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ORIGINAL ARTICLE

Identification of QTLs controlling somatic embryogenesis using RI population of cultivar × weedy soybean

Pilson Choi · Yoshiro Mano · Atsuko Ishikawa · Masashi Odashima · Taishi Umezawa · Tatsuhito Fujimura · Yoshihito Takahata · Takao Komatsuda

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Abstract Quantitative trait loci (QTLs) controlling ability of somatic embryogenesis were identified in soybean. A frame map with 204-point markers was developed using an RI population consisting of 117 F_{11} lines derived from a cross between cultivar 'Keburi' and a weedy soybean 'Masshokutou Kou 502'. The parents differed greatly in their abilities of somatic embryogenesis using immature cotyledons as explants. The ability of somatic embryogenesis was evaluated in five different experiments: the F_{11} (evaluated in 1998) and F_{15} (2002) generations cultured on basal media supplemented with 40 mg l^{-1} 2,4-D (2,4-D1998 and 2,4-D2002), F_{14} (2001) generation on medium with 40 mg l^{-1} 2,4-D and high sucrose concentration [2,4-D2001 (30 g l^{-1} sucrose)], and the F_{11} (1998) and F_{12} (1999) generations on medium with 10 mg l^{-1} NAA (NAA1998 and NAA1999). The RILs showed wide and continuous variations in each of the five experiments. In the composite interval mapping analysis, 2 QTLs were found in group 8 (D1b + W, LOD = 5.42, $r^2 = 37.5$) in the experiment of 2,4-D1998 and in group 6 (C2, LOD = 6.03, $r^2 = 26.0$) in the experiment of 2,4-D2001 (high concentration sucrose). In both QTLs, alleles of 'Masshokutou Kou 502' with high ability of somatic embryogenesis contributed to the QTLs. For the other three experiments, no QTL was detected in the criteria of LOD >3.0, suggesting the presence of minor genes.

Keywords *Glycine max* (L.) Merr. · Quantitative trait loci · Recombinant inbred line (RIL) · Somatic embryogenesis · Weedy soybean

P. Choi · Y. Mano · A. Ishikawa · T. Komatsuda
National Institute of Agrobiological Sciences (NIAS), 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

Present Address:
P. Choi (✉)
Department of Oriental Pharmaceutical Development, Nambu University, 864-1 Wealkye-dong, Gwangsan-gu, Gwangju City, Korea
e-mail: cps6546@hanmail.net

Present Address:
Y. Mano
National Institute of Livestock and Grassland Science, 768 Senbonmatsu, Nishinasuno, Tochigi 329-2793, Japan

A. Ishikawa · Y. Takahata
Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan

M. Odashima · T. Umezawa · T. Fujimura
Institute of Agriculture and Forestry, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8572, Japan

Present Address:
M. Odashima
Iwate Agricultural Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan

Present Address:
T. Umezawa
RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

Introduction

Soybean is one of the most important economic crops in the world and has been subjected to genetic transformation to improve economically important traits such as resistances to diseases and pest, and tolerances to abiotic stresses. It is essential to select genotypes or cultivars with high ability of somatic embryogenesis and shoot differentiation in transgenic plant study. Suitable culture conditions for soybean in vitro culture are also needed for the efficient

acquisition of transgenic plants (Ritala et al. 1994; Tingay et al. 1997). Several authors have investigated culture conditions and explant sources of soybean, but no culture condition suitable for all genotypes has been established. Genetic studies of tissue culture traits such as somatic embryogenesis and differentiation will make it possible to transfer genes controlling desirable tissue culture traits into recalcitrant crops or species.

