Induction of Rhizophagy by yeast Saccharomyces cerevisiae in roots of lettuce Lactuca sativa

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Abstract

This work is part of a scientific project that aims to study the effect of adding yeast (Saccharomyces cerevisiae) on growth, productivity and nutritional value of two varieties of lettuce (Lactuca sativa), planted in a hydroponic system. Here we extend experimental approaches to detect the Rhizophagy process induced by added Saccharomyces cerevisiae nutritive solution. An Laboratory anatomical study was conducted to detect and investigate the occurrence of Rhizophagy phenomenon induced by yeast cells. Results showed that the yeast cells aggregate around the root cells of lettuce, and then enter the cells, followed by digestion and disappearance.

Keywords: Rhizophagy, Saccharomyces cerevisiae, hydroponic systems, lettuce, Lactuca sativa.

Introduction

All societies around the world consume Lettuce (Lactuca sativa) widely. Because it contains low calories, fat, and sodium. and it is a good source of fiber, iron, folic acid, and vitamin C. in addition to many health-promoting bioactive compounds (Kim et.al.,2016). Since the last decades, farmers used to add sewage wastes as fertilizers for this plant (Al-Azawi and Alarazah; 2015). These wastes are known to contain heavy elements that affect the soil properties, underground water, agricultural crops and the whole environment as an integrated unit (Angin and Yaganoglu; 2011).

The danger of heavy elements lies in their containment of many micro elements, which are among the most important environmental pollutants due to their high stability and unlimited periods of survival, as the concentrations of these elements can be multiplied through the food chain to make the plants or animals that feed on them poisonous and dangerous (Polle and Schutzendubel, 2002).
The increased interest in the environment made it imperative to seek alternatives fertilizers that are more environmentally friendly in agriculture. For example, integrative agriculture where mineral and organic fertilizers are used together. One of the most important means for developing plant nutrition and fertilization with an organic valley is the use of the extract of baking yeast (Saccharomyces cerevisiae), it is safe in the environment, cheap and economically feasible. It has proven its effectiveness on many crops by improving and increasing the characteristics of growth and yield (Thierry et al., 2014). Yeast is one of the eukaryotic single-celled microorganisms. Reproduce by simple division or budding (Barnett et al., 1990).

A new dimension of plant – microorganism relationships had attracted the interest of many researchers in the field of plant nutrition. As it has proven its efficiency in harnessing non-pathogenic microbes to extract the maximum nutrients from microbes for increasing growth, in a phenomenon similar to phagocytosis called Rhizophagy (Paungfoo-Lonhienne et al., 2013).

Rhizophagy, means "root eating", represents a mechanism for nutrients transmission from symbiotic microbes (bacteria and fungi) to host plant roots (White et al., 2018). Rhizophagy means the decay of symbiotic microbes within the root cells, this process happens frequently in which plants extract nutrients from symbiotic microorganisms that transit between the internal phase within the root cells and the free soil stage. Microorganisms obtain soil nutrients at free soil stage; and then these nutrients are extracted from them in the intracellular phase (Paungfoo-Lonhienne et al., 2013).

It has been found that plants grow microbes and then extract nutrients from them. Plant root secretes sugars, proteins, and vitamins to create cultural media suitable for microbes growth. The microbes grow and enter the root apex, where the root cell lacks cellulosic walls, then become trapped in plant cells and exposed to reactive oxygen (superoxide). in which it breaks down the cells of microbes and extracts nutrients from them effectively (White et al., 2018).

In order to explore the potential of yeast to induce Rhizophagy, this investigation came to detect the phenomenon of Rhizophagy induced by yeast in the roots of two varieties of lettuce plant.

