Analysis of genotype-environment interaction in fennel using Sudoku design

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Abstract

Two trials were carried out over two locations, Ramadi and Fallujah included nine genotypes of Fennel, in order to assess the interaction between genotypes (G) and locations (E) using real data from the two experiments. The nine genotypes of fennel were randomly distributed under with Sudoku square design using type-I and model-I for combined analysis. The methodologies of this type and model solution were explained in details in the current study. Genotypes were significantly differed (P>0.01) in the first location. In the combined analysis, the genotype by location (G X E) interaction was also significant (P<0.01) which is due to the effect of the genotype. It can be concluded that Sudoku square design is suitable for selecting better genotypes as a honeycomb design and to test the multi-environment trial yield. Therefore, augmented studies should be conducted to extract the efficiency of this design using other types and models embedded in this design.

Keywords: GxE interaction, Fennel, Locations, Sudoku design

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Introduction

Soil microorganisms services have significant Sudoku is a popular combinatorial puzzle and it is the short form of the Japanese sentence “Suji Wa Dokushin Ni Kagiru” which means that the number should appear only once (Danbaba and Shehu, 2016). Sarkar and Sinha (2015) cited that Wayne Gould from Hong Kong discovered this puzzle while visiting Japan in 1997 and commenced to program computer software for this purpose, then he shared it with TIMES in London (UK) where it was first published in 2004 and ever since it was well known therefore; it consequently, had appeared in newspapers and magazine in Australia, Canada, Eastern Europe, India, and USA. Subramani and Ponnuswamy (2009) proposed the construction of Sudoku designs of order \( k = m^2 \). For applying Sudoku design in agricultural trials, they speculated linear models as a tool to analyze the data resulted from their design. Recently, Sudoku design may have been revealed as partial or NP-complete (nondeterministic polynomial time) (Béjar et al. 2012; Mahdian and Mahmoodian 2015). Likely, Danbaba and Dauran (2016) proposed a linear model and procedure for analyzing data of partially balanced Sudoku designs. In addition, Donovan et al. (2017) and Kumar et al. (2015) investigated the Sudoku extracted space-filling designs. Furthermore,
Thus, Sudoku design could be used as experimental design as special Latin square design with additional variable (internal block) without maximizing the experimental units (Sarkar and Sinha, 2015) which is represented as orthogonal Graeco-Latin square design (Subramani, 2013; Subramani, 2012). Li et al. (2016) proposed a facilitative and effective construction method of uniform designs based on Sudoku applied by a mixed level of factors. Recently, Subramani (2018) explained the rectangles for the construction and analysis of the Sudoku square designs. Experiments could be repeatedly laid out at years, locations (environments) or season with treatments as genotypes then data of interaction of treatments and locations, years or season extracted from these experiments could be analyzed using combined analysis. In response with this particularity, Danbaba and Shehu (2016) and Danbaba (2016) stated combined analysis to analyze data resulted from experiments conducted using Sudoku square design. Consequently, Shehu and Danbaba (2018) applied the analysis of variance method to derive the variance components for the four Sudoku square designs models. For covariance, least square method was applied to derive the sum of the square of the different effects and covariance of Sudoku square design (Shehu and Danbaba, 2018). From the other hand, a multivariate extension of various variables laid out at Sudoku designs could be extracted and done (Shehu and Danbaba, 2018). As Sudoku can be used in many situations in life, the current study was carried out by using Sudoku design in order to evaluate the performance of nine Fennel genotypes over to environments.

**Material and Methods**

Tow field experiments were conducted at two locations viz; Ramadi and Fallujah west of Baghdad, Iraq. Sudoku square design type I was used to randomly distribute nine fennel genotypes (Figure 1).

![Figure-1: Sudoku puzzle (left) and solved puzzle (right) indicated how the nine (1-9) fennel genotype randomly distributed in the two locations of the study.](image)

Nine genotypes of the fennel were used in the current study (Table 1).

