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# Effects of ionizing radiation decontamination on botanical collections in herbaria

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ABSTRACT

Herbaria collections are very sensitive to attacks from microorganisms and insects. Therefore, preservation strategies and appropriate treatments are essential to manage these artifacts. Decontamination by ionizing radiation has become an effective strategy to preserve cultural heritage objects and archived materials, achieving excellent results. Therefore, this work aimed to study the effects of Co-60 gamma radiation on botanical collections. To accomplish this, samples of exsiccates, including botanical pressed and dehydrated specimens from Asteraceae and Solanaceae families, collected on diferentes dates were selected from the Dom Bento José Pickel Herbarium (SPSF), located in São Paulo (Brazil). Irradiation was performed at the Multipurpose Gamma Irradiation Facility at IPEN, applying absorbed doses of 1 kGy, 6 kGy and 10 kGy. Gamma radiation effect was analyzed using colorimetry with CIELAB color space scale, scanning electron microscopy (SEM) and attenuated total reflectance with Fourier transform infrared (ATR-FTIR) spectroscopy. Results showed no significant colorimetric changes, or changes in the morphological properties of samples, indicating that this decontamination method can be used as an alternative treatment to eliminate insects and micro-fungi of botanical collections without the use of toxic substances.

## 1. Introduction

Herbaria preserve botanical materials, such as exsiccatae, fungi, algae and wood samples, as a dynamic database maintained within a custodial framework that requires knowledge, specialized staff and adequate management practices (IBGE - Fundação Instituto Brasileiro de Geografia e Estatística and Departamento de Recursos Naturais e Estudos Ambientais, 1992; Peixoto and Morim, 2003; Peixoto and Maia, 2013; Thiers, 2021). Herbarium collections act as a repository of information, and curators certify plant biological diversity, essential for studies in various fields of research (Besnard et al., 2018; Bieker and Martin, 2018; Carine et al., 2018; Espinosa and Castro, 2018; Funk, 2003; Heberling and Isaac, 2017; James et al., 2018; Rocchetti et al., 2021). Herbaria curators gather specimens that are unique and irreplaceable biodiversity samples collected in a manner specific to location, condition and time. As such, these specimens cannot be reproduced. Monteiro et al. (2009) emphasized that preventive procedures for contamination reduction of botanic specimen start at field collection. In order to assure low level of microbiological contamination and/or insects of exsiccate for herbaria collection several procedures may be applied during preparation, drying rooms and collection assembly, to stored materials and building (Guarino et al., 2020; Hall, 1988).

Consequently, curators must guarantee the preservation and perennial protection of specimens in their custody (Rabeler et al., 2019). More specifically, these collections require constant monitoring, and many of them are vulnerable to storage conditions in environments with no thermal or humidity control, presence of dirt, low protection or inadequate pest control. Curators must also be mindful of the intrinsic condition of the cycle of natural life and fragility of this material (Bridson and Forman, 1992; Hall, 1988; Joseph, 2021; Mori et al., 1989). For disinfection and disinfestation of cultural heritage artifacts and document collections using gamma rays from Cobalt-60 is an alternative and has also been used effectively for different materials. Radiation processing can help preserve these records against microorganismal attack (Adamo et al., 2004; Cappitelli et al., 2020; De Tassigny and Brouqui, 1978; IAEA, 2017; Santos, 2017).

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However, it is well known that the interaction of radiation transfers energy to materials and can cause changes in molecular and physico-chemical properties. In response, researchers have investigated the effects of ionizing radiation on objects composed of different materials to guarantee the safety of this practice. In general, based on treatment research reported in the scientific literature, it has been established that irradiation, in a dose range between 1 kGy and 10 kGy necessary to eradicate insects with fungicidal effect, carries minimal risk to the preservation of cultural heritage collections and are effective in reducing microbiological contamination in cellulose material. (Adamo et al., 2004; Baccaro and Cemmi, 2017; Cappitelli et al., 2020; Cortella, 2019; Cortella et al., 2020; FAO - Food and Agriculture Organization for the United Nations, 2003; IAEA, 2017; IAEA, 2022; Kodama, et al., 2016; Magaudda, 2004; Moise et al., 2017; Moise et al., 2019; Nagai et al., 2021; Ponta et al., 2017; Severiano et al., 2010; Silva et al., 2006).

For this study, the possible effects of irradiation on the color of processed herbaria materials were investigated. The CIE Lab L*a*b* system, published in 1976 by the Commission Internationale d'Eclairage (CIE), has become the universally accepted colorimetric reference system for quantifying and communicating color (ASTM International, 2016; CIE, 2004; Minolta, 2007). Analysis of chromatic changes is a sound scientific method for evaluating the secondary effects of gamma radiation treatment on different cultural heritage artifacts. The vast majority of these artifacts are composed of natural organic materials of animal origin (proteins) or plant origin (cellulose) (IAEA, 2017).

Scanning electron microscopy (SEM) is also a valuable tool for leaf surface analysis in systematic studies to identify particular structures of different families and species. With the use of this technique applied to plant characterization, Chen et al. (2015) showed that SEM could be successfully used to analyze the morphology (length, density and shape) of hair, stomatal level and appearance of the leaf surface characteristics of the herborized species *Timonius* DC. (Rubiaceae). Budel et al. (2018) used this technique to investigate the comparative leaf anatomy and micro-morphology of six species of Baccharis and identified differences important and unique in the cuticle, stomata, trichomes and tissues.

Fourier-transform infrared (FTIR) spectroscopy provides information on the vibrational modes of chemical bonds in the organic compounds of botanical samples. Studies have shown that this technique is an important resource in the discrimination of specimens from herbaria, characterizing and quantifying their organic compounds (Botelho, 2017; Wang, 2012).

Thus, this study will employ all of these tools to investigate the effects of ionizing radiation on disinfestation and decontamination processes in exsiccates from herbaria. Samples of exsiccates were irradiated using gamma rays with absorbed doses between 1 kGy and 10 kGy and were evaluated to verify any changes in color, chemical, molecular and morphological aspects.

