Note

Plant Biotechnology, **16** (5), 419–421 (1999)

Genotype × Sucrose Interactions for 2,4-D-induced Somatic Embryogenesis in Soybean (*Glycine max* L.)

Minako ITO*, **, Takao KOMATSUDA*[1], Yoshihito TAKAHATA**, and Norihiko KAIZUMA**

** National Institute of Agrobiological Resources, Department of Biotechnology, Tsukuba 305-8602, Japan*
*** Iwate University, Faculty of Agriculture, Morioka 020-8550, Japan*
[1] To whom correspondence should be addressed.

Received 4 June 1999; accepted 13 September 1999

Abstract

Cotyledons isolated from immature embryos of 11 varieties of soybean were cultured on embryogenesis media containing 2,4-D at 40 mg l^{-1} and sucrose at 10, 20, 40, or 60 g l^{-1}. The number of somatic embryos per cotyledon was counted after 5 weeks of culture. Analysis of variance showed that the effects of genotype, sucrose concentration, and genotype × sucrose interaction were all highly significant. Further analysis of the genotype × sucrose interaction showed that the heterogeneity of regression among the varieties was significant. The variety mean and regression were significantly correlated, indicating that the 2 factors are not under separate genetic control. Nevertheless, these results suggest that embryogenic response to 2,4-D and NAA seems to be under separate genetic control in soybean.

Somatic embryogenesis of soybean has been developed by using immature embryos as explants (for a review, see Komatsuda 1995). Komatsuda and Ohyama (1988) found a large genotypic difference in the response of immature embryos to naphthaleneacetic acid (NAA, 10 mg l^{-1}) and 2,4-dichlorophenoxyacetic acid (2,4-D, 2 mg l^{-1}). When NAA was used as a hormone, significant interaction between genotype and sucrose concentration was found (Komatsuda *et al.* 1991). A higher concentration of 2,4-D (40 mg l^{-1}) was reported to generate proliferative embryogenesis in soybean (Finer 1988). The proliferative embryogenic cultures were good target tissue for transformation via particle bombardment (Finer and McMullen 1991, Sato *et al.* 1993). In this study, using 40 mg l^{-1} 2,4-D as a hormone, we estimated the genotype × sucrose interaction needed to produce somatic embryos on media containing different sucrose concentrations.

Eleven soybean varieties selected from previous studies (Komatsuda and Ohyama 1988, Komatsuda *et al.* 1991) were tested (**Table 1**). Immature seeds half the length of mature seeds, aged approximately 2 to 3 weeks post anthesis, were excised from pods. After the seed coats and embryonic axes were removed, the cotyledons were placed adaxial side up, 10 pairs (20 cotyledons) per 90 mm × 20 mm plastic dish (Terumo, Tokyo). Each dish contained 30 m*l* of embryogenesis medium composed of MS salts (Murashige and Skoog 1962), B5 organics (Gamborg *et al.* 1968), 40 mg l^{-1} 2,4-D (Finer 1988), and sucrose (10, 20, 40, or 60 g l^{-1}). The medium was adjusted to pH 5.8 and supplemented with 2.2 g l^{-1} gellan gum (Gelrite, San-ei, Osaka, Japan) before autoclaving. The dishes were sealed with surgical tape (Micropore, 3M, St. Paul, MN, USA) and incubated at 25 ℃ under a 16-h photoperiod of dim light (~30 lux).

Formation of somatic embryos usually occurred 4-5 weeks after culture initiation. Secondary somatic embryos were formed from the primary somatic embryos, as described by Finer (1988). The number of primary somatic embryos, counted 5 weeks after culture initiation, was used to evaluate the embryogenic ability of each variety (**Table 1**). Genotypic variation was considerable, ranging from 0.07 (Orihime) to 1.10 (American Jellow), as usually recognized in plant regeneration from tissue culture. In general, the media containing 20 or 40 g l^{-1} sucrose produced more somatic embryos than the media containing 10 or 60 g l^{-1} sucrose. When NAA was used instead of 2,4-D, the media containing 5 or 10 g l^{-1} sucrose produced more somatic embryos than the media containing 20 or 30 g l^{-1} sucrose (Komatsuda *et al.* 1991). This indicates that somatic embryogenesis with 2,4-D requires a higher concentration of sucrose than that with NAA.

The experimental design consisted of 4 concentrations of sucrose (fixed effect) and 11 varieties (fixed effect). Each experiment had 2 replications

Table 1. Number of somatic embryos per cotyledon averaged over 2 replications and standard deviation (in parentheses) in soybean varieties. The medium contained 40 mg l^{-1} 2,4-D.

