A Grid-enabled Application for the Simulation of Plant Tissue Culture Experiments

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Abstract

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. These may be organs (roots, stems, shoot tips, leaves and fruit); tissues; cells (suspension cultures) and special tissues and organs such as embryos, anthers, pollen and protoplasts. Plant tissue culture is a laborious and time-consuming technique. Potentially, modeling or computer simulation can provide a useful method for gaining insight into these complex processes by reducing the time needed to screen numerous hormonal combinations. We present a simulation application based on multiple regression models deployed on a grid computing infrastructure. The application simulates the plant tissue culture experiments and predicts the amount and combinations of auxins and cytokinins needed to yield optimal growth of propagules. The results obtained from the simulation showed over 67% prediction accuracy as compared to the laboratory experiments.

Key Words

Explants, In Vitro, Micropropagation, Plant Tissue Culture, Simulation.
I. INTRODUCTION

This collaborative research work was necessitated by the project: ‘Sustaining the Research and Grid Computing Components of the University of Nigeria’s UNESCO – HP Brain Gain Project’ awarded to the University of Nigeria through a competitive bid process under the UNESCO-Hewlett Packard Brain Gain Initiative (UNESCO-HP BGI). As a follow-up to the ongoing plant tissue culture research activities, the project developed a simulation application to predict the outcome of various combinations of plant growth hormones. This was aimed at reducing the number of laboratory trials, time and the cost of the experiment. The application is deployed on the University of Nigeria Grid Computing infrastructure. A comparative analysis of this application and the results of the laboratory work are presented in this paper. The rest of the paper is organized as follows. Section II presents a brief review of related works. Section III shows details of the laboratory work, as well as the design of the software with a brief description of linear regression model on which this software is based. And section IV shows the results from the comparison of the software and experimental data, as well as sample output of the application.

II. REVIEW OF RELATED WORKS

Grid computing is a form of distributed and parallel computing, whereby a 'super and virtual computer' is composed of a cluster of networked, loosely coupled computers acting in concert to perform very large tasks [1]. Foster and Kesselman [2], defined a computational grid as a hardware and software infrastructure that provides dependable, consistent, pervasive, and inexpensive access to high-end computational capabilities.” Thanks to the growth of the Internet and high speed data networks, geographically distributed resources, such as storage devices, data sources, and supercomputers, are interconnected and can now be exploited by users around the world as single, unified resource. Apart from hardware resources, application software programs can now be shared. The grid has thus drastically reduced computing cost, as well as made scare resources available to researchers who were technologically disadvantaged a few years back. One of the most common grid applications is simulation application. Simulation is widely used for modeling real world processes in many different application areas, including manufacturing, construction, and computer science. It provides the study of various issues, such as feasibility, behaviour and performance without building the actual system, thus saving time, cost and effort [3], [4]. It is based on the applicability of the grid to many fields of research that we developed a simulation application to model the processes involved in plant tissue culture experiments.

Plant tissue culture is still in its empirical stage, involving a lot of trials and error. The experiment is time and material intensive, running into several months of laboratory efforts in trying to build hormonal combinations that will be best for mass propagation of a particular species. According to [5], 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 that can run into hundreds. The growth regulators are important in determining the developmental pathway of plant cells. Modeling or computer simulation will readily be of great help in reducing the time needed to screen the numerous hormonal combinations.
Thus due to the potentials of plant tissue culture technique, innovative approaches to reduce labour requirements and costs are being developed.

According to Afreen [6], using machines to accomplish the various steps of micropropagation will help to cut down the production costs. Sluis [7], 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 [8] 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.

Methods that could be used for the routine propagation of all kinds of trees have not yet been developed, despite much research [9]. This fact has been reiterated recently by [10] 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 [11], also wrote 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) [12]. 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.

