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Short Communication

Interactions in the *Agrobacterium*-soybean system and capability of some Brazilian soybean cultivars to produce somatic embryos

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Abstract

Twenty-five Brazilian soybean cultivars were studied for susceptibility to four strains of *Agrobacterium tumefaciens* (C58, Ach5, Bo542 and A281) and for their ability to produce somatic embryos. Twelve plants of each cultivar were inoculated in a greenhouse at 4-6 weeks of age, using 12 inoculation sites per plant. The number of galls formed on plants were counted 8-10 weeks after inoculation. To study ability to produce somatic embryos, immature cotyledons, 4-6 mm in length, were plated onto N10 medium for induction of somatic embryogenesis, using four Petri dishes with 20 cotyledons for each cultivar. The embryogenic tissues were transferred onto new N10 medium six times at 15-day intervals and the number of somatic embryos per cultivar determined. Significant interaction between soybean cultivars and *A. tumefaciens* strains was observed; the most virulent strain was A281. The opine type apparently had no effect on strain virulence, and the most embryogenic cultivars were IAS-5, Cristalina, FT-Cometa, IAC-7 and OC-3.

INTRODUCTION

Soybean (G*lycine max* L. Merrill) cultivars differ in susceptibility to *Agrobacterium tumefaciens* strains (Byrne *et al.*, 1987; Delzer *et al.*, 1990; Bailey *et al.*, 1994; Mauro *et al.*, 1995). Although no relationship exists between a given soybean cultivar susceptibility and its somatic embryo susceptibility to *A. tumefaciens*, information about cultivar susceptibility to *A. tumefaciens* strains may be useful in studies related to specificity between soybean cultivars and *A. tumefaciens* strains.

Several studies have shown differences among soybean cultivars *in vitro* regeneration via somatic embryogenesis (Owens and Cress, 1985; Komatsuda and Okyama, 1988; Delzer *et al.*, 1990; Mauro *et al.*, 1995). Previous studies developed by Bodanese-Zanettini *et al.* (1993) and Mauro *et al.* (1994a) found cultivar IAS-5 among the most embryogenic. Successful *Agrobacterium*-mediated transformation of the soybean depends on embryogenic capability, since somatic embryos can easily be infected and transformed by strains of *A. tumefaciens*. Few Brazilian soybean cultivars have been studied so far for their interactions with strains of *A. tumefaciens* or for their ability to produce somatic embryos. The objectives of this research were to study strain and cultivar specificity in the *Agrobacterium*-soybean system and to identify some Brazilian soybean cultivars with good ability to produce somatic embryos.

MATERIAL AND METHODS

Plant material and bacterial strains

The soybean cultivars used in this study were: IAC-1, IAC-4, IAC-6, IAC-7, IAC-17, FT-Cometa, Cristalina, Savana, Viçoja, Peking, Paraná, IAS-5, Bossier, IAC PL-1, IAC-100, CAC-1, OC-4, OC-14, OC-3, JAB-11, UFV-15, UFV-14, UFV-10, UFV-1, UFV-9 and UFV-5. The cultivar Peking was used as susceptible (Delzer *et al.*, 1990).

The wild-type strains of *A. tumefaciens* C58 (nopaline), Ach5 (octopine), Bo542 (agropine) and A281 (agropine) were used for inducing gall formation on plants, since according to Petit *et al.* (1983), different opines could be responsible for pathogenicity observed in tumor formation. The experimental plots consisted of pots containing four plants of each genotype; three replications were used in a randomized block experimental design. The *Agrobacterium* cultures were initiated on LB solid medium. To prepare the inoculum a small amount of each bacterial colony was taken from the solid medium with a sterile loop and transferred to 250-ml flasks containing 25 ml of LB liquid medium (Sambrook *et al.*, 1989).

To reach log phase growth (OD value ranging from 0.7 to 0.8 at 600 nm) the flasks were incubated overnight at 28ºC in a rotary shaker at 180 rpm. After incubation, the content of each flask was poured into sterile centrifuge tubes and centrifuged at 10,000 rpm (12,062 *g*) for

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10 min at 4°C. The supernatant was discarded, 15 ml of MSO liquid medium (Murashige and Skoog, 1962) added and the pellet resuspended.

Somatic embryogenesis induction

Plants of each cultivar were cultivated in the field and when immature cotyledons were 4 to 6 mm long 40 pods of each cultivar were collected. Pods were washed thoroughly but gently in a 1% neutral detergent solution, rinsed in deionized water, placed in 70% isopropanol solution for 30 s, and drained. They were then placed in 20% NaOCl for 15 min and rinsed three times with sterile water (Lazzeri *et al.* 1985). After asepsis, the cotyledons were excised and plated on solid N10 medium (medium MS of Murashige and Skoog, 1962, supplemented with 10 mg/l of NAA) solidified with Phytagel (0.2%). Four Petri dishes (60 x 15 mm), each containing 20 immature cotyledons, were used per cultivar and considered as replications in a randomized block experimental design.