Variety	Concentration of sucrose (g l^{-1})				Mean[1)]	Regression[1,2)]
	10	20	40	60		
American Jellow	0.48	3.84	4.67	1.11	1.10	2.07
	(0.19)	(0.88)	(0.08)	(0.11)		
Cha Masshokutou	0.46	1.71	1.12	0.24	0.57	1.15
	(0.30)	(0.86)	(0.45)	(0.06)		
Keburi	0.04	0.14	0.43	0.09	0.15	0.35
	(0.06)	(0.09)	(0.02)	(0.04)		
Kimusume Ibarai 1	0.03	0.20	0.93	0.17	0.25	0.63
	(0.04)	(0.07)	(0.29)	(0.12)		
Masshokutou Kou 502	0.58	2.28	0.19	0.11	0.48	0.88
	(0.16)	(0.39)	(0.10)	(0.10)		
Orihime	0.13	0.11	0.09	0.00	0.07	0.04
	(0.06)	(0.10)	(0.03)	(0.00)		
T34	0.82	1.31	0.50	0.08	0.48	0.67
	(0.23)	(0.48)	(0.02)	(0.06)		
Tachisuzunari	0.27	1.30	2.38	0.10	0.59	1.73
	(0.17)	(0.28)	(1.00)	(0.12)		
Toyosuzu	0.00	0.22	5.57	0.96	0.67	1.20
	(0.00)	(0.31)	(3.01)	(0.25)		
Wasesuzunari	2.00	5.30	1.93	0.20	0.94	1.44
	(1.05)	(5.77)	(2.01)	(0.14)		
Yamashiratama	0.31	1.53	0.34	0.11	0.39	0.86
	(0.35)	(0.71)	(0.39)	(0.15)		
Mean[1)]	0.32	0.76	0.76	0.22	0.52	1.00

[1)] These values were calculated based on ln(x+1) transformed data.

[2)] Coefficient of regression of individual yield on the mean yield of all genotypes at each sucrose concentration.

Table 2. Analysis of variance for 11 soybean varieties and 4 sucrose concentrations.

Source	df	MS	F
Genotype(G)	10	0.79	11.46**
Sucrose(S)	3	1.82	26.74**
G × S	30	0.30	4.34**
(Heterogeneity of regression)	10	0.35	5.07**
(Deviations from regression)	20	0.26	3.77**
Error	44	0.07	

** Significant at the 0.01 probability level.

(date of culture initiation), with each replicate containing 3 dishes. The data (x) were transformed into the form $y = \ln(x + 1)$ to make mean and variance mutually independent before analysis of variance. The analysis of variance (**Table 2**) showed that the effects of genotype, sucrose concentration, and genotype × sucrose interaction were all highly significant ($P < 0.01$). This result is in good agreement with a previous study that used NAA instead of 2,4-D (Komatsuda *et al.* 1991).

Because the analysis of variance revealed a significant interaction between genotype and sucrose concentration, a regression analysis (Finlay and Wilkinson 1963) was used to evaluate the genotypic stability across sucrose concentrations. For each genotype, a linear regression of individual yield on the mean yield of all genotypes at each sucrose concentration was computed (**Table 1**). Then the variance of the genotype × sucrose interaction shown in **Table 2** was partitioned into heterogeneity of regression and deviations from regression. The analysis showed that heterogeneity of regression was significant ($P < 0.01$), indicating a difference in genotypic response to sucrose concentration. Deviations from regression were also significant ($P < 0.01$), indicating the presence of other factors besides the regression coefficient. The ability of cultivars to produce somatic embryos, therefore, can be explained by at least 2 factors: the variety mean and the regression coefficient (**Table**

Table 3. Correlation coefficients between meen and regression.

	Regression(2,4-D)	Mean(NAA)	Regression(NAA)
Mean(2,4-D)	0.91***	0.58ns	0.05ns
Regression(2,4-D)		0.49ns	0.13ns
Mean(NAA)			0.37ns

*** Significant at the 0.001 probability level.

Data obtained no media containing 2,4-D (40 mg l^{-1}, this study) and NAA (10 mg l^{-1}, Komatsuda *et al.* 1991).

1). The correlation between these 2 factors, however, was significant ($r = 0.91$, $P < 0.001$) (**Table 3**). Therefore, we could not conclude that the 2 factors are under separate genetic control.

Comparison of this study and that of Komatsuda *et al.* (1991) allowed analysis of the correlation coefficients (**Table 3**). In this table, apart from the significant correlation between mean (2,4-D) and regression (2,4-D), there was no significant correlation between the 4 factors. This result is in good agreement with the different responses by soybean varieties to 10 mg l^{-1} NAA and 2 mgl^{-1} 2,4-D (Komatsuda and Ohyama 1988) and suggests that embryogenic response to 2,4-D and NAA is under separate genetic control in soybean.

We thank Dr. Pil-Son Choi for his critical reading of the manuscript.

References

Finer, J.J., 1988. Apical proliferation of embryogenic tissue of soybean [*Glycine max* (L.) Merrill]. Plant Cell Rep. **7**:238-241.

Finer, J.J., McMullen, M.D., 1991. Transformation of soybean via particle bombardment of embryogenic suspension culture tissue. *In Vitro* Cell. Dev. Biol. **27**:175-182.

Finlay, K.W., Wilkinson, G.N., 1963. The analysis of adaptation in a plant-breeding programme. Aust. J. Agric. Res. **14**:742-754.

Gamborg, O.L., Miller, R.A., Ojima, K., 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. **50**:151-158.

Komatsuda, T., 1995. Somatic embryogenesis in soybean (*Glycine* species). In: Bajaj, Y. (ed.) Somatic Embryogenesis and Synthetic Seed II, pp. 239-255. Springer-Verlag, Berlin.

Komatsuda, T., Ohyama, K., 1988. Genotypes of high competence for somatic embryogenesis and plant regeneration in soybean *Glycine max*. Theor. Appl. Genet. **75**: 695-700.

Komatsuda, T., Kaneko, K., Oka, S., 1991. Genotype × sucrose interactions for embryogenesis in soybean. Crop Sci. **31**: 333-337.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. **15**:473-497.

Sato, S., Newell, C., Kolacz, K., Tredo, L., Finer, J., Hinchee, M., 1993. Stable transformation via particle bombardment in two different soybean regeneration systems. Plant Cell Rep. **12**:408-413.