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Inheritance of Rose-Flowered Mutation in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Mutations are the most important phenomenon in the living organisms including plant species due to creating variation. Variation in crop plants can be increased by induced mutations. The present study deals with inheritance of an induced mutation with rose-flowered in the cultivated chickpea (*Cicer arietinum* L.). A mutant having rose-shaped flowers without male and female organs in the cultivated chickpea was isolated in M_2 and harvested as a single plant with its sisters (sibs). In M_3, inheritance of the rose-flowered mutant was studied in the segregated rows because hybridization between the mutant and parent or the other genotype of the cultivated chickpea was not possible. Results indicated that the rose-flowered mutation was controlled by a single recessive gene (*rs*). This study is an alternative approach on inheritance studies if hybridization is impossible due to sterility.

Keywords: *Cicer arietinum;* Mutagenesis; Rose-flowered mutant

Nohutta (*Cicer arietinum* L.) Gül-Çiçekli Mutasyonun Kalıtımı

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ÖZET

Mutasyonlar, bitki türlerini de içeren canlı organizmalarda varyasyon yaratmak için en önemli varyasyon kaynağıdırlar. Bitkilerde varyasyon yapay mutasyonlarla artırılabilmektedir. Bu çalışma kültürü yapılan nohutta (*Cicer arietinum* L.) gül-çiçekli yapay mutasyonun kalıtımıyla ilgilidir. Erkek ve dişi organları olmayan gül-çiçekli nohut mutantı M_2 generasyonunda belirlenmiş ve kardeşleriyle birlikte tek bitki hasadı yapılmıştır. Gül-çiçekli mutantın kalıtımı, mutant ve ebeveyn ya da diğer genotiplerle döllenmenin olanaksız olduğu M_3 generasyonunda açılan sıralarda çalışılmıştır. Sonuçlar gül-çiçeklilik özelliğinin resesif bir genle (*rs*) idare edildiğini göstermektedir. Bu çalışma kısırlıktan dolayı döllenmenin mümkün olmadığı durumlar için alternatif bir yaklaşımdır.

Anahtar Kelimeler: *Cicer arietinum*; Mutagenesis; Gül-çiçekli mutant

1. Introduction

Cicer L. consists of 49 taxa including 9 annuals with the cultivated chickpea (*Cicer arietinum* L.) and 40 perennials (van der Maesen et al 2007; Donmez 2011; Ozturk et al 2011; 2013; Smykal et al 2015). Among the 49 taxa, *Cicer arietinum* L. is solely species under cultivation. It is diploid with 2n= 2x= 16 chromosomes (van der Maesen 1972) and its genome size ~738-Mb with 28,269 genes (Varshney et al 2013). Its solitary flowers are borne in an axillary raceme (Cubero 1987) despite being from 2 to 9 flowers on the same node (Gaur & Gour 2002; Yasar et al 2014). Flowers in the cultivated chickpea are completely bisexual and self-pollinated owing to its cleistogamic (closed) flowers shape (Cubero 1987), while there was low level of outcrossing (Toker et al 2006).

