Funded by European Union
Horizon 2020
Grant agreement No 771367

D 5.7

Final publishable report on WP5

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SECURITY (DISSEMINATION LEVEL)	Public
CONTRACTUAL DATE OF DELIVERY	29 February 2024
ACTUAL DATE OF DELIVERY	29 January 2024
DELIVERABLE NUMBER	D 5.7
TYPE	Deliverable
STATUS AND VERSION	Final
NUMBER OF PAGES	28
WP CONTRIBUTING TO THE DELIVERABLE	WP5
LEAD BENEFICIARY	CRI
AUTHOR(S)	Adela María Sánchez Moreiras, Yedra Vieites Álvarez, Meiliang Zhou, Kaixuan Zhang, Paul Bilsborrow, Petra Hlásná Čepková, Tomáš Čermák, Gabriela Mühlbachová, Maria Bernhart, Primož Titan, Aleš Kolmanič, Barbara Pipan, Lovro Sinkovič, Vladimir Meglič, Dagmar Janovská
KEYWORDS	Organic agriculture; Buckwheat genetic resources; Allelopathic weed control; Phosphorus mineralisation; GWAS; Breeding buckwheat varieties
ABSTRACT (FOR DISSEMINATION)	This comprehensive study, which is part of the ECOBREED project, focuses on promoting the use of buckwheat (*Fagopyrum esculentum* Moench and *F. tataricum* (L.) Gaertn.) in sustainable agriculture, with particular emphasis on organic agriculture in the EU. Recognising the vulnerabilities of conventional agriculture, the project promotes agrobiodiversity, which is essential for maintaining healthy agricultural ecosystems and resilience to climate change. Key objectives included identifying genetic variation for agronomic and nutritional traits, screening for allelopathic activity and increasing phosphorus mobilisation in the soil. The study included comprehensive phenotyping of buckwheat accessions, analysis of allelopathic activities and field trials on phosphorus mineralisation. Key findings include the identification of genotypes with high allelopathic activity against aggressive weeds, the ability of certain buckwheat varieties/accessions to effectively mobilise soil phosphorus, and the discovery of significant genetic diversity among the accessions. The study also emphasises the

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	potential of buckwheat in weed control and soil nutrient dynamics, contributing to sustainable agricultural practices. The results of the ECOBREED project not only enhance our understanding of the role of buckwheat in sustainable agriculture, but also set the stage for future breeding and improvement efforts aimed at realising the full potential of this crop in the context of global food security and environmental sustainability.
DOCUMENT ID	D 5.7_ Final publishable report on WP5

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Executive summary

The ECOBREED project, funded by the European Union, has put buckwheat in the spotlight and demonstrated its potential to enhance sustainable and organic agriculture. Known for its nutritional value and gluten-free nature, buckwheat is more than just a healthy food option. It is a versatile plant with unique properties that are beneficial for environmentally friendly agriculture.

A key property of buckwheat is its natural ability to control weeds through allelopathy, a process in which it produces chemicals that inhibit weed growth. This feature could significantly reduce reliance on synthetic herbicides in conventional agriculture, which are harmful to the environment and support weed management in low-input and organic agriculture. In addition, buckwheat enhances biodiversity in agricultural systems as it is a valuable resource for pollinators such as bees by providing abundant nectar.

Another notable characteristic of buckwheat is its role in improving soil health, particularly by making phosphorus in the soil more accessible to subsequent crops. Phosphorus is essential for plant growth, but often remains locked up in the soil and unavailable to plants. Buckwheat shows promise when it comes to releasing this essential nutrient and thus improving soil fertility and plant health.

As part of the ECOBREED project, different buckwheat varieties and accessions from genebanks were analysed and their growth, yield and nutrient content evaluated.

These findings are of great importance. Utilising the natural properties of buckwheat can lead to more sustainable and environmentally friendly farming practises that benefit not only the environment, but also the long-term viability and productivity of agricultural land.

Looking to the future, the results of the ECOBREED project lay the foundation for future efforts to improve and promote the use of buckwheat in agriculture. This includes breeding new varieties to maximise the positive characteristics of buckwheat and to better integrate buckwheat into European agricultural systems.

To summarise, the ECOBREED project highlights the potential of buckwheat as a versatile and sustainable crop that might be crucial for the future of agriculture. Its role is particularly important given the environmental challenges and the growing need for sustainable food production systems. Buckwheat is therefore a promising solution in the search for sustainable agricultural practices.

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What is the problem?

Conventional agriculture often relies on a limited number of species, which can lead to vulnerabilities such as disease outbreaks or climate-related impacts. In contrast, organic agriculture, which is based on the principles of sustainability, promotes the use of a variety of crops and breeds. This diversity can not only make the agricultural system more resilient, but also helps to conserve and utilise genetic resources that are crucial for future food security.

Biodiversity in agriculture (agrobiodiversity) is essential for maintaining healthy agricultural ecosystems. It supports a range of Ecosystem Services, including soil protection, water quality maintenance and pollination all of which are essential for sustainable agriculture. In the face of climate change, fostering biodiversity in agricultural systems contributes to greater environmental resilience.

The transition to greater agrobiodiversity is not without its challenges. It requires support at multiple levels, from policy change and research investment to consumer education and market development. However, the potential benefits i.e. a more resilient agricultural system, a healthier environment and greater food choice for consumers make this goal worth pursuing.

As the EU remains committed to organic agriculture, integrating agrobiodiversity into this paradigm is not just an option, but a necessity for a sustainable, resilient and vibrant agricultural future in the EU.

Choosing the right crops and varieties can help avoid the use of synthetic herbicides, which, despite their quick and effective weed control, contribute to environmental pollution and pose health risks. Allelopathy, a natural phenomenon where plants synthesise chemicals that impact surrounding plants, microorganisms, or animals, offers an eco-friendly alternative. These substances, known as allelochemicals, are released through various methods including volatilisation, leaching, root exudates, and plant residues. Present in all plant tissues, allelochemicals continue to affect the germination and growth of other plants even after the plant's life cycle. Once in the soil, microorganisms can modify these chemicals, altering their effects on other plants. While some allelochemicals inhibit the growth of nearby species, giving the allelopathic plant an advantage, others can benefit neighbouring plants.

