



# The Desirability Optimization Methodology; a Tool to Predict Two Antagonist Responses in Biotechnological Systems: Case of Biomass Growth and Hyoscyamine Content in Elicited *Datura stramonium* Hairy Roots

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**Background:** The use of the desirability function approach combined with the response surface methodology (RSM), also called Desirability Optimization Methodology (DOM), has been successfully applied to solve medical, chemical, and technological questions. It is particularly efficient for the determination of the optimal conditions in natural or industrial processes involving different factors leading to the antagonist responses.

**Objectives:** Surprisingly, DOM has never been applied to the research programs devoted to the study of plant responses to the complex environmental changes, and thus to biotechnological questions.

**Materials and Methods:** In this article, DOM is used to study the response of *Datura stramonium* hairy roots (HRs), obtained by genetic transformation with *Agrobacterium rhizogenes* A<sub>4</sub> strain, subjected to the jasmonate treatments.

**Results:** Antagonist effects on the growth and tropane alkaloid biosynthesis are confirmed. With a limited number of experimental conditions, it is shown that 0.06 mM jasmonic acid (JA) applied for 24 h leads to an optimal compromise. Hyoscyamine levels increase by up to 290% after 24 h and this treatment does not significantly inhibit biomass growth.

**Conclusions:** It is thus demonstrated that the use of DOM can efficiently - with a minimized number of replicates - leads to the optimization of the biotechnological processes.

**Keywords:** Desirability Optimization Methodology (DOM); Hyoscyamine; Jasmonic Acid; Multiresponse Optimization; Response Surface Methodology (RSM).

## 1. Background

When using a complex system, several experimental factors have to be optimized so it is essential to evaluate alternative analytical procedures according to multiple criteria (1, 2). The determination of the optimum conditions for the input variables requires the simultaneous consideration of all responses. This is called a multiresponse problem (3, 4) based on Multicriteria Decision Making. To obtain a satisfactory compromise, the desirability approach is used as a

powerful tool in multi-response systems (4). The desirability function, defined by Harrington (1965) (5) and by Derringer and Suich (1980) (3), is one of the approaches used for factor optimization in such systems (6-8). It is based on the transformation of all the obtained responses from different scales into a scale-free value. The values of desirability functions lie between 0 and 1. The value 0 is attributed when the factors give an undesirable response, while the value 1 corresponds to the optimal performance for the studied factors (3, 4).

As an example in biological sciences, an optimization strategy based on the desirability function approach has been used for the microbial glutamine production (9). Furthermore, for studying the behavior of the complex systems, the use of the desirability function approach combined with the classic experimental designs enables the following matters (10-12):

A reduction in the number of modalities to be tested and a determination of the optimal compromise between different levels of environmental factors leading to the antagonist responses.

Among the classic experimental designs, Response Surface Methodology (RSM) is widely used because of its simplicity and effectiveness. The goal of RSM is to explore the relationship between the response (a dependent variable) and the studied factors (an independent variable) involved in an experiment. The mathematical model of RSM is a polynomial equation (Eq.) of the second degree which has the advantage of being easy to estimate and then to apply for the approximation of the response (13-15).

The combined application of RSM and the desirability approach gives rise to a more powerful method for finding an optimal balance between the responses, including antagonist responses (multicriteria optimization). This combination of the RSM and desirability function has resulted in a new method called "Desirability Optimization Methodology or DOM" (16). Although quite commonly used in industrial microbiology, biochemistry, and chemical analysis (7, 10, 17), to the best of our knowledge, such a method has never been applied to the plant biotechnological studies and, more specifically, to the study of the *in vitro* response of a plant secondary metabolism to the environmental factors.

Because of the interest in the biotechnological production of the plant secondary metabolites, we chose to evaluate the potential of DOM for studying the metabolic changes induced by elicitation in the hairy roots (HRs) of *Datura stramonium*. As widely described (18-20), hairy root cultures of *Datura* species produce tropane alkaloids. Their biosynthesis can be modulated by the culture medium composition or by elicitor treatments. Such treatments can lead to tropane alkaloid levels as high as, and even higher than those measured in the whole plants. Because of their rapid growth capacity, HRs are also a good experimental system.

