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RESEARCH ARTICLE

Response Surface Methodology for optimization of gamma irradiation of faba bean (Vicia faba L.)

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Abstract

Creating new mutants of faba bean for improving crop was carried out using gamma rays irradiation. The effect of operational parameters such as dose, dose rate and post irradiation storage time was studied using Response Surface Methodology (RSM) to optimize the conditions of maximum rate germination of faba bean. Optimal operating conditions were determined to be 150Gy dose, 18 Gy min^{-1} dose rate, and 1 to 2 days post irradiation time, respectively. After several tests, the desired mutants were found, and RSM techniques were able to predict the optimal rate germination of faba bean as a function of irradiation process condition.

INTRODUCTION

Tunisia as a Mediterranean region growing faba bean to cover it's human and animal needs (Larralde, 1982). The presence of the parasitic weed, *Orobanche* spp., in some faba bean growing areas is considered as a limiting factor to the expansion of the crop (Stoddard et al., 2010). Diseases and pests have been reported as recurrent problems in Tunisia (Kharrat et al., 1991). Genetic resistance using ^{60}Co gamma rays irradiation is considered as the most desirable control method since it is more cost effective and environmentally friendly than the use of chemicals (Borzouei et al., 2010). Though, gamma radiation-induced breeding greatly depends on the selection of the appropriate radiation dose. Simultaneously, some report indicated that the dose rate is also a key question in the induced radiation (Yamaguchi et al., 2008).

The Genetic mutation process using gamma irradiation is fairly multifaceted, because the process is influenced by several irradiation factors. Owing to the complexity of the process, it is difficult to be modeled by ordinary method. The RSM as the commonest technique used in the optimization of several process such as chemical, geotechnical, food and biochemistry (Ravikuma et al., 2006; Rodrigues et al., 2006), is considered as promising approache due to their simplicity against prediction, and modeling (Ravikuma et al., 2006; Rodrigues et al., 2006). The RSM technique allows for simultaneous variation of several factors and reduces the number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time consuming than other approaches.

The objective of the present paper is to establish the optimum of the Genetic mutation process as a function of gamma irradiation conditions (dose, dose rate, humidity and post irradiation storage time) of faba bean seed through Response Surface Methodology.

Materials and methods

Seeds Irradiation

The irradiations were carried out at the Tunisian semi-industrial ^{60}Co gamma-irradiation facility. The dose rate was established using Fricke dosimetery system. Three dosimeters were irradiated for each point of measurement. Before irradiation the water moisture content of faba bean seeds is fixed at 8%.

Seeds germination

After irradiation a calcium hypochlorite (10% for 30 minutes) is used to seeds surface-sterilize, and then seeds, were rinsed using sterile water. The incubation procedure is carried out at 28°C in the dark for 5 days in Petri dishes. Non treated seeds were used as controls. Germination rate was calculated according to the following formula (Hegazi and Hamideldin, 2010).

$$Germination\quad rate = \frac{(G1 \times N1) + (G2 \times N2) + ...(Gn \times Nn)}{G1 + G2 + ...Gn}$$

Where: G = Number of germinated seeds in a definite day, n = Number of this definite day.

Factorial analysis and design of experiments

The applied optimization approach is based on a Central Composite Design (CCD) (Myer and Montgomery, 2002) with (2^k+2k+N0) runs where k is the number of studied factors, 2^k are the points from the factorial design, 2k the face-centred points and N0 the number of experiments carried out at the centre.

In our case, 2^3+6+3 experiments were performed in random order to minimize the effect of systematic errors. The relative humidity is considered constant (40 to 60 %) (Table 1). The parameter levels and coded values were given in Table 2.

Model of fitting

A full second-order polynomial model was used to evaluate the response as a function of independent variables and their interaction (Clarke and Kempson, 1997).

$$Y = a_0 + \sum a_i.X_i + \sum a_{ii}.X_i^2 + \sum a_{ij}.X_i.X_j$$

Where: Y = Percentage of germination (%)

ai, aj, aij. = coefficients determined from the results of the experiment variable parameters;

Xi and Xj= are coded experimental levels of the variables.

The coefficients of the model are calculated using multiple regression analysis. The fit quality of the model was judged from their coefficients of determination (R^2) which provide a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions, the better empirical model fits the actual data is provided when R^2 approaches unity (100%).

Data analysis

NemrodW Software (Mathieu and Phan-Tan-Luu, 1997) was used for the regression analysis of the experimental data and the response surfaces. The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2, and its statistical significance checked by a Fisher F-test (Cornell, 1990). The significance of the regression coefficient was tested by a Student's t-test. The level of significance was given as values of the probability less than 0.05.