Buckwheat (*Fagopyrum esculentum* Moench) originated in China and has been a staple food in the European diet for centuries. Its adaptability and nutritional value make it a favourite choice in various food cultures across the continent. From a nutritional point of view, buckwheat is a real treasure trove. Rich in high-quality protein, fibre and a range of vitamins and minerals, it offers significant health benefits. Its gluten-free nature makes it an excellent food for people with gluten intolerance or coeliac disease, further emphasising its value in food production. Apart from its agricultural and nutritional benefits, buckwheat has a special place in the traditional cuisines of Europe. In Italy,

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buckwheat flour is used for 'pizzoccheri', a savoury, rustic dish from Lombardy. The French enjoy 'galettes' - savoury crêpes made from buckwheat flour, especially in Brittany. In Eastern Europe, buckwheat is used for kasha, a staple food that reflects the versatility of this humble grain. However, many other European countries have even more traditional dishes. Buckwheat is a very suitable crop for expanding the range of crops grown in agriculture. It is an excellent source of pollen for pollinators, a potential crop with allelopathic effects and potential to increase the phosphorus availability in soils. Buckwheat has also the potential to improve food and nutrition security but faces cultivation challenges such as indeterminate growth, unstable yields, high flower abortion, palatability issues, short shelf-life and poorly characterised genetic variation available to potential breeders, researchers and other users. In addition, the impact of climate change on agriculture is increasing the need for resilient crop varieties.

The main objectives of the ECOBREED project focused on buckwheat were therefore to:

- Identify genetic variation in agronomic and nutritional traits that can be used in the development of new elite varieties suited to organic production.
- Identify genotypes with increased allelopathic activity.
- Screen selected common buckwheat genotypes for P-mineralisation capacity.
- Perform crosses between and within common buckwheat (*Fagopyrum esculentum* Moench.) genotypes and self-compatible species of the genus *Fagopyrum* to identify fertile progenies that can be used in organic buckwheat breeding programmes.

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What did we do?

Involved partners:

Phenotyping

In our comprehensive study, we carried out an extensive analysis of 215 buckwheat accessions and commercial varieties. These were carefully selected from genebanks and seed companies to represent a broad spectrum of genetic diversity.

They were grown under different environmental conditions in the Czech Republic, Slovenia, Austria and the United States, with additional comparative analyses conducted in China with their own accessions. Our approach was multi-faceted and involved a thorough examination of a variety of factors.

Fig 1 Buckwheat samples from the genebank ready to be sent to partners for phenotyping as part of the ECOBREED project.

To gain a comprehensive understanding of the characteristics of these buckwheat accessions, we carried out detailed phenotyping. This included the evaluation of morphological traits such as plant height, branching pattern and flowering behaviour, which are critical to understanding growth habits and yield potential. In addition to these

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physical traits, we also assessed the occurrence of abiotic or biotic stress factors, an essential aspect for ensuring stable yields under changing climatic conditions.

An important part of our study was the analysis of yield components. We carefully measured aspects such as seed size, weight and the rate of flower abortion, which have a direct impact on the overall productivity and economic profitability of buckwheat cultivation. We also placed great emphasis on analysing the nutrient composition of the seed. This included quantifying protein, rutin and phenolic content, as well as assessing antioxidant activity, which is an important factor given the growing interest in buckwheat as a health-promoting functional food.

Our methodology was robust and included state-of-the-art techniques. We used liquid chromatography-mass spectrometry (LC-MS) for a detailed metabolite profile and ultra-high performance liquid chromatography-electrospray ionisation tandem mass spectrometry (UHPLC-ESI-MS/MS) to analyse the phenolic compounds, with a particular focus on rutin due to its recognised health benefits. We also used the Kjeldahl method for protein analysis.

From this extensive dataset, we selected 96 genotypes that showed promising traits for further genetic diversity studies. This selection was based on a combination of factors such as yield potential, nutritional profile and resistance to environmental stresses. The aim was to identify candidates for future breeding programmes that could improve the sustainability and nutritional value of buckwheat cultivation.

Allelopathic activities

To this end, we investigated the allelopathic potential of 37 buckwheat accessions from two different species, common and Tartary buckwheat, against two very aggressive and herbicide-resistant weeds in Europe, common purslane (*Portulaca oleracea* L.) and annual ryegrass (*Lolium rigidum* Gaud). The study aimed to test how the different accessions can affect the individual weeds. After co-cultivation of the buckwheat accessions with the weeds, the germination rate, total weight, shoot and root length and plant height of the weeds were measured to obtain information on the ability of the weed species to colonise and occupy the space through germination and development. Seedling Vigour Index (SVI), which provides information on how the weed plants can develop in the next generation, and the specific plant length (SPL), which provides information on the plant's ability to respond to environmental stress, were calculated. Shoot and root length and weight of each buckwheat accession were also measured. In addition, allelochemicals from shoots, roots and root exudates were quantified to assess the accumulation or release of these compounds by the different buckwheat accessions in front of the weeds.

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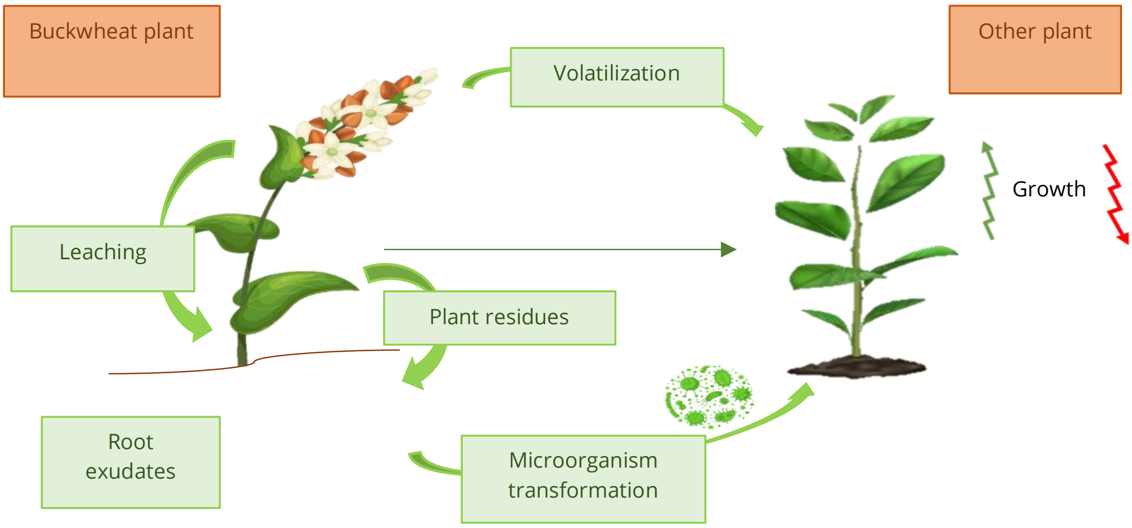

Fig 2 Diagram of the possible effects of plants on each other.

