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# Response surface methodology used evaluation of the combined influence of pH, sucrose and BAP on *in vitro* propagation of *Momordica cochinchinensis* (Lour.) Spreng. (Cucurbitaceae)

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***Abstract***

*Momordica cochinchinensis* (Lour.) Spreng. is a tropical dioecious plant belonging to the family, Cucurbitaceae also known as Gac, baby jack fruit, cochinchin gourd and sweet gourd. The plant is noted for its densely orange fruit, rich in nutrients, especially with 70 times more lycopene content than tomatoes. It possesses medicinal properties and the seeds are used in traditional Chinese medicine called *Mubiezhi* to treat fluxes, liver and spleen disorders, wounds and hemorrhoids. This study evaluated the effect of combination of pH, sucrose and BAP concentration on *in vitro* multiple shoot induction response of *M. cochinchinensis* according to guidelines of design of experiment. For those three factors, Response surface methodology (RSM) using the Box-Behnken design (BBD) was chosen and applied to the design matrix. The response shoot number/explant was evaluated by *in vitro* culture under variable conditions given in the design matrix. The response shoot number/explant was evaluated using *in vitro* culture under variable conditions given in the design matrix. The outcome of this method is an equation that calculates the shoot number as a function of pH, sucrose, and BAP concentration This study created response surface plots and contour plots for each factor, by analysing each factor's level of influence on the response shoot induction.

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***Keywords: Momordica cochinchinensis, Response surface methodology, micropropagation, Box Behnken design, Multiple shoot induction***

## INTRODUCTION

*Momordica cochinchinensis* (Lour.) Spreng. (MC) belonging to the family Cucurbitaceae is commonly known as Gac, a tropical dioecious perennial vine native to south east Asian regions. The Gac fruits are rich in nutrients, especially with 70 times higher lycopene content than tomatoes. Seeds were used in traditional Chinese medicine named Mubiezhi (1). For the propagation of this plant, germination by seeds *ex situ* is agriculturally not suitable because of dormancy due to trypsin inhibitors and is not possible to distinguish between male and female plants before flowering. Tissue culture is the technique to culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions (*in vitro*).
Plant tissue culture is a modern technique for the propagation and conservation of plant species. It is highly valuable in the industrial domain for plant propagation, disease prevention, plant enhancement, and secondary

metabolite production, in addition to their use as research tools. Hundreds of thousands of plants can be continuously grown in an aseptic culture using small pieces of tissue known as explants. Regardless of the season or weather, a single explant can be multiplied into several plants in a comparatively short amount of time and space under controlled conditions, on a year-round basis (2). Micropropagation assumes greater significance in species which cannot be regenerated or are difficult to regenerate by conventional methods such as seeds and vegetative propagation, where conventional methods are inadequate to meet the demand of planting materials and vast variability exists in seed raised progenies.

In culture media, sucrose serves as the primary carbon energy source for *in vitro* cells. Because they are unable to autotrophic, plant cells and tissues in culture media require external carbon sources to provide energy (3). When an external carbon source is added to the medium green shoot regeneration and cell proliferation are enhanced (4, 5). The adequate concentration of sucrose in a medium should be adequate to satisfy the basic energy requirements for cell division/ differentiation without imposing any negative osmotic effects on shoot formation (6). This suggests that sucrose serves as an osmotic regulator, and variations in concentrations of sucrose are one of the factors controlling the shoot induction and growth (7, 5).

Likewise for growth and development in cultures, plant tissues and cells need an optimum pH. Plant enzymatic and hormonal activity, as well as nutrient intake, are influenced by pH (8). The optimal pH balance controls the cytoplasmic activity that influences cells division and the growth of shoots and does not interfere with the buffered pH of the cytoplasm and the function of the cell membrane (9). The pH of a medium also determines the state of the solidifying agent: a pH higher than 6 generates a very hard media, whereas a pH less than 5 does not sufficiently solidify the medium (8). The pH change in cells or organs is due to ions absorbed from the media (10). Hence, it is necessary to optimize the sucrose concentration and pH level for maximum shoot regeneration because the sucrose concentration and pH level directly influence shoot regeneration.

