using different tillage and conditioner applications in soil and climatic conditions, can maximize the agronomic and nutritional potential of buckwheat. Such strategies are particularly relevant for sustainable and ecological farming systems, where the balance between yield, quality, and soil conservation is important.

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IZVLEČEK

Vpliv različnih tehnologij pridelave na spremembe količinskih in kakovostnih parametrov ajde

Pri poskusih na lokaciji Milhostov (Slovaška) so bile pri različnih načinih obdelave tal med letoma 2013 in 2015 ugotovljene spremembe kvantitativnih in kvalitativnih parametrov ajde. Poskus je bil izveden z dvema načinoma obdelave tal: konvencionalna obdelava in zmanjšana obdelava, ter tremi načini nanašanja pripravkov: talni kondicioner PRP SOL, kombinacija talnega kondicioniranja PRP SOL in pomožne snovi za rastline PRP SOL+EBV ter kontrola. Spremljane so bile tudi osnovne fizikalne lastnosti. Značilno višji pridelki ajde so bili doseženi z zmanjšanim obdelovanjem tal. Pomembne razlike so bile ugotovljene v pridelku ajde med posameznimi leti. Najnižje pridelke ajde so ugotovili v suhem in izjemno vročem letu 2015. V različici s konvencionalno obdelavo tal so bile ugotovljene ustreznje vrednosti osnovnih fizikalnih lastnosti tal v primerjavi z zmanjšano obdelavo. Z uporabo kondicionerjev so bili ugotovljeni bistveno višji pridelki ajde kot pri kontrolni različici. Uporaba pomožne snovi PRP SOL+EBV pri različici s PRP SOL ni bistveno povečala pridelkov. Vsebnost dušikovih snovi v zrnju ajde je bila odvisna od možnosti gnojenja. V kontrolnem vzorcu je bila v zrnju ajde ugotovljena višja vsebnost dušikovih snovi kot pri uporabi izboljševalcev. Med pridelkom in dušikovimi snovmi v zrnju ajde je bila ugotovljena negativna korelacija ($r = -0,74$).

Research paper

Interactive Effects of Rice Husk Biochar and Zinc Oxide Nanoparticles on Physio-biochemical Traits, and Yield of Buckwheat (*Fagopyrum esculentum*) under Salinity Stress

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ABSTRACT

Salinity stress negatively affects the physiological and biochemical processes of plants, leading to reduced yields. This study addresses the knowledge gap regarding effective strategies to mitigate salinity-induced damage and enhance productivity in buckwheat. We hypothesized that zinc oxide nanoparticles (ZnO NPs) and rice husk biochar could improve salinity tolerance in buckwheat by modulating its physiological and biochemical responses. To test this, common buckwheat plants were grown under irrigation with well-watered (0 mM salinity) and moderate saline water (75 mM salinity) following a completely randomized design (CRD) with three replications. Results showed that the application of 50 g/kg rice husk biochar and 200 ppm ZnO NPs, either separately or in combination, significantly enhanced the yield and improved key physiological and biochemical traits, including relative water content, photosynthetic rate, stomatal conductance, chlorophyll content, and antioxidant activity. The combination of ZnO NPs and rice husk biochar led to improvements in the plants' relative water content, photosynthetic rate, chlorophyll levels, membrane stability index (MSI), proline, antioxidant activity (DPPH), and seed yield by 18.32, 15.29, 40.18, 14.54, 38.56, 6.87, and 40.78%, respectively, compared to untreated salinity plants. Moreover, this treatment reduced oxidative stress indicators such as hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) by 25.56 and 35.0%, respectively. These results show that ZnO NPs, when combined with rice husk biochar, significantly improve salinity tolerance in common buckwheat, providing a viable strategy to increase crop yields in saline environments. In view of climate change, this study emphasizes the potential of combining biochar with nanomaterials for sustainable agricultural practices.

INTRODUCTION

Salinity is one of the most critical abiotic stresses limiting crop productivity worldwide. High soil salinity disrupts plant water uptake, ionic balance, and nutrient acquisition, often leading to osmotic stress, ion toxicity, oxidative damage, and reduced photosynthetic efficiency (Askari-Khorasgani et al., 2021; Lu et al., 2023). Among salt-sensitive crops, common buckwheat (*Fagopyrum esculentum*) is highly susceptible also to drought stress, which adversely affects germination, growth, and yield quality due to impaired physiological and biochemical processes (Selwal et al., 2022; Sah et al., 2025).

Under saline conditions, plants often accumulate reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), which induce lipid peroxidation, protein oxidation, and enzyme inactivation, ultimately compromising cellular function and productivity (Singh, 2022). To counteract these effects, plants employ antioxidant enzymes and compatible solutes, such as proline, to maintain redox homeostasis and osmotic balance. However, these innate mechanisms are often insufficient under moderate-to-high salinity stress, necessitating external interventions to enhance stress tolerance.

Soil amendments like biochar have emerged as a promising strategy to mitigate salinity-induced damage. Biochar, a carbon-rich product derived from pyrolysis of biomass, improves soil water-holding capacity, nutrient retention, and microbial activity, and reduces ionic toxicity, thereby enhancing plant growth and yield under stress conditions (Yadav et al., 2023; Mannan et al., 2025). Specifically, rice husk biochar has been reported to enhance photosynthetic pigments, relative water content, and antioxidant capacity in various crops subjected to abiotic stress (Safari et al., 2023; Sah et al., 2025).

Nanotechnology provides another avenue for enhancing crop tolerance to salinity. By stimulating hormonal signalling, root activity, water uptake, and antioxidant activities (Ahmad et al., 2017), the application of NPs enhances photosynthetic efficiency, synthesis of secondary metabolites and chlorophyll, and antioxidant activity, improving plant growth during drought (Djanganuraman et al., 2018; Zahedi et al., 2018; Van Nguyen et al., 2022). Engineered nanoparticles, such as zinc oxide nanoparticles (ZnO NPs), have been shown to improve nutrient use efficiency, modulate antioxidant defense, enhance photosynthesis, and stabilize membranes under stress (Qian et al., 2024). Zinc, in particular, is an essential micronutrient that regulates enzyme activity, ROS

scavenging, and osmotic balance, making ZnO NPs a valuable tool to counteract salt-induced oxidative stress.

Despite the promising roles of biochar and ZnO NPs individually, little is known about their combined effects on salinity tolerance in buckwheat. Considering the complementary mechanisms - biochar improving soil physicochemical properties and ZnO NPs enhancing plant physiological and biochemical processes - integrated application may exert synergistic effects to improve crop performance under saline conditions.

Therefore, in this study, we hypothesized that the combined application of rice husk biochar and ZnO NPs would enhance salinity tolerance in common buckwheat by improving water relations, photosynthetic efficiency, antioxidant defense, and osmolyte accumulation, thereby increasing yield. The objective of this study was to evaluate the individual and combined effects of rice husk biochar and ZnO nanoparticles on physiology, biochemical traits, and yield of buckwheat under salinity stress.

