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Deploying Plant Tissue Culture Simulation use case for E-Infrastructures

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ABSTRACT

E-Infrastructures can be defined as networked tools, data and resources that support a community of practice (CoP), broadly including all those who participate in and benefit from research. During the UNESCO-HP BGI project (2009-2012), the University of Nigeria team conducted experiments on plant tissue culture under the theme, "sustaining the plant tissue culture component of grid computing". Plant Tissue culture is a method for plant propagation under in vitro conditions. Different types and parts of plants (known as explants) may be cultivated in vitro. Because plant tissue culture is still in its empirical stage, it is time-consuming, cost-intensive and man-power demanding. These necessitated the design and development of a plant tissue culture simulation application that predicts explant yields using multiple regression models. The initial version that was developed during the BGI project with a prediction accuracy of about 67%, unfortunately was not deployable on an e-infrastructure like the grid or the cloud. Hence, during the Sci-GaIA project (2014-2017), a use case for the development of a newer version, Plantisc2, that is deployable on e-infrastructure was proposed. The outcome of which is reported in this paper.

Keywords: *Plant Tissue Culture; Simulation; E-Infrastructure; Prediction, Auxins, Science Gateway*

1. INTRODUCTION

E-Infrastructures can be defined as networked tools, data and resources that support a community of researchers, broadly including all those who participate in and benefit from research [1]. According to [1], the term e-Infrastructure comprises very heterogeneous projects and institutions within the scientific community. E-Infrastructures include services as diverse as the physical supply of backbone connectivity, single- or multi-purpose grids, supercomputer infrastructure, data grids and repositories, tools for visualization, simulation, data management, storage, analysis and collection, tools for support in relation to methods or analysis, as well as remote access to research instruments and very large research facilities.

In 2011 the University of Nigeria Brain Gain Initiative (BGI) project successfully set up the first ever Grid Computing in Nigeria (The Lion Grid), under the funding of UNESCO and HP [2][3], which has further been redesigned and developed into a community cloud computing infrastructure [4]. During the UNESCO-HP BGI project the University of Nigeria team conducted experiments on plant tissue culture and subsequently developed a simulation application which achieved about 67% prediction accuracy [5]. Plant Tissue culture is a method for plant propagation under *in vitro* conditions. Different types and parts of plants (known as explants) may be cultivated *in vitro*. Thus explants could be sections of organs such as roots, stems, shoot tips, leaves and fruit or tissues / cells (suspension cultures) or special tissues and organs such as embryos, anthers, pollen and protoplasts. According to [6], the *in vitro* technique is also known as micropropagation due to the minute size of the explants.

Lineberger [7] observed that the advantages of micropropagation are:

- Multiplication of a plant into several thousand plants in less than one year
- Plant multiplication can continue throughout the year irrespective of the season
- With most species, the excision of the ex-plant does not destroy the parent plant

The technique is widely used for large scale plant multiplication (mass propagation), for efficient disease elimination and for production of secondary metabolites [8].

One of the drawbacks of this technique is that there is no one protocol that could be used for the propagation of all kinds of plants. This fact has been reiterated recently by [9] who reported that cultural requirements for the process of plant tissue culture differ from species to species. The most appropriate conditions for a given species must always be evolved out of experimentation.

Bhojwani and Razdan [10] noted that the formulation of a suitable medium for an untested species, would naturally start with a well-known basal medium such as Murashige and Skoog (MS) [11]. Furthermore, they noted that by making minor

qualitative or quantitative changes through a series of experiments, a new medium may be evolved to accommodate specific requirements of the plant material in question.

One of the most variable or critical factors in plant tissue culture media are growth regulators or hormones especially auxins and cytokinins which are usually used in various combinations. The growth regulators are important in determining the developmental pathways of plant cells. Screening of these hormonal combinations is time and material intensive running into several months of laboratory efforts in trying to develop a protocol that will be best for mass propagation of a particular species.

Modeling or computer simulation will readily be of great help in reducing the time needed to screen the numerous hormonal combinations. Due to the potentials of plant tissue culture technique, innovative approaches to reduce labour requirements and costs are being developed.

According to [12], using machines to accomplish the various steps of micropropagation will help to cut down the production costs. Sluis [13], however, was of the opinion that automation of micropropagation work is not technologically simple and also not readily achievable economically. He further noted that the human eye-hand-brain combination is both highly sophisticated, technologically and incredibly inexpensive when considered on a global scale. Warren [14] had earlier reported that human operators are proving difficult to supersede because much judgment is required concerning the best tissue to transfer and the optimum timing of the various steps.

