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INHERITANCE OF FEMALE STERILITY IN INDUCED *Cicer* SPECIES

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ABSTRACT

Mutations play an important role to induce new genes, creating variation. The present study deals with inheritance of female sterilities in the induced mutants of the cultivated chickpea (*Cicer arietinum* L.) and its progenitor (*Cicer reticulatum* Ladiz.). Air-dried seeds of *Cicer* species were irradiated with 200, 300 and 400 Gy gamma rays. Two induced mutations conferring open flower and determinate growth habit with small leaf characteristics in the cultivated chickpea and its progenitor were discovered in M_2 generation. These mutant chickpeas were female sterile. Inheritance study showed that the female sterility in the induced mutants was controlled by a single recessive gene (*fs*). The female sterility, determinate growth habit, open flower and small leaf characteristics were first reported for *C. reticulatum* Ladiz. Results indicated that a joint segregation of the female sterility, open flower, determinate growth habit and small leaf characteristics might be linked in both of induced mutants of the cultivated chickpea and its progenitor. The new gene discovered on the sterility in the induced mutants may be useful for gene mapping in *Cicer* species.

Key words: Chickpea, *Cicer arietinum*, *Cicer reticulatum*, mutagenesis, mutant, sterility

INTRODUCTION

The number of species in the genus *Cicer* L. has reached up to 45 taxa with a new endemic perennial species, *C. uluderensis* Donmez (van der Maesen *et al*., 2007; Donmez, 2011), recently found in Southeast Turkey. In the genus *Cicer* L., *Cicer arietinum* L. is only cultivated species, with 2n = 16 chromosomes, and self-pollinated species due to its cleistogamic flowers (Cubero, 1987). The cultivated chickpea is a derivative from its wild progenitor species, *C. reticulatum* Ladizinsky (Zohary and Hopf, 2000; Toker, 2009). *C. reticulatum* Ladiz. is originated from South-Eastern Turkey (Ladizinsky and Adler, 1976), and it can easily be crossed with the cultivated chickpea (Abbo *et al*., 2007). The morphological variations in *C. reticulatum* Ladiz. are narrower than those of the cultivated chickpeas (Robertson *et al*., 1997).

Induced mutations are useful for genetic exploitation of crop plants when the genetic variation is narrow. So far, several morphological mutants have been found and utilized in chickpea improvement as well as in linkage studies (Dahiya *et al*., 1984; Pundir and Reddy, 1998; Gaur and Gour, 2002; McNeil *et al*., 2007; Rajesh *et al*., 2007; Salimath *et al*., 2007; Srinivasan *et al*., 2006; Wani and Anis, 2008; Ali *et al*., 2010; Kharkwal *et al*., 2010; Si *et al*., 2010; Wani, 2011). The aim of the present study is to report inheritance of induced male and female sterilities in the cultivated chickpea and its progenitor.

MATERIALS AND METHODS

Air-dried seeds of two *Cicer* species including the cultivated chickpea genotype namely '*macrosperma*' chickpeas, 'CA 2969' (Rubio *et al*., 2003), and a genotype of *C. reticulatum* Ladiz 'AWC 612' (Toker, 2005; Toker *et al*., 2007; Canci and Toker, 2009) were irradiated with 200, 300 and 400 Gy of gamma rays from a ^{60}Co source in Turkish Atomic Energy Agency (TAEK), Ankara, Turkey (Toker *et al*., 2005). There were approximately 500 seeds in each treatment level, after which they were stored at 4^{o}C. The field was fertilized with N, P and K at a rate of 20 kg per ha prior to sowing. The irradiated seeds were sown in plots with 45 cm row and 5 cm plant spacing at Antalya location (30^{o} 38' E, 36^{o} 53' N, 32 m from sea level) in the spring of 2005. Weed control was done by hand during seedling stage. Additional irrigation was not practiced because of sufficient rainfall.

The seeds from M_1 generation were individually harvested in the summer of 2005. M_2 generation was grown as single plant progenies in separate rows at the same location in 2006. After germination, M_2 plants were

carefully screened for all morphological mutations until harvest. Putative mutants in M_2 generation were isolated and individually harvested. Furthermore, the sibs of selected putative mutants were also harvested as single plants. For observation of mutant and segregation ratios, the selected mutants and their sibs were grown as M_3 generation at the same location in the following year. The normal and female sterile plants were counted in segregating rows for M_3 generation. The mode of inheritance was performed using chi-square (χ^2) test according to the formula: $\chi 2 = \Sigma(O\text{-}E)^2/E$, where O and E are observed and expected values, respectively (Toker *et al*., 2012b).