MATERIALS AND METHODS

Experimental location, soil, treatments and design

The study was carried out in a semi-controlled vinyl house at the Department of Agronomy, Gazipur Agricultural University, Bangladesh, between November 2023 and February 2024. At latitude $24^{\circ} 5' 23''$ N and longitude $90^{\circ} 15' 36''$ E, the experimental site is 8.4 meters above mean sea level. Figure 1 shows the average maximum and minimum temperatures as well as relative humidity during the growing season (GAU, 2024). The experimental soil was composed of 52.99% sand, 33.00% silt, and 13.21% clay. It had a sandy loam texture and a pH of 6.3. The values for soil organic carbon, accessible P, total N, exchangeable K, CEC, and EC were 0.55%, 0.06 mg/100 g, 0.07%, 0.73 cmol/kg dry soil, 12.75 cmol/kg dry soil, and 0.02 dS/m, respectively. Approximately 30% of the soil's moisture content is retained at field capacity (FC). A 4:1 mixture of soil and cow dung was placed into each 30 cm long by 24 cm wide plastic pot. It contained six kg of blended soils that had been allowed to air dry. Two components made up the experiment. Factor A: salinity levels: i) well water irrigation (0 mM NaCl) and ii) saline water irrigation (75 mM NaCl). Factor B consists of the following four treatments: i) control (no treatment); ii) rice husk biochar (BC) at 50 g/kg soil; iii) foliar application of ZnO NPs at 200 ppm concentration (ZnO NPs);

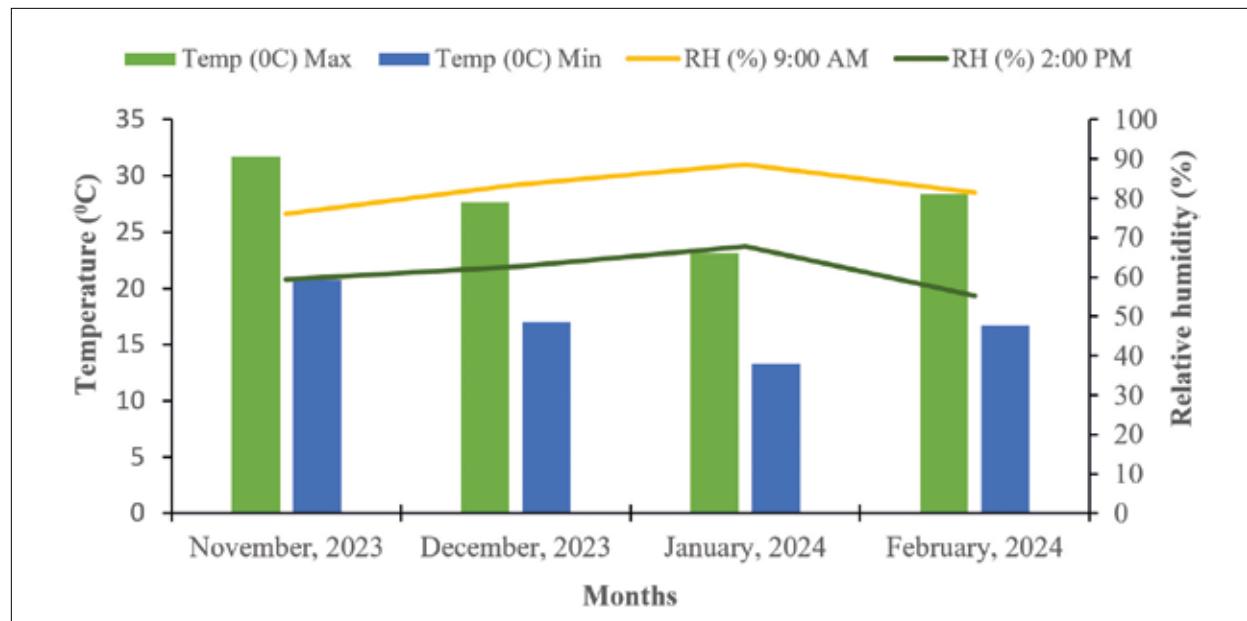


Figure 1. Temperature and relative humidity during experimentation

and iv) a combination of biochar application and foliar application of ZnO NPs (BC + ZnO NPs). Three replications of a Completely Randomized Design (CRD) were used in the experiment.

Rice husk biochar

The process outlined by Islam et al. (2018) was used to create the rice husk charcoal in a biochar burner. Rice husk biochar has the following chemical composition: pH 7.1, N 2.51%, P 0.23%, K 0.235%, Ca 1.012%, Mg 0.446%, S 0.326%, and EC (Exchangeable cation) 1.23 mS/cm.

ZnO nanoparticle solution preparation

Nanoparticle solutions were made using zinc oxide nanopowder, which has an average particle size of less than 50 nm, a specific surface area of at least 30 m²/g, a molecular weight of 81.39 g/mol, a white colour, and X-ray diffraction that conforms to structure (Sigma Aldrich, 2016). One litre of distilled water was mixed with 200 milligrams of this material to create 200 ppm nano-ZnO solutions. A hot plate and a magnetic stirrer were used to heat the mixture to 60 °C for sixteen hours. To ensure the solution could easily pass through the plant leaves during

application, it was then placed in a sonication bath with constant vibration to uniformly mix all the particles into the water (Sandhya et al., 2021). After that, these solutions were stored in a plastic bottle at room temperature. A hand sprayer was filled with the required volume before the solution was applied to the plant.

Treatments, imposition, and cultural practices

In pots treated with biochar, the rice husk biochar was uniformly combined with soil at a rate of 50 g/kg soil. After being sterilised with 1% sodium hypochloride, the common buckwheat seeds genotype NB1 (collected from Nepal) were repeatedly rinsed with distilled water. The seeds were sterilised and then placed on a sanitised bench to dry overnight. Ten seeds, equally spaced, were placed in each container. A small amount of water was supplied to the pots to promote consistent germination. Five days after seeding, seeds began to germinate. Throughout the growing season, twelve pots that were in the fourth leaf stage were regularly irrigated with tap water (0 mM NaCl solution). Throughout the growing season, the remaining 12 pots were irrigated in a salinity-stressed environment with a salinity of 75 mM NaCl. The leaves of the salt-treated and control plants were sprayed with a 200 ppm concentration of nano-ZnO solution after seven

days of the fourth leaf stage. Two sprayings were applied to each plant, separated by seven days.

Data collection

Data on physio-biochemical parameters were made on both control and salt-treated leaves during the flowering stage. Yield-related data were recorded at maturity.

Relative water content (RWC)

To determine the relative water content (RWC), five fully expanded upper leaves from each treatment were randomly collected, placed in polyethylene bags, and immediately transported to the laboratory. Fresh weight (FW) was recorded promptly to minimize moisture loss. For measuring turgid weight (TW), the leaves were immersed in distilled water and kept overnight. After 24 hours, the samples were removed, surface moisture was blotted gently, and the turgid weight was recorded. The leaves were then oven-dried at 65 °C for 72 hours to obtain the dry weight (DW). The RWC for each treatment was calculated using the following equation (Mannan et al., 2013):

$$RWC (\%) = [(FW - DW) / (TW - DW)] \times 100$$

where FW, DW, and TW refer to the fresh weight, dry weight, and turgid weight of the leaf samples, respectively.

Photosynthetic rate measurement

Photosynthetic rate (Pn) was measured using a portable photosynthetic gas exchange system (LI-COR 6400, LI-COR Biosciences, Lincoln, NE, USA). Measurements were conducted on clear, sunny days between 11:00 a.m. and 1:00 p.m., when ambient light intensity was stable and near its natural peak. Fully expanded uppermost leaves from each pot were selected to ensure uniformity in physiological status. Prior to recording, the leaves were allowed to acclimate inside the chamber to stabilize temperature, CO₂ concentration, and light conditions. Photosynthetic rate was then recorded under these steady-state conditions to ensure accurate and comparable measurements across treatments.

Leaf chlorophyll content measurement

A fully expanded leaf from the apex of each plant was collected following the procedure of Mannan et al.

(2023) to quantify chlorophyll content for each replication. Approximately 20 mg of fresh leaf tissue was placed into vials containing 20 mL of 80% acetone and kept in complete darkness for 72 hours, with the vials wrapped in aluminum foil to prevent pigment degradation. After extraction, absorbance was measured at 663 nm and 645 nm using a double-beam spectrophotometer (Thermo Fisher Scientific, Model 20020). Total chlorophyll concentration was calculated using the equation:

$$\text{Total chlorophyll (mg g}^{-1} \text{ FW}) = [20.2 (A645) - 8.02 (A663)] \times (V / 100 \times W)$$

where A₆₆₃ and A₆₄₅ represent the absorbance of the extract at 663 nm and 645 nm, respectively; V is the final volume (mL) of 80% acetone containing the extract; and W is the fresh weight (g) of the leaf sample.

Cell membrane stability (MSI) measurement

Cell membrane stability was assessed following the protocol described by Rady (2011), with minor modifications. For each treatment, two identical sets of leaf discs (10 discs per set) were prepared using a cork borer, ensuring uniform size and avoiding major veins. The discs were rinsed gently with distilled water to remove surface-adhered electrolytes before incubation.