Embryogenic tissue was transferred to fresh N10 medium at 15-day intervals for 90 days, and kept in a growth chamber at 26 °C with a 23/1-hour light/dark photoperiod. Petri dishes containing embryogenic tissue of each cultivar were examined every 15 days for the presence of somatic embryos and the somatic embryo number per cultivar per replication was counted.

Plant inoculations and statistical analysis

Inoculations were performed when plants reached V_8 growth stage (Fehr and Caviness, 1980) using a hypodermal syringe (5 ml). Slight pressure was used to form a drop of bacterial suspension at the tip of the needle, which was used to make a cut across the epidermis and cortex. Four sites per internode, in the three first internodes, approximately 1 cm apart were inoculated, resulting in 12 wound sites per plant. Four to six weeks after inoculation, the plants were scored by counting the number of galls larger than 0.5 cm in diameter, per genotype, per replication, per strain of *A. tumefaciens*. The suggestions of Mauro *et al.* (1994b) were always followed, since the authors found better *Agrobacterium tumefaciens* infection results by performing the inoculations 5 h after inoculum preparation.

The number of galls derived from inoculations as well as the number of somatic embryos were transformed to $(x + 0.5)^{1/2}$ and submitted to variance analysis according to procedures suggested by Snedecor and Cochran (1989). Comparisons among means were performed through the Scott-Knott and Tukey tests, at 5% probability level.

RESULTS AND DISCUSSION

The variance analysis results for the number of galls derived from inoculation (Table I) showed differences in soybean cultivar susceptibility and in pathogenicity degree of *A. tumefaciens* strain in the soybean cultivars. Interaction between soybean cultivars and *A. tumefaciens* strain was also observed. Similar interactions were previously reported by Byrne *et al.* (1987), suggesting possible specificity between strains and cultivars. The 17.15% variation indicates reliable results.

Table I - Analysis of variance for the number of galls resulting from inoculation of soybean cultivars with four strains of *A. tumefaciens* (CV% = 17.15; Mean = 1.7127).

Source of variation	d.f.	SS	MS	F-test
Rep./Strains	52	8.3674	0.1609	-
Cultivars (Cult.)	25	207.3779	8.2951	96.1820*
Strains (Str.)	3	15.9182	5.3061	61.1820*
Cult. x Str.	75	116.7861	1.5571	18.0550*
Cult./Str.1	25	75.8933	3.0357	35.2161*
Cult./Str.2	25	70.4060	2.8162	32.6705*
Cult./Str.3	25	71.1045	2.8442	32.9954*
Cult./Str.4	25	106.7602	4.2704	49.5406*
Error	156	13.4541	0.0862	-

*Significant at the 1% level of probability. d.f. = Degrees of freedom; SS = sum of squares; MS = mean square.

The mean number of galls derived from inoculations with each strain of *A. tumefaciens* on the soybean cultivars (Table II) showed IAS-5 susceptibility to strain A281, FT-Cometa to strain Ach5, and IAC-7 to strain Bo542. IAC PL-1 was susceptible to strains C58 and A281, IAC-4 susceptible to strains Ach5 and Bo542 but resistant to strain C58, and IAC-100 susceptible to strain Ach5 only. Cultivars OC-14, IAC-17, OC-4, Paraná and UFV-14 were resistant to all strains of *A. tumefaciens;* Peking, as expected, was highly susceptible to all four strains.

Table II also shows that strain A281 was the most virulent, followed by strains Bo542, Ach5 and C58, with no significant differences among them. Bailey *et al.* (1994) and Mauro *et al.* (1995) also reported for strain A281 high virulence to the soybean cultivars tested. The results also suggested that additional factors apart from opine type may contribute to pathogenicity degree of the *Agrobacterium* strains since significant differences were observed between strains carrying the same opine (A281 and Bo542).

Table III summarizes the results of numerical analysis of somatic embryos produced by each cultivar. The F-test showed differences in somatic embryogenesis capability among cultivars; comparisons among means for the number of somatic embryos produced by each soybean cultivar confirmed these differences. Cultivars IAS-5, Cristalina, FT-Cometa, IAC-7 and OC-3 produced the highest numbers of somatic embryos and could therefore be indicated as embryogenic parentals for studies related to the genetics of embryogenic capability in the soybean. Cul-

Table II - Mean number of galls $(x + 0.5)^{1/2}$ in each soybean cultivar produced by *Agrobacterium tumefaciens* strains.