Eight promising buckwheat accessions were also tested under semi-natural conditions in the greenhouse. These plants were grown in pots with soil and irrigated in two ways: with manual irrigation to test how root exudates behave in the soil and can affect weeds, and with artificial rain to measure the allelopathic potential of leaf leachate. The weed and buckwheat parameters were measured and the soil solution was extracted to analyse the presence and abundance of allelochemicals.

Our methodology was comprehensive and utilised advanced analytical techniques. We conducted detailed measurements of plant length, seedling vigour indices and root and shoot growth to quantify the allelopathic impact. Additionally, sophisticated analytical tools like Liquid Chromatography-Mass Spectrometry (LC-MS) were employed to analyse the chemical profile of polyphenols in buckwheat root exudates. These analyses were critical for identifying specific compounds responsible for the allelopathic effects and understanding their mechanisms of action.

The integration of controlled environment experiments with detailed chemical and biological analyses allowed us to systematically evaluate the allelopathic potential of buckwheat varieties. This approach not only provided insights into the allelopathic properties of buckwheat but also set the stage for future research into optimising these properties for practical agricultural applications.

Phosphorus mineralisation

As part of our study, a comprehensive field trial was conducted in Slovenia and the Czech Republic, focussing on 11 buckwheat varieties, including 10 common and 1 Tartary buckwheat varieties. The main objective was to investigate the ability of buckwheat to mobilise soil phosphorus (P), a key nutrient for plant growth, under different environmental conditions.

To collect a broad spectrum of data, we carried out the experiments during both spring and summer sowing. This allowed us to assess the influence of seasonal variation on

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buckwheat performance and its impact on soil P availability for subsequent crops. The choice of sowing date was crucial as it directly affects the growth and nutrient uptake of the plant and provides information on the optimal conditions for each variety.

Throughout the trial period, we meticulously collected soil and plant samples at all sites. These samples were analysed for their phosphorus content to measure the effectiveness of buckwheat in mobilising this important nutrient. We also analysed the concentrations of other essential minerals such as magnesium (Mg), potassium (K) and calcium (Ca) to understand the overall impact of buckwheat cultivation on soil nutrient dynamics.

Fig 3 Flowering buckwheat stands for the evaluation of phosphorus uptake, Czech Republic, can you recognise differences in buckwheat varieties and species?

Our experimental design included a detailed assessment of agronomic traits such as plant height, flowering time and yield. In addition to these physical measurements, we also conducted analyses of grain quality, focusing on key nutrient components such as rutin and protein content. These analyses were crucial for understanding the relationship between buckwheat varieties and their potential impact on grain quality and nutritional value.

To account for external influences, we closely monitored environmental factors, particularly weather conditions, during the trial period. To draw meaningful conclusions from our investigations, it was important to understand how these factors interact with the growth and phosphorus mobilisation of buckwheat.

Using advanced methods, we measured the phosphorus content in the soil before and after buckwheat cultivation. Both standard and innovative techniques were used to ensure accurate and comprehensive data collection. In addition, we used a comprehensive set of descriptors when evaluating plant traits and yield, providing a holistic view of each variety's performance under different conditions.

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In summary, our approach was multidimensional, combining field trials, detailed plant and soil analyses and environmental monitoring. This methodology was developed to gain a comprehensive understanding of how different buckwheat varieties affect and are affected by soil phosphorus levels, providing valuable insights into sustainable agricultural practices.

In **Slovenia**, the phosphorus content of the soil varied between the varieties. **For spring field trials**, Kora and Čebelica had the highest phosphorus contents according to the Mehlich-3 method, while Zoe and KIS Doris had the lowest values. The NH_4 acetate method showed similar results for Zoe, with La Harpe having the highest phosphorus content. The highest-yielding variety for both parameters was Kora (0.59±0.28 t/ha seed and 1.33±0.63 t/ha straw). **For summer field trials**, the highest phosphorus content in the plants was achieved by the variety La Harpe, and the lowest by the variety Zita. The highest phosphorus uptake (combination of P content in plants and yield) was recorded in the variety La Harpe and the lowest in Tartary buckwheat variety KIS Doris. In **Czech Republic** the situation was different. For **spring field trials**, phosphorus uptake by buckwheat showed that one of the best varieties was Zamira, with higher phosphorus content in dry matter and higher yields, while phosphorus content in soils was low or medium, indicating increased availability for the variety Zamira. On the other hand, Hruszowska generally had one of the lowest phosphorus contents in its tissues, very low yields, and also relatively higher residual phosphorus content in the soils, indicating lower mobilisation of phosphorus from the soils.

Genotyping

In our comprehensive genotyping study, we used advanced genotyping by sequencing (GBS) and RNA sequencing (RNA-seq) to investigate the genetic diversity of buckwheat. We focussed on a broad range of European and Chinese buckwheat accessions (total of about 570 accessions). This selection was strategically made to cover a broad genetic spectrum and to capture the most important variations within the species.

Our main goal was to create a high-quality reference genome for common buckwheat (*Fagopyrum esculentum*). This involved sequencing and assembling the genome using state-of-the-art technology. This was a crucial step to enable more detailed and accurate genetic analysis. In parallel, we created a comprehensive database of genomic variations which helped identify genetic loci associated with important agronomic traits such as rutin content, flowering time and environmental adaptability.

The study involved a comprehensive phenotypic and molecular characterisation of common and Tartary buckwheat accessions from a collection of global accessions. We meticulously recorded and analysed a range of traits, from physical characteristics to biochemical profiles, and linked these to the genomic data obtained through GBS and RNA-seq. This approach enabled us to establish meaningful correlations between genetic make-up and observable traits.

