Uses of X-ray 3D-Computed-Tomography to Monitor the Development of Garlic Shooting Inside the Intact Cloves

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X-ray high resolution three-dimensional computed tomography (XHR3DCT) is a non-invasive technique to monitor the inner morphology of an object. It permits to obtain a series of horizontal stack of the structure that allows its 3D reconstruction of images by a computer post-processing analysis. This technology is commonly used for medical analysis on human or rarely on animals and its utilization in the plant field has been recently discussed. As we are engaged in the investigation on the possibility to use XHR3DCT for monitoring the storage quality and/or post-harvest development of fresh produces such as vegetables, here we report on minimal demonstration performed on garlic bulbs. In particular, immediately after the harvest from the soil, cloves of garlic bulbs have been maintained under different conditions differed in temperature and humidity, with and without irradiation by red (660 nm) or infra-red (735 nm) lights. At an intermediate time, some cloves have been non-invasively monitored by XHR3DCT to predict the changes in the size (volume) of growing inner shoots (sprouts). To determine the sprout volume based on the XHR3DCT-scanned images, several mathematical approaches have been tested. With approximation of the garlic sprout shape as a parabolic cone, estimation of shoot volume could be readily achieved. By analyzing the inner shoot size in garlic clove kept under different conditions, increase in the shoot size under red light or under higher temperature and relative humidity could be monitored non-invasively, suggesting that XHR3DCT can be used for monitoring of inner structure within the clove of garlic without damaging the samples. Future applications of this technique in during post-harvest managements of a wide range of fresh produces are expected.

Keywords: garlic, plant morphology, sprout volume, X-ray computed tomography

INTRODUCTION

The X-ray scan technology, initially born as 2D computed tomography and lately evolved as X-ray high resolution 3D computed tomography (XHR3DCT), attracted the attention of researchers in the last three decades. This non-invasive technique has many applications including the structure of biological sample. Since its invention in 1973 by Godfrey Hounsfield, this technique has been mainly applied mainly to medical analysis and rarely to animals. Recently, this technique found its application even in the field of botany such as detection and visualization of the root system hidden below ground as growth of roots are central to the eco-physiology of terrestrial plants including agriculturally important crops and forest trees (Pires et al., 2010). However, therefore, XHR3DCT should be used only if the plants are grown in the laboratory-sized small pots. Due to the size of equipment and other restriction of the use of X-ray in the open space, the use of X-ray for scanning of root cannot be readily employed on-site in the most plant producing facilities. For the purpose of below-ground plant tissue sensing, we have recently employed acoustic probes by developing a novel non-invasive sensing technology for detection of belowground plant tissues based on sound propagation in the soil (Iwase et al., 2015a; 2015b).

Apart for root visualization in the pots, the utilization of XHR3DCT has been limited until its potential use for analyzing the inner plant tissue structure emerged out (Stuppy et al., 2003). Recently, the applications and capabilities of XHR3DCT have moved forward with the improvement of the available technologies (Mooney et al., 2012). The time required for entire analysis and images-processing has been largely shortened while high resolution of the data acquisition and the ability of precisely scanning the large-sized objects have been enhanced year by year. Furthermore, recent effort for manufacturing the short-sized devise may allow application of XHR3DCT in fresh agricultural product on-site (Dhondt et al., 2010). Past studies demonstrated the ability of X-ray to perform real-time monitoring of the growing plant tissue (Brodersen et al., 2010; Ferreira et al., 2010) and although the ionizing effect of the X-rays should be taken in account, sporadic examinations can be a perfect approach to monitor the quality and the development of living plant material, especially in...
case of monitoring a limited number of samples to be used non-destructively in the long-term experiments. However, even today, XHR3DCT remains strongly underused in plant sciences despite its high potential in delivering detailed 3D phenotypical information because of the low X-ray absorption of most plant tissues (Staedler et al., 2013).