## 2. Experimental

### 2.1. Sample preparation

Six representative botanical samples of exsiccates were selected from vouchers of the Dom Bento José Pickel Herbarium (SPSF). Samples from the Asteraceae and Solanaceae families were selected from replicates of the collection, while the main specimen of the collection remained untouched. In the herbaria, these selected taxa are more often susceptible to attack by pests than others, especially Asteraceae. Exsiccatae represent a food source for a variety of insect pests that can cause damage during feeding, defecating and burrowing. (Hall, 1988; Mori et al., 1989; Bridson and Forman, 1992; Guarino et al., 2020). Table 1 and Fig. 1 show the main identification and classification of the selected materials.

### 2.2. Gamma ray irradiation

Samples of exsiccates were irradiated with gamma rays from Co-60 at the Multipurpose Gamma Irradiation Facility of the Nuclear and Energy Research Institute (IPEN) of the National Nuclear Energy Commission (CNEN) in São Paulo. The currently installed Cobalt-60 activity of the facility is 10.4 PBq (320 kCi).

**Table 1**
Samples of exsiccates: main identification and general classification.

| Sample No. | Botanical family | Species | Collection date |
|---|---|---|---|
| SPSF-4021 | Asteraceae | *Baccharis crispa* Spreng. | 1946 |
| SPSF-10516 | Asteraceae | *Critoniopsis quinqueflora* (Less.) H. Robinson | 1984 |
| SPSF-10553 | Asteraceae | *Baccharis regnellii* Sch. Bip. ex Baker | 1986 |
| SPSF-4074 | Solanaceae | *Solanum swartzianum* Roem. & Schult. | 1953 |
| SPSF-08821 | Solanaceae | *Solanum pseudoquina* A. St.-Hil. | 1984 |
| SPSF-39975 | Solanaceae | *Solanum mauritianum* Scop. | 2007 |

Parts of leaves, flowers and branches from exsiccate samples were prepared and packed in paper envelopes. Then, in January of 2019, they were irradiated by gamma rays with radiation absorbed doses of 1 kGy, 6 kGy and 10 kGy, with 5–6 kGy $h^{-1}$ dose rate. Selected doses were applied taking into consideration insect eradication and disinfection (IAEA, 2017). The polymethylmethacrylate (PMMA) dosimetry system and UV-VIS spectrophotometry were used in order to estimate the absorbed dose in the irradiated samples.

### 2.3. Colorimetric measurements

For this study, the CIE Lab L*a*b* system was used to detect color changes in samples and confirm the possible effects of irradiation on the color of processed material from herbaria. In equation (1), ΔE represents the variation between the initial reading of the non-irradiated sample and the reading after the ionizing radiation. In order to analyze the results, values of Δ were calculated for each point of the sample, where ΔE* represents the total color difference, ΔL* represents the difference in lightness and darkness (+= lighter, - = darker), and Δa* and Δb* represent red to green and yellow to blue, respectively (ASTM International, 2016; CIE, 2004; Minolta, 2007).

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (1)$$

For measurements, a PCE-CSM8 spectrophotometer was used. A white tile was used as base for samples measurements, with a circle mark to keep always the same geometric position of colorimeter in relation to the sample's points. Window of colorimeter measurement was 1 cm diameter. Readings were taken at three selected points in each sample (A = flowers/inflorescences, B = leaves/branches (adaxial surface) and C = leaves/branches (abaxial surface) (Fig. 2). Each point was measured by 10 consecutive readings, mean value and error were calculated using PCE software.

### 2.4. Field-emission gun scanning electron microscopy (FE-SEM)

Scanning electron microscopy was used to analyze and characterize the non-irradiated (0 kGy) and irradiated (10 kGy) exsiccates. SEM images were obtained using Jeol JSM-6701F equipment with a field emission gun operating at 2 kV and 3 kV accelerating voltages. Sample pieces were fixed in a sample holder with a conductive double-sided carbon tape and then carbon coated to improve conductivity and reduce electron charging effect.


**Fig. 1.** Samples of exsiccates used for irradiation. Asteraceae: (a) *Baccharis crispa* (SPSF-4021), (b) *Critoniopsis quinqueflora* (SPSF-10516) and (c) *Baccharis regnellii* (SPSF-10553); Solanaceae: (d) *Solanum swartzianum* (SPSF-4074), (e) *Solanum pseudoquina* (SPSF-08821) and (f) *Solanum mauritianum* (SPSF-39975).

### 2.5. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

Infrared spectroscopy was used to analyze the changes or formation of chemical groups before and after the processing of botanical samples by ionizing radiation. ATR-FTIR spectra were acquired on Nicolet 6700 equipment (Thermo Scientific®). Analyses were conducted with ATR mode (Pike Miracle®) using a diamond crystal. Constant pressure was applied to samples to ensure good contact between the crystal and the sample. Data were obtained from 3200 to 600 $cm^{-1}$ (medium infrared) and mediating 64 scans with 4 $cm^{-1}$ resolution. In the six botanical specimens, spectral readings were performed on both sides of leaves (abaxial and adaxial) and on flowers.

## 3. Results and discussion

### 3.1. Colorimetric measurements

Table 2 and Fig. 3 present the colorimetric analysis at three points: A - flower/inflorescence, B - leaf (adaxial surface) and C - leaf (abaxial surface) from samples irradiated with different doses: 1 kGy, 6 kGy and 10 kGy. For color change evaluation after irradiation, we used the criteria proposed by Hardeberg et al. (1999) to interpret color change according to the ΔE value (Eq. (1)). For our samples, results indicate that color change was predominantly *"hardly perceptible"* based on $\Delta E < 3$, and *"perceptible, but acceptable"* based on $3 < \Delta E < 6$ for the flower of sample SPSF-08821, after irradiation at 10 kGy, which reached a ΔE value of 3.72. None of the measured points of the samples had a *"un-acceptable"* color change ($\Delta E > 6$). The table presents the mean values, considering 10 sequential measurements of each set of points and mean value was calculated by PCE software.

