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X-ray Fluorescence Microscopy Computed Tomography Reveals Internal Distribution of Selenium in Hyperaccumulator Plant Seeds

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Selenium hyperaccumulators are plants able to accumulate over 1000 μg selenium g^{-1} in their shoots while growing in their natural environments. The most studied selenium hyperaccumulators are *Astragalus bisulcatus*, *Neptunia amplexicaulis* (Fabaceae), and *Stanleya pinnata* (Brassicaceae). To date, several studies have investigated selenium distribution in these species, most of which have focused on the leaves and roots. Other plant organs, such as seeds, remain poorly investigated. In this study, we determined selenium distribution in the seeds of these three key hyperaccumulating species using X-ray fluorescence microscopy computed tomography (XFM-CT). Seeds of *A. bisulcatus*, *S. pinnata*, and *N. amplexicaulis* were also analyzed for total elemental content. Single seeds of each species were examined using XFM-CT at beamline P06 of PETRA III (Deutsches Elektronen-Synchrotron, DESY) to generate detailed elemental maps. Selenium concentrations were 1800 ± 180 , 1240 ± 170 , and $870 \pm 100 \mu\text{g g}^{-1}$ DW in *N. amplexicaulis*, *A. bisulcatus*, and *S. pinnata* seeds, respectively. In general, selenium is enriched in the cotyledon parenchyma and radicle cortex of all seeds. Selenium is generally absent from the seed coat and endosperm. Selenium is present in the inner part of the seed coat (tegmen). The distribution of selenium in tissues that are especially sensitive to herbivory appears to support the defense function of this metalloid in hyperaccumulator plants.

1 | Introduction

Selenium (Se) hyperaccumulators are plants able to accumulate this element in their shoots at concentrations $> 1000 \mu\text{g}$ Se g^{-1} while growing in their natural environments (van der Ent et al. 2013). They were first discovered in the prairies of central western USA, characterized by Se-rich soils, also called 'seleniferous' soils (Beath et al. 1941). Selenium hyperaccumulators occur in many families, particularly in the Fabaceae, Brassicaceae, and Asteraceae (El Mehdawi and Pilon-Smits 2012). The genus *Astragalus* (Fabaceae) has the highest number of Se-hyperaccumulating species, comprising 25 taxa (White 2016). Among them, *Astragalus bisulcatus* (Figure 1a)

is the strongest, able to accumulate up to $13,685 \mu\text{g}$ Se g^{-1} DW when growing on seleniferous soils (de Jong et al. 2015). Among the Brassicaceae, *Stanleya pinnata* (Figure 1c) is also a notable hyperaccumulator, able to accumulate $4000 \mu\text{g}$ Se g^{-1} DW when growing on soils containing only $2\text{--}10 \mu\text{g g}^{-1}$ Se (Beath et al. 1941; Galeas et al. 2007). Selenium hyperaccumulators are also found in seleniferous soils in central Queensland (Australia), where *N. amplexicaulis* (Fabaceae, Figure 1b) can accumulate $\geq 4000 \mu\text{g}$ Se g^{-1} under natural conditions, mainly in the form of selenocystathionine (Knott and McCray 1959; Peterson and Butler 1967). Given their worldwide distribution across different plant taxa, Se hyperaccumulation has likely evolved independently in response to

