

INDUCTION AND INHERITANCE OF COMPOUND LEAF AND CONE STIPULE IN THE CULTIVATED CHICKPEA (*Cicer arietinum* L.)

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ABSTRACT

The cultivated chickpea (*Cicer arietinum* L.) has multipinnate, simple and normal leaves, caused by mutations. About 1500 air-dried seeds (500 seeds per dose) of chickpea genotype ICC 6119 were irradiated with 200, 300 and 400 Gy gamma rays. Two new induced characteristics, compound leaf and cone stipule on internodes, were isolated in a single mutant chickpea in the M₃ generation. Inheritance of the new characteristics was studied in the segregating populations of the M₄ and the results indicated that the new characteristics were governed by two recessive genes designed as *ccl* and *cscs* symbols. These characteristics could be linked since they were jointly segregated. The new genes identified may be useful for gene mapping of *Cicer* species.

Keywords: Chickpea, *Cicer arietinum*, mutagenesis, mutant, leaf types

INTRODUCTION

The cultivated chickpea (*Cicer arietinum* L.) is a diploid species with $2n = 16$ chromosomes. It is self-pollinated species due to its cleistogamic flowers (van der Maesen, 1972; Cubero, 1987). Rachis length of the cultivated chickpea is 25-75 mm ending a leaflet. Stipules are ovate to oblique-triangular incised with 2-4 teeth. Leaves are often pseudo-imparipinnate (Cubero, 1987) and typically bear 11-15 leaflets, with teeth covering nearly 2/3 of the foliar blade (van der Maesen, 1972). This shape of the leaves in the cultivated chickpea is referred to as normal leaf (Pundir et al., 1990). However, different leaf derivatives have been reported in the cultivated chickpea. Rao et al. (1980) illustrated four different leaf shapes; normal, bipinnate, simple and tiny leaves. Muehlbauer and Singh (1987) indicated five different leaves and leaflets; normal leaf, narrow leaflets, simple leaf, tinny leaflets and bipinnate leaf. After that, Toker and Cagirgan (2004) reported an additional different leaf shape, isolated from an induced mutant chickpea. Despite of these reports, Pundir et al. (1990) showed that the cultivated chickpea consisted of three major leaf shapes (e.g., normal, simple and multipinnate).

Normal leaf in the cultivated chickpea is referred to as imparipinnate, alternifolia, bunchy leaves, filicoid, and

narrow leaf. Multipinnate leaf is called tiny, fasciculifoliate, bipinnate and decomposed leaf. Simple leaf is known as chrysanthefolia leaf (Pundir et al., 1990). These leaves were reported to be under the control of genetic factors (Muehlbauer and Singh, 1987). Similarly, Toker et al. (2012b) reported genetics of three kinds of leaves in the cultivated chickpea. Chickpea with the normal leaf is predominantly grown in the world (van der Maesen, 1972; Cubero, 1987).

Variation in the leaves of the cultivated chickpea has been induced by spontaneous or induced mutations (Muehlbauer and Singh, 1987; Salimath et al., 2007; Toker et al., 2012b). Induced mutations are one of the most effective approaches to create variation and new genes in the chickpea (Yildirim et al., 2013). The purpose of the present study was to study the inheritance of a new leaf shape and stipule in the cultivated chickpea induced by gamma rays.

MATERIALS AND METHODS

About 1500 air-dried seeds (500 seeds per dose) of chickpea genotype ICC 6119 were irradiated with 200, 300 and 400 Gy of gamma rays from a ⁶⁰Co source in the Turkish Atomic Energy Agency (TAEK), Ankara, Turkey (Toker et al., 2005). Morphological characteristics of ICC 6119 as well as mutant were given in Table 1.

Table 1. Morphological characteristics of the parent chickpea genotype ICC 6119 and the mutants, ACC 3204, and ACC 3204-1 with compound leaf and cone stipule on internodes

Characteristics	ICC 6119	ACC 3204	ACC 3204-1
Selected generation	-	M ₂	M ₃
Flower color	Pink	White	White
Flower per peduncle	1	2	1
Flower shape	Cleistogamic	Cleistogamic or open	Open
Seed color	Brown	Cream	Cream
Pigmentation	Pigmented	Non-pigmented	Non-pigmented
Stipule shape	Small with 2-3 teeth	Small with 2-3 teeth	Giant with 2-6 teeth
Stipule in internodes	Absent	Absent	Cone-stipulated
Leaf shape	Multipinnate	Multipinnate	Compound
Petioles	Short	Short	Too long
Fe-deficiency	Fe-deficient	Fe-efficient	Fe-efficient

In 2005, the M₁ plants were grown at Antalya location (30° 38' E, 36° 53' N, 32 m from sea level) in the spring of 2005 and harvested individually in the beginning of summer. In 2006, the M₂ was grown as single plant progeny rows at the same location. In 2007, the selected mutants and their sibs were grown as the M₃ at the same location. Certain and additional mutant chickpeas were confirmed and selected in the M₃ generation, respectively. In 2008 and in 2009, the progenies of the mutant plants were grown in the separate rows as single plant progeny in the M₄ and the M₅ at the same location. The mutant 'ACC 3204-1' was confirmed in the M₄ and the M₅.

Genetic materials and generations were grown in plot with 45 cm row and 5 cm plant spacing. Materials were fertilized with N, P and K at the rate of 20 kg per ha prior to sowing. Weed control was done by hand during the seedling stage.

