Renewable Agriculture and Food Systems

cambridge.org/raf

Research Paper

Cite this article: Jiménez-Rosado M, Di Foggia M, Rosignoli S, Guerrero A, Rombolà AD, Romero A (2023). Effect of zinc and protein content in different barley cultivars: use of controlled release matrices. *Renewable Agriculture and Food Systems* **38**, e34, 1–10. https://doi.org/10.1017/S1742170523000297

Received: 4 September 2022
Revised: 23 May 2023
Accepted: 30 May 2023

Keywords:
barley; controlled release; matrices; proteins; zinc

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Effect of zinc and protein content in different barley cultivars: use of controlled release matrices

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Abstract

Barley is one of the most consumed cereals, with many different cultivars available worldwide. Like other crops, its yield has been affected by climate change and soil degradation. This work proposes controlled-release protein-based matrices with incorporated zinc to improve barley seed germination and zinc content in the plant. Thus, the main objective of this study was to investigate the use of controlled-release protein-based matrices for massive crops, such as barley. Different barley cultivars of barley were studied: Barke, Golden Promise, Morex, WB-200, WB379, and WB-446. The seeds of each cultivar were also analyzed in order to explain the behavior of plants observed during the growth. To this end, the physico-chemical (FT-IR, Raman spectroscopy, and Zn concentration) and microstructural (SEM) properties of the different seeds were firstly evaluated to establish differences between the studied cultivars. In addition, the use of controlled-release soybean protein-based matrices without zinc (M) or with zinc incorporated (MZ) was evaluated as fertilizers in the different barley cultivars. In this sense, the use of these matrices as a zinc carrier improved seed germination and zinc content in the plants, indicating that the use of matrices improves the amount of zinc assimilated by the crops (up to 30 and 50% with M and MZ, respectively) and allows the proper root growth of all cultivars of barley. In conclusion, this article shows the potential of controlled-release protein-based matrices as substitutes for conventional fertilization.

Introduction

Barley (*Hordeum vulgare*) is an annual monocotyledonous plant. It is a cereal of great importance for both humans and animals due to its energy and amino acid content (Briggs, 2012). It is the fourth most cultivated cereal globally, only surpassed by corn, wheat, and rice (Tadele, 2018). In this way, 145 million tons of barley were cultivated in 2021 (last data collected) (Food and Agriculture Organization of the United Nations, 2023). Barley is a strategy II species (Jolley et al., 1996; Shirley et al., 2011). It can secrete phytosiderophores, non-proteinogenic amino acids capable of complexing micronutrients such as zinc (Ueno et al., 2007), which improve its availability in deficient soils (Suzuki et al., 2006).

Among the uses of barley, human consumption stands out through a roasting and grinding process (*máchica*) or in bread (Jaeger et al., 2021). However, its main use is the production of beer, whiskey, and gin from its malt (Düzgün, 2021). Besides its nutritional properties, barley also has antispasmodic, astringent, digestive, and fever-reducing properties, which is why it is often used in certain medications (Ó'Nualláin, 2019).

Climate change, together with the growing demand for crops, has harmed the yield of crops such as barley (a reduction of 5% in yield is observed in barley production in the last 5 years [Food and Agriculture Organization of the United Nations, 2023]) due to abiotic stress and soil degradation (Khalid, 2020). Abiotic stress refers to environmental factors that alter the physiological and metabolic processes of plants, such as droughts or extreme temperatures. On the other hand, soil degradation is known as the decrease in the capacity of the soil to provide the necessary services for humans and the ecosystem (Zhang et al., 2022). This occurs as a consequence of the depletion of its natural resources, which cannot be regenerated due to soil overexploitation (Bindraban et al., 2012). All this has led to damages on crops (reduction in yield), less nutritious crops or acceleration in the spread of pests and diseases, which affects humans (Haq et al., 2022).

Zinc deficiency is crop plants' most pervasive nutritional problem (Alloway, 2009). Zinc is a micronutrient. However, its use is crucial for the proper functioning of the organism (Frassinetti et al., 2006) since it stabilizes many proteins present in crops and humans (Zhao and Bai, 2012). Cell membranes must have zinc to allow for plant growth (Hart et al., 2002); otherwise, these membranes lose their stability, leaking various carbon-rich compounds (e.g., sugars and amino acids) from the roots into the soil. These compounds may attract some pathogens, thereby increasing the susceptibility of the crop plant to pathogen attack (Moreira et al., 2018). On the other hand, plants that develop in conditions of zinc deficiency are not able to use all the light energy absorbed during the photosynthesis process. This is because the enzymatic activity of superoxide dismutase, whose function is to prevent oxidative stress, is highly dependent on zinc (Wang and Jin, 2007). Thus, the energy that the plant cannot use generates free oxygen radicals that damage chlorophyll and lipids, causing chlorosis and necrosis (Cakmak, 2000; Huang et al., 2000). In addition, zinc participates in the biosynthesis of indoleacetic acid since it is essential for the synthesis of tryptophan, which is the precursor of this phytohormone (Cakmak et al., 1999). Thus, it is involved in the growth and elongation of the cell (Liu et al., 2011). Therefore, zinc deficiency causes a reduction in the elongation of shoots, the growth of internodes, and the formation of leaves (Hafeez et al., 2013).