Fig. 3 shows the behavior of each sample in response to irradiation in terms of color change. No specific similarities were identified among the three species of Asteraceae when compared with the three species of the Solanaceae. The aging effect also showed no correlation when analyzing the species according to their dates of collection: 1940–60 (SPSF-4021 and SPSF-4074), 1980–90 (SPSF-10516, SPSF-08821, SPSF-10553) and 2007 (SPSF-39975). Importantly, no significant changes were noted in the properties of irradiated samples upon application of absorbed doses up to 10 kGy. Furthermore, it is important to cite that herbaria samples consisted by small flowers, leaves with veins, stems with narrow leaves may interfere on the colorimeter positioning due to its flat base. This


**Fig. 2.** Identification of colorimetric points and PCE colorimeter measurement set-up.

**Table 2**
Results of colorimetric analysis.

| Sample | Dose (kGy) | Point A (ΔE) | Point B (ΔE) | Point C (ΔE) |
|---|---|---|---|---|
| SPSF-4021 | 1 | 1.751 | 0.206 | 1.152 |
| | 6 | 1.655 | 0.994 | 0.009 |
| | 10 | 0.913 | 1.038 | 0.095 |
| SPSF-10516 | 1 | 0.376 | 1.798 | 0.754 |
| | 6 | 0.402 | 1.520 | 0.674 |
| | 10 | 0.015 | 1.986 | 0.618 |
| SPSF-10553 | 1 | 1.742 | 0.384 | 0.402 |
| | 6 | 1.616 | 0.475 | 0.616 |
| | 10 | 2.153 | 0.800 | 0.108 |
| SPSF-4074 | 1 | 0.702 | 0.175 | 0.449 |
| | 6 | 0.228 | 0.149 | 0.362 |
| | 10 | 0.062 | 0.375 | 0.688 |
| SPSF-08821 | 1 | 0.454 | 0.052 | 0.298 |
| | 6 | 1.545 | 0.583 | 0.385 |
| | 10 | 3.724 | 0.639 | 0.434 |
| SPSF-39975 | 1 | 1.144 | 0.484 | 1.308 |
| | 6 | 0.532 | 0.199 | 1.108 |
| | 10 | 1.640 | 0.369 | 1.240 |

error can be minimized by set a fixed position of measurement. By using the same sample for each step of irradiation process.

Other similar studies corroborate these results, including, for example, Gonzales et al. (2002) who demonstrated that a radiation dose around 14 kGy did not cause changes in the color of the irradiated paper. Furthermore, Choi et al. (2012) and Moise et al. (2019) reported no occurrence of significant changes in color properties on irradiated paper with absorbed doses of radiation up to 50 kGy. Vieira et al. (2019) studied ionizing radiation in doses appropriate for the disinfection of feather specimens from the collection of the Museum of Archeology and Ethnology of the University of São Paulo and no secondary effect on colors was observed. However, consensus on this subject is still uncertain since some studies did find some changes in the color of material caused by irradiation. For instance, Bicchieri et al. (2016) and Drábková et al. (2018) detected color change and yellowing of their tested paper samples with gamma irradiation doses between 2 and 9 kGy. Owing to their composition, some materials may suffer changes, as determined by Ponta et al. (2017) who confirmed color changes in glass objects and transparent stones, as well as browning and yellowing in bones and ivories. Additionally, in the experiment of Vujcic et al. (2019), results indicated a darkening of the color in samples with dyes in wool, linen, silk and cotton when exposed to gamma radiation doses from 0.5 to 25 kGy. Therefore, the peculiar characteristics of each material and the color changes that naturally occur over time must also be taken into account in the analysis and reproduction of these results.

### 3.2. Scanning electron microscopy (SEM) analysis

Fig. 4 depicts SEM images of exsiccate samples before and after submission to irradiation at a dose of 10 kGy. A comparison showed

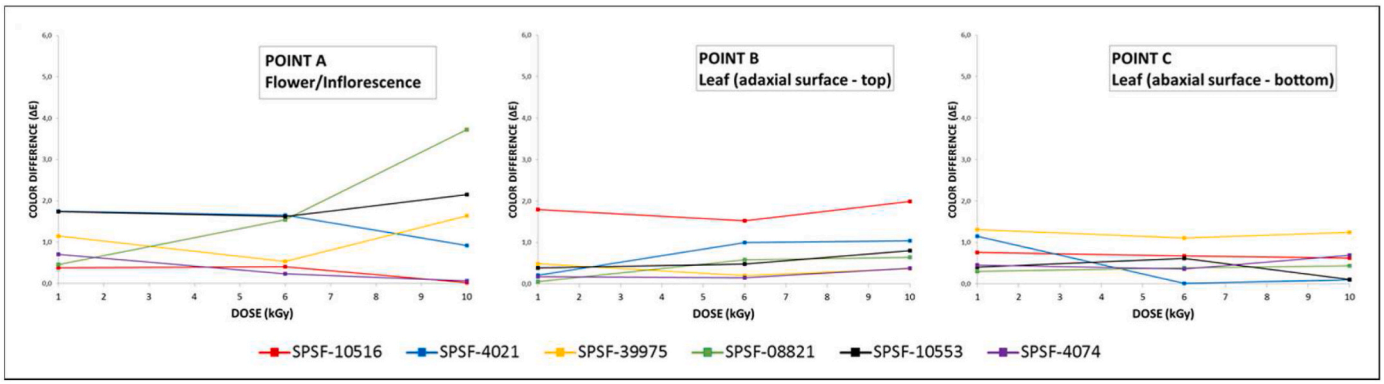


**Fig. 3.** Diagram of total color difference for exsiccate samples irradiated with different doses of gamma rays (1 kGy, 6 kGy and 10 kGy).

similar structures to be perfectly preserved after irradiation. The topographic structures of irradiated samples did not suffer significant damage when a 10 kGy dose was applied, making it possible to perform taxonomic and anatomical studies, for example, on herborized specimens after irradiation.