A balance of both auxin and cytokinin will often produce an organized growth of cells, or callus because both cell division and cell expansion occur in actively dividing tissue, but morphology of the outgrowth will depend on the plant species as well as medium composition.

*In vitro* regeneration of female *M. cochinchinensis* has been established. Callus was established on Murashige and Skoog's (MS) agar gelled medium supplemented with 2mg/L 2, 4-D and Coconut milk (15% v/v). Multiple shoot buds were developed from the callus when they were cultured on MS containing 4mg/l 6 Benzylaminopurine (BAP) and CM (15% v/v) (11).

However, to our knowledge, no studies have examined the combined influence of the three factors; pH, sucrose and BAP on micropropagation of *Momordica cochinchinensis*. To study the combined effects of these parameters, different combinations were investigated using statistically designed experiments. However, when using a statistical synthesis method, the number of experiments can be very large so finding an optimal statistical method is essential. Here response surface methodology (RSM) has been selected and used.

The main objective of this study was to advance a clear understanding of the effects of the culture condition, especially the influencing factors, on multiple shoot induction. SM, according to the DOE (design of experiments), is a set of statistical and mathematical tools for experimental design and optimization of the effect of process variables (12,13). RSM reduces the number of trials and recognises the influence of component parameters on corrosion current density. To obtain the input statistics of multiple shooting from RSM, tests were carried out to formulate an equation that calculates the multiple shooting/ explants using the parameters of pH, sucrose concentration, and BAP concentration. After that, the equation was confirmed to be reliable by analysis of variance (ANOVA), and the level of influence of each factor on corrosion current density was investigated by T-test. Finally, to determine the interactions between the factors, surface contour plots of each factor were created.

## MATERIALS AND METHODS

Nodal explant from two-year-old healthy female plants of *M. cochinchinensis* were collected from a grower in Trivandrum, Kerala, India and established in the Department of Botany, University of Kerala. Young shoot cuttings were selected and the older leaves were trimmed off and only the upper five most distal nodes were used as initial explant. Those were subjected to different sterilization treatment experiments. To study the *in vitro* multiplication responses, modified Murashige and Skoog medium (MS) was used (14).

Investigation Scheme: Design of Experiment

To achieve the desired aim, the investigation was planned in the sequence shown in Figure 1.

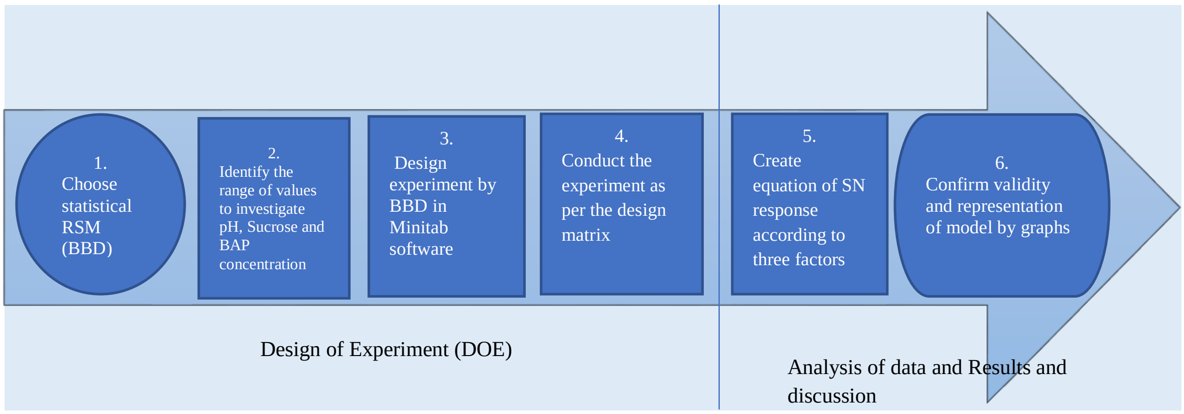


**Figure 1.** Six steps were implemented to investigate the influence of pH, sucrose concentration and BAP concentration on the invitro multiple shoot induction response in *Momordica cochinchinensis*.

