MAGNESIUM AND PHOSPHORUS DISTRIBUTIONS IN DEVELOPING TARTARY BUCKWHEAT COTYLEDONS

RAZPOREDITEV MAGNEZIJA IN FOSFORJA V RAZVIJAJOČIH SE KLIČNIH LISTIH TATARSKE AJDE

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ABSTRACT

Magnesium and phosphorus distributions in developing tartary buckwheat cotyledons

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is a gluten-free pseudocereal with good mineral element composition of grain. In particular, Tartary buckwheat grains have greater magnesium (Mg) concentration than cereal grains. In (pseudo)cereal grain, Mg is bound to phytic acid, a phosphorus (P)-rich compound responsible for poor availability of Mg in the diet. The aim of the study was to evaluate changes in cell-type specific distribution of Mg and P in cotyledons of Tartary buckwheat grain and in 5-day-old and 8-day-old sprouts. Low-energy-X-ray-fluorescence mapping revealed considerable co-localisation of Mg and P in globoid-like structures in cotyledons. In the grain these globoid-like structures were numerous and appeared in mesophyll cells of the cotyledons. In 5-day-old sprouts less Mg-P co-localisation was seen in mesophyll cells. In 8-day-old sprouts co-localisation of Mg and P was less confined to globoid-like structures in the mesophyll cells indicating the degradation of these globoids and release of Mg, presumably for different metabolic processes that occur during seedling development. The extent to which Mg release from phytic acid in sprouts correlates with the bioavailability of Mg during digestion remains to be investigated.

Keywords: *Fagopyrum tataricum*, X-ray fluorescence micro-spectroscopy, sprouts, phytic acid, germination

IZVLEČEK

Razporeditev magnezija in fosforja v razvijajočih se kličnih listih tatarske ajde

Tatarska ajda (*Fagopyrum tataricum* (L.) Gaertn.) je nepravo žito, ki ne vsebuje glutena in ima za prehrano ugodno mineralno sestavo. Zrnje tatarske ajde vsebuje na primer več magnezija (Mg) kot zrna pravih žit. V zrnju pravih, kot nepravih žit, je Mg močno vezan na fitinsko kislino, organsko spojino, ki je zgrajena iz velikega števila atomov fosforja (P). Omenjena vezava je odgovorna za slabo topnost Mg in slabo biodostopnost Mg v prehrani. Namen študije je bil ovrednotiti spremembe v razporeditvi Mg in P v razvijajočih se kličnih listih tatarske ajde. Preučevali smo klične liste v zrnu, v pet in osem dni starih kalicah tatarske ajde. S pomočjo nizko-energijske rentgensko fluorescenčne mikrospektroskopije smo na celičnem nivoju v kličnih listih zrn potrdili močno kolokalizacijo Mg in P v globoidnih strukturah. V kličnih listih pet dni starih kalic se je stopnja kolokalizacije zmanjšala in globoidne strukture so bile manj številne. Stopnja kolokalizacije v kličnih listih osem dni starih kalic se je še dodatno zmanjšala, kar je nakazovalo na razgradnjo kompleksov fitinske kisline in Mg, verjetno kot posledica metabolnih potreb kalice po Mg. Potrebne so nadaljnje raziskave, s katerimi bi ovrednotili povezavo med postopnim sproščanjem Mg s fitinske kisline v kalicah in biodostopnostjo Mg v prebavilih.

Ključne besede: Fagopyrum tataricum, rentgensko fluorescenčna mikro-spektroskopija, kalice, fitinska kislina, kalitev

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1 INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is a good source for secondary metabolites and mineral elements (Bonafaccia et al. 2003a, b), particularly in people with coeliac disease, as it is a gluten-free pseudocereal. Tartary buckwheat grain and sprouts contain greater concentration of magnesium (Mg) than wheat (*Triticum aestivum* L.) grain and sprouts (Pongrac et al. 2016a), which is strongly linked to the phylogenetic origin of the two species. Tartary buckwheat belongs to the family *Polygonaceae* within the order Caryophyllales, while wheat belongs to the *Poaceae* within the Poales. Species from the Caryophyllales (and Oxalidales) generally have greater concentrations of Mg in shoots compared to other orders within Angiosperms, including Poales (White et al. 2015). Since Mg is highly phloem mobile (Hawkesford et al. 2012), greater shoot Mg concentration will result in greater Mg concentration of phloem-fed tissues, such as grains. Considering that Mg is one of the mineral elements often lacking in human diets (White & Broadley 2009), identification of crops and plant species that are likely to have greater shoot Mg concentration could augment human and animal diets (White et al. 2015, Nielsen 2015), even though the final Mg concentrations in shoots will ultimately depend on available soil Mg concentrations.