The first set of discs was placed in test tubes containing a fixed volume of distilled water and incubated in a water bath at 40 °C for 30 minutes. After incubation, the electrical conductivity of the bathing solution (EC₁) was measured using a calibrated conductivity meter.

The second set of discs, representing total electrolyte leakage, was immersed in an equal volume of distilled water and incubated at 100 °C for 10 minutes to ensure complete membrane disruption. After cooling to room temperature, the electrical conductivity (EC₂) was recorded.

The membrane stability index (MSI) was calculated using the formula:

$$MSI (\%) = [1 - (EC_1 / EC_2)] \times 100$$

A higher MSI value indicates greater cell membrane integrity under the given treatment conditions.

Malondialdehyde (MDA) measurement

Malondialdehyde (MDA) content, an indicator of lipid peroxidation, was quantified following the thiobarbituric acid (TBA) reaction method described by Rao and Sresty (2000). 0.5 g of fresh leaf tissue was homogenized

in 0.1% (w/v) trichloroacetic acid (TCA) under chilled conditions. The homogenate was centrifuged, and an aliquot of the supernatant was combined with 20% (w/v) TBA prepared in 0.1% TCA to generate the TBA–MDA reaction complex. The mixture was incubated in a water bath to facilitate chromogen development and subsequently cooled to room temperature before a second centrifugation. Absorbance of the clarified supernatant was recorded at 530 nm and 600 nm using a UV–visible spectrophotometer (Shimadzu UV-1201, Kyoto, Japan). The MDA concentration was calculated by subtracting the non-specific absorbance at 600 nm from the TBA–MDA absorbance peak at 530 nm, providing a precise estimate of lipid peroxidation intensity under both control and salinity stress conditions.

Hydrogen Peroxide (H_2O_2) measurement

The H_2O_2 content was quantified following a modified protocol of Velikova et al. (2000). 300 mg of frozen leaf powder was homogenized with 2 mL of ice-cold 0.1% (w/v) trichloroacetic acid (TCA) and the mixture was centrifuged at $12,000 \times g$ for 15 minutes at 4°C . Each sample was processed in triplicate. To 0.5 mL of the resulting supernatant, 1 mL of 1 M potassium iodide and 5 mL of 10 mM potassium phosphate buffer (pH 7.0) were added. The blank contained 0.1% TCA instead of the sample extract. Absorbance was recorded at 390 nm using a Cary 100 Bio spectrophotometer (Varian, Australia), under identical conditions applied to the H_2O_2 standard.

Estimation of total antioxidant (2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was assessed spectrophotometrically following the protocol of Okonogi et al. (2007). This assay is based on the reduction of the purple DPPH radical to a yellow-colored product upon reaction with antioxidants. Leaf extracts were prepared as 10 mg/mL stock solutions in methanol. For the assay, 1,000 μL of each extract dilution was mixed with 5,000 μL of DPPH solution (150 μM in methanol), followed by vigorous shaking and incubation in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm to determine the remaining DPPH, with each sample analyzed in triplicate. Radical scavenging activity (%) was calculated as:

$$\text{DPPH radical-scavenging (\%)} = \frac{\text{A}_0 - \text{A}_1}{\text{A}_0} \times 100$$

where A_1 is the sample's absorbance and A_0 is the control's absorbance. A sample's IC₅₀ value indicates the concentration needed to scavenge 50% of the DPPH free radicals. The reaction mixture's lower absorbance suggests a higher degree of free radical scavenging activity.

Proline estimation

Proline content was determined following the method of Bates et al. (1973). 2.0 mL of proline extract was mixed with 2.0 mL of acid ninhydrin and 2.0 mL of glacial acetic acid. The reaction mixture was incubated according to the original protocol, and the absorbance was measured at 520 nm. A standard curve was generated using L-proline of known concentrations to quantify the proline content in the samples.

Estimation of yield and yield contributing parameters

At maturity, three plants from each pot were harvested, and the number of grains per plant, 1,000-grain weight, and grain yield per plant were determined.

Statistical analysis

The obtained data were statistically analyzed for each parameter using analysis of variance (ANOVA), and differences between treatment means were assessed with the least significant difference (LSD) test at $p = 0.05$ (Gomez and Gomez, 1984). Statistical analyses were conducted using CropStat 7.2, and graphs were generated in Microsoft Excel 2016.

RESULTS

Relative water content

Salinity stress markedly reduced the relative water content (RWC) of buckwheat leaves across all treatments. In the control plants, RWC declined from approximately 82% under non-saline conditions (0 mM NaCl) to nearly 67% at 75 mM NaCl. The addition of biochar provided a modest improvement in leaf hydration, maintaining RWC at about 83% in non-saline conditions and 74% under salinity. Plants treated with ZnO nanoparticles (ZnO NPs) exhibited a further increase in water retention, with RWC values reaching approximately 84% at 0 mM NaCl and 76% under saline conditions. Notably, the combined

application of biochar and ZnO NPs resulted in the highest RWC, achieving roughly 87% in the absence of salinity and 79% under saline stress (Figure 2). These findings

clearly indicate that both biochar and ZnO NPs- individually and synergistically-ameliorate the negative effects of salinity on buckwheat leaf water status.

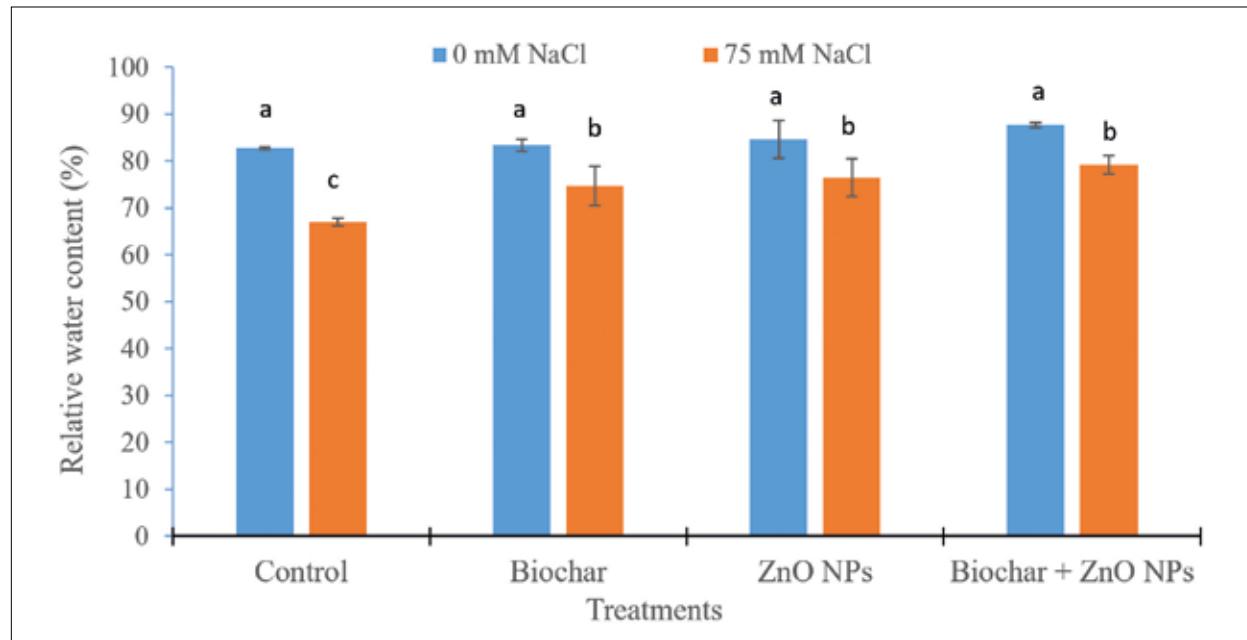


Figure 2. Effects of biochar and ZnO NPs on relative water content of buckwheat leaf under salinity. Bar indicates (mean \pm SE). Different letters indicate a significant difference between treatments according to Tukey's test at $P \leq 0.05$.