Because of the afore-mentioned challenges involved in plant tissue culture experiments, our team has developed a cloud-based software model for predicting plant yields using a repository of experimental data. This application predicts the right combinations of growth hormones and their expected yields before the researcher goes to the laboratory. This in effect, reduces time of trials, costs of reagents and man-hour. Section 2 describes how the plant tissue culture experiment was conducted, while section 3 briefly describes the application and section 4 shows the deployment of the application on the e-infrastructures like the Catania Future Gateway and the UNN Cloud.

2. DESIGN OF LABORATORY EXPERIMENTS

The laboratory experiments were carried out at the Plant Tissue Culture Laboratory of National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria. Shoot tip explants were excised from aseptically germinated buds of cocoindia (a variety of cocyam) on basal MS media. Multiple shoot induction from these explants were investigated on two culture media which were: Schenk and Hildebrandt (SH) [15]; Arnold and Eriksson (AE) [16]. To these two respective basal media were added, 30g of sucrose, 10mg/l L-cysteine, 100mg/l myo-inositol, and vitamins.

Different concentrations of an auxin, Naphthalene acetic acid (NAA) and cytokinins, 6-Benzyl amino purine (BAP) and 6-furfuryl amino purine (Kinetin) were also added to each of the medium. The concentrations were, 0.0, 0.05, 0.1, 0.5, and 1.0mg/l of NAA and 0.0, 2.0, 4.0, 6.0, 8.0mg/l of BAP and Kinetin respectively. NAA was combined in all possible combinations with BAP to give 25 treatments and likewise NAA plus Kinetin. Therefore, a total of 50 treatment combinations were obtained for each medium.

Each treatment combination was replicated 10 times thus giving a total of 500 culture vessels for each medium. Thirty milliliters of the respective medium were dispensed into each culture vessel.Three shoot tip explants were seeded into each culture vessel thus giving a total of 1500 explants per medium; however, data analysis was performed with the mean of the three with respect to the attributes in question. The attributes studied included: number of shoots, number of leaves, number of roots and plant height.

The culture vessels were sealed with paraffin and aluminum foil and placed on shelves in a growth room. The vessels were exposed to a 16 hour photoperiod which was provided by white fluorescent tubes. The temperature in the growth room was maintained at 28+ 2^{o}C by air conditioning units. A separate rooting stage media were not prepared because the plantlets rooted while still in the respective multiplication media.

The whole experiment lasted for twelve months. The first four months were used to generate the required number of shoot tips while the last eight months were used to screen the hormonal combinations for their effects on multiple shoot induction from the shoot tip explants.

3. SOFTWARE DESIGN

In this section, we present the design and implementation for the predictive and simulation application.

A. Predictive Module

The system is designed to first predict the desired concentration mixture of auxin and cytokinin before simulating for other concentrations.

A. *Input Data*

The system accepts input from two sources which are:

a. Users' input data pertaining to their experiment

b. Data downloaded from the site's repository.

The two sources make use of an excel template (data.xls) through which the input is made into the system. A typical layout of the template is given in Table 1 below:

Table I: Input Data Template

Auxin concentration	Auxin Name	Cytokinin Concentration	Response/Yield

Auxin is constant while cytokinin varies from BAP to Kinetin and the variation is accommodated in the software portal.

User Interface:-Every user is expected to register and log in to use the portal. After a successful log in, a user then proceeds to upload data using either of the mentioned approaches. A user cannot make use of data uploaded by another person except through download and subsequent upload through the template. Once a user is logged on, the user has access to data download, data upload, single auxin and cytokinin concentration prediction, general simulation, simulation result and data export to excel. The export is necessary if further statistical tests and analysis is required on the data.

Prediction:-The prediction is done using a multiple regression model with two independent variables and a dependent variable given as:

$$\hat{Y} = a + b_1 X_1 + b_2 X_2 \qquad (1),$$

Where

$\hat{Y}$ = predicted value of Y which is dependent on the values of X_1 and X_2variable.

a = The $\hat{Y}$ Intercept, b1 = The change in Y for each 1 increment change in X_1, b2 = The change in Y for each 1 increment change in X_2.
X_1 = Auxin concentration (Independent Variable) for which we wish to predict a
value of Y. X_2 = Cytokinin concentration (Independent Variable) for which we wish to predict a value of Y.