Synthesized from linolenic acid, jasmonate is a signal molecule involved in the response to the different kinds of stress, and more specifically, to the elicitation; it thus leads to the plant defense reactions (21-24). It stimulates the accumulation of the secondary metabolites such as tropane alkaloids in solanaceous plants (25). The

use of elicitors or phytohormones, such as jasmonate and its volatile derivative methyl jasmonate (26, 27), is a possible approach for increasing tropane alkaloid biosynthesis in *Datura* species (25, 28). However, plant growth is then inhibited (25, 29, 30). This kind of antagonist response is often observed during stress (31-35). In order to develop an efficient *in vitro* technology to produce secondary metabolites using elicitation, optimum conditions must be determined *i.e.* elicitor concentration and elicitation time and duration. In *Datura* hairy root cultures, because growth decreases when alkaloid levels increase, determining an optimal treatment condition thus becomes a difficult challenge (36-38).

## 2. Objectives

This study investigates how efficiently DOM can be used to study the antagonist response (growth *vs.* alkaloid synthesis) of *Datura stramonium* HRs subjected to jasmonate treatments. Subsequently, i) elicitation conditions are optimized for hyoscyamine production and ii) this mathematical modeling using DOM may serve as an example for other studies devoted to the plant biology and biotechnology.

## 3. Materials and Methods

### 3.1. Plant Material

The HRs of *Datura stramonium*, obtained by genetic transformation with *Agrobacterium rhizogenes* A<sub>4</sub> strain on hypocotyls, were examined for their performance: growth (dry weight), alkaloid content, and reaction to elicitation (14, 20). Then, the best hairy root line was selected and subcultured every 20-25<sup>th</sup> day in fresh B5 Gamborg hormone-free culture medium containing 3% sucrose (35). All hairy root cultures were grown at 26 ± 1 °C in the dark. For experiments, root tips (0.3 g fresh weight) were cultured in Petri dishes containing 20 mL of hormone-free medium (Gamborg) (39) with 3% sucrose.

### 3.2. Elicitation

The solution of jasmonic acid (JA) [(–)jasmonic acid from Sigma-Aldrich] was prepared by dissolving jasmonic acid in an adequate volume of ethanol. It was then filtered through a membrane filter (pore size: 0.2 µm Nalgene) (14). Five concentrations of the jasmonic acid (JA): 0.0172, 0.0250, 0.0500, 0.0750, and 0.0830 mM and five exposure times (ET): 1.6, 8, 28, 48, and 54.4 hours were tested according to CCDO, (see section RSM: experimental design). The choice of concentrations and ETs was based on a literature study (25, 30) and on our previous works. The elicitation experiments were initiated after the transfer of roots to

the fresh B5 medium (20 mL) on the 12<sup>th</sup> day of culture (logarithmic phase of growth according to Amdoun *et al.*, 2009 (20).

All cultures were grown in Petri dishes containing 20 mL of B5 medium and in darkness at  $26 \pm 1$  °C. The control (ethanol-water solution without JA) was the same hairy root line in the same conditions of the culture. All culture experiments were carried out in the experimental setup design of triplicates.

### 3.3. Determination of Biomass and Hyoscyamine Content

Applying a precision balance, the biomass of the correspondent dry weight of HRs, obtained after oven drying for 48 h at 40 °C, was measured. The alkaloids were extracted using a method described by Amdoun *et al.* (2009) (20). Hyoscyamine was analyzed by the GC-MS method previously reported by Kartal *et al.* (2003) (40).

