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# Optimization of shoot regeneration using leaf explants from *Cichorium intybus* by Box-Behnken Design

**Dakshayini*, C. G. Louella, C. R. Vaman, P. Ujwal**

*Department of Biotechnology Engineering, NMAM Institute of Technology, Udupi, Karnataka, India.*

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***ABSTRACT***
*Chicory (Cichorium intybus), a typical coffee substitute, is also a medicinally important plant used to treat ailments ranging from jaundice to tumors and cancer. The levels of growth factors and conditions for 3 independent variables were selected for optimization (concentration of Kn, indole-3-acetic acid, and CH) which affect the shoot generation from the leaf explants of C. intybus. The $R^2$ value was determined as 0.94338 which means that 94.38% of the experimental data of the length of the shoots and $R^2$=0.90575 which means 90.75% for number of the shoots for shoot generation was compatible with the data predicted by the model.*

***Key words:*** *Cichorium intybus, In vitro shoot regeneration, Response surface methodology and optimization.*

## 1. INTRODUCTION

Medicinal plants are being used in the treatment of diseases from time immemorial. Plant-based medicines are found to be safe for consumption with fewer side effects than the synthetic drugs. *Cichorium intybus* is one such medicinal plant that is of great importance in Eurasia and parts of Africa [1]. Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is affected by several independent factors and the objective is to optimize this response [2,3]. The present work aimed at employing RSM to optimize the direct shoot generation from leaf explants of *C. intybus*.

## 2. EXPERIMENTAL

Seeds of *C. intybus* were air dried and washed thoroughly with soap solution and soaked in 0.1% mercuric chloride for 10 min. The seeds were then germinated in pots for 2 months. The leaves of germinated plantlets were soaked in distilled water for 1 h. They were later rinsed with tween 20 for 20 min and 70% ethanol for an hour**.** The leaves were dipped in 0.1% mercuric chloride for 10 min in the laminar air flow chamber and were used for the optimization experiments.

*Corresponding Author:
*E-mail: dakshayinikandoor2@gmail.com*

## 3. RESULT AND DISCUSSION

### *3.1. Optimization of Factors for Obtaining Maximum Number of Shoot Regenerated from Leaf Explants of C. intybus by Box-Behnken Design*

Initially, the experiments were carried out to measure the induction of shoots from leaf explants, using combinations and concentration of plant growth regulators. Induction of shoots was developed on the 25th day using 1.5 $mgl^{-1}$ Kn + 0.5 $mgl^{-1}$ indole-3-acetic acid (IAA) + 500 mg casein hydrolysate. The yield obtained was approximately 5-7 shoots per explant. It was employed to determine the optimum levels of the three selected variables that resulted in the maximum length of the shoots and number of shoots from leaf explants of *C. intybus*. The observed response ($Y_1$ and $Y_2$) is shown in Tables 1 and 2 which is in close agreement with other report [1]. The regression analysis performed on the results obtained leads to mathematical model represented by the following second order equation for number of shoots ($Y_1$) as a function of Kn ($X_1$), IAA ($X_2$), and CH concentration ($X_3$) for the length of the shoots.

