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Fertilizing model for Djulis (*Chenopodium formosanum* Koidz.) using mixture design approaches

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Abstract

Djulis (*Chenopodium formosanum* Koidz.), a cereal plant native to Taiwan, was used for wine making, insect repellant, and health promotion. Therefore, using appropriate experimental design and modeling approach is of importance to predict the effect of the interaction among nitrogen (N), phosphorus (P), and potassium (K) on djulis yields. In this study, a mixture design approach was applied to investigate the effects of interactions among nitrogen, phosphorus and potassium on the grain yields of djulis plants. Based on a three-variable including N, P, and K with each maximum of them was of 200 g (6 m^2)$^{-1}$, a mixture design approach was employed by 42 experiments in 14 study plots to obtain an optimal combination of N : P : K= 100 : 200 : 100 (g / 6 m^2)$^{-1}$) to predict an optimal dry djulis yield of 52.80 g / plant in this study. This study revealed that the linear mixture of N, P, and K and the interaction of P and K had marked effects on the yields of djulis. Moreover, the results were fitted to a quadratic polynomial equation using a multiple regression analysis. Our data showed this mixture design is a reliable approach to develop a model that can be used to predict the djulis yields.

Keywords: *Chenopodium formosanum* Koidz.; mixture design approaches; multiple regression analysis; left out test; Djulis.
Abbreviations: DFFITS-Difference in fit standardized; ICP-MS-inductively coupled plasma-mass spectrometry.

Introduction

Djulis (*Chenopodium formosanum* Koidz.) is a native crop plant to Taiwan and, mostly, was used for wine making, insect repellant (Chio and Yang, 2008), and health promotion. The important compound of djulis was betalains and was reported recently on its antioxidant and anti-tuber bioactivities (Chyau et al. 2015; Khan and Giridhar, 2015; Tsai et al., 2010). In 2017, the djulis cultivation area in Taitung County was about 200 ha and the production was about 300 tons. Nitrogen uptake in cassava tuber was significantly affected by interaction between N and K and might be suggested that nitrogen (N) and potassium (K) had a synergistic effect on the nutrient uptake of cassva (Thummanatsakun and Yampracha, 2018). Hou et al. (2019) reveal that an adequate amount of K from the exchangeable K pool in the soil is necessary, if N was to be used wisely and efficiently in rice production for achieving its full role in increasing grain yield. In the general mixture problem, the measured response is assumed to depend only on the proportions of the ingredients percent in the mixture and not on the amount of the mixture (Cornell, 2002). The reason for mixing together ingredients in experiments is to investigate whether there exists mixture of two or more ingredients that produce more product properties than obtained from single ingredient individually (Cornell, 2002). Traditionally, the models used in mixture experiments are Scheffé's canonical polynomials (Cornell, 2002) and is as follows:

$$Q(\beta,X)=\beta_0+\sum_{i=1}^{q}\beta_i\ X_i+\sum\sum_{i<j}^{q}\beta_{ij}\ X_i\,X_j+\sum\sum_{i<j<k}^{q}\beta_{ijk}\ X_i\,X_j\,X_k+\cdots$$

Where the β's are the models parameter coefficients and X_i, X_j, X_k are original components. Mixture methods have been used to successfully optimize conditions in structural ceramics, antifreeze performances, rice noodle quality, self-compacting concrete ,and ice cream manufacture (Coronado et al. 2014; BahramParvar et al. 2015; Shi et al. 2015; Loubes et al. 2016; Liu et al. 2016). In this study, a mixture method was applied to investigate the effects of proportional combinations of nitrogen (N), phosphorus (P), and potassium (K) that would produce the optimal djulis yields.

Results and discussion

Multi-regression tests

The results of the mixture experiments to determine the effects of three variables on yields of djulis are shown in Table 1. The variables include nitrogen (X_1), phosphorus (X_2), and potassium (X_3). By applying a multiple regression analysis on the experimental data, the predicted canonical model was obtained using following polynomial function:

Dry-weight = $-8.58\ X_1 + 60.27\ X_2 + 38.22\ X_3 - 2.35\ X_1X_2 + 14.22\ X_1X_3 - 49.33\ X_2X_3$

Model fitness

The F-value of this model was 7.79 (Table 2), which implied that the model was significant and there was less than a 0.61% chance that a large F-value could occur due to random error. The p-value in the model was less than 0.05, which confirms the adequacy of the quadratic model. The data in Table 2 indicated that djulis dry weight was affected by the linear combination of nitrogen (X_1), phosphorus (X_2), and potassium (X_3) with their quadratic terms of (X_2X_3) ($p < 0.05$). Furthermore, the X_1, X_2, X_3, and X_2X_3 values were the main factors affecting the djulis grains dry weight.