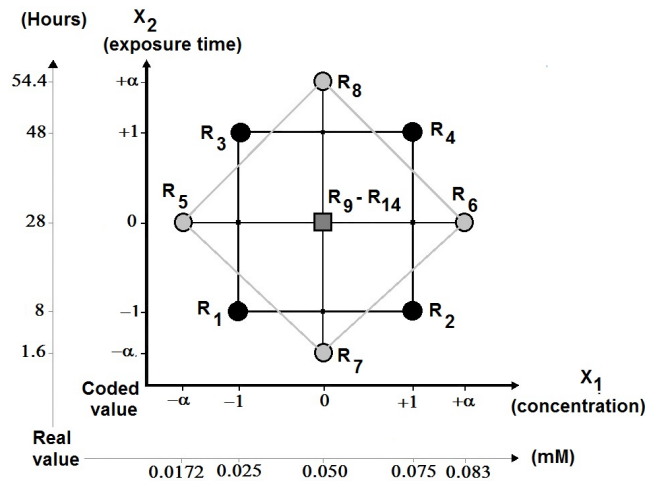
### 3.4. RSM: Experimental Design

The CCDO, described by Box and Wilson (1951) (13), was chosen for this work. It is composed of a factorial design  $2^k$ , with at least one experimental central point and a star designs whose axial points:  $-\alpha$  and  $+\alpha$  are placed on the axis of each factor. The *ET* and jasmonic *JAC* experiments which influence the biomass and hyoscyamine content were carried out according to the CCDO. Only fourteen experiments ( $R_1$  to  $R_{14}$ ) were necessary to model the *JAC* and *ET* influence on biomass and alkaloid production. The CCDO, the variables (*JAC*; *ET*) and their studied levels (5 concentrations and 5 elicitation times) are presented in **Figure 1**. Each point ( $R_1$ ... $R_{14}$ ) corresponds to one experiment. The points  $R_1$  to  $R_4$  represent the factorial design. The points  $R_5$  to  $R_8$  are the star design. The points  $R_9$  to  $R_{14}$  represent the experiments carried out in the central experimental design. Six repetitions ( $R_9$  to  $R_{14}$ ) were performed and the optimal criterion was orthogonality. In this case,  $\pm\alpha = \pm 1.32$  was chosen respect to the criterion.

The levels of variable  $X_i$  were coded from the equation  $X_i = (A_i - A_0) / \Delta A_i$ , where  $X_i$  is the independent variable coded value,  $A_i$  is the independent variable real value,  $A_0$  is the independent variable real value on the central point, and  $\Delta A_i$  is the step change value.

### 3.5. Expression of Measured Responses and Results

The biomass growth was represented by a growth index (GI) calculated from equation 1. For a  $GI = 1$ , hairy root growth was not affected by JA. On the other hand, a  $GI < 1$  has revealed an affected growth. At the same time, the increase in hyoscyamine content after elicitation was represented by the elicitation index (EI). It was



**Figure 1.** The central composite design orthogonal (CCDO): variables and their studied levels. Each point corresponds to one experiment. The points  $R_1$  to  $R_4$  are the factorial design, the points  $R_5$  to  $R_8$  are the star design, the points  $R_9$  to  $R_{14}$  are the experiments carried out in the central experimental design (with coded value  $\pm \alpha = \pm 1.32$  for the orthogonality criterion).

calculated from equation 2. If the hyoscyamine content was increased, the EI was  $> 1$ .

$$GI = \frac{\text{biomass of HR elicited (g DW.l}^{-1}\text{)}}{\text{biomass of HR controls (g DW.l}^{-1}\text{)}} \quad (\text{Eq. 1})$$

$$EI = \frac{\text{specefic production of hyoscyamine (g.g}^{-1}\text{ DW) of HR elicited}}{\text{specefic production of hyoscyamine (g.g}^{-1}\text{ DW) of HR controls}} \quad (\text{Eq. 2})$$

### 3.6. Mathematical Modeling of Measured Responses: Growth Index ( $Y_{GI}$ ) and Elicitation Index ( $Y_{EI}$ )

The results (i.e., the measured GI and EI) obtained from the experiments defined in Figure 1 (CCDO) were used for the mathematical model expression of the effects of *JAC* and elicitation time (*ET*) on GI and EI. The results of the CCDO were used for the computation of the coefficients of the two quadratic models Eq. 3 and Eq. 4 by the method of the least squares (41).