*3.1.1. Second-order model for length of the shoots*

$$Y_1=156.1+144.433X_1 35.533X_1^2+42.377X_2 46.208X_2^2+47.233X_3 17.896X_3^2+1.050X_1 X_2 13.4X_1X_3-2.062X_2X_3$$

*3.1.2. Second-order model for number of the shoots*

$$Y_2=64.687537.0X_1+8.0X_1^2 35.0X_2+37.5X_2^2 31.875X_3 +6.2500X_3^2 10.0X_1X_2+5.0X_1X_3+31.25X_2X_3$$

The $R^2$ value was determined as 0.94338 which means that 94.38% of the experimental data of the length of the shoots (Table 2) and $R^2$=0.90575 which means 90.75% for a number of the shoots for shoot generation was compatible with the data predicted by the model (Table 3). Thus, the number of shoots produced from leaf explants of *C. intybus* could be predicted by this model. The response surface plots show the variation of length and number of shoots with two interacting factors keeping the other factor fixed at its center value. It was observed that the number of shoots produced, decreased with increase in IAA and CH concentration whereas, in case of the length of shoots (Figure 1a and b), they increased with IAA and CH concentration. In general, auxin is used for elongation of the shoots and a very high increase in auxin will inhibit the formation of shoots. An appropriate amount of IAA and CH was mixed to obtain cell elongation. The length of shoots increased with increase in Kn concentration up to 2 $mgl^{-1}$, beyond which a decrease was observed, whereas in the case of number of shoots a continuous increase was observed with increase in Kn concentration (Figure 1c and d). This is in close agreement with the earlier report [4] where they proved increasing concentrations of Kn lead to the formation of shoots and higher rate of this hormone lead to a decrease in shoots (Figure 1e and f). The growth rate was more when Kn was used alone or in combination with the IAA. It stimulates cell division for length of shoots ($Y_1$) as a function of Kn ($X_1$), IAA ($X_2$), and CH concentration ($X_3$) for the length of the shoots were determined by desirability profile generated by the software (Figure 2a) and were found to be 1.75 $mgl^{-1}$ Kn, 0.4 $mgl^{-1}$ IAA, and 0.5 $mgl^{-1}$ CH concentration and for number of shoots it was found to be 1.75 $mgl^{-1}$ Kn, 0.7 $mgl^{-1}$ IAA, and 0.7 mgl CH concentration (Figure 2b). Figure 3a and b show the initiation of shoot and multiple shoot generation from the explants of *C. intybus*. The regenerated shoots developed rooting on the same media after 2 months of culture. These elongated shoots were well established in the field (Figure 3c).

**Table 1:** Box-Behnken design with 3 factors 15 experiments.

| $X_1$ | $X_2$ | $X_3$ | $Y_1$ | $Y_2$ |
|---|---|---|---|---|
| 1.75 | 0.3 | 0.5 | 3.77 | 7 |
| 2.25 | 0.3 | 0.5 | 2.26 | 3 |
| 1.75 | 0.7 | 0.5 | 2.59 | 8 |
| 2.25 | 0.7 | 0.5 | 1.29 | 2 |
| 1.75 | 0.5 | 0.3 | 4.34 | 5 |
| 2.25 | 0.5 | 0.3 | 3.21 | 2 |
| 1.75 | 0.5 | 0.7 | 5.35 | 5 |
| 2.25 | 0.5 | 0.7 | 1.54 | 3 |
| 2 | 0.3 | 0.3 | 3.7 | 7 |
| 2 | 0.7 | 0.3 | 2.73 | 3 |
| 2 | 0.3 | 0.7 | 5.4 | 4 |
| 2 | 0.7 | 0.7 | 4.1 | 5 |
| 2 | 0.5 | 0.5 | 6.76 | 3 |
| 2 | 0.5 | 0.5 | 6.7 | 3 |
| 2 | 0.5 | 0.5 | 6.18 | 3 |

$X_1$=Kn, $X_2$=IAA, $X_3$=CH, $Y_1$=Length, $Y_2$=Number, IAA=Indole-3-acetic acid

**Table 2:** ANOVA table showing the significance results for $Y_1$ (Length).

| Factors | SS | df | MS | F | p |
|---|---|---|---|---|---|
| $X_1$ (L) | 7.50781 | 1 | 7.50781 | 14.9469 | 0.01181 |
| $X_1$ (Q) | 18.2108 | 1 | 18.2108 | 36.255 | 0.00182 |
| $X_2$ (L) | 2.44205 | 1 | 2.44205 | 4.86175 | 0.0786 |
| $X_2$ (Q) | 12.6142 | 1 | 12.6142 | 25.1129 | 0.00407 |
| $X_3$ (L) | 0.72601 | 1 | 0.72601 | 1.44538 | 0.2831 |
| $X_3$ (Q) | 1.892 | 1 | 1.892 | 3.76669 | 0.10996 |
| $X_1$ by $X_2$ | 0.01103 | 1 | 0.01103 | 0.02195 | 0.88801 |
| $X_1$ by $X_3$ | 1.7956 | 1 | 1.7956 | 3.57477 | 0.11725 |
| $X_2$ by $X_3$ | 0.02723 | 1 | 0.02723 | 0.0542 | 0.82514 |
| Error | 2.51149 | 5 | 0.5023 | | |
| Total SS | 44.359 | 14 | | | |
| $R^2$=0.94338 | | | | | |