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An important aspect of our research was the comparative analysis between Chinese and European germplasm. By analysing the genetic relationship and differences between these groups, we wanted to understand how mating systems and geographical origin have shaped the genetic diversity and structure of buckwheat. This analysis was crucial for the development of efficient breeding programmes, as it provided insights into how best to use the available genetic diversity to improve the crop.

Fig 4 For genetic analysis, plants were grown in pots and then DNA isolated.

Genome-wide association studies (GWAS) were conducted to complement our genetic studies. These studies were pivotal in identifying specific genes and genetic markers associated with key traits and responses to environmental conditions. By mapping these associations, we gained a deeper understanding of the genetic basis underlying the adaptability and nutritional traits of buckwheat.

Overall, our methodology was multi-faceted and thorough, combining state-of-the-art genomic technologies with detailed phenotypic analyses. This comprehensive approach was designed to uncover the rich genetic diversity of buckwheat and set the stage for future breeding and improvement efforts aimed at realising the full potential of this crop.

Breeding new varieties

Our approach to increasing buckwheat yields involved an innovative crossing programme that exploited the genetic diversity of different *Fagopyrum* species. We utilised the knowledge and genetic resources we had gathered in previous tasks from genebanks and embarked on a strategic breeding effort.

The first step in our breeding programme was the careful selection of parent plants. We selected different species and accessions of buckwheat, each with unique characteristics that could contribute to increased yield and adaptability. This selection was based on extensive genetic and phenotypic evaluations from previous phases of research in the ECOBREED project to ensure that we have a broad and potent gene pool for crosses.

To facilitate the generation of productive crosses, we have utilised emasculation techniques. This process, which is crucial for controlling pollination and ensuring the desired genetic combinations, involves manually removing the male flower parts from the

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plants before pollen maturation. This technique has been essential for creating specific crosses between different *Fagopyrum* species, especially for development of varieties suitable for organic agriculture.

An important aspect of our breeding strategy was to focus on heterosis, i.e. hybrid vigour. Heterosis has already been successfully used to increase yields in various cross-pollinated crops, and we wanted to try and replicate this success in buckwheat. By creating hybrid plants through strategic crossing, we sought to combine the best traits of different varieties to improve yield, disease resistance and adaptability to different environmental conditions.

Another focus was the development of synthetic varieties. These varieties are created by crossing several genetically different parents to produce seeds with a wide range of desirable traits. The concept behind synthetic varieties is to combine the benefits of genetic diversity and heterosis in a single plant to improve the overall performance of the crop.

The breeding process also involved converting self-incompatible genotypes into self-compatible ones. Self-compatibility is crucial for stable seed production, especially in organic farming systems. This conversion was achieved through wide crosses, especially between *Fagopyrum esculentum* and other buckwheat species.

Throughout the project, we meticulously documented and evaluated the results of these crosses. This included the evaluation of morpho-agronomic traits, grain yield and environmental adaptability of the resulting hybrids. The ultimate goal was to identify high-performing crosses that could be further developed into elite varieties suitable for organic agriculture. Our buckwheat breeders (RGA and SZG), who were also involved in phenotyping to select the best parent material of the buckwheat accessions, have made more than 90 crosses since 2019 between common buckwheat accessions. Progeny from the first year of crosses is now in the F_4 stage. In addition, crosses were made in RGA with other species of the genus *Fagopyrum* (70 crosses). Thus, after the 2023 harvest, we had F_3 plants from 16 crosses, F_2 plants from 13 crosses and F_1 plants from 41 inter-specific crosses forming a solid foundation for development of new buckwheat varieties suitable for organic farming.

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What results have we obtained?

Phenotyping

One of the most striking results was the significant variation in morphological and phenological characteristics observed under different environmental conditions. This variability was evident in important growth parameters such as plant height, number of branches, number of flowers and seed weight. Thus, some accessions showed remarkable adaptability to changing climatic conditions, maintaining stable growth and yield, while others showed greater sensitivity, especially in terms of flower development and seed formation. This suggests that certain genotypes of buckwheat may be better suited to certain environments or agricultural practices while others have greater stability across varied environmental conditions and soil conditions. From the set of evaluated genetic resources from the genebanks as well as commercial varieties, the following promising materials for breeding have been identified: **Czech Republic** - Billy, CD 7272, Čebelica, Dozhdik, Emka, Chishiminskaya, Kara Dag, La Harpe, Lehnická krajová, Pulawska II, Pyra, Rubra, Sweden-1, Tempest, Tokushima Zairai, and Zita, **Austria** - Bamby, Česká krajová, Emka, Hara Zairai, Chishiminskaya, Kora_CV, Krasnostreletskaya, Lada, Pyra, Rubra, Skorospelaya, Sweden-1, and Vychodoslovenská krajová, **Slovenia** - Sweden-1, Tempest, Dozhdik.

In terms of nutrient content, we found remarkable differences between the different locations and years. Protein content, for example, varied significantly, from 12.91% to 14.70% across environments and years, which is of great interest given the growing demand for plant protein sources.

An important aspect of our research focussed on the antioxidant activity of buckwheat, in particular the presence and concentration of rutin, a phenolic compound known for its health benefits. Our results show that rutin content varies greatly between different accessions and under different environmental conditions. This finding is particularly important for the functional food industry and for consumers interested in the health-promoting properties of buckwheat.

In addition, our study has revealed a potential trade-off between seed weight and other growth parameters, a finding that could have implications for breeding programmes to optimise yield and nutritional quality. For example, accessions with heavier seeds tended to have a lower number of branches and flowers, suggesting that breeding for higher seed weight may be at the expense of other desirable traits.

Overall, our results highlighted the great genetic diversity within buckwheat accessions and their adaptability to a range of environmental conditions. This diversity is a valuable resource for the development of new varieties that can meet the challenges of climate change, fulfil nutritional requirements and contribute to sustainable agriculture.

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Allelopathic activities

Our research revealed significant insights into the allelopathic properties of buckwheat. Of the 37 buckwheat accessions tested, the majority showed a growth inhibitory effect on at least one of the weed species, *Lolium rigidum* and *Portulaca oleracea*. This result is of crucial importance, as it emphasises the potential of buckwheat as a natural herbicide in agriculture.