Grain is a major component of daily diets and, as such, a major source of essential mineral elements. In grain mineral elements are tightly bound to phosphorus (P)-rich phytic acid (*myo*-inositol hexakisphosphate) forming phytates. This enables appropriate storage environment for mineral elements in dormant grains. However, when grains are consumed mineral elements bound to phytic acids are poorly bioavailable to monogastric animals (Bohn 2008). Magnesium and potassium (K) are typical counter-ions in phytate salts (Raboy 1997). Using techniques for visualisation of mineral element distribution in plant material, co-localisation of P, Mg and K in (pseudo)cereal grain has been demonstrated (Pongrac et al. 2013a, Wu & Becker 2012, Regvar et al. 2011). In addition, absorption of Mg from white wheat bread was significantly impaired by the addition of phytic acid, in a dose-dependent manner, at amounts similar to those naturally present in whole-meal and brown bread (Bohn et al. 2004). These observations support strong interaction of Mg and P in (pseudo)cereal grain and in the diets.

During grain soaking and germination, phytates are enzymatically broken down by the enzyme phytase leading to the release of mineral elements required by the growing embryo and the developing seedling. Consequently, soaked grains and seedlings (sprouts) are believed to be nutritionally advantageous (Holtz and Gibson 2007, Nelson et al. 2013). In addition, sprouting was shown to result in increased concentrations of mineral elements (Pongrac et al. 2016a, Lintschinger et al. 1997) although the extent of the increase depended on plant species and the mineral-element composition of the water used for sprout cultivation (Lintschinger et al. 1997, Liu et al. 2007, Pongrac et al. 2016a). The observed increase in mineral element concentration is arguably a result of the conversion of the dry matter and the uptake of mineral elements by developing roots. Dry matter of the grain (mostly non-fibrous storage carbohydrates) is converted to energy for growth of the embryo and seedling. With activation of metabolic processes requirements of mineral element change and this can be observed as a change in the distribution of mineral elements during sprouting, especially for sulphur (S), K, calcium (Ca) and Fe (Pongrac et al. 2016a, b). No redistribution of P and Mg was seen in hydrothermally processed grains (groats) of Tartary buckwheat, but a slight redistribution was seen in 8-day-old sprouts when compared to the grain (Pongrac et al. 2016b). Thus, it appears that hydrothermal processing does not affect phytate-mineral-element complexes in cotyledons significantly, but germination does.

The aim of the study was to resolve the distribution of Mg and P in developing cotyledons of Tartary buckwheat. We hypothesised that it is possible to demonstrate degradation of P-Mg complexes in developing Tartary buckwheat cotyledons by visualising mineral element distributions. Since analysis of Mg distribution using X-ray fluorescence (XRF)-based techniques is limited by low fluorescence yield of low-Z elements (e.g. sodium, Mg, aluminium, silicon and P; Vogel-Mikuš et al. 2012) the use of specialised facilities providing spatially resolved information for low-Z elements was required. For this purpose, the low-energy XRF microscopy beamline (TwinMic) of the synchrotron Elettra, Trieste, Italy, which enables simultaneous determination of Mg and P distribution in plant material (Kaulich et al. 2009) was utilised.

2 MATERIALS AND METHODS

Grain of Tartary buckwheat was obtained from Mlin Rangus (Dolenje Vrhpolje at Šentjernej, Slovenia). Mature, air-dried grain was kept in paper bags in the dark at room temperature. Sprouts were grown in an automatic sprouter (EasyGreen® MicroFarm System, EasyGreen Factory Inc., Nevada, USA) where they were watered by misting every 3 h during the day (five times), and twice during the night (with a 4-h and 5-h gap). Five-day-old and 8-day-old sprouts were removed from the sprouter. These two stages were selected, as on the fifth day sprouts were in a shedding stage in which cotyledons are not yet fully developed and are still enclosed in the husk. Prior to the analysis, husks were manually removed from the cotyledons. On the eight day, cotyledons were fully unfolded and sprouts at this stage could be directly consumed (the husk has fallen off the cotyledon). A schematic illustration of the material investigated is provided in *Fig. 1*.

For bulk mineral element analyses, sprouts were washed in bidistilled water, roots were removed and cotyledons and hypocotyls were dried at 60 °C for three days. Mature whole grains and dried (root-less) sprouts were homogenised in liquid nitrogen, using a pestle and a mortar, and kept at -20 °C in air-tight containers. Homogenised material was wet digested in a microwave oven (Ethos 1, Milestone, Sorisole, Italy) and concentrations of P and Mg were measured with inductively coupled plasma-mass spectrometry and inductively coupled plasma-optical emission spectroscopy as described previously (Pongrac et al. 2013b).