SS=Sum-of-squares, MS=Mean square

**Table 3:** ANOVA table showing the significance results for $Y_2$ (number).

| Factors | SS | df | MS | F | p |
|---|---|---|---|---|---|
| $X_1$ (L) | 28.125 | 1 | 28.125 | 29.6053 | 0.00285 |
| $X_1$ (Q) | 0.92308 | 1 | 0.92308 | 0.97166 | 0.36953 |
| $X_2$ (L) | 1.125 | 1 | 1.125 | 1.18421 | 0.32615 |
| $X_2$ (Q) | 8.30769 | 1 | 8.30769 | 8.74494 | 0.03162 |
| $X_3$ (L) | 0 | 1 | 0 | 0 | 1 |
| $X_3$ (Q) | 0.23077 | 1 | 0.23077 | 0.24291 | 0.64299 |
| $X_1$ by $X_2$ | 1 | 1 | 1 | 1.05263 | 0.35195 |
| $X_1$ by $X_3$ | 0.25 | 1 | 0.25 | 0.26316 | 0.62981 |
| $X_2$ by $X_3$ | 6.25 | 1 | 6.25 | 6.57895 | 0.05034 |
| Error | 4.75 | 5 | 0.95 | | |
| Total SS | 50.4 | 14 | | | |

SS=Sum-of-squares, MS=Mean square

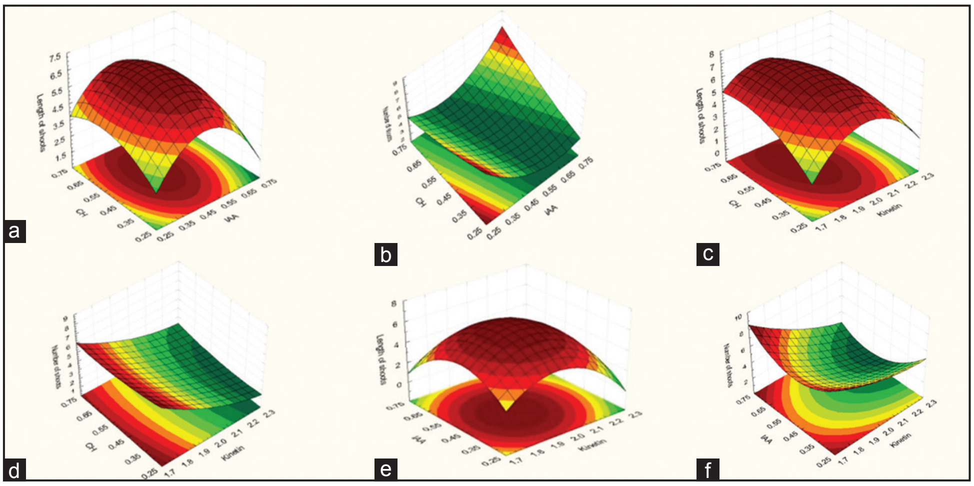

**Figure 1:** Three-dimensional plot showing the variation of length and number of shoots from leaf explants of *Cichorium intybus.* (a) CH and indole-3-acetic acid (IAA) concentration for length of the shoots, (b) CH and IAA concentration for number of the shoots, (c) Kn and CH concentration for length of the shoots, (d) Kn and CH concentration for number of the shoots, (e) Kn and IAA concentration for length of the shoots, (f) Kn and IAA concentration for number of the shoots.

