Tr. J. of Botany
22 (1998) 313-316

Research Article

Susceptibility of Different Lentil (*Lens culinaris* Medik.) Cultivars to *Agrobacterium tumefaciens* (Smith & Townsend) Conn

Aziz KARAKAYA
University of Ankara, Faculty of Agriculture, Department of Plant Protection, Ankara-TURKEY

Sebahattin ÖZCAN
University of Ankara, Faculty of Agriculture, Department of Field Crops, Ankara-TURKEY

Received: 08.01.1998
Accepted: 05.06.1998

Abstract: *Agrobacterium tumefaciens* (Smith& Townsend) Conn is commonly used in gene transfer to plants. The effectiveness of bacterial strains and suitable plant genotypes facilitate this process. In this study, 8 lentil cultivars grown in Turkey were assessed for their susceptibility to two wild-type *A. tumefaciens* strains, A281 and A136NC. Both strains induced tumors in lentil cultivars, while differing in the ability to form tumors. Although most of the cultivars had some degree of susceptibility to *A. tumefaciens* infection, cultivars Yeşil 21 and Pul 11 had higher frequencies of tumor formation in both strains than the other cultivars tested.

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

Değişik Mercimek (*Lens culinaris* Medik.) Çeşitlerinin *Agrobacterium tumefaciens* (Smith & Townsend) Conn'e Hassasiyetlerinin Belirlenmesi

Özet: *Agrobacterium tumefaciens* (Smith & Townsend) Conn bitkilere gen aktarımında yaygın olarak kullanılmaktadır. Uygun bitki genotipleri ve bakteriyel suşların belirlenmesi bu işlemi kolaylaştırmaktadır. Bu çalışmada, Türkiye'de yetiştirilen 8 mercimek çeşidinin doğal tip *A. tumefaciens* suşları A281 ve A136NC'ye karşı hassasiyetleri belirlenmiştir. Her iki suş da kullanılan çeşitlerin çoğunda ur oluşturmuş olup, çeşitler arasında önemli farklılıklar gözlenmiştir. Çeşitlerin çoğu *A. tumefaciens* enfeksiyonlarına hassasiyet göstermelerine karşın, Yeşil 21 ve Pul 11 çeşitleri test edilen diğer çeşitlere oranla her iki bakteri suşuyla da daha yüksek oranlarda ur oluşturmuşlardır.

Anahtar Sözcükler: *Agrobacterium tumefaciens,* ur oluşumu, mercimek

Introduction

Agrobacterium tumefaciens (Smith & Townsend) Conn is a gram-negative soil-borne bacterium that causes crown gall disease in numerous crop species (1). Tumor formation is mediated by the Ti plasmid which is found in the bacterial cell (2, 3). Three components, two plasmid and one chromosomal, are required for plant cell transformation (4). A small portion of this plasmid, called T-DNA, integrates the nuclear DNA of plant cells (5). Artificial plasmid vectors have been developed since the T-DNA genes are not essential for gene transfer and DNA inserted between border sequences may be transferred to the plant genome (6, 7). These engineered vectors are now used routinely to introduce antisense, modifying, insect-, disease- and herbicide-resistance genes into many plant species.

The lentil is an important grain legume crop, providing protein for millions of people in the Mediterranean region, India, Pakistan and South America. Recently, this crop has also been used for reducing fallow areas in Turkey. The improvement of the lentil by genetic engineering techniques is of great importance. Before starting a transformation work via *A. tumefaciens,* successful elucidation of effective strains and suitable genotypes is necessary. In this study, 8 different lentil cultivars grown widely in Turkey were assessed in order to determine their susceptibility to two different wild-type strains of *Agrobacterium tumefaciens.*

Materials and Methods

Seeds of the following lentil cultivars were obtained from the Field Crops Central Research Institute, Ankara, Turkey: Yeşil 21, Malazgirt, Yerli Kırmızı, Sultan, Kırmızı 51, Kayı 91, Pul 11 and Erzurum 89. *Agrobacterium tumefaciens* strains A281 and A136NC were obtained

from Leicester University, England. Eight seeds of each lentil cultivar were sown in pots. After germination, they were thinned out to 4 plants/pot. Plants were maintained in the greenhouse with a 25±5°C and 15±5°C day/night temperature regime. One month after planting, the lentil cultivars were inoculated with *A. tumefaciens* strains A281 and A136NC. For inoculation, strains were grown overnight at 28°C and diluted to 1:50 ($1x10^8$ cells/ml) in liquid MSO (MS mineral salts and vitamins, 3% sucrose, pH 5.6). Thereafter, stems were wounded by stabbing four times at different locations using a syringe needle dipped in the 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. Six weeks after inoculation, the tumors were counted and their diameters were measured. Results were subjected to analysis of variance and means were separated by Duncan's Multiple Range Test (8). Data given in percentages were subjected to arcsin transformation before statistical analysis.

