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GERMPLASM

Registration of AWC 612M Chickpea Mutant Germplasm Line Resistant to Leaf Miner (*Liriomyza cicerina*)

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Abstract

Chickpea leaf miner (*Liriomyza cicerina* Rond.) (Diptera: Agromyzidae) is a distinctive, important insect pest of chickpea (*Cicer arietinum* L.) that can cause substantial yield losses if it is not adequately controlled. Host plant resistance to leaf miner is one of the best control options in sustainable farming. Since there are insufficient resistant sources in cultivated chickpea, an accession (AWC 612) of *C. reticulatum* Ladiz., crossable with cultivated chickpea, was subjected to 200, 300, and 400 Gy gamma rays to increase variation through mutating, and the mutant germplasm line AWC 612M (Reg. No. GP-305, PI 688421), with a multipinnate leaf type, was developed and selected in the M_3. AWC 612M was selected for its resistance against leaf miner under natural epidemic conditions in the field for 10 yr from 2007 to 2016. AWC 612M was free from damage, while the susceptible chickpea line ILC 3397 had numerous mines in >91% of leaflets and leaf drop >31%. AWC 612M, derived from *C. reticulatum*, which is compatible with the cultivated chickpea, represents a new source for breeding programs of resistance to leaf miner.

Chickpea leaf miner (*Liriomyza cicerina* Rond.) (Diptera: Agromyzidae) is a major insect pest affecting chickpea (*Cicer arietinum* L.) from the seedling through flowering and podding stages. The pest shows widespread distribution in western Asia, northern Africa, and Europe and causes up to 40% yield loss depending on severity of infestation, chickpea genotype, and planting time (Reed et al., 1987; Chen et al., 2011; Cikman et al., 2008).

Leaf miner damage is initiated by females during the feeding and oviposition processes, which can result in a stippled appearance of the leaflets. The major form of damage is the mining of leaflets by larvae, which results in destruction of leaf mesophyll. The pattern of mining is an irregular zigzag appearing with whitish stains. Both stippling and mining of leaflets can greatly depress the level of photosynthesis in the plant. Extensive mining also causes premature leaf drop, which results in lack of shade and sun scalding of pods. Wounding of the leaflets also allows entry of bacterial and fungal diseases. The infestation and damage process continues during the entire vegetative growth period (Reed et al., 1987; Chen et al., 2011).

Control of chickpea leaf miner generally depends on the use of chemical insecticides, but insecticide resistance is a major problem. In addition, efficacy of insecticides varies widely among leaf miner populations, and their susceptibility to insecticides is directly related to frequency of insecticide application. Use of many insecticides exacerbates problems by killing parasitoids of leaf miners as the parasitoids often provide effective suppression of the pest when disruptive insecticides are not used. Host plant resistance to manage insects is an effective, economical, and environmentally friendly method of pest control for sustainable farming systems. In this context, thousands of chickpea genotypes of the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) have been screened for resistance to leaf miner under natural invasive field conditions (Reed et al., 1987; Chen et al., 2011, Sharma et al., 2014). After screening 9500 genotypes from the

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Abbreviations: ICARDA, International Center for Agricultural Research in the Dry Areas; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics.

germplasm collection and breeder materials for resistance to leaf miner (Reed et al., 1987), three lines were determined to have resistance to leaf miner (Malhotra et al., 1996). Subsequently, seven lines were developed through hybridization of these lines (Malhotra et al., 2007). Separately, 200 accessions of annual wild *Cicer* species were screened; about 30 of these showed resistance, of which *C. judaicum*, *C. pinnatifidum*, and *C. cuneatum* performed well. Because *C. reticulatum* and *C. echinospermum* are readily crossable with the cultivated chickpea (Ladizinsky and Adler, 1976; Kazan et al., 1993; Singh and Ocampo, 1997; Singh et al., 2005; Koseoglu et al., 2017; Adak et al., 2017), accessions of these two wild *Cicer* species are more desirable over other annual species in interspecific hybridization studies (Talip et al., 2017). Leaf miner resistance was found in the *C. reticulatum* line designated AWC 612M (Reg. No. GP-305, PI 688421), selected after induced mutation (gamma rays) was defined. This germplasm line may be used to develop pest-resistant chickpea genotypes through interspecific hybridization.