For X-ray fluorescence (XRF) microscopy, mature grain, pre-soaked for 4 hours at 4 °C, and pieces of cotyledons were frozen in propane cooled with liquid nitrogen and 25 μm thick sections were cut using a cryotome at -25 °C. Sections were freeze-dried (Alpha Christ 2-4, Osterode am Harz, Germany) at -30 °C and 0.210 mbar for three days and mounted between two layers of pioloform (Vogel-Mikuš et al. 2009, 2014) stretched onto aluminium holders. Cross-sections were scanned in vacuum at the 2200 eV on TwinMic beamline, synchrotron Elettra, Trieste, Italy. The beam was focused to 1.2 μm^2 using a scanning X-ray microscope zone plate. X-rays were used to image structural make-up of Tartary buckwheat cotyledons and X-ray fluorescence was used to determine spatial distributions of Mg and P. X-ray fluorescence spectra were fitted with PyMCA (Solé et al. 2007) and quantified using a software for quantitative micro-XRF analysis developed by P. Kump (Jožef Stefan Institute, Ljubljana, Slovenia). Sample thickness was calculated in each pixel on the basis of absorption measurements as measured using a CCD system positioned behind the sample (Kaulich et al. 2009). Cellulose was taken as a matrix in the quantification procedure (Koren et al. 2013). Quantitative maps and two coloured co-localisation images were generated using PyMCA software (Solé et al. 2007). Concentrations of Mg and P in cotyledons were extracted from the quantitative distribution maps using ImageJ software (Abràmoff et al. 2004; Vogel-Mikuš et al. 2014). Statistically significant differences in concentrations of Mg and P between developmental stages were determined by Student-Newman-Keuls post-hoc tests (at $p<0.05$) after analysis of variance using GenStat software (64-bit Release 17.1; VSN International Ltd, Oxford, UK).

3 RESULTS AND DISCUSSION

Distributions of Mg and P were studied in developing cotyledons of Tartary buckwheat. Three stages in the development of Tartary buckwheat cotyledons were selected (*Fig. 1*). The first stage was mature grain, the second was shedding stage and the third was ready-to-eat stage. Bulk concentrations of Mg and P in dry matter increased during cotyledon development (*Fig. 2*) in line with previous results (Pongrac et al. 2016a). This increase is due to the conversion of dry matter (storage compounds) to energy used in growth and seedling development, and of root uptake of mineral elements from the solution used for irrigation.

Tartary buckwheat grain contained greater average bulk concentration of Mg (2110 mg kg^{-1} dry weight) than reported for 125 Tartary buckwheat accessions from China (1523 mg kg^{-1} dry weight) but was within the minimum – maximum range, namely 729 - 3104 mg kg^{-1} (Huang et al. 2014). This indicates relative stability of Mg concentrations in Tartary buckwheat grain, regardless of the environmental conditions.

Phosphorus concentration in cotyledon was greater than Mg concentrations at all stages of development; however the P to Mg (P/Mg) concentration ratio decreased during the cotyledon development (*Fig. 2*). This is in line with our previous observations of Tartary buckwheat grains and 8-day-old sprouts (*Table 1*; Pongrac et al. 2016a). Considering that total grain P and P in phytic acid are highly and positively correlated (Raboy 1997), total P can be used as proxy for estimating phytate concentration in plants. Hence a lower P/Mg ratio indicates lower phytate to Mg ratio, which might positively affect solubility and bioavailability of Mg. Indeed, at molar ratios of phytic acid to Mg below 0.16 an increased solubility of Mg was demonstrated at pH>6 (Cheryan et al. 1983). In grain and seed of different crops P/Mg ratio is typically above 1 (*Table 1*). In potato (*Solanum tuberosum* L.) tubers, another phloem-fed tissue, P/Mg ratio was 1.3 (Kärenlampi & White 2009). In wheat sprouts irrigated with tap water the ratio was much greater than in grains, namely 5.1, and much greater than in Tartary buckwheat sprouts (*Table 1*) which was a consequence of unchanged Mg concentration and increased concentration of P in wheat sprouts (Pongrac et al. 2016a). When the same sprouts were irrigated with a solution containing 5.6-times greater Mg concentration, P/Mg ratio decreased in both species (*Table 1;* Pongrac et al. 2016a). In leaves, lower P/Mg ratios have been reported (*Table 1*). Among grains noticeably lower P/Mg ratios have been reported for both buckwheat species and quinoa (these are pseudocereals) compared to cereal grain and edible seeds from Fabales (pulses: e.g. bean, pea, lentil, chickpea). Presumably this is a result of the phylogenetically-dependent greater Mg concentrations in Caryophyllales species (buckwheat and quinoa) than cereal (Poales) species (White et al. 2015). As argued previously, this might positively affect availability of Mg from grains of plants from Caryophyllales (buckwheats and quiona) in comparison to cereal grains and edible seeds from Fabales (pulses). Furthermore, from a dietary perspective, leaves that have lower P/Mg ratios might be advantageous for ensuring increased Mg intakes. For further discussion on plant-sourced Mg and Mg requirements in human diets see Nielsen (2015).