Results and Discussion

Tumor formation started about two weeks after inoculation and was scored after six weeks (Figure 1). No tumor formation was observed in non-inoculated control plants. With respect to the *A. tumefaciens* strains used, the percentage of tumor formation induced by A136NC appeared to be higher than that induced by A281 (Table 1). Most of the cultivars tested showed a high percentage of tumors with strain A136NC, whereas cultivar Malazgirt had very low tumor formation. A higher variation was observed in tumor formation with *A. tumefaciens* strain A281, which produced a higher percentage of tumor formation in cultivars Yeşil 21, Yerli Kırmızı, Kırmızı 51, Kayı 91 and Pul 11, than in the other 3 cultivars tested. No tumor formation was observed in the cultivar Malazgirt. Analysis of variance for tumor diameter revealed that strain and strain and lentil interaction were not significant (P>0.01). Although, most cultivars developed large tumors, tumor diameters measured in the cultivars Sultan, Malazgirt and Erzurum 89 were lower than in the other cultivars tested (Table 2). These results clearly indicate that these cultivars are more resistant to *Agrobacterium* infection and may have problems via *A. tumefaciens*-mediated transformation.

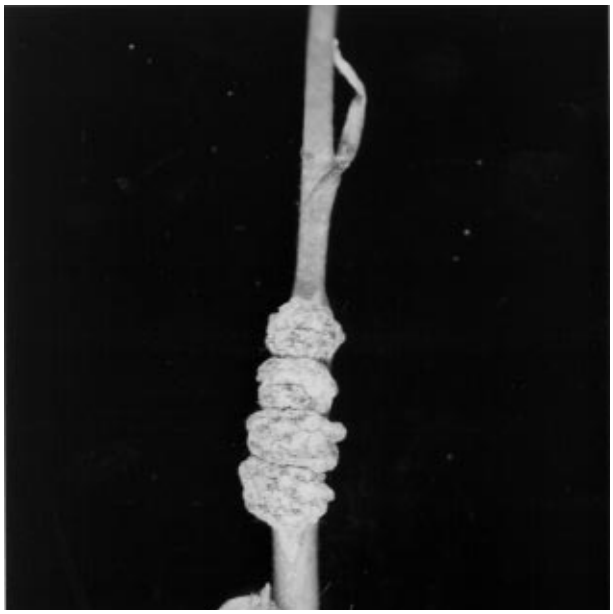

Figure 1. Tumor formation on lentil cultivar Pul 11 six weeks after inoculation with *Agrobacterium tumefaciens* strain A281.

Table 1. *In vivo* tumor formation on lentil stems infected with *Agrobacterium tumefaciens* strains A281 and A136NC six weeks after inoculation

Cultivars	A281		A136NC	
	Arcsin√P	%	Arcsin√P	%
Yeşil 21	0.32[1] A	100	0.30 A	88
Malazgirt	0.00 D	0	0.07 B	19
Yerli Kırmızı	0.19 AB	38	0.32 A	100
Sultan	0.04 CD	6	0.32 A	100
Kırmızı 51	0.31 A	94	0.24 A	75
Kayı 91	0.28 AB	81	0.27 A	75
Pul 11	0.31 A	94	0.32 A	100
Erzurum 89	0.17 BC	38	0.32 A	100

[1] Arcsin√Percentage of wound site on stems from 4 plants forming tumors. There were four wound sites for each plant. Means followed by different letters in the same column are statistically significant (P<0.01) according to Duncan's Multiple Range Test.

Lentil cultivars	Tumor diameter (mm) ± SE		
	A281	A136NC	Average
Yeşil 21	2.36 ± 0.16	2.15 ± 0.37	2.26* A
Malazgirt	0.00 ± 0.00	0.05 ± 0.05	0.03 C
Yerli Kırmızı	1.21 ± 0.49	2.52 ± 0.54	1.87 AB
Sultan	0.30 ± 0.30	0.38 ± 0.13	0.34 C
Kırmızı 51	1.49 ± 0.15	1.82 ± 0.74	1.66 AB
Kayı 91	2.24 ± 0.55	1.08 ± 0.28	1.66 AB
Pul 11	2.44 ± 0.76	1.66 ± 0.22	2.05 AB
Erzurum 89	0.69 ± 0.34	1.19 ± 0.36	0.94 BC

Table 2. Diameter of tumors formed 6 weeks after stem inoculation of lentil cultivars with *Agrobacterium tumefaciens* strains A281 and A136NC. Since only lentil cultivars were significant ($P<0.01$) in the analysis of variance, average values were also given. Numbers are the mean of 4 replications.