Table-1: Genetic background and some morphological characteristics the fennel genotypes used in the study.

| Symbol | Genotype | Origin | Introduction | Information |
|--------|----------|--------|--------------|-------------|
| G1     | Romanesco | Italian| Introduced by the first author from Poland in 2010 | Bulbossum, long stem hollow. The long diameter of the main umbel |
| G2     | Amigo    | Italian| By Elsahookie from Azuricum. Name derived from Elsahookie | Bulbossum, semi-long. The low diameter of main umbel |
| G3     | Sahoo    | improved| From the local market | long stem hollow Low diameter of the main umbel |
| G4     | Azuricum | Germany| Cultivated in Dollap town 135Km west Baghdad | Short stem. The low diameter of the main umbel |
| G5     | Sajjet   | Iranian| By Researcher Hannan from Dept. of Horticulture named on his memory | long stem hollow Low diameter of the main umbel |
| G6     | Dollap   | local | By irradiation | Short stem. The low diameter of the main umbel |
| G7     | Hannan   | Jordan | Introduced by the first author from Poland in 2010 | Bulbossum, long stem hollow. The long diameter of the main umbel |
Table 2: Data of secondary umbel of nine fennel genotypes grown in Ramadi (L1) and Fallujah (L2) locations.

| Column box | (Ramadi-L1) | (Fallujah-L2) |
|------------|-------------|---------------|
|            | CB1         | CB2           | CB3           | CB1         | CB2           | CB3           |
| Row box    | C1          | C2            | C3            | C4          | C5           | C6            | C7            | C8           | C9          | C1          | C2           | C3           | C4           | C5           | C6           | C7           | C8           | C9          |
| RB1        | 43          | 42            | 21            | 17          | 20           | 43            | 34            | 47           | 23          | 22          | 23           | 17            | 39            | 47           | 34           | 45           | 17           | 44          |
| RB2        | 19          | 16            | 20            | 43          | 47           | 30            | 43            | 39           | 24          | 48          | 40           | 16            | 42            | 43           | 16           | 25           | 35           | 25          |
| RB3        | 41          | 32            | 46            | 35          | 22           | 24            | 42            | 15           | 16          | 35          | 45           | 44            | 24            | 23           | 15           | 16           | 48           | 40          |
| R4         | 47          | 14            | 45            | 33          | 23           | 18            | 24            | 43           | 36          | 45          | 35           | 25            | 15            | 24           | 47           | 44           | 38           | 17          |
| R5         | 15          | 31            | 27            | 24          | 44           | 24            | 21            | 43           | 47          | 17          | 48           | 25            | 21            | 43           | 36           | 35           | 45           | 15          |
| R6         | 21          | 23            | 45            | 44          | 35           | 44            | 15            | 15           | 35          | 15          | 44           | 38            | 35            | 15           | 43           | 25           | 25           | 48          |
| R7         | 12          | 48            | 40            | 22          | 40           | 43            | 16            | 21           | 42          | 26          | 18           | 48            | 42            | 35           | 43           | 40           | 16           | 26          |
| R8         | 41          | 28            | 16            | 25          | 43           | 38            | 47            | 35           | 16          | 44          | 16           | 34            | 16            | 39           | 21           | 48           | 26           | 43          |
| R9         | 43          | 42            | 23            | 47          | 20           | 20            | 39            | 22           | 43          | 40          | 26           | 43            | 47            | 16           | 22           | 18           | 44           | 34          |

Thus, the analysis for location one will be \( \frac{(844)^2 + (836)^2 + (872)^2}{3} - 80403.75 = 26.47 \)

4. Calculating the column box sum of squares (SSCB) (Table 4)

\[ SSCB = \frac{\sum_{j=1}^{n} CB^2_j}{n^3} - c.f. =; \]

5. Calculating the row sum of squares (SSR); (Table 5)

\[ SSR = \sum_{i=1}^{n} \frac{R^2_i}{n^2} - c.f. =; \]

Table 3: Sum of row box data - Location 1

| Row box | \( \Sigma RB_l \) |
|---------|-----------------|
| RB1     | 844             |
| RB2     | 836             |
| RB3     | 872             |

Table 4: Sum of row box data - Location 1

| Column box | CB1 | CB2 | CB3 |
|------------|-----|-----|-----|
| \( \Sigma CB_l \) | 841 | 868 | 843 |

Consequently,

\[ SSCB = \frac{(844)^2 + (868)^2 + (843)^2}{3} - 80403.75 = 16.77 \]