Model sensitivities

A model sensitivity analysis ($R^2 = 0.8295$; adjusted $R^2 = 0.7230$; predicted $R^2 = 0.5350$; adequate precision = 10.530; AAD (%) = 2.91) revealed that the model fit reasonably close to the experimental values (Table 3). It is generally considered that an adequate precision value greater than 4 is desirable (Carpinteyro and Torres, 2013). In this study, the adequate precision value was 10.530, which indicates that an adequate signal from this model can be used to steer the design space. The relationship between the actual response (experimental data) and the predicted values was displayed linearly. The predicted R^2 was 0.5350, indicating that it agreed reasonably well with the adjusted R^2 value of 0.7230.

The actual value and predicted value were shown in Table 4. A two-tailed independent t-test was performed to confirm the adequacy of the model tested in this study. The results showed that the experimental responses agreed closely with the predicted values (p = 0.99). The linearity between actual and predicted values was shown in Fig. 1.

Optimization of the process

The main objective of this study was to find the optimal combination conditions of nitrogen, phosphorus, and potassium that would give the optimal djulis yields. The 2D contour plots and 3D response surfaces were used to represent the regression functions. The 2D contour plots and 3D response surfaces showed the combinational effect of N, P, and K on the djulis yields and were shown Fig. 2 and Fig. 3.

The model predicted that the optimal combination of the variables in this study would be a nitrogen: phosphorus: potassium = 100: 200: 100 g/ (6 m^{-2})., which together would result in a optimal djulis yields of 52.80 g / plant in dry weight. Under these optimal conditions, the experimental data demonstrated that maximum actual djulis yields was of 53.91 g / plant, which was greater than the predicted value (52.80 g / plant). The maximum experimental value was of 53.91 g / plant and the combination of the independent variables was an N: P: k = 100: 200: 100 g/ (6 m^{-2}). This might come from some outliers in the repeated experiments. However, the statistical results in this study showed that the data confirmed that these conditions of N: P: k = 100: 200: 100 g/ (6 m^2) were optimal to produce a maximal djulis yield and near the maximum experimental value in this study.

Thummanatsakun and Yampracha (2018) and Hou et al. (2019) revealed that when the proportion of N and K was of 1:1 the yields of cassava and rice might reach an optimal high value. In this study, the data showed that when N: K= 1:1 the yields of djulis reached an optimal high value. It was in consistent to the reports of Hou et al. and Thummanatsakun and Yampracha.

According to the contour plots in Figure 2, each curve represents a region of constant response. A steep slope or curvature in the plot indicates the sensitivity of the response to a particular factor. The 3D figures in Figure 3 show no saddle points on the graphs, indicating that the maximum data point is stable. The results of this study show that the experimental responses were closely agree with predicted values. Meanwhile, the result confirmed that the conditions found in this study was optimal to get highest djulis yield.

The left out test of the model

DFFITS (Difference in fit standardized) was a diagnostic tool to show how influential a point was in a statistical regression. It was obtained when that point was left out of the regression and was calculated by dividing by the estimated standard deviation of the fit at that point:

$$\text{DFFITS} = (y_i - y_{io}) / [s_{io} \times (h_{ii})^{1/2}]$$

where y_i and y_{io} were the prediction for point i with and without point i included in the regression, s_{io} was the standard error estimated without the point in question, and h_{ii} was the leverage for the point. For a perfect experimental design, the leverage for each point was p/n, the number of factors divided by the number of points.

When investigating those points, the DFFITS was suggested greater than the limit, $2 \times (p/n)^{1/2}$. The DFFITS was shown in Table 5. When the DFFITS value exceeded the limit and greater than 2, they were the most influential cases (Aboobacker and Chen, 2009; Meloun and Militký, 2001; Belsley et al., 1980). The data in Table 6 showed that the DFFITS value in run order of 5 and 12 were exceeded the limit of DFFITS and greater than 2. The standard order 5 and 12 could be identified as powerful influence runs.

Outlier detections

The internally studentized residual quantifies how large the residuals were and was calculated by the residual divided by the estimated standard deviation of that residual.

The externally studentized residual was calculated by:

$$t_i = (e_i)/ [MSE_i \times (1-h_{ii})]^{1/2}$$

where e_i was the residual for the ith observation and MSE_i was the mean squared error for the regression model if the ith observation was left out.