It is noteworthy that the common buckwheat varieties generally showed a stronger inhibitory effect on *L. rigidum* compared to *P. oleracea*. Among them, certain genotypes such as Arihira zairai and Chernigovskaya 17 stood out for their significant reduction in *L. rigidum* growth parameters. This included a large decrease in weed height, root length and total biomass, indicating a strong allelopathic potential that could be utilised for weed control in agriculture.

Tartary buckwheat accessions showed a weaker overall reduction effect, but some accessions effectively suppressed both *L. rigidum* and *P. oleracea*. This suggests that different buckwheat species could be used selectively to control specific weed species, providing a targeted approach to weed control.

Chemical analysis of the buckwheat varieties revealed different polyphenolic profiles. There were significant differences in the accumulation of specific compounds such as dihydroxybenzoic acid and luteolin, particularly in response to the presence of weeds. This finding is of crucial importance as it provides a chemical basis for the observed allelopathic effects and opens possibilities for breeding buckwheat varieties with enhanced allelopathic properties.

The greenhouse studies added another layer of understanding. They demonstrated the effectiveness of certain buckwheat varieties in controlling weed growth under simulated environmental conditions, such as rainfall. This aspect is of particular importance for practical application in agriculture, as it demonstrates the potential effectiveness of buckwheat allelopathy in real agricultural scenarios.

Overall, the results of our study paint a comprehensive picture of the allelopathic capabilities of buckwheat. They not only confirm the presence of allelopathic effects in buckwheat but also provide a nuanced understanding of how these effects vary between different genotypes and under different environmental conditions. This knowledge is invaluable for the development of sustainable agricultural practices that utilise natural methods of weed control.

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Phosphorus mineralisation

The field trials conducted in Slovenia and the Czech Republic with 11 buckwheat varieties yielded important findings on the ability of these plants to mobilise soil phosphorus (P).

One of the most important results was the identification of marked differences in the ability to mobilise P between the buckwheat varieties tested. Varieties such as Kora and Čebelica, for example, showed high levels of phosphorus in their plant tissue, indicating efficient P uptake and mobilisation. These varieties performed particularly well in spring sowing, highlighting the importance of sowing time in maximizing nutrient mobilisation.

The variety Zita, on the other hand, performed best in the summer trials, suggesting that different varieties may be better suited to specific seasonal conditions. This finding is crucial for farmers and agricultural planners, as it guides selecting the right buckwheat variety for a specific sowing time to optimize P utilisation.

In addition, the study showed the significant influence of annual weather conditions on the performance of buckwheat varieties. For example, the variety Kora achieved optimal yields under climatic conditions in Slovenia in spring, while the variety Zamira was found to be more consistent across different phosphorus levels in the soil in the Czech Republic. These differences emphasise the need for region-specific variety selection and illustrate the adaptability of buckwheat to different environmental conditions.

The studies also showed that certain varieties such as Zamira and KIS Doris are particularly effective at mobilising and uptaking phosphorus, even in soils with low to medium P content. This ability makes them valuable candidates for cultivation in soils where the availability of phosphorus is a limiting factor.

Fig 5 Harvest of buckwheat field trials in Slovenia.

In addition, the trials shed light on the relationship between buckwheat cultivation and nutrient dynamics in the soil. The data suggest that buckwheat not only mobilises phosphorus for its growth but may also improve P availability for subsequent crops, which could have significant implications for crop rotation and soil health management.

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To summarise, our results provide a detailed picture of how different buckwheat varieties interact with phosphorus in the soil under different environmental conditions. These findings are of key importance for the development of sustainable agricultural practices that optimise phosphorus use, reduce dependence on external P inputs and enhance overall soil fertility.

Genotyping

The genotyping study of buckwheat accessions, which used advanced sequencing technologies, led to several important findings.

The assembly of the genome of *Fagopyrum esculentum* var. *homotropicum* was an important achievement of our research. This genome was characterised by a low level of heterozygosity and a high content of repetitive elements. The successful assembly of this genome represents a decisive step in genetic research on buckwheat and provides an important reference for future studies.

In our analysis of genetic diversity among buckwheat accessions, we uncovered considerable variation. The accessions were divided into major groups based on phylogenetic analyses. These groups were not only geographically coherent but also showed correlations with certain traits. For example, we observed that certain groups were characterised by different patterns in flower development, metabolic processes and stress response mechanisms. This genetic clustering provides a better understanding of the evolutionary and functional diversity within buckwheat accessions.

Our genome-wide association studies (GWAS) were particularly revealing. We identified several genes associated with key agronomic traits and environmental adaptations. These included loci related to flavonoid metabolism, which is directly linked to rutin content in buckwheat, a compound of great nutritional and medicinal interest. The GWAS results also shed light on genetic factors that influence maturity timing and flowering time, which are critical in breeding for specific climatic conditions and agricultural practices.

One of the most fascinating discoveries was the identification of genes related to fertility differences between buckwheat species. This discovery is crucial for breeding self-compatible lines, which can lead to more consistent yields and improved crop performance. Understanding these genetic factors opens the door to developing new buckwheat varieties with improved fertility and better adaptability to different agricultural environments.

In summary, our results from the genotyping study provide a comprehensive genetic map of buckwheat. They show the complex interplay of genetic factors that influence key buckwheat traits and highlight the genetic diversity and adaptability of the plant. These findings will be invaluable for future breeding efforts aimed at developing improved buckwheat varieties with enhanced nutritional profiles and agronomic traits.