Zinc is also important in plant fertility. It acts in the processes of fruit formation and growth and seed production, and it favors the formation, viability, and fertility of pollen (Laware and Raskar, 2014). Zinc deficiency could reduce the yield by up to 20% (Castellanos-Ramos and Santiago-Rodríguez, 2014). The zinc concentration in barley and other cereals is essential for human nutrition (Cakmak, 2008). Lastly, zinc enables seed germination and root growth, which allows for the development of the crop, as it is the part that assimilates most of the nutrients (Marschner et al., 1986; Brown et al., 1993).

Usually, zinc deficiency is mitigated in crops through conventional or foliar fertilization, using zinc sulfate or EDTA-chelated zinc as fertilizers (Cakmak and Kutman, 2018). However, this fertilization is ineffective, causing major pollution problems in the subsoil and groundwater (Rengel et al., 1995). This effectivity improves with the use of superabsorbent polymer matrices that can retain nutrients and supply them in a controlled way (Projar, 2020). Nevertheless, using these polymers in the long run can contaminate the soil with microplastics or acrylamide during their degradation, which can worsen the soil quality and discard its crops for human consumption (Chen et al., 2022).

A recent alternative that has been tested to enhance plant growth is the use of protein-based biostimulants (Jolayemi et al., 2022). Among them, Jiménez-Rosado et al. improved zinc fertilization using soy-controlled-release protein-based matrices (Jiménez-Rosado et al., 2021). These matrices allow having the biostimulating effect of proteins, as well as provide an extra amount of zinc in a controlled way that plants can use during their growth. In addition, these matrices solve the drawbacks during their degradability by being completely degradable in natural elements present in the soil (mainly nitrogen, but also carbon and water), being more sustainable (Jiménez-Rosado et al., 2022).

However, few studies evaluate the use of these protein-based matrices in crops. For this reason, the novelty of this work is the evaluation of the use of these matrices in a crop as important as barley. Furthermore, the relationship between the physico-chemical and morphological properties of seeds and their germination and root growth was also evaluated.

Among the most interesting parameters to determine the prosperity of crops, the root system is of great importance (Fageria, 2012). Thus, long roots facilitate the absorption of nitrogen and other nutrients (Marschner et al., 1986). In addition, a large number of separate roots is also important to capture the nutrients present in a larger area of the soil (Jackson et al., 1997). Finally, these roots also allow for the absorption of water, which is essential for crop development (Ahuja et al., 2000). Thus, many authors have evaluated the roots of different crops under different growth conditions (Marschner et al., 1986; Jackson et al., 1996; Williams, 2001; Hodge, 2004; Wasson et al., 2012). However, no one has evaluated the effect produced by controlled-release protein-based matrices on root growth.

The main objective of this work was to address the use of controlled-release protein-based matrices for crops such as barley. This study evaluated how these matrices affect different barley cultivars' root growth and zinc content of different cultivars of barley. Furthermore, proteins have a biostimulant effect on plants (Colla et al., 2014, 2017). Thus, it is important to evaluate not only the effect of the controlled release of zinc from the matrix but also the effect of the matrix on the plant. Therefore, the matrix without incorporated zinc (M) was also evaluated. The seeds of each cultivar were also analyzed in order to correlate their properties with the behavior observed during the root growth. To this end, the physico-chemical (FT-IR, Raman spectroscopy, Zn concentration) and microscopic (SEM) properties of the seeds of different cultivars of barley (Barley, Golden Promise, Morex, WB-200, WB-379, and WB-446) were evaluated. Finally, the root growth and plant physico-chemical properties (FT-IR profile and zinc content) were analyzed after undergoing the different treatments (reference, i.e., without treatment; matrix without zinc [M]; and matrix with zinc [MZ]).

Materials and methods

Materials

Six different cultivars of barley (*H. vulgare*) were used in this work (Talamè et al., 2008; Rosignoli and Salvi, 2020): Barke (a German-bred two-row spring brewing barley), Golden Promise (a classic British spring barley), Morex (a six-row malting cultivar from America), WB-200 (Genebank identifier HOR7531, an advanced/improved spring cultivar from Poland), WB-379 (Genebank identifier HOR11123, a traditional spring landrace from Italy), and WB-446 (Genebank identifier BCC1576, a traditional spring landrace from Spain). These cultivars were chosen for their physico-chemical differences (explained in the results section). In this way, it was possible to evaluate the efficacy of the matrices in a wide range of possibilities.

Controlled-release soybean protein-based matrices with and without zinc (MZ and M, respectively) were used to provide zinc to the plants in a controlled manner. They were processed following the protocol optimized in a previous work (Jiménez-Rosado et al., 2021). To this end, soybean, glycerol, and zinc sulfate monohydrate (45:45:10) were homogenized in a rotating mixer (Polylab QC, ThermoHaake, Germany) at 50 rpm for 10 min, and they were subsequently injected in a MiniJet Piston Injection Molding System II (ThermoHaake, Germany; parameters used: 40 and 90°C in the cylinder and mold, 600 bar of injection pressure for 20 s, and 300 bar of holding pressure for 300 s) to obtain the bioplastic matrices. This system was immersed in 300 mL of ethanol for 24 h to remove the glycerol. The controlled-release protein-based matrices were obtained after a freeze-drying process (LyoQuest, Telstar, Spain), with a controlled-release time and biodegradability of 40 days. In addition, the protein-based matrices biodegrade providing mainly nitrogen, carbon, and water, all of which plants can take up.