$$Y_{GI} = \alpha_0 + \alpha_1 JAC + \alpha_2 ET + \alpha_{11} JAC^2 + \alpha_{22} ET^2 + \alpha_{12} JACET \quad (\text{Eq. 3})$$

$$Y_{EI} = \alpha_0 + \alpha_1 JAC + \alpha_2 ET + \alpha_{11} JAC^2 + \alpha_{22} ET^2 + \alpha_{12} JACET \quad (\text{Eq. 4})$$

Where,  $Y_{GI}$  and  $Y_{EI}$  are the responses (biomass or hyoscyamine level), *JAC* is the jasmonic acid concentration, *ET* is the elicitation time, and  $\alpha_0, \alpha_1, \dots, \alpha_{12}$  are the model's coefficients. After the overall evaluation ( $R^2$ , ANOVA) followed by adjustment of the model, they were used for the simultaneous optimization of  $Y_{GI}$  and  $Y_{EI}$  with the desirability function approach.

### 3.7. Desirability Function

The desirability function approach transforms an estimated response into a scale-free value, called desirability (3, 4, 5). The objectives of optimization can be used to maximize, minimize, or obtain the target value of the response (42). Depending on the objective different desirability functions can be employed. Derringer and Suich (1980) (3) applied a special function for the transformation of the  $Y_i$  responses to the desirability  $d_i(Y_i)$ . So, two transformations are proposed:

The one-sided transformation used to maximize or minimize  $Y_i$ ; the two-sided transformation (equation 5) used to obtain the target value  $t_i$  for  $Y_i$  where  $l_i$  and  $u_i$  are the lower and upper bounds on the studied response (such that  $l_i < t_i < u_i$ ).

The powers  $s$  and  $t$  correspond to the weighted factor.  $s$  and  $t$  are the parameters that determine the shape of  $d_i(Y_i)$ . For  $s = t = 1$ , the desirability function increases linearly to  $t_i$ . If  $s < 1$  and  $t < 1$ , the function is concave and if  $s > 1$  and  $t > 1$ , the shape is convex (4, 42).

$$d_i(Y_i) = \begin{cases} 0, & Y_i < l_i, \\ \left(\frac{Y_i - l_i}{t_i - l_i}\right)^s, & l_i \leq Y_i \leq t_i, \\ \left(\frac{Y_i - u_i}{t_i - u_i}\right)^t, & t_i \leq Y_i \leq u_i, \\ 0, & Y_i > u_i \end{cases} \quad (\text{Eq. 5})$$

The overall desirability function  $D$  is defined as the geometric average of the individual desirability functions of each response  $d_i(Y_i)$  (equation 6) where  $n$  is the number of responses. The optimal solutions are determined by maximizing  $D$  (4).

$$D = \left( \prod_{i=1}^n d_i(Y_i) \right)^{\frac{1}{n}} \quad (\text{Eq. 6})$$

### 3.8. Statistical Analyses

ANOVA (Fisher Test) was used for an overall evaluation of the models. Tests at 5% risk were considered significant. The coefficient of determination  $R^2$  has to be taken into account. It represents the ratio of the variance suggested by the mathematical model with respect to the overall variance and varies from 0 to 1. It was calculated from equation 7 where  $SCE$  is the sum of the squares of the residuals and  $SCT$  is the total sum of the squares. It is also necessary to take into account the Absolute Average Deviation (AAD), whose value must be low. The  $R^2$  analysis alone is not

a measure of the model's accuracy even if the value is large. The AAD is calculated by the equation 8 where  $Y_{i,mes}$  and  $Y_{i,pre}$  are the measured and predicted responses, respectively, and  $n$  is the number of the experimental run. The simultaneous evaluation of these two values ( $R^2$  and AAD) shows the overall predictive capability of the mathematical model (43).

$$R^2 = \frac{SCE}{SCT} \quad (\text{Eq. 7})$$

$$AAD = \left\{ \left[ \sum_{i=1}^n \left( \frac{|Y_{i,mes} - Y_{i,pre}|}{Y_{i,pre}} \right) \right] / n \right\} \times 100 \quad (\text{Eq. 8})$$

## 4. Results

### 4.1. Modeling the Growth Index ( $Y_{GI}$ ) and Elicitation Index ( $Y_{EI}$ )

The results obtained after the experiments according to the CCDO were used to compute by the least squares method; the coefficients of the two quadratic models (equations 3 and 4) are expressing the effects of  $JAC-ET$  on  $GI$  and  $EI$ .