RESULTS AND DISCUSSION

The mutants selected from 'CA 2969' and 'AWC 612' was similar to each other for some respects. They had lesser number of stamens than those of parents, and both were female sterile bearing no stigma. Also, flowers in these mutants were open as shown in Figure 1. Furthermore, these mutants were determinate growth habit, while their parents were indeterminate growth habit (Table 1). The sterile mutant selected from 'CA 2969' and 'AWC 612' had smaller leaf with 4-7 leaflets than those of parents (Table 1).

Table 1. Characteristics of the mutants and their parent in *Cicer* species

Characteristics	*C. arietinum* L.		*C. reticulatum* Ladiz.	
	CA 2969	Mutant	AWC 612	Mutant
Flower color	White	White	Pink	Pink
Flower shape	Normal	Open	Normal	Open
Flower per peduncle	2	3-6	1	1
Stamens	9+1	<10	9+1	<10
Stigma	Yes	Absent	Yes	Absent
Leaflet per leaf	16-18	4-7	14-16	4-7
Leaf shape	Normal	Normal	Normal	Normal
Growth habit	Indeterminate	Determinate	Indeterminate	Determinate

Figure 1. Female sterile mutants with open flower, small leaf and determinate growth habit in *C. arietinum* L. (a and c) and in *C. reticulatum* Ladiz. (b and d).

Although flowers of the mutants were pollinated by hand, no pods were obtained from the crosses between the induced female sterile mutants (♂) and their parents (♀). Apparently pollen fertilities were deficient confirmed by with negative pollen staining (data not shown).

In M_1 generation, some morphological mutants were observed in plots. In M_2 generation, there were two female sterile mutants in plots. The ratio of the fertile sibs to the sterile mutants were 20:4 in 'CA 2969' and 17:3 in 'AWC 612' in M_2 generation, respectively (Table 2). The mutant plants in M_2 rows had no pods and seeds due to female sterility, whereas normal sibs were fertile. Therefore, only fertile sibs were sown in M_3 generation. In M_3 generation, the ratios of the fertile sibs to the mutants in 'CA 2969' and 'AWC 612' 'were recorded as 121:35 and 110:39, respectively (Table 2). Chi-square analysis of data from these mutants indicated that segregation ratios fit well to the ratio of 3:1 (i.e. 3 fertile sibs: 1 sterile mutant). Therefore, all these mutations described here were governed by a single recessive gene.

Table 2. Inheritance of induced sterility in *Cicer* species

Parents	M_2		M_3		$\chi 2$	*P*
	No. of filials	F: S*	Observed F: S*	Expected F: S*		
CA 2969	24	20:4	121:35	3:1	0.5	0.90-0.75
AWC 612	20	17:3	110:39	3:1	0.1	0.90-0.95

* F and S are the induced fertile sibs and the induced sterile mutant, respectively.

The nomenclature guidelines proposed by Muehlbauer and Singh (1987) were used for gene symbols. The gene symbol *fs* was designed for the induced female sterility in 'CA 2969' and 'AWC 612'. A similar study on inheritance of the induced sterility was reported by Ashri (1968) in peanut (*Arachis hypogeae* L.). van Rheenen *et al*. (1994) found a sterile mutant in the cultivated chickpea. Although male sterility in the cultivated chickpea has been reported in different studies (Sethi 1979; Muehlbauer and Singh, 1987), the sterility has not been used as sustainable so far.

The induced mutants and their sibs segregating in M_2, M_3, and latter generations can be used for gene mapping. These sterile mutations indicated that useful male sterile chickpea could be induced if chemical mutagen could have been used instead of gamma rays. Large chromosomal aberrations are possible in the present study because physical mutagens are one of the reasons for chromosome aberrations (Salimath *et al*., 2007).

