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PYRAMIDING OF THE RESISTANCE TO FE-DEFICIENCY CHLOROSIS AND LEAF MINER (*Liriomyza cicerina* ROND.) IN CHICKPEA (*Cicer arietinum* L.) BY MUTATION BREEDING

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ABSTRACT

Considerable yield losses due to iron (Fe) deficiency chlorosis in chickpea (*Cicer arietinum* L) may occur when susceptible genotypes are grown in calcareous soils with high pH. The most efficient practical and economical solution to overcome Fe-deficiency chlorosis in chickpea is through the utilization of genetic resistance. In this study, ICC 6119, which is leaf miner [*Liriomyza cicerina* Rond. (Diptera:Agromyzidae)] resistant but susceptible to Fe-deficiency chlorosis, was irradiated with 200, 300 and 400 Gy gamma rays. Mutated populations were evaluated for resistance to Fe-deficiency chlorosis and leaf miner using a visual scale from M_1 to M_5 generations. In the M_3 generation, one mutant was selected for resistance to Fe-deficiency chlorosis and leaf miner from a single seed descent (SSD) set. Active Fe and chlorophyll content in Fe-efficient mutants were found higher than in the parent genotype ICC 6119. The identified Fe-efficient and leaf miner resistant mutant may be useful in chickpea breeding programs to develop cultivars suitable for a niche environment.

Keywords: Chickpea, *Cicer arietinum*, iron deficiency, leaf miner, *Liriomyza cicerina*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is grown on nearly 12 million ha areas and produced about 11 million t, with average yield of 913 kg per ha, in the world. In Turkey, chickpea is grown on 446 218 ha areas and is produced 530 634 t with average yield of 1 189 kg per ha (FAOSTAT 2010). It is the first rank among food legumes and one of the most important crops in Turkey (Sepetoglu et al., 2008; Ozalkan et al., 2010; Cagirgan et al., 2011; Toker et al., 2012). It is divided into two groups as '*macrosperma*' or '*kabuli*' and '*microsperma*' or '*desi*' on the basis of plant characteristics (Toker et al., 2012a).

Abiotic stresses such as drought, low and high temperatures and nutrient deficiencies, and biotic stresses such as diseases, weeds and pests prevent obtaining high yield. Among abiotic stresses, nutrient imbalance is one of the most important stresses (Toker et al. 2007; Toker et al. 2010a). Globally, nitrogen (N), phosphorus (P) and micronutrients deficiencies in chickpea have been reported to cause yield losses of 709 000, 653 000 and about 360 000 t per year, respectively (Ryan 1997). Iron (Fe) deficiency in high pH and calcareous soils is a common problem in legume growing areas in some parts of the world (Saxena and Sheldrake, 1980; Srinivasarao et al. 2003). Fe-deficiency has been reported when susceptible genotypes of chickpea were grown on calcareous soils with high pH (Saxena et al. 1990; Bejiga et al. 1996). Yield reduction in chickpea due to Fe-deficiency was estimated to be 44 % in Syria and Lebanon (Saxena and Sheldrake, 1980) and about 24-50 % in India (Sakal et al. 1987). Fe is essential for the establishment of effective legume symbioses (Gupta and Gupta, 2005) and adequate nutrition of human population (Graham and Welch, 2000). Fe-deficiency initially appears as an interveinal chlorosis in younger leaves (Marschner 2003), since Fe cannot be readily mobilized from older to younger leaves (Gupta and Gupta, 2005). Leaflets of Fe-deficient plants of chickpea become yellow-green (Ahlawat et al., 2007). White or light-colored necrotic patches develop on the leaflets of young leaves when Fe-deficiency becomes more severe. Acute Fe-deficiency causes leaflets to wither and die (Toker et al., 2010a). Although soil application of 20 kg Fe per ha has been proposed (Srinivasarao et al., 2003), soil application is usually not feasible due to high soil pH (Ahlawat et al., 2007). Also, application of a foliar spray (1 % $FeSO_4$, 250 L ha^{-1}) solution was found to enhance chickpea yield (Ahlawat et al. 2007). However, applications may not be a viable long term solution since chickpea is usually grown in marginal areas (Toker et al., 2007). An alternative method would be to modify the genotypes with respect to Fe-efficiency by plant breeding.