**Materials and Methods**

This study was conducted in the laboratories of the College of Science / Department of biology/ University of Qadisiyah. It aimed to investigate and detect the phenomenon of Rhizophagy induced by yeast in the roots of two varieties of lettuce plant, included the local variety and the Paris island (USA) variety.
Lettuce seeds were planted on October 20, 2019 in small containers containing peat moss, intended for obtaining seedlings, after four weeks of germination the seedlings were transferred to the laboratory for the purpose of conducting the laboratory study.

For the laboratory Experiment Lettuce seedlings were removed from the small containers and washed with running water in order to remove soil residues. The washing process was done slowly and quietly to avoid the destruction of the thin root hairs. After that, the seedlings were transferred to test tubes filled by the hydroponic nutrient solution that contains 1% active yeast cells. The root of lettuce seedlings was immersed in the hydroponic -yeast solution. Microscopic slides of root hairs were prepared using the whole loading method with the yeast solution, without any kind of stains to avoid there effect on yeast viability. Microscopic slides were prepared sequentially at the rate of five minutes intervals for the same plant sample. The prepared microscopic slides were examined under compound microscope; pictures were captured by the IS 95 IXUS Cannon camera.

Results and Discussion

Before starting the laboratory experiment, a microscopic image of the yeast solution used in the experiment was taken (plate 1).

plate1: Saccharomyces cerevisiae solution used in the experiment(40X)

By examining and studying the serial microscopic slides that attended at regular intervals, some behaviors related or similar to the phenomenon of Rhizophagy were observed. The first of which, the aggregation of yeast cells around the root hairs. Especially around the root apical cells, Shown in the plate2 below. This is due mainly to root exudations, which contain sugars and proteins, that attract yeast cells to root hairs (White et.al., 2018).
Plate 2: the aggregation of S.cerveasea yeast cells around the root hair of lettuce *Lactuca sativa* (400X)

a- local variety  
b- Paris island (USA) variety.

The entering of yeast cells through the apical root cells was also observed. In which, an inward invagination was seen in the apical root cells so the yeast cell passes through it. And that was shown clearly in plate 3. It is worth mentioning that the mechanism of the microbe entry into root cells varies according to the plant species, where Pawlowski and Demchenko (2012) found, in their study on symbioses between soil aerobic Filamentous actinobacteria and a diverse group of plants from eight dicotyledonous families, that the entrance mechanism of actinobacteria *Frankia* into the plant root hair is determined by the host plant species. In which it remains hosted inside the plant cells.

Plate 4 shows a group of yeast cells that have passed into the plant tissue, where the number of yeast cells increases over time and moves inside the root hair.

By continuously studying serial microscopic slides and by observing the behavior of yeast cells within plant tissues, the movement of yeast cells through plant cells has been established. A variation in the movement of yeast cells is observed through plant tissue, some of which continue to move and others disappear. plate 5 shows some yeast cell that move through root hair to reach the hypodermis layer. That was noticed also by Cocking et.al. (2005). They demonstrated that inoculation of root meristem cells of some non-legume crops with low numbers of bacteria, can lead to Intracellular Colonization of the non-rhizobial microbes.
These observations are consistent with those found by grunert et al. (2013) on other Asteraceae plants. They confirmed that tomato (Lycopersicum esculentum) take up yeast cells of Saccharomyces cerevisiae into their root cells, digest them and use as a nutrient source.

plate 3: the inward invagination in the root apical cells of lettuce Lactuca sativa and the entering of Saccharomyces cerevisiae cells through it. (400X)

a- local variety b- Paris island (USA) variety.

The ability of roots to harness microbes activates the mechanism of up taking nutrients and This activated mechanism can be used to develop plant nutrition and fertilization management.
**Plate 4:** the increase in number of *Saccharomyces cerevisiae* cells in root hairs of lettuce *Lactuca sativa* over time (400X).

a- local variety  
b- Paris island (USA) variety

**Plate 5:** *Saccharomyces cerevisiae* cells reaching the hypodermis of lettuce *Lactuca sativa* (400X).

a- local variety  
b- Paris island (USA) variety
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