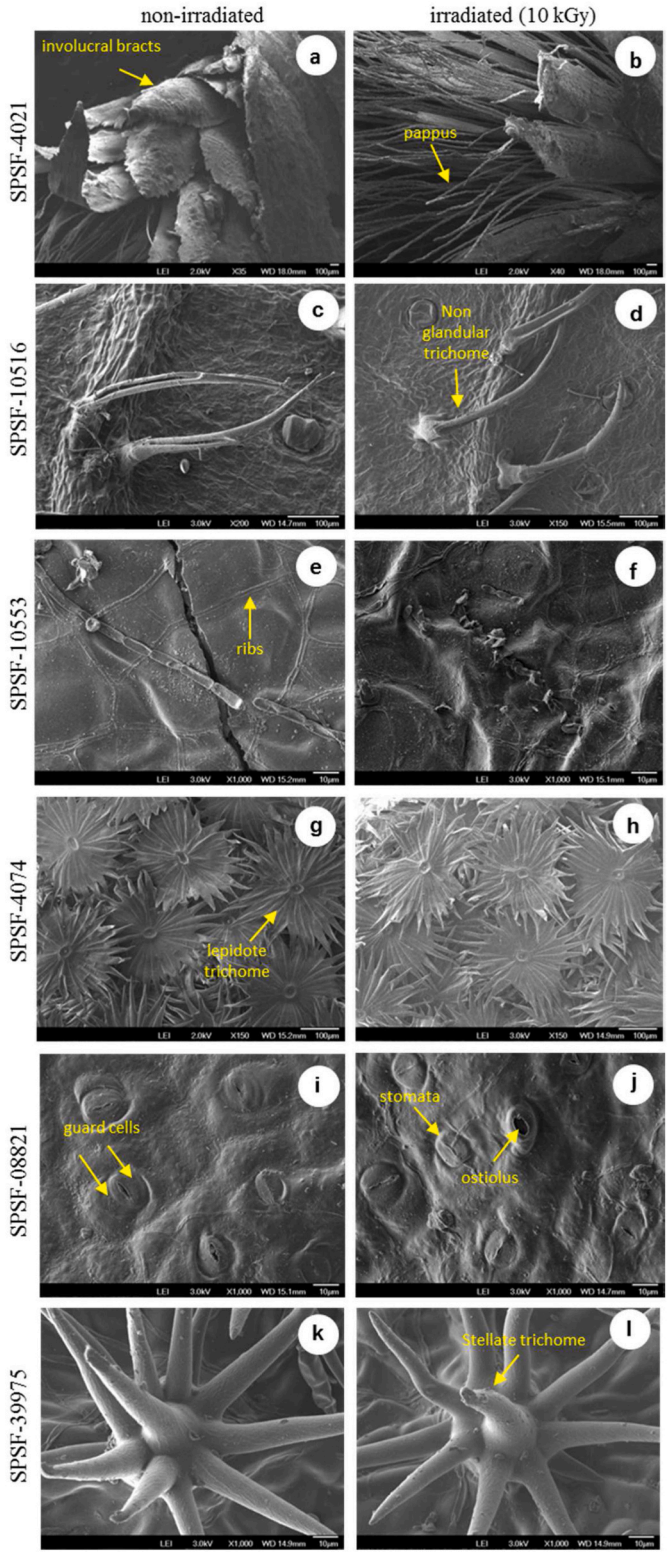


**Fig. 4.** SEM micrographs of non–irradiated (0 kGy) and irradiated (10 kGy) samples: SPSF-4021(a,b), SPSF-10516 (c,d), SPSF-10553 (e,f), SPSF-4074 (g,h), SPSF-08821 (i,j) and SPSF-39975 (k,l).

In Fig. 4 (a), which shows sample SFSP-4021 (*Baccharis crispa*), the calyx of the flower is composed of involucral bracts that protect the internal reproductive elements of the capitulum (inflorescence of the Asteraceae). In Fig. 4 (b), in addition to the bracts, it is possible to see the marginal filaments with bristly papus. Note that the structures are intact and preserved. In Figures (c) and (d), which show sample SPSF-10516 (*Critoniopsis quinqueflora*), non-glandular trichomes are observed. In sample SPSF-10553 (*Baccharis regnellii*) on the adaxial face (e) and (f), articulated hairs and a segmented structure and broken hair fragments can be seen. The leaf reticulum is formed by veins of various orders, which can also be open or closed (Esau, 1974). These characteristics are very important in the identification of plants in herbaria, and in the images observed, these structures are clearly visualized in the irradiated samples without compromising their identification. In sample SPSF-4074 (*Solanum swartzianum*) (g) and (h), the abaxial face has a lepidote surface, densely covered by trichomes or stellate scales. In sample SPSF-08821 (i) and (j) (*Solanum pseudoquina*), stomata can be seen on both adaxial and abaxial sides, a common character of this species (Stehmann et al., 2015). In sample SPSF-39975 (k) and (l) (*Solanum mauritianum*), stellate hairs and leaf organelles are microscopically shown to be perfectly preserved in the samples irradiated with 10 kGy.

### 3.3. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

Fig. 5 shows the FTIR spectra of exsiccate samples in the range of 3200 to 600 $cm^{-1}$. The wavenumber and assignments of the bands for plant-based samples are listed in Table 3, according to the literature (Atkins and Paula, 2002; Celino et al., 2014; Derrick et al., 1999; Luz, 2005; Silverstein et al., 1994; Wang, 2012). It is expected that changes in the chemical bond structure caused by radiation might be detected by ATR-FTIR analysis, although it could be insensitive to detect very small changes.

According to the results of non-irradiated and 10 kGy irradiated samples (Fig. 5), ATR-FTIR analysis indicates no displacement of the functional groups, in turn suggesting the absence of reactions with significant formation of new organic groups. Just a few variations in the vibrational intensity of some characteristic bands of samples were observed. Comparing the non-irradiated SPSF-4021 (black line) with the 10 kGy irradiated sample (blue line), no displacement of vibrational bands was observed after irradiation on both leaf surfaces (a-adaxial and b-abaxial). At this dose, the ionizing radiation did not promote a significant increase in intensity for either the 1000 $cm^{-1}$ region, which could be attributed to C-O stretching vibration and aromatic ring, or the 2918 and 2884 $cm^{-1}$ bands of $CH_2$ and $CH_3$ asymmetric stretching modes, respectively. Furthermore, no significant increase was noted in band intensity of the C=O carbonyl group vibrations of pectin at the 1622 $cm^{-1}$ region or C-OH vibration of cellulose at 1037 $cm^{-1}$ present in vegetable leaves. Such changes would be expected to occur by oxidation reactions between free radicals formed by the interaction of radiation with these compounds and the oxygen present in the air. For sample SPSF-10516, the adaxial surface (a) showed a slight reduction in absorption intensities of the $CH_2$ and $CH_3$ asymmetric stretching bands at 2918 and 2884 $cm^{-1}$ regions, respectively, for the sample irradiated with 10 kGy, that could suggest the bond breaking of some aliphatic chains from lipids, proteins, carbohydrates or nucleic acids of the vegetable structures.

