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Multi-Elemental Analysis and 2D Image Mapping within Roots, Leaves and Seeds from *O. glaberrima* Rice Plants Using Micro-PIXE Technique

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Abstract

Understanding metal accumulation at organ level in roots, leaves and seeds in *O. glaberrima* (OG) is crucial for improving physiological and metabolic aspects in growing Asian and African rice in salted areas. The micro-analytical imaging techniques are required to reveal its accumulation and distribution within plant tissues. PIXE studies have been performed to determine different elements in rice plants. The existing microbeam analytical technique at the iThemba LABS will be applied for the 2D image mapping of fresh rice tissues to perform a concentration of low atomic mass elements (such as Al, Si, P, S, Cl, Ca, Ti, Mn, Fe, Cu, Br, Zn and K) with detection limits of typically 1 - 10 μg/g. Comparison of the distribution of the elements between leaves, root and seed samples using uptake and distribution of elements in particular environmental conditions with potential amount of salt in water have been performed. We are also expecting to indicate metal exclusion as salt tolerance strategies from leaves, root, and seed compartments using matrix correlation between samples and between elements on rice species.

Keywords

PIXE, 2D Mapping, Rice, Concentration, Elemental Analysis

1. Introduction

This *Oryza glaberrima* Steud (OG) plant was domesticated 3500 years ago in the

inland delta of the upper Niger River in present-day Mali [1]. From there it spread across all of West Africa and adapted to very different environments from the desert region of Mali to the humid forests of Sierra Leone [2]. *O. glaberrima* species show tolerance to drought and flood, pest and diseases, and exhibit the ability to grow in acid and phosphorus-deficient soils, alkaline soils, and thrive without the application of chemical fertilizer. Many of these environments and conditions are not suitable for high-yielding Asian rice species, such as *O. sativa* that came to dominate rice production in West Africa [3]. OG is largely used for household consumption and rarely sold on the market. It is a very important food security crop, as it has a short production cycle and can be harvested during the "hunger period" thus providing food when other rice crops such as Asian rice (Taiwan region) are in the germination and maturation stages.

The proposed research addresses the investigation of the metal accumulation at organ level in roots, leaves and seeds in African rice by performing 2D elemental image mapping and by determination of their elemental composition (such as Al, Si, P, S, Cl, Ca, Ti, Mn, Fe, Cu, Br, Zn and K). To facilitate the elemental spatial analysis of rice, the use of micro-analytical imaging techniques are required to reveal its accumulation and distribution within plant tissues and deduce the mechanisms underlying metal decommissioning and compartmentalization on the cellular, tissue and organ levels in case of contaminated area or hazardous genetic effect of the rice plant [4] [5]. Several micro-analytical techniques can be used for the spatial investigation of elements within biological tissues, such as microbeam X-ray diffraction, synchrotron based micro X-ray fluorescence (SR-XRF) [6], energy dispersive X-ray spectrometry (EDXS) [7] and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) [8]. The scanning microbeam PIXE technique will be applied for the 2D image mapping of rice dried tissues. The proton induced X-ray emission spectroscopy (PIXE) technique will be used to measure the concentration of low atomic mass elements from Na to U in the rice plants with detection limits of typically 1 - 10 μg/g [9] [10]. The samples to be supplied to the Tandetron Laboratory (TL) for PIXE analysis will be in dried form, prepared by freeze-drying and milling for sample homogenization. The main objective of this trial and the results obtained in this study, is to conduct a characterization of a mini-core collection of OG in irrigated lowland conditions. This mini-core collection is representative of the genetic diversity of *O. glaberrima*. Specifically, 1) determine the yield and yield components of a diversity panel 317 OG accessions under irrigated lowland conditions. 2) Determine the possible associations of phonological and morphological traits with yield and yield components amongst a panel of 317 OG accessions under irrigated lowland conditions; 3) identify OG accessions with high yield potential traits and/or traits conferring tolerance to salinity, cold, anaerobic germination or heat stress. And to cover all these aspects described above, we focused on the responses of plants in term of bioaccumulation and spatial distribution of metals in different compartmentalization of OG.