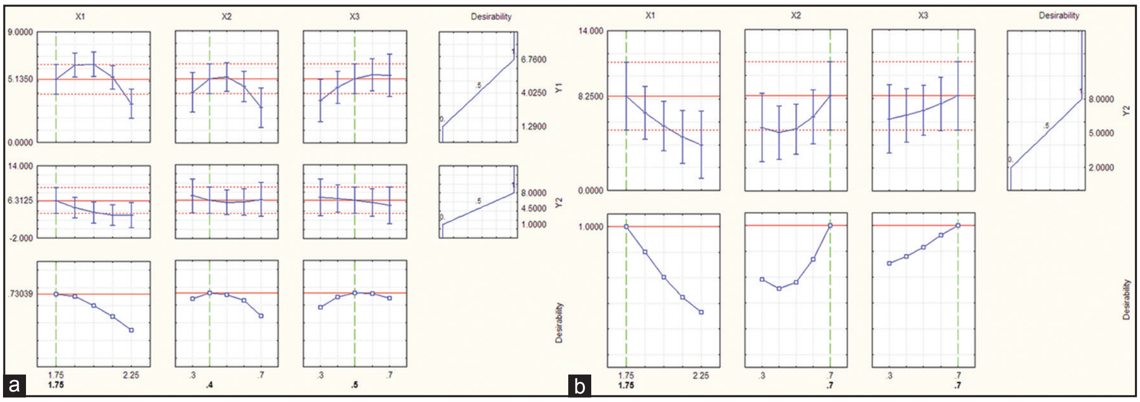


**Figure 2:** Profiles for desirability levels of factors. (a) Length optimum conditions, (b) number of the shoots i.e., Kn ($X_1$), indole-3-acetic acid ($X_2$), CH concentration ($X_3$) and number desirability for obtaining maximum number of shoots.

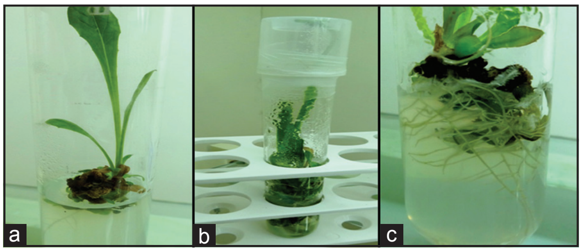

**Figure 3:** Regeneration of the plant from leaf explant of *Cichorium intybus* using Box-Behnken design. (a) Induction of shoots from the leaf explant of *C. intybus,* (b) multiple shoot formation from the leaf explants of *C. intybus* at the optimized conditions, (c) rooting of regenerated shoots.

Second order model:

$$Y_1=156.1+144.433X_1 35.533X_1^2+42.377X_2 46.208X_2^2+47.233X_3 17.896X_3^2+1.050X_1X_2 13.4X_1X_3-2.062X_2X_3$$

$$Y_2=64.687537.0X_1+8.0X_1^2 35.0X_2+37.5X_2^2 31.875X_3+6.2500X_3^2 10.0X_1X_2+5.0X_1X_3+31.25X_2X_3$$

## 4. CONCLUSION

*C. intybus* has a long history of traditional use with great potential as medicine. Optimization studies in the field of plant micropropagation are scarce. In this context, an attempt was made to optimize the direct shoot generation of *C. intybus* using its leaf explants by using Box-Behnken. RSM was found to be exemplary in describing the effect of significant factors and modeling the process of shoot generation of *C. intybus.* The significance of this study is that the optimized conditions obtained from this study can be used for the large-scale micropropagation of *C. intybus* in a short span of time.

## 5. REFERENCES

1. R. A. Street, J. Sidana, G. Prinsloo, (2013) *Cichorium intybus*: Traditional uses,

phytochemistry, pharmacology, and toxicology, ***Evidence-Based Complementary and Alternative Medicine,*** **1:** 1-13.

2. D. C. Montgomery, (2001) Design and analysis of experiments, In: K. H. Anderson, E. Aiello, (Eds.), ***Response Surface Methods and other Approaches in Process Optimization***, New York: John Wiley and Sons Inc., p427-430.
3. D. Bas, H. Boyaci, (2007) Modeling and optimization I: Usability of response surface methodology, ***Journal of Food Engineering,*** **78:** 836-845.
4. S. Nandagopal, B. R. Kumari, (2006) Adenine sulphate induced high frequency shot organogenesis in callus and *in vitro* flowering of *Cichorium intybus* L. cv. Fokus – A potent medicinal plant, ***Acta Agriculturae Slovenica,*** **87:** 415-425.