Table 5: Sum of row data – Location 1 & 2

| Ramadi- L1 | Fallujah- L2 |
|------------|--------------|
| Row | \( \Sigma R_l \) | Row | \( \Sigma R_l \) |
| R1      | 290          | R1   | 288          |
| R2      | 281          | R2   | 290          |
| R3      | 273          | R3   | 290          |
| R4      | 283          | R4   | 290          |
| R5      | 276          | R5   | 285          |
| R6      | 277          | R6   | 288          |
| R7      | 284          | R7   | 294          |
| R8      | 289          | R8   | 287          |
| R9      | 299          | R9   | 290          |
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Thus, the analysis will be;
\[
SSR = \frac{(290)^2 + (291)^2 + \cdots + (299)^2}{3^2} - 80403.75 = 58.69
\]
a. Extract the column sum of squares (SSC) (Table 6)
\[
SSC = \sum_{p=1}^{n^2} \frac{c^2p}{n^2} = c.f. =
\]

**Table-6: Sum of column data – Location 1&2**

| Location | Column | Ramadi - L1 | Fallujah - L2 |
|----------|--------|-------------|---------------|
|          | C1     | C2          | C3            | C4  | C5  | C6  | C7  | C8  | C9  |
|          | 282    | 276         | 283           | 290 | 294 | 284 | 281 | 280 | 282 |

Consequently,
\[
SSC \approx \frac{(282)^2 + (276)^2 + \cdots + (282)^2}{3^2} - 80403.75 = 25.80
\]

6- Extract the sum of the square of row box (Table 7)
\[
SSS = \sum_{q=1}^{n^2} S^2q.. - c.f. =
\]

**Table-7: Sum of sub-squares data or boxes – Location 1**

| Location | Ramadi - L1 | Fallujah - L2 |
|----------|-------------|---------------|
|          | CB1         | CB2           | CB3           |
| sub-square | 280         | 281           | 283           | 290 | 283 | 295 |
| RB1      | 268         | 289           | 279           | 292 | 279 | 292 |
| RB2      | 293         | 298           | 281           | 295 | 281 | 295 |
| RB3      |             |               |               |     |     |     |

Thus,
\[
SSS = \frac{(280)^2 + (281)^2 + \cdots + (281)^2}{3^2} - 80403.75 = 68.47
\]

Extract the genotypes sum of squares (SSG) (Table 8)
\[
SSG = \sum_{k=1}^{n^2} G^2k.. - c.f. =
\]

**Table-8: Sum of nine genotypes data over two locations**

| Genotypes | Ramadi- L1 | Fallujah- L2 | SUM |
|-----------|------------|--------------|-----|
| G1        | 386        | 344          | 730 |
| G2        | 386        | 305          | 691 |
| G3        | 309        | 290          | 599 |
| G4        | 336        | 303          | 639 |
| G5        | 149        | 290          | 439 |
| G6        | 208        | 305          | 513 |
| G7        | 156        | 346          | 502 |
| G8        | 202        | 196          | 398 |
| G9        | 420        | 223          | 643 |
| SUM       | 2552       | 2602         | 5152 |

Accordingly,
\[
SSG = \frac{(386)^2 + (386)^2 + \cdots + (420)^2}{3^2} - 80403.75 = 9971.14
\]

8- Calculating the error sum of squares (SSE)
\[
SSE = \sum_{k=1}^{n_2} \sum_{j=1}^{n_1} r_{ij}^2 + \sum_{k=1}^{n_1} \sum_{j=1}^{n_1} r_{ij}^2 + \sum_{k=1}^{n_1} \sum_{j=1}^{n_1} r_{ij}^2 + \sum_{k=1}^{n_1} \sum_{j=1}^{n_1} r_{ij}^2 + \sum_{k=1}^{n_1} \sum_{j=1}^{n_1} r_{ij}^2 + \sum_{k=1}^{n_1} \sum_{j=1}^{n_1} r_{ij}^2 + \sum_{k=1}^{n_1} r_{ij}^2 + \sum_{k=1}^{n_1} r_{ij}^2 + \sum_{k=1}^{n_1} r_{ij}^2
\]

So, SSE will be equal to
\[
= 90896 - 80430.22 - 80430.25 - 80462.44 - 80429.56 - 90374.89 - 80472.22 + 20455.80 = 324.91
\]

The same aforementioned steps were applied to get the same statistical information for location 2 (Fallujah); therefore ANOVA table constructed for both locations as below (Table 9);