For sample SPSF-10553, the adaxial surface (a) showed a low intensity for the characteristic $CH_2$ and $CH_3$ vibrational bands at 2923 and 2850 $cm^{-1}$ regions, respectively, and the saturated ester C=O stretch at 1735 $cm^{-1}$. The 10 kGy radiation dose promoted slight changes at the 1727 $cm^{-1}$ region of saturated ester C=O stretch and 1614 $cm^{-1}$ pectin C=O stretch correlated with a double carbonyl band, which can be seen in vegetable leaves, as well as a more pronounced C-O stretch band of cellulose at 1060 $cm^{-1}$. However, for abaxial surface samples (b), the

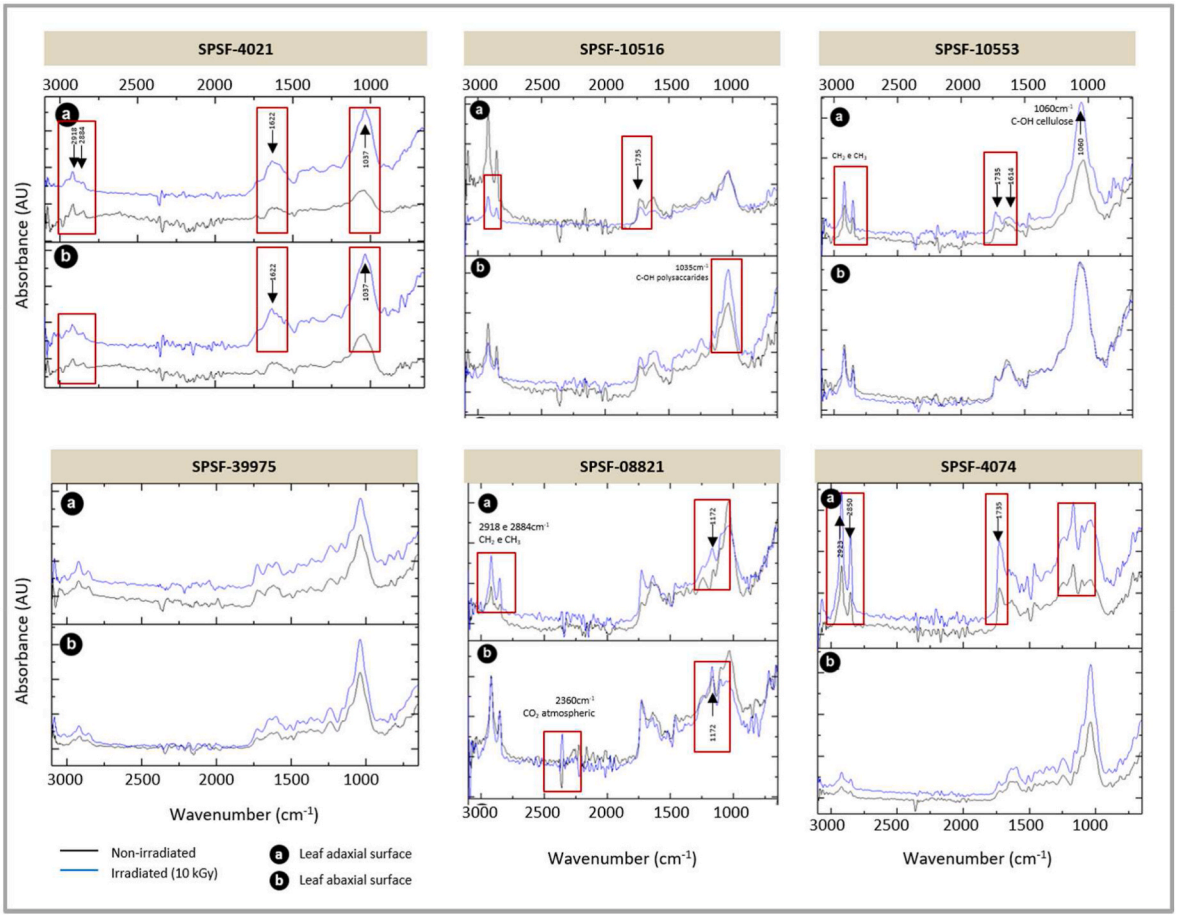


**Fig. 5.** FTIR spectra, ranging from 3200 to 600 $cm^{-1}$, obtained from botanical samples. Black lines in the figure indicate the spectral results of the non-irradiated samples, and the blue lines indicate the 10 kGy irradiated samples. (a) and (b) indicate the adaxial and abaxial surfaces of the leaf, respectively.

**Table 3**
General band assignments of the average FTIR spectrum of plants based on the literature.

| Wavenumber ($cm^{-1}$) | Definition of spectral assignments |
|---|---|
| 2923–2884 | $CH_2$ and $CH_3$, asymmetric stretching: mainly lipids with a little contribution from proteins, carbohydrates, and nucleic acids |
| 2366 | $CO_2$ stretch of atmospheric carbon dioxide |
| 1735–1727 | Saturated ester C=O stretch: phospholipids, cholesterol esters, hemicellulose, and pectin |
| 1640–1614 | Amide I (protein C=O stretch): protein and pectin, O-H bending: sample's residual water |
| 1604 | C=O aromatic stretching: lignin |
| 1403 | O-H bending: cell wall polysaccharides, alcohols and carboxylic acids |
| 1261–1224 | Amide and amines (C=N and N-H stretching): mainly proteins |
| 1170 | Symmetric stretching modes of aliphatic $CH_2$, OH or CO of various groups: cell wall polysaccharides |
| 1145 | Cellulose (β-1,4 glucan) |
| 1050–1020 | C-O stretching: cell wall polysaccharides (glucomannan) OH and C-OH stretching: cell wall polysaccharides (arabinan, cellulose) |

ionizing irradiation at 10 kGy did not cause any perceptible changes in vibrational spectra.