The substrate used during the tests was provided by Vigor Plants (Italy). This substrate is obtained from the mixture of blonde and brown peat and bark humus. Its load of humid and fulvic acids and the richness of organic substances improve the structure of orchard soil. Its chemical composition is min. 30 wt.% organic carbon, min. 7 wt.% humid and fulvic acids, min. 80 wt.% organic nitrogen (C/N ratio 50% max.). Its pH is neutral (6.5–7.0) and it has 45–65% of humidity. In addition, its porosity is 87% and its apparent density 180 kg m^{-3}.

Finally, all the reagents used during the characterization (i.e., H_2O_2 and HNO_3) were purchased from Sigma Aldrich (Germany) in analytical grade.

Growth conditions

The seeds were sterilized in 1.2% of sodium hypochlorite for 5 min and rinsed with distilled water. Then, they were incubated at 20°C to promote germination (Hoy et al., 1981). Only the germinated seeds were used for experiments. They were sown in rhizoboxes (30 × 60 cm, width × high), as shown in Figure 1, to facilitate root growth analysis. The growth was carried out following a previous work (Kirschner et al., 2021), keeping the roots in the dark at an angle of 30°. Two germinated seeds were planted (6 cm from each wall and 1 cm deep) in each rhizobox. Three different experiments were performed for each barley cultivar to evaluate the effect of incorporating the matrix in the crops: reference (without matrix), matrix without zinc (M), and matrix with zinc (MZ). The matrices were buried next to the seeds (one matrix between the wall and the seed with the upper end at a depth of 1 cm). In total, four replicates per treatment and barley cultivar were carried out, allocating them in a randomized block design. All samples were placed in a greenhouse for 20 days at a temperature of 23 ± 5°C, 50–60% relative humidity, and a photoperiod of 10 h of day. The substrate was kept at 100% of water holding capacity. After the test, the plants with their roots were carefully extracted, removing the substrate with gentle strokes, and individually stored in plastic bags at 4°C until their characterization.

Characterization techniques

Fourier-transform infrared spectroscopy (FT-IR)

The physico-chemical structure of the endosperm and hull of the different seeds was evaluated through FT-IR. For this, the seeds were cut longitudinally into two pieces with a scalpel to expose the endosperm. An Alpha compact FT-IR spectrometer (Bruker, USA) was used with an ATR detector and a diamond crystal to evaluate both parts (endosperm and hull). The spectra were obtained between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1}; each spectrum was the average of 64 scans. The spectra of each plant's leaves, stems, and roots were also obtained with the same procedure described for the seeds. Leaves and roots were superficially analyzed, while the stems were measured inside, cutting a section of them with a scalpel.

Raman spectroscopy

The chemical bonds of the endosperm were also evaluated by Raman spectroscopy for more detailed information on its physico-chemical properties using a Jasco NRS-2000C spectroscope (Japan) with a 160 K frozen digital CCD detector. The Raman spectra were obtained between 3200 and 100 cm^{-1} with 4 cm^{-1} resolution and 15 mW of power. Each spectrum was the average of 16 scans. Due to the high fluorescence background, it was not possible to register any Raman spectrum of the hull.

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES)

The matrices release zinc that the plant can assimilate. Thus, it is important to evaluate the amount of zinc that the seeds present, as well as the content in the plants. Zinc determination in seeds and plants was carried out by ICP-AES spectroscopy in the different seeds. Before these measurements, the whole plant was dried by freeze-drying (−80°C and <0.01 mbar) and ground. Then, 0.2 g of the sample were digested in a microwave using 10 mL of acids (1:7 H_2O_2:HNO_3). The resulting liquid was made up to 25 mL adding distilled water. These samples were pulverized in argon and subjected to 6000 K in a plasma torch of an ICP SpectroBlue TI device (Spectro, Germany), where the zinc content was obtained due to its characteristic wavelength (213.86 nm).

Scanning electron microscopy (SEM)

The microstructure of the endosperm was also evaluated, as it plays a role in seed germination. For this, the seeds were cut and lyophilized in order to remove their water content without altering their microstructure. Then, they were coated with a thin film of palladium/gold (>10 nm) to improve their electrical conductivity and, thus, the micrographs' quality. This step was carried out in a Leica ACE600 unit (Germany) mounted on aluminum stubs with carbon glue. Then, the endosperm microstructure of the different barley cultivars was observed through SEM

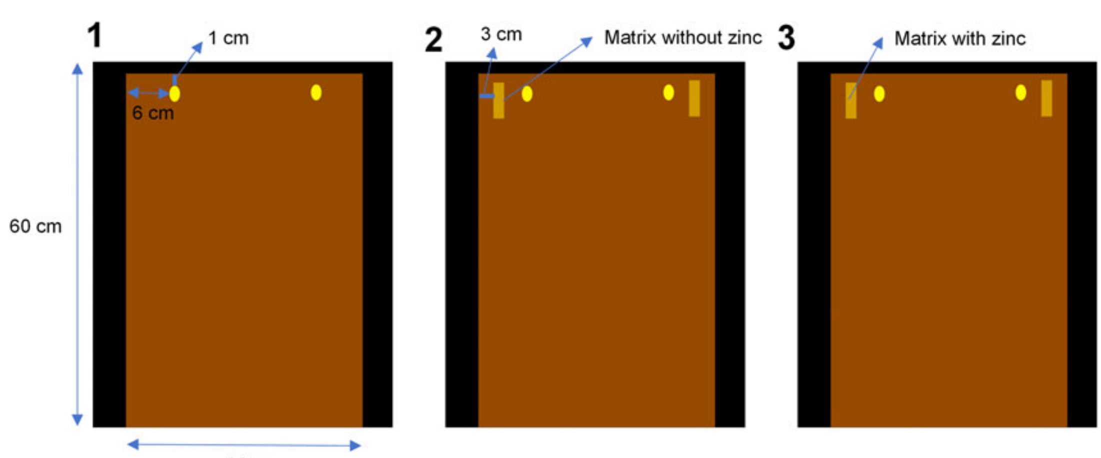

Figure 1. Rhizobox scheme. (1) Reference; (2) matrix without zinc; (3) matrix with zinc.