Results

Lethal Dose effect (LD_{50})

In order to find out the LD_{50} (the semi-lethal dose at 50%) for irradiated faba bean seeds, the response curve (percentage of seed germination versus dose) was measured in the dose range 50-700 Gy (Figure 1). All data for the dose response curve were immediately acquired after irradiation sowing in Petri dishes on a sterile filter paper imbibed with distilled water and allowed to germinate at 28°C in the dark for 7 days. As expected, the percentage of seed germination decay as function of irradiation dose. This fading process seems to follow a simple first-order exponential kinetic fit (y = Ger0+a exp (-D/t)), where t is the first order decay rate constant, (Ger0) and (a) are fitting parameters. The coefficient of correlation (R^2) was 0.977. Fitting parameters where grouped in Table 3. The $LD_{50}$was found to be within 150Gy at normal conditions (19.34 Gy min^{-1}, sowing time (7days)).

The relative germination rate of seeds irradiated with small doses shows no significant effect. A general downward trend of the rate is observed with increasing dose (Figure 1). A significant correlation was found between the dose and relative germination rate. The results were in accordance with some previous reports, which showed that the higher exposures are usually inhibitor on seed germination (Khawar et al., 2010; Thapa, 2004; Melki and Marouani, 2010). Maximum decrease in germination percentage was observed from 600 Gy for faba bean seeds. Whereby there was no significant difference in germination percentage of irradiated and non irradiated seedlings of hard wheat at low doses but at height doses a maximum decrease in germination percentage was observed.

Determination of lethal dose at 50%: Appropriate radiation dose is the premise for the production of more effective variations (Dehpour et al., 2011). Materials treated with appropriate dose of radiation can gain valuable mutants in a short time and enrich germplasm sources. It is an important indicator to determine the effects of radiation damage. The critical dose and semi-lethal dose is commonly used to determine appropriate dose of radiation breeding. Semi-lethal dose is a main indicator to determine the radiosensitivity, as well as the reference of suitable radiation dose for induced mutation breeding (Li et al., 2012). The semi-lethal dose of faba bean seeds was calculated according to the linear regression equation, which were 150 Gy.

Optimisation of LD_{50} using Response Surface Methodology (RSM)

The results of the statistical analysis corresponding to the two-level Central composite design are given in Table 1. Using the experimental results, analysis of variance and fitting quadratic response model are determined by ANOVA. This statistical tool is required to test the significance and adequacy of the model.

The Fischer variation ratio (F-value) is a statistically valid measure of how well the factors describe the variation in the data about its mean. It can be calculated from ANOVA as follows: F-value=MS (due to the model variation) /MS (due to error variance). The greater F-value from unity explains adequately the variation of the data around its mean, in addition the estimated factor effects are real (Myer and Montgomery, 2002) .

In general, the calculated F-value should be several times greater than the tabulated value for the model to be considered good. In fact, the calculated F-value corresponding to the irradiated faba bean germination response model is 21.266 and exceeds the tabulated F-value ($F^{0.05}_{(9,7)}$ = 4.06) at the 5% level indicating that the treatment differences are highly significant (Table 3). The probability p-value is relatively low, indicating the significance of the model. On the other hand, the regression coefficients are estimated acceptable (R^2=0.98). The R^2 value gives a good agreement between the experimental and predicted values of the fitted model. It implies that 98% of the variability in the response could be explained by the model.

The multiple regression results and the sorted significance of regression coefficients for the irradiated faba bean germination response model are in the Table 4 where significant parameters are sorted based on t and p-values. The p-values were used as a tool to check the significance of each of the coefficients which in turn are necessary to understand the pattern of mutual interactions between the test variables. With a low p-value and a high absolute t-value, the corresponding coefficient is highly significant.

The linear, quadratic and interaction terms of dose, dose rate (X1X2) in the model have the largest effect statistically on the percentage of germination related to irradiated faba bean at 95% confidence level of significance (probability < 0.001) and the highest absolute t-values. Next, the interaction terms of dose, dose rate and post irradiation time (X1X3) and (X2X3) showed a lowest t-value. However, the effect of this term on the percentage of germination related to irradiated faba bean seeds model is low significant statistically.

The comparison between the obtained and calculated values (Table 5) reveals that the results are in good agreement with the modelling equation in our experimental field.

The graphical representations of the distribution of these experimental points are given in Figure 2. The measured response was defined seeds germination rate (% Ger).