The following sections will provide a detailed explanation of the aforementioned steps.
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Response Surface Methodology and Box–Behnken Design
To investigate the influence of several factors on a response, a conventional statistical method can be very useful. However, this approach requires a large number of combinations of different parameter values to determine the effects of those combinations. For example, in this study using the three factors of pH, sucrose, and BAP to obtain the multiple shootings, the maximum, median, and minimum were determined for each factor; therefore, the number of experiments would be at least 3 × 3 × 3 = 27 experiments and thus time-consuming. To simplify the procedure, RSM, a multivariate statistical tool that offers a modern approach to investigating the combined variables, was implemented because it has several advantages (15,16). Firstly, RSM provides accuracy and process optimization with a good perspective for predictive model innovation; secondly, response surfaces are graphical representations used to illustrate the interactive effects of factors effects on response; and most importantly, a limited number of experiment runs are designed, in comparison to a conventional approach for the same number of estimated factors (16) RSM is useful for analyzing the effects of parameters and their interactions with each other.
Estimation of coefficients, prediction of responses, and confirmation of the adequacy of the model are process optimization.
The response is represented by Equation (1) (15
Y = f (X1, X2,..., Xn) ± E, (1)
Here, Y is the response, f is the response function, X1 . . . Xn are the variables of action called factors, n is the number of variables, and E is the experimental error.
These mathematical models are typically polynomials with an unknown structure; however, to fully present linear interactions and z effect, it is preferable to employ a second order polynomial model (17). Experiment design could have a significant effect on the accuracy of approximation as well as the cost of building the response surface (18). In this study, X1, X2, and X3 are the three experimental variables studied, that is, pH, sucrose concentration, and BAP concentration, respectively.
The response (SN) was related to the selected variables by the second-order polynomial

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j>i}^{n} \beta_{ij} X_i X_j + E, (2)$$

Here, $\beta_0$ represents the intercept or regression coefficient; $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ represent the linear, quadratic, and interaction parameters, respectively; $X_i$ and $X_j$ are the coded values of the process variables.
Different designs can be used in statistical experiments; the variation between these designs is determined by the number of runs (experiments) and the experiment points chosen. Central composite design (CCD) and Box–Behnken design (BBD) are two extremely helpful and common for fitting a second-order model. Simple factorial or fractional designs are used to create both designs. Simple factorial or fractional factorial designs are used to create both designs. Both designs are built up from simple factorial or fractional factorial designs. BBD is a spherical, three-level fractional factorial design including a center point and middle point of edges of a circle circumscribed on a sphere while CCD is a fractional factorial design. The advantage of BBD is that it just requires a reasonable group of factors to determine the complex response function and voids experiments performed under extreme conditions, and BBD was selected and applied to analyze the influence of combination factors on the response (19). Response surface methodology has recently been a widespread

approach to optimize and analyze diverse products because of the availability of various computer software programs, such as Design Expert, Statistica, MATLAB, and Minitab, which make the application of RSM easier.

Identifying the Range of the Values to Investigate and Preparing Reagents

Before designing the experiment, the effective factors of a system and the range of these factors must be identified. In this study, three factors (pH, sucrose, and BAP) were used and the tested range of these components was determined based on a standardization experiment. (pH: 5.8; Sucrose: 30; BAP: 5) (11).