Flowers of the cultivated chickpea are different colors viz., white, pink or purple and blue in color (Kumar et al 2000; Yasar et al 2014). Flowers have papilionaceous corolla and five petals, which are generally polypetalous i.e., occurring of standard (vexillium), wings, and keel. The vexillum is obovate shaped, 8-11 mm long, and 7-10 mm wide. The wings are obovate with short pedicels, 6-9 mm long and about 4 mm wide with an auriculate base. The keel is rhomboid, 6-8 mm long, with a pedicel 2-3 mm long. Androecium forms from 10 stamens in diadelphous (9+1) condition. The anthers burst longitudinally, and the adult pollens are orange colored. Gynoecium consists of the stigma, style and ovary. The ovary is ovate with a pubescent surface, 2-3 mm long and 1-15 mm wide. It contains 1-4 ovules. The style is linear shaped, upturned, and glabrous except at the bottom with 3-4 mm long. The stigma is globose and capitate (van der Maesen 1972; Cubero 1987; Singh & Diwakar 1995). Despite of being cleistogamous flowers, some different induced mutants with open flowers were reported (Pundir & Reddy 1998; Srinivasan & Gaur 2012; Yildirim et al 2013). There are two different flower shapes as (i) cleistogamous flowers and (ii) open flowers in the cultivated chickpea when the available literature has been searched (ISI 2015). This type of flowers was created by spontaneous (Pundir & Reddy 1998; Srinivasan & Gaur 2012) or induced (Yildirim et al 2013) mutations. Mutations are referred to as the most important phenomenon in the living organisms including plant species due to creating variation (van Harten 1998). Mutation breeding is considered as the most efficient powerful method to create variation compared to those hybridizations, and also it takes shorter time to the release of the mutant cultivars than those of hybridizations (Salimath et al 2007). Both of qualitative and quantitative traits, previously the known alleles or unknown alleles and linkage among genes can be altered by mutation breeding (van Harten 1998; Tomlekova 2010). Genetic variability in the cultivated chickpeas has been narrowed by using pure line selection or hybridization followed by the pedigree method, the bulk method, the single seed descent, or modification of these methods for a longer period (Salimath et al 2007). To create genetic variability in chickpea, the most efficient way is mutagenesis (Salimath et al 2007; Toker & Uzun 2010; Wani et al 2014). Considerable traits in chickpeas have been induced by Toker (2014) by mutagenesis. One of them was rose-flowered mutant chickpea (Toker 2014). Therefore, the present study deals with inheritance of the rose-flowered mutation in the cultivated chickpea.

2. Material and Methods

2.1. Selection of rose-flowered mutant

The rose-flowered mutant in the present study was identified in M_2 generation of irradiated seeds of the cultivated chickpea (*Cicer arietinum* L.) cv. Sierra (Toker et al 2010; Toker 2014). Irradiation was applied on seeds with 12% moisture as 200, 300 and 400 Gy of gamma rays with rate was 1.66 kGy^{-1} from a ^{60}Co source in Turkish Atomic Energy Agency (TAEK), Ankara, Turkey (Toker et al 2005). Approximately 500 seeds each treatment level were used and irradiated seeds were stored at 4 ^{o}C up to sowing date. M_1 plants were harvested as a single plant and then each M_1 plant was grown as single plant progeny in M_2. M_2 plants were screened for all kind of morphological mutations and morphologically different plants from Sierra were isolated as putative mutants. The putative mutants and their sister plants (sibs) were grown as single plant progeny in M_3. In M_3, the mutants were confirmed for whether they were true mutant or not. Number of mutants in the segregating rows was recorded for chi-square test.

2.2. Growing of rose-flowered mutant

The row-to-row and plant-to-plant spacing was 50 cm and 10 cm, respectively. The fertilizers with N, P and K were applied at a rate of 20 kg per ha prior to sowing. The all generations were advanced at Antalya location (30° 38' E, 36° 53' N, 32 m from sea level) from 2005 to 2008 crop seasons. Weeds were controlled pulling by hand during seedling period. Plants were grown without additional irrigation under rainfed conditions.

2.3. Goodness of fit 3 to 1

The rose-flowered mutant plants were counted in segregating rows of M_2 and results confirmed in M_3 generation. Chi-square (χ^2) test was performed for goodness of fit of 3:1 ratio according to the Equation 1 (Steel & Torrie 1980).

$$\chi^2 = \Sigma(O-E)^2/E \quad (1)$$

Where; *O* and *E*, observed and expected values, respectively.

3. Results and Discussion

3.1. Morphological characteristics of rose-flowered mutant

Flowers of the rose-flowered mutant look like flowers of the genus *Rosa* L., whereas flowers of the parent cv. Sierra are cleistogamic papilionaceous. The mutant had many petals from 27 to 35 petals (Figure 1), while Sierra had five petals namely one standard, two wings and two keels (Figure 1). It was isolated from 200 Gy treatment.