Chickpea leaf miner [*Liriomyza cicerina* Rond. (Diptera:Agromyzidae)] is one of the common pests in Mediterranean region including Turkey (Reed et al. 1987; Cikman and Civelek, 2006). The female chickpea leaf miner lays up to six eggs on the basis of infestation level. After four days of incubation period, newly hatched larvae mine serpentine tunnels through the parenchyma resulting in a loss of photosynthetic capacity. Yield losses due to leaf miner in chickpea may reach up to 40 %. Although there are several recognized methods to control insect pests in chickpea (Reed et al., 1987), host plant resistance with cultural practices are preferred due to effective and economical reasons. Host plant resistance is also known as environmentally friendly practice. In this point of view, selection of Fe-efficient cultivars seems to be crucial for calcareous soil with high pH. The study was aimed to select Fe-efficient chickpea mutants from a leaf miner resistant source and to compare the mutants with check and parent for resistance to leaf miner and Fe-deficiency chlorosis.

MATERIALS AND METHODS

Genetic material

ICC 6119 is susceptible to Fe-deficiency (Toker et al., 2010b) and resistant to leaf miner due to its very small leaflets (Toker et al., 2010c). Air-dried seeds were irradiated with 200, 300 and 400 Gy of gamma rays from a ^{60}Co source in the Turkish Atomic Energy Agency (TAEK), Ankara, Turkey (Toker et al., 2005). Sierra, which is resistant to Fe-deficiency chlorosis (Toker et al., 2010b) and susceptible to leaf miner (Toker et al., 2010c), was used as check in comparison to mutants and their parent, ICC 6119. Details on the irradiated materials were given by Toker et al. (2012b).

Mutated generations and agronomic practices

Mutant generations from the M_1 to M_5 were grown at Antalya location (approximately 30^o 44’ E, 36^o 52’ N, 51 m from sea level) from 2005 to 2011, and the M_1 and the M_3 generations were grown at the same experimental area. The M_4 in 2008 and the M_5 in 2011 were evaluated for resistance to leaf miner and Fe-deficiency chlorosis. The M_5 generations was grown in a single row of 4 m length with 45 cm row and 5 cm plant spacing as screening nursery. A randomized complete block design with two replications was used. A fertilizer with N, P and K (20:20:20) were applied at a rate of 20 kg per ha prior to sowing. Experimental areas were cleaned from weeds by hand during seedling stage.

Screening for resistance to Fe-deficiency chlorosis

For screening the chickpea, Saxena et al. (1990) proposed a 1-9 visual scale, where l =Very Highly Resistant, plant free of any Fe-deficiency symptoms; 5 = Intermediate; 41-60 % leaflets and some plants yellow; and 9 = Very Highly Susceptible, all plants showing severe chlorosis with the youngest leaves completely bleached and necrotic (Bejiga et al., 1996). The screening was completed before the flowering period owing to transient of Fe-deficiency chlorosis in ICC 6119.

Assessment of active Fe and chlorophyll contents

Active Fe and chlorophyll contents were evaluated in the M_5 generation in 2011. Chickpea plant samples taken from the parent genotype ICC 6119 and its mutant ACC 3204 were representatively collected (Singh and Diwakar, 1995) and then were washed with distilled water. Plant samples were dried at 65^{o}C for 48 h and ground with a stainless steel mill. Active Fe determination was performed as described by Sonmez and Kaplan (2004). 10 ml of 1 N HCl was added to 1 g plant samples and was kept 24 hours; afterwards were filtered and then active Fe was measured with ICP-OES. Chlorophyll content was determined using a chlorophyll meter in all chickpea genotypes.

Screening for resistance to leaf miner

Plants were screened for resistance to leaf miner under natural insect infestation in the field during spring using a 1–9 scale, where 1 = Very Highly Resistant, free from any damage; 5 = Intermediate, mines present in 31 to 40 % of the leaflets, some defoliation in the lower half of plants; and 9 = Very Highly Susceptible, many mines in almost all the leaflets (90 %) and defoliation of greater than 31 % (Toker et al., 2010c).

Soil analyses

Soil had low organic matter (1.87 %) and total nitrogen (0.106 %) levels. Soil texture was loam with a pH of 7.96. Electrical conductivity and $CaCO_3$ were 0.93 dS m^{-1} and 26.5 % (Table 1.).