To study the distribution of Mg and P in developing Tartary buckwheat cotyledons the low-energy XRF beamline TwinMic, synchrotron Elettra, Trieste, Italy, was utilised. An excitation of 2200 eV provided information on the structure of cross-sectioned plant material and access to spatially resolved information on P and Mg distribution at the cell-type specific level. Moreover, presence of these two mineral elements was determined simultaneously (*Fig. 3*). Magnesium and P co-localised in globoid structures densely dispersed within the cells of cotyledon in the grain (*Fig. 4 top panels*). These globoids have previously been reported to contain zinc (Zn), Fe, copper and also manganese (Pongrac et al. 2013a). Similarly, in the aleurone layer of wheat grain such globoids were composed of P, Mg, Zn, Fe and sodium (Regvar et al. 2011) and of K (Lott & Spitzer 1980). These globoids are discrete, electron-dense globular inclusions in single-membrane storage micro-bodies, which can contain crystalline or amorphous storage protein deposits with functions resembling those of the vacuole in non-storage cells (Raboy 1997). In 5-day-old Tartary buckwheat sprouts fewer globoids were seen (*Fig. 4 middle panels*) which is likely a result of the digestion of globoids and dilution as seedlings grow. These cotyledons are still enclosed in the husk, which means the cotyledons have not yet been in direct contact with the light. The 8-day-old sprouts had fully developed cotyledons (*Fig. 1*), which were of darker green colour and had a more defined epidermal layer (*Fig. 4 bottom left panels*). Here the distribution of Mg and P was no longer confined to globoid structures but instead Mg and P were spread more evenly in mesophyll cells (*Fig. 4 bottom panels*) indicating degradation of phytate globoids. In co-localisation images Mg was indicated in green and P in red (*Fig. 4 right panels*). A mixture of these two colours, i.e. co-localisation in particular pixel, yields a yellow colour. In grain yellow colour, hence the co-localisation of P and Mg appears throughout the cotyledons, while in the sprouting cotyledons, there are fewer co-localisations with less intensity.

In cotyledons of grains and 5-day-old and 8-day-old sprouts little Mg was located in the epidermal layer (*Fig. 4*), which is in line with the function of Mg as an essential component of chlorophyll and protein synthesis and functioning (Hawkesford et al. 2012). Cotyledons of 5-day-old sprouts do not photosynthesise yet since they have not been exposed to light, hence it can be argued that extensive remobilisation of Mg from phytate globoids was not required at this stage. By contrast, cotyledons of 8-day-old sprouts have been fully exposed to the light, thus location of Mg at this developmental stage might be a result of increased requirement for photosynthetic activity and protein synthesis (Hawkesford et al. 2012). Light-triggered reactions increase the concentration of Mg of the stroma and increase pH (Hawkesford et al. 2012). In combination these changes are sufficient to increase the activity of ribulose-1,5-bisphosphate (RuBP) carboxylase and also of other stromal enzymes which depend on high Mg concentrations and have a pH optimum above 6 (Hawkesford et al. 2012).

Concentrations of P and Mg in cotyledons were extracted from P and Mg distribution maps using ImageJ software (Abràmoff et al. 2004; Koren et al. 2013). These concentrations are referred to as cotyledon-specific Mg and P concentrations, because only part of the cotyledons was mapped in this study. As seen in bulk data (*Fig. 2*) the P concentrations in cotyledons were greater than Mg at all stages of development, although P/Mg ratio decreased (*Fig. 5*). However, the greatest concentrations of P were seen in grain cotyledon. This in contrast to bulk P concentrations (*Fig. 2*) and is a consequence of whole grain containing tissues which contain lower P concentrations (endosperm and husk; Pongrac et al. 2013a). In cotyledon-specific analyses reported in *Fig. 5* only cotyledons were analysed. Cotyledons of 5-day-old sprouts contained smaller P and Mg concentrations than cotyledons in the grain, presumably a result of translocation of P and Mg for the growth of roots and hypocotyl, which represent sinks for P and Mg. The Mg concentration in 8-day-old sprouts was greater than in 5-day-old sprouts, which indicates increased needs for Mg in chlorophyll synthesis accompanied by mineral element uptake by newly developed roots.

4 CONCLUSIONS

Tartary buckwheat grain (and other grain from plants belonging to Caryophyllales) was identified as having a more favourable P/Mg ratio than cereal grains or pulses. During cotyledon development a decrease in P/Mg concentration ratios was observed. Using low-energy XRF mapping for simultaneous determination of the distribution of Mg and P in developing cotyledons of Tartary buckwheat relocation of Mg and P was visualised. The observed changes in the distributions of Mg and P are likely a result of metabolic changes taking place during development from dormant grains to growing seedlings. The extent to which potential release of Mg from P groups in phytate globoids in the developing Tartary buckwheat cotyledons is linked to increased bioavailability of Mg in the diet remains to be investigated.

5 POVZETEK

5.1 Uvod

Tatarska ajda (*Fagopyrum tataricum* (L.) Gaertn.) je nepravo žito, ki ne vsebuje glutena in ima za prehrano ugodno mineralno sestavo. Na primer, zrnje tatarske ajde ima večjo koncentracijo magnezija (Mg) kot zrnje žit. V zrnju pravih in nepravih žit je Mg močno vezan na fitinsko kislino, organsko spojino, ki je zgrajena iz velikega števila atomov fosforja (P). Zaradi omenjene vezave je Mg slabo topen in slabo biodostopen v prehrani. Namen študije je bil določiti koncentracije Mg in P v zrnju in kličnih listih kalic tatarske ajde in ovrednotiti spremembe v razporeditvi Mg in P v razvijajoči se kličnih listih tatarske ajde.

5.2 Materiali in metode

Preučevali smo klične liste v zrnu, v pet in osem dni starih kalicah tatarske ajde (*Slika 1*). Za določitev skupnih koncentracij Mg in P smo zrna in kalice posušili in razklopili v mikrovalovki. Koncentracije Mg in P smo izmerili z masno spektrometrijo z induktivno sklopljeno plazmo. Razporeditev Mg in P na celičnem nivoju smo določili s pomočjo nizko-energijske rentgensko fluorescenčne mikroskopije na prečnih prerezih kličnih listov, ki so bili pripravljeni s hitrim zamrzovanjem in liofilizacijo.