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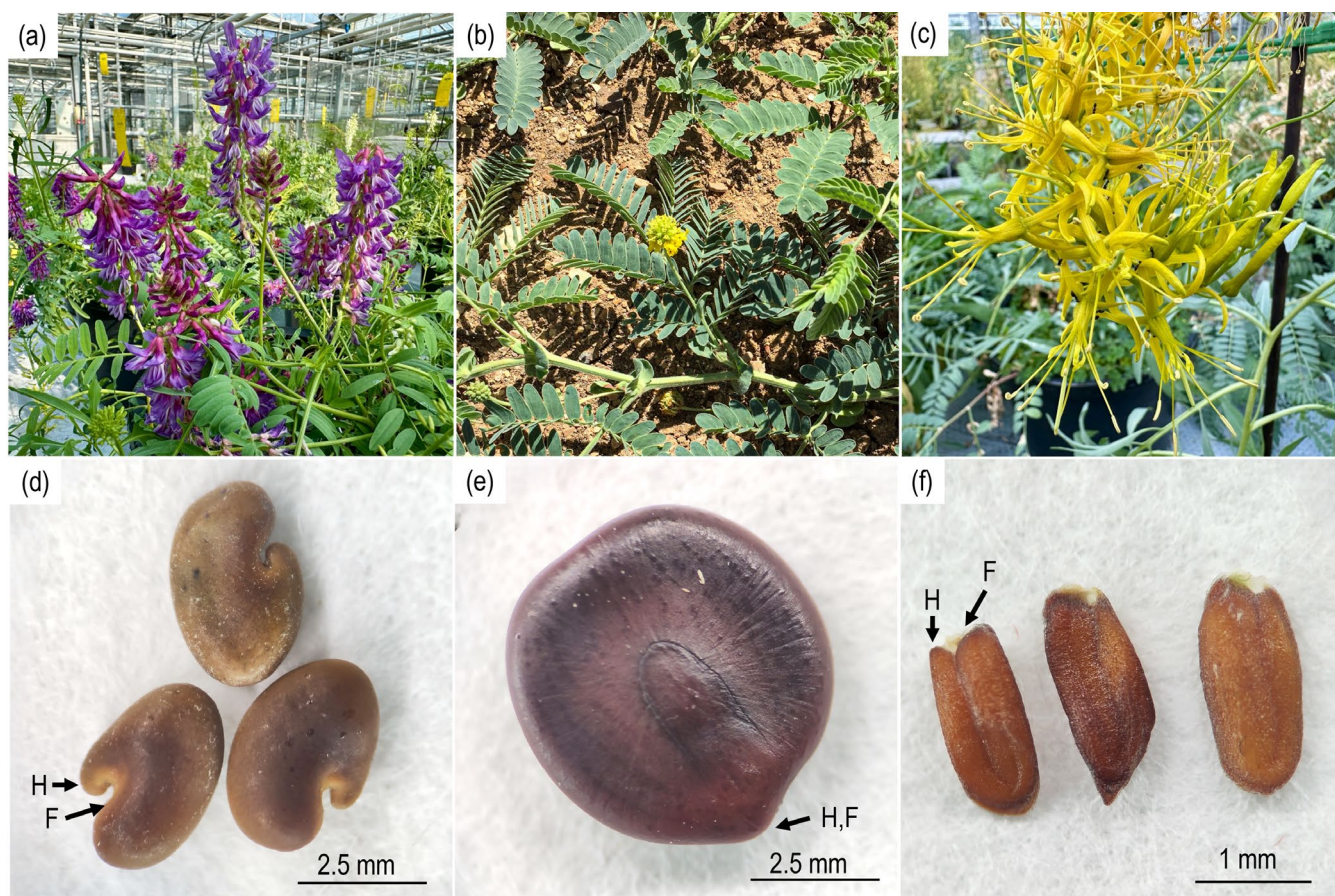


FIGURE 1 | Selenium hyperaccumulator plants and seeds. (a) *Astragalus bisulcatus*; (b) *Neptunia amplexicaulis*; (c) *Stanleya pinnata*; (d) *Astragalus bisulcatus* seeds; (e) *Neptunia amplexicaulis* seed; (f) *Stanleya pinnata*. F: Funiculus; H: Hilum.

similar environmental pressures, such as the presence of Se-enriched substrates and dry conditions (Pilon-Smits 2017). In general, the accumulation of high quantities of Se is posited to protect plants against herbivores and pathogens (El Mehdawi and Pilon-Smits 2012). In accordance with this hypothesis, a common trait among Se hyperaccumulators is the storage of Se in actively growing tissues, especially in young leaves and flowers (Pilon-Smits 2017).

Interest in elucidating the physiological mechanisms contributing to the hyperaccumulation and detoxification of Se in plants has increased over the last decades to improve understanding of the ecophysiology of these species and to anticipate future applications in Se-biofortification strategies (Xie et al. 2023). Selenium, in fact, is an essential element for humans, with a recommended dietary intake of 55–200 µg/day for adults (Wu et al. 2015). Conversely, it is not considered an essential element for higher plants; however, at low concentrations it can have several beneficial effects, including stimulation of plant growth and enhancement of cellular antioxidant activity due to the hormesis effect (Hartikainen 2005). As previously observed in Se hyperaccumulator species, this metalloid confers protection against herbivores and pathogens and provides allelopathic advantages over competing plant species (El Mehdawi and Pilon-Smits 2012). Plants primarily take up Se as selenate (SeO_4^{2-}), which is highly bioavailable under oxidizing conditions, and to a lesser extent, as selenite (SeO_3^{2-}), the predominant form in reducing environments (Dinh et al. 2019).

Several studies have investigated the distribution of Se in plant tissues at the cellular level; however, most of these studies have focused on above-ground tissues, particularly leaves, which act as the major sink for the accumulated Se, and roots (Valdez Barillas et al. 2012; Harvey et al. 2020; van der Ent et al. 2023). On the other hand, other plant organs, such as seeds, have been poorly studied. These organs are of primary importance for plant fitness and reproduction and constitute an important Se sink in hyperaccumulator species. A recent study of Se spatial distribution in a single seed of the Se hyperaccumulator *Cardamine violifolia* reported prominent Se accumulation in the cotyledons, with overall concentrations of 1730 µg g⁻¹ (Xie et al. 2023).