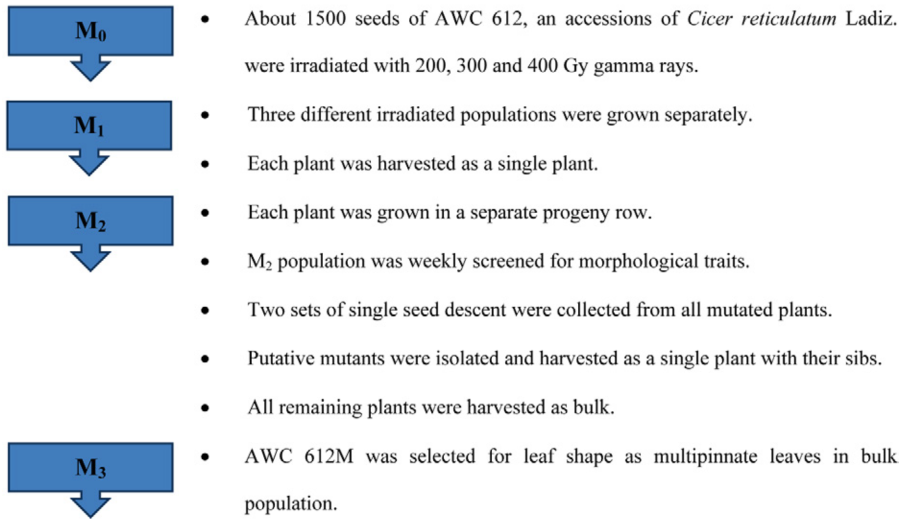

Fig. 1. Process for irradiation of seeds, growing and harvesting details of mutant plants, and selection of chickpea germplasm AWC 612M.

Methods

Source Material and Irradiation

For each dose, approximately 500 air-dried seeds of accession AWC 612 of *C. reticulatum* were irradiated with 200, 300, and 400 Gy gamma rays using a ^{60}Co source in the Turkish Atomic Energy Agency, Ankara, Turkey (Toker et al., 2005). Although most of the mutants were selected in M_2 for morphological traits, AWC 612M was selected in M_3 on the basis of leaf shape (Toker, 2014). The selection procedure leading to AWC 612M is detailed in Fig. 1. As shown in Fig. 2, AWC 612M has multipinnate leaves, indicating qualitative inheritance (Muehlbauer and Singh, 1987) was selected differently from the respective parent having a fern leaf type (Toker, 2014).

Measuring Leaf Miner Resistance

Of the 14 different accessions evaluated in the present study, AWC 612 and AWC 612M are of *C. reticulatum* and the remaining 12 (Sierra, CA 2969, ICC 6119, ICC 4951, FLIP 2005-1C, FLIP 2005-7C, LMR 60, LMR 154, ILC 3397, YAR, ACC 3305 and ACC 5305) are cultivated chickpea accessions. ACC 3305 and ACC 5305 were previously selected as mutant lines based on their morphological appearance after gamma ray treatment of their parental lines, ICC 6119 and ICC 4951, respectively (Ikten et al., 2015). Other lines, ICC 6119 and LMR 154, have multipinnate leaves, while AWC 612, ILC 3397, FLIP 2005-1C, FLIP 2005-7C, LMR 60, CA 2969, YAR, and ICC 4951 have fern, or normal, leaves. Sierra, ACC 5305, and ACC 3305 have simple leaves (Toker et al., 2012; Ikten et al., 2015). Improved lines (FLIP and LMR) from ICARDA were screened for resistance to leaf miner over 5 yr from 2011 to 2016, while mutants (ACC lines) were screened for 9 yr from 2007 to 2015.

The germplasm were evaluated for resistance against leaf miner under natural infestation conditions in the field using a visual 1-to-9 scale recommended by Singh and Weigand (1994) and modified by Toker et al. (2010), where 1 = very highly resistant and 9 = very highly susceptible. This scale is based on visual observations of the two main damages caused by leaf miner: (i) the extent of mines on chickpea leaflets and (ii) leaf drop rate. Ratings were completed three times, at the seedling, flowering, and mid-pod setting stages, with the highest visual scores among the three stages reported for each genotype (Table 1).