**Table 1.** Lists the initial concentration of the solution and range of the factors that influence *in vitro* propagation of MC.

| Investigated Factor | pH | Sucrose (g) | BAP (μM) |
|---|---|---|---|
| Initial value | 5.8 | 30 | 5 |
| Investigated value range | 5-6.6 | 20-40 | 1-9 |

Design of Experiment and Design Matrix

Based on the testing range, the design of the experiment was carried out using the Minitab 18 software. For the RSM using Box–Behnken design, an experiment number of N = k2 + k + cp was required, where k is the factor number and cp is experiment runs at the center point. For the statistical calculations, the variables were coded as the minimum, central/median, and maximum levels of each variable, designated as −1, 0, and 1, respectively, as shown in Table 2. Two ways to collect enough data is to use replicates and add just a few center points. Replicating could be time-consuming; therefore, this design includes 3 experiment runs at the center point. In this study, the number of factors, k, was 3 and cp was 3 times the number of central points (red point in Figure 2); therefore, the total number of experiments was 15 at three levels, as shown in Table 3. This number was significantly reduced compared with the conventional methods.

**Table 2.** Actual investigated values of factors and their corresponding coded levels.

| Variables | Code values | | |
|---|---|---|---|
| | -1 minimum | 0 median | +1 maximum |
| X1 pH | 5 | 5.8 | 6.6 |
| X2 Sucrose | 20 | 30 | 40 |
| X3 BAP | 1 | 5 | 9 |

The results were input into the Minitab 18 software for further analysis. On examining the fit summary, the quadratic model was found to be statistically significant for the response.

**Table 3.** Box-Behnken Design Table (Randomized)

| StdOrder | Run | PtType | Blk | A | B | C |
|---|---|---|---|---|---|---|
| 11 | 1 | 2 | 1 | 0 | -1 | 1 |
| 3 | 2 | 2 | 1 | -1 | 1 | 0 |
| 14 | 3 | 0 | 1 | 0 | 0 | 0 |
| 13 | 4 | 0 | 1 | 0 | 0 | 0 |
| 6 | 5 | 2 | 1 | 1 | 0 | -1 |
| 10 | 6 | 2 | 1 | 0 | 1 | -1 |
| 1 | 7 | 2 | 1 | -1 | -1 | 0 |
| 4 | 8 | 2 | 1 | 1 | 1 | 0 |
| 7 | 9 | 2 | 1 | -1 | 0 | 1 |
| 9 | 10 | 2 | 1 | 0 | -1 | -1 |
| 12 | 11 | 2 | 1 | 0 | 1 | 1 |
| 8 | 12 | 2 | 1 | 1 | 0 | 1 |
| 5 | 13 | 2 | 1 | -1 | 0 | -1 |
| 2 | 14 | 2 | 1 | 1 | -1 | 0 |
| 15 | 15 | 0 | 1 | 0 | 0 | 0 |

## RESULTS AND DISCUSSION

### Data analysis using Box Behnken design

Effect of three independent variables pH (X1), Sucrose (X2), BAP (X3) on in vitro shoot induction in Momordica cochinchinensis (Lour.) Spreng. were assessed using a three level and three factors as per the response surface methodology of the Box Behnken design. The design comprises 15 experiments arranged randomly consisting of three centre points and twelve factorial points. The three replicate centre points (runs 3, 4 and 15) were applied to access the pure error sum of squares. A regression model was built to evaluate the shoot induction and multiple shooting(Y1), presented through a second order polynomial equation:

$$SN\ (Y1) = -47.7 + 12.25\ pH + 0.797\ Sucrose + 1.038\ BAP - 0.898\ pH * pH - 0.01075\ Sucrose * Sucrose + 0.2641\ BAP * BAP - 0.0313\ pH * Sucrose - 0.4531\ pH * BAP + 0.01875\ Sucrose * BAP$$

where, X1, X2, X3 define the actual value and concentrations of pH, sucrose and BAP respectively.

Statistical significance of regression model was analyzed using F distribution value. Model can be validated if P value in F distribution analysis was less than 0.05. F value of the model was 129.14 and P value 0.000, which imply that model was significant with chances of F value being this large due to noise in each case. Therefore, we reject the null hypothesis of no relationship between the dependent and the independent variables. Therefore, the full quadratic model of the pH, Sucrose, BAP (independent variables) significantly affects the response SN (dependent variable). Analysis of variance (ANOVA) for multiple shooting SN was presented in Table 5. Results imply that model was significant as p-values are less than 0.05. The P-value for the linear terms for the factors, pH, Sucrose, and concentration of BAP, are also lower than the level of significance. Therefore, the linear terms significantly affect SN. The P-value for the quadratic terms for the three factors is also observed to be lower than the level of significance. Therefore, the quadratic terms for the temperature and the humidity significantly affect comfort. The interaction between the pH and sucrose is observed to be insignificant with respect to SN.

