

Susceptibility of Different Bean (*Phaseolus vulgaris* L.) Cultivars to *Agrobacterium tumefaciens*

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Abstract : Although several direct gene transfer techniques have been developed, *Agrobacterium tumefaciens* is still preferred for the transfer of foreign genes into many plant species. Perhaps the greatest weakness of *A. tumefaciens* is the host range limitation. Therefore, in this study, the susceptibility of different bean cultivars to oncogenic *A. tumefaciens* strain A281 was assessed. The cultivars 4F-2409, Karacaşehir-90, Akman-98 and Eskişehir-855 resulted in higher frequencies of tumor formation than the other cultivars tested. Tumor formation was not observed in Afyon-3, IVD-10, Göynük-98, Yunus-90 or Şehirali-90 bean cultivars. Tumor formation was observed on both the hypocotyl and epicotyl portions of the plants.

Key Words: *Agrobacterium tumefaciens*, gene transfer, tumor formation, bean

Farklı Fasulye (*Phaseolus vulgaris* L.) Çeşitlerinin *Agrobacterium tumefaciens*'e karşı Duyarlılıklarının Belirlenmesi

Özet : Farklı doğrudan gen aktarım teknikleri geliştirilmesine karşın, *Agrobacterium tumefaciens* birçok bitki türüne yabancı genlerin aktarımında tercih edilmektedir. Belki de *A. tumefaciens*'in en büyük dezavantajı konukçusunun sınırlı olmasıdır. Bu yüzden, bu çalışmada farklı fasulye çeşitlerinin doğal tip *A. tumefaciens* streyni A281'e karşı duyarlılıkları belirlenmiştir. 4F-2409, Karacaşehir-90, Akman-98 ve Eskişehir 855 çeşitleri test edilen diğer çeşitlere oranla daha yüksek oranlarda ur oluşturmuşlardır. Afyon-3, IVD-10, Göynük-98, Yunus-90, ve Şehirali-90 çeşitlerinde ur oluşumu gözlenmemiştir. Bitkilerin hem hipokotil ve hem de epikotil kısımlarında ur oluşumu gözlenmiştir.

Anahtar Sözcükler: *Agrobacterium tumefaciens*, gen aktarımı, ur oluşumu, fasulye

Introduction

Seed legumes are extremely important crop plants, which are widely cultivated in the Middle East, Southern Asia and throughout the tropical and subtropical regions. Grain legumes have commonly twice and sometimes even three times the protein content of cereals (1). Among these, the bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*) provide a large proportion of the dietary protein requirement in many developing countries. With an annual production of around 17 million tons, the bean has also become one of the more important agricultural crop species, as an eminent protein source for human nutrition. In addition, *Phaseolus vulgaris*, as a member of *Leguminosae*, possesses the valuable property of enriching soil fertility by virtue of its capacity to fix atmospheric nitrogen.

Although conventional breeding programs have enhanced certain agronomic characters of the bean, further improvement of this crop generally includes such features as improved yield, protein quality and quantity, and insect and disease resistance. It seems to be extremely difficult to improve such characters by conventional breeding due to genetic linkage and sexual hybridization barriers. However, foreign genes coding for important traits can be efficiently introduced into many crop species via gene transfer techniques such as particle bombardment and *Agrobacterium tumefaciens* mediated indirect gene transfer. On the other hand, transformation of the bean by *A. tumefaciens* is relatively difficult due to host range limitation (2). Therefore, in the present study we assessed the susceptibility of 16 different bean cultivars grown in Turkey to oncogenic *A. tumefaciens* infection with the ultimate aim of introducing foreign genes into the bean genome using non-oncogenic *A. tumefaciens* strains.

Materials and Methods

Seeds of bean cultivars 4F-2409, Afyon-3, 4F-675/I, IVD-10, Karacaşehir-90, Önceler-98, Göynük-98, Yunus-90, IVD-7, Şehirali-90, 4F/1286, Akman-98, 4F-2714, 4F/89, Eskişehir 855 and IVD-9 were obtained from Muzaffer Işık, Anatolian Agricultural Research Institute, Eskişehir, Turkey. The *A. tumefaciens* strain A281 (pTiBo542: : pBI121.1) was obtained from Leicester University, England. PTiBo542 contains agropine synthase and oncogenes, whereas binary vector pBI121.1 harbors neomycin phosphotransferase II (NPT II) and *b*-glucuronidase (GUS) marker genes. Six seeds of each bean cultivar were sown in pots containing soil. After germination, they were thinned out to 4 plants/pot. The plants were maintained in a growth room with a 22±2°C and 15±2°C day/night temperature regime. Two weeks after planting, the bean cultivars were inoculated with the *A. tumefaciens* strain A281.

For inoculation, the bacterial strain was grown overnight and diluted to 1:50 in liquid MS (Murashige and Skoog) medium (3) containing 3% sucrose (MSO). Thereafter, the hypocotyl and epicotyl portions of each cultivar were stabbed separately 4 times using a syringe needle dipped in the bacterial solution. The bacterial concentration was 1×10^8 cells/ml, which was

determined by dilution plating. 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. The plants were observed daily. Eight weeks after inoculation, tumors were counted and tumor diameters were measured.