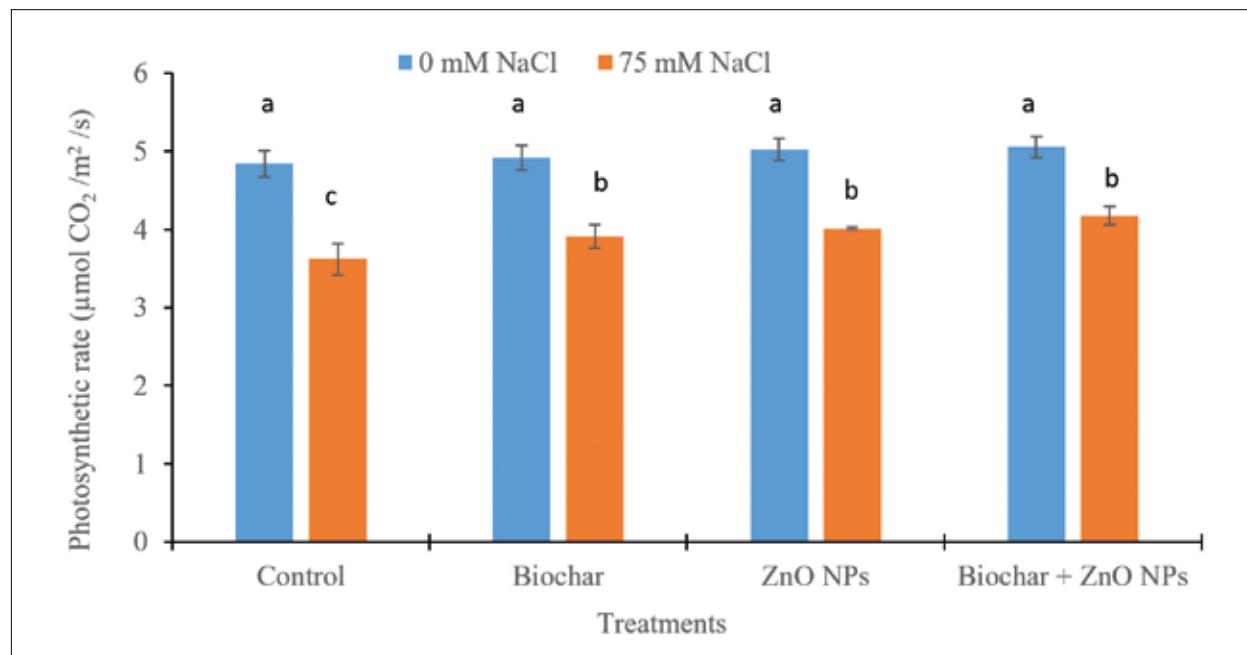


Figure 3. Effects of biochar and ZnO NPs on photosynthetic rate of buckwheat leaf under salinity. Bar indicates (mean \pm SE). Different letters indicate a significant difference between treatments according to Tukey's test at $P \leq 0.05$.

Photosynthetic rate

Salinity stress (75 mM NaCl) reduced the photosynthetic rate of buckwheat leaves across all treatments compared with non-saline conditions (0 mM NaCl) (Figure 3). Under non-saline conditions, the highest photosynthetic rate was recorded in the biochar + ZnO NPs treatment ($5.06 \mu\text{mol m}^{-2} \text{ s}^{-1}$), followed closely by ZnO NPs ($5.03 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and biochar alone ($4.91 \mu\text{mol m}^{-2} \text{ s}^{-1}$), while the control exhibited the lowest value ($4.84 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Exposure to 75 mM NaCl markedly decreased photosynthetic activity; however, the combined biochar + ZnO NPs treatment maintained the highest rate ($4.18 \mu\text{mol m}^{-2} \text{ s}^{-1}$), followed by ZnO NPs ($4.01 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and biochar ($3.91 \mu\text{mol m}^{-2} \text{ s}^{-1}$). The control plants showed the greatest reduction under salinity, recording the lowest photosynthetic rate ($3.62 \mu\text{mol m}^{-2} \text{ s}^{-1}$). The combined application of biochar and ZnO NPs demonstrated the most pronounced protective effect on maintaining photosynthetic capacity under salinity stress.

Total Chlorophyll

When compared to non-saline conditions (0 mM NaCl), the total chlorophyll content of buckwheat leaves

under salinity stress (75 mM NaCl) was significantly lower in all treatments (Figure 4). The biochar + ZnO NPs treatment had the highest chlorophyll content under non-saline conditions (2.86 mg/g FW), followed by ZnO NPs alone (2.63 mg/g FW) and biochar (2.31 mg/g FW), while the control showed the lowest value (2.18 mg/g FW). The combination biochar + ZnO NPs treatment maintained the highest value (1.99 mg/g FW), followed by ZnO NPs (1.80 mg/g FW) and biochar (1.55 mg/g FW), despite a significant decrease in chlorophyll content following exposure to 75 mM NaCl. The control plants had the lowest chlorophyll content (1.42 mg/g FW) and the biggest loss under salinity. The most noticeable protective impact on preserving chlorophyll content under salinity stress was shown by the combination application of biochar and ZnO NPs.

Membrane stability index and malondialdehyde

Salinity stress (7.5 mM NaCl) significantly reduced the membrane stability index (MSI) and increased malondialdehyde (MDA) accumulation in buckwheat leaves compared with the non-saline control (Table 1). Under 0 mM NaCl, MSI ranged from 72.59% in the

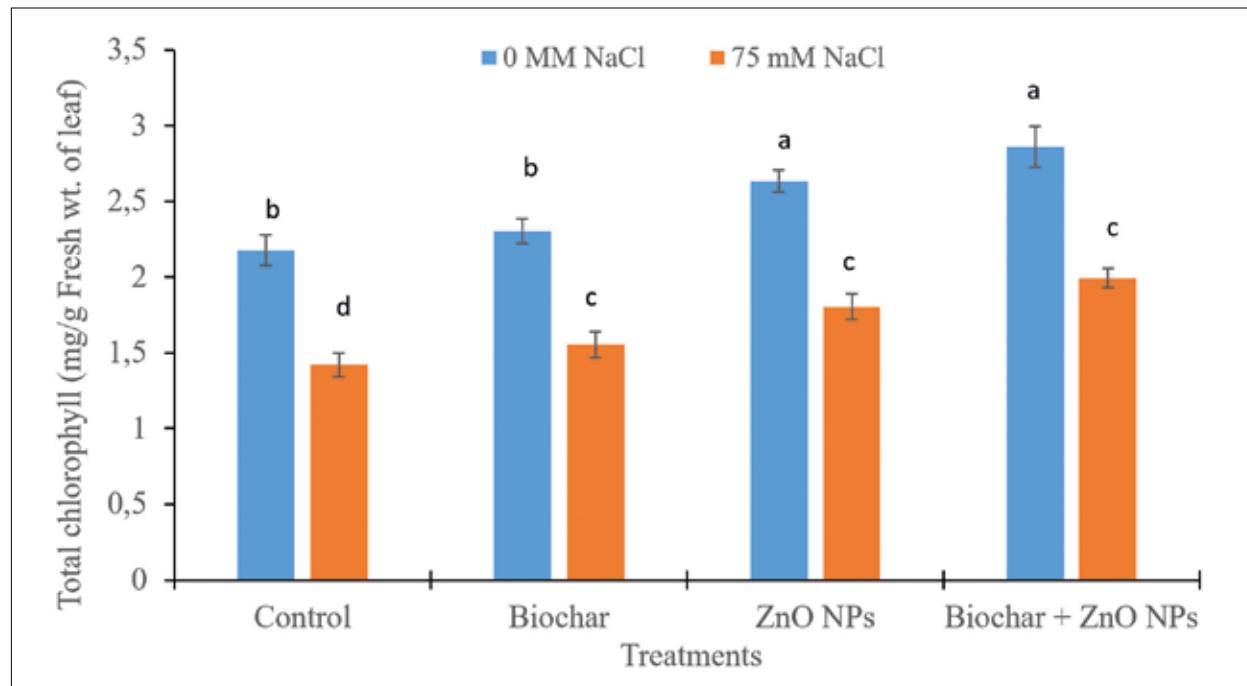


Figure 4. Effects of biochar and ZnO NPs on total chlorophyll of buckwheat leaf under salinity stress. Bar indicates (mean \pm SE). Different letters indicate a significant difference between treatments according to Tukey's test at $P \leq 0.05$.