In case of multiple shooting SN (Y1), lack of ft of F-value is 4.50, and P- value 0.187 which is insignificant relative to pure error. An insignificant lack of fit indicates that the model perfectly fits the experimental values. Therefore, the quadratic model with the predictor variables pH, sucrose, BAP significantly predict SN of MC. Lack of fit of regression model is insignifcant and to determine if model fts experimental values the R2 coeffcient was measured. As per model summary Table 6, The standard deviation of the difference between the fitted and data values is represented by S. It indicates the deviation of the data values from the fitted values and is expressed in response variable units. The lower the value of S (0.609918), the better the model describes the response.

R-sq is the percentage of variation in the response that is explained by the model, here it is 99.57. The higher the R-sq value, the better the model fits the data.

The percentage of the variation in the response that is explained by the model is denoted as Adjusted $R^2$, is adjusted for the number of predictors in the model relative to the number of observations. And it is 98.80, This adjusted $R^2$ can be used to compare models that have different numbers of predictors. $R^2$ always increases when you add a predictor to the model, even when there is no real improvement to the model. The adjusted $R^2$ value incorporates the number of predictors in the model to help you choose the correct model.

PRESS (The prediction error sum of squares) is a measure of the deviation between the fitted values and the observed values. PRESS is similar to the sum of squares of the residual error (SSE) here it is 26.46 which is the summation of the squared residuals and estimating the regression equation. PRESS is to use to assess the model's predictive ability, the smaller the PRESS value, the better the model's predictive ability.

By systematically eliminating each observation from the data set, estimating the regression equation, and assessing how well the model predicts the eliminated observation, Predicted $R^2$ is calculated. The value of predicted $R^2$ is 93.91%. We can use predicted $R^2$ to determine how the model predicts the response for new observations. Here the model that has larger predicted $R^2$ values means it has better predictive ability. Predicted $R^2$ can also be more useful than adjusted $R^2$ for comparing models because it is calculated with observations that are not included in the model calculation

**Evaluation of the Effects of pH, Sucrose, and BAP Concentrations on the invitro shoot induction in MC.**

An F-test can show if a group of variables is jointly significant and a T-test can indicate if a variable is statistically significant. To precisely determine the effect of each factor on the multiple shooting in MC (i.e., which influence is reliable and which is not), the Pareto chart in Figure 4 shows the standardized effects of pH, sucrose, and BAP concentrations on the multiple shooting evaluation. The T-value, which is used to measure how large the coefficient is in relationship to its standard error, is equal to the coded coefficient divided by its standard error. The Pareto chart was used to determine the magnitude and importance of the effects. Each bar represents the T-value for a type of factor; the height of the bar represents any important factors. Therefore, the biggest influence on multiple shoot induction in MC is BAP concentration. On the Pareto chart, bars that cross the reference line are statistically significant. In Figure 3, the bars that represent factors B, BB, AC, A, CC, C cross the reference line at 2.57. These factors are statistically significant at the 0.05 level in terms of the current model. Because the Pareto chart displays the absolute value of the effect, it determines large effects; however, it cannot determine effects that increase or decrease the response. A normal probability plot of the standardized effect is used to examine the magnitude and direction of the effects on one plot. In this case, all parameters have a significant influence on the corrosion current density when their p-value is less than 0.05. If the p-value is greater than 0.10, the model terms are insignificant. In this case, the p-value of pH*pH is 0.130, pH*Sucrose is 0.450 and Sucrose*BAP is 0.57, which are inefficient model terms as shown in Table 5. However, the p-value of the model shown in Table 5 &6 is 0.000; therefore, removing them is not necessary.