**Table-9: ANOVA Table of Sudoku design of type-1 of the mean square for both locations (Ramadi-L1 and Fallujah-L2)**

| Source Of Variance | DF | L1 | L2 | L1 | L2 | MS | F cal. (Observed) |
|--------------------|----|----|----|----|----|----|------------------|
| genotypes          | n^2-1 | 8  | 8  | 9971.14 | 2221 | 1246.39 | 277.62 | 168.79** | 1.43 |
| Row blocks         | n-1     | 2  | 2  | 26.47  | 1.21  | 13.24  | 0.61  | 1.79  | 0.003 |
| Column blocks      | n-1     | 2  | 2  | 16.77  | 33.36 | 8.38   | 16.68 | 1.14  | 0.09  |
| Rows               | n^2-1   | 8  | 8  | 58.69  | 5.65  | 7.34   | 0.71  | 0.99  | 0.004 |
| Columns            | n^2-1   | 8  | 8  | 25.80  | 39.21 | 3.23   | 4.90  | 0.44  | 0.03  |
| Sub-squares (boxes)| n^2-1   | 8  | 8  | 68.47  | 36.32 | 8.56   | 4.54  | 1.16  | 0.02  |
| Error              | (n-1)(n+1)(n^2-3)-2 | 44 | 44 | 324.91 | 8546.03 | 7.38   | 194.23 |
| Total              | n^4-1   | 80 | 80 |        |       |        |        |        |
Data presented in ANOVA table indicated that genotypes possessed highly significant effect in location 1 which had F calculated of 168.79 and mean squares of 1246.39 secondary umbels per the main umbel. Error mean square was extracted the low value of 7.38. The other components of variance were not significant whereas, at Location of Fallujah (L2) all components of variance were not significant. Thus, error mean square was very high whose value was 194.23 secondary umbels per main umbel (table 9). As least significant difference (LSD$_{0.05}$) can be used only if F test is significant, therefore it was calculated for Location of Ramadi (L1) as it was significant for the treatments (genotypes) as follow:

$$L.S.D_{0.05} = t_{	ext{ad}fe} \sqrt{2 \text{mse}/9},$$
$$= 2.035\sqrt{2 \times 7.38}/9, = 2.61$$ for genotypes grown over location 1, figure 2 showed the significant differences among genotypes at the probability of 0.05.

### Figure 2. Number of secondary umbel per main umbel for the nine fennel genotypes in Ramadi location (L1) which indicated that G9 was superior overall genotypes under study (P<0.05)

**Combined analysis of Sudoku**

Data were recorded from two locations for nine genotypes of fennel conducted in Sudoku square design as mentioned above. Thus, the linear model was shifted to be involved in the multi-location experiment as in equation:

$$y_{ijmlx} = \mu + \theta x + \alpha i + \beta jx + \delta lx + \gamma m + \gamma mx + (\alpha \theta)x + \epsilon(ij)lmlx$$

The components of the model interpreted as below:

- $y_{ijmlx}$ represents the value which observed in each plot for $ih$ row and $mth$ column, this value is subjected to the $ith$ genotypes, $jth$ sub-square (box) of the $xth$ experimental location; $\mu$ is the general mean, the symbols of $ai, bjx, \delta lx, \gamma mx, \theta x$ and $(\alpha \theta)ix$ represent the main effects that influenced by $ith$ genotype, $jth$ sub-square, $mth$ column, $x$ location and genotype by location interaction, respectively. Residual or random error is signed by $\epsilon_{ijmlx}$.

To complete combined analysis, the sum square of experimental location would be SSEL, SSG is the total genotype sum of squares for both locations, SSR is sum of squares of total rows for both locations, SSC is sum of squares of total columns for both locations, SSS is sum of squares of total sub-squares (boxes or sub-blocks) for both locations, SSL is sum squares of genotypes by locations and SSL is the total sum of squares of both location. To calculate these statistics indices, the following equations were applied:

$$T1 = \frac{y^2}{ek^2}, T2 = \frac{\Sigma x y^2}{k^2}, T3 = \frac{\Sigma \Sigma x y^2 \ldots l \ldots x}{k}, T4 = \frac{k^2}{\Sigma m \Sigma x y^2 \ldots m x}, T5 = \frac{k^2}{\Sigma i y^2 i \ldots},$$

$$T6 = \frac{k^2}{\Sigma x \Sigma y^2 i \ldots x}, T7 = \frac{\Sigma x \Sigma y^2}{k}, T8 = \frac{\Sigma i \Sigma j \Sigma m y^2 ij l m x}{ek}$$

(Danbaba, 2016, Danbaba and Shehu, 2016)

Therefore, $T1 = \frac{5154^2}{162} = 163973.6$.