In the present paper a case of study where the XHR3DCT has been applied to monitor garlic sprout is shown. The aim of the work was to investigate long-term garlic storage. In fact, due to many problems related to the sprout growth the majority of the growers sell their garlic as a fresh harvest at markets today. Only limited portion of total garlic are commercially available over the winter time due to difficulty to maintain the fresh unfrozen cloves of garlic without development of sprout during storage without the use of chemicals, despite indication in public guidelines suggesting that garlic can be kept for 6–7 months if it is stored at 0°C at 65–70% of relative humidity (RH) (Bachmann et al., 2008). In this study, we tested the utilization of the XHR3DCT for non-invasive monitoring of the growth and development of garlic sprout within the cloves, under different model storage conditions. Furthermore, mathematically assisted estimation of the size (volume) of growing inner shoots (sprouts) has been performed in order to evaluate if the use of XHR3DCT can be a promising approach permitting the analysis of hidden growth stages without interfering the physiological state in the living samples.

MATERIALS AND METHODS

Garlic was obtained freshly immediately after the harvest from growers in Mizumaki town, situated in Fukuoka prefecture, Japan. For the analysis of garlic storage, several intact garlics cloves covered by outer tunic layer were placed under different conditions. Cold incubator maintaining the samples at ca. between 10 and 13°C were used for low temperature storage of garlic cloves. One group has been subjected to storage in the cold incubator a temperature of 13.09±0.5°C with a RH of 83±11.5%, another group to 10±1.15°C with a RH of 21±11%. The whole cloves have been scanned after a period of 130–150 days for each treatment. The scanning procedure has been done in one day during the storage by transporting the garlic samples in insulated bag to minimize the shock due to temperature changes.

Some sub-groups of cloves have been maintained under completely dark condition whilst others have been irradiated at a photon flux density of 25 μmol m⁻² s⁻¹ under different light emitting diodes (LEDs), namely, red LED (peak emission at 660 nm) and infra-red LED (peak emission at 735 nm). In the end of storage, the garlic bulbs have been sliced by knife in half and analyzed.

The X-ray scanning device (Fig. 1) used for the experiment was a HMX225-ACTIS+3 (X-Tek Systems Ltd., Tring, Hertfordshire, United Kingdom) was available at Fukuoka Industrial Technical center (Kitakyushu, Japan). The scanning conditions were as follows: Source voltage 100 (kV), Source current (80 μA), and Scan width 0.2 mm. Images were post-processed with the Actis Multi Planar Reconstruction software. Measurements of morphologic parameters on the images have been performed by using the software Image J.

**Shoot volume estimation after XHR3DCT**

For the estimation of the garlic sprout volume, several simplified mathematical approaches (Table 1) have been tested. The easiest way to calculate the garlic sprout volume is to assume the shape of shoot as a cone with an oval

![Fig. 1](image1.png) The X-ray scanning apparatuses used for the experiment. HMX225-ACTIS+3 (left). Set-up for scanning of a garlic clove (right).

![Fig. 2](image2.png) Different models for simplified 3D representations of the garlic sprout shape. (A) 3D representation of the garlic cut by horizontal planes. (B) 3D approximation where the volume is given by the sum of all conical frustum plus the top part as oval cone. (C) 3D parabolic cone model. (D) Scans showing the lateral and horizontal section of a garlic clove used for the calculation of the volume using the area (a, i=1, 2, 3….) and the height (b, i=1, 2, 3….) of each section.
base (occasionally circle) which volume can be calculated with the subsequent formulations:

1) **Oval cone model**

   \( V = \frac{\pi d D h}{12} \)

   Where \( d \) is the minor diameter and \( D \) is the major and \( h \) is the height of the cone.

2) **Section area-based cone model**

   \( V = \frac{h' (a_1 + a_2 + \sqrt{a_1 a_2})}{3} \)

   Where \( a_1 \) is the area of the bottom section and \( a_2 \) is the area of the upper section.