**Interactive effect of pH and BAP and Sucrose concentration on Multiple shoot induction**

Figure 4 show Effect of three independent variables pH (X1), Sucrose (X2), BAP (X3) on *in vitro* shoot induction in *Momordica cochinchinensis* (Lour.) Spreng. were assessed using a three level and three factors as per the response surface methodology of the Box Behnken design. The design comprises 15 experiments arranged randomly consisting of three centre points and twelve factorial points. The three replicate centre points (runs 3, 4 and 15) were applied to access the pure error sum of squares. A regression model was built to evaluate the shoot induction and multiple shooting(Y1), presented through a second order polynomial equation:

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**Interactive effect of pH and BAP and Sucrose concentration on Multiple shoot induction**
Figure 4 shows response surface and contour plots showing the effect of pH and BAP on multiple shoot induction response (SN) in MC. The number of shoots increased when the concentration of BAP increasing from 1 to 9 is dependent on the changes in pH with a hold value of sucrose concentration 30g. Figure 5 shows response surface and contour plots showing the effect of Sucrose and BAP on multiple shoot induction response (SN) in MC with a hold value of pH 5.8. Here the Sucrose concentration related variation in the response SN is considered to be negligible and the concentration of sucrose is the independent variable having least effect on the response.

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**Table 4.** Experimental observations for response variable obtained according to the BBD.

| RunOrder | PtType | Blocks | pH | Sucrose | BAP | SN |
|---|---|---|---|---|---|---|
| 1 | 2 | 1 | 5.8 | 20 | 9 | 11.2 |
| 2 | 2 | 1 | 5 | 40 | 5 | 3.2 |
| 3 | 0 | 1 | 5.8 | 30 | 5 | 3.2 |
| 4 | 0 | 1 | 5.8 | 30 | 5 | 3.2 |
| 5 | 2 | 1 | 6.6 | 30 | 1 | 0.4 |
| 6 | 2 | 1 | 5.8 | 40 | 1 | 0.4 |
| 7 | 2 | 1 | 5 | 20 | 5 | 1.8 |
| 8 | 2 | 1 | 6.6 | 40 | 5 | 1.2 |
| 9 | 2 | 1 | 5 | 30 | 9 | 16.6 |
| 10 | 2 | 1 | 5.8 | 20 | 1 | 0.2 |
| 11 | 2 | 1 | 5.8 | 40 | 9 | 14.4 |
| 12 | 2 | 1 | 6.6 | 30 | 9 | 10.8 |
| 13 | 2 | 1 | 5 | 30 | 1 | 0.4 |
| 14 | 2 | 1 | 6.6 | 20 | 5 | 0.8 |
| 15 | 0 | 1 | 5.8 | 30 | 5 | 3.8 |

**Table 5.** Response Surface Regression: SN versus pH, Sucrose, BAP Analysis of Variance

| Source | DF | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|
| Model | 9 | 432.369 | 48.041 | 129.14 | 0.000 |
| Linear | 3 | 345.880 | 115.293 | 309.93 | 0.000 |
| pH | 1 | 9.680 | 9.680 | 26.02 | 0.004 |
| Sucrose | 1 | 3.380 | 3.380 | 9.09 | 0.030 |
| BAP | 1 | 332.820 | 332.820 | 894.68 | 0.000 |
| Square | 3 | 75.579 | 25.193 | 67.72 | 0.000 |
| pH*pH | 1 | 1.221 | 1.221 | 3.28 | 0.130 |
| Sucrose*Sucrose | 1 | 4.267 | 4.267 | 11.47 | 0.020 |
| BAP*BAP | 1 | 65.910 | 65.910 | 177.18 | 0.000 |
| 2-Way Interaction | 3 | 10.910 | 3.637 | 9.78 | 0.016 |
| pH*Sucrose | 1 | 0.250 | 0.250 | 0.67 | 0.450 |
| pH*BAP | 1 | 8.410 | 8.410 | 22.61 | 0.005 |
| Sucrose*BAP | 1 | 2.250 | 2.250 | 6.05 | 0.057 |
| Error | 5 | 1.860 | 0.372 | | |
| Lack-of-Fit | 3 | 1.620 | 0.540 | 4.50 | 0.187 |
| Pure Error | 2 | 0.240 | 0.120 | | |
| Total | 14 | 434.229 | | | |