More than 10 years ago, there was a report that some wild and weedy soybeans exhibited higher competence of somatic embryogenesis and plant regeneration than cultivated soybeans (Komatsuda and Ohyama 1988; Komatsuda and Ko 1990). Sucrose concentration and genotype × sucrose concentration had a significant interaction on the culture media that included naphthaleneacetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D), and a cultivar 'Keburi' and a weedy form of soybean 'Masshokutou Kou 502' showed extremely low and high somatic embryogenesis ability at all sucrose concentrations (Komatsuda et al. 1991; Ito et al. 1999). This finding was of great interest for mapping quantitative trait loci (QTLs). For such a purpose, recombinant inbred lines (RILs) at the F_{11} generation derived from a cross between 'Keburi' and 'Masshokutou Kou 502' (Komatsuda, unpublished) were developed. Heritability for somatic embryogenesis was shown to be high in a preliminary experiment (Endo et al. 1998). In addition, we constructed AFLP–SSR-based linkage map with 600-point markers of the RILs (Choi et al. 2003).

Tissue culture traits are quantitatively controlled, and restriction fragment-length polymorphism (RFLP) markers linked to genes controlling somatic embryogenesis or shoot differentiation ability have been identified in cereal crops such as maize (Armstrong et al. 1992), rice (Taguchi-Shiobara et al. 1997), wheat (Ben Amer et al. 1997), and barley (Komatsuda et al. 1993; Mano et al. 1996; Mano and Komatsuda 2002). But little is known about mode of inheritance and chromosome location of gene(s) controlling somatic embryogenesis in soybean. Here, we report the identification of QTLs for somatic embryogenesis using RILs in several culture conditions. The information obtained in this study will help to establish an efficient transformation system.

Materials and methods

Plant materials

A recombinant inbred population consisting of 117 F11 lines (RILs) was developed from the cross between 'Keburi' and 'Masshokutou Kou 502' by single seed descent method. Keburi is a Japanese cultivar, and Masshokutou Kou 502 (synonymous with Mo-shi-dou Gong 502) is a weedy soybean originating from Manchuria (Fukuda 1933). The parents were chosen based on the diversity of somatic embryogenesis ability from their immature cotyledons (Komatsuda et al. 1991; Endo et al. 1998; Ito et al. 1999).

Tissue culture

Immature cotyledons were cultured in five experiments: the F_{11} (evaluated in 1998) and F_{15} (2002) generations were cultured on MS basal medium containing 40 mg l^{-1} 2,4-D (2,4-D1998 and 2,4-D2002); F_{14} (2001) generation on the medium with 2,4-D and high sucrose concentration [2,4-D2001 (30 g l^{-1}, high conc. sucrose)]; and the F_{11} (1998) and F_{12} (1999) generations on the medium plus 10 mg l^{-1} NAA (NAA1998 and NAA1999). A total of 117 RILs and their parents were grown in a greenhouse under natural daylight at constant temperature of 25°C. Pods (approximately 2 weeks post-anthesis) of each RIL and parent were surface sterilized with 70% ethanol for 1 min. Immature cotyledons were separated from the immature zygotic embryos (2.5–3.0 mm in size) and placed on 25 ml solid culture medium to induce somatic embryos. The somatic embryogenesis medium (SEM) consisted of MS salt (Murashige and Skoog 1962) supplemented with B5 vitamins, 40 mg l^{-1} 2,4-D and 20 g l^{-1} sucrose, or 10 mg l^{-1} NAA and 10 g l^{-1} sucrose. For the experiment of 2,4-D2001 (high concentration sucrose), 30 g l^{-1} sucrose was supplied. All media were adjusted to pH 5.8, then 4 g l^{-1} Phytagel was added, and they were autoclaved at 121°C for 15 min. Ten cotyledon explants were placed in each Petri dish and sealed with Parafilm. Three Petri dishes (replications) for each RIL were cultured at 25°C under dark conditions. Periodically, cultures were observed under a dissecting microscope. After incubation for 6 weeks, contaminated embryos were discarded, and then the number of immature embryo with somatic embryo and number of somatic embryos per explant were recorded.

Map construction and QTL analysis

The linkage map with 600-point markers for the RILs at the F_{11} generation was developed with an average interval of 3.7 cM/locus, having considerable marker clustering in certain regions (Choi et al. 2003). A subset of 204 markers, to provide 5- to 10-cM intervals without marker clustering was reconstructed as a "base map" by using MAPMAKER 3.0 (Lander et al. 1987; Lincoln et al. 1992) and used for QTL analysis. Since the tissue culture response was affected by growth stage of explant and culture condition, QTL mapping of somatic embryogenesis was separately carried out in five experiments.