Table 1. Effects of biochar and ZnO NPs on membrane stability index and melondealdehyde (MDA) in buckwheat leaf under salinity stress. Values are presented as mean \pm SE ($n = 3$).

| Treatments | Membrane stability index (%) | | MDA (nano mole/g fresh wt. of leaf) | |
|------------------|------------------------------|-------------------|-------------------------------------|-------------------|
| | 0 mM NaCl | 75 mM NaCl | 0 mM NaCl | 75 mM NaCl |
| Control | 72.59 \pm 2.14a | 60.18 \pm 2.99c | 33.11 \pm 1.42c | 54.99 \pm 2.03a |
| Biochar | 74.97 \pm 2.54a | 64.92 \pm 2.91b | 31.85 \pm 0.53c | 49.82 \pm 2.08a |
| ZnO NPs | 75.42 \pm 2.34a | 67.29 \pm 1.09c | 33.21 \pm 0.26c | 42.42 \pm 3.17b |
| Biochar +ZnO NPs | 77.24 \pm 1.40a | 68.96 \pm 1.59b | 32.19 \pm 1.37c | 35.67 \pm 1.31c |
| CV (%) | 5.8 | | 7.7 | |

Different letters indicate a significant difference between treatments according to Tukey's test at $P \leq 0.05$.

control to 77.24% in the biochar + ZnO NPs treatment. A similar trend was observed under salinity, where MSI decreased across all treatments but remained highest in the biochar + ZnO NPs treatment (68.96%), followed by ZnO NPs (67.29%) and biochar (64.92%). The lowest MSI was recorded in the control (60.18%).

Conversely, MDA content increased markedly under salinity (Table 1). The control exhibited the highest MDA levels at both 0 mM (33.11 nmol /g FW) and 7.5 mM NaCl (54.99 nmol /g FW). Treatments containing ZnO NPs effectively reduced lipid peroxidation under stress, with the combined biochar + ZnO NPs treatment showing the lowest MDA concentration (35.67 nmol /g FW), followed by ZnO NPs alone (42.42 nmol /g FW). Biochar alone also lowered MDA compared with the control. Overall, the combined application of biochar and ZnO NPs offered the greatest protection by enhancing membrane stability and minimizing oxidative damage under salinity stress.

Hydrogen Peroxide (H_2O_2) content and Total antioxidant contents

Hydrogen peroxide (H_2O_2) levels increased markedly under salinity stress across all treatments (Table 2). In the control plants, H_2O_2 content rose from $4.09 \pm 0.10 \mu\text{mol} / \text{g FW}$ at 0 mM NaCl to $6.36 \pm 0.67 \mu\text{mol} / \text{g FW}$ at 75 mM NaCl (Table 2). Application of biochar or ZnO NPs alone effectively reduced H_2O_2 accumulation under salinity, with values of 5.42 ± 0.38 and $5.06 \pm 0.33 \mu\text{mol} / \text{g FW}$, respectively, compared with the stressed control. The combined application of biochar and ZnO NPs produced the strongest reduction, lowering H_2O_2 content to $4.75 \pm 0.07 \mu\text{mol} / \text{g FW}$ under 75 mM NaCl, indicating enhanced mitigation of oxidative stress.

Total antioxidant activity (expressed as IC_{50}) decreased under salinity in all treatments, reflecting stress-induced reduction in antioxidant potential (Table 2). The control exhibited a decline from $168.21 \pm 4.03 \text{ mg / ml}$ (0 mM NaCl) to $153.39 \pm 2.28 \text{ mg / ml}$ (75 mM NaCl).

Table 2. Effects of biochar and ZnO NPs on hydrogen peroxide (H_2O_2) and total antioxidant in buckwheat leaf under salinity stress. Values are presented as mean \pm SE ($n = 3$).

| Treatment | Hydrogen peroxide ($\mu\text{mol/g}$ fresh wt. of leaf) | | Antioxidants ($IC_{50} = \text{mg/ml}$) | |
|------------------|--|------------------|---|--------------------|
| | 0 mM NaCl | 75 mM NaCl | 0 mM NaCl | 75 mM NaCl |
| Control | 4.09 \pm 0.10c | 6.36 \pm 0.67a | 168.21 \pm 4.03a | 153.39 \pm 2.28c |
| Biochar | 3.76 \pm 0.11c | 5.42 \pm 0.38b | 173.52 \pm 1.51a | 157.46 \pm 1.51b |
| ZnO NPs | 3.87 \pm 0.06c | 5.06 \pm 0.33b | 173.76 \pm 2.19a | 161.58 \pm 1.16b |
| Biochar +ZnO NPs | 3.57 \pm 0.16c | 4.75 \pm 0.07b | 171.21 \pm 2.08a | 163.88 \pm 2.16b |
| CV (%) | 11.6 | | 2.4 | |

Different letters indicate a significant difference between treatments according to Tukey's test at $P \leq 0.05$.

Both biochar (157.46 ± 1.51 mg /ml) and ZnO NPs (161.58 ± 1.16 mg /ml) improved antioxidant capacity under salinity compared with the stressed control. The highest antioxidant activity (lowest IC_{50}) under salinity was recorded in the combined biochar + ZnO NPs treatment (163.88 ± 2.16 mg /ml), demonstrating a synergistic effect in enhancing the antioxidant defense system of buckwheat.

Proline content

Proline content in buckwheat leaves showed non-significant variation among treatments under both non-saline and mild salinity (7.5 mM NaCl) conditions (Figure 5). Under control conditions, proline concentration remained unchanged between 0 mM and 7.5 mM NaCl ($0.16 \mu\text{mol g}^{-1}$ FW). Application of biochar slightly increased proline at 0 mM NaCl ($0.19 \mu\text{mol g}^{-1}$ FW), although the value declined marginally under salinity ($0.18 \mu\text{mol g}^{-1}$ FW). ZnO nanoparticles also enhanced proline accumulation compared to the control at 0 mM NaCl ($0.18 \mu\text{mol g}^{-1}$ FW), with no change observed under saline conditions ($0.18 \mu\text{mol g}^{-1}$ FW). Notably, the combined application of biochar and ZnO NPs resulted in the highest proline accumulation under 7.5 mM NaCl ($0.22 \mu\text{mol g}^{-1}$ FW).

FW), indicating a synergistic effect that improved osmotic adjustment under salinity stress.

Yield and its components of buckwheat

Significant variation was observed in the reproductive traits of buckwheat in response to rice husk biochar (BC) and ZnO NPs under both non-saline and saline (75 mM NaCl) conditions (Table 3). Salinity markedly reduced the number of grains per plant, 1000-grain weight, and grain yield compared to the non-saline control. Under 0 mM NaCl, the number of grains per plant ranged from 155.67 in the control to 159.67 in the BC + ZnO NPs treatment. Under 75 mM NaCl, all treatments exhibited a reduction in grain number, with the lowest in the control (114.67) and the highest in the combined BC + ZnO NPs treatment (133.67).

The 1000-grain weight also declined under salinity, dropping from 16.13 g in the control to 9.30 g. Among the amendments, BC + ZnO NPs produced the highest 1000-grain weight at both 0 mM (17.40 g) and 75 mM NaCl (13.33 g).