The correlation coefficient R is the combined correlation between auxin concentration and cytokinin concentration with the response yield. R is programmed and computed for every prediction using the equation

$$R = \sqrt{\frac{\left[(r_{y,x1})^2+(r_{y,x2})^2\right]-(2r_{y,x1}r_{y,x2}r_{x1,x2})}{1-(r_{x1,x2})^2}} \quad (2)$$

Where

$r_{y,x1}$ = Correlation between auxin and response yield
$r_{y,x2}$ = Correlation between cytokinin and response yield

$r_{x1,x2}$ = Correlation between auxin and cytoknin concentration
Similarly, the regression model components were programmed using equations 3 and 4

$$b_1 = \left(\frac{r_{y,x1}-r_{y,x2}r_{x1,x2}}{1-(r_{x1,x2})^2}\right)\left(\frac{SD_y}{SD_{x1}}\right) \quad (3)$$

$$b_2 = \left(\frac{r_{y,x1}-r_{y,x1}r_{x1,x2}}{1-(r_{x1,x2})^2}\right)\left(\frac{SD_y}{SD_{x2}}\right) \quad (4)$$

$a = \bar{Y} - b_1\bar{X}_1 - b_2\bar{X}_2$
$\bar{Y}$ = mean of the dependent variable (response/yield)
$\bar{X}_1$ = mean of auxin
$\bar{X}_2$ = mean of cytokinin

B. Simulation

The system computes the line of fit for the given data and uses it to simulate for other concentration mixture which are not in the data set. The essence of this is to ensure a deeper search having established the model of fit using the input data. The simulation is achieved by taking the range between the lowest and the highest concentration of auxin and cytokinin respectively and using the range to generate and predict other concentration data sets which were not included in the original input data. The simulation ensures that each auxin concentration exhausts all possible cytokinin concentration within the input data set range. This is almost impossible in the laboratory due to cost, time and labour. A typical layout of the simulated data set is given in Table 2 below:

Table II: Simulated Data Layout

Auxin concentration	Cytokinin concentration	Simulated Auxin concentration	Simulated Cytokinin concentration
0	0	0	0
0.05	2	0.1	1
0.1	4	0.2	2
0.5	6	0.3	3
1	8	0.4	4
		0.5	5
		0.6	6
		0.7	7
		0.8	8
		0.9	
		1	

Layout of the simulation Technique

From the layout, it could be seen that 0.1 auxin concentration will be compared with 0, 1, 2,3,4,5,6,7,8 cytokinin concentration respectively which were not in the original input data set.

C. SCREEN SHOTS

Figure 1 show already uploaded data sets in the application repository which individuals can download and use for their own prediction. Figure 2 shows a screenshot of the parameters selection window for the simulation, while figures 3 and 4 show simulation details and final predicted rsults, respectively.

Fig. 1: Existing data Download

Fig. 2: Simulation Window

Fig. 3: Prediction Window

Fig. 4: Output Window

4. APPLICATION DEPLOYMENT

Figure 5 depicts the architectural framework of the Plantisc2 application, showing the process models and the deployment infrastructures.

The Plantisc2 app is deployed on the Catania Future Gateway using Ansible Playbook. FutureGateway is a project to develop an API and related services which will allow web applications to interact with back-end distributed infrastructure of almost any kind. Its design principles are to provide a platform-agnostic bridge between front-end (web) interfaces and back-end interfaces [17].

It adopts the philosophy of using open standards when it comes to implementation choices as far as possible, and thus relies on the SAGA standard - specifically the jSAGA implementation for interacting with grid, cloud and HPC sites. For different cloud stacks, the gateway uses OCCI

The reliance on standards means that the front-end developer needs to implement far less functionality, and has access to far more potential resources, as compared to having to implement native functionality for each different kind of backend - different grid, cloud, HPC stacks.

Ansibel, on the other hand, is an IT automation and configuration management tool written in yaml code. It uses playbooks to deploy, manage, build, test and configure anything from full server environments to websites to custom compiled codes for applications. The codes for the app were uploaded to GitHub from where Ansibel pulls them into a playbook. The palybook is then used to deploy the app on the e-infrastructure independent of platform.

Plantisc2 is deployed both on the Lion Cloud, University of Nigeria Cloud Computing Infrastructure (https://astakos.unn.edu.ng, http://cloud.unn.edu.ng , as well as on the Catania Future Gateway.