Soybean cultivars	*A. tumefaciens* strains				Mean[1]
	C58	Ach5	Bo542	A281	
IAC-1	2.10C	2.26A	2.84B	2.91D	2.53A
IAC-4	0.88D	3.18A	3.28A	3.76B	2.78A
IAC-6	1.00D	2.20A	2.11C	2.26E	1.89B
IAC-7	0.71D	2.47A	3.65A	2.41D	2.31B
IAC-17	0.71D	0.71B	0.71E	0.71F	0.71C
FT-Cometa	1.86C	2.26A	1.00D	1.00F	1.53C
Cristalina	2.26C	0.71B	1.93C	2.67D	1.89B
Savana	0.71D	2.74A	1.10D	0.71F	1.31C
Viçoja	0.71D	1.74B	0.71E	1.95E	1.28C
Peking (C)	3.83A	3.89A	3.29A	4.38A	3.84A
Paraná	0.71D	0.71B	0.71E	1.00F	0.78C
IAS-5	0.71D	0.71B	0.71E	3.77B	1.48C
Bossier	0.71D	0.71B	0.71E	2.03E	1.04C
IAC PL-1	3.87A	1.39B	2.73B	3.90B	2.97A
IAC-100	0.71D	3.31A	1.18D	0.71F	0.83C
CAC-1	1.00D	0.71B	1.86C	2.59D	2.19B
OC-4	1.11D	1.17B	0.71E	0.71F	0.93C
OC-14	0.71D	0.71B	0.71E	0.71F	0.78C
OC-3	1.93C	0.71B	1.00D	0.71F	1.09C
JAB-11	2.53C	0.71B	2.46C	2.67D	2.09B
UFV-15	1.93C	1.43B	1.29D	2.53D	1.80B
UFV-14	0.71D	1.00B	0.71E	0.71F	0.71C
UFV-10	1.81C	0.71B	1.65D	1.05F	1.31C
UFV-1	0.71D	1.17B	0.71E	2.20E	1.20C
UFV-9	2.85B	1.67B	2.79B	3.29C	2.65A
UFV-5	2.90B	2.48A	1.86C	3.22C	2.62A
Mean[2]	1.53b	1.59b	1.63b	2.10a	

[1] Means followed by the same capital letter did not differ by the Scott-Knott test at the 5% level of probability. [2] Means followed by the same lower case did not differ by the Tukey test at the 5% level of probability.

Table III - Total (TSE) and mean (MSE) number of somatic embryos $(x + 0.5)^{1/2}$ derived from each soybean cultivar (F-test = 25.37[1]; C.V.% = 15.78).

Soybean cultivar	TSE	MSE[2]
IAC-1	0	0.71D
IAC-4	8	1.44C
IAC-6	4	1.18C
IAC-7	32	2.92B
IAC-17	4	1.18C
FT-Cometa	33	2.95B
Cristalina	36	3.08B
Savana	0	0.71D
Viçoja	0	0.71D
Peking	0	0.71D
Paraná	0	0.71D
IAS-5	100	5.04A
Bossier	0	0.71D
IAC PL-1	6	1.40C
IAC-100	6	1.35C
CAC-1	8	1.56C
OC-4	7	1.49C
OC-14	0	0.71D
OC-3	27	2.74B
JAB-11	5	1.27C
UFV-15	0	0.71D
UFV-14	1	0.84D
UFV-10	7	1.44C
UFV-1	0	0.71D
UFV-9	0	0.71D
UFV-5	0	0.71D

[1]Significant at the 1% level of probability. [2]Means followed by the same letter did not differ by the Scott-Knott test at the 5% level of probability.

tivar IAS-5 produced the highest individual number of somatic embryos, as also reported by Bodanese-Zanettini *et al.* (1993) and by Mauro *et al.* (1994a). On the other hand, IAC-1, Savana, Viçoja, Peking, Paraná, Bossier, OC-14, UFV-15, UFV-1, UFV-9 and UFV-5 were non-embryogenic. The variation was 15.78%, indicating reliability.

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RESUMO

Vinte e cinco cultivares brasileiros de soja foram estudados quanto à suscetibilidade a quatro linhagens de *Agrobacterium tumefaciens* (C58, Ach5, Bo542 e A281) e quanto à capacidade de produzir embriões somáticos. Doze plantas de cada cultivar foram inoculadas, na casa de vegetação, 4-6 semanas após a semeadura, sendo efetuadas 12 inoculações por planta. O número de galhas derivadas dessas inoculações foi contado 8-10 semanas após as inoculações. Para avaliar a capacidade de produção de embriões somáticos, cotilédones imaturos com 4-6 mm de comprimento foram cultivados em meio N10 para indução de calos embriogênicos, sendo empregadas, para cada cultivar, quatro placas de Petri contendo 20 cotilédones cada. Os tecidos embriogênicos foram transferidos 6 vezes, a cada 15 dias de intervalo, para novo meio N10, sendo contado o número de embriões somáticos por cultivar. Foi observada uma interação significativa entre cultivares de soja e linhagens de *A. tumefaciens* e a linhagem mais virulenta foi a A281. O tipo de opina aparentemente não teve efeito sobre a virulência das linhagens e os cultivares mais embriogênicos foram IAS-5, Cristalina, FT-Cometa, IAC-7 e OC-3.

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