The obtained two quadratic models  $Y_{GI}$  and  $Y_{EI}$  show large coefficients of determination ( $R^2 = 0.80$  for  $Y_{GI}$  and  $R^2 = 0.90$  for  $Y_{EI}$ ) with values  $< 10\%$  for AAD (6.5% for  $Y_{GI}$  and 6.0% for  $Y_{EI}$ ). Thus, these two models explain 80% and 90%, respectively, of the  $Y_{GI}$  and  $Y_{EI}$  variability; they are predictive overall. Following the variance analysis (**Table 1**), evaluation and diagnostic of these two models, all the insignificant terms were removed (14, 44).

In this way, the adjusted mathematical models became:

$$\hat{Y}_{GI} = 0.72 - 0.11JAC - 0.14ET + 0.01ET^2 \quad (\text{Eq. 9})$$

$$\hat{Y}_{EI} = 1.76 - 0.30JAC - 0.60ET + 0.25JAC^2 + 0.22ET^2 \quad (\text{Eq. 10})$$

### 4.2. Simultaneous Optimization by the Desirability Function Approach

Equation 5 was used for the two-sided transformation of the responses to an individual desirability scale where they increased linearly from 0 to 1. They were noted  $d_{GI}$  for  $GI$  and  $d_{EI}$  for elicitation index ( $EI$ ). Equation 6 led to the overall desirability function  $D$  defined to find an optimal solution for both  $JAC-ET$  factors. **Figures 2** and **3** show the response surface and the contour plot of the desirability function in the space of the factor  $JAC$  and  $ET$  for the two models.

For biomass, the maximum desirability values are: 0.7 for  $GI$  (target values for  $GI$ ), between small values of  $ET$



**Table 1.** Analysis by ANOVA for the quadratic models of the growth and elicitation indexes. The results in bold are significant at 5% risk: \* significant; \*\* highly significant; \*\*\* very highly significant.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
<b>Growth Index (GI)</b>						
Model	0.304	5	0.060	8.753	<b>0.004</b>	**
<i>JAC</i>	0.084	1	0.084	12.106	<b>0.008</b>	**
<i>ET</i>	0.150	1	0.150	21.661	<b>0.001</b>	**
<i>JAC x ET</i>	0.010	1	0.010	1.437	0.264	
<i>JAC</i> <sup>2</sup>	0.001	1	0.001	0.261	0.623	
<i>ET</i> <sup>2</sup>	0.057	1	0.057	8.297	<b>0.020</b>	*
Residual	0.055	8	0.006			
Cor Total	0.360	13				
<b>Elicitation Index (EI)</b>						
Model	4.014	5	0.802	15.560	<b>0.000</b>	***
<i>JAC</i>	0.655	1	0.655	12.714	<b>0.007</b>	**
<i>ET</i>	2.686	1	2.686	52.067	<b>0.000</b>	***
<i>JAC x ET</i>	0.002	1	0.002	0.048	0.831	
<i>JAC</i> <sup>2</sup>	0.375	1	0.375	7.278	<b>0.027</b>	*
<i>ET</i> <sup>2</sup>	0.293	1	0.293	5.695	<b>0.044</b>	*
Residual	0.412	8	0.051			
Cor Total	4.427	13				

