

Susceptibility of some Brazilian soybean genotypes to three strains of *Agrobacterium tumefaciens*

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ABSTRACT

Twenty-six soybean (*Glycine max* (L.) Merrill) genotypes were screened for susceptibility to three strains of *Agrobacterium tumefaciens*. The genotype Peking, the most susceptible cultivar known, was used as the control. The strains of *A. tumefaciens* used included: C-58 (nopaline), Bo-542 (agropine), and Ach-5 (octopine). The cultivars were evaluated in the greenhouse in a randomized complete block design, with two replications. The genotypes IAC-5, IAC-14, and Peking were the most susceptible to strain C-58; IAC-4, IAC-10, and Peking were susceptible to strain Ach-5; and IAC-5, IAC-7 and IAC-12 were the most susceptible to strain Bo-542, with susceptibility greater than for Peking. Strong and significant interaction between genotypes and strains of *A. tumefaciens* was also observed.

INTRODUCTION

De Cleene and De Ley (1976) reported that soybean (*Glycine max* (L.) Merrill) was not a host for *Agrobacterium tumefaciens*. Later studies, however, have shown that tumors form on soybean in response to infection with *A. tumefaciens* (Matthysse and Gurlitz, 1982; Pendersen *et al.*, 1983; Hood *et al.*, 1984; Owens and Cress, 1985; Wyndaele *et al.*, 1985). Owens and Cress (1985), Byrne *et al.* (1987), Delzer *et al.* (1990), and Mauro *et al.* (1992) reported differences among soybean genotypes regarding their response to infection by *A. tumefaciens*. Peking was the genotype found most susceptible to *A. tumefaciens* and Byrne *et al.* (1987) reported a soybean genotype x *A. tumefaciens* strain interaction. Mauro *et al.* (1992) and Bailey *et al.* (1994)

studied the inheritance of susceptibility of soybean genotypes to *A. tumefaciens*, and both studies concluded that the susceptibility of soybean to *A. tumefaciens* is a quantitative trait, probably related to two or more genes.

Agrobacterium mediated gene transfer is routine in dicotyledon species in many laboratories. There are protocols for different species, including soybeans. Weising *et al.* (1988), Uchimiya *et al.* (1989), and van Wordragen and Dons (1992) obtained transgenic plants by vector-mediated transformation in *Glycine max* (L.) Merrill, *Pisum sativum*, *Vitis vinifera*, *Gossypium hirsutum* and others.

There are few studies on susceptibility of Brazilian soybean genotypes to *A. tumefaciens*. Knowledge of suitable soybean genotypes for *Agrobacterium* mediated transformation would be a powerful tool for the improvement of soybean in Brazil, the second biggest world producer of soybeans. The first step for applying these transformation techniques is to determine the genotypes capable of

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transformation. The main objective of this study was to verify the susceptibility of some Brazilian soybean genotypes to three strains of *A. tumefaciens*.

MATERIAL AND METHODS

Twenty six soybean genotypes, including 25 Brazilian (IAC-1, IAC-2, IAC-3, IAC-4, IAC-5, IAC-6, IAC-7, IAC-8, IAC-9, IAC-10, IAC-11, IAC-12, IAC-13, IAC-14, IAC-15, IAC-16, IAC-17, IAC-18, IAC-19, IAC-31, FT-Cometa, BR-15, Cristalina, Savana and Viçoja) and one susceptible control (Peking), were selected for this study. Soybean is an autogamous species, consequently genetic variability among plants within cultivars is not expected, meaning that few plants of each cultivar can be used to study the susceptibility to *A. tumefaciens*. Studies conducted by Mauro et al. (1994) also suggested that there are no differences among inoculators in the number of galls resulting from inoculations. Three *A. tumefaciens* strains C-58 (nopaline), Bo-542 (agropine), and Ach-5 (octopine) were used to induce tumors on the soybean plants. The experimental plots were pots containing three plants of each genotype. The experimental design was a randomized complete block with two replications, and the pots containing three plants of each genotype (one pot per strain of *A. tumefaciens*) were randomized in each replication. A 3:1 mixture (soil:sand) was used as substrate.