Knowledge of Se distribution in seeds may provide important insights into hyperaccumulators, as accumulation of Se in the endosperm, germ, or seed coat may indicate differences in bioaccessibility and, therefore, different levels of potential toxicity for ecological partners. In particular, when concentrated in seed coats, Se may act as a protective barrier against pathogens or herbivores, whereas accumulation in storage tissues may serve to deter herbivory (Freeman et al. 2012). Furthermore, Se localization in seeds may indicate possible roles of Se in early germination, enzyme protection, or defense against oxidative stress.

Micronutrients mapping has been widely used in crop seeds to evaluate nutritional properties or possible contamination. In these samples, the distribution of metals and metalloids has been investigated using a variety of techniques (Lombi

et al. 2011). Ockenden et al. (2004) used scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) to map micronutrients in barley. However, SEM-EDS techniques are characterized by relatively high detection limits, typically 0.1 wt. % for zinc (Zn) and Se (Lombi et al. 2011), and do not provide detailed elemental distribution maps. Synchrotron-based techniques are considered more reliable, with higher sensitivity and a resolution down to the subcellular level (Kachenko et al. 2009). These include X-ray fluorescence microscopy (XFM) and micro-proton-induced X-ray emission (micro PIXE), which are widely used for metal mapping in plant seeds (Lombi et al. 2011; Kopittke et al. 2018). However, for all the above-cited techniques, sample preparation for analysis often includes physical sectioning, with possible dislocation of elements into neighboring tissues. To solve this problem, X-ray fluorescence microscopy computed tomography (XFM-CT) can be employed to produce virtual sections of samples, without any physical sectioning. One of the limitations of this technique is the shape and diameter of samples that can be analyzed, which should be more or less round and smaller than 2 mm in diameter. For analysis of seeds, these limitations are usually not problematic, and this technique is ideal for gathering precise information about elemental distribution (Spiers et al. 2022).

In this study, we aim to determine the distribution of Se in the seeds of three key hyperaccumulating species, *A. bisulcatus*, *S. pinnata*, and *N. amplexicaulis*, using X-ray fluorescence microscopy computed tomography (XFM-CT). This study can help to advance our knowledge of the physiological mechanisms that are responsible for Se hyperaccumulation in these species.

2 | Materials and Methods

2.1 | Seeds Material Collection and Ionomics

Seeds of *A. bisulcatus* (Figure 1a,d) and *S. pinnata* (Figure 1c,f) were purchased from the specialized seed supply company Prairie Moon Nursery (Minnesota, USA). Seeds of *N. amplexicaulis* (Figure 1b,e) were collected from plants grown hydroponically at Wageningen University (Netherlands) with Se spiked (selenate 50 mg/L) modified Hoagland's solution as described in Mohtadi and Schat (2024). In addition to the purchased seeds, *S. pinnata* seeds collected from plants growing in seleniferous soil located in the Pine Ridge Natural Area, Fort Collins (Colorado, USA), were analyzed for Se distribution and compared with commercially sourced seeds from plants grown in non-seleniferous soil (data shown in Figure 3). For this location, only a few seeds were available; therefore, bulk elemental analysis was not possible, and based on the literature (Lima et al. 2022), they contained on average $\sim 5600 \mu\text{g Se g}^{-1}$ DW. Prior to the synchrotron analysis, 1 g of seeds from each species was dried in a dehydrating oven at 60°C for 4 days and then ground to a fine powder ($< 200 \mu\text{m}$) in an impact mill (IKA TubeMill 100 Control). Then, 0.5 g subsamples were inserted into custom XRF sample holders and covered with a 6 μm polypropylene thin film (Chemplex Industries Inc.) for XRF analysis, which was performed using a Z-Spec JP500 instrument (Z-Spec Inc., New York, USA). The instrument uses monochromatic X-ray fluorescence excitation at 17.48 keV to analyze elements from $Z = 13$ (Al) to $Z = 39$ (Y)

on the K-lines and up to $Z = 92$ (U) on the L-lines with optimum sensitivity for elements Cu–Se and Hg–Tl–Pb with LODs ranging from 0.009 to $0.025 \mu\text{g g}^{-1}$. Samples were analyzed for 30 s in plant mode (Zhang et al. 2025). Quality controls included NIST SRM 1570a and NIST SRM 1573a, which had recovery rates of 95%–105%.