For leaf miner damage observations, all 14 accessions were repeatedly grown at Antalya, Turkey, for 10 yr from 2007 to 2016 under rainfed conditions. Trials were fertilized each year with 20 kg ha^{-1} N–P–K (20–20–20) before planting. All the accessions were sown by hand into 2-m-long rows using a row spacing of 50 cm and plant-to-plant spacing of 10 cm within a row. ILC 3397 and YAR germplasm were grown every 10 or 20 rows as a susceptible control. At the screening nurseries, the experiments were arranged as a randomized complete block design with two replications each year, with the exception of six replications in 2012.

Fig. 2. Leaf miner damage on simple leaf of Sierra chickpea (right) and no leaf miner damage on multipinnate leaf of AWC 612M chickpea (left).

Table 1. Visual rating scale to classify chickpea resistance to leaf miner.

Score	Reaction to leaf miner	Appearance of genotypes
1	Very highly resistant	Free from damage
2	Highly resistant	Few mines evident with careful observation
3	Resistant	Few mines in <20% of leaflets, no leaf drop
4	Moderately resistant	Mines present in 21–30% of leaflets, no leaf drop
5	Tolerant	Mines present in 31–40% of leaflets, some leaf drop in the lower half of plant
6	Moderately susceptible	Many mines in 41–50% of leaflets, leaf drop of 10% of the lower leaflets
7	Susceptible	Many mines in 51–70% of leaflets, leaf drop of 11–20% of the leaflets
8	Highly susceptible	Many mines in 71–90% of leaflets, leaf drop of 21–30% of the leaflets
9	Very highly susceptible	Many mines in >91% of leaflets and leaf drop >31%

Soil and Climate Conditions

The experimental area is in the Mediterranean climate belt, with dry summers and rainy winters. Rainfall is generally irregular in the spring. According to 90-yr climate data, some days exhibit subzero temperatures (to −4.6°C) between December and March. For the remaining months, low temperatures range from 0 to 14.8°C, while the highest temperatures were recorded as 44.8 and. 45.0°C in June and July, respectively.

Soil in the experimental field at a depth of 0 to 30 cm has low level of organic matter and N and is terrarosa-type soil with loam texture. Calcium carbonate was 26.5% (w/w), and pH was 7.69. Both Fe and Zn were found to be deficient due to the high pH.

Statistical Analyses

Visual scores obtained during the 10-yr cultivation period of germplasm were converted to means with standard errors. After subjecting the germplasm mean scores converted from numerical data to percentages for variance analysis, the differences among the germplasm were determined based on Duncan's multiple range test ($P \leq 0.05$) using the XLSTAT package program.

AWC 612M Resistance

Significant differences for resistance to leaf miner were found among the germplasm ($P \leq 0.05$). However, genotype × year interactions were not significant at $P \leq 0.05$ (data not shown). Leaf miner resistance scores for the germplasm from 2007 to 2016 are given in Fig. 3. The period of winter sowing is more suitable for leaf miner proliferation than alternative sowing times due to longer vegetative and generative stages. The susceptible controls, ILC 3397 and YAR, had scores of 9 and 8.5 against leaf miner damage, respectively, indicating that insect spread was adequate for monitoring damage. Among the germplasm tested, ACC 3305 (score 8.4) and Sierra (score 7.9) were highly susceptible, whereas CA 2969 (6.9 score), ICC 4961 (6.9 score), and ACC 5305 (6.5 score) were moderately susceptible. Lines developed earlier that had high leaf miner resistance had scores of 4 or less. FLIP 2005-7C and FLIP 2005-1C, previously developed from ICARDA materials, were moderately resistant and highly resistant, with scores of 4.1 and 2.4, respectively. Other resistance germplasm were LMR 60 (4) and LMR 154 (3.6). AWC 612, an accession of *C. reticulatum*, was resistant, with a score of 3. AWC 612M, which scored the lowest rate (1.1) among all the germplasm, was classified as very highly resistant. AWC 612M was free from leaf miner damage, while ILC 3397, one of the susceptible checks, had numerous mines in >91% of leaflets and leaf drop >31% (Fig. 3).