The h_{ii} and externally studentized residual (t_i) were useful for detecting potential outliers that probably deserve additional attention. When t_i was greater than 3, the data may be identified as an outlier (Gary and Woodall, 2012). Therefore, when the model was applied, those cases must be watched carefully. The DFFITS value and externally studentized residual identified that run order 12 in Table 5 as an outlier and had significant higher predicted values than the actual responses.

Limitation and applicability

It is possible to use a model equation with a higher degree than the second-order; however, a second-order equation is useful because it has only one stationary point. If a poor fit is caused by third or higher order terms, the second-order model representation would be inadequate (Arshad and Gilmour, 2012).

Materials and Methods

Soil

The average mineral contents in the planted soil were measured using an inductively coupled plasma-mass spectrometry (ICP-MS) by Taitung Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan, Taiwan and were of (in mg / kg of soil) P_2O_5: 19.60 ± 1.03; K_2O: 80.54 ± 4.12; CaO:

Table 1. Mixture design arrayfor djulis (*Chenopodium formosanum* Koidz.) yields.

Run	Nitrogen	Phosphorus	Potassium	Yields (g/plant)
1	0[†]	1	1	48.4
2	0.5	0.5	1	46.17
3	0.5	1	0.5	53.91
4	1	1	0	49.38
5	1	0.5	0.5	36.38
6	0.333	0.833	0.833	52.2
7	0.5	0.5	1	46.17
8	0	1	1	48.4
9	0.833	0.333	0.833	41.23
10	0.833	0.833	0.333	43.53
11	1	1	0	49.38
12	0.667	0.667	0.667	37.5
13	1	0	1	43.6
14	1	0	1	43.6

[†] The codes in the table were: 0: 0 g/6m^2 and 1:200 g/6m^2; meanwhile, the other codes were the proportions from 1.

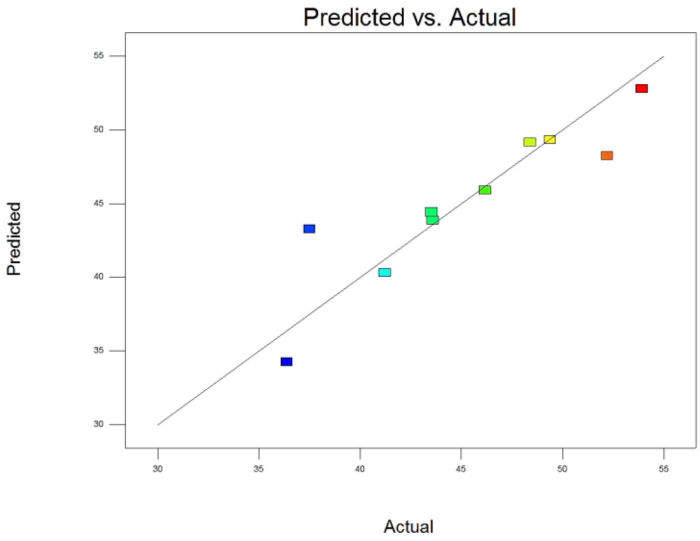

Fig 1. Linearity between actual and predicted responses.

Table 2. ANOVA for mixture factorsthat affected the yields of *Chenopodium formosanum* Koidz.

Source	Sum of Squares	df	Mean Square	FValue	p-value
Model	281.52	5	56.30	7.79	0.0061
Linear Mixture	118.67	2	59.34	8.20	0.0115
AB	0.51	1	0.51	0.071	0.7965
AC	13.05	1	13.05	1.80	0.2160
BC	157.06	1	157.06	21.72	0.0016

[†]A: nitrogen, B: phosphorus, C: potassium. ‡ The major factors of this study were linear mixture and the interaction of phosphorous and potassium

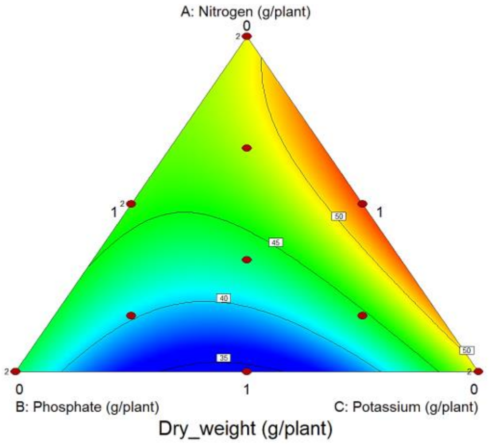

Fig 2. Contour plot of the mixture factors effect on the yields of djulis in dry weight. The red color region approaches higher yields and blue color region approaches lower yields.