2.2 | Synchrotron-Based Micro-X-Ray Fluorescence Computed Tomography

The X-ray fluorescence microscopy experiments were undertaken at PETRA III (Deutsches Elektronen-Synchrotron, DESY), a 6-GeV synchrotron radiation source, specifically at the hard X-ray microprobe at the undulator beamline P06 (Boesenberg et al. 2016; van der Ent et al. 2023; Garrevoet 2025). P06 is equipped with a cryogenically cooled double-crystal monochromator with Si (111) crystals. An ion chamber upstream of the sample is used to monitor the incoming flux, while a 500- μm -thick Si PIPS diode with a 19-mm-diameter active area [PD300-500CB, Mirion Technologies (Canberra) GmbH, Germany], located downstream of the sample, can be used to record the transmitted X-ray intensity in order to extract absorption data. Multiple X-ray fluorescence (XRF) detectors allow the measurement of XRF data. The data presented in this manuscript were measured at an incident X-ray energy of 16 keV. A pair of KB (Kirkpatrick-Baez) mirrors were used to focus the x-ray beam down to about $600 \text{ nm} \times 500 \text{ nm}$ (horizontal $\times$ vertical), resulting in a flux of about 3.4×10^{10} photons s^{-1} on the sample. Detection of XRF was performed by a four-element Vortex silicon drift detector (Hitachi High-Tech, USA) and a 16-element Ardesia silicon drift detector (Politecnico di Milano), both with Xspress 3 pulse processors (Quantum Detectors, UK), placed at 90° and 270° angles, with the forward direction of the X-ray beam defined as 0°. Seeds were glued onto a top 0.5 mm diameter Kapton capillary and mounted under a cryostream for analysis to reduce possible beam damage. The tomographic measurements were conducted in single-slice tomography mode.

2.3 | Data Processing and Statistics of the XRF Data

Data acquisition was handled by a custom workflow (Garrevoet 2025), and the XRF data were processed using nonlinear least-squares fitting as implemented in PyMCA (Solé et al. 2007). Energy calibration was achieved by measuring various thin film deposited foils with XRF emission lines well-spaced over the significant energy range (Micromatter Technologies Inc., Canada). The elemental calibration for other elements was calculated using the results of the foil measurements in combination with elemental parameters from *xraylib* (Schoonjans et al. 2011). This produced 32-bit .tiff files with pixel values corresponding to $\mu\text{g cm}^{-2}$ or $\mu\text{g cm}^{-3}$ of the element in question. The figures were prepared in ImageJ (Schneider et al. 2012) by changing the lookup table (LUT) to “Fire,” adjusting the maximum values, and adding length scales (van der Ent et al. 2023). The tomographic data were aligned using tomographic consistency methods to correct for unwanted sample movement in the horizontal direction. All

datasets were reconstructed using a maximum-likelihood expectation–maximization (MLEM) algorithm (Bruyant 2002). The tomograms shown were calibrated and quantified, apart from self-absorption effects.

3 | Results

The concentration of Se was 1800 ± 180 , 1240 ± 170 , and $870 \pm 100 \mu\text{g g}^{-1}$ DW in *N. amplexicaulis*, *A. bisulcatus*, and *S. pinnata* seeds, respectively (Table 1). Sulfur was also very high in all three species, with values of almost 18 mg g^{-1} DW in *N. amplexicaulis*. Seeds of this species were generally more concentrated in all analyzed elements except Mg and Ni compared with the other two species (Table 1). Seed morphology and structure of the three analyzed species were determined using x-ray absorption (Figure 2a,d,g), as x-rays are absorbed differently depending on the density of the seed structures. Zinc is commonly used as a reference in spatial analysis, as it exhibits patterns contrasting with those of other elements. Based on the contrasting patterns between Zn and Se, additional information highlighting different seed tissues was extracted. Seeds of *A. bisulcatus* and *S. pinnata* are campylotropous and are characterized by a curved shape (C-shape), with the hilum (the opening of the seed) near but separated from the funiculus (the stalk attaching the seed to the placenta) (Figure 1d,f). Conversely, *N. amplexicaulis* seeds are anatropous, with a straight shape. In these seeds, the funiculus and the hilum overlap at the same end (Figures 1e and 2d).

The Zn and Se maps of the three seeds were obtained using XFM-CT, and a single virtual slice for each seed is shown in Figure 2. In campylotropous seeds, virtual slices are perpendicular to the cotyledons and radicle, allowing detailed visualization of the vasculature (Figure 2a–c,g–i). In anatropous seeds, the virtual slice is parallel to the cotyledons, enabling sectioning

of the radicle and plumule but limiting detailed observation of the vasculature (Figure 2d–f).

In *A. bisulcatus*, Zn was particularly enriched in the main vasculature of the radicle (Figure 2b, top) and in the vascular bundles of the cotyledons (Figure 2b, bottom). A closer examination revealed that Zn was mostly localized in the outer regions of the vasculature, predominantly in the phloem. In contrast, the radicle cortex and cotyledon parenchyma were Zn-depleted, and Zn was completely absent from the endosperm and seed coat. Selenium distribution was opposite to that of Zn: it was more concentrated in plant reserve tissues, such as the radicle cortex and cotyledon parenchyma. Interestingly, the epidermis of both organs contained very low Se, and no Se was detected in the endosperm and the seed coat. Longitudinal virtual sections of *A. bisulcatus* seeds confirmed this distribution pattern (Figure S1).