Cor Total: Corrected Total sum of squares

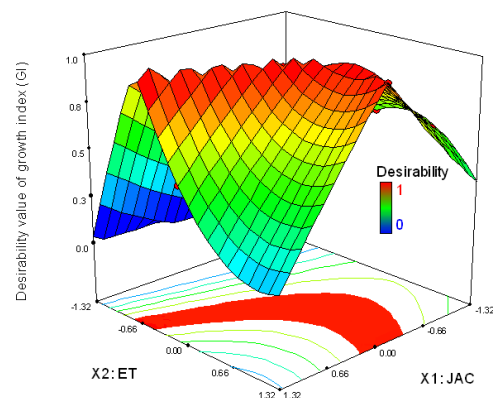
and mean values of *JAC*. The desirability is high for EI = 2.15 (target values for EI) ( $d_{EI}=1$ ), even at low *JAC* (Fig. 3). The optimal solutions fit with all the intersection points between *JAC* and *ET* and correspond to the values of the maximized desirabilities for GI and EI, so  $d_{GI} = 1$  and  $d_{EI} = 1$ , respectively (Figs. 2 and 3). These two responses, individually, show an area of optimal solutions for  $d_i = 1$ . The level of difficulty of multiresponse optimization increases when the optimal areas of responses are too distant and do not cross. It is therefore necessary to find an optimal compromise between all the responses taken into consideration. Thus, the overall desirability function *D* is the most used and significant approach (45).

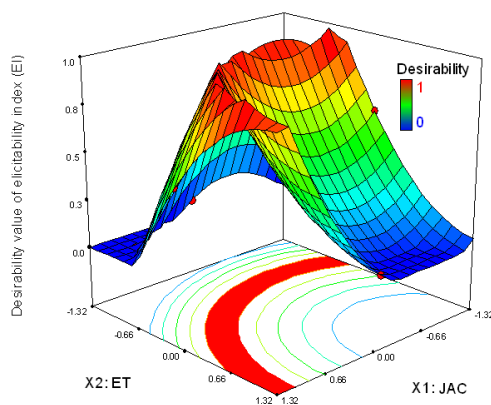
**Figure 4** shows the resulting overall desirability *D* with respect to *JAC* and *ET*. The area of optimal solutions decreases when the two responses GI and EI are taken simultaneously for the optimization.

This response surface analysis is due to the conflict of *JAC* and *ET* on the biomass and hyoscyamine level. So, the optimal combinations *JAC* and *ET* are presented by the intersection between  $X_1$  and  $X_2$  (red zone in Fig. 4) where  $D = 1$ . The projected area corresponds to the optimal solutions (compromise) between the two antagonist responses (Fig. 4). This area has been defined with a limited number of experiments, thanks to the use

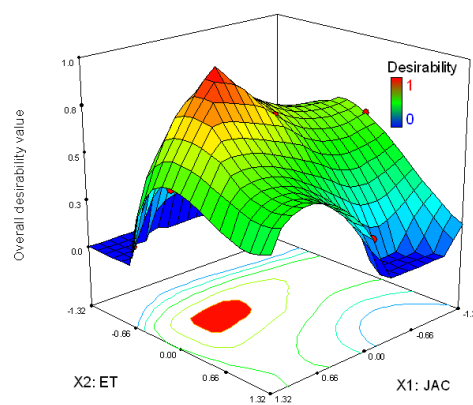
of DOM. Thus, these results confirm the usefulness of DOM for finding the compromises between antagonist responses and, more specifically here, between hyoscyamine levels and the growth preservation.

To obtain the target values GI = 0.7 and EI = 2.15, one of the solutions which maximizes  $D (=1)$  corresponds to 0.06 mM (coded 0.55) for *JAC* and 23.2 h for *ET* (coded -0.24).

**Figure 2.** Response surfaces and estimated contours of the desirability value of the biomass as a function of *JAC* and elicitation time (*ET*).



**Figure 3.** The response surfaces and estimated contours of the desirability value of hyoscyamine content as a function of *JAC* and elicitation time (*ET*).



**Figure 4.** The response surfaces and estimated contours of the overall desirability as a function of *JAC* and *ET*.

#### 4.3. Optimization Levels by the Application of DOM

To investigate the optimization level by DOM, a number of experiments were carried out with *JAC* = 0.06 mM and *ET* = 23.2 h (Table 2). For practical reasons, the *ET* was adjusted to 24 h; the value that was included in the optimal solutions. Before elicitation experiments on the 12<sup>th</sup> day of culture, the roots were transferred to a fresh B5 medium (20 mL). The responses on biomass and hyoscyamine level were studied at 24 h and 120 h after elicitation.