Bacterial inoculum was prepared by growing the strains in solidified LB media (10 g Trypticase + 10 g NaCl + 5 g Yeast extract + 15 g Bacto-Agar, pH = 7.5, per liter of solution) in Petri dishes in a growth chamber, at 28°C for four days. To prepare the inoculum, a barely visible amount of each strain was taken with a sterile loop and put in sterilized 250 ml flasks, each containing 25 ml of LB liquid media and properly identified by the code of the strain. The flasks were put on a rotary shaker for 24 hours at 200 rpm, at 28°C, for bacterial growth. When the strains reached the log phase ("od" value ranging from 700 to 800, at 600 nm), the content of each flask was poured into sterile centrifuge tubes containing the bacterial solution were centrifuged at 10,000 rpm for 10 minutes at 4°C (12,062 g). The supernatant was discarded and 15 ml of the liquid media MSO (Murashige and Skoog, 1962), which was used as vehicle, was added, and the pellet was resuspended. The tubes were left at room temperature approximately (26°C) for one hour. Studies conducted by Mauro et al. (in press) showed that better results in terms of pathogen virulence and gall formation were obtained one hour after the preparation of the inoculum.

The inoculations were performed when the plants reached the V₈ growth stage (Fehr and Caviness, 1977). The three first internodes of each genotype were inoculated four times, 1 cm apart, on each internode, with a total of 12 inoculation sites per plant. A disposable syringe and needle were used for the inoculations. The needle was used to wound the stem across the cortex. A drop of the inoculum was applied through the wound across the stem with a slight pressure on the needle. Four to six weeks after inoculation the plants of each genotype were scored by counting number of galls larger than 0.5 cm in diameter, per genotype per replication. Data were transformed to $\sqrt{x + 0.5}$ and submitted to analysis of variance, according to procedures suggested by Snedecor and Cochran (1989). The sources of variation among genotypes and the interaction of genotypes by strains were pooled into effects among genotypes within each strain of *A. tumefaciens* to evaluate the significance or not of the interaction among genotypes within each strain.

RESULTS

Table I contains the analysis of variance for the number of galls observed in each genotype screened for susceptibility to three strains of *A. tumefaciens*. There were significant differences among the genotypes

Table I - Analysis of variance for the number of galls observed in each genotype screened for susceptibility to three strains of *Agrobacterium tumefaciens*.

Source	D.F.	S.S.	M.S.	F
Rep./Strains	3	0.6997	0.2332	-
Genotypes	25	90.0558	3.6062	2.6520**
Strains	2	7.5941	3.7971	2.7957 ^{ns}
Gen. x Str.	50	67.9122	1.3582	18.3560**
Gen./Str.1	25	44.2200	1.7688	23.9027**
Gen./Str.2	25	48.4100	1.9364	26.1676**
Gen./Str.3	25	65.6100	2.6244	35.4649**
Error	75	5.5497	0.0740	
C.V.% = 14.05				Mean = 1.9363

ns - Not significant.

** - Significant at the 1% level of probability.

Rep. = Replications.

Gen. = Genotypes.

Str. = Strains.

evaluated, but no differences were observed in the number of galls produced by the three strains. As previously reported by Byrne *et al.* (1987), an interaction between genotypes and *A. tumefaciens* strains was observed ($P < 0.01$).

Table II contains the means of galls, transformed to square root of $x + 0.5$, and the tumor index (TI) per genotype per strain of *A. tumefaciens*. The tumor index (TI) expresses the percentage of galls in relation to the total number of inoculations. Peking was more susceptible to strains C-58 and Ach-5 according to the Scott-Knott test, but the genotypes IAC-5 and IAC-14 were as susceptible as Peking to the strain C-58. These three genotypes showed a tumor index of 30. For strain Ach-5, the most susceptible genotypes were Peking, IAC-4, and IAC-10, which also showed a tumor index of 30.

The genotypes IAC-5, IAC-7, and IAC-12 were more susceptible to strain Bo-542 of *A. tumefaciens* than Peking (Table II). None of the 26 genotypes was susceptible to all three strains of *A. tumefaciens*. IAC-17 and IAC-13 were the only genotypes resistant to all three strains, while BR-15 and Viçoja were only susceptible to strain Ach-5.