5.3 Rezultati in razprava

Koncentraciji Mg in P sta naraščali od zrnja do osem dni starih kalic, medtem ko je razmerje med koncentracijami P in Mg padalo (*Slika 2*). Manjše razmerje med P/Mg je zaželeno v naši prehrani, saj je koncentracija P dobro merilo za količino fitinske kisline, ki, kot je bilo že omenjeno, omejuje absorpcijo Mg v naši prebavi. Med zrni in semeni imajo zrna rastlin, ki sodijo v red klinčnikovcev (Caryophllales) bolj ugodno razmerje P/Mg kot zrna pravih žit in stročnic (*Tabela 1*). Najbolj ugodno razmerje med P in Mg je v listih rastlin.

Tehnika nizko-energijske rentgensko fluorescenčne mikroskopije omogoča analizo morfološke zgradbe analiziranega tkiva in hkratno določitev razporeditve Mg in P (*Slika 3*). V kličnih listih zrnja smo z omenjeno tehniko pokazali močno kolokalizacijo Mg in P, ki pa se je tekom razvoja kličnih listov postopno manjšala (*Slika 4*).

5.4 Zaključki

Rezultati potrjujejo, da lahko s tehnikami vizualizacije razporeditve Mg in P orišemo kemijske spremembe v rastlinskem tkivu, v tem primeru razgradnjo kompleksov fitinske kisline in Mg. Potrebne so nadaljnje raziskave, s katerimi bi ovrednotili povezavo med postopnim sproščanjem Mg s fitinske kisline v razvijajočih se kličnih listih tatarske ajde in biodostopnostjo Mg v prebavi.

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Table 1. Phosphorus (P) and magnesium (Mg) concentration ratios in grain, seed, tubers, sprouts and leaves of some crop plant species.
Tabela 1. Razmerja med koncentracijami fosforja (P) in magnezija (Mg) v zrnju, semenih, gomoljih, kalčkih in listih nekaterih kmetijskih rastlin.

Plant species	*Plant part*	*P/Mg ratio*	*Reference*
Rice *Oryza sativa* L. n=1755 genotypes [flooded] n=1729 genotypes [unflooded]	Grain	Flooded: 2.4 Unflooded: 2.5	Pinson et al. 2015
Pearl millet *Pennisetum glaucum* (L.) R. Br. n=225 genotypes	Grain	3	Bashir et al. 2014
Wheat *Triticum aestivum* L. n=1 genotype	Grain	2.8	Pongrac et al. 2016a
Common buckwheat *Fagopryrum esculentum* Moench n=1 genotype	Grain	1.4	Pongrac et al. 2016b
Tartary buckwheat *Fagopyrum tataricum* (L.) Gaertn n=1 genotype	Grain	1.8	Pongrac et al. 2016a, b
Quinoa *Chenopodium quionoa* Willd. n=7 genotypes	Grain	1.1 – 2.2	Prado et al. 2014
Lentil *Lens culinaris* Medik. n=39 genotypes	Seed	3.9	Karaköy et al. 2012
Pea *Pisum sativum* L. n=481 genotypes	Seed	3.1	Grusak 2002*

Chickpea *Cicer arietinum* L. n=239 genotypes	Seed	2.6	Grusak 2006*
Potato *Solanum tuberosum* L. n=21 genotypes	Tubers	1.3	Kärenlampi & White 2009
Wheat *Triticum aestivum* L. n=1 genotype	Sprouts	Tap water: 5 High-Mg solution**: 3.5	Pongrac et al. 2016a
Tartary buckwheat *Fagopyrum tataricum* (L.) Gaertn. n=1 genotype	Sprouts	Tap water: 1.2 High-Mg solution**: 0.8	Pongrac et al. 2016a
Spinach *Spinacia oleracea* L. n=327 genotypes	Leaves	0.78	Grusak 2003*
Cabbage, kale, broccoli, cauliflower, Brussels sprouts *Brassica oleracea* L. n=260 genotypes	Leaves	Low-P soil: 0.3 High-P soil: 0.5	Broadley et al. 2008, Broadley et al. 2010
Potato *Solanum tuberosum* L. n=21 genotypes	Leaves	0.5	Kärenlampi & White 2009

*Data for chickpea, pea and spinach genotypes was obtained from the Germplasm Resources Information Network (GRIN) website (https://npgsweb.ars-grin.gov/gringlobal/descriptors.aspx?). **High-Mg solution contained 5.6-times greater Mg concentration than tap water.