The distribution of Zn in *N. amplexicaulis* was difficult to observe due to the orientation of the virtual section parallel to the cotyledons. Nevertheless, high Zn accumulation at the edges of the cotyledons and in the radicle tip was clearly evident, while Zn was completely absent from the central region of the cotyledon parenchyma, although this may be due to self-absorption effects. In this seed, Zn was also present in the seed coat, but only in the hilum area (Figure 2e). Se was evenly distributed throughout the cotyledon, although higher levels were observed in the outer regions, similar to Zn. The radicle tip and the plumule showed Se levels similar to those in the rest of the seed. In the radicle tip, Se was primarily localized in the epidermis (Figure 2f). Additional virtual slices shown in Figure S2 confirm this distribution pattern.

In *S. pinnata*, Zn was highly localized in the main vasculature of the radicle (Figure 2f, bottom right) and in the vascular bundles of the cotyledons (Figure 2b, top left), similar to that

TABLE 1 | Seed ionomes of the three Se hyperaccumulator species investigated.

Elements	<i>Astragalus bisulcatus</i>	<i>Stanleya pinnata</i>	<i>Neptunia amplexicaulis</i>
Mg	4120 ± 150	4210 ± 170	2270 ± 530
Si	70 ± 10	80 ± 20	880 ± 200
P	2410 ± 650	2950 ± 570	5890 ± 1690
S	4320 ± 400	5400 ± 810	$18,000 \pm 5100$
Cl	1380 ± 320	510 ± 80	5900 ± 680
K	9780 ± 590	6330 ± 950	$19,200 \pm 3070$
Ca	2580 ± 260	3480 ± 510	2920 ± 230
Mn	15 ± 2	19 ± 2	33 ± 2
Fe	70 ± 7	46 ± 4	280 ± 40
Ni	16 ± 2	1.4 ± 0.2	1.9 ± 0.3
Cu	10.7 ± 0.9	5.2 ± 0.5	8.5 ± 0.6
Zn	39 ± 4	71 ± 5	62 ± 3
Se	1240 ± 170	870 ± 100	1800 ± 180

Note: Values are given in $\mu\text{g g}^{-1}$ DW as mean $\pm$ standard deviation. The concentrations reported for *Stanleya pinnata* seeds refer to commercially sourced seeds.