QTL mapping for somatic embryogenesis was performed using composite interval mapping (CIM) implemented by computer program of QTL Cartographer Version 1.14 (Basten et al. 2000). CIM was run with the default setting for model 6 in the program (five background markers and a window size of 10 cM). The inclusion of background markers makes the analysis more precise and permits efficient mapping of QTLs. A log-likelihood (LOD) score threshold of 3.0 was used to identify regions containing putative loci associated with ability of somatic embryogenesis.

Results and discussion

Immature cotyledon explants excised from the pods of parents and 116 RILs except one missing line were cultured on the medium containing MS salts, B5 vitamins, 40 mg l^{-1} 2,4-D, and 20 g l^{-1} sucrose. Somatic embryos were directly formed on the brownish surface of the cotyledon explants or arose from the yellowish callus produced on the surface of the explants. The number of somatic embryos produce from per cotyledon varied greatly among lines, which were 0.0–0.20 for Keburi and 3.92–3.05 for Masshokutou Kou 502 following the culture conditions of 2,4-D1998 (F_{11}) and 2,4-D2002 (F_{15}), respectively (Table 1). The RILs also showed an extremely wide range and continuous frequency distribution for somatic embryogenesis in 2,4-D1998 (0.0–2.100, average 0.646) and 2,4-D2002 (0.0–2.195, average 0.340) conditions, respectively, suggesting multiple genes would be involved in the expression of this trait. For the condition of 2,4-D2001 (high concentration sucrose), the surface of immature cotyledon explants based on lines also became deep-brownish in color or dedifferentiated into yellowish calli, and then somatic embryos were formed at low sucrose concentration (2,4-D1998 and 2,4-D2002). The number of somatic embryos per explant also showed an extreme difference of 0.27 for Keburi and 1.65 for Masshokutou Kou 502, respectively, but the number of 1.65 in the case of Masshokutou Kou 502 decreased in the comparison of 2,4-D1998 and 2,4-D2002. Frequency distribution of somatic embryogenesis of RILs in 2,4-D2001 (0.0–2.253, average 0.480) was nearly the same as at low sucrose concentration (2,4-D1998 and 2,4-D2002). In the conditions of NAA1998 and NAA1999, the number of somatic embryo per explant also showed significant differences of 0.41 and 0.13 for Keburi, and 2.79 and 2.47 for Masshokutou Kou 502, respectively. The RILs in the 2,4-D experiment showed a wide frequency of somatic embryogenesis in NAA1998 (0.019–1.450, average 0.694) and NAA1999 (0.019–1.690, average 0.746) conditions, respectively. Average value of somatic embryos among the RILs in the NAA experiment was nearly the same (0.694 in NAA1998 vs. 0.746 in NAA1999), whereas that in the 2,4-D experiment was decreasing in the order: 2,4-D1998 (0.646) > 2,4-D2001 (0.480) > 2,4-D2002 (0.340) (Table 2). Heritability of plant differentiation trait has been reported on somatic embryogenesis in soybean (Endo et al. 1998), and shoot regeneration in barley (Komatsuda et al. 1998). In our study, the ability of somatic embryogenesis between the cultivars was extremely low or high, and showed wide and continuous variations in the RILs (Fig. 1).

Table 1 Somatic embryogenesis ability in parent varieties

Parents	Number of cotyledons cultured	Number of explants with somatic embryo (%)	Number of somatic embryos per cotyledon
Keburi			
2,4-D1998	60	0 (0)	0
2,4-D2002	60	2 (0.03)	0.20
Masshokutou Kou 502			
2,4-D1998	60	55 (92)	3.92
2,4-D2002	60	52 (87)	3.05