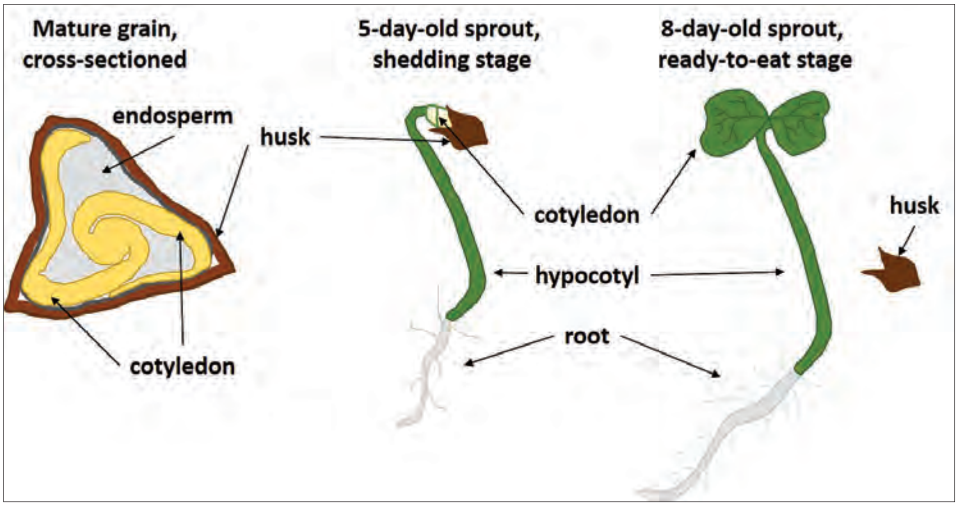

Figure 1. Development of Tartary buckwheat from seed to sprouts.
Slika 1. Razvojne faze tatarske ajde - od semena do kalice.

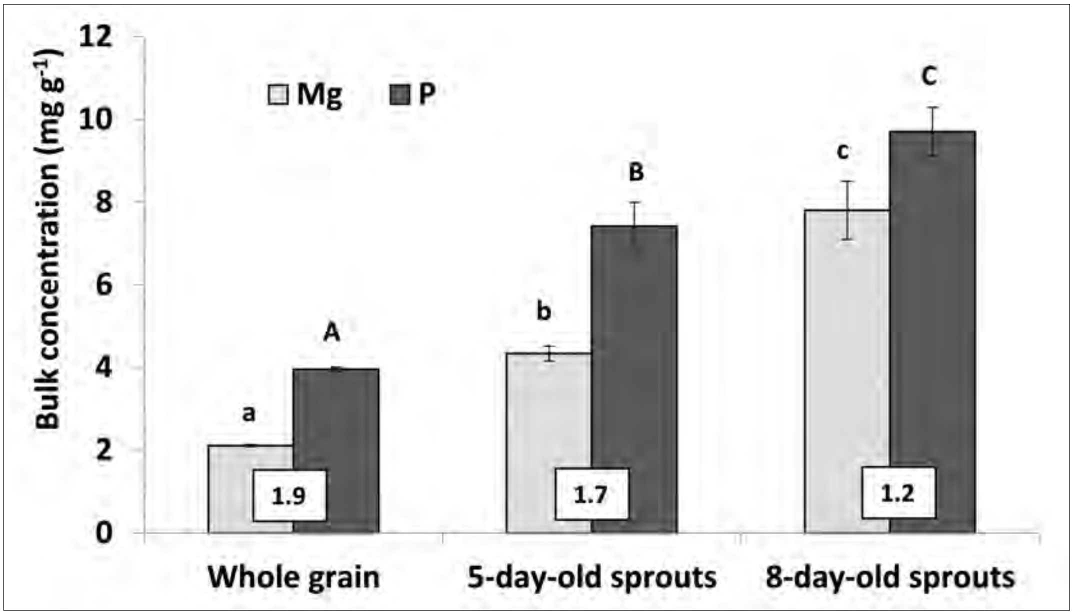

Figure 2. Bulk concentrations (mg g^{-1} dry matter) of magnesium (Mg) and phosphorus (P) in grain and sprouts of Tartary buckwheat and P/Mg concentration ratio (in squares). Shown are means (n=3 for each developmental stage) and standard deviations. Different letters above columns indicate statistical differences for each mineral element separately (Student-Newman-Keuls post-hoc test at p<0.05).

Slika 2. Skupne koncentracije (mg g^{-1} suhe snovi) magnezija (Mg) in fosforja (P) v zrnju in kalicah tatarske ajde in razmerje med koncentracijama P in Mg (podano v kvadratkih na stolpcih). Prikazana so povprečja (n=3 za vsako razvojno fazo) in standardne deviacije. Različne črke nad stolpci predstavljajo statistično značilne razlike za vsak posamezen mineralni element (Student-Newman-Keuls post-hoc test, p<0.05).