**Table 6.** Model Summary

| S | R-sq | R-sq(adj) | PRESS | R-sq(pred) |
|---|---|---|---|---|
| 0.609918 | 99.57% | 98.80% | 26.46 | 93.91% |

**Table 7.** Estimated coefficients with coded units for SN

| Term | Coef | SE Coef | T-Value | P-Value | VIF |
|---|---|---|---|---|---|
| Constant | 3.400 | 0.352 | 9.66 | 0.000 | |
| pH | -1.100 | 0.216 | -5.10 | 0.004 | 1.00 |
| Sucrose | 0.650 | 0.216 | 3.01 | 0.030 | 1.00 |
| BAP | 6.450 | 0.216 | 29.91 | 0.000 | 1.00 |
| pH*Ph | -0.575 | 0.317 | -1.81 | 0.130 | 1.01 |
| Sucrose*Sucrose | -1.075 | 0.317 | -3.39 | 0.020 | 1.01 |
| BAP*BAP | 4.225 | 0.317 | 13.31 | 0.000 | 1.01 |
| pH*Sucrose | -0.250 | 0.305 | -0.82 | 0.450 | 1.00 |
| pH*BAP | -1.450 | 0.305 | -4.75 | 0.005 | 1.00 |
| Sucrose*BAP | 0.750 | 0.305 | 2.46 | 0.057 | 1.00 |

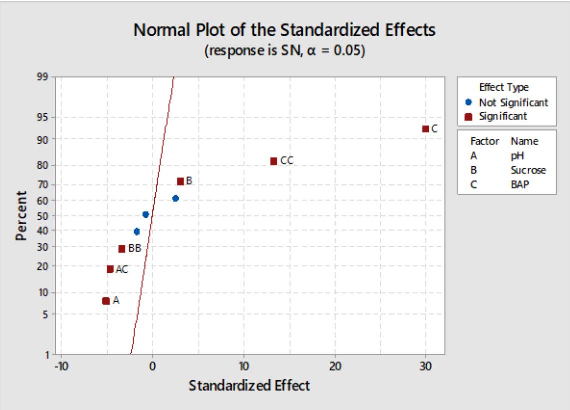


**Fig. 2.** Normal probability plot

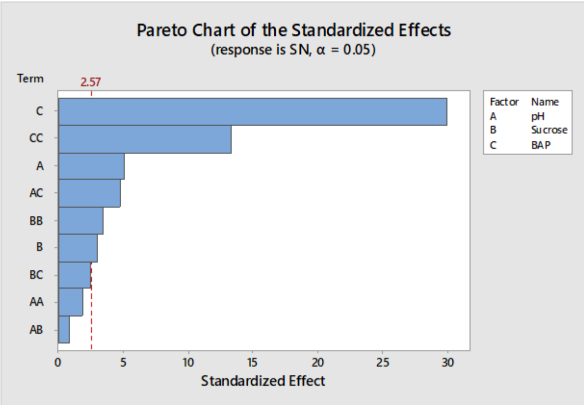


**Fig. 3**. Pareto chart for the standardized effects of pH, Sucrose and BAP concentration on the response SN on invitro culture of MC