For the adaxial surface of sample SPSF-08821 (a), comparing the non-irradiated and the 10 kGy irradiated sample, a slight increase in the intensity of the 1172 $cm^{-1}$ band was observed, which could be attributed to the symmetric stretching modes of aliphatic $CH_2$, OH, or CO of various groups of cell wall poly-saccharides. For the abaxial surface, a band appeared in the 2360 $cm^{-1}$ region of the 10 kGy irradiated sample, typically associated with the atmospheric carbon dioxide $CO_2$ band (Derrick et al., 1999). The appearance of this band is not reported in the literature for compounds present in plant leaves, so much so that they did not appear in the other samples analyzed. Although this band was derived from the irradiated sample, it cannot be claimed that the degradation actually occurred because this region is very sensitive to the $CO_2$ formation that results from manipulation of the sample or background misdirected compensation. Furthermore, no other bands present in the sample showed any significant changes.

The spectra of SPSF-4074 adaxial (a) and abaxial (b) surface samples irradiated with 10 kGy did not show changes in the vibrational intensities of the $CH_2$ and $CH_3$ bands at 2923 and 2850 $cm^{-1}$ regions, the saturated ester C=O stretch at 1735 $cm^{-1}$, the C=N stretching of proteins at 1240 $cm^{-1}$ or the C-C, C-OH, and C-O bands of the cellulose at 1072-1025 $cm^{-1}$.

## 4. Conclusion

The results showed no significant changes in the measured properties of irradiated samples when applying absorbed doses of 1 kGy, 6 kGy and 10 kGy. Color changes between non-irradiated samples and irradiated samples in the highest absorbed dose are noticeable, but acceptable, according to the adopted scale. The analysis of microscopic images of non-irradiated and 10 kGy irradiated samples did not show significant differences in the topographic morphology of exsiccate samples. According to the ATR-FTIR results of non-irradiated and 10 kGy irradiated samples, the spectra showed some slight changes for the adaxial side of three of them, but we can consider, in general, no significant

displacements of functional groups or significant molecular changes after irradiation. Thus, the present study demonstrated that gamma radiation applied to botanical collections, such as exsiccate for fungicide purposes, is safe up to a radiation absorbed dose of 10 kGy without significantly modifying, the optical, morphological, and topological properties and characteristics. After decontamination of the material by the ionizing radiation method, it is important that the treated specimens be properly stored to avoid new contamination. Although safe for the treatment of cultural heritage collections, adequate care must still be taken going forward since the absorbed doses are cumulative, and repeated processing may damage organic heritage material. It should also be noted that the use of ionizing radiation is not a readily available technology easy to access since radiactive installations are complex, require specialized technicians, safety and security requirements to be attended, likely making this technique unavailable to herbaria in remote areas with limited resources.

## CRediT authorship contribution statement

**Leni Meire Pereira Ribeiro Lima**: Conceptualization, Data Curation, Methodology, Validation, Formal Analysis, Investigation, Writing – Original Draft, Writing - Review and Editing, Visualization. **Yasko Kodama**: Data Curation, Investigation, Resources, Writing - review. **João Batista Baitello**: Data Curation, Validation, Resources. **Larissa Otubo**: Data Curation, Investigation, Resources, Writing - review. **Paulo de Souza Santos**: Data Curation, Investigation, Resources. **Pablo A.S. Vasquez**: Conceptualization, Methodology, Formal Analysis, Supervision, Project Administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

Adamo, M., Magaudda, G., Tata, A., 2004. Radiation technology for cultural heritage restoration. Restaurator 25 (3), 159–170. https://doi.org/10.1515/REST.2004.159.

ASTM International, 2016. Standard Practice for Calculation of Color Tolerances and Color Differences from Instrumentally Measured Color Coordinates. ASTM, United States (Designation D2244 – 16).

Atkins, P., Paula, J., 2002. Físico-Química. Tradução de Edilson C. da Silva, Márcio J.E. M. Cardoso, Oswaldo E. Barcia. Rio de Janeiro, 2. LTC Livros Técnicos e Científicos Editora S.A.

Baccaro, S., Cemmi, A., 2017. Radiation activities and application of ionizing radiation on cultural heritage at ENEA Calliope gamma facility (Casaccia R.C., Rome, Italy). Nukleonika 62 (4), 261–267. https://doi.org/10.1515/nuka-2017-0038.

Besnard, G., Gaudeul, M., Lavergne, S., Muller, S., Rouhan, G., Sukhorukov, A.P., Vanderpoorten, A., Jabbour, F., 2018. Herbarium-based science in the twenty-first century. Botany Letters 165 (3–4), 323–327. https://doi.org/10.1080/23818107.2018.1482783.

Bicchieri, M., Monti, M., Piantanida, G., Sodo, A., 2016. Effects of gamma irradiation on deteriorated paper. Radiat. Phys. Chem. 125, 21–26. https://doi.org/10.1016/j.radphyschem.2016.03.005.

Bieker, V.C., Martin, M.D., 2018. Implications and future prospects for evolutionary analyses of DNA in historical herbarium collections. Botany Letters 165 (3–4), 409–418. https://doi.org/10.1080/23818107.2018.1458651.

Botelho, I.M.S., 2017. Uso da espectroscopia no infravermelho próximo (FT-NIR) como ferramenta na discriminação de espécies herborizadas de Burseraceae oriundas de diferentes locais da Amazônia Legal. Dissertação (Mestrado em Botânica). Instituto Nacional de Pesquisas da Amazônia, Manaus, p. 75.

Bridson, D., Forman, L., 1992. The Herbarium Handbook. Richmont: Royal Botanic Gardens, Kew.

Budel, J.M., Raman, V., Monteiro, L.M., Almeida, V.P., Bobek, V.B., Heiden, G., Takeda, I.J.M., Khan, I.A., 2018. Foliar anatomy and microscopy of six Brazilian species of Baccharis (Asteraceae). Microsc. Res. Tech. 81, 832–842. https://doi.org/10.1002/jemt.23045.

Cappitelli, F., Catto, C., Villa, F., 2020. The control of cultural heritage microbial deterioration. Microorganisms 8 (10), 1542. https://doi.org/10.3390/microorganisms8101542.

Carine, M.A., Cesar, E.A., Ellis, L., Hunnex, J., Paul, A.M., Prakash, R., Rumsey, F.J., Wajer, J., Wilbraham, J., Yesilyurt, J.C., 2018. Examining the spectra of herbarium uses and users. Botany Letters 165 (3–4), 328–336. https://doi.org/10.1080/23818107.2018.1482782.