Similarly, flowers of Sierra consisted of a stigma but flowers of the mutant contained ovary and style without stigma. Moreover, the female organ (gynoecium) was absent in rose-flowered mutant, while Sierra had 10 (9 fused+1 free) female organs. Flower structure of the rose-flowered mutant is quite different compared to flowers of Sierra since flowers of the mutant is open while flowers of Sierra is normal cleistogamous viz., closed (Table 1).

Figure 1- Rose-flowered mutant with open flower, 27-35 petals and without stigma and stamens (left) and normal cleistogamous flower of Sierra (right)

Şekil 1- Sierrada dişi ve erkek organları bulunmayan 27-35 taç yapraklı gül-çiçekli mutant (sol) ve normal kleistogamik çiçek (sağ)

Table 1- Characteristics of rose-flowered mutant and its parent

Çizelge 1- Gül-çiçekli mutant ve ebeveynlerin karakteristikleri

Characteristics	*Parent (Sierra)*	*Rose-flowered mutant*
Flower color	White	White
Flower shape	Cleistogamous (closed)	Open
No. of petals	5	27-35
Stamens	9+1	Nil (0)
Stigma	Yes	Absent

Open-flowered mutants in the cultivated chickpea (*Cicer arietinum* L.) and its progenitor (*C. reticulatum* Ladiz.) were reported prior to our study (Pundir & Reddy 1998; Srinivasan & Gaur 2012; Yildirim et al 2013). Previously reported open flower mutants were given as ICC 16341, ICC 16129 and OFM-3 by Srinivasan & Gaur (2012). Like flower shape, flower color mutations were reported in the cultivated chickpea (Gaur & Gour 2001; Atta et al 2003) and *C. reticulatum* Ladiz. (Toker

2009). In M_2, 15 plants, sister of rose-flowered mutants, were harvested as single plant. These 15 single plants were grown as single plant progeny in M_3. Of 15 rows, 9 rows in M3 were segregated as rose-flowered mutant and normal plants. A total of 89 plants in 9 segregated rows were counted as 69 normal and 20 rose-flowered in M_3.

3.2. Inheritance and genetics of the rose-flowered mutant

Chi-square test indicated that both of segregation in M_3 generation fit in well to the expected ratio of 3:1, indicating that the rose-flowered characteristic was governed by a single recessive gene (Table 2).

Results in the present study were in agreement with findings of previous reports (Pundir & Reddy 1998; Srinivasan & Gaur 2012) since open-flower trait was controlled by a single recessive gene. The gene symbol '*rs*' was designed for the induced rose-flowered characteristic. Results indicated that genotypic formula of the rose-flowered mutant and Sierra were assumed as '*rsrs*' and '*RsRs*', respectively. The new approaches could be used for all traits in mutants of crop plants. Srinivasan & Gaur (2012) pointed out that the genes controlling open-flower trait in ICC 16341, ICC 16129 and OFM-3 were '*ofl-1*', '*ofl-2*' and '*ofl-3*', respectively. These genes were non-allelic genes (Srinivasan & Gaur 2012).

Table 2- Inheritance of rose-flowered trait

Çizelge 2- Gül-çiçekliliğin kalıtımı

Parents	M_3		χ^2	*P*
	Observed	*Expected*		
	No. of fertile plants (F): No. of sterile mutants (S)	F:S*		
Sierra	69:20	3:1	0.31	0.90-0.75

*, F is the induced fertile sib and S is the induced sterile mutant

In conclusion, a quite unique mutant and a new gene controlling rose-flowered trait viz., '*rs*' in the cultivated chickpea cv. Sierra was created with gamma rays in the present study. The present study is an alternative approach on inheritance studies if hybridization is impossible due to sterility.

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