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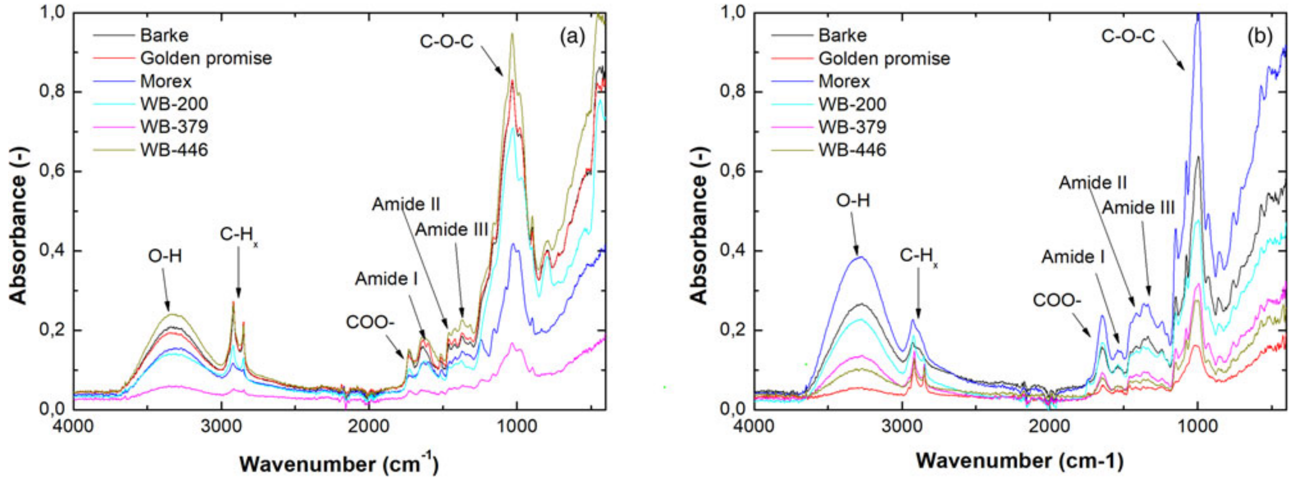

Figure 2. FT-IR spectra of seeds. (A) Hull; (B) endosperm.

using an EVO microscope (Zeiss, Germany), at an acceleration voltage of 10 kV and a magnification of 300×. The images were analyzed in the Image-J program (free software). This technique was also used to determine the hull thickness.

Root growth analysis

This analysis was performed to evaluate the effect of the matrices on the plant roots. To this end, the protocol proposed by Rosignoli and Salvi (2020) was followed. The roots of each replicate were scanned (P2600 A3 scan, Plustek OpticSlim, USA) in the rhizoboxes every 5 days to evaluate their growth. Finally, images were analyzed in the Image-J program to obtain the number of roots, root size, and angle.

Statistical analysis

At least four replicates of each measurement were carried out to determine the possible deviation of the results. Thus, the standard deviation was calculated using Excel (Microsoft software). The results were presented as mean values and standard deviation. In addition, different letters were used to show significant differences ($P < 0.05$ in a Tukey test).

Results and discussion

Seeds

Figure 2 shows the spectra of the hulls and endosperms of the different seeds. As can be seen, both parts have a similar profile. In this way, the vibration band of the O-H group (3320 cm^{-1}) is observed, which is due to the residual water present in the seeds (Shih et al., 2012). The polysaccharides (cellulose, hemicellulose, and lignin) present a band between 1260 and 870 cm^{-1} due to the vibration mode of the C-O-C groups (Yin et al., 2010). Another band was found between 2970 and 2840 cm^{-1}, corresponding to the C-H_x vibrations of lipid content in the seeds (Siano et al., 2018). These lipids could also be observed in the band at 1780 cm^{-1}, corresponding to ester compounds (Ogbu and Ajiwe, 2016). In addition, the spectra show protein structures in the samples, which can be observed in the bands between 1520 and 1280 cm^{-1}, corresponding to amide groups (Yu et al., 2008). All these peaks have also been identified in previous works, being the typical profile of barley seeds (Gürsoy, 2019).