After Muehlbauer and Singh (1987), many induced and some spontaneous genes in the cultivated chickpea and its progenitor were identified (Dahiya *et al*., 1984; Pundir and Reddy, 1998; Gaur and Gour, 2002; McNeil *et al*., 2007; Rajesh *et al*., 2007; Salimath *et al*., 2007; Srinivasan *et al*., 2006; Wani and Anis, 2008; Ali *et al*., 2010; Kharkwal *et al*., 2010; Si *et al*., 2010; Wani, 2011; Toker *et al*., 2012a). Pundir and Reddy (1998) isolated mutant having few leaflets. Open flower was also reported as a useful trait in the cultivated chickpea because it will reduce the time needed for cross pollinations (Pundir ve Reddy, 1998). van Rheenen *et al.* (1994) isolated an induced mutant chickpea having indeterminate growth habit. The symbol *cd* was designed for the allele conditioning for determinancy and *Dt* for the allele expressing the determinate trait (van Rheenen *et al*., 1994). To our knowledge, the mutant having open flower and determinate growth habit with small leaf is the first report in *C. reticulatum* Ladiz. (Figure 1 and Table 1). The mutant selected in 'CA 2969' was identical with the mutants reported by van Rheenen *et al*. (1994) and Pundir and Reddy (1998). These results suggested that the same loci could be induced again and also it was more inducible than the others.

A joint segregation of the induced sterility and open flower in 'CA 29169' and 'AWC 612' indicated that these characteristics may be linked to each other. Gaur and Gour (1999) reported similar results on linked genes in the cultivated chickpea.

The results indicated that there was a parallelism for the induced sterility between the cultivated chickpeas and *C. reticulatum* Ladiz., since both had the same mutation. The known genes related to determinate growth habit and small leaf was also induced in the cultivated chickpea and *C. reticulatum* Ladiz. Although the induced female sterility in the cultivated chickpeas and *C. reticulatum* Ladiz. is undesirable, this new gene can be useful for gene mapping.

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LITERATURE CITED

Abbo, S., R.J. Redden, S.S. Yadav, 2007. Utilization of wild relatives. In: Yadav, S.S., Redden, R., Chen, W., Sharma, B. (eds), Chickpea Breeding and Management. CAB Int. pp. 338–354.

Ali, H., T.M. Shah, N. Iqbal, B.M. Atta, M.A. Haq, 2010. Mutagenic induction of double-podding trait in different genotypes of chickpea and their characterization by STMS marker. Plant Breed. 129: 116–119.

Ashri, A., 1968. Morphology and inheritance of sterile brachytic dwarfs in peanuts, *Arachis hypogaea*. Crop Sci. 8: 413–415.

Canci, H., C. Toker, 2009. Evaluation of annual wild *Cicer* species for drought and heat resistance under field conditions. Genetic Resour. and Crop Evol. 56: 1-6.

Cubero, J.I., 1987. Morphology of chickpea. In: Saxena, M.C., Singh, K.B. (eds), The Chickpea. CAB Int. pp. 35–66.

Dahiya, B.S., V.S. Lather, I.S. Solanki, R. Kumar, 1984. Useful spontaneous mutants in chickpea. Int. Chickpea Newslet. 11: 4–7.

Donmez, A.A., 2011. *Cicer uludereensis* Donmez: a new species of *Cicer* (Chickpea) (Fabaceae) from around the Fertile Crescent, SE Turkey. Turk. J. Bot. 35: 71–76.

Gaur, P.M., V.K. Gour, 1999. An induced fasciated mutant of chickpea (*Cicer arietinum* L.). Indian J. Genet.Pl. Br. 59: 325–330.

Gaur, P.M., V.K. Gour, 2002. A gene producing one to nine flowers per flowering node in chickpea. Euphytica 128: 231–235.

Kharkwal, M.C., M.I. Cagirgan, C. Toker, T. Shah, M.M. Islam, H. Nakagawa, X. Xu, P. Si, 2010. Legume mutant varieties for food, feed and environmental benefits. In: 5th International Food Legumes Research Conference (IFLRC V) and 7th European Conference on Grain Legumes (AEP VII), 26-30 April 2010, Antalya, Turkey, pp: 196.

Ladizinsky, G., A. Adler, 1976. The origin of chickpea *Cicer arietinum* L. Euphytica 25: 211–217.

McNeil, D., F. Ahmad, S. Abbo, P.N. Bahl, 2007. Genetics and cytogenetics. In: Yadav, S.S., Redden, R., Chen, W., Sharma, B. (eds), Chickpea Breeding and Management. CAB Int. pp. 321–337.

Muehlbauer, F.J., K.B. Singh, 1987. Genetics of chickpea. In: Saxena, M.C., Singh, K.B. (eds), The Chickpea. CAB Int. pp. 99–125.

