Susceptibility of Chickpea (*Cicer arietinum* L.) Cultivars to *Agrobacterium tumefaciens* (Smith and Townsend) Conn

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Abstract: Twelve chickpea cultivars grown in Turkey were assessed for susceptibility to wild-type *Agrobacterium tumefaciens* strains A281 and A136NC. Although all chickpea cultivars showed susceptibility to infection from both *A. tumefaciens* strains, Diyar-95, Flip 87-8, İspanyol, Gökçe, Damla-89, and Aziziye-94 had higher frequencies of tumor formation from both strains than the other cultivars tested.

Key Words: *Agrobacterium tumefaciens*, tumor formation, chickpea

Introduction

Genetic engineering techniques provide valuable tools for improving crop plants (1). Gene transfer to higher plants via engineered *Agrobacterium tumefaciens* strains is now a routine technology (2). *Agrobacterium tumefaciens* is the causative agent of crown gall disease in numerous crops (3). Tumor formation is mediated by the Ti plasmid found in the bacterial cell (4, 5). Two plasmid and one chromosomal components are required for plant cell transformation (6). A small portion of this plasmid, which is called T-DNA, integrates the nuclear DNA of plants (7). Artificial transformation vectors have been developed since T-DNA genes are not essential for gene transfer, and DNA inserted between border sequences may be transferred to plant genomes (8, 9). These engineered vectors are now used routinely to introduce
The chickpea is an important legume crop, providing protein for millions of people in the world, and is also very important in Turkey. Therefore, genetic engineering techniques to improve this crop will be of great value.

Before transformation work via *A. tumefaciens* can begin, successful elucidation of effective strains and suitable genotypes is necessary. In this study, 12 different chickpea cultivars grown widely in Turkey were assessed for susceptibility to 2 different strains of *Agrobacterium tumefaciens*.

### Materials and Methods

Seeds of the following chickpea cultivars İzmir-92, Gökçe, Menemen-92, Diyar-95, Akçin-91, Eser-87, Damla-89, Canitez-87, Flip 87-8, Aydın-92, Aziziye-94 and İspanyol were obtained from the Field Crops Central Research Institute, Ankara, Turkey. *Agrobacterium tumefaciens* strains A281 and A136NC were obtained from Leicester University, England. Six seeds of each chickpea cultivar were sown in pots containing soil. No fertilizer was used. After germination, they were thinned out to 4 plants/pot. Plants were maintained in a greenhouse with a 25+5°C and 15+5°C day/night temperature regime. Two weeks after planting, the chickpea cultivars were inoculated with the *Agrobacterium tumefaciens* strains A281 and A136NC.

For inoculation, strains were grown overnight and diluted to 1:50 in liquid MSO (MS mineral salts and vitamins, 3% sucrose, pH 5.6). Thereafter, each stem was stabbed four times using a syringe needle dipped in the appropriate bacterial solution. As a negative control, a needle was dipped in liquid MSO and stabbed into stems. The stems were then wrapped with cotton wool soaked in the inoculum for four days. Four plants were inoculated for each cultivar strain combination. Plants were observed daily. Five weeks after inoculation, tumors were counted and tumor diameters were measured.

### Results and Discussion

All cultivars tested developed tumors; however, the percentage of tumor formation and tumor size varied (Table 1). Tumor formation was not observed in non-inoculated control plants. With the cultivars İspanyol, Flip 87-8 and Diyar-95, a high tumor formation, irrespective of the bacterial strain used, was observed. The inoculation of *A. tumefaciens* strain A281 caused tumor formation in 50% or more of the plants of chickpea cultivars Gökçe, Diyar-95, Akçin-91, Damla-89, Flip 87-8, Aziziye-94 and İspanyol. In contrast, tumor formation was observed to be low in the cultivars İzmir-92, Menemen-92, Eser-87, Canitez-87 and Aydın-92. Most of the galls produced were 0.5-1 mm in diameter. Galls produced by the cultivars Menemen-92, Canitez-87 and İspanyol were greater than 1 mm in diameter (Figure 1). The inoculation of *A. tumefaciens* strain A136NC caused tumor formation in 50% or more of the plants of chickpea cultivars Gökçe, Diyar-95, Damla-89, Canitez-87, Flip 87-8, Aziziye-94 and İspanyol. Tumor formation in İzmir-92, Menemen-92, Akçin-91, Eser-87, and Aydın-92 was lower. Seven cultivars...
developed tumors that ranged from 0.5 to 1 mm. In most cultivars, tumor formation began in about 2 weeks; however, in İspanyol and Flip 87-8 inoculated with A. tumefaciens strain A136NC, tumor formation was visible after 1 week. Tumor formation with Diyar-95, Flip 87-8, İspanyol, Gökçe, Da MLA-89 and Aziziye-94 was high following inoculation with the both Agrobacterium strains.

Table 1. Response of chickpea cultivars 5 weeks after stem inoculation with Agrobacterium tumefaciens strains A281 and A136NC

| Chickpea cultivars | Tumor formation (%)  | All size**
|--------------------|----------------------|---------
|                    | A281 | A136NC | A281 | A136NC |
| İzmir-92           | 25   | 25     | ++   | ++     |
| Gökçe              | 50   | 56     | ++   | ++     |
| Menemen-92         | 25   | 13     | +++  | +      |
| Diyar-95           | 88   | 88     | ++   | ++     |
| Akçin-91           | 69   | 38     | ++   | +      |
| Eser-87            | 25   | 13     | ++   | ++     |
| Damla-89           | 50   | 88     | ++   | ++     |
| Canıtez-87         | 19   | 63     | +++  | ++     |
| Flip 87-8          | 81   | 81     | ++   | ++     |
| Aydın-92           | 31   | 38     | +    | +      |
| Aziziye-94         | 69   | 50     | ++   | +      |
| İspanyol           | 69   | 100    | +++  | ++     |

*Percentage of wound site on stems from 4 plants forming tumors. There were 4 wound sites per plant.
** +=<0.5 mm diameter, ++=0.5-1 mm diameter, +++=> 1 mm diameter

Although A. tumefaciens can be used routinely to transfer foreign genes into many crop species, perhaps the greatest weakness of this system is the host-range limitation. Therefore, the susceptibility of different chickpea cultivars grown widely in Turkey to wild-type A. tumefaciens was assessed in this study with the ultimate aim of obtaining transgenic chickpea plants. Cultivar and genotype response to A. tumefaciens strains may vary. Strain and genotype differences were reported previously in many seed legumes such as the soybean (10, 11), pea (12), chickpea (13) and lentil (14). The data in the present study also indicates that different chickpea cultivars have various levels of susceptibility to A. tumefaciens infection. The cultivars Diyar-95, Flip 87-8, İspanyol, Gökçe, Damla-89 and Aziziye-94 appeared to be the most susceptible to both strains used. Therefore, these cultivars can be used for the production of transgenic chickpea cultivars via disarmed A. tumefaciens strains.
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Figure 1. Tumor formation on chickpea cultivar Menemen-92 five weeks after inoculation with Agrobacterium tumefaciens strain A281.

References