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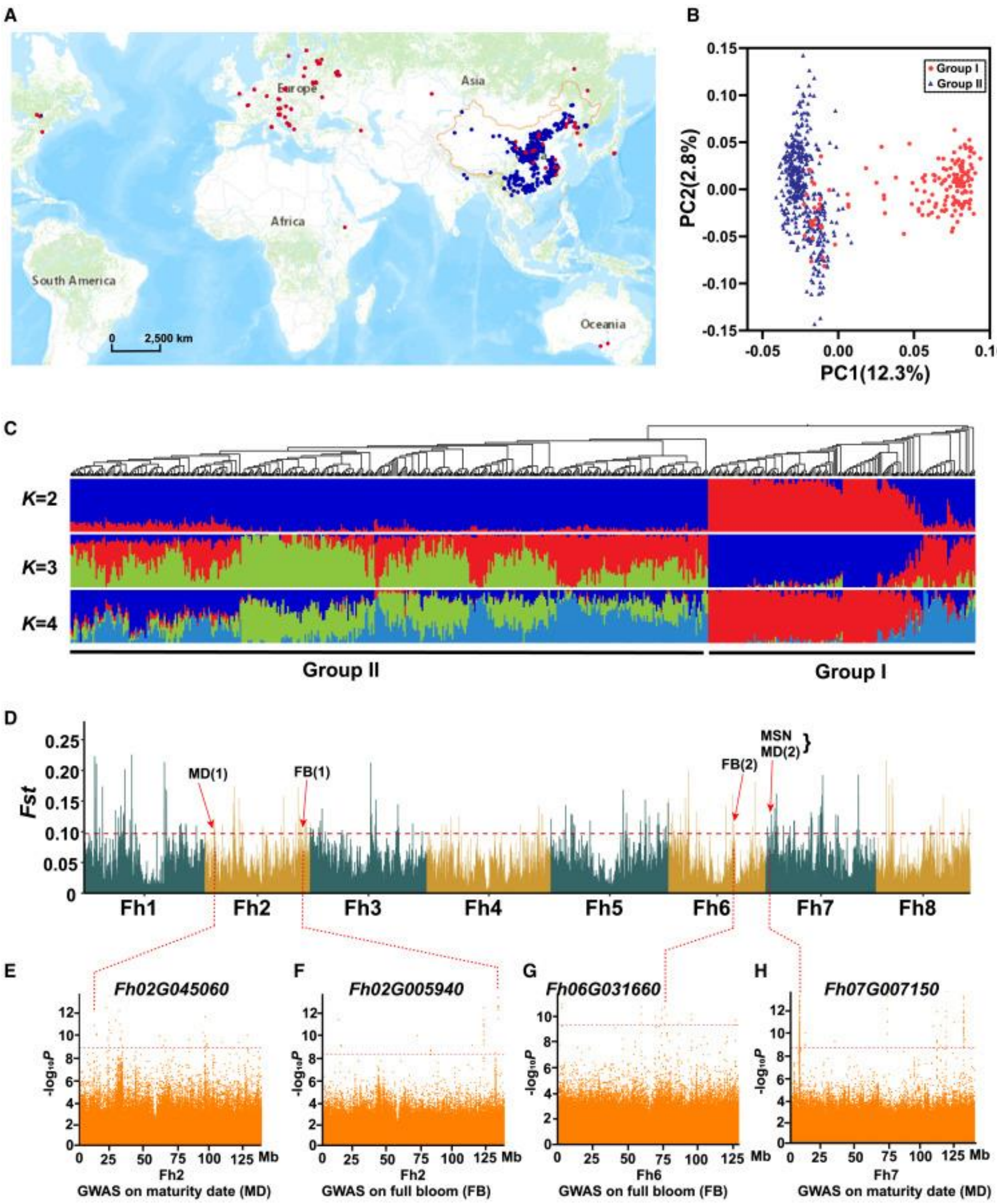

Fig 6 Population feature and divergence of common buckwheat.

(A) Geographic distribution of common buckwheat accessions used in the population genomic analysis. Accessions found in Groups I and II are shown in red and blue, respectively.

(B) Principal-component analysis of common buckwheat accessions, showing the first two components. Colours correspond to Group I and Group II.

(C) Population structure analysis with different numbers of clusters (K = 2, 3, and 4) with the phylogenetic tree superimposed on top.

(D) Highly divergent genomic regions between Group I and Group II. The horizontal dashed line indicates the top 1% of F_{ST}. Red vertical lines indicate high-divergence regions that overlapped GWAS signals.

(E–H) Local Manhattan plots of GWAS signals overlapping with high-divergence genomic regions for maturity date **(E and H)** and full bloom **(F and G)**.

For more information see: Zhang, K. et al. (2023). Comparative and population genomics of buckwheat species reveal key determinants of flavor and fertility. Molecular Plant, 16(9), 1427-1444. doi:10.1016/j.molp.2023.08.013

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Breeding new varieties

The extensive crossing programme we have carried out has led to many important findings and developments in buckwheat breeding.

In 2019, we successfully created 55 experimental crosses, which are the result of a careful selection of parent plants according to their different and complementary traits. These crosses were developed to capitalise on the potential of heterosis and improve the overall yield and adaptability of buckwheat.

Fig 7 Ripening fruits of plants from crosses between selected parents in SZG, Austria.

The following year, we sowed these crosses and began the first phase of data collection. This phase aimed to evaluate the extent of heterosis in these crosses by analysing various morpho-agronomic traits and most importantly, grain yield. The results were promising, as we observed significant improvements in these traits compared to the parent plants. This initial success indicated the potential of our crossing approach to effectively increase the yield of buckwheat.

We used the accessions with a high combining ability to produce Syn0 seeds in subsequent years. These seeds represented the first generation of our synthetic varieties, which combined the genetic diversity and desirable traits from multiple parental lines. The creation of these synthetic varieties was a crucial step towards the development of buckwheat varieties with improved yields and adaptability, particularly tailored for organic agriculture.

An important focus of our research was the transformation of self-incompatible buckwheat genotypes into self-compatible varieties. This conversion was crucial to ensure consistent and stable seed production, a key challenge in organic buckwheat production.

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We achieved this through various inter-specific crosses, especially between *Fagopyrum esculentum* and other species. The resulting hybrids showed promising traits, such as reduced shattering and restored fertility, which are essential for commercial cultivation.

Fig 8 One of our breeders Dr Primož Titan crossing buckwheat.

Among the crosses, those with *Fagopyrum homotropicum* were particularly noteworthy. These crosses exhibited high levels of heterosis for grain yield, a critical trait for commercial success. However, we also encountered challenges such as male sterility and complex seed production processes, which are common in inter-specific hybridisation. Despite these challenges, the crosses have produced promising material that offers potential for further development and refinement.

In summary, our crossing efforts have yielded significant results, demonstrating the feasibility and effectiveness of utilising heterosis and inter-specific crosses in buckwheat breeding. The experimental crosses and subsequent generations of synthetic varieties represent a significant advance in the development of high-yielding, environmentally adaptable buckwheat varieties for organic agriculture.

What knowledge does this provide?