The obtained results show that JA elicitation (0.06 M for 24 hours) led to a 2.9-folds improvement in the hyoscyamine content 24 h after elicitation. However, the same hyoscyamine content was obtained in the control and elicited hairy roots (*EI* = 1.0) 120 h after elicitation. Nevertheless, the biomass was slightly affected (*GI* = 0.9) 120 h after elicitation.

## 5. Discussion

### 5.1. Modelization of $Y_{GI}$ and $Y_{EI}$

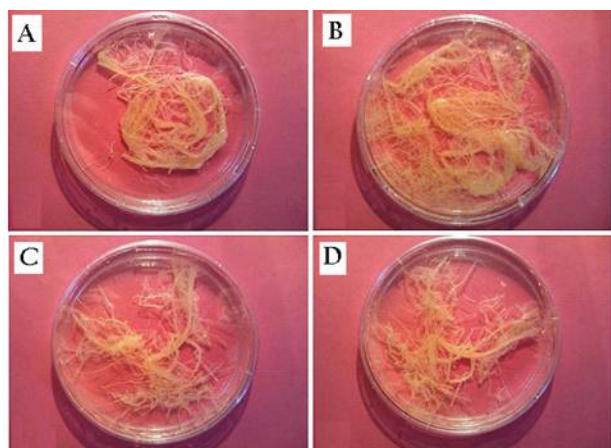
For the  $\hat{Y}_{GI}$  model, it can be seen that *JAC* (-0.11) and *ET* (-0.14) show a negative effect on hairy root growth (equation 9). So, if *JAC-ET* factors are increased, hairy root growth is inhibited (Fig. 5). In contrast, if *JAC-ET* factors are low, hairy root growth is not inhibited.

These results are similar to those obtained by other authors. For example, Kang *et al.* (2004) (30) reported that 1 mM methyl jasmonate treatment did not have a negative effect on the growth of adventitious roots of *Scopolia parviflora*. However, when the adventitious root cultures were treated with 2.0 mM, the root *GI* decreased to 63% of the control culture. After 72 h of elicitation, the roots were severely damaged and died (Kang *et al.*, 2004). Similar results have been reported by Deng (2005) (25) on the root growth inhibition of *Datura stramonium* using methyl jasmonate at a concentration of  $> 10^{-5}$  M.

An analysis of the  $\hat{Y}_{EI}$  model (equation 10) shows a positive effect of *JA* (+0.30) and a negative effect of *ET* (-0.60) on the hyoscyamine level. The effect of *JA* on the production of tropane alkaloids comes from the results which suggest that jasmonates regulate the expression of the key enzymes *PMT* (*Putrescine N-Methyl Transferase*) and *H6H* (*Hyoscyamine 6  $\beta$ -Hydroxylase*) and the biosynthesis of the tropane alkaloids. The evaluation of jasmonate effects on the tropane alkaloid biosynthesis has been suggested by western blotting on adventitious root cultures of *Scopolia parviflora* (30). In *Datura stramonium*, methyl jasmonate was shown to increase phenylalanine levels, one of precursors of

**Table 2.** Optimization of the hyoscyamine content and biomass growth at 24 and 120 h after elicitation (results are rounded to one decimal point).

Jasmonic acid (mM)	Hyoscyamine (mg.g <sup>-1</sup> DW)		Biomass (g DW.L <sup>-1</sup> )	
	24 h	120 h	24 h	120 h
0 (control)	0.3 ± 1.9	6.3 ± 0.2	5.0 ± 0.2	0.2 ± 6.3
0.06	0.5 ± 5.6	6.5 ± 0.2	0.2 ± 5.0	0.2 ± 5.6
GI and EI	EI = 2.9	EI = 1.0	GI = 1.0	GI = 0.9



**Figure 5.** Elicited and control hairy root cultures. **A:** hairy root control on 13<sup>th</sup> day of culture. **B:** hairy root control on 17<sup>th</sup> day of culture. **C:** elicited hairy root culture on 13<sup>th</sup> day of culture (0.0830 mM jasmonic acid; 24 h after elicitation). **D:** elicited hairy root culture on 17<sup>th</sup> day of culture (0.0830 mM jasmonic acid; 120 h after elicitation).

tropine alkaloids, at  $10^{-8}$  M. Subsequently, tropinone, tropine and  $\phi$ -tropine levels were greatly increased by methyl jasmonate at  $10^{-7}$  and  $10^{-6}$  M (25).