Table 3. Analysis of variance (ANOVA) for the fitted quadratic polynomial model of the strawberry fruit weight.

Standard deviation	Mean	C.V.%	R^2	Adjusted R^2	Predicted $R^{2\dagger}$	Adequate precision‡	AAD (%)
2.690	45.700	5.880	0.830	0.723	0.535	10.530	2.91

[†] Predicted R^2, calculated by $\{1- [\Sigma\, (y_{iexp}–y_{ipre})^2 / SS_T]\}$, where y_{iexp} is the experimental value, y_{ipre} is the predicted value, and SS_T is the corrected total. ‡ Adequate precision, the signal to noise ratio.

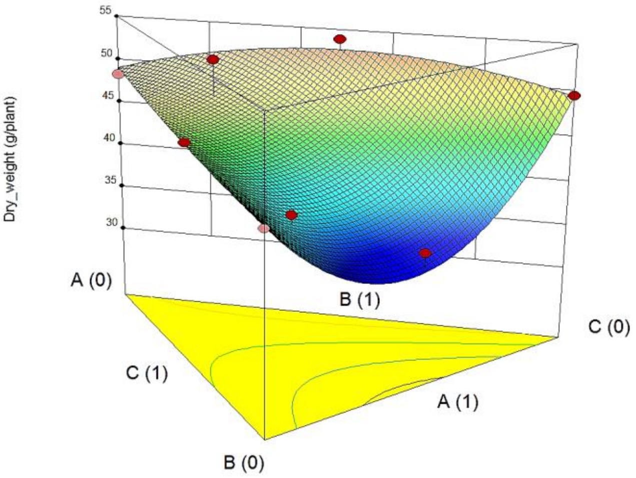

Fig 3. 3D graphs of the mixture factors affected the yields in dry weight. The pink spot is the design point below predicted value and the red spot is the design point above predicted value. Where A: Nitrogen, B: Phosphorus, C: Potassium.

Table 4. Actual value vs. Predicted value.

Run Order	Actual Value	PredictedValue
1	48.40	49.16
2	46.17	45.92
3	53.91	52.80
4	49.38	49.33
5	36.38	34.26
6	52.20	48.25
7	46.17	45.92
8	48.40	49.16
9	41.23	40.30
10	43.53	44.42
11	49.38	49.33
12	37.50	43.29
13	43.60	43.85
14	43.60	43.85

[†] The unit of actual and predicted value were of g / plant.

Table 5. Diagnostic case statistics of djulis(*Chenopodium formosanum* Koidz.)yields in difference in fit.

Runs Order	Residual	Leverage	Internally Studentized Residual	Externally Studentized Residual	Cook's Distance	Influence on Fitted Value DFFITS
1	-0.76	0.482	-0.392	-0.370	0.024	-0.357
2	0.25	0.423	0.123	0.116	0.002	0.099
3	1.11	0.715	0.774	0.753	0.250	1.192
4	0.049	0.481	0.025	0.023	0.000	0.023
5	2.12	0.715	1.476	1.618	0.911	2.564 *
6	3.95	0.195	1.637	1.877	0.108	0.925
7	0.25	0.423	0.123	0.116	0.002	0.099
8	-0.76	0.482	-0.392	-0.370	0.024	-0.357
9	0.93	0.195	0.384	0.362	0.006	0.179
10	-0.89	0.222	-0.377	-0.356	0.007	-0.190
11	0.049	0.481	0.025	0.023	0.000	0.023
12	-5.79	0.223	-2.441	-4.517 **	0.285	-2.420 *
13	-0.25	0.482	-0.131	-0.123	0.003	-0.119
14	-0.25	0.482	-0.131	-0.123	0.003	-0.119

† * Exceed limits (when DFFIT value was greater more than 2, the runs may have power to influence model result). ‡ When externally studentized residual is greater than 3, the data was identified as an outlier.

770.63 ± 4.50; MgO: 154.39 ± 7.02; Fe: 39.8 ± 8.58; Mn: 39.90 ± 6.32. The organic compounds content and pH value of the soil were of 3.72 ± 0.45% and 5.39 ± 0.09 respectively.

The straight fertilizers including N, P_2O_5, and K_2O were bought from Taiwan Fertilizer Company LTD., Taiwan.

