Application of micro-PIXE (particle induced X-ray emission) to study buckwheat grain structure and composition

Paula PONGRAC1,*, Mitja KELEMEN1, Primož VAVPETIČ1, Katarina VOGEL-MIKUŠ1,2, Marjana REGVAR2, Primož PELICON1

1 Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
2 Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
* Corresponding author:
Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
Tel: +38651222963.
E-mail addresses: paula.pongrac@ijs.si; mitja.kelemen@ijs.si; primoz.vavpetic@ijs.si; katarina.vogelmikus@bf.uni-lj.si; marjana.regvar@bf.uni-lj.si; primoz.pelicon@ijs.si

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ABSTRACT

Tartary buckwheat (Fagopyrum tataricum Gaertn.) is a gluten-free pseudo-cereal crop with a grain nutrient profile that makes it an excellent alternative foodstuff. The distribution of calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S) was investigated by micro-PIXE (particle induced X-ray emission) to resolve allocation and concentration of the elements in nine distinct grain tissues. Magnesium, P and S were preferentially allocated to the cotyledons and the embryonic axis (both inner and outer tissues), and Ca was predominant in the pericarp where two Ca-rich layers were observed. Allocation of P and S to aleurone suggests that this layer of cells, although not as prominent as in cereal grain, is rich in phytate and proteins. Quantitative information on spatial distribution of mineral elements in the edible grain may be useful in the technological processing of the grain and particularly in reducing the amount of mineral-element loss during milling.
INTRODUCTION

Over four decades ago, an interdisciplinary research team comprising scientists from the Jožef Stefan Institute and the Biotechnical Faculty, University of Ljubljana began a fruitful collaboration with the aim to study elemental composition of edible crop tissues, in particular the presence of S in protein-rich seeds (Budnar et al., 1980; Kump et al., 1977, 1976; Rupnik et al., 1977). Among other goals, they planned to employ a recently developed technique, the particle induced X-ray emission (PIXE), which enables a quantitative analysis of biologically relevant elements in plant tissue. The technique was at its infancy and two main obstacles prevented significant progress: i) the size of the analytical beam (in millimetre range) exceeded the size of the regions of interest several-fold and ii) the energy range of the beam was inappropriate, leading to irreparable radiation damage to biological samples. Eventually, the beamline was equipped with magnetic lenses to focus the ion beam into the micrometre scanning resolution (micro-PIXE), which enables a quantitative analysis of biologically relevant elements in plant tissue. The technique was at its infancy and two main obstacles prevented significant progress: i) the size of the analytical beam (in millimetre range) exceeded the size of the regions of interest several-fold and ii) the energy range of the beam was inappropriate, leading to irreparable radiation damage to biological samples. Eventually, the beamline was equipped with magnetic lenses to focus the ion beam into the micrometre scanning resolution (micro-PIXE), following the elegant original demonstration on wheat (Triticum aestivum L.) grain (Mazzolini et al., 1985). Along with that the beam energy profile was optimized. First high-quality element distribution maps were acquired almost thirty years after the discouraging initial attempts. The case study was common buckwheat (Fagopyrum esculentum Moench) grain (Vogel-Mikuš et al., 2009), followed by the analysis with improved lateral resolution (Pongrac et al., 2011). The detailed element distributions in Tartary buckwheat grain corroborated observations in common buckwheat grain in which the largest concentrations of Mg, P, S, potassium (K), iron (Fe) and zinc (Zn) were found in cotyledons and that of Ca in pericarp (Pongrac et al., 2013a). By contrast, 7-day-old cotyledons of Tartary buckwheat sprout relocated Ca to inter-vascular mesophyll, Mg to mesophyll and S to epidermis (Pongrac et al., 2016a). The described progress was accompanied using complementary techniques such as scanning electron microscopy and fluorescence microscopy (Francisco and Kreft, 1989; Javornik and Kreft, 1980; Kreft and Kreft, 2000) and synchrotron radiation micro X-ray fluorescence mapping (Pongrac et al., 2013a; 2016b; 2016c; 2017). This short review emphasises tissue-specific allocation of Ca, Mg, P and S in Tartary buckwheat grain and specifically focuses on elemental composition of aleurone and embryonic axis.

MATERIALS AND METHODS

Tartary buckwheat grain was provided by a local grower (cultivar ‘Zlata’, Mlin Rangus, Dolenje Vrhpolje at Šentjernej, Slovenia) in 2018. The grain was soaked for 4 h in Milli-Q water at 4°C, hand-cut into 2-mm-thick cross-sections (perpendicular to the embryonic axis) with a sharp stainless-steel platinum-coated razor blade, frozen in liquid nitrogen and freeze dried for 2 days at −24°C and 0.120 mbar. The hand-cut dried sections were mounted between two layers of Fiolofrom foil stretched over aluminium frames (Vogel-Mikuš et al., 2009, 2014). The spatial distribution of the mineral elements was determined using micro-PIXE set-up of the Jožef Stefan Institute, Slovenia, as described previously (Lyubenko et al., 2012; Pongrac et al., 2013b). The quantitative mineral element distribution maps were generated using the GEOPIXE II software package (Ryan, 2000) and tissue-specific concentrations were extracted from the numerical matrices obtained with the GEOPIXEII software, using the ImageJ programme (Abramoff et al., 2004).