Grain yield displayed a similar trend, with combined BC + ZnO NPs showing the greatest improvement. Under non-saline conditions, the highest grain yield (2.66 g/

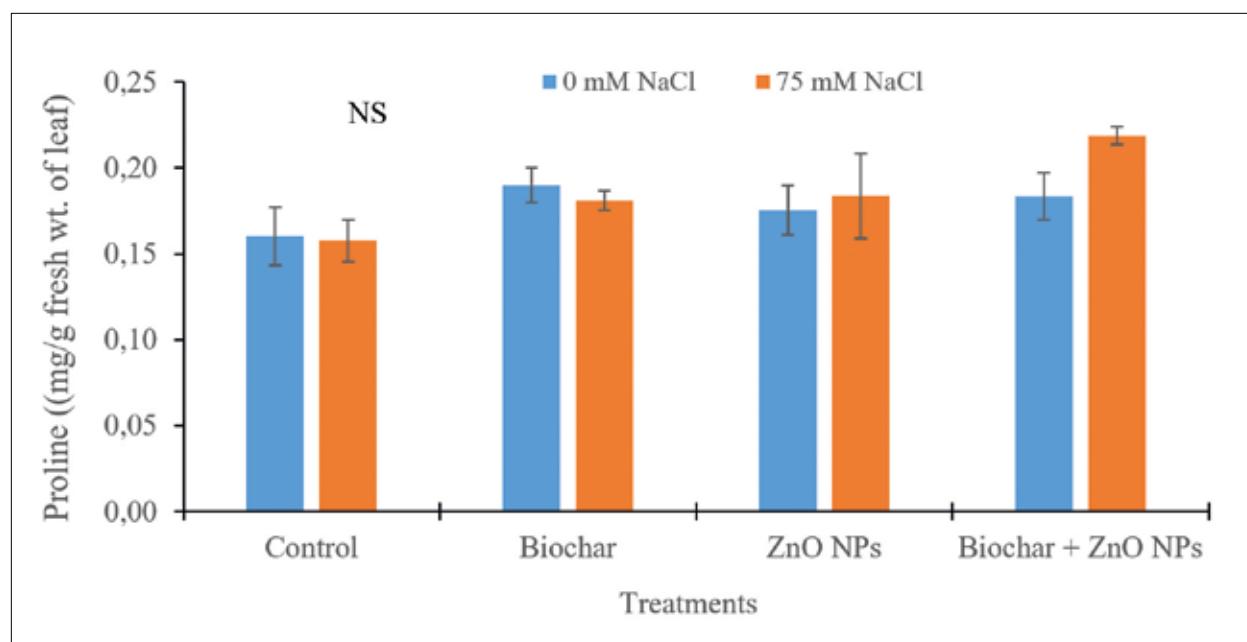


Figure 5. Effects of biochar and ZnO NPs on proline content of buckwheat leaf under salinity. Bar indicates (mean \pm SE). NS= Non significant

Table 3. Effects of biochar and ZnO NPs on the number of grains/plants, 1000-grain weight, and grain yield of buckwheat under saline conditions. Values are presented as mean \pm SE ($n = 3$)

| Treatments | Number of grains/plants | | 1000-grains weight (g) | | Grain yield (g/plant) | |
|-------------|-------------------------|--------------------|------------------------|-------------------|-----------------------|------------------|
| | 0 mM NaCl | 75 mM NaCl | 0 mM NaCl | 75 mM NaCl | 0 mM NaCl | 75 mM NaCl |
| Control | 155.67 \pm 2.97a | 114.67 \pm 2.91c | 16.13 \pm 0.94a | 9.30 \pm 0.40d | 2.33 \pm 0.10b | 1.11 \pm 0.02d |
| BC | 157.67 \pm 6.23a | 125.00 \pm 1.73b | 16.20 \pm 0.46a | 10.59 \pm 0.19c | 2.36 \pm 0.07b | 1.26 \pm 0.05d |
| ZnO NPs | 156.00 \pm 4.73a | 131.00 \pm 3.22b | 16.63 \pm 0.72a | 10.90 \pm 0.55c | 2.58 \pm 0.01a | 1.41 \pm 0.05c |
| BC+ ZnO NPs | 159.67 \pm 5.18a | 133.67 \pm 3.29b | 17.40 \pm 0.50a | 13.33 \pm 0.78b | 2.66 \pm 0.08a | 1.57 \pm 0.04c |
| CV (%) | 4.9 | | 7.7 | | 5.5 | |

Different letters indicate a significant difference between treatments according to Tukey's test at $P \leq 0.05$.

plant) was recorded in the BC + ZnO NPs treatment, followed by ZnO NPs alone (2.58 g/plant). Salinity reduced yield drastically in the control (1.11 g/plant), but the BC + ZnO NPs treatment maintained the highest yield under stress (1.57 g/plant).

Overall, the combined application of rice husk biochar and ZnO NPs demonstrated the most pronounced positive effects in mitigating salinity-induced reductions in buckwheat grain production metrics.

Discussion

Salinity induces osmotic stress, disrupts water balance, and reduces cellular hydration, leading to a marked decline in relative water content (RWC) (Munns & Tester, 2008). In this study, buckwheat exposed to 75 mM NaCl showed a substantial reduction in RWC, confirming its sensitivity to salt-induced water deficit. However, biochar, ZnO nanoparticles (ZnO NPs), and their combined application significantly mitigated this decline. Biochar improved water retention and soil physical properties (Lehmann & Joseph, 2015), consistent with earlier reports showing enhanced leaf water status under salinity (Akhtar et al., 2015; Su et al., 2024). ZnO NPs further supported water balance - likely through improved membrane stability, antioxidant activity, and osmolyte accumulation (Gupta et al., 2024; Dimkpa & Bindraban, 2018). The combined treatment was most effective, indicating complementary soil improvement and physiological protection, in agreement with studies reporting synergistic benefits of biochar–nanoparticle integration under stress (Elshayb et al., 2022).

Photosynthetic rate was also strongly reduced by salinity, reflecting osmotic imbalance, ion toxicity, and stomatal constraints typical of glycophytic species (Gupta et

al., 2014). Biochar improved photosynthetic performance under stress by enhancing water availability and reducing Na^+ uptake, similar to previous findings (Zonayet et al., 2023). Zinc supplied through ZnO NPs further increased photosynthesis via its role in chlorophyll synthesis, enzyme activation, and oxidative stress mitigation (Hassan et al., 2024). Again, the combined amendment produced the greatest improvement, supporting earlier evidence that integrating soil conditioners and nanoparticles enhances chlorophyll retention and gas exchange efficiency under salinity (Wang et al., 2022).

Chlorophyll content declined sharply under salinity, confirming that NaCl stress disrupts pigment biosynthesis and accelerates chlorophyll degradation (Parida & Das, 2005). Biochar partially alleviated this decline by improving nutrient availability and reducing ionic toxicity (Lehmann & Joseph, 2015). ZnO NPs further enhanced chlorophyll levels in both saline and non-saline conditions through improved chloroplast stability and antioxidant regulation (Broadley et al., 2007; Rizwan et al., 2019a). The highest chlorophyll content was observed under the combined biochar + ZnO NP treatment, demonstrating synergistic enhancement of both soil-mediated and physiological processes, consistent with previous reports (Rizwan et al., 2019b).

Salinity also compromised membrane integrity, evidenced by reduced membrane stability index (MSI) and elevated malondialdehyde (MDA) levels due to ROS-induced lipid peroxidation (Hasanuzzaman & Fujita, 2023). Biochar reduced these adverse effects by improving water balance and decreasing Na^+ accumulation (Murtaza et al., 2024). ZnO NPs strengthened membrane stability through activation of antioxidant enzymes and improved osmoprotection (Ashraf et al., 2019). The combined treatment produced the lowest

MDA and highest MSI under salinity, demonstrating strong cellular protection and agreeing with reports of synergistic ROS mitigation using biochar and nanoparticles (Rahman et al., 2022).

Salinity-induced oxidative stress was further evident from elevated hydrogen peroxide (H_2O_2) levels in control plants (Wang et al., 2016). Biochar and ZnO NPs individually reduced H_2O_2 accumulation by alleviating osmotic stress and enhancing antioxidant capacity (Sultan et al., 2025). Their combined application produced the greatest reduction, indicating enhanced ROS scavenging and improved redox homeostasis, consistent with previous findings (Bao et al., 2023). A similar trend was observed for total antioxidant capacity, with the combined treatment supporting the strongest antioxidant response (Sharma et al., 2012).