3.1) **Compartment model: conical frustum**

   \( V_{cf} = \frac{\pi h (d_1 D_1 + d_2 D_2 + \sqrt{d_1 d_2 D_1 D_2})}{12} \)

   Volume of a single conical frustum (\( V_{cf} \)). Where \( d_1 \) is the minor diameter and \( D_1 \) is the major diameter of the base at the bottom and \( d_2 \) and \( D_2 \) the diameters of the upper base section.

3.2) **Compartment model: Sum of compartments**

   \( V = \sum_{n} V_{cf} \frac{\pi d D h}{12} \)

   Where \( n \) is the number of conical frusta assumed.

4) **Parabolic cone model**

   \( V_s = \frac{\pi d D h}{8} \)

5) **Elliptical cone model**

   \( V_s = \frac{\pi d D h}{8} \)

**Table 1** List of formulae used for estimation of shoot volume based on the size data from XHR3DCT images of intact garlic.

| Model                        | Formula                  | Description                                                                 |
|------------------------------|--------------------------|----------------------------------------------------------------------------|
| 1) Oval cone model           | \( V = \frac{\pi d D h}{12} \) | Where \( d \) is the minor diameter and \( D \) is the major and \( h \) is the height of the cone. |
| 2) Section area-based cone model | \( V = \frac{h' (a_1 + a_2 + \sqrt{a_1 a_2})}{3} \) | Where \( a_1 \) is the area of the bottom section and \( a_2 \) is the area of the upper section. |
| 3.1) Compartment model: conical frustum | \( V_{cf} = \frac{\pi h (d_1 D_1 + d_2 D_2 + \sqrt{d_1 d_2 D_1 D_2})}{12} \) | Volume of a single conical frustum (\( V_{cf} \)). Where \( d_1 \) is the minor diameter and \( D_1 \) is the major diameter of the base at the bottom and \( d_2 \) and \( D_2 \) the diameters of the upper base section. |
| 3.2) Compartment model: Sum of compartments | \( V = \sum_{n} V_{cf} \frac{\pi d D h}{12} \) | Where \( n \) is the number of conical frusta assumed. |
| 4) Parabolic cone model      | \( V_s = \frac{\pi d D h}{8} \) |                                                                 |
| 5) Elliptical cone model     | \( V_s = \frac{\pi d D h}{8} \) |                                                                 |

RESULTS AND DISCUSSION

XHR3DCT is a non-invasive tool to determine the structure of an object exploiting the characteristic of an X-ray beam when it pass through a material and reach a detector. In particular it is possible to monitor the bulk compactness of the sample by its intrinsic ability to attenuate the X-Ray beam depending on the bulk compactness. For this reason, it is possible to obtain a 2D image of the section cut by the X-ray beam having different gray tonalities proportional to the density, derived by the interactions between the beam and the object. This operation is repeated for all the height of the object to obtain a stack of images of all the cross-section that are finally combined to form the 3D representation.

Here, the quality of the images permitted a reconstruction and the calculation of all standard parameter (e.g. perimeter, area, etc...) for the cloves morphology determination. As shown in Fig. 3, The XHR3DCT resulted in a stack of images that can be analyzed singularly or processed through a multi planar reconstruction (MPR) to obtain all the views of the sprout. After the MPR, it is possible to manually modulate and analyze the size of cutting plane from the horizontal, vertical and lateral views, eventually to obtain all the desirable oriented sections as showed in Fig. 4. The quality of the obtained scan was considered accurate if the number of section is big enough, but is not really suited for the analysis of multiple samples because it requires frustum time-consuming frustum steps where all the morphological parameters have to be manually measured. For these reason, these calculation has been compared with the simplified assumption that the volume of the garlic sprouts can be considered as the volume of a parabolic or an elliptical cones. These calculations are relatively simple and requiring only the step for determination of the diameters at the base and the height of the whole sprout. The calculation of the volume as parabolic cone and elliptical shape of the sprout have been performed using the parabolic cone model: \( V_{par} = \frac{\pi d D h}{8} \), and the elliptical cone model: \( V_{ell} = \frac{\pi d D h}{6} \).
high enough for structural analysis defining the size of shoot tissue. For example, it is possible to observe and identify shape of single leaves being developed, with a good balance between the luminosity and the contrast. In Fig. 5 two examples where the number and shape of the leaves clearly detected are shown and compared with conventional anatomical sections.