RESULTS AND DISCUSSION

Quantitative distribution maps of Ca, Mg, P and S in Tartary buckwheat grain are shown in Fig. 1. Allocation of Ca to pericarp (Fig. 1A), where two distinct Ca-rich layers (one in inner pericarp and another in outer pericarp) were observed in agreement with previous observations in common buckwheat (Pongrac et al., 2011; Vogel-Mikuš et al., 2009) and other crops: different cereal grain (Antonini et al., 2018; Pongrac et al., 2013c; Ren et al., 2007; Singh et al., 2014) and legume seed (Cominelli et al., 2020). A poor mobility of Ca in phloem (White and Broadley, 2003) and consequently the limited translocation of Ca from maternal (pericarp) to filial (embryo and endosperm) grain tissues may explain this observation. On average 3,000 mg Ca kg⁻¹ was found in pericarp, which was 20 times more than in endosperm and 4 times more than in cotyledons (Fig. 2).

Calcium is the only element to exhibit such distinct allocation to pericarp (Fig. 1). Magnesium is allocated to cotyledons and embryonic axis, with some Mg found also in the outer layer of pericarp (Fig. 1B). In pericarp the average Mg concentration was 4 times smaller than in cotyledons (8,600 mg kg⁻¹ dry weight); and in endosperm 4 times smaller (Fig. 2). Phosphorus is clearly allocated to cotyledons and the embryonic axis (Fig. 1C). The largest P concentration is found in the outer layer of embryonic axis.

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Phosphorus distribution can be used as an approximation of the location of phytate, a salt of phytic acid which strongly binds (even immobilizes) some essential mineral elements (mainly the divalent cations), such as Mg, manganese, (Mn), Fe and Zn in grain and seed (Hallberg et al., 1987; Pongrac et al., 2013a; Regvar et al., 2011). The co-localisation of Mg and P in Fig. 1 illustrates the fact. During germination these mineral elements are being enzymatically released to become available for the growth of the seedling. Endosperm and pericarp contain around 30-times less P per unit mass than the embryo (Fig. 2). Sulphur, on the other hand, can be used as an indicator of proteins (Budnar et al., 1980; Kump et al., 1976), being present in two common amino acids, methionine and cysteine. In wheat grain, S was mainly located in sub-aleurone layer reflecting a significant presence of proteins in these cells (Pongrac et al., 2013c; Singh et al., 2014; Tosi et al., 2009), whereas in Tatary buckwheat grain, S is allocated mainly, to cotyledons and the embryonic axis (Fig. 1D; (Pongrac et al., 2013a)). However, there is a thin layer enriched in Mg, S and P just under the pericarp, surrounding the endosperm. This is aleurone, which is in contrast to cereal grain, a layer of small cells (approximately 10-15 µm in thickness) in buckwheat grain (Javornik and Kreft, 1980). Because aleurone of buckwheat grain is so inconspicuous (often strongly attached to the cotyledons) it is seldom mentioned, although it has been previously noticed in common buckwheat (Vogel-Mikuš et al., 2009). In buckwheat grain the aleurone is known to contain large concentration of pro-
teins, as also supported by the S distribution maps shown here.

For optimum evaluation of nutritional quality of buckwheat grain the elemental distribution maps should be complemented with distributions of secondary metabolites as accessible with MeV secondary ion mass spectrometry, currently being developed at the nuclear microprobe at the Jožef Stefan Institute. Because buckwheat grain contains large concentrations of rutin and quercetin (Fabjan et al., 2003), antioxidants exhibiting positive impact on human health, understanding their allocation will have important consequences in planning milling fractions and further grain processing.

CONCLUSIONS

Results demonstrate that in Tartary buckwheat grain Mg, P and S, are preferentially allocated to the cotyledons and the embryonic axis (both inner and outer tissues), while Ca presence is predominant in the pericarp, where two Ca-rich layers can be observed. Phosphorus and S distributions can be used as indicators for phytate and protein distribution, respectively. Understanding the quantitative distribution of mineral elements is essential for the technological processing of the grain, with an impact on the amount of mineral-element loss during milling.

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IZVLEČEK

Namen raziskave je bil proučiti razporeditev esencialnih elementov kalcija (Ca), magneziija (Mg), fosforja (P) in žvepeła (S) v tkivih znara tatarske ajde s tehniko mikro-PIXE (z delci inducirana emisija rentgenskih žarkov), ki omogoča kvantitativno analizo elementov z ločljivostjo enega mikrometra. Kemijska priprava znara pri tehniki mikro-PIXE ni potrebna. Največje koncentracije Ca smo izmerili v luski, v kateri sta bili jasno vidni dve s Ca bogati plasti. Magnezija, P in S je bilo največ v kličnih listih in v tkivih embrionalne osi. Ker lahko razporeditev P in S uporabimo kot oceno razporeditve fitatov in beljakovin, sklepamo, da je s P in S bogat tanek sloj, ki obdaja celotno zrno in je jasno viden predvsem na delu, kjer meji na endosperm, v bistvu sloj alevronskih celic, v katerih so prisotni fitati in zlasti beljakovine. Kvantitativne informacije o prostorski porazdelitvi mineralnih elementov v znaru so koristne pri razvoju tehnološke predelave znara in pri zagotavljanju zmanjšanja izgube mineralnih elementov med mletjem.