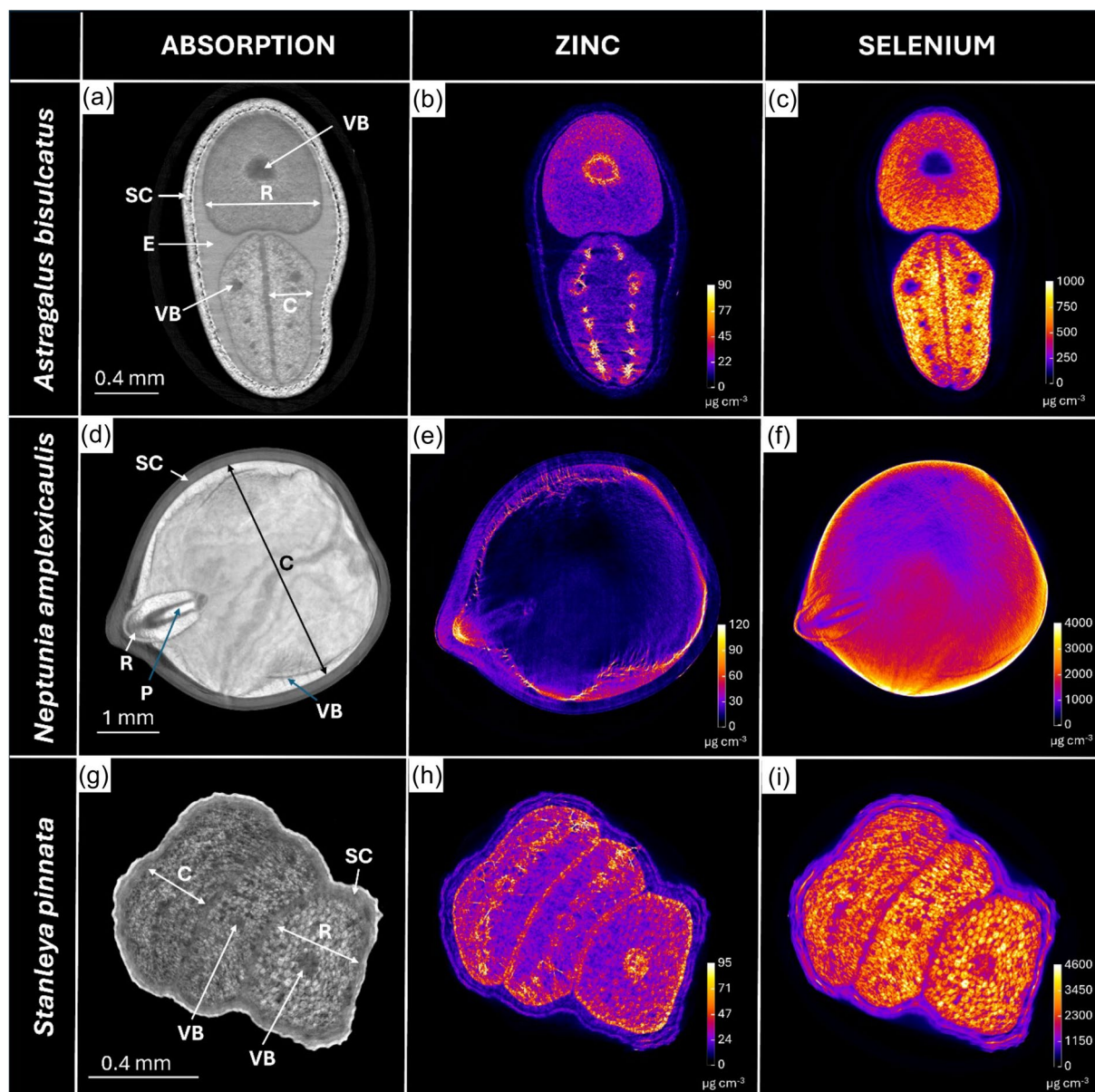


FIGURE 2 | Selenium and zinc XFM-CT virtual maps in three hyperaccumulator seeds. (a–c) *Astragalus bisulcatus* seeds absorption, zinc map, and selenium map; (d–f) *Neptunia amplexicaulis* seed absorption, zinc map, and selenium map; (g–i) *Stanleya pinnata* seeds absorption, zinc map, and selenium map. Elemental concentrations are expressed as $\mu\text{g cm}^{-3}$. C: cotyledon; E: endosperm; P: plumule; R: radicle; SC: seed coat; VB: vascular bundle.

observed in *A. bisulcatus* seeds. The seed coat showed a distinct pattern, with the inner layer (tegmen) being Zn-depleted and the outer layer (testa) more enriched. The radicle cortex was also enriched in Zn, especially in its outer region. Zinc was also present at high concentrations in the epidermis of both the radicle and cotyledons. Selenium exhibited a distribution pattern opposite to that of Zn. It was primarily localized and evenly distributed in plant reserve tissues, such as the radicle cortex and cotyledon parenchyma, similar to observations in *A. bisulcatus* seeds. In the seed coat, Se accumulates in the outer part of the tegmen, but not in the testa.

Stanleya pinnata seeds from both non-seleniferous and seleniferous soils were also analyzed using 2D imaging mode (Figure 3). The distribution of Zn was similar to that observed

in the XFM-CT images, with Zn appearing more concentrated in the seed vasculature, especially in plants from seleniferous soils (Figure 3a). Selenium was present in seeds of both the seleniferous and non-seleniferous accessions, but its overall distribution was different. In seeds from the non-seleniferous accession, Se appeared localized in a crust-like formation on the seed surface. This Se accumulation may originate from the phloem connection to the mother plant and could consist of dried phloem sap. However, it cannot be excluded that these Se hotspots result from contamination. In seeds from the seleniferous accession, Se was distributed throughout the seed, appearing in the cotyledons and radicle. This distribution was similar in seeds of the non-seleniferous accession but was less apparent due to scaling with hotspots in the Se-enriched ‘crusts’.