Table 1. Soil properties, macro and micro nutrients in the experimental area

pH	7.96	Exchangeable Na (me 100 g^{-1})	0.15
Electrical conductivity (dS m^{-1})	0.93	Exchangeable Ca (me 100 g^{-1})	37.71
CaCO3 (%)	26.5	Exchangeable Mg (me 100 g^{-1})	7.12
Organic matter (%)	1.87	Available Fe (mg kg^{-1})	3.56
Total N (%)	0.106	Available Zn (mg kg^{-1})	0.75
Available P (mg kg^{-1})	9.37	Available Mn (mg kg^{-1})	23.16
Exchangeable K (me 100 g^{-1})	0.61	Available Cu (mg kg^{-1})	1.37

Climatic conditions

Weather in the region was characteristically warm and temperature increased gradually during spring months. Rainfall was irregular and drastically reduced in spring months as a typical Mediterranean climate. Maximal temperatures were about 30 ^{o}C during the flowering period and 35 ^{o}C during the pod filling period.

Statistical analyses

Analysis of variation (ANOVA), means ± standard errors, correlations were performed using MINITAB 13.1 program (MINITAB, 2000).

RESULTS

Mutants

All plants were observed for all possible mutations from the M_1 to the M_3 generation. Population size in the M_2 generation was about 4 000 plants and 198 rows. Some chimeric structures and chlorophyll deficiency mutations were noticed in the M_1 and the M_2 generation, respectively. The chimeras selected in the M_1 were not confirmed in the M_2. Some morphologic mutations were found and isolated individually in the M_2 and the M_3 generations. Viable mutants in the M_3 with 200, 300 and 400 Gy treatments were confirmed as 7, 10 and 8 mutants, respectively. ACC 3305-1 and ACC 3305-2 had simple leaves, whereas ACC 3204 and ACC 3224 had multipinnate leaves with tinny leaflets (Table 2.).

Table 2. Qualitative characteristics in leaf miner resistant mutant, the parent genotype ICC 6119 (Leaf miner resistant) and check variety Sierra (Leaf miner susceptible)

Genotypes	Kabuli/Desi	Leaf shape	Flower color	Pod per peduncle
Sierra	Kabuli	Simple	White	Single
ICC 6119	Desi	Multipinnate	Pink	Single
ACC 3204	Kabuli	Multipinnate	White	Double
ACC 3224	Desi	Multipinnate	Pink	Single
ACC 3305-1	Kabuli	Simple	White	Single
ACC 3305-2	Desi	Simple	Pink	Single

Assessment of Fe-efficient mutants

In the M_3 generation, three Fe-efficient mutants were selected from a SSD set. These mutants, ACC 3204, ACC 3305-1 and ACC 3305-2 were free from Fe-deficiency chlorosis having the score 1, while the parent ICC 6119 and the mutant ACC 3224 were susceptible to Fe-deficiency chlorosis having the score 9 and 8 on the scale, respectively (Figure 1. and Figure 2.).

Figure 1. Fe-efficient and leaf miner resistant mutants (left side) selected from single seed decent set and Fe-deficiency in ICC 6119 (right side).

There was statistically significant differences (*$P < 0.05$*) among genotypes but interaction between genotypes and year was insignificant in the M_4 and the M_5 generations. Active Fe concentrations of Fe-deficiency parent ICC 6119 was 35.48 mg kg^{-1}, whereas the healthy mutant (ACC 3204) contained 40.03 mg kg^{-1}. Chlorophyll content of the selected mutants was found between 16 and 60, whereas it was found as 12.7 in the Fe-deficiency parent (Fig 2). Simple correlation analysis indicated that chlorophyll content was significantly related with resistance to Fe-deficiency chlorosis -0.970.

Assessment of leaf miner resistant mutants

The mutants, ACC 3305-1 and ACC 3305-2, had the score 7 and 8, respectively; whereas ACC 3204 and ACC 3224 had the score 3 on 1-9 scale. Check variety Sierra was susceptible to leaf miner having the score 9 on the scale, while the parent genotype ICC 6119 was resistant to leaf miner having the score 3 (Fig 2.).

Type of Fe-deficiency chlorosis

Fe-deficiency chlorosis in ICC 6119 and its mutant ACC 3224 were transient, the deficiency symptoms disappearing during reproductive growth.