Djulis seedlings

The 35 cm height djulis seedlings were collected from the Taitung Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan, Taiwan.

Mixture Experimental design

The mixture experiments are Scheffé's canonical polynomials (Cornell, 2002). Scheffé's Cubic Model was listed in *Introduction* section.

When levels of factors in an experiment were changed, it could affect the mixing properties of the ingredients. Fourteen 2 m x 3 m x 35 cm square plots were made and each plot was separated by 3 m. Ten djulis seedlings were planted along the fringe on each plot and each seedling was separated by 1 m. Fertilizer mixtures were made according to the mixture design array (Table 1) and the fertilizers were sprayed averagely on the middle line of each plot once only from the seedling were planted to harvested.

This study were conducted from November to March next year and were from 2014 to 2017, respectively. The djulis were harvest on March and was dried in sun and the dry weight were measured each year.

Mixture method was used to determine the mixture effects of the variables including nitrogen (N) , phosphorus (P) , and potassium (K) and all in g/(6m^2). The coded mixture variables used in the mixture method design were listed in Table 1. The maximum weight of fertilizers that combined with N, P, and K was 600 g/(6 m^2). A three-variable and fourteen runs were applied to investigate the best portion combination of N, P, and K that would reach highest djulis yields. The study were repeated three times.

Adequacy tests

The significant terms were found using an analysis of variance (ANOVA) for each response and were subjected to an F-test at a probability level less than 0.05.

Baş and Boyaci (2007) reported that a large total determination coefficient value (R^2) does not necessarily imply that the regression model is adequate. The addition of variables always increases R^2 regardless of whether the added variables are statistically significant. The application of the absolute average deviation (AAD) can eliminate these types of errors. The AAD is calculated using the following equation:

$$\text{AAD} = \{ [\sum_{i=1}^{p} (|y_{i\exp} - y_{ipre}|) / y_{i\exp}] / \text{P}\} \times 100$$

where y_{iexp} represents the experimental value, y_{ipre} represents the predicted value, and P represents the number of runs.

Sensitivities tests

In total, the model was evaluated using the R^2, adjusted-R^2, AAD, and adequate precision values. A good model generally has a high R^2, a low AAD, and a high adequate precision value.

The R^2 value is the coefficient of determination and is calculated by $\Sigma (y_{ipre} - y_{avg})^2 / SS_T$, where y_{ipre} is the predicted value, y_{avg} is the average value, and SS_T is the corrected total which generally equals to the total of the sum of squares of both model and residual. The R^2 is always positive. The higher is the coefficient of determination, the better the variance that the dependent variable is explained by the independent variable. The coefficient of determination is the overall measure of the usefulness of a regression.

. The adjusted R^2 value is calculated by $\{1 - [(n - 1) / (n - p)] \times (1 - R^2)\}$, where n represents the observations and p represents the number of parameters. The predicted R^2 is calculated by $\{1- [\Sigma (y_{iexp} - y_{ipre})^2 / SS_T]\}$, where y_{iexp} represents the experimental value, y_{ipre} represents the predicted value, and SS_T represents the corrected total that generally equals the sum of the model squares and residual squares. SS_T represents the sum of squares that was not calculated from zero, but was instead corrected by summing the squared distances of each individual response value from its entire average. Totally, predicted R^2 represents the predictive capacity of the model.

The adequate precision value is the signal-to-noise ratio and is calculated by

$$[(\text{Max}(YP) - \text{Min}(YP)] / (p\sigma^2 / n)^{1/2}$$

Where YP is the predicted values at the run settings, p is the parameters (including intercept (β_0) and any block coefficients) number of the model, σ^2 is the residual mean square from ANOVA table, and n is the number of runs in the experiment.

Statistics

Design Expert Version 9 (Stat-Ease, USA) software was used to calculate the mixture method design in this study. A two-tailed independent t-test in SPSS Version 22 (IBM SPSS, USA) was used to evaluate the significant difference between the experimental and predicted groups.

Conclusion

The djulis yields could be sufficiently described by the second-order polynomial model generated in this study. The independent variables included nitrogen, phosphorus, and potassium can be estimated using the contour and 3D surface plots in mixture method design. Moreover, the graphical estimation method in mixture methodology was adopted to estimate the optimal djulis yields conditions. Following the optimal conditions, the mean experimental djulis yields were corresponded well with the predicted values. Therefore, the model generated in this study is an important tool for predicting djulis yields.

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