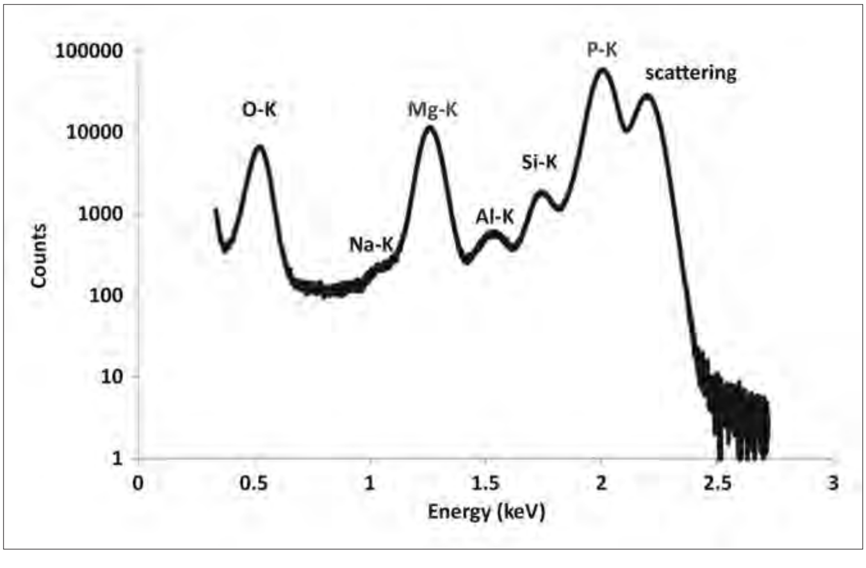

Figure 3. X-ray fluorescence sum spectrum (66 x 66 pixels) of the cross-sectioned cotyledon of a 8-day-old sprout (Figure 5) recorded at the TwinMic beamline, synchrotron Elettra, Trieste, Italy. Excitation energy was 2200 eV. The spectrum was extracted with PyMCA software (Solé et al. 2007). Characteristic K-lines are shown together with scattering peak.

Slika 3. Skupni rentgensko fluorescenčni spekter (vsota intenzitet rentgenskih fluorescenčnih črt na matriki velikosti 66 x 66 slikovnih pik) posnet na prečnem prerezu kličnega lista osem dni stare kalice na žarkovni liniji TwinMic na sinhrotornu Elettra v Trstu, Italija. Vzbujevalna energija je bila 2200 eV. Spekter je bil izrisan s programsko opremo PyMCA (Solé et al. 2007). Prikazani so vrhovi karakterističnih K-črt in sipanje.

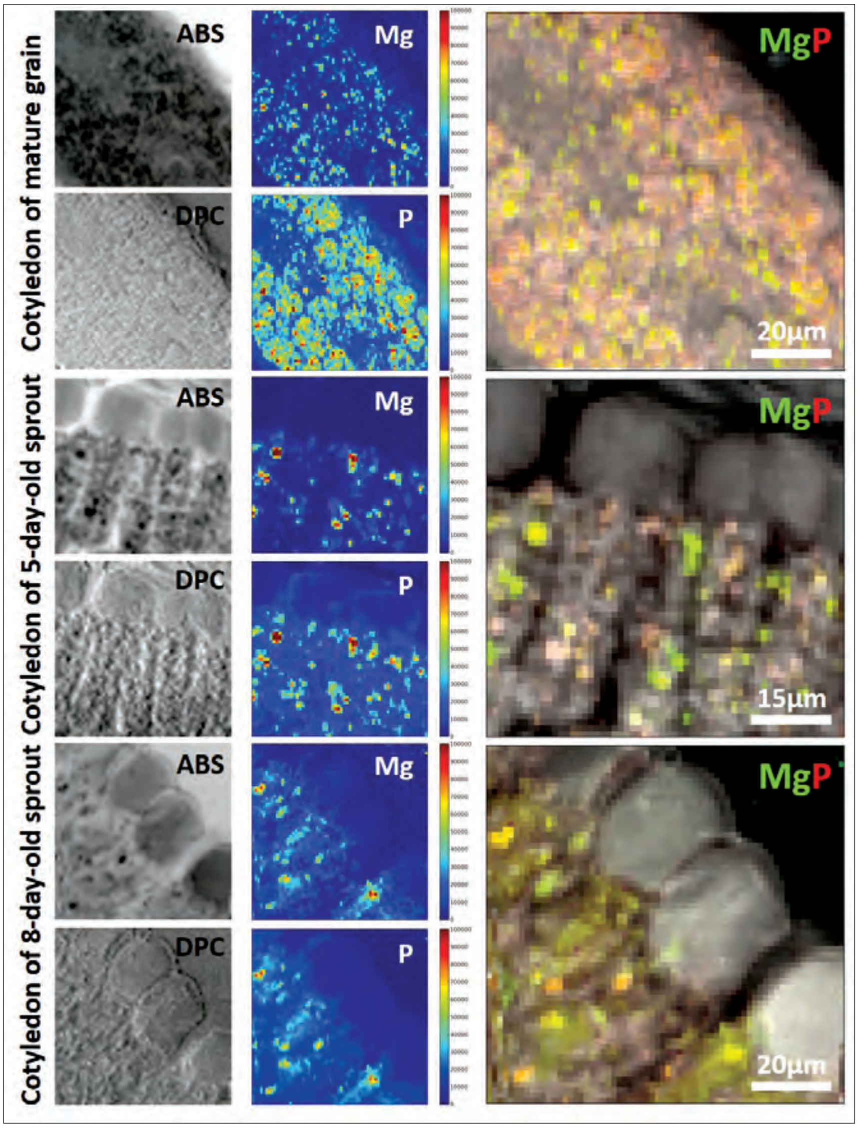

Figure 4. X-ray imaging of structural make-up (left panels), quantitative distributions of magnesium (Mg) and phosphorus (P) (middle panels) and co-localisation images (right panels) of Tartary buckwheat cotyledons. Representative sample from n=3 for each developmental stage is shown. Structure of cotyledons is indicated with absorption (ABS) and differential phase contrast (DPC) images. In co-localisation images, co-localisation of Mg (shown in green) and P (shown in red) is indicated as yellow (a mix of red and green).