The response surface plots were generated using NEMROUD software to reveal the effect of gamma rays dose, dose rate and post irradiation time on the seeds germination percentage (% Ger). In order to simplify representations, the color code is based on the Fact that more the student value is higher more the representative color of the range is hot (from pink towards red).

Experiments show that all the studied factors have an effect on the faba bean seeds germination. A reduction in the post-irradiation storage time induces a remarkable increase in (Ger. %) (Figure 2) while an increase of the dose rate gives the same result. These evolutions are more marked to a dose value sets up (150Gy) (centers domain).

The evolutions of the faba bean seeds germination percentages induced by the modification of the dose rate are less important than the dose and the post-irradiation storage time.

Critical values predicted from the data analyzed with the statistical technique are shown in Table 6. The obtained results indicate that adequate seed germination percentage (50%) for maximal mutagenesis is achieved and it’s in good agreement with the predicted value.

Table 1. Experimental matrix for the three factor-two level Central composite design

Expriment	Dose (kGy)	Dose rate (Gy min^{-1})	Post-Irradiation time (days)	Y_{LD50} (%)
1	-1	-1	-1	85
2	+1	-1	-1	50
3	-1	+1	-1	80
4	+1	+1	-1	70
5	-1	-1	+1	95
6	+1	-1	+1	65
7	-1	+1	+1	90
8	+1	+1	+1	70
9	- α	0	0	100
10	+ α	0	0	65
11	0	- α	0	55
12	0	+ α	0	70
13	0	0	- α	55
14	0	0	+ α	70
15	0	0	0	50
16	0	0	0	50
17	0	0	0	45

Table 2. Factors levels and coded values used in the experimental design

level	Dose (kGy) X1	Dose rate (Gy min^{-1}) X2	Post-Irradiation time (Days) X3
-α =- 1,68	23.87	28	7.36
-1	75	12	2
0	150	18	4
+1	225	24	6
+α = 1,68	276.13	7	0.64

Table 3. Analysis of Variance (ANOVA) for the selected quadratic model

Sources of variations	Sum of Squares (SS)	Degree of freedom (DF)	Mean Squre (MS)	F Value	Probability P (>F)
Model	4.37811E+0003	9	4.86457E+0002	21.2662	0.0001
Error	1.60123E+0002	7	2.28747E+0001		
total	4.53824E+0003	16			

$F0,05(9,7) = 4.06$ (Tabled value); $R^2 = 0.98$

The mean squares (MS) are obtained as follows: MS=SS/DF

where: SS=sum of squares (SS) of each variation sources; DF=the respective degrees of freedom (DF).

Table 4. Estimated quadratic regression coefficients of central composite design on faba bean seed germination

Coefficients	Estimated Parameter	Standard error coefficient	Computed t-value	Significance level (%)
a_0	47.999	2.756	17.42	***
a_1	-11.266	1.294	-8.71	***
a_2	2.946	1.294	2.28	*
a_3	4.410	1.294	3.41	*
a_{11}	13.233	1.424	9.29	***
a_{22}	6.162	1.424	4.33	**
a_{33}	6.162	1.424	4.33	**
a_{12}	4.375	1.691	2.59	*
a_{13}	-0.625	1.691	-0.37	72.1%
a_{23}	-1.875	1.691	-1.11	30.5%

α significance level <0.05

Table 5. Calculated differences between the experimental and estimated % of germination related to irradiated faba bean seeds

Experiment	Observed YLD50 (%)	Computed YLD50(%)	residue
1	85.000	79.341	5.659
2	50.000	49.308	0.692
3	80.000	80.232	-0.232
4	70.000	67.699	2.301
5	95.000	93.161	1.839
6	65.000	60.628	4.372
7	90.000	86.552	3.448
8	70.000	71.519	-1.519
9	100.000	104.375	-4.375
10	65.000	66.479	-1.479
11	55.000	60.473	-5.473
12	70.000	70.381	-0.381

13	55.000	58.010	-3.010
14	70.000	72.844	-2.844
15	50.000	47.999	2.001
16	50.000	47.999	2.001
17	45.000	47.999	-2.999