From table 8, $T2$ was calculated:

$$T2 = \frac{(2552^2)+(2602^2)}{(9)^2} = 163989;$$

From table 5 $T3$ was calculated: $T3 = \frac{(290^2)+(281^2)+\ldots+(290^2)}{9} = 164053.3$.

Thus, SSR = $T3 - T2$; $= 164053.3 - 163989 = 64.35$

From table 6 $T4$ was calculated: $T4 = \frac{(282^2)+(276^2)+\ldots+(292)^2}{9} = 164054$

Thus, SSL = $T4 - T2$; $= 164054 - 163989 = 65.01$
From Table 8, T5 was calculated: $T5 = \frac{(730)^2 + (691)^2 + \ldots + (643)^2}{9} = 169847.2$

Thus $SSG = T5 - T2$;

=169847.2-163989 = 5858.24

From the same Table T6 was calculated: $T6 = \frac{(386)^2 + (344)^2 + \ldots + (223)^2}{9} = 176181.1$

From Table 7, T7 was calculated: $T7 = \frac{(280)^2 + (281)^2 + \ldots + (295)^2}{9} = 164093.8$

Thus, $SSS = T7 - T2$;

=164093.8-163989= 104.79

$SSL = T2 - T1$;

=163989-163973.6= 15.4321

From Table 2, was calculated: $T8 = (43)^2 + (42)^2 + \ldots + (44)^2 + (34)^2 = 185364$

Thus, SST = $T8 - T1$;

=185364-163973.6= 21390.44, an $SS = T6 - T2 - T5 + T1$;

=176181.1-163989-169847.2+163973.6= 6318.45

Thus, SSE could be found by subtraction as below:

$SSE = T8 - T1 - T3 - T4 - T6 - T7 + 4(T2)$;

=185364-163973.2-164053.3-164254-174181.1-164093.8+4(163989) = 8964.17

**Table 10:** ANOVA table revealed combined analysis of Sudoku square design for nine fennel genotypes

| Source                        | df | Sum of squares | Mean squares | Fcal. (observed) |
|-------------------------------|----|----------------|--------------|-----------------|
| Location                      | 1  | 15.43          | 15.43        | 0.16            |
| Genotypes k=1-9-1=8           | 8  | 5858.24        | 732.28       | 7.84*           |
| Rows                          | e(k-1) = 2(9-1) = 16 | 64.35        | 4.02         | 0.04            |
| Columns                       | e(k-1) = 2(9-1) = 16 | 65.01        | 4.06         | 0.04            |
| Sub-squares                   | e(k-1) = 2(9-1) = 16 | 104.79       | 6.55         | 0.07            |
| Genotype X Location           | (e-1)(k-1) = (2-1)(9-1) = 9 = 96 | 6318.46  | 1120.58      | 1200**          |
| Error                         | e(k-1)(k-3) = 2(9-1)(9-3) = 161 | 8964.60   | 93.38        |                 |
| Total                         | ek² - 1 = 2(9²) - 1 = 161 | 21390.44   |              |                 |

**Results**

**Analysis of variance (ANOVA)**

The Analysis of variance (Table 9) and Table 10 illustrated that there is a significant difference among fennel genotypes that grown over location 1 (Ramadi).

Thus, G9 was superior which showed the highest average of umbellate number per the main umbel of 46.67, followed by G1 and G2 of 42.89 and 42.89 umbellate umbel$^1$, respectively, for each one. Whereas, G5 has the lowest number of umbellate per umbel of 16.56 umbellates umbel$^1$. While the genotypes that sown over location 2 did not show significant differences in the number of secondary umbel per main umbel (Table 11).