Pundir, R.P.S., G.V. Reddy, 1998. Two new traits – open flower and small leaf in chickpea (*Cicer arietinum* L.). Euphytica 142: 357–361.

Rajesh, P.N., K.E. McPhee, R. Ford, C. Pittock, J. Kumar, F.J. Muehlbauer, 2007. Ciceromics: Advancement in genomics and recent molecular techniques. In: Yadav, S.S., Redden, R., Chen, W., Sharma, B. (eds), Chickpea Breeding and Management. CAB Int. pp. 445–457.

Robertson, L.D., B. Ocampo, K.B. Singh, 1997. Morphological variation in wild annual *Cicer* species in comparison to the cultigen. Euphytica 95:309–319.

Rubio, J., M.T. Moreno, C. Martinez, J. Gil, 2003. Registration of CA2969, an ascochyta blight resistant and double-podded chickpea germplasm. Crop Sci. 43: 1567–1568.

Salimath, P.M., C. Toker, J.S. Sandhu, J. Kumar, B. Suma, S.S. Yadav, P.N. Bahl, 2007. Conventional breeding methods. In: Yadav, S.S., Redden, R., Chen, W., Sharma, B. (eds), Chickpea Breeding and Management. CAB Int. pp. 369–390.

Sethi, S.C., 1979. Male sterility. Int. Chickpea Newslet. 1: 4.

Si, P., Y. Chen, S. Weerakoon, J. Quealy, S. Powles, W. Erskine, 2010. Chickpea (*Cicer arietinum* L.) breeding lines tolerant to metribuzin applied post-emergence. In: 5th International Food Legumes Research Conference (IFLRC V) and 7th European Conference on Grain Legumes (AEP VII), 26-30 April 2010, Antalya, Turkey, pp: 216.

Srinivasan, S., P.M. Gaur, S.K. Chaturvedi, B.V. Rao, 2006. Allelic relationships of genes controlling number of flowers per axis in chickpea. Euphytica 152: 331–337.

Toker, C., 2005. Preliminary screening and selection for cold tolerance in annual wild *Cicer* species. Genet. Resour. and Crop Evol. 52: 1–5.

Toker, C., B. Uzun, H. Canci, F.O. Ceylan, 2005**.** Effects of gamma irradiation on the shoot length of *Cicer* seeds. Radiat. Phys. Chem. 73: 365–367.

Toker, C., H. Canci & T. Yildirim, 2007. Evaluation of perennial wild *Cicer* species for drought resistance. Genetic Resour. and Crop Evol. 54**:** 1781–1786.

Toker, C., 2009. A note on the evolution of kabuli chickpeas as shown by induced mutations in *Cicer reticulatum* Ladizinsky. Genet. Resour. and Crop Evol. 56: 7–12.

Toker, C., H. Canci, N.E. Inci, F.O. Ceylan, 2012a. Improvement in imidazolinone resistance in *Cicer* species by induced mutation. Plant Breeding 131: 535-539.

Toker, C., F.O. Ceylan, N.E. Inci, T. Yildirim, M.I. Cagirgan, 2012b. Inheritance of leaf shape in the cultivated chickpea (*Cicer arietinum* L.). Turkish J. of Field Crops 17: 16–18.

van Rheenen, H.A., R.P.S. Pundir, J.H. Miranda, 1994. Induction and inheritance of determinate growth habit in chickpea (*Cicer arietinum* L.). Euphytica 78: 137–141.

van der Maesen, L.J.G., N. Maxted, F. Javadi, S. Coles, A.M.R. Davies, 2007. Taxonomy of the genus *Cicer* revisited. In: Yadav, S.S., Redden, R., Chen, W., Sharma, B. (eds), Chickpea Breeding and Management. CAB Int. pp. 14–46.

Wani, A.A., 2011. Spectrum and frequency of macromutations induced in chickpea (*Cicer arietinum* L.). Turkish. J. of Biol. 35: 221–231.

Wani, A.A., M. Anis, 2008. Gamma ray- and ems-induced bold-seeded high-yielding mutants in chickpea (*Cicer arietinum* L.). Turkish J. of Biol. 32: 161–166.

Zohary, D., M. Hopf, 2000. Domestication of Plants in the Old World: the origin and spread of cultivated plants in West Africa, Europe, and the Nile Valley, 3. ed. Oxford: Clarendon Press, 316 p.