Characteristics

AWC 612M has multipinnate leaves, with leaflets per leaf counted up to 72, while its parent (AWC 612) had normal or fern-like leaves, up to 7 pairs per leaf. AWC 612M has purple flowers like its parent, but with darker pigmented leaves, leaflets, and stems especially under stress conditions than those of AWC 612. AWC 612M has a semi-spreading plant habit, with a plant height of 61 cm and plant canopy width of 65 cm when grown as a winter crop. One hundred seed weight of AWC 612M was 18. 9 g, with a reticulate seed shape and brown seed color. AWC 612M had the lowest visual leaf miner damage score of 1.1 among all the germplasm tested and was found to be very highly resistant to leaf miner (Fig. 2).

Although most leaf miner–resistant chickpea germplasm have a multipinnate leaf type, AWC 612M exhibited higher resistance against leaf miner than even the other multipinnate germplasm tested. *Cicer* species have exudates on green parts consisting of organic acids such as malic, oxalic, succinic, citric, and quinic acids (Khanna-Chopra and Sinha, 1987; Toker et al., 2004). Among these acids, oxalic acid was reported by Yoshida et al. (1995) to inhibit larvae of pod borer [*Helicoverpa armigera*

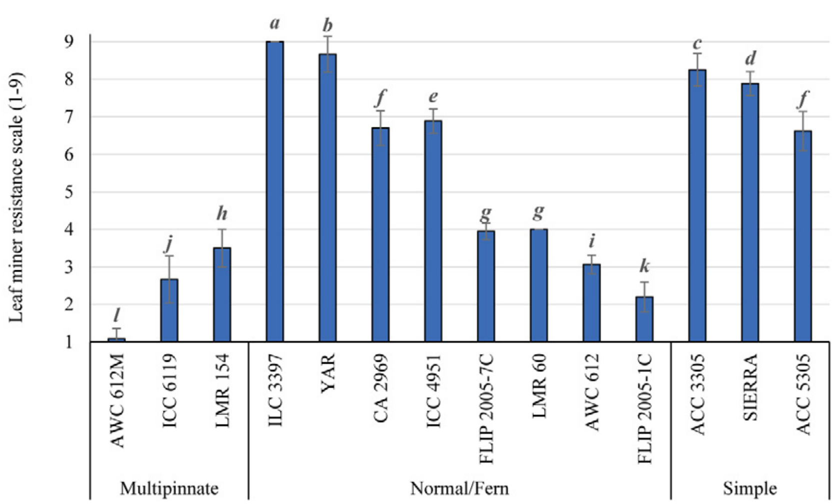

Fig. 3. Leaf miner resistance scores of chickpea lines based on leaf shapes. Bars indicate mean ± standard errors and Duncan's multiple range test. Each letter on the bars represents statistical different groups. 1–4 = resistant, 5 = tolerant, and 6–9 = susceptible on a visual rating scale.

(Hubner)] (Lepidoptera: Noctuidae). Resistance in AWC 612M may be dependent on high levels of organic acids, although we did not study this aspect. Resistance to insects has been previously described by Weigand and Pimbert (1993) as (i) nonpreference (ii) antibiosis, and (iii) tolerance; while Edwards and Singh (2006) described insect resistance mechanism in legumes as (i) structural defense, (ii) secondary metabolites, and (iii) antinutritional compounds. The resistance mechanism found in AWC 612M can be described as nonpreference or structural defense due to the multipinnate leaf shape and leaf structure. Also, AWC 612M has dense pigmentation on its stem and leaves. AWC 612M, derived from *C. reticulatum*, is an important germplasm source for leaf miner resistance because it can be crossed with cultivated chickpea.

Availability

One hundred seeds of AWC 612M will be provided upon request to plant breeders by ICRISAT. Seed may be requested from Hari D. Upadhyaya, head of gene bank, located in the ICRISAT. Seed of AWC 612M has also been deposited in the USDA–ARS National Plant Germplasm System, where it will be available 20 years after publication.

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