2. Materials and Methods

2.1. Plant Materials

Plant materials were collected from trials conducted using two different sets of germ Plasm. One set comprised of a diversity panel of 317 accessions of *O. Glaberrima* received from Africa Rice Genetic Resources Unit and checks: FL478 (salt tolerance), IR 29 (salt sensitive), Sahel 210 and Sahel 134. The second is comprised of an indica diversity panel of 330 genotypes received from IRRI. Both sets were evaluated under control conditions where they were grown under irrigated lowland conditions using non-saline river water (EC < 1 $dS \cdot m^{-1}$) and also under saline conditions in a concrete-lined field where salinity was maintained at 4 $dS \cdot m^{-1}$. Rice genotypes were classified into three different classes, namely, tolerant, moderately tolerant, and susceptible, based on root tolerance index. The method of hydroponic culture was modified, and elaborated [11] [12].

The check varieties used for the salinity stress were FL478 (IR 66946-3R-178-1-1), called the salt tolerant check; IR 29, the salt sensitive check; Sahel 134 (IR 31851-96-2-3-2-1) characterized by a high yield potential (between 11 and 12 tonnes of paddy per hectare), the long grain type and moderate tolerance to salinity; and Sahel 210 (ECIA 31-6066) medium duration and characterized by a potential yield of 12 tonnes of paddy per hectare with long grain and soft cooking traits.

MAGIC-indica Reference Panel Trial: The current global rice array "Reference Panel Population" is composed of an *indica* diversity panel of 330 genotypes. The trial for the reference panel was laid out using a partially replicated (P-rep) design with 10 rows (blocks) and 41 columns (plots) for each block. Out of the 325 *Magic-indica* subset, 65 selected entries (representing 20% of the population) were be replicated twice while the remaining 260 were planted in single plots. There were be 4 global checks and 1 local check (Sahel 108) replicated 4 times. This gave a total of (260 lines × 1 rep) + (65 lines × 2 reps) + (5 checks × 4 reps) = 410 elementary plots.

The plots size was be 4 m^2 (5 rows of 4 m) making a total of 5 × 20 hills = 100 hills per plot. Planting was done at a spacing of 20 cm between and within rows and 1 m alleys between blocks. Two rows of filler plants were planted around (at the beginning and end) the field to minimize border effect.

Site characteristics: The trials were conducted at the experimental farm of the Africa Rice Senegal Regional Center, Saint Louis, Senegal located in the village of Ndiaye (16˚12'05"N, 16˚16'00"W and 7 m altitude). Ndiaye is located in the Delta of the Senegal River characterized by: 1) low rainfall (200 - 300 mm), irregular, spread over two to three months period (2 - 3 months) between end of July and end of September 2) a dry season with cycles of warm winds and Dry dust-laden, often evaporative air transfer carriers; 3) very saline soils resulting from marine deposits at the soil surface coming from the water table at approximately. According to the FAO Classification (2006), soil is of the Gleysol type with a percentage of clay between 40% and 54% composed of Smaltites and

Kaolinites.

Cultural practices: Treatment to break the dormancy was done on the 20th July 2019; the nursery was sown on the 23rd July 2019 in a wet nursery and transplanted on August 17th 2019. The field was continuously irrigated after transplanting until maturity by maintaining a water depth of around 3 - 5 cm. Weeds were controlled with a mixture of 6 l/ha Propanyl (360 g/l propanyl) and 1.0 l/ha of Wee done (480 g/l of 1 2,4-D-amine) and hand weeding. A fertilizer rate of NPK 120-60-60 kg/ha and zinc (5 kg/ha) was applied in 3 applications. For all fertilizer application, 40% N, 100% P (phosphorus), 100% K (potassium) and 100% Zn were broadcast at early tillering stage. The remaining N dose was split-applied at panicle initiation (40%) and at booting stage (20%). At the time of application, the water level was reduced to 3 cm. For salinity treatment, the salinity was maintained at 4 $dS{\cdot}m^{-1}$ by applying measured quantities of ordinary table salt to the standing water when salinity levels decrease or by irrigating with fresh river water when salinity levels increase beyond 4 $dS{\cdot}m^{-1}$.

2.2. Sample Preparation

The preparation of the test samples will be performed in the starting from fresh living plants in the maturation stage, exported from Senegal. Pellets (diameter: 16 mm) made of roots, leaves, whole brown rice seeds and white rice for PIXE analysis (beam diameter: 1 - 2 cm) will be provided (4 type of pellets and 3 aliquots for each rice type; total 24 samples). For the microprobe analysis, slicing of the freeze-dried roots, leaves and whole brown rice seeds by microtome will be made for obtaining samples with dimensions inferior to 1 mm^3 (several cross-sectioned parts of 3 types for each rice; total 6 samples).