Thus, the two models: (9) and (10) confirm that JA is a sensitive elicitor with two antagonist responses: it increases the hyoscyamine content with growth inhibition of the HRs. So, a compromise between *JAC* and *ET* is essential in order to improve the hyoscyamine level with no inhibition on the root growth by using the desirability approach.

To obtain a satisfactory compromise, the optimal solution (*JAC-ET* combination) is defined by the target values ( $t_i$ ) of the each response. The average values  $t_i$  of both bounds  $l_i$  and  $u_i$  are the target values for GI,  $t_i = 0.7$  ( $l_i = 0.4$  and  $u_i = 1$ ), and for *ET*,  $t_i = 2.15$  ( $l_i = 1.3$  and  $u_i = 3$ ), respectively. The values of the bounds  $l_i$  and  $u_i$  of the GI and EI present the bounds of the experimental values obtained after application of the CCDO. To solve the conflict established between biomass and hyoscyamine content, the use of the average values as target values allows the solutions to be maximized and a satisfactory compromise to be established. The fitted models (9) and (10) were used for the simultaneous optimization of the  $Y_{GI}$  and  $Y_{EI}$  using the desirability function. In addition, the two-sided transformation was used in this work for multiresponse optimization and for both simultaneous responses.

### 5.2. Application of DOM

For various species like *Scopolia parviflora* (30) and *Brugmansia suaveolens* (46), the *ET* for the best

production of hyoscyamine was 24 hours. The same authors have claimed the optimal *JAC* varies from 0.01 mM (30) to 2 mM (46). Zabetakis *et al.* (1999) (26) have reported that 0.1  $\mu$ M of methyl jasmonate was the optimal concentration for the elicitation in the root culture of *D. stramonium* and increased the hyoscyamine production 1.8-fold compared to the control. In other species, Kang *et al.* (2004) (30) have reported the elicitation of the adventitious roots of *Scopolia parviflora* with 0.01 mM with an elicitation time of 24 h increased the hyoscyamine content 1.8-fold compared to the control over the same time without growth inhibition. In the same way, elicitation of the HRs of *Brugmansia suaveolens* by 2 mM JA for 24 h improved the yield of hyoscyamine 25 folds (46). However, these authors did not report the effect of *JAC* on the biomass.

Several studies on jasmonic acid effects on the tropane alkaloid content and biomass growth show conflicting responses. It is to be expected that the experimental conditions which lead to an optimum for one of these factors are not the same as for another. So, the optimization of these two parameters is rather difficult. However, in this paper we have shown that DOM (i.e., RSM combined with the desirability approach) is a satisfactory compromise for multiresponse optimization. DOM is useful and easy to adapt to the complex biological systems such as hairy root cultures. In our case, it led to an optimal solution for the parameters *JAC-ET* with a 2.9-fold improvement in hyoscyamine content and a slight growth inhibition; 10% less than the control. It seems that 90% of the hairy root biomass is retained after 5 days of elicitation (120 h). Thus, the maximum overall desirability function is reached for: *JAC* = 0.06 mM and *ET* = 24 hours. The present study was performed in a closed system, so the inhibitory effect of JA on biomass at low concentrations could be due to its prolonged presence in the culture medium. We can suppose that this effect will be absent in a system where the medium is changed regularly, such as a continuous or semi-continuous regime. In such a system, we anticipate that the high concentrations of JA (with a short *ET*) will not affect the biomass.

DOM has proved its efficiency for the development of the products in which different properties need to be associated (16). In this article we have shown that DOM is also extremely efficient for determining even antagonist responses in the plant biotechnological processes. These results thus show the way and opens up new opportunities for the other kinds of uses in the wide area of plant sciences and technologies.



## Abbreviations

<i>B5</i>	Gamborg culture medium
<i>SCE</i>	Sum of the squares of residuals
<i>SCT</i>	Total sum of the squares
<i>YEI</i>	Response of hyoscyamine content
<i>YGI</i>	Response of growth level

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