Celino, A., Gonçalves, O., Jacquemin, F., Fréour, S., 2014. Quantitative and qualitative assessment of water sorption in natural fibres using ATR-FTIR spectroscopy. Carbohydr. Polym. 101, 163–170. https://doi.org/10.1016/j.carbpol.2013.09.023.

Chen, J., Tan, H.T.-W., Wong, K.M., 2015. All that shimmers is not the same: silvered leaves and micromorphological diversity in Timonius DC. (Rubiaceae). Plant Systemat. Evol. 301 (2), 841–848. https://doi.org/10.1007/s00606-014-1114-9.

Choi, J., Chung, Y.J., Kang, D.I., Lee, K.S., Lee, J., 2012. Effect of radiation on disinfection and mechanical properties of Korean tradicional paper. Hanji. Radiat. Phys. Chem. 81, 1051–1054. https://doi.org/10.1016/J.RADPHYSCHEM.2011.11.019.

CIE (Comission Internationale de l'Éclairage), 2004. Colorimetry. CIE Technical Report 15, 3ª ed. CIE Central Bureau, Vienna.

Cortella, L., 2019. Gamma radiation processing for cultural heritage preservation - biocide treatment of organic materials and consolidation of wooden degraded artifacts by radiation curing resins. In: Radioactive Techniques for Diagnosis and Conservation of Cultural Heritage, International Symposium on Non-destructive Testing of Cultural Heritage, Daejeon, 4 September 2019. National Research Institute of Cultural Heritage, Daejeon, Republic of Korea, pp. 130–144.

Cortella, L., Albino, C., Tran, Qk, Froment, K., 2020. 50 years of French experience in using gamma rays as a tool for cultural heritage remedial conservation. Strasbourg: 19th International Meeting on Radiation Processing (IMRP). Radiat. Phys. Chem. 171 https://doi.org/10.1016/j.radphyschem.2020.108726.

Preprints De Tassigny, C., Brouqui, M., 1978, October 1978/17/5. Adaptation à la désinfection de la momie de Ramsés II du procédé de radio-stérilisation gamma, 1–8. ICOM Committee for Conservation 5th triennial meeting, Zagreb, pp. 1–16 (In French).

Derrick, M.R., Stulik, D., Landry, J.M., 1999. Infrared Spectroscopy in Conservation Science. Scientific Tools for Conservation, 248p. Getty Conservation Institute, Los Angeles, CA. https://doi.org/10.1017/CBO9781107415324.004.

Drábková, K., Durovic, M., Kucerová, I., 2018. Influence of gamma radiation on properties of paper and textile fibres during disinfection. Radiat. Phys. Chem. 152, 75–80. https://doi.org/10.1016/j.radphyschem.2018.07.023.

Esau, K., 1974. Anatomia das plantas com sementes. Tradução de Berta Lange de Morretes, São Paulo, p. 293. Edgard Blücher.

Espinosa, F., Castro, M.P., 2018. On the use of herbarium specimens for morphological and anatomical research. Botany Letters 165 (3–4), 361–367. https://doi.org/10.1080/23818107.2018.1451775.

FAO - Food and Agriculture Organization for the United Nations, 2003. Guidelines for the use of irradiation as a phytosanitary measure. International Standards for Phytosanitary Measures. ISPM n. 18. Roma. https://www.fao.org/3/a0785e/a0785e00.htm.

Funk, V., 2003. 100 Uses for an herbarium (Well at least 72). Am. Soc. Plant Taxonomists Newslett. 17 (2), 17–19.

Gonzales, M.E., Calvo, A.M., Kairiyama, E., 2002. Gamma radiation for preservation of biologically damaged paper. Radiat. Phys. Chem. 63, 263–265. https://doi.org/10.1016/S0969-806X(01)00510-2.

Guarino, S., Basile, S., Caimi, M., Carratello, A., Manachini, B., Peri, E., 2020. Insect pests of the Herbarium of the Palermo botanical garden and evaluation of semiochemicals for the control of the key pest Lasioderma serricorne F. (Coleoptera: Anobiidae). J. Cult. Herit. 43, 37–44. https://doi.org/10.1016/j.culher.2019.10.009.

Hall, A.V., 1988. Pest control in herbaria. Taxon 37 (4), 885–907. https://doi.org/10.2307/1222094.

Hardeberg, J.Y., 1999. Acquisition et reproduction d'images couleur: approches colorimétrique et multispectrale. Interface homme-machine [cs.HC]. Thèse (Docteur Specialité: Signal et Images). Ecole Nationale Supérieure des Télécommunications - Télécom ParisTech, Français, p. 253.

Heberling, J.M., Isaac, B.L., 2017. Herbarium specimens as exaptations: new uses for old collections. Am. J. Bot. 104 (7), 963–965. https://doi.org/10.3732/ajb.1700125.

IAEA, 2017. Uses of Ionizing Radiation for Tangible Cultural Heritage Conservation. In: IAEA Radiation Technology Series N., 6. International Atomic Energy Agency, Vienna.

IAEA, 2022. International Database on Insect Disinfestation and Sterilization. International Atomic Energy Agency, Vienna. http://www-ididas.iaea.org.

IBGE - Fundação Instituto Brasileiro de Geografia e Estatística, Departamento de Recursos Naturais e Estudos Ambientais, 1992. Manual Técnico da Vegetação Brasileira. (Série Manuais Técnicos em Geociências), 1. IBGE, Rio de Janeiro, p. 92.

James, S.A., Soltis, P.S., Belbin, L., Chapman, A.D., Nelson, G., Paul, D.L., Collins, M., 2018. Herbarium data: global biodiversity and societal botanical needs for novel research. Applications Plant Sci. 6 (2) https://doi.org/10.1002/aps3.1024.

Joseph, E., 2021. Microorganisms in the Deterioration and Preservation of Cultural Heritage. Springer, p. 367. https://doi.org/10.1007/978-3-030-69411-1 [recurso eletrônico].

Kodama, Y., Rodrigues Jr., O., Garcia, R.H.L., Santos, P.S., Vasquez, P.A.S., 2016. Study of free radicals in gamma irradiated cellulose of cultural heritage materials using Electron Paramagnetic Resonance. Radiat. Phys. Chem. 124, 169–173. https://doi.org/10.1016/j.radphyschem.2016.02.018.