Phenotyping

Firstly, our results emphasise the remarkable adaptability of buckwheat to different climatic and environmental conditions. This adaptability is crucial in the context of global climate change, as it suggests that buckwheat could play an important role in sustainable agriculture. Its ability to thrive in different environments makes it a promising crop for regions facing climatic challenges, contributing to global food security efforts.

Fig 9 Beautiful flowering buckwheat varieties Billy, Bamby and KIS Doris.

In addition, the genetic diversity observed in buckwheat, particularly in terms of morphological and nutritional traits, opens up new possibilities for plant breeding. The variation in protein, and especially in phenolic compounds such as rutin represents a valuable genetic resource for the development of new varieties. These varieties could be tailored to specific nutritional needs, making buckwheat an even more important crop in the context of food security and the growing demand for functional foods. Our study also emphasises the potential for the use of buckwheat in organic farming systems. The crop's resilience to various biotic and abiotic stress factors fits well with the principles of organic agriculture, which emphasises environmental sustainability and the reduction of chemical inputs. This alignment with organic farming practices is particularly important in the context of the European Union's priorities for biodiversity and sustainable agriculture.

In addition, the observed trade-offs in growth traits such as seed weight versus branch and flower development provide valuable insights for breeding strategies. These findings could lead to the development of buckwheat varieties that strike a balance between high yields and desirable nutritional properties, thus meeting both farmers' needs and consumers' preferences.

To summarise, our research makes an important contribution to the fields of plant science, agriculture and food technology. It highlights the untapped potential of buckwheat as a resilient, nutritious and versatile crop that is well-suited to meet the challenges of modern agriculture and changing dietary habits. This study not only contributes to a better understanding of buckwheat but also supports wider efforts for crop improvement and sustainable food production.

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Allelopathic activities

The results of our study on the allelopathic properties of buckwheat provide several important insights with significant implications for agriculture, environmental sustainability and crop management.

Firstly, the study emphasises the considerable potential of buckwheat as a natural herbicide. The varying degrees of inhibition of *Lolium rigidum* and *Portulaca oleracea* by different buckwheat accessions indicate the possibility of developing targeted, environmentally friendly strategies for weed control. This is especially important in the context of sustainable agriculture, where reducing reliance on chemical herbicides is a priority. By selecting and cultivating buckwheat varieties with specific allelopathic properties, farmers could potentially control weed populations more naturally and sustainably.

In addition, the discovery of the different polyphenol profiles in different buckwheat genotypes and their different effects on weed suppression provides valuable information for plant breeders. This knowledge could be used to breed new buckwheat varieties that optimise specific allelopathic traits. Such breeding efforts could lead to more effective and environmentally friendly solutions for weed control in agriculture, in line with the global trend towards more sustainable farming practices.

Our study also contributes to the understanding of allelopathy as a phenomenon. The observed variation in allelopathic effects under different environmental conditions, such as simulated rainfall, provides insight into how these interactions might occur in natural ecosystems. This knowledge is crucial for predicting and managing the ecological impact of introducing or growing certain buckwheat varieties in specific environments.

Furthermore, the results emphasise the importance of considering both plant interactions and the role of environmental factors in crop management and agricultural planning. The ability to harness and optimise these natural processes could lead to more resilient agricultural systems that are better adapted to changing environmental conditions.

To summarise, our research on the allelopathic properties of buckwheat not only improves our understanding of this crop but also contributes to the broader field of agricultural science. It opens new pathways for the development of sustainable, environmentally friendly farming practices that can utilise the natural interactions between plants, change weed control methods and contribute to more sustainable agricultural ecosystems.

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Phosphorus mineralisation

The results of our field trials in Slovenia and the Czech Republic, which focus on the ability of buckwheat to mobilise phosphorus, provide important insights with far-reaching implications for sustainable agriculture and environmental protection.

Firstly, the study enhances our understanding of genetic variation in buckwheat concerning phosphorus uptake and mobilisation. This knowledge is important for identifying buckwheat varieties that can be strategically used in crop rotations to improve phosphorus availability in the soil. Such an approach could lead to a reduction in the need for phosphorus fertilisers, which is not only a finite resource but also contributes to environmental problems such as eutrophication and soil degradation.