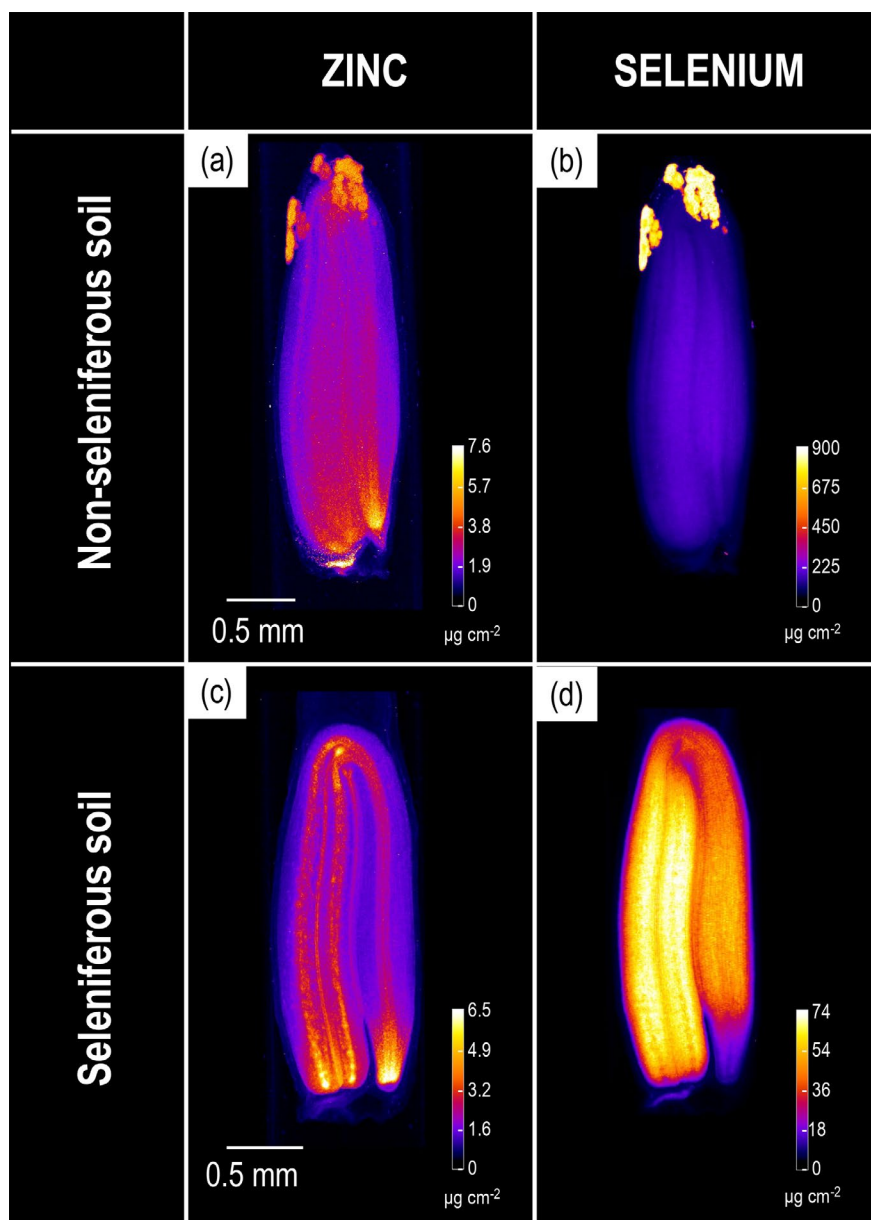


FIGURE 3 | Selenium and zinc 2D elemental maps in *Stanleya pinnata* seeds. (a, b) *S. pinnata* seeds from non-seleniferous soil. (c, d) *S. pinnata* seeds from seleniferous soil. Elemental concentrations are expressed as $\mu\text{g cm}^{-2}$.

4 | Discussion

The concentration of Se in seeds of *A. bisulcatus* ($\sim 1240 \mu\text{g g}^{-1}$ DW) was 4.6-fold lower than previously reported by Freeman et al. (2012; $5750 \mu\text{g g}^{-1}$ DW), yet remained consistent with the strong accumulation capacity previously documented in the leaves and roots of this species. Indeed, Se concentrations in vegetative tissues of *A. bisulcatus* commonly exceed the hyperaccumulation threshold, frequently reaching $6000 \mu\text{g g}^{-1}$ DW in young leaves of field-grown plants (Freeman et al. 2012). The lower Se concentration observed in seeds relative to leaves likely reflects tightly regulated partitioning of Se into reproductive tissues rather than a reduced uptake capacity, a pattern commonly observed in Se hyperaccumulators. In seeds of *N. amplexicaulis*, Se concentration ($\sim 1800 \mu\text{g g}^{-1}$ DW) was in line with that found in the hyperaccumulator *C. violifolia*, which can reach up to $1730 \mu\text{g g}^{-1}$ (Xie et al. 2023). This relatively high seed Se content

is consistent with recent reports indicating that *N. amplexicaulis* can accumulate extraordinarily high Se concentrations in young leaves, reaching up to $\sim 13,600 \mu\text{g g}^{-1}$ DW in cultivation (Harvey et al. 2020, 2025). Root tissues in this species also exhibit substantial Se accumulation with concentrations exceeding $8000 \mu\text{g g}^{-1}$ DW (van der Ent et al. 2023), highlighting an exceptionally efficient root uptake system and a strong capacity for internal Se redistribution to shoots and reproductive structures. In contrast, *S. pinnata* seeds, despite being highly enriched in Se, did not reach the hyperaccumulation threshold of $1000 \mu\text{g g}^{-1}$, showing a concentration lower than that reported for the same species by Freeman et al. (2012; $3293 \mu\text{g Se g}^{-1}$) and Lima et al. (2022; up to $\sim 5500 \mu\text{g Se g}^{-1}$). This reduced seed enrichment did not reflect limited uptake capacity, as *S. pinnata* is known to accumulate substantial Se concentrations in vegetative tissues, with leaves typically containing $2000\text{--}4000 \mu\text{g Se g}^{-1}$ DW and roots around $600\text{--}800 \mu\text{g g}^{-1}$ DW under field conditions, and up to

~12,000 $\mu\text{g g}^{-1}$ DW under controlled conditions (Valdez Barillas et al. 2012; Lima et al. 2022; van der Ent et al. 2023). Rather, the lower seed Se concentrations in this study were more likely due to the origin of the seeds, which were obtained from a commercial nursery and not collected from wild populations naturally growing in Se-rich environments.