DISCUSSION

The results show that there is a strong soybean genotype by strain of *Agrobacterium* interaction. These genotypic differences in gall formation do not necessarily represent the frequency of T-DNA expression in the host tissue (van Wordragen *et al.*, 1992). Bailey *et al.* (1994) discussed the tumorigenesis

Table II - Mean galls ($\sqrt{x + 0.5}$) and tumor index (TI) for each soybean genotype and strain of *Agrobacterium tumefaciens*.

Soybean genotype	<i>Agrobacterium tumefaciens</i> strain means* and Tumor index (TI)					
	C-58	TI	Ach-5	TI	Bo-542	TI
01. IAC-1	2.11B	11.11	2.34C	13.89	2.99B	23.61
02. IAC-2	0.97D	0.11	2.72B	19.44	2.99B	26.39
03. IAC-3	2.24B	12.50	2.24C	12.50	2.83C	20.83
04. IAC-4	0.71D	0.00	3.32A	29.17	3.38B	30.56
05. IAC-5	3.45A	31.94	2.65B	18.06	3.87A	40.28
06. IAC-6	1.15C	2.78	2.24C	12.50	2.00D	9.72
07. IAC-7	0.71D	0.00	2.65B	18.06	3.94A	41.67
08. IAC-8	1.55C	5.56	0.97E	1.39	2.64C	18.06
09. IAC-9	1.55C	5.56	0.97E	1.39	2.34C	13.89
10. IAC-10	2.45B	15.28	3.53A	33.33	3.16B	26.39
11. IAC-11	2.55B	16.67	1.73D	6.94	1.85D	8.33
12. IAC-12	1.73C	6.94	1.73D	6.94	4.42A	52.78
13. IAC-13	0.71D	0.00	0.71E	0.00	0.97E	1.39
14. IAC-14	3.29A	27.78	1.73D	6.94	3.32B	29.17
15. IAC-15	0.97D	1.39	0.71E	0.00	1.55E	5.56
16. IAC-16	2.45B	15.28	0.97E	1.39	1.55E	5.56
17. IAC-17	0.71D	0.00	0.71E	0.00	0.71F	0.00
18. IAC-18	2.00B	9.72	0.97E	1.39	1.40E	4.17
19. IAC-19	0.97D	1.39	0.97E	1.39	2.45C	15.28
20. IAC-31	1.29C	4.17	0.71E	0.00	0.71F	0.00
21. FT-Cometa	2.00B	9.72	2.45C	15.28	0.71F	0.00
22. BR-15	0.71D	0.00	2.83B	20.83	0.71F	0.00
23. Cristalina	2.65B	16.06	0.71E	0.00	2.00D	9.72
24. Savana	0.71D	0.00	2.65B	18.06	1.40E	4.17
25. Viçoja	0.71D	0.00	2.00C	9.72	0.71F	0.00
26. Peking	3.77A	38.89	3.93A	41.67	3.39B	30.56

* - The means in the same column followed by the same letter did not differ by the Scott-Knott test at the 5% probability level.

phenomenon in soybean and concluded that in some cases transformation may occur even without gall formation.

Before studying the effectiveness of transformation or the expression of the T-DNA, it is necessary to know if the tissue or plant is visibly susceptible to a specific strain of *A. tumefaciens* by increasing the T-DNA expression, through the use of specific enzymes or other means. The interactions among soybean genotypes and strains of *A. tumefaciens* may involve many factors, and may constitute a complex system.

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RESUMO

Vinte e seis genótipos de soja foram avaliados quanto a suscetibilidade a três linhagens de *Agrobacterium tumefaciens*, a saber: C-58 (nopalina), Bo-542 (Agropina) e Ach-5 (octopina). Como padrão de suscetibilidade foi empregado o genótipo Peking, considerado altamente suscetível ao agente transformante. O delineamento experimental empregado foi o em blocos ao acaso com duas repetições. Os resultados obtidos evidenciaram que existem diferenças genotípicas no que concerne à suscetibilidade a *Agrobacterium*. IAC-5, IAC-14 e Peking revelaram-se mais suscetíveis à linhagem C-58; IAC-4, IAC-10 e Peking foram as mais suscetíveis à linhagem Ach-5, enquanto que os genótipos IAC-5, IAC-7 e IAC-12 evidenciaram maior suscetibilidade à linhagem Bo-542 que o padrão Peking. Também foi observada interação altamente significativa entre os genótipos e as linhagens de *A. tumefaciens* estudadas.

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