Results and Discussion

In previous studies, the oncogenic *A. tumefaciens* strain A281 appeared to be the most virulent strain for seed legumes (4-6) including the bean (2). Stem inoculation of young seedlings with bacteria has also been the primary technique used to assess the susceptibility of a plant genotype to *A. tumefaciens* in earlier studies (4-7). Therefore, in the present study, the epicotyl and hypocotyl portions of two-week-old plantlets were infected with A281 to determine the susceptibility of 16 different bean cultivars to oncogenic *A. tumefaciens*.

Small tumors were visible at the infection sites of some cultivars after two weeks of inoculation. In contrast, tumor formation was not observed in the cultivars Afyon-3, IVD-10, Göynük-98, Yunus-90 or Şehirali-90, or in non-inoculated control plants. With the cultivars Önceler-98, 4F-1286 and 4F-89, no tumor formation was observed on the hypocotyl portion of the plants (Table). In these cultivars, only limited tumor formation was observed following inoculation of the epicotyl portion of the plants. It appears that these cultivars are more resistant to *Agrobacterium* infection and may have problems via *A. tumefaciens*-mediated transformation.

The inoculation of *A. tumefaciens* strain A281 caused tumor formation in 50% or more of the plants of the bean cultivars 4F-2409, Karacaşehir-90, Akman-98 and Eskişehir-855. In contrast, tumor formation was found to be low in the cultivars 4F-675/I, IVD-7, 4F-2714, and IVD-9 (Table). Generally, tumors were formed on both the hypocotyl and epicotyl portions of these cultivars. Most of the tumors produced by the cultivars 4F-2409, Karacaşehir-90, Akman-98 and Eskişehir-855 were greater than 1 mm in diameter (Figure). These cultivars appear to be the most susceptible to *Agrobacterium* infection. Therefore, these cultivars can be used for the production of transgenic bean cultivars via disarmed *A. tumefaciens* strains.

Tumor formation provides clear evidence for the transfer of tumor-inducing genes located in T-DNA from *Agrobacterium* to plant cells (8). Therefore, screening for susceptibility of different plant genotypes to *Agrobacterium* at the tumor production level has been found to be an easy and reliable method in previous studies (4,5,9). Many researchers reported that tumor formation by *Agrobacterium* may vary not only in different species but also in different genotypes of the same species (4,8,10). According to Potrykus (10), plant genotypes differ in their wound response and only genotypes with a pronounced wound response develop larger populations of wound-adjacent competent cells for *Agrobacterium* infections. In accordance with the observations of many researchers, a large degree of variation among bean genotypes

| Bean cultivars | Tumor formation (%*) | | Gall size** | |
|----------------|----------------------|----------|-------------|----------|
| | Hypocotyl | Epicotyl | Hypocotyl | Epicotyl |
| 4F-2409 | 92 | 83 | +++ | ++ |
| Afyon-3 | - | - | - | - |
| 4F-675/I | 6 | 19 | +++ | ++ |
| IVD-10 | - | - | - | - |
| Karacaşehir-90 | 67 | 83 | +++ | +++ |
| Önceler-98 | - | 13 | - | ++ |
| Göynük-98 | - | - | - | - |
| Yunus-90 | - | - | - | - |
| IVD-7 | 25 | 38 | ++ | ++ |
| Şehirali-90 | - | - | - | - |
| 4F-1286 | - | 13 | - | ++ |
| Akman-98 | 63 | 75 | +++ | ++ |
| 4F-2714 | 13 | 13 | + | + |
| 4F-89 | - | 6 | - | ++ |
| Eskişehir-855 | 50 | 50 | +++ | +++ |
| IVD-9 | 42 | 25 | + | + |

Table

Response of bean cultivars 8 weeks after hypocotyl/epicotyl inoculation with *Agrobacterium tumefaciens* strain A281.

* Percentage of wound site on stems from 4 plants forming tumors. There were 4 wound sites on hypocotyl and epicotyl portions of the plants, separately.

**+ = < 0.5 mm diameter, ++ = 0.5-1 mm diameter, +++ = > 1 mm diameter

was observed in response to *Agrobacterium* infections in the present study. McClean et al. (2) screened the susceptibility of 19 dry bean genotypes to *A. tumefaciens* and they found variation of susceptibility expressed by the genotypes to infection by A281 (agropine catabolism), A208 (nopaline catabolism) and LBA 4001 (octopine catabolism). In their study, it was also revealed that the dry bean had a high level of tumor formation caused by the A281 strain, similar to the results obtained from our study. The large degree of variation was also observed among chickpea (9,11), soybean (8,12), pea (5) and lentil (6) genotypes. Considerable variation exists in plant cultivars to pathogens and virulence of pathogen races to different cultivars. Different kinds and probably different numbers of resistance genes may be responsible for the variation observed in host susceptibility (13).



Figure Tumor formation on epicotyls of bean cultivars 4F-2409 (left), Akman-98 (middle) and Karacaşehir-90 (right), eight weeks after inoculation with *Agrobacterium tumefaciens* strain A281.

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