Leaves sample preparation, for micro-PIXE analysis, was performed directly by sectioning leaves using stainless steel scissors without any prior treatment and separate into inside and outside part.

Nikon SMZ1500 stereomicroscope fitted with a digital camera (Nikon DS Fi2) was used to visually inspect and photograph each sectioned leave sample, to ensure that major anatomical features were clearly distinguishable in all samples selected for micro-PIXE analysis.

Roots sample preparation from different plants material were sectioned from the whole plant using stainless steel scissor and cleaned with tap and distilled water to remove all of the soil particles then dried in an oven at 105 degree. Dried samples were then milled to a fine homogeneous powder in an analytical drill milling and pressed in a hydraulic press into pellets into size of about 1 cm in diameter for further analysis.

Seeds sample preparation were dried and milled to homogeneous powder in an analytical drill milling and pressed in a hydraulic press into pellets form under pressure of 15 Tons for further analysis.

All leave samples were coated with thin layer of carbon before mounting in the nuclear microprobe chamber for μ-PIXE analysis. We have used double-sided adhesion carbon tape to be able to stuck samples on 3 mm thick aluminum

frame.

Thin layer of carbon was deposited on front surface of all samples using high quality compact desktop vacuum coating system. This was for conductivity enhancement and charge build-up prevention. Carbon coating enables good atomic number contrast, without spurious x-ray peaks and with minimal increase of low-energy x-rays from the samples. All precautions were taken to avoid cross contamination.

2.3. Experimental Setup

Elemental analysis of the samples was carried out using micro-PIXE available at the nuclear microprobe facility, MRD, iThemba LABS, Somerset West, South Africa. The measurement of the samples was carried out with a 3 MeV proton beam generated by a 3 MV Tandetron accelerator. The Oxford magnetic quadruple triplet was used for beam focusing to a 2.5 × 2.5 μm^2 spot with an approximate current kept around ~100 pA. Scanned areas were typically analyzed in a square pattern, of up to 128 × 128 pixels.

PIXE spectra were recorded between 1 and 30 keV x-ray energy range using a Si (Li) detector placed at a take-off angle of 135˚. The effective energy resolution of the PIXE system (for the Mn Kα line) was about 159 eV. The 125 μm Be absorber inserted between the sample and detector in order to shield the detector from scattered protons. Data were recorded in event-by-event mode using a new data acquisition system based on MIDAS data acquisition framework. . The whole system linked to a PC running control system software for stage stepper motors, scanning coils and beam on demand deflecting coils. The PIXE count rate was kept below 1000 count/s to circumvent pulse pile-up and to achieve satisfactory counting statistics. The accumulated PIXE spectra were analyzed using GeoPIXE III software [13]. Elemental mapping was performed using Dynamic Analysis method which uses K, L or M X-ray line to generate elemental image. Concentration of elements was reported quantitatively in μg/g.

3. Results and Discussions

The types of samples analyzed were fresh plants, roots, leaves and seeds. All samples were prepared following different protocols to make the samples ready for analysis. From the bulk, samples were divided in leave (inside/outside), roots and seeds (pellet/sectioned).Two different phenotypes of glaberrima were cultivated in fresh water with one Indica rice type which is widely used in Senegal named Sahel-134 and approved moderately as tolerate to salinity. We also compared the nutrient uptake by the plants in fresh and salted water. The concentrations of Al, Si, P, S, Cl, Ca, Ti, Mn, Fe, Cu, Br, Zn and K were determined in different parts of rice plants. The polished rice fraction is essentially the rice grain endosperm and the bran contains most of the embryo and aleurone layer; which is highlighted as seed samples [14]. Leave sides samples were analyzed for all batch tolerance, sensitive moderate and salted samples as shown by **Figure**

1(a) & Figure 1(b). And, the responses of seed and root samples based on their affinity with salt were carried out as matrix correlation between salted, sensitive moderate and tolerance samples in Figure 1(c) & Figure 1(d). on can see in Figure 1(a) the outside leave does not suffer with the amount of salt within samples. The correlation is between 0.92 - 0.98. In Figure 1(b) the correlation matrix of inside rice samples oscsillating between 0.8 - 0.4 on can see tolerance salt indica (179_1(9), 315_2, FL478, FL478_(5b)) are in good correlation with the glaberrima rices (v3211R29 and WAB100_3).