Comparing both parts, it can be seen that the hull (Fig. 2a) generally has more cellulose, hemicellulose, and lignin than the endosperm, except for Morex and WB-379 (Fig. 2b). This is

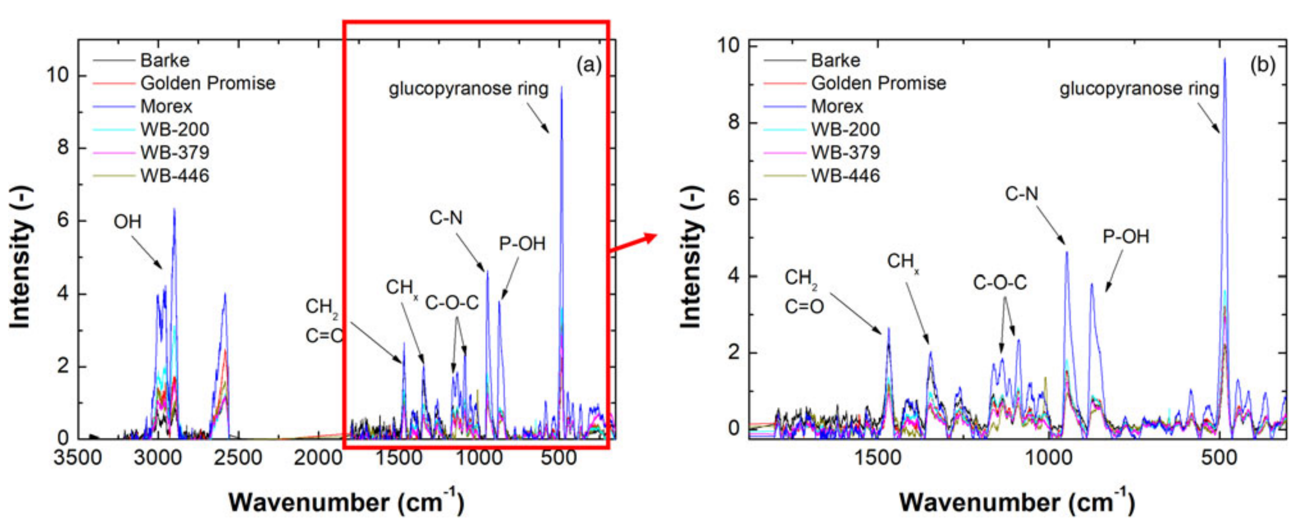

Figure 3. Raman spectra of endosperm of the different barley cultivars. (A) Entire spectra; (B) magnification.

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Table 1. Zinc content (ppm) in barley seeds and plants treated with matrices

		Plants		
Cultivars	Seeds	R	M	MZ
Barke	48^{a}	223^{A}	270^{C}	335^{E}
Golden Promise	33^{b}	–	–	280^{F}
Morex	47^{a}	–	272^{C}	331^{E}
WB-200	54^{c}	–	–	362^{G}
WB-379	39^{d}	194^{B}	235^{D}	291^{H}
WB-446	40^{d}	196^{B}	–	294^{H}

R, reference (without matrix); M, matrix without zinc; MZ, matrix with zinc. Different letters mean significant differences between the systems ($P < 0.05$). Different symbols were used in different non-comparative parameters.

Table 2. Data obtained from SEM images

Cultivars	Granules (nm)	Hull thickness (nm)
Barke	15.7^{a}	25.4^{A}
Golden Promise	8.7^{b}	25.2^{A}
Morex	12.4^{a}	15.4^{B}
WB-200	17.2^{c}	20.8^{C}
WB-379	13.3^{a}	7.2^{D}
WB-446	13.9^{a}	26.1^{E}

Different letters mean significant differences between the systems ($P < 0.05$). Different symbols were used in different non-comparative parameters.

explained by the protective function of the hull, which must be strong enough to ensure that the seed is not damaged by insects, bacteria, or weather conditions. On the other hand, the endosperm is used as a source of nutrients for the embryo during germination, thus, it has more proteins and lipids (Yan et al., 2014).

Regarding the different barley cultivars, WB-379 has the least amount of polysaccharides in its hull and, therefore, it is the most unprotected. Morex also has a low amount of polysaccharides in the hull compared to the other cultivars. However, it has the highest nutritional source in the endosperm. The least nutritious endosperms are Golden Promise, WB-446, and WB-379, thus they have less energy to thrive on their own to form plants.

Figure 3 shows the Raman spectra of the different seeds' endosperm. Barley endosperm has starch granules embedded in a protein matrix and cell walls composed of arabinoxylan and glucans. It also contains cellulose, phenolic acids, and heteromannans (Fincher and Stone, 1986). All of these compounds are manifested in Raman spectra. Thus, polysaccharides are observed in the C-O-C stretch (1160–1080 cm^{-1}). More specifically, starch has a band at 485 cm^{-1}, corresponding to the glucopyranose unit. Proteins are represented by the characteristic peak of the peptide bond (C-N) at 947 cm^{-1}. Finally, the phosphorous components are manifested in the vibration of the OH groups (3020–2880 cm^{-1}) and the narrowing of P-OH (875 cm^{-1}). Galvis et al. (2015) obtained similar profiles for native and malted barley seeds (Galvis et al., 2015).

All barley cultivars have a similar Raman profile. However, small differences are observed, similar to those discussed in the FT-IR profiles. In this way, polysaccharides and proteins are present in the endosperm of each seed. Lipids are also present in both profiles (Raman and FTIR). Morex shows the best nutritional reserve (proteins and lipids), with the endosperm of Golden Promise, WB-446, and WB-379 being the least nutritious.

The zinc concentration of the different seeds can be observed in Table 1. As can be seen, Golden Promise is the one with the worst zinc concentration, followed by WB-379 and WB-446. It is interesting to emphasize that the seeds that presented a lower protein profile in FT-IR were those that showed a lower amount of zinc. This observation suggests that zinc is involved in protein chains (Genc et al., 2002).