DISCUSSION

Extreme maximum temperatures, recorded more than 30 and 35 ^{o}C in the flowering and the pod filling stages, respectively, caused the abortion of flowers and pods. Abortion of flowers and pods in chickpea were attributable to the negative impact of heat stress (Toker et al., 2007). Chickpea is especially susceptible to high

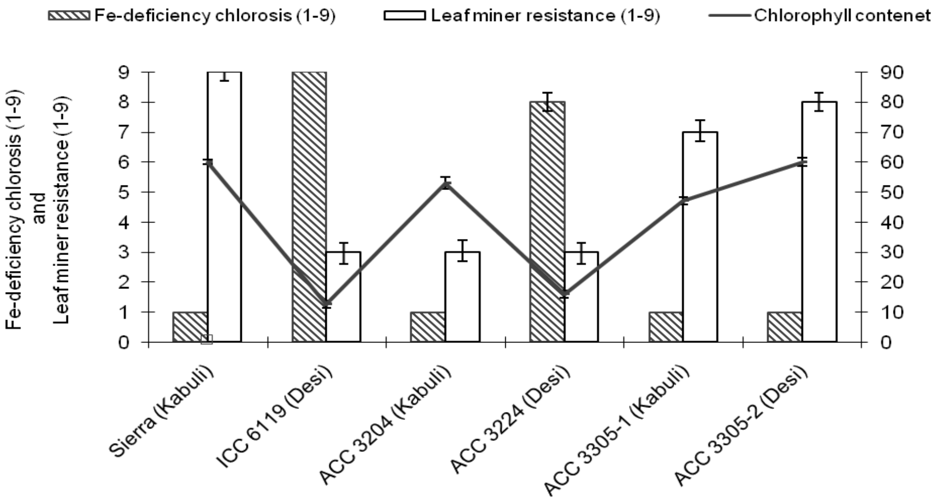

Figure 2. Comparison of Fe-deficiency chlorosis, leaf miner resistance and chlorophyll content in mutants, ICC 6119 (Leaf miner resistant) and Sierra (Leaf miner susceptible). Bars and line indicate means ± standard errors.

temperatures in the reproductive stages (Canci and Toker, 2009ab).

Chimeric mutations indicated that chimeras were due to physiologic damage, since they were not noticed in the later generations. Cagirgan (2009) observed 3 visible chimeric mutations in the M_1 generation of fast-neutron irradiated durum wheat cultivar (*Triticum durum* L.), Kunduru-1149. The frequency of chlorophyll mutations in M_2 corresponded to the occurrence of morphological mutants in M_2. This was in agreement with the results of previous studies in kabuli chickpea (Kharkwal 1999; Toker and Cagirgan, 2004). Maximum viable mutations were obtained with 300 Gy treatments.

Available Fe and Zn in experimental area (Table 1) were found to be at low levels (Lindsay and Norwell, 1978). To ensure elimination of environmental fluctuations, selection for resistance to Fe-deficiency chlorosis was initiated in the M_3 generation in spite of the fact that resistance is thought to be governed by a single dominant gene (Gowda and Rao, 1986; Saxena et al. 1990; Toker et al. 2010b). A negative selection to discard the susceptible lines from breeding program with hybridization was recommended as an effective strategy in chickpea (Saxena et al. 1990; Bejiga et al. 1996; Toker et al. 2010b). Gumber et al. (1997) induced Fe-deficiency chlorosis in chickpea via irrigation. The recovering ability of chickpea from Fe-deficiency chlorosis might be based on soil temperatures and day length since the trait was transient during the pod filling period. This result was in agreement with report in lentil (Erskine et al., 1993). The Fe-deficiency chlorosis in chickpea with Fe-inefficient cultivars may occur on calcareous soil. The most effective and economical way to overcome the problem is to modify the cultivar by plant breeding. In chickpea, hybridization (Gowda and Rao, 1986; Saxena et al. 1990; Toker et al. 2010b) and mutation breeding (Toker et al., 2011) can be preferred to improve cultivars. In sesame (*Sesamum indicum* L.) and wheat (*Triticum durum Desf.*), useful mutants were also reported by Toker et al. (2009) and Kusaksiz and Dere (2010), respectively.

Fe and chlorophyll contents are important for green leaves as vegetable (Yadav et al., 2007). The World Health Organization (WHO) has concerned on micronutrient deficiencies in human populations in many countries. Due to this reason, the mutant ACC 3204 with high level active Fe and chlorophyll content will be useful to overcome Fe deficiency in human nutrition.

According to our knowledge, the mutant ACC 3204, found to be resistant to leaf miner in the naturally contaminated environment, was isolated by mutation breeding for the first time. The mutant ACC 3204 with double and white flowers, and cream seeds was '*macrosperma*' chickpea selected from a '*microsperma*' chickpea (Table 2). It appears feasible to increase Fe content through breeding of new varieties.

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