Prasad and Gupta [13] described the various applications of artificial neural networks (ANN) in in vitro plant culture systems. They observed that ANN can play central role as highly potential predictive modeling tool in in vitro plant culture studies. They further reported that neural computing offers reliable and realistic approach for describing in vitro culture of plant species even with minimal available information. The successes obtained after applying neural network technology have been phenomenal with a relatively modest experimental effort while consuming minimum amount of time. ANN based prediction of the behaviour of the in vitro derived plants in terms of their ex vitro survival rate and their rooting or organogenic ability could also be useful in large scale propagation.

III. MATERIALS AND METHODS

In this section, we present the laboratory experiment design and execution. We shall also briefly discuss the software design methodology.

A. Design of 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 cocoyindia on basal MS media. Multiple shoot induction from these explants were investigated on two culture media which were: Schenk and
Hildebrandt (SH) [14]; Arnold and Eriksson (AE) [15]. 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 cytokinin, 6-Benzyl amino purine (BAP) and 6-furfuryl amino purine (Kinetin) were also added to each of the media. 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 media.

Each treatment combination was replicated 10 times thus giving a total of 500 culture vessels for each media. Thirty milliliters of the respective media 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 include: number of shoots, number of leaves, number of roots and plant height. Thus for NAA x Kinetin, 500 pieces of data were obtained for each of the attributes.

But for NAA x Kinetin, 250 pieces of data (corresponding to 250 culture vessels) were obtained for the attributes; number of shoots and number of leaves. 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°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.

B. Software design

The system design and implementation for the simulation application is presented in this section. We review briefly the theory of linear regression which the application uses to estimate the yield of the propagules. A matrix formulation of the multiple regression model is shown in the equation below.

In the multiple regression setting, it is more efficient to use matrices to define the regression model and the subsequent analyses. This is because of the potentially large number of predictors.

Here, we review basic matrix algebra, as well as learn some of the more important multiple regression formulas in matrix form. Starting with the simple case first, consider the following simple linear regression function:
\[ y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \epsilon_i \quad \text{for } i = 1, \ldots, n \]  

Let us assume \( i = 1 \) to \( n \), we then obtain equations as below:

\[ \begin{align*}
  y_1 &= \beta_0 + \beta_1 x_{11} + \beta_2 x_{21} + \epsilon_1 \\
  y_2 &= \beta_0 + \beta_1 x_{12} + \beta_2 x_{22} + \epsilon_2 \\
  y_n &= \beta_0 + \beta_1 x_{1n} + \beta_2 x_{2n} + \epsilon_n 
\end{align*} \]

At a glance, one would easily make out that the equations would be cumbersome to handle in cases of numerous trials (where \( n \) is large), and we would also agree that a pattern seems to be observed. With the help of matrix, we can translate the following linear regression functions into a matrix notation:

\[
\begin{bmatrix}
  y_1 \\
  y_2 \\
  \vdots \\
  y_n
\end{bmatrix} = 
\begin{bmatrix}
  1 & x_{11} & x_{21} \\
  1 & x_{12} & x_{22} \\
  \vdots & \vdots & \vdots \\
  1 & x_{1n} & x_{2n}
\end{bmatrix} 
\begin{bmatrix}
  \beta_0 \\
  \beta_1 \\
  \beta_2
\end{bmatrix} + 
\begin{bmatrix}
  \epsilon_1 \\
  \epsilon_2 \\
  \vdots \\
  \epsilon_n
\end{bmatrix} \tag{v}
\]

One can now see that the matrix notation still gives us a simple linear regression that can be expressed as:

\[ y = \chi \beta + \epsilon \]  

We can now express the multiple \( n \) equations in a simple linear form, and the linear regression function now becomes even shorter and simpler, as shown in (v).

Now \( y \) is the response variable in equation (v), while \( \chi \) is the control variable and in this case we have two variables (\( x_1 \) and \( x_2 \)) representing auxin and cytokinin concentrations respectively, the parameters to be estimated from the data, including the constant of interception \( \beta \). These constants of interception are obtained by computation of the matrix, using the already known parameters (\( y \) for expected result, \( x_1 \) for auxin concentration, and \( x_2 \) for cytokinin concentration, and \( \epsilon \) is the random error variable which is assumed to be zero. Once the constant of interception has been successfully calculated, over a wide range of trials, we could now trust the constant to help in the estimation of response values (yield), when other concentration of auxin and cytokinin are introduced.