The correlation coefficient on somatic embryogenesis between 2,4-D1998 and 2,4-D2002 was 0.425 (significant at the 1% level), indicating the repeatability of the experiment was moderate. In the experiment of 2,4-D1998, the CIM analysis detected a single locus in group 8 (D1b + W), with an LOD score of 5.75. The peak LOD was located in the e16m29-9-1–e13m20-8-1 interval. This QTL accounted for 37.5% of the total phenotypic variance, and tbe additive effect was estimated as 0.35 (Table 3, Fig. 2a). Alleles in Masshokutou Kou 502 with high ability of somatic embryogenesis were attributed to this QTL. In the experiment of 2,4-D2002, no significant QTL was found in the criteria of LOD >3.0 (Fig. 2a). The CIM analysis detected single locus controlling ability of somatic embryogenesis in group 6 (C2) (Fig. 2b), and 'Masshokutou Kou 502' alleles increased the frequency of somatic embryos. The peak LOD score (satt277–e08m31-6-2 interval) was 6.03, and this QTL accounted for 26.0% of the total phenotypic variance (Table 3, Fig. 2b).

However, the correlation coefficient of somatic embryogenesis between NAA1998 and NAA1999 was 0.828 (significant at the 1% level), indicating the repeatability of the experiment was high. In the CIM analysis, no significant QTL was detected in either NAA1998 or NAA1999 culture condition in the criteria of LOD >3.0, suggesting minor genes were present. Also, no QTL was found when the criteria was decreased to LOD >2.0. Since the high value of correlation coefficient between NAA1998 and NAA1999, and the average value of somatic

Table 2 Range and average of somatic embryos per immature cotyledon in the RILs of Keburi × Masshokutou Kou 502

Experiments	Generations of RILs	Concentrition of plant growth regulators (mg l^{-1})	Sucrose level (%)	Range of somatic embryos per cotyledon (average)
2,4-D1998	F_{11}	40	20	0.0–2.100 (0.646)
2,4-D2001	F_{14}	40	30	0.0–2.253 (0.480)
2,4-D2002	F_{15}	40	20	0.0–2.195 (0.340)
NAA1998	F_{11}	10	10	0.019–1.450 (0.694)
NAA1999	F_{12}	10	10	0.019–1.690 (0.746)

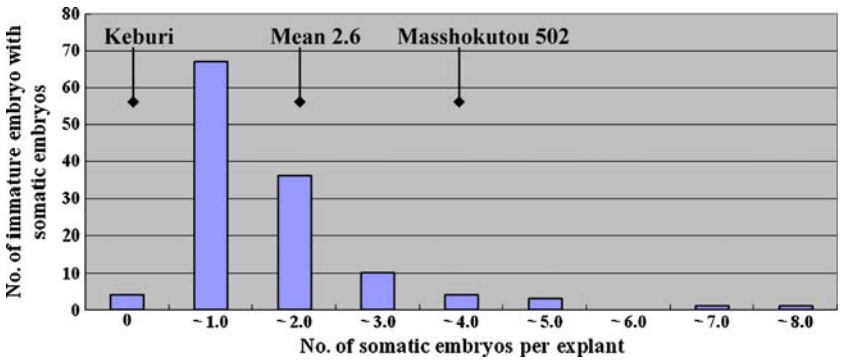

Fig. 1 Frequency distribution for somatic embryogenesis in the RILs (2,4-D1998) derived from Keburi × Masshokutou Kou 502

Table 3 Chromosomal location of QTLs on somatic embryogenesis evaluated by five experiments in the RILs derived from Keburi × Masshokutou Kou 502

Chromosome	Nearest marker	LOD for SE (allele)[a]	
		2,4-D (1998, F_{11}) R^2 (%)	2,4-D (2001, F_{14}) R^2 (%)
Group8 (D1b + W)	(309) e13m20-8-1	5.75 (M) 20.6	
	(32) satt172	6.05 (M) 20.6	
	(287) e12m26-6-1	4.71 (M) 47.6	
	(447) e16m29-9-1	4.71 (M) 37.5	
Group6 (C2)	(343) e13m21-11		3.59 (M) 21.0
	(545) e08m31-6-2		3.79 (M) 18.0
	(24) satt277		6.03 (M) 26.0

[a] *M* 'Masshokutou Kou 502' indicating parent contributing a higher-value allele

embryogenesis among the RILs were nearly the same, data from the two experiments were pooled, and the resulting dataset was used for CIM analysis. A minor QTL with an LOD score of 2.70 was found in group 6 (C2, satt277–e08m31-6-2 interval) which explained 9.1% of the total phenotypic variance (data not shown). The position of the QTL was identical to that found in group 6 (C2) in the experiment of 2,4-D2001 (high concentration sucrose).