Sulfur and Se are chemical analogues, and Se hyperaccumulators typically exhibit higher Se:S ratios than non-hyperaccumulators across different organs, including seeds (Galeas et al. 2007). The Se:S ratio in seeds reflects how much Se the plant can partition into reproductive tissues while still ensuring sufficient S for essential and storage functions. A high Se:S ratio in hyperaccumulator seeds may confer protective benefits, such as the protection of embryos from pests and pathogens (Freeman et al. 2012). In our study, the Se:S ratio was highest in *A. bisulcatus* (0.28) and lower in *S. pinnata* (0.16) and *N. amplexicaulis* (0.10); in non-accumulator plants, this ratio is usually <0.01. These data are consistent with previous reports (Freeman et al. 2012), which showed that *A. bisulcatus* is more efficient than *S. pinnata* in translocating Se from leaves to reproductive structures, resulting in more Se-enriched seeds. Specifically, Freeman et al. (2012) reported Se:S ratios of 0.48 in *A. bisulcatus* seeds and 0.11 in *S. pinnata* seeds. The lower Se:S ratio observed in *N. amplexicaulis* may indicate a high ability of this species to translocate both elements, likely through different pathways (Harvey et al. 2020). In addition, differences in seed Se levels among the three hyperaccumulators suggest that species-specific regulation of whole-plant Se accumulation and allocation patterns also contributes to the reduced Se enrichment observed in *S. pinnata* seeds. In particular, while *A. bisulcatus* exhibited strong reproductive allocation of Se, consistent with its high seed Se:S ratios, *S. pinnata* appeared to maintain more conservative reproductive partitioning, reflecting differential modulation and transporter affinities involved in S/Se translocation. These divergent allocation strategies likely represent evolutionary trade-offs shaped by selective pressures in seleniferous environments, balancing elemental defense with reproductive success.

Moreover, the higher concentrations of Se, S, and other elements (e.g., P, K, Si, Mn, and Fe) in *N. amplexicaulis* seeds were likely due to the hydroponic cultivation of the mother plants prior to seed collection, in contrast to the other two species. The higher concentrations of Mg and Ca in seeds of *S. pinnata* and *A. bisulcatus*, which were grown in soil, may confer greater resilience in Se-rich environments. In particular, enrichment of these elements in seeds can enhance seedling establishment, providing hyperaccumulator species with a reproductive advantage in such environments.

The localization of Zn in the vascular bundles of the radicle and cotyledons of *A. bisulcatus* and *S. pinnata* is a common trait of these two hyperaccumulators. This distribution observed in our study aligns with previous findings showing that Zn and Fe are mostly localized in vascular tissues but completely absent in the seed coat of these two species (Freeman et al. 2012). The distribution of Zn in *N. amplexicaulis* was difficult to observe because the virtual section was oriented parallel to the cotyledons; nevertheless, high accumulation in the radicle tip was clearly evident, as also described for *S. pinnata* (Freeman et al. 2012). In general, Se was evenly distributed in the parenchyma and

root cortex of all three seed embryos. These tissues constitute most of the seed volume and are subject to seed herbivory; thus, the uniform distribution of this element may provide an effective defense (Freeman et al. 2012). High concentrations of Se in the cotyledons and low levels in the seed coat have also been reported for *C. violifolia* seeds (Xie et al. 2023). Thanks to the non-destructive imaging technique (XFM-CT), some previously unobserved features were detected: (i) In *S. pinnata*, the inner layer of the seed coat shows a band enriched in Se, which may serve as an additional defense line against herbivores; (ii) *A. bisulcatus* seeds contained abundant endosperm, which lacked Se. For the latter feature, a possible explanation is that the endosperm mainly serves as a nutrient reserve for the embryo; therefore, Se may be excluded to prevent potential toxicity to the developing embryo or to avoid interference with early metabolic processes driven by Se-sensitive enzymes.

5 | Conclusions

Synchrotron XFM-CT analysis enabled detailed mapping of Se and Zn in the seeds of three Se hyperaccumulator plants, revealing similarities as well as some differences among the species. The uniform distribution of Se across the cotyledons and radicle parenchyma appears to support the defense function of this metalloid in hyperaccumulator plants. Future studies linking the spatial distribution of Se to the expression of specific genes in seeds will provide deeper insight into the physiology of these species.

Author Contributions

M.S., A.V.D.E., M.S., and M.-A.H. conducted the synchrotron X.F.M. experiment. M.S. conducted the elemental analysis. D.B. processed the synchrotron X.F.M. data. All authors contributed to write, review, and edit the manuscript.

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Conflicts of Interest

A.V.D.E. serves as a Guest Editor of this special feature, but had no involvement in the peer review and decision-making processes for this article.

Data Availability Statement

The data that support this study will be shared upon reasonable request to the corresponding author.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Longitudinal virtual sections of *Astragalus bisulcatus* seeds: (a) seed absorption; (b) zinc map; (c) selenium map. Elemental concentrations are expressed as $\mu\text{g cm}^{-3}$. **Figure S2:** Longitudinal virtual sections of *Neptunia amplexicaulis* seeds. First and third columns: seed absorption; second and fourth columns: selenium maps. Elemental concentrations are expressed as $\mu\text{g cm}^{-3}$.