Proline accumulation, a key osmoprotective mechanism (Szabados & Savouré, 2010; Kishor et al., 2005), showed treatment-dependent variation. Mild salinity alone did not significantly induce proline, consistent with earlier observations that moderate NaCl levels may not strongly trigger osmotic stress (Santos et al., 2021). Biochar and ZnO NPs slightly increased proline under non-saline conditions through enhanced metabolic activity and stress signaling (Lehmann & Joseph, 2015; Rizwan et al., 2019). The highest proline accumulation occurred under the combined treatment at 75 mM NaCl, demonstrating improved osmotic adjustment. Similar synergistic enhancement of osmolyte production has been reported with combined soil amendments and nanoparticles (Ali et al., 2019).

Reproductive traits were highly sensitive to salinity, as shown by reductions in grain number, 1000-grain weight, and grain yield. Such declines are commonly attributed to impaired pollination, restricted assimilate flow, and reduced seed filling under salt stress (Munns & Tester, 2008; Zörb et al., 2019). Biochar improved reproductive performance by enhancing soil structure, aeration, and nutrient retention (Hossain et al., 2020). ZnO

NPs supported grain development through improved chlorophyll content, enzyme activation, and nutrient uptake (Singh et al., 2020). Their combined application produced the strongest improvements in yield components under both saline and non-saline conditions. The enhanced grain weight and yield under salinity suggest efficient carbohydrate translocation and improved reproductive resilience, consistent with earlier studies reporting the benefits of integrating organic amendments with nanoparticles under stress (Zhao et al., 2023; Rizwan et al., 2019b).

Overall, the findings demonstrate that combining biochar with nanoparticles ZnO strengthens physiological, biochemical, and reproductive tolerance mechanisms in buckwheat, providing a promising strategy for enhancing crop resilience and productivity under saline conditions.

CONCLUSION

The application of rice husk biochar and ZnO nanoparticles significantly enhanced the physio-biochemical processes, grain yield, and overall salinity tolerance of common buckwheat. Although salinity stress typically disrupts buckwheat physiology, plants treated with 200 ppm ZnO NPs and rice husk biochar displayed markedly improved physiological and biochemical responses under salt stress. Moreover, the combined application of ZnO NPs and biochar produced a synergistic effect, resulting in better plant performance and ultimately higher buckwheat yields. Among the treatments, soil amendment with rice husk biochar, combined with 200 ppm ZnO NPs, proved most effective in mitigating the detrimental impacts of salinity. Therefore, integrating nanoparticles with biochar may serve as a promising strategy to alleviate salt-induced damage in buckwheat. Future research should focus on elucidating the molecular mechanisms that drive the beneficial and complementary actions of biochar and ZnO nanoparticles under saline growing conditions.

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IZVLEČEK

Interaktivni učinki biooglja iz riževih luščin in nanodelcev cinkovega oksida na fiziološko-biokemične lastnosti in pridelek ajde (*Fagopyrum esculentum*), gojene v stresnih razmerah povečane slanosti

Stres zaradi visoke koncentracije soli negativno vpliva na fiziološke in biokemijske procese rastlin, kar vodi v zmanjšane pridelke. Študija se osredotoča na omilitveni vpliv nanodelcev oksida cinka (ZnO NPs) in biooglja iz riževih luščin na rastline, ki so uspevale v razmerah povečane slanosti. Avtorji so predvidevali, da lahko nanodelci oksida cinka (ZnO NPs) in biooglje iz riževih luščin izboljšajo odpornost ajde na povečano slanost, tako da vplivajo na njene fiziološke in biokemijske odzive. Avtorji so rastline ajde gojili z zalivanjem z vodo (0 mM slanosti) in zmerno slano vodo (75 mM slanosti) po randomiziranem načrtu (CRD) s tremi ponovitvami. Rezultati so pokazali, da je uporaba 50 g/kg biooglja iz riževih ostankov in 200 ppm ZnO NPs, bodisi posamezno ali v kombinaciji, pomembno povečala pridelek in izboljšala ključne fiziološke in biokemične lastnosti navadne ajde, vključno z relativno vsebnostjo vode, fotosintežno učinkovitostjo, vsebnostjo klorofila in antioksidativno aktivnostjo. Kombinacija ZnO NPs in biooglja iz riževih luščin je vodila k povečanem indeksu stabilnosti membran (MSI), prolinu, in pridelku semen za 18,32, 15,29, 40,18, 14,54, 38,56, 6,87 in 40,78 % glede na netretirane rastline prizadete zaradi slanosti. Poleg tega je navedeno tretiranje zmanjšalo kazalnike oksidativnega stresa, kot sta vodikov peroksid (H_2O_2) in malondialdehid (MDA), za 25,56 oz. 35,0 %. Ti rezultati kažejo, da je ZnO NPs v kombinaciji z bioogljem iz riževih luščin bistveno izboljšal toleranco navadne ajde na povečano slanost, kar predstavlja možno strategijo za povečanje pridelka v razmerah stresa zaradi povečane slanosti. Glede na podnebne spremembe ta raziskava poudarja potencial kombiniranja biooglja z nanomateriali za trajnostno kmetijstvo.

Empowering buckwheat, revitalizing the future

16th International Buckwheat Conference

June 24–28, 2026

Xichang · China

Information on the 16th International Buckwheat Conference in 2026 in Xichang, Sichuan, China

The information is published by Dr. Meiliang Zhou. The 16th IBC dates will be 24 June to 28 June 2026, the host city is Xichang, Sichuan, China. 24 June 2026 is the registration day, and 25 June to 27 June is the academy congress, 28 June is the visiting buckwheat field day. The meeting place is Qionghai Hotel, which is close to Qionghai lake in Xichang. Xichang is the city of Tartary buckwheat, and have many buckwheat processing factories.

More details are available on <https://ibra26.org/#/welcome>

History & Mission

The International Buckwheat Conference has long advanced global buckwheat research and industry development—spanning germplasm resources, breeding, processing, and nutrition-driven innovation.

This edition aims to bridge frontier science and real-world needs through cross-disciplinary dialogue and international collaboration, accelerating the translation of research into standards, products, and sustainable value chains.

FOUNDING

The founding of IBRA

International Buckwheat Research Association (IBRA) was established during the First International Symposium on Buckwheat in Ljubljana, Slovenia, on September 3rd, 1980. Founding members were Marek Ruszkowski (Puławy, Poland), Toshiko Matano (Ina, Japan), Takashi Nagatomo, Taiji Adachi (both from Miyazaki, Japan), Björn O. Eggum (Roskilde, Denmark) and Ivan Kreft (Ljubljana, Slovenia).

The overall profile and task of IBRA

The International Buckwheat Research Association (IBRA) is a global academic organization dedicated to advancing scientific research, academic exchange, and international collaboration in the fields of *Fagopyrum* (buckwheat) plants and related disciplines. Its core activities include organizing international conferences, publishing specialized journals, and facilitating the sharing of research resources, all conducted under strict adherence to academic and non-political principles.

International Buckwheat Research Association (IBRA)

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PAST CONFERENCES

Across four decades, tracing the global footprint of buckwheat science

1st Symposium, 1980

The 1st International Symposium on Buckwheat was organized in Ljubljana, Slovenia, in September 1-3, 1980, the main organizers are I. Kreft, B. Javornik, B. Vombergar and colleagues. The symposium was followed by the excursion to the typical traditional buckwheat growing area at Dolenjska, Slovenia, old buckwheat mill at Višnja Gora and buckwheat fields at Vrhtrebnje hill village.

2nd Symposium, 1983

The 2nd Symposium was organized in Miyazaki, Japan in September 7 – 10., 1983 by Takashi Nagatomo, Taiji Adachi and colleagues. Miyazaki Symposium was followed by excursion around Kyushu island and to Kyoto. In the first years of IBRA a lot of support was, besides the establishing members, given as well by Ohmi Ohnishi (Kyoto University, Japan), Hyoji Namai (Tsukuba University, Japan), Kiyokazu Ikeda, Sayoko Ikeda (Kobe Gakuin University, Japan), and Riichiro Shiratori (Shiratori Milling Co.).

3rd Symposium, 1986

The 3rd Symposium was performed in Puławy, Poland in July 7-12, 1986 by Marek Ruszkowski and colleagues. The symposium was followed by the excursion to buckwheat experiments and fields in Poland. At this symposium, by acclamation basic rules of IBRA were confirmed, the rules were afterwards published in FAGOPYRUM journal.