* Means followed by the different letters are significantly different ($P<0.01$) according to Duncan's Multiple Range Test

Although *A. tumefaciens* can be used rountinely 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 lentil cultivars grown widely in Turkey to wild-type *A. tumefaciens* was assessed in this paper with the ultimate aim of obtaining transgenic lentil plants. Cultivar and genotype response can vary from strain to strain. Strain and genotype differences have been reported previously in many seed legumes such as the soybean (9, 10), pea (11), chickpea (12) and lentil (13). The data in this study also indicate that different lentil cultivars showed various susceptibility levels to *A. tumefaciens* infection. The cultivars Yeşil 21, Yerli Kırmızı, Kırmızı 51, Kayı 91 and Pul 11 appeared to be the cultivars most susceptible to both strains used. Therefore, these cultivars can be used for the production of transgenic lentil cultivars via disarmed *A. tumefaciens* strains.

Acknowledgements

The authors thank Dr. E. Başpınar for his help on the statistical analysis and Ayşegül Gürbüz for providing the lentil seeds used in the study.

References

1. Agrios, G., Plant Pathology, 3rd. Ed. Academic Press. 803 pp. (1987).
2. Zaenen, I., Van Larebeke, N., Teuchy, H., Van Montagu, M. and Schell, J., Supercoiled circular DNA in crown gall inducing Agrobacterium strains. J. Mol. Biol. 86, 109-127, (1974).
3. Watson, B., Currier, T.C., Gordon, M.P., Chilton, M.D., and Nester, E.W., Plasmid required for virulence of Agrobacterium tumefaciens J. Bacteriol. 123, 255-264, (1975).
4. Citovsky, V., McLean, B.G., Greene, E., Horward, E., Kuldau, G., Thornstenson, Y., Zupan, J., Zambryski, P., Agrobacterium - plant cell interaction: induction of vir genes and T-DNA transfer. Molecular Signals in Plant-Microbe Communication. Verma, D.P.S., (Ed.) London, CRC Press. 170-199, (1992).
5. Chilton, M.D., Saiki, R.K., Yadav, N., Gordon, M.P., and Quetier, F., T-DNA from Agrobacterium Ti plasmid is the nuclear fraction of crown gall tumor cells. Proc. Natl. Acad. Sci. USA. 77, 4060-4064, (1980).
6. Leemans, J., Langenakens, J., De Greve, H., Deblaere, R., Van Montagu, M., and Schell, J., Broad host range cloning vectors derived from the W-plasmid Sa. Gene, 19, 361-364, (1982).
7. Schell, J. and Van Montagu, M., The plasmids as natural and practical gene vectors for plants. Bio/Technology, 1, 75, (1983).
8. Steel, R.G.D. and Torrie, J.H., Principles and Procedures of Statistics A Biometrical Approach, 2nd Ed. McGraw-Hill Book Company. 633 pp. (1980).
9. Owens, L.D., and Cress, D.E., Genotypic variability of soybean response to Agrobacterium strains harboring the Ti or Ri plasmids. Plant Physiol., 77, 87-94, (1985).
10. Bryne, M.C., McDonnell, R.E., Wright, M.S., and Carnes, M.G., Strain and cultivar specificity in the Agrobacterium-soybean interaction. Plant Cell, Tissue and Organ Culture, 8, 3-15, (1987).

11. Özcan, S., Assessment of the susceptibilty of different pea (Pisum sativum L.) genotypes to Agrobacterium tumefaciens. Tr. J. of Botany, 19, 417-422, (1995).

12. Islam, R., Malik, T., Husnain, T., Riazuddin, S., Strain and cultivar specificity in the Agrobacterium-chickpea interaction. Plant Cell Reports, 13, 561-563, (1994).

13. Warkentin, T.D., and McHughen, A., Crown gall transformation of lentil (Lens culinaris Medik.) with virulent strains of Agrobacterium tumefaciens. Plant Cell Reports, 10, 489-493, (1991).