For root and seed samples there is no correlation in term of salt tolerance between glaberrima and indica species. The amount of salt dos not affected the elemental composition of samples within rice species between 179_1(9) and v3211R29 and 315_2 [15]. The high accumulation rate of Cu (2×) for tolerance salt sample FL_478, Al (80×) salt sentivity sample 315_2, Cl (18×) for salt sensitivity sample 315_2 and Fe (≥2000×) in the indica rice roots relative to the seeds indicates the existence of low translocation factors for these metals from root to leaves probably due to the existence of plant mechanisms to limit their transport and to preferentially accumulate P, K and Zn in the rice seeds for three indica

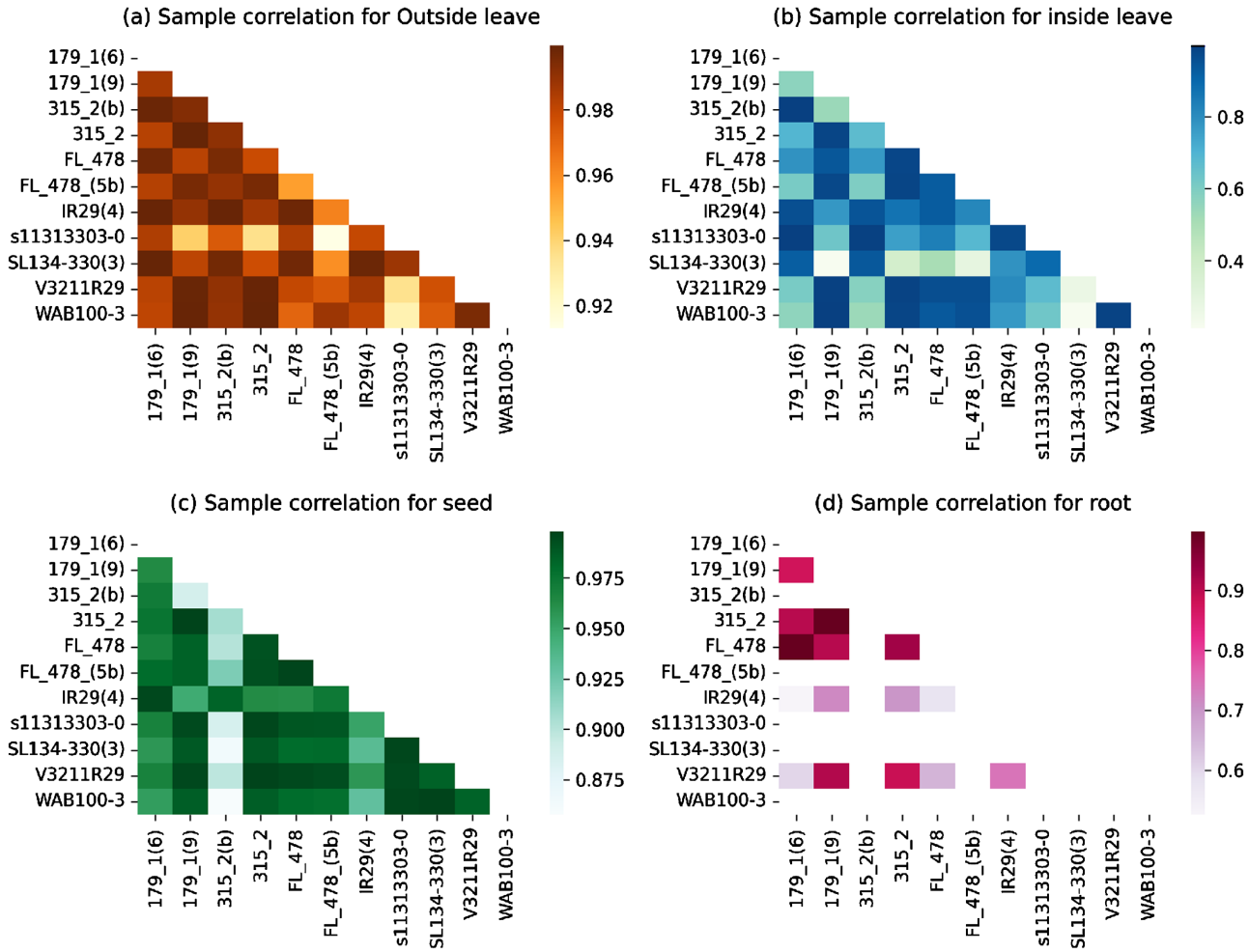

Figure 1. Leave sides rice and Matrix correlation between salted, sensitive moderate and tolerance samples.

phenotypes (FL_478, IR29(4) and IR29(4)) and one glaberrima species v3211R29. Other group of elements P and K have shown high accumulation in seed than roots samples for 179_1(6) and 179_1(9) [16].