All the methodologies tested here for the estimation of the garlic shoot volume have been compared in order to select the most suited approach for this work. It is obvious to us that the methodology summing conical frustum described in the formula (2) is the most accurate if the number of section is large enough, and it is also obvious to us that such approach is time-consuming and requires higher resolution by spending longer scanning time with finer special intervals, thus no-longer non-invasive. Including the simplified parabolic cone model and the elliptical cone model, all the methodology has been compared. The differences among all calculations have been compared as the ratio respect the formula (2) that is considered 100% of the volume (Table 2).

The comparison of different approaches for evaluation of the shoot volume in the garlic cloves showed that the one most close to the calculation by formula (2) was shown to be the approach using formula (3.2), which gave a 92% of the volume. The Elliptical cone volume calculation (5) was shown to be less accurate showing its tendency to overestimate by 50%, whilst the simple oval cone model

![Fig. 4](image1.png)

**Fig. 4** Different views (i.e. front, side lateral and transversal) of the same garlic bulbs with several cut plane sections.

![Fig. 5](image2.png)

**Fig. 5** Comparison between the actual slice of garlic cloves and XHR3DCT images showing the presence of hidden shoots. Garlic cloves sliced after harvest (left) and non-destructively obtained XHR3DCT image (middle and right). The number and the shapes of the leaves can be clearly detected (right).

![Fig. 6](image3.png)

**Fig. 6** Changes in garlic sprout size under different lighting and humidity controls. (A) Measured fresh weight of shoot after 210 d of storage (left, n=6, bars=SEM). (B) XHR3DCT-based shoot volume increase estimation after 130-150 d of storage (% of dark control).
showed tendency to underestimate the volume by 25%.

The parabolic cone calculation (4), which is a simple and easy method to estimate the total volume of the sprout, resulted in a good approximation at about 112% compared to the calculation by formula (2). For this reason, further calculation of the shoot volume was performed with this model which is much rapid than the use of formula (3.2).

Impact of light
The dark treatment under cold condition resulted in lower size and weight of garlic sprouts inside the cloves. Samples harvested at the end 210 d of storage under red and infra-red light treatments showed greater fresh weight compared to dark control, thus reflecting the light-dependent changes in volume (Fig. 6A). Compared to 735 nm infra-red light treatment, the 660 nm red light treatment resulted in much greater enhancement in the size of the sprouts (Fig. 6A). By XHR3DCT-based shoot volume calculation using formula (4), non-destructively performed during storage (130–150 days of storage), the increment in fresh weight due to the treatments by light (especially red light) could be well predicted (Fig. 6B). As predicted by XHR3DCT-based shoot volume estimation prior to harvest, enhanced shooting under red light was anatomically examined by harvesting the sample at the end of storage (Fig. 7).

CONCLUSION
XHR3DCT successfully detected the inner structure in garlic cloves and permitted us to perform estimation of shooting status under different conditions. Among the model we examined, the simple mathematic model based on approximation of the garlic sprout shape as parabolic cone was shown to be the most easiest and reliable approach for estimation of garlic shoot volume.

The cold storage under high RH caused an increase in the sprout growth and development in all treatments. In both the light treatments, the growth of the cloves resulted enhanced respect the dark control. In the absence of light, the garlic development at both 13.09 ± 0.5°C with a RH of 83 ± 11.5% and at 10 ± 1.15°C with a RH of 21 ± 11% were shown to be minimal. The XHR3DCT-based prediction of shoot volume showed similar trend with the actual increase in fresh weight measured on fresh garlic. Therefore we can conclude that it would be possible to predict the development of the sprout hidden inside the cloves without destroying the sample.

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