**Table 11:** Means of nine fennel genotypes grown in location 1&2

| Genotypes | Ramadi - L1 | Fallujah - L2 |
|-----------|-------------|---------------|
| G1        | 42.89       | 38.22         |
| G2        | 42.89       | 33.89         |
| G3        | 34.33       | 32.22         |
| G4        | 37.33       | 33.67         |
| G5        | 16.56       | 32.22         |
| G6        | 23.11       | 33.89         |
| G7        | 17.33       | 38.44         |
| G8        | 22.44       | 21.78         |
| G9        | 46.67       | 24.78         |
| LSD 0.05  | 2.61        | N.S.          |

**Table 12:** Means of umbellate per umbel of nine fennel genotypes over two locations

| Genotypes | Ramadi-L1 | Fallujah-L2 | Means |
|-----------|-----------|-------------|-------|
| G1        | 42.89     | 38.22       | 40.56 |
| G2        | 42.89     | 33.89       | 38.39 |
| G3        | 34.33     | 32.22       | 33.28 |
| G4        | 37.33     | 33.67       | 35.50 |
| G5        | 16.56     | 32.22       | 24.39 |
| G6        | 23.11     | 33.89       | 28.50 |
| G7        | 17.33     | 38.44       | 27.89 |
| G8        | 22.44     | 21.78       | 22.11 |
| G9        | 46.67     | 24.78       | 35.73 |
| LSD 0.05  | 8.92      | 6.31        |       |
| Means     | 31.51     | 32.12       |       |
| LSD 0.05  | N.S.      |             |       |

**Combined analysis of Sudoku**

Analysis of covariance (Table 10 and 12) indicated that the genotypes of fennel significantly differed. Consequently, G1 showed the highest performance of umbellate per umbel of 40.56 umbellates umbel$^1$, followed by G2 of 38.39 umbellates umbel$^1$. Whereas, G8 has the lowest performance which gave the lowest number of umbellate of 22.11 umbellates umbel$^1$. Effect of location did not achieve a significant difference. Moreover, the interaction between
genotype X location displayed significant differences. Where, G9*L1 gave the highest number of umbellate of 46.67 umbellates umbel\(^{-1}\) followed by G1*L1 and G2*L1 of 42.89 umbellate umbel\(^{-1}\) for each one. While G5*L1 had the lowest number of umbellate of 16.56 umbellates umbel\(^{-1}\). Furthermore, G7*L2 achieved the highest number of umbellate per umbel of 38.44 umbellates umbel\(^{-1}\), followed by G1*L2 of 38.22 umbellate umbel\(^{-1}\). While G8*L2 gave the lowest number of umbellate per umbel of 21.78 umbellates umbel\(^{-1}\).

**Discussion**

Not many agricultural researchers have the time or ability to master the details of the complex mathematical operations, yet they all are looking forward to gain a general understanding of the logic of the designs they used. The understanding of the basics and principles of statistics will help to properly design an experiment and get logic conclusions from gained results. The current research was achieved according to real data were recorded on secondary umbel number of nine fennel genotypes that were sown in two locations in the Iraqi environment. Sudoku square design type I was used in order to detect the variation among those genotypes over the two locations. Analysis of variance of nine fennel genotypes over two locations revealed that genotypes in location1 were significantly varied at the probability of 5% (\(\alpha=0.05\)) when used univariate Sudoku model proposed by Subramani and Ponnuswamy (2009). ANOVA technique was efficiently enhanced by Shehu and Danbaba (2018). The analysis of variance was effective to extract the effect significance variations among genotypes which supported by Subramani (2018) who used Sudoku square designs with rectangles to be applied in many fields of science. The properties of Sudoku designs made those designs were simple and efficient when used in a factorial experiment (Li et al., 2016). The combined analysis of genotypes X location (G*L) was efficient in revealing the significant differences of some variation components nevertheless location variation effect was not significant. However, genotypes (G) and genotype X location (G*L) were significant may due to the properties of analysis of covariance. Shehu and Danbaba (2018) discussed the analysis of variance for Sudoku models and significance of treatment who indicated that the use of this combined analysis is a good tool to detect the variation over multil locations especially when they share common treatments.

**Conclusion**

Sudoku square design Model Type I assumed as Latin square design in terms of rows, columns, and treatments (genotypes). In the current study, the aforementioned experimental design was used to derive the variance components over multi-environments. Based on results of the current study, although G9 showed the highest number of secondary umbels per the main umbel in Ramadi (L1) only, G1 showed great stability over the two locations used in this study according to Sudoku Square design type I yet, support studies should be applied over more divergent environments in order to detect the variation among used treatments.

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Al-Mehemdi AF: Designed the study and put the hypothesis, performed the measurement and statistical analysis
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Al-Issawi MH: Performed the measurement and statistical analysis, manuscript writing