Because of their importance as toxic parameter arsenic (As) was measured and compared among rice species and Out/Inside leaves to check potential contamination from airborne particulate matter, only FL478_5 indica specie have shown 5 µg/g of arsenic in their leaves [17] [18] [19]. our analysis of the metal composition in seeds and roots revealed consistency higher phytoextractor of P, Al, Si, K, Mn and Zn in IR29(4) specie; that genotype of rice varieties do not content arsenic in the seeds.

In **Figure 2(a)** & **Figure 2(b)**, the results of the elemental correlation matrix, for out/inside leaves, showed that there were significant correlations among most of mineral element contents. Ca, Al, Si, Cl, K, S and P contents were significantly correlated with most of the other mineral element contents, while Br,

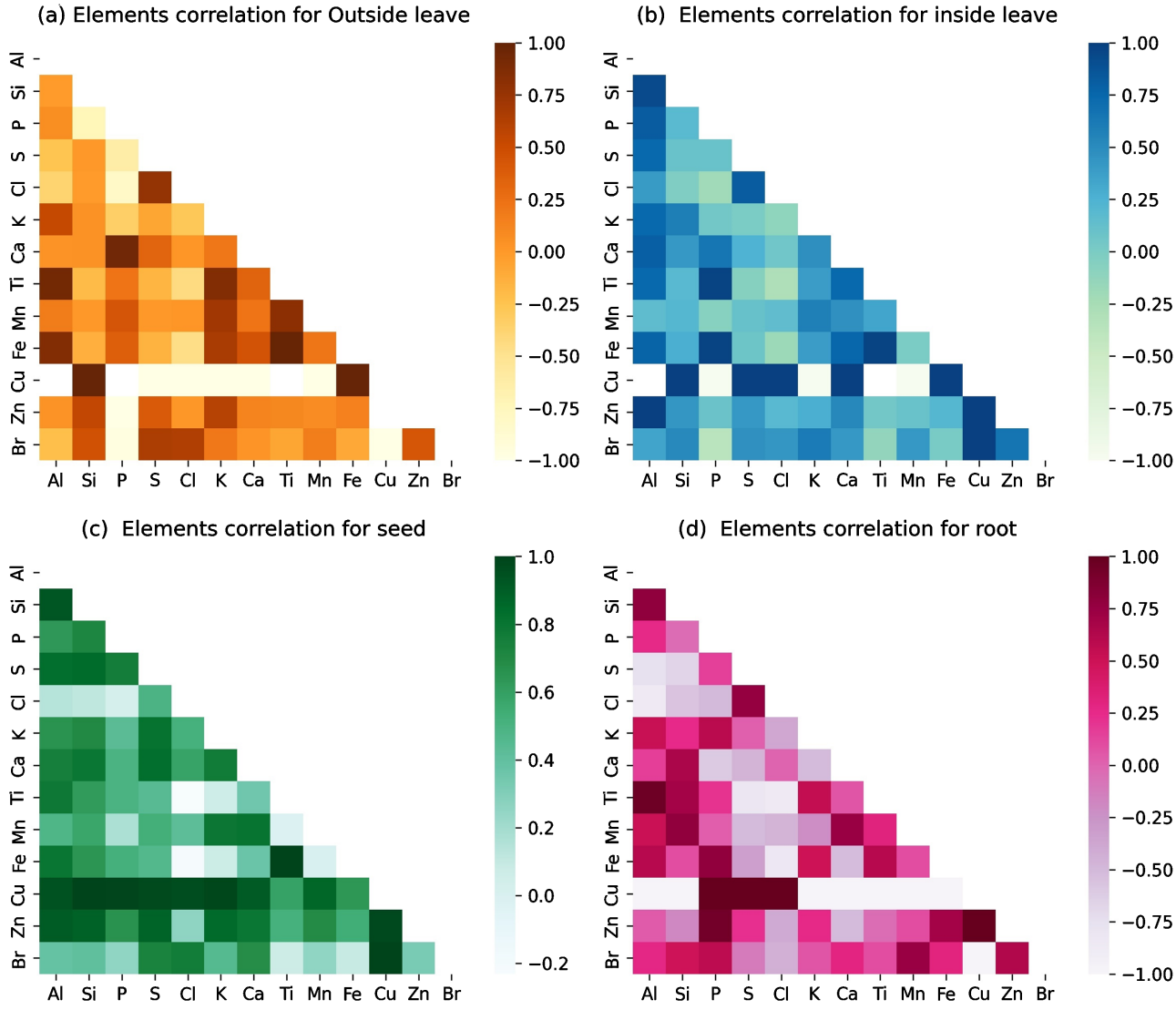

Figure 2. Elemental composition of leave sides rice and matrix correlation of rice species.

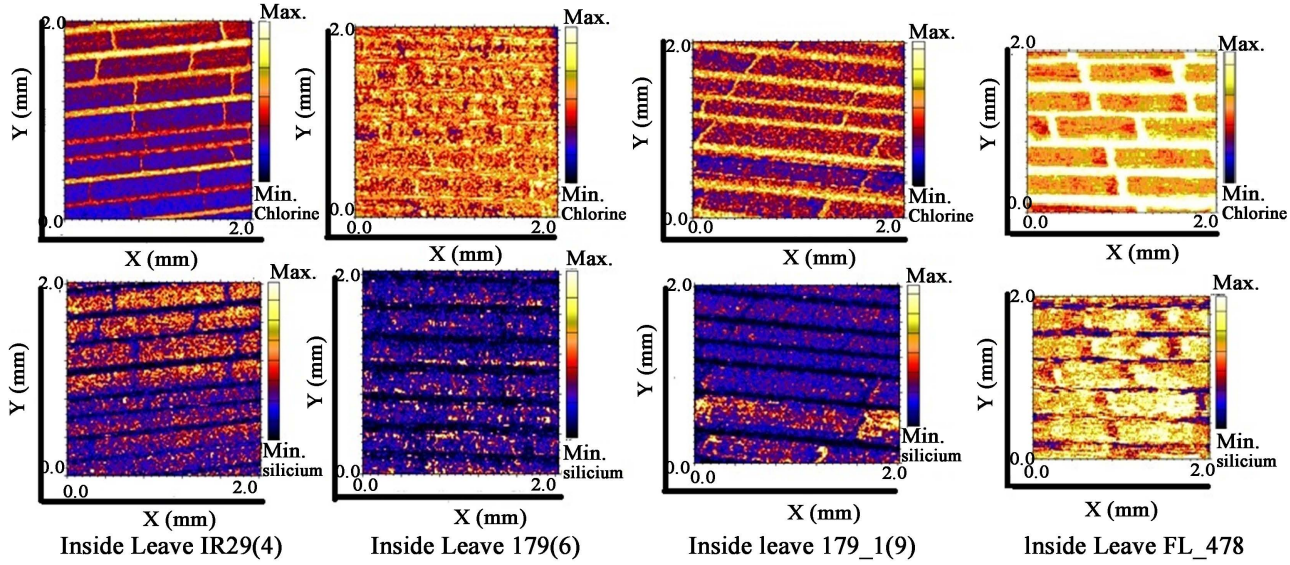

Figure 3. 2D μ-PIXE image elemental mapping of Silicium & Chlorine distribution inside leave rices organ.

Cu, Zn, Ti, Mn and Fe content had significantly negative associations with the S, Cl and Ca contents of rice side leaves. Furthermore, significant associations were found in **Figure 2(c)** & **Figure 2(d)** between seed and root Cu, Fe, Mn, Ti, Ca, Al, Si, Cl, K, S and P contents. The 2D image highlighted in **Figure 3** represents the spatial distribution of chlorine and silicium within leaves and their bio localization at organ level. The presence on the special part of the leaves can be explained by their play role against stress.

4. Conclusion

Species FL-478 and SL-134 which are salt tolerance and moderate tolerance salinity, respectively, in term of absorption of microelements P and K present no variability vis-à-vis to the study environment. Species 315(6) and 315(2) Si is higher in seed 100× than leave for SL_134. Micro PIXE analysis have shown the elemental distribution of Cl, Si, K localization of the epicuticular on the flag leaves whereas others elements S, Zn, Cu, P, Mn, Ca and Ti are on the cytoplasm.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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