The mapping population used in this study is suitable for QTL analysis of somatic embryogenesis, because their parents showed extremely different somatic embryogenesis characteristics, and the RILs provided sufficient seeds for replicated experiments. By QTL analysis throughout the soybean genome in several cultural conditions, we identified two QTLs that had significant effects in controlling somatic embryogenesis (Table 3, Fig. 2). Though replication tests were preformed at the condition with 2,4-D (2,4-D1998 vs. 2,4-D2001), there was no QTL in common. Berrios et al. (2000) reported four (*tee*) QTLs and seven (*ete*) QTLs on somatic embryogenesis using composite interval mapping in sunflower. Kwon et al. (2001) reported some initial results on the study of regeneration ability with molecular markers. Nishimura et al. (2005) demonstrated the possibility of altering regeneration ability by genetic engineering. Like these, QTL mapping studies could detect chromosomal locations not only for somatic embryogenesis but also for regeneration ability, such as organogenesis or callus differentiation (Mano and Komatsuda 2002). The identification of QTLs could pave a way to clone some related specific genes for crop improvement.

In conclusion, the present study identified some regions in the chromosomes of weedy soybean 'Masshokutou Kou 502', which were involved in the control of somatic embryogenesis. This results obtained from this study will be useful for improving soybean germplasm and help to establish efficient transformation system of this plant.

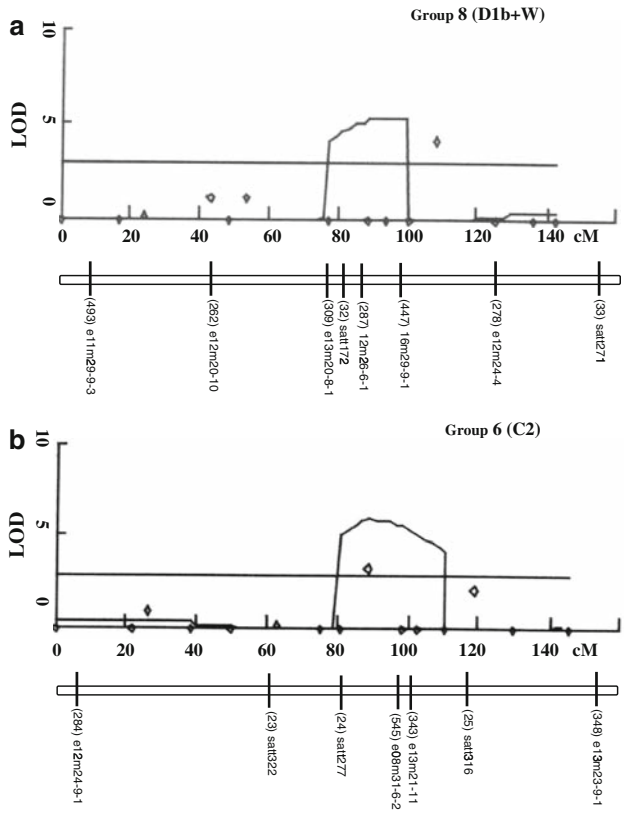

Fig. 2 Chromosomal locations of QTLs for controlling somatic embryogenesis in the RILs of Keburi × Masshokutou Kou 502 in group 8 (D1b + W) in the experiment of 2,4-D1998 (**a**) and in group 6 (C2) in the experiment of 2,4-D2001 (high concentration sucrose) (**b**). Short arms are on the *left*. QTL analysis of somatic embryogenesis was performed in the F_{11} and F_{14} generation

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