4th Symposium, 1989

In 1989 the 4th symposium was organized in Orel, Russia by N.V. Fesenko and his colleagues on July 11-15, 1989. Field experiments with buckwheat were visited.

5th Symposium, 1992

The 5th symposium was organized by Lin Rufa et al. on August 20-26, 1992, in Taiyuan, Shanxi, China. The symposium included the excursion to buckwheat growing areas in Shanxi, north from Taiyuan.

6th Symposium, 1995

The 6th Symposium was organized by Toshiko Matano, Akio Ujihara and their colleagues in August 24-29,

1995 at Shinshu University, Ina Campus, Nagano-ken in Japan. Field experiments with buckwheat were visited

7th Symposium, 1998

On August 12-14, 1998, Clayton Campbell, Roman Przybylski, and colleagues organized the 7th Symposium in Winnipeg, Manitoba, Canada. The symposium included excursions to buckwheat fields.

8th Symposium, 2001

On August 30 – September 2, 2001 the 8th Symposium was organized by Cheol Ho Park, in Korea (South). The symposium started in Chunchon City (Kangwon) and included the visit to Buckwheat Exhibition, where several buckwheat growing countries were presented, with their respective booths. After the Symposium, excursion to buckwheat festival in Bongpyeong took place.

9th Symposium, 2004

In August 18 – 22, 2004, in Prague, Czech Republic, the 9th Symposium was organized by Zdeněk Stehno, Anna Michalová et al. After the Symposium, excursion was organized to Czech buckwheat growing area. A post-symposium trip was organized by train to Vienna, Austria, and to Maribor, Slovenia. In Maribor post-symposium Buckwheat Conference at the Higher Vocational College of the Education centre Piramida Maribor was organized by Blanka Vombergar. Further, many international participants travelled by train to Sondrio and Teglio (Valtellina, Italy) to taste Italian buckwheat dishes and to observe buckwheat growing and utilization practice in Italy.

10th Symposium, 2007

In August 14-18, 2007, in Yangling, Shaanxi, China, 10th Symposium was organized by Chai Yan and colleagues. Before the symposium a welcome program for foreign participants, visiting places with connection to buckwheat and culture in Yangling area was organized. After the Symposium, excursion was organised to buckwheat growing areas of Shaanxi, including the Yellow river area.

11th Symposium, 2010

In July 19-23, 2010, 11th Symposium was organized in Orel, Russia, by Galina Suvorova, V.I. Zotikov and colleagues. After the Symposium, excursion was organised to buckwheat experimental field and buckwheat growing area in Orel region.

12th Symposium, 2013

The 12th Symposium was organized in August 21 – 25, 2013 in Laško, Slovenia by Blanka Vombergar, Mateja Germ, Maja Vogrinčič and Ivan Kreft, after the Symposium excursions to buckwheat fields, Rangus Mill in Šentjernej, to Slovenian Adriatic coast and Bled lake were organized, to taste diverse regional Slovenian buckwheat dishes.

13th Symposium, 2016

The 13th Symposium was organized in September 9 – 11, 2016, Chungbuk and Pyeongchang in South Korea by Sun-He Woo and Cheol Ho Park, including visit to Bongpyeong Buckwheat Festival, tasting buckwheat dishes, and visit to experimental factory of buckwheat food products in Chunchon.

14th Symposium, 2019

The 14th Symposium was organized in September 3 – 6, 2019 in Shillong, Meghalaya, India, by Nikhil

Chrungoo and colleagues, the Department of Botany, North-Eastern Hill University (NEHU), Shillong, India in collaboration with ICAR-National Bureau of Plant Genetic Resources (NBPGR), India, and DBT-Institute of Bioresources and Sustainable Development (IBSD), India. Symposium was followed by the excursions to buckwheat field experiments, in Meghalaya, and to Sikkim.

15th Symposium, 2023

The 15th Symposium was organized in July 2 – 8, 2023 in Puławy, Poland, by Grażyna Podolska, Krzysztof Dziedzic, Jacek Kwiatkowski and colleagues, in the Institute of Soil Science and Plant Cultivation, Puławy, Poland. Symposium was followed by the excursions to buckwheat field and factory in Nieznanice and Polanowice.

BRIEF INTRODUCTION TO XICHANG

Xichang is located in the hinterland of the Anning River Plain on the western Sichuan Plateau, serving as the seat of the prefectural government of Liangshan Yi Autonomous Prefecture, Sichuan Province. Situated in the cross-radiation area of the three major cities of Chengdu, Chongqing and Kunming, it acts as an important inland channel radiating to Southwest China and Southeast Asia. The city covers a total area of 2,882.9 square kilometers and has a permanent resident population of 966,000.

With an altitude of 1,500 meters, which is deemed the most suitable for human habitation, Xichang boasts an average annual temperature of 18°C, more than 2,500 hours of annual sunshine, an average annual oxygen content of 95%, and over 360 days of excellent air quality per year. Characterized by warm winters, cool summers, spring-like weather all year round, abundant sunshine and beautiful scenery, it is known as „A City Where Spring Resides“. The city has a well-developed transportation network consisting of railways, highways and civil aviation. Located in the renowned Panxi Rift Valley metallogenic belt, Xichang ranks among the top in China and Sichuan Province in terms of the proven reserves of non-ferrous metals and vanadium-titanium magnetite. It is the core area of the National Strategic Resource Innovation and Development Pilot Zone.

WARM REMINDER

The conference organizing committee will assist with hotel reservations and shuttle services to and from the station/airport during the conference. Please be sure to complete and submit your conference registration information in full, to facilitate the committee's vehicle arrangements and communication with the hotel. Hotel expenses are to be borne by the participants themselves.

Address of organizers of the 16th IBRA Conference:

Cereal Research Institute, CAAS, No. 12, Zhongguancun South Street, Haidian District, Beijing

PHONE: +86 10 82106367 | **E-MAIL:** YMQMZWH@163.COM | **WWW:** <https://ibra26.org/#/welcome>

INSTRUCTIONS FOR AUTHORS, FAGOPYRUM

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Manuscript should be written in standard English and submitted to the Editorial office as a word (.doc) document. Figures (photographs) should be IN SEPARATE FILE each in jpg or other original file, not imbedded in word .doc document or in PDF. Submission shall be sent to the email:

ivan.kreft@guest.arnes.si.

After reviewing by two reviewers and accepting the paper, the editorial office will ask the authors to provide the original figures if the submitted ones will not be adequate. Your manuscript should be sent to the Editor-in-Chief (Prof. Ivan Kreft). E-mail: **ivan.kreft@guest.arnes.si**

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Manuscripts should be typed in one column (we will transfer later the text to two columns). All pages, including the tables, legends and references, should be numbered consecutively. The manuscript should be arranged in the following order, or other suitable similar order:

1. Title page (page 1)
 - Title (the title should be as short as possible, but should contain adequate information to indicate the contents)
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2. Key words/Running head (not to exceed 50 letters including spaces) (page 2)
 - Key words (maximum of 8, in alphabetical order, suitable for indexing)
3. Abstract (brief and informative, not to exceed 250 words).
4. Main text
 - Introduction, Material and Methods, Results, Discussion
 - The relative importance of headings and subheadings should be clear.
5. The approximate location of figures and tables could be indicated in the margin or in the text.
 - The use of footnotes is to be avoided.
6. After the main text
 - Acknowledgements (also grants, support etc., if any) should follow the text and precede the references.
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Abstract in Slovenian will be for foreign authors made by the editors.

Review papers are welcome, main text has to be organised according to authors' suggestion.

The literature references should be arranged alphabetically, in the text referred to as: author and year of publication, e.g., Budagovskaya (1998), (Inoue et al., 1998). At the end of each literature citation at references should be, when possible a doi number in this way (for example <https://doi.org/10.3986/fag0040>)

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The papers received by editor latest on **July 30, 2026**, will be published in the autumn issue 2026 of FAGOPYRUM.

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Best regards,
Ivan Kreft