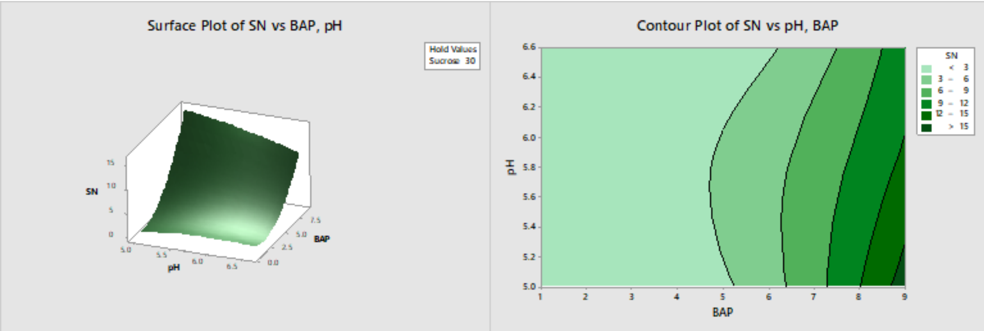


**Fig. 4.** Response surface and contour plots showing the effect of pH and BAP on multiple shoot induction response (SN) in MC

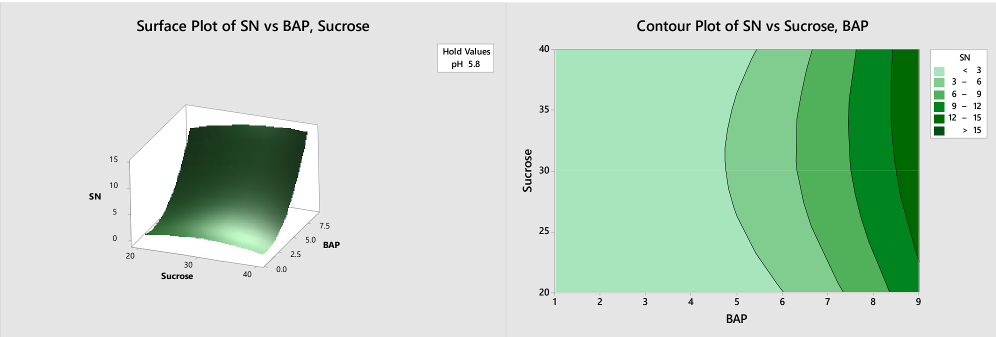


**Fig. 5.** Response surface and contour plots showing the effect of Sucrose and BAP on multiple shoot induction response (SN) in MC

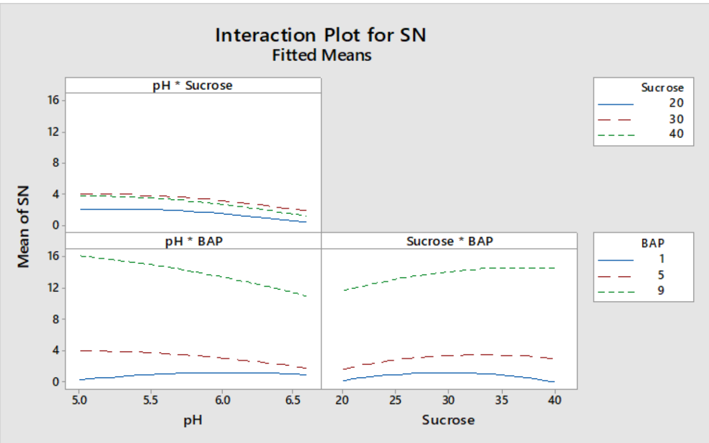


**Fig. 6.** Interaction Plot of pH, Sucrose, BAP concentration for SN

**Fig. 7:** Fig. 7.1. Habit of MC, Fig. 7.2.-7.5 showing regenerated multiple shoots of experiment with experiment run order 3,8,11,9

## CONCLUSIONS

Based on the results, the following conclusions could be drawn:

The effects of pH, sucrose concentration and BAP concentration on multiple shoot induction in *Momordica cochinchinensis* (Lour.) Spreng. were investigated by statistical method RSM. The results suggest that BAP is an independent variable positively affecting multiple shoot induction in MC, followed by pH and sucrose concentration.

The effect level of independent variables on multiple shoot induction/explant was found to follow an increasing sequence of sucrose concentration < pH < BAP concentration.

The results show that the model was successful; however, it has a limitation because it can only be used within the experimental ranges. As BAP was the critical factor influencing multiple shoot induction further research with expanded BAP concentration ranges is necessary.

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