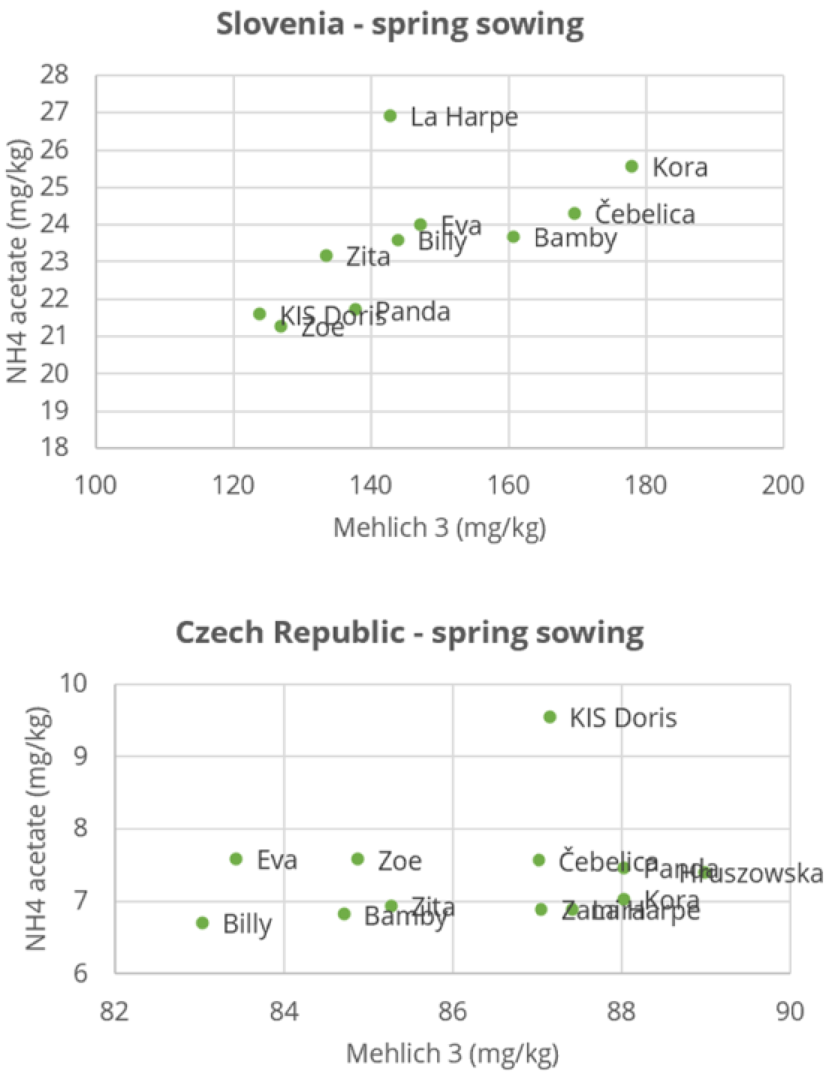

Fig 10 Comparison of buckwheat varieties evaluated in a spring field trial in the Czech Republic and Slovenia, multi-year averages.

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The identification of specific buckwheat varieties that mobilise particularly high levels of phosphorus under different environmental conditions and at different sowing times will provide farmers with valuable information. This knowledge enables more informed crop selection decisions, which ultimately leads to higher crop yields, better soil health and more efficient use of available resources. By selecting the appropriate buckwheat variety for specific conditions, farmers can maximise the environmental benefits of this crop, contributing to more sustainable and environmentally friendly agricultural practices.

In addition, the study's findings on how seasonal and annual weather variations affect buckwheat performance underline the importance of adaptive agriculture in the face of climate change. Understanding and utilising the natural variability of crops such as buckwheat can help to develop agricultural systems that are more resilient to climatic uncertainties and variability, thus ensuring food security and the sustainable use of natural resources.

Additionally, the research contributes to a broader scientific understanding of plant-soil interactions, particularly in the context of nutrient cycling. By shedding light on the mechanisms by which certain buckwheat varieties enhance phosphorus availability in the soil, the study opens new avenues for research in soil science and plant physiology. This could lead to the development of novel agricultural strategies that utilise natural processes for nutrient management, reduce dependence on chemical inputs and promote ecological balance.

In summary, our research provides important insights into the optimisation of phosphorus use in agriculture through the strategic selection and cultivation of buckwheat varieties. This knowledge not only supports sustainable farming practices but is also in line with global efforts towards environmental protection, resource efficiency and sustainable food production systems.

Genotyping

The results of our study on the genotyping of buckwheat accessions using advanced sequencing techniques have provided a wealth of knowledge with significant implications for agricultural science, plant breeding and food security.

First and foremost, the creation of a reference genome for *Fagopyrum esculentum* var. *homotropicum* is a ground-breaking achievement. This reference genome is an important tool for future genetic studies and breeding efforts. It enables a more precise identification of genetic traits and their association with phenotypic characteristics. This understanding is essential for targeted breeding programmes aimed at developing buckwheat varieties with specific desirable traits, such as increased rutin content for better nutritional value or improved stress tolerance for better adaptability to different environmental conditions.

The discovery of significant genetic diversity among common buckwheat accessions and the identification of specific genetic loci associated with important agronomic traits have

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several practical applications. For example, this knowledge can be used to breed buckwheat varieties that are better suited to specific climatic regions or cultivation methods, thus optimising yields and sustainability. Furthermore, understanding the genetic basis of traits such as flowering time and environmental adaptation can help to develop varieties that are more resilient to climate change and other environmental stressors.

Identifying genes related to fertility and mating systems in buckwheat opens new opportunities for breeding more productive and efficient varieties. By developing self-compatible buckwheat lines, it may be possible to achieve higher and more stable yields, which is crucial for ensuring food security. This is particularly important in regions where buckwheat is a key staple food and source of nutrients.

In addition, the knowledge gained from our GWAS on flavonoid metabolism and other biochemical pathways is invaluable to the functional food industry. By understanding the genetic factors that influence the concentration of compounds such as rutin, breeders can develop varieties that offer enhanced health benefits and meet the growing demand for functional foods that support health and well-being.

In summary, our buckwheat genotyping study not only improves our understanding of the genetic make-up of this important crop but also provides the basis for innovative breeding strategies. These strategies have the potential to produce buckwheat varieties with improved nutritional profiles, greater adaptability to the environment and higher yields. Ultimately, this research contributes to the broader goals of sustainable agriculture, improved food security and improved nutritional quality of crops.

Breeding new varieties

The results of our comprehensive breeding programme in buckwheat provide crucial insights with far-reaching implications for plant breeding, sustainable agriculture and food security.

Firstly, the successful production of hybrid varieties through our crossing programme shows that heterosis can help improve buckwheat yields. This finding is of key importance for buckwheat breeding as it shows that the principles of hybrid vigour can be effectively applied to buckwheat, a crop that has traditionally struggled with low yields and complex flowering systems. The observed improvements in morpho-agronomic traits and grain yield in the hybrids compared to their parental lines emphasise the potential of strategic crosses in enhancing crop performance.

The development of synthetic varieties that combine traits from multiple parental lines represents a significant advance in buckwheat breeding. These varieties combine the benefits of genetic diversity and result in plants that are not only higher yielding, but also more adaptable to different environmental conditions. This is particularly important in the context of climate change and the growing need for crops that can withstand different climatic conditions.

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Our research also makes a significant contribution to the field of organic and low-input agriculture. The resulting self-compatible hybrids allow for more stable and predictable seed production that is consistent with organic farming principles and reduces dependence on external inputs.

The inter-specific crosses, especially those with *Fagopyrum homotropicum*, have shown promising traits such as reduced shattering and restored fertility. These traits are critical to the economic viability of buckwheat and may lead to varieties that are not only more productive but also easier to harvest and process.

In addition, the study provides valuable insights into the genetic potential of buckwheat. Understanding the genetic basis of traits such as yield, shattering and fertility paves the way for future breeding efforts that focus on further optimising these characteristics. It also opens the opportunities for the development of buckwheat varieties with specific traits tailored to the needs of different regions and cropping systems.

In summary, our buckwheat crossing programme makes an important contribution to the promotion of sustainable agriculture. By developing high-yielding, environmentally adaptable and self-compatible buckwheat varieties, this research supports efforts to increase food security and promote sustainable farming practices worldwide.

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