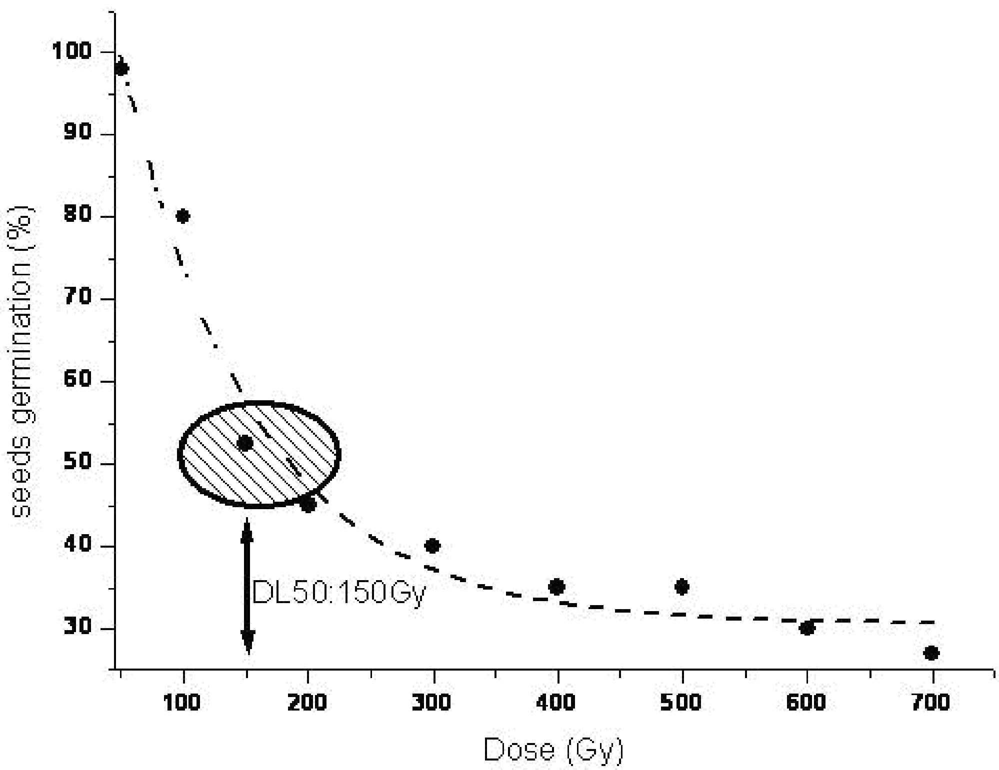

Figure 1. The correlation between the relative seed germination rate and irradiation dose.

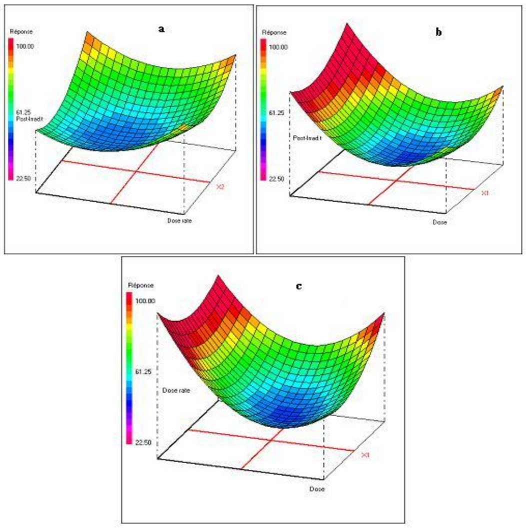

Figure 2. Three-dimensional plots for seeds germination percent (% Ger) as a function of : (a) dose rate and post irradiation time, (b) dose and post irradiation time, (c) dose and dose rate.

Discussion

Previous studies taken under hard wheat indicated that gamma radiation had no significant effect on final germination percentage (Melki and Marouani, 2010). Irradiated wheat seeds kept their germination capacity compared to the control. Maximum decrease in germination percentage of wheat seeds was observed with 300Gy; in contrast faba bean reaches 700Gy. Chaudhuri (2002) reported that in higher radiation dose, the (%Ger) was reduced while, in lower dose i.e., 0.1 kGy the (Ger %) was not significantly different from control. In another study by Kiong et al., (2008) , it was found that radiation increases plant sensitivity to gamma rays and this may be caused by the reduced amount of endogenous growth regulators, especially the cytokines, as a result of breakdown, or lack of synthesis, due to radiation.

Concerning the dose rate effects, these results are in agreement with the findings of Chaomei and Yanlin (1993) on wheat, who noticed that treating seeds with high rates of gamma radiation reduced germination with a corresponding decline in growth of plants.

Conclusion

Response Surface Methodology is used to optimize the lethal dose at 50% of germination rate of gamma irradiated faba bean seeds. Graphical response surface is used to locate the optimum point. A central composite design was applied to establish second order model relating to the percentage of germination of an irradiated faba bean seeds. The validity of the polynomial model was proven by the coefficient R^2 (0.98) and the F-test. The proposed model can be used to predict the effect of gamma irradiation on faba bean seeds in order to induce mutation breeding.

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