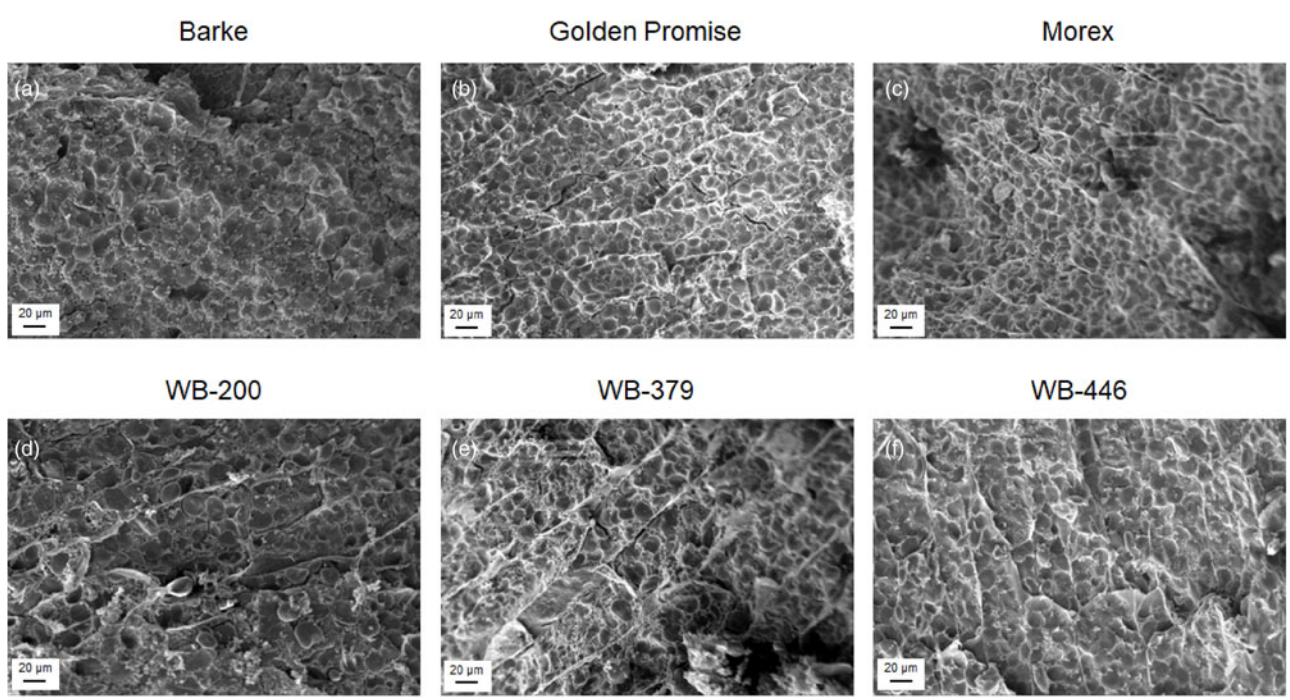

Figure 4. SEM images of the endosperm of the different barley seeds. (A) Barke; (B) Golden Promise; (C) Morex; (D) WB-200; (E) WB-379; (F) WB-446.

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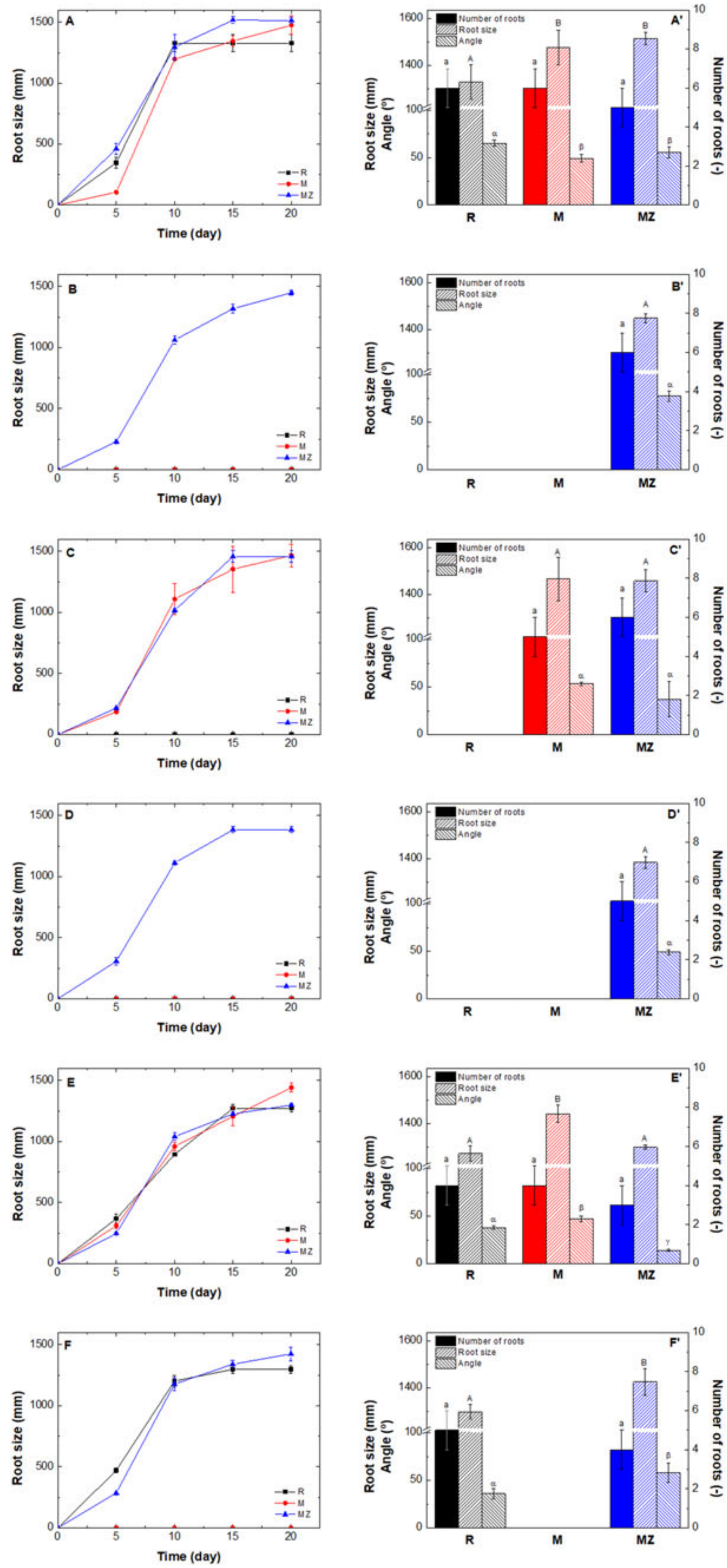