Chi-squares (χ^2) test was performed for goodness of fit of 15 multipinnate leaves to 1 compound leaf and cone stipule on internodes ratio in the segregating population in the M₄ generation using the formula:

$$\chi^2 = \sum (O-E)^2/E,$$

where O and E are observed and expected values, respectively (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The parent genotype 'ICC 6119' produced pigment on the aerial part of plants with pink flowers and brown

seeds, while the mutant selected in the M₂ generation (ACC 3204) was free of pigmentation with white flowers and cream seeds. The parent genotype 'ICC 6119' had single flower per peduncle, while 'ACC 3204' had double flowers per peduncle. The parent genotype 'ICC 6119' and the mutant 'ACC 3204' had multipinnate leaves. The mutant 'ACC 3204-1' selected from the mutant 'ACC 3204' in the M₃ had cone stipules, open flowers and compound leaves with long petioles (Figure 1). As seen in Table 1, the mutant 'ACC 3204-1' had considerably different characteristics from the parent genotype 'ICC 6119' and the mutant 'ACC 3204'. It had compound leaves with long petioles like the genus *Lupinus* L (Cowling, 1998a, 1998b). Also, it had stipules like cone on internodes (Figure 1). A joint segregation of the compound leaf and cone stipules in the mutant 'ACC 3204-1' indicated that these characteristics could be linked. Leaves of the mutant 'ACC 3204-1' were different from those described in following reports (Rao et al., 1980; Muehlbauer and Singh, 1987; Pundir et al., 1990; Toker et al., 2012b). According to our knowledge, stipules and leaves of the mutant 'ACC 3204-1' were the first report in the cultivated chickpea. The mutated characteristics in the mutant 'ACC 3204-1' proved that mutations could affect more than one locus. The similar and useful mutations in the progenitor (*C. reticulatum* Ladiz.) were also reported (Toker et al., 2012a).

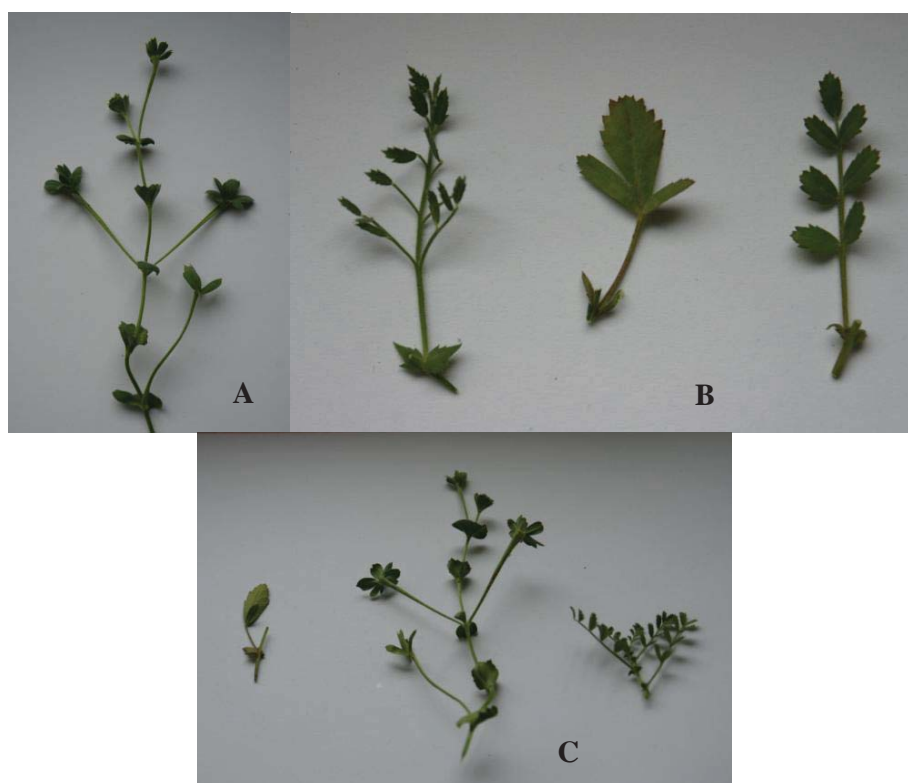


Figure 1. A: The mutant 'ACC 3204-1' with compound leaves with long petioles and cone stipules on internodes. B: Leaf derivatives in the cultivated chickpea (LtoR: multipinnate, simple and normal leaves. C: LtoR: simple, compound and normal leaves).

In the M_4 generation, the segregation ratio of the mutant 'ACC 3204' to the mutant 'ACC 3204-1' was found as 90 : 9 (Table 2). The chi-square test indicated that segregation in the M_4 generation fit well to the expected ratio of 15 : 1 (i.e. 15 multipinnate leaves : 1 compound leaf). The results showed that the compound leaf and cone stipule on internodes were governed by two recessive

genes, which show supplementary gene action. Pundir et al. (1990) studied inheritance of leaf shapes in the cultivated chickpea. Multipinnate, simple and normal leaves were occurred ml^+sl/sl , ml/ml and ml^+sl^+/\dots , respectively (Pundir et al., 1990). The gene symbols $clcl$ and $cscs$ were designed for compound leaves and cone stipules, respectively.

Table 2. Inheritance of compound leaf and cone stipule in an induced mutant chickpea

No. of filials	M_4		Expected	χ^2	P
	Observed	No. of filials			
Leaf and stipule type					
99	Multipinnate leaf and normal stipule	90	15 : 1	1.35	0.25-0.10
	Compound leaf and cone stipule	9			

In conclusions, two new genes were the first report of a discovery by using gamma rays. These genes will be useful for the gene mapping studies in *Cicer* species.

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