In the implementation of this work, we used python programming language because of its ability to handle the manipulation of data, even at large scale. This gives us the ability to efficiently perform the matrix operations required in less time. With the help of python libraries such as numpy and scipy, the matrix operations were handled and results confirmed to be accurate.

From the model equation in (v) above, we used the existing data obtained from real case experiment to obtain our \( \beta \) values and assuming \( \epsilon \) to be 0 at all times in equation (vi), we could easily predict the outcome of the yield of propagules (\( y \)) with a given combination of growth conditions.
hormones ($\chi_1$ and $\chi_2$).

At the time of this report, we had 750 test cases, and this helps in the estimation of the $\beta$ values, also giving room for more experimental values to be added, which would increase the accuracy of the predictions.

**IV. RESULTS AND DISCUSSIONS**

In this section, we use statistical analysis tools to compare the results obtained from the experimental data with that obtained from the software simulations. Regression graphs and tables were used to find the coefficients of determination ($R^2$) between the experimental and simulation data. These graphs are shown in figures 1 - 12.

![Figure 1](image1.png)

**Figure 1: Number of Shoots in AE Medium (NAA x KINETIN)**

![Figure 2](image2.png)

**Figure 2: Number of shoots in SH medium (NAA x KINETIN)**
**Figure 3** Number of Leaves in AE Medium (NAA X KINETIN)

\[ y = 0.6901x + 0.8424 \]

\[ R^2 = 0.0083 \]

**Figure 4** Number of Leaves in SH Medium (NAA X KINETIN)

\[ y = 1x + 2E-05 \]

\[ R^2 = 0.2098 \]
Figure 5: Plant height in Ae medium (NAA x Kinetin)

y = 0.9323x + 0.4077
R² = 0.2661

Figure 6: Plant height in Sh medium (NAA x Kinetin)

y = 1.0049x - 0.0518
R² = 0.1938
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**Figure 7** Number of Roots in Ae Medium (NAA x Kinetin)

\[ y = 1x + 1E-06 \]
\[ R^2 = 0.614 \]

**Figure 8** Number of Roots in Sh Medium (NAA x Kinetin)

\[ y = 1x - 2E-06 \]
\[ R^2 = 0.2814 \]
**Figure 9** Number of shoots in AE medium (NAA X BAP)

\[ y = 0.9964x + 0.015 \]
\[ R^2 = 0.1672 \]

**Figure 10** Number of shoots in SH medium (NAA X BAP)

\[ y = 0.9659x + 0.1075 \]
\[ R^2 = 0.0236 \]
Figures 1 to 12 are graphical explanations of variations in the attributes studied in the laboratory as predicted by the software. The slopes of the regression lines in figures 3, 10, 11 and 12 are parallel to the X-axis, thus there are no relationships between the laboratory and the software data. On the other hand, the slopes of the other figures depict some linear relationships between the two sets of data.

The coefficients of determination (R²) authenticate the above results. The R² values for figures 3, 10, 11 and 12 were 0.008 (0.8%), 0.023 (2.3%), 0.000 (0%) and 0.004 (0.4%) respectively.
follows that some 98%, 97.7%, 100%, and 96% respectively of the variations in laboratory data shown in those figures are not accounted for by the software. In contrast, 61.4% of the variations in the number of roots obtained in the laboratory on AE medium + NAA + Kinetin were predicted by the software.