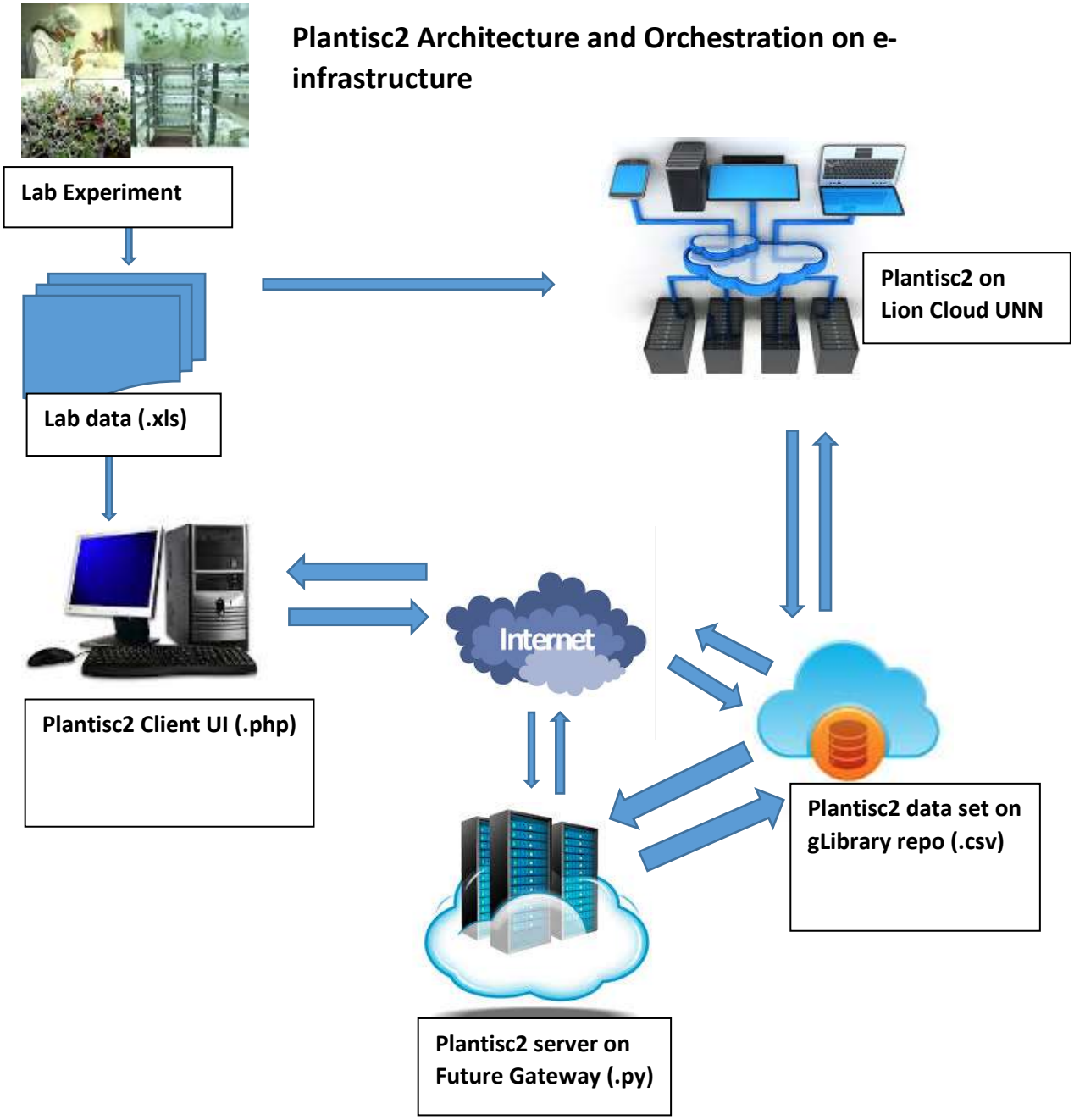

Fig. 5: Deployment architecture of Plantisc2 application

Figures 6 and 7 show the code deployment on Github and the Ansible playbook, respectively.