Luz, B.R., 2005. Propriedades espectrais das plantas no infravermelho termal (2,5 - 14 μm): da química ao dossel. Tese (Doutorado em Ciências) Instituto de Biociências da Universidade de São Paulo, p. 188.

Magaudda, G., 2004. The recovery of biodeteriorated books and archive documents through gamma radiation: some considerations on the results achieved. J. Cult. Herit. 5 (1), 113–118. https://doi.org/10.1016/2Fj.culher.2003.07.003.

Minolta, 2007. Precise Color Communication: Color Control from Perception to Instrumentation. Minolta Co. Ltd., Japan, p. 59.

Moise, I.V., Ene, M., Negut, C.D., Cutrubinis, M., Manea, M.M., 2017. Radiation processing for cultural heritage preservation – Romanian experience. Nukleonika 62 (4), 253–260. https://doi.org/10.1515/nuka-2017-0037.

Moise, I.V., Manea, M.M., Vasilca, S., Pintilie, C., Virgolici, M., Cutrubinis, M., Stanculescu, I.R., Meltzer, V., 2019. The crosslinking behaviour of cellulose in gamma irradiated paper. Polym. Degrad. Stabil. 160, 53–59.

Monteiro, S.S., Siani, A.C.A., 2009. Conservação de Exsicatas em Herbários: Contribuição ao Manejo e Preservação. Revista Fitos, [S.l.], 4, pp. 24–37.

Mori, S.A., Silva, L.A.M., Lisboa, G., Coradin, L., 1989. Manual de manejo do herbário fanerogâmico. 2, Ilhéus. Centro de Pesquisas do Cacau, p. 104.

Nagai, M.L.E., Santos, P.S., Otubo, L., Oliveira, M.J.A., Vasquez, P.A.S., 2021. Gamma and electron beam irradiation effects for conservation treatment of cellulose triacetate photographic and cinematographic films. Radiat. Phys. Chem. 171 (182) https://doi.org/10.1016/j.radphyschem.2021.109395.

Peixoto, A.L., Maia, L.C., 2013. Manual de procedimentos para herbários [recurso eletrônico]/Organizadoras: Ariane Luna Peixoto e Leonor Costa Maia [escritos]: Pedro da Costa Gadelha Neto... [et al.] – Recife: Ed. Universitária da UFPE, 52p. http://inct.florabrasil.net/wp-content/uploads/2013/11/Manual_Herbario.pdf.

Peixoto, A.L., Morim, M.P., 2003. Coleções botânicas: documentos da biodiversidade brasileira. Cienc. Culto. São Paulo 55 (3), 21–24. http://www.jstor.org/stable/3179346.

Ponta, C.C., Havermanns, J.B.G., Tran, Q.K., Cortella, L., 2017. Effects of ionizing radiation on materials. In: IAEA, 2017. Uses of Ionizing Radiation for Tangible Cultural Heritage Conservation. IAEA Radiation Technology Series N. 6. International Atomic Energy Agency, Vienna, pp. 61–91.

Rabeler, R.K., Svoboda, H.T., Thiers, B., Prather, L.A., Macklin, J.A., Lagomarsino, L.P., Majure, L.C., Ferguson, C.J., 2019. Herbarium practices and Ethics, III. St. Louis, EUA: American society of plant taxonomists. Syst. Bot. 44 (1), 7–13. https://doi.org/10.1600/036364419X697840.

Rocchetti, G.A., Armstrong, C.G., Abeli, T., Orsenigo, S., Jasper, C., Joly, S., Bruneau, A., Zytaruk, M., Vamosi, J.C., 2021. Reversing extinction trends: new uses of (old) herbarium specimens to accelerate conservation action on threatened species. Tansley review. 2020. New Phytologist 230, 433–450. https://doi.org/10.1111/nph.17133.

Santos, P.S., 2017. Estudo e otimização dos parâmetros de processamento por radiação gama em escala industrial considerando fatores operacionais. Dissertação (Mestrado em Tecnologia Nuclear). Instituto de Pesquisas Energéticas e Nucleares, São Paulo, p. 92.

Severiano, L.C., Lahr, F.A.R., Bardi, M.A.G., Santos, A.C., Machado, L.D.B., 2010. Influence of gamma radiation on properties of common Brazilian wood species used in artwork. Prog. Nucl. Energy 52 (8), 730–734.

Silva, M., Moraes, A.M.L., Nishikawa, M.M., Gatti, M.J.A., Alencar, M.A.V., Brandão, L. E., Nóbrega, A., 2006. Inactivation of fungi from deteriorated paper materials by radiation. Int. Biodeterior. Biodegrad. 57, 163–167. www.elsevier.com/locate/ibiod.

Silverstein, R.M., Bassler, G.C., Morril, T.C., 1994. Identificação espectrométrica de compostos orgânicos, 5ª ed. Guanabara Koogan, Rio de Janeiro.

Stehmann, J.R., Mentz, L.A., Agra, M.F., Vignoli-Silva, M., Giacomin, L., Rodrigues, I.M. C., 2015. Solanaceae. Lista de Espécies da Flora do Brasil [recurso eletrônico]. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB225.

Thiers, B.M., 2021. The World's Herbaria 2021: a Summary Report Based on Data from Index Herbariorum - a Global Directory of Public Herbaria and Associated Staff. Botanical Garden's Virtual Herbarium, New York, NY, USA [Recurso eletrônico]. http://sweetgum.nybg.org/ih/.

Vieira, A.C.D., Kodama, Y., Otubo, L., Santos, P.S., Vasquez, P.A.S., 2019. Effect of ionizing radiation on the color of featherwork. In: International Nuclear Atlantic Conference, October 21-25, 2019, Santos, SP. Proceedings. Associação Brasileira de Energia Nuclear, Rio de Janeiro, pp. 2662–2676, 2019.

Vujcic, I., Masic, S., Medic, M., Milicevic, B., Dramicanin, M., 2019. The influence of gamma irradiation on the color change of wool, linen, silk, and cotton fabrics used in cultural heritage artifacts. Radiat. Phys. Chem. 156, 307–313. https://doi.org/10.1016/j.radphyschem.2018.12.001.

Wang, Y., 2012. Application of fourier transform infrared microspectroscopy (FTIR) and thermogravimetric analysis (TGA) for quick identification of Chinese herb Solanum lyratum. Plant Omics J. 5 (6), 508–513, 1836-3644.