Figure 5. Data obtained from root analysis of the different plants. (A, A') Barke; (B, B') Golden Promise; (C, C') Morex; (D, D') WB-200; (E, E') WB-379; (F, F') WB-446. Different letters in bars mean significant differences between the systems ($P < 0.05$). Different symbols were used in different non-comparative parameters.

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Figure 4 shows the SEM images of the endosperm of the different seeds. All of them present a similar microstructure made up of granules. This microstructure was also obtained in previous works (Wijngaard et al., 2007; Nair et al., 2011). The granules observed have different sizes (Table 2), with those of Golden Promise being the smallest and those of WB-200 being the highest. Generally, the more protein and zinc present in the endosperm, the larger the size of the granules. This follows a linear trend for the zinc content (granule size = 0.332·[Zn] − 0.9108).

Generally, the more protein and zinc in the endosperm, the larger the size of the granules. This generally follows a linear trend with the zinc concentration presented in the seeds (granule size = 0.332·[Zn] − 0.9108; R^2 = 0.957).

Regarding the hull thickness, the higher the polysaccharide content observed in FT-IR spectra, the higher the hull thickness. Thus, WB-379 is the one that presents the lowest hull thickness, and, in contrast, WB-446 is the one with the greatest hull thickness.

Plants

Figure 5 shows the data obtained from the root analysis of the different plants. Firstly, it is observed that the physico-chemical and microstructural properties of the seeds influence the prosperity of the final plants. In this way, comparing the reference systems, in which no treatment was carried out, it can be observed that some seeds did not manage to form a plant. Golden Promise, Morex, and WB-200 did not prosper, due to any of these factors: (i) the thick hull that surrounds them, which does not allow the hatching of the seed; (ii) the poor nutritional source that they present in the endosperm; or a combination of both. Thus, Golden Promise has one of the thickest hulls and a low presence of protein and zinc in the endosperm. WB-200 has a thick shell and a moderate presence of protein in the endosperm, not enough to thrive on its own. However, Morex has a narrow hull and a good reserve of protein and zinc. Therefore, in this case, its non-growth does not have a physico-chemical origin, and may be due to the irregularity observed in the granules observed by SEM, which are also small compared to the others.

Regarding the different treatments, the zinc-free matrices (M) improved the quality of the protein reservoir, possibly due to the biostimulation generated by the amino acids (Radu et al., 2010; Souri and Bakhtiarizade, 2019), allowing the plants of the Morex cultivar to develop. In addition, the presence of these matrices increases the amount of nitrogen (a biodegradation