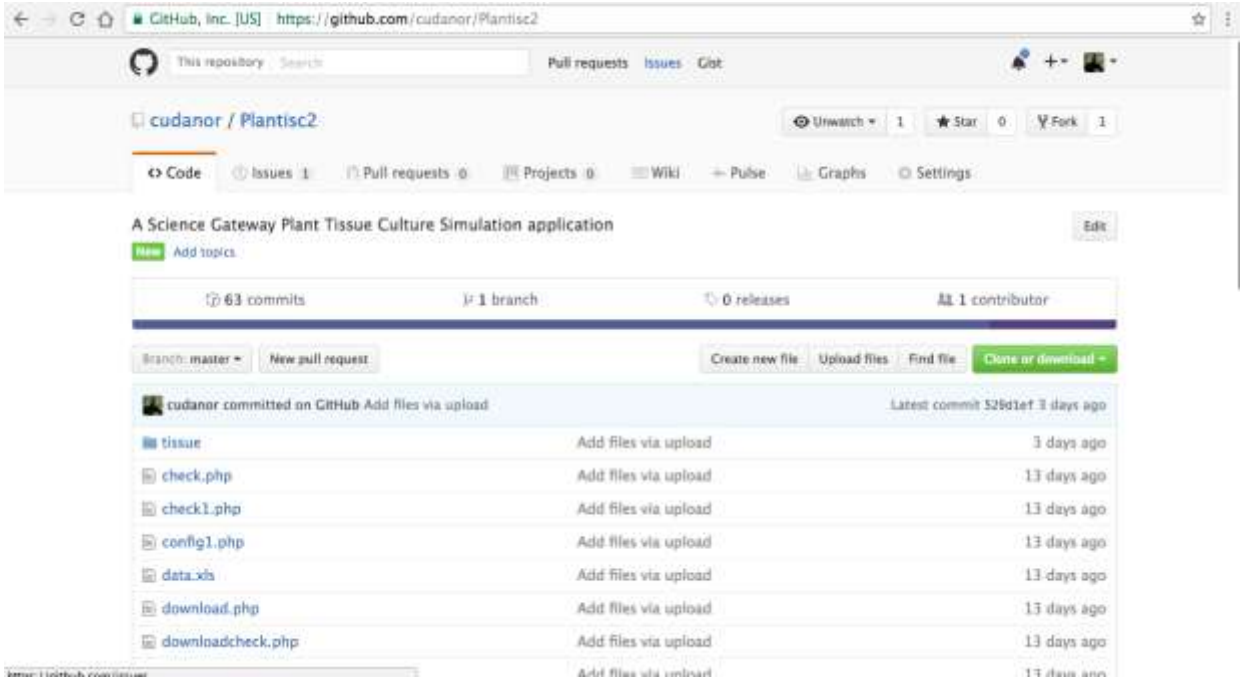

Fig. 6: The Plantisc2 codes on Github

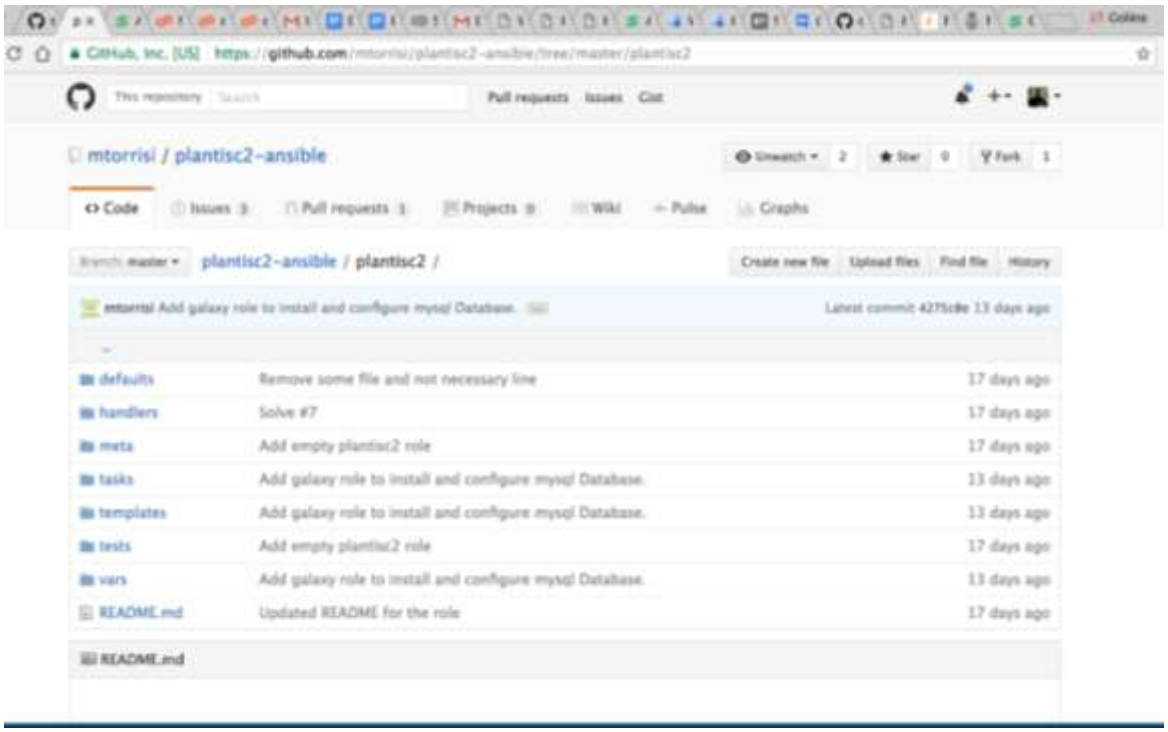

Fig. 7: The Ansibel playbook of Plantisc2.

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