### Table 1: Estimates of Linear Regression Coefficients for Attributes Studied

<table>
<thead>
<tr>
<th>Attributes</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE (NAA x Kinetin)</td>
<td>5.005</td>
<td>15.585***</td>
</tr>
<tr>
<td>No. of shoots</td>
<td>0.321</td>
<td></td>
</tr>
<tr>
<td>SH (NAA x Kinetin)</td>
<td>2.254</td>
<td>5.926*</td>
</tr>
<tr>
<td>No. of shoots</td>
<td>0.380</td>
<td></td>
</tr>
<tr>
<td>AE (NAA x Kinetin)</td>
<td>0.096</td>
<td>0.192 NS</td>
</tr>
<tr>
<td>No. of leaves</td>
<td>0.500</td>
<td></td>
</tr>
<tr>
<td>SH (NAA x Kinetin)</td>
<td>3.611</td>
<td>6.108*</td>
</tr>
<tr>
<td>No. of leaves</td>
<td>0.591</td>
<td></td>
</tr>
<tr>
<td>AE (NAA x Kinetin)</td>
<td>10.189</td>
<td>8.341**</td>
</tr>
<tr>
<td>Plant height</td>
<td>1.221</td>
<td></td>
</tr>
<tr>
<td>SH (NAA x Kinetin)</td>
<td>17.705</td>
<td>5.528*</td>
</tr>
<tr>
<td>Plant height</td>
<td>3.203</td>
<td></td>
</tr>
<tr>
<td>AE (NAA x Kinetin)</td>
<td>157.036</td>
<td>36.585***</td>
</tr>
<tr>
<td>No. of roots</td>
<td>4.292</td>
<td></td>
</tr>
<tr>
<td>SH (NAA x Kinetin)</td>
<td>33.437</td>
<td>9.009**</td>
</tr>
<tr>
<td>No. of roots</td>
<td>3.712</td>
<td></td>
</tr>
<tr>
<td>AE (NAA x BAP)</td>
<td>3.248</td>
<td>4.619*</td>
</tr>
<tr>
<td>No. of shoots</td>
<td>0.703</td>
<td></td>
</tr>
<tr>
<td>SH (NAA x BAP)</td>
<td>0.050</td>
<td>0.555 NS</td>
</tr>
<tr>
<td>No. of shoots</td>
<td>0.090</td>
<td></td>
</tr>
<tr>
<td>AE (NAA x BAP)</td>
<td>0.017</td>
<td>0.012 NS</td>
</tr>
<tr>
<td>No. of leaves</td>
<td>1.496</td>
<td></td>
</tr>
<tr>
<td>SH (NAA x BAP)</td>
<td>0.009</td>
<td>0.106 NS</td>
</tr>
<tr>
<td>No. of leaves</td>
<td>0.082</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: * = significant at 5% level of probability, ** = significant at 1% level of probability, *** = significant at 0.1% level of probability, NS = not significant.

The regression mean squares in Table 1 above show the amount of total variation in the laboratory data that can be explained by the software model. The effectiveness of the model was greatest in AE and SH media supplemented with NAA and Kinetin for the following attributes in that order – number of roots per explant and plant height. The error mean square shows the amount of variation in the laboratory data that are left unexplained by the model and this was found to be highest in the AE and SH media supplemented with NAA and Kinetin for the number of roots per explants.
The table also shows that there were no significant relationships between the laboratory and software data for the following attributes: No. of leaves for AE medium supplemented with NAA x Kinetin, No. of leaves for AE and SH media supplemented with NAA and BAP, and No. of shoots for SH media supplemented with NAA and BAP. The rest showed some linear relationships and the maximum was observed with AE medium + NAA + Kinetin on number of roots.

V. CONCLUSION

Out of 12 tests conducted as indicated in Table 1, only 4 results did not give significant coefficient of determination, while 8 were significant. This means that the software has 66.67% overall ability to predict the outcome of the laboratory trials. Future work will focus on the development of a newer version of the software that will increase the prediction accuracy up to 90% and above.

APPENDIX

In this section, we show some sample screen shots from Software.

Appendix A: THE HOME PAGE
Appendix B: FILE UPLOAD PAGE

Appendix C: FILE LIST PAGE

Appendix D: APPLICATION PAGE
Appendix E: THE RESULT PAGE

ACKNOWLEDGMENT

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