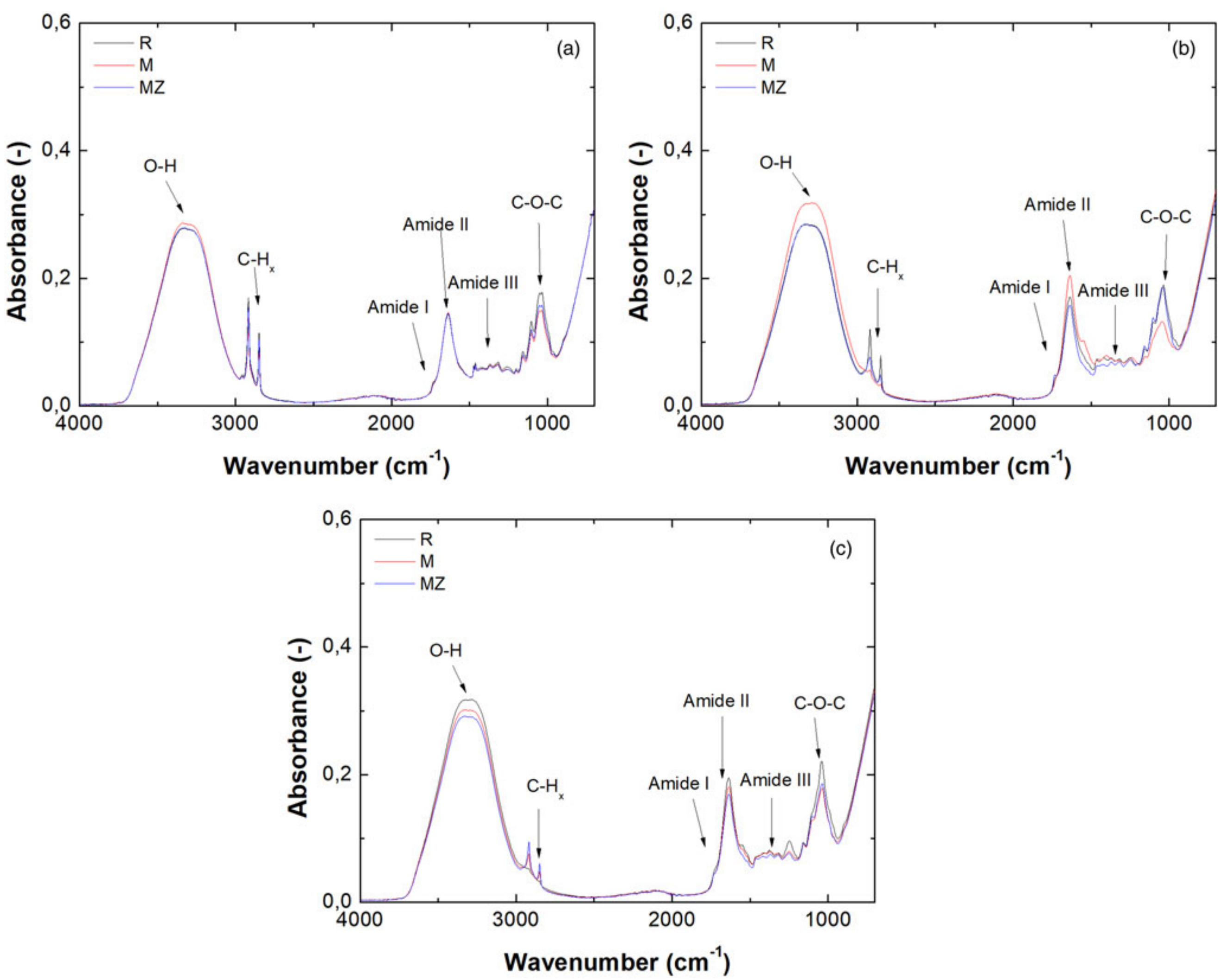

Figure 6. FT-IR spectra of leaves (A), stems (B), and roots (C) of Barke plants. R, reference (without matrix); M, matrix without zinc; MZ, matrix with zinc.

element of the matrices) present in the medium, improving the plants' prosperity and growth (Mattsson et al., 1991). On the other hand, matrices with zinc (MZ) enable the performance of all cultivars of barley, regardless of their physico-chemical properties and morphology. This indicates that the zinc present in the matrix penetrates the seed to cause its hatching regardless of its thickness. In addition, it improves the biostimulation generated by the protein. However, the morphology of the roots is not altered by the inclusion of the matrices. No significant differences were observed in the size, number, or angle of the roots (Fig. 5a'–F').

The FT-IR spectra of a representative plant are shown in Figure 6. All the plants have the same profile regardless of the treatment. This is consistent with the results obtained in the root analysis. Thus, it can be concluded that the inclusion of the matrices to improve seed germination does not alter their structure.

Finally, the zinc concentration of the plants is shown in Table 1. The incorporation of matrices, even without zinc, improves zinc absorption from the medium. In this way, the plants treated with M had a better zinc absorption from the medium. This behavior could be due to two effects: (i) the amino acids present in the matrix are able to complex zinc, increasing its availability to be assimilated by plants; or (ii) the amino acids could stimulate the synthesis of phytosiderophores in order to improve the assimilation of zinc (Nakib et al., 2021; Northover et al., 2021). However, the MZ treatment is the most prosperous, obtaining the plants with the highest zinc capacity (50% more), possibly due to the highest amount of zinc present in this case.

In addition, the zinc assimilated by the plant through the matrix with zinc presents a linear correlation with the zinc contained in the seed ($\text{Zn-plant} = 4.858 \cdot \text{Zn-seed} + 102$, $R^2 = 0.998$). In this way, the more zinc the seed has, the better it is assimilated by the plant, which is reflected in its final analysis.

Conclusions

To sum up, it was possible to identify the physico-chemical and microstructural differences between different cultivars of barley seeds and relate them to the performance of the crop. In addition, a treatment was found through soybean protein-based matrices with zinc incorporated that improves seed germination, obtaining plants with a higher zinc content during their growth due to the biostimulation of the plants and the great availability of zinc present in the medium. However, after this initial work, it has become clear that future studies are needed to evaluate some specific issues, such as the inclusion of this type of matrices in large-scale systems and their implications for human health, as well as the extrapolation with different nutrients (Mn, Fe, Cu, etc.) and protein-based matrices (potato, wheat, and crambe, etc.) that could generate similar effects. In addition, it would be worthwhile to compare the starting seeds and the seeds produced after crop plant growth.

Acknowledgements. Grant PID2021-124294OB-C21 funded by MCIN/AEI/10.13039/501100011033 and by 'ERDF A way of making Europe'. The authors gratefully acknowledge the financial support of MCI/AEI/FEDER, EU with the research project PID2021-124294OB-C21. In addition, the authors thank MEFP for the predoctoral grant of Mercedes Jiménez-Rosado (FPU17/01718) and her research stay (EST21/00238). Furthermore, the authors acknowledge Professor Silvio Salvi for providing us with the different cultivars of barley. Finally, the authors acknowledge the assistance in microscopy and microanalysis services of CITIUS (Universidad de Sevilla).

Competing interests. None.

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