Slika 4. Slika anatomskih lastnosti (levi del slike), kvantitativne razporeditve magnezija (Mg) in fosforja (P) (srednji del slike) in kolokalizacije Mg in P (desni del slike) v kličnih listih tatarske ajde. Za vsako razvojno stopnjo je prikazan izbran vzorec izmed treh analiziranih tkivnih rezin. Anatomske lastnosti kotiledona so prikazane na sliki posneti v načinu absorpcije rentgenskih žarkov (ABS) in diferencialnega faznega kontrasta rentgenskih žarkov (DPC). Na kolokalizacijskih slikah je Mg prikazan z zeleno, P pa z rdečo bravo. Njuno mešanje, torej prisotnost obeh mineralnih elementov na posamezni slikovni piki, je prikazano z rumeno barvo.

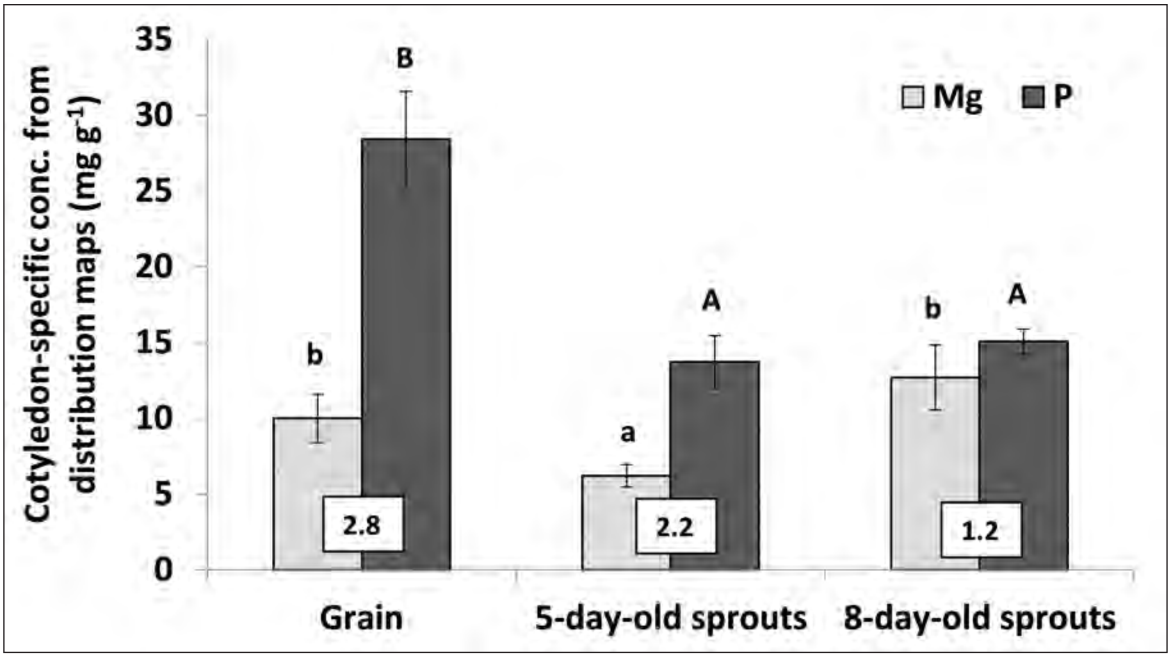

Figure 5. Cotyledon-specific concentrations (mg g^{-1} dry matter) of magnesium (Mg) and phosphorus (P) in grain and sprouts of Tartary buckwheat and their P/Mg concentration ratio (in squares). Concentrations were extracted from quantitative distribution maps (representative maps are shown in Figure 4) using ImageJ software (Abràmoff et al. 2004; Vogel-Mikuš et al. 2014). Means (n=3 for each developmental stage) and standard deviations are shown. Different letters above columns indicate statistical differences for each mineral element separately (Student-Newman-Keuls post-hoc test at p<0.05).

Slika 5. Koncentracije (mg g^{-1} suhe snovi) magnezija (Mg) in fosforja (P) v kličnih listih v zrnu in kalicah tatarske ajde in razmerje koncentracij P in Mg (podano v kvadratkih na stolpcih). Koncentracije so bile pridobljene iz kvantitativnih map razporeditve P in Mg (ena izmed slik je prikazan na Sliki 4) s pomočjo programske opreme ImageJ (Abràmoff et al. 2004; Vogel-Mikuš et al. 2014). Prikazana so povprečja (n=3 za vsako razvojno stopnjo) in standardni odkloni. Različne črke nad stolpci predstavljajo statistično značilne razlike za vsak posamezen mineralni element (Student-Newman-Keuls post-hoc test pri p<0.05).