Factors Affecting the Production of a BC₁F₁ Population and Characterization of the BC₁F₁ Progenies Obtained from the Interspecific Crosses between a Cultivated Tomato and the *peruvianum*-complex

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The present study was conducted to evaluate the factors (e.g. cultivar (V), wild species line (L), year (Y) and their interactions) affecting the production of a BC₁F₁ population (cultivar × F₁ (cultivar × '*peruvianum*-complex')), a breeding strategy for using many cultivars as pistillate parents for the production of a BC₁F₁ population and for the segregation of some characteristics such as sweet aroma of leaf, morphological traits and self-incompatibility in the BC₁F₁ population. Ovule selection and culture method was used for the production of progenies. The interaction between the cultivar and the wild species line (V × L) was found to be significant. Differences in the number of GOFs (Germinated Ovules per Fruit) among 19 tomato cultivars crossed with F₁ (cultivar × *L. peruvianum* LA1554) were significant. Differences in the number of GOFs among wild species lines (as F₁ pollen parents) were also significant and much more pronounced than those of the cultivars. Significant variation was observed within-year and affected the interaction (V × Y) which was variable. The present study suggests that the probability of obtaining a stable, consistent average number of GOFs is high for the production of a BC₁F₁ population if many cultivars are used at a time as seed parents. The segregation of some characteristics in the BC₁F₁ population was evaluated. Most of the morphological traits resembled those of the wild species parents. Thirty six percent of the BC₁F₁ population inherited self-compatibility from the *L. esculentum* parent. A sensory panel composed of 8 members evaluated the leaf aroma of 100 BC₁F₁ plants (19 cultivars × F₁ derived from LA1554) and two BC₁F₁ plants were found to have the same sweet aroma as the F₁ (pollen parent). In the present study a large BC₁F₁ population with an expected number of plants was obtained. Sensory attributes of some BC₁F₁ plants suggested that the expected quantitative trait had successfully been introduced into the BC₁F₁ population.

Key Words: *Lycopersicon peruvianum*, *Lycopersicon esculentum*, tomato, production of BC₁F₁

plants, introgression, aromatic-fragrance, quantitative traits.

Introduction

The strong breeding barrier of cross incompatibility between cultivated tomato (*Lycopersicon esculentum* Mill.) and *Lycopersicon peruvianum* is the major problem to be overcome for the introgression of the valuable traits of *L. peruvianum* into cultivated tomato. Many researchers had attempted to overcome the strong cross incompatibility barrier between this cross combination by using various methods. These included a) pre-fertilization techniques such as the use of cross-compatible *L. peruvianum* accessions (Rick 1986), chronic gamma irradiation of pollen (Yamakawa 1971), development of bridge lines (Poysa 1990); and b) post-fertilization techniques such as embryo culture (Smith 1944), embryo callus culture (Thomas and Pratt 1981, Segeren *et al.* 1993), ovule selection and culture (Imanishi 1988) and embryo culture via embryo rescue (Chen and Adachi 1992). Regarding the cross-incompatibility for the production of F₁ hybrids, significant genetic differences among the cultivars of *L. esculentum* as seed parents were reported (Imanishi 1988, Chen and Imanishi 1991, Sacks *et al.* 1997, Takashina *et al.* 1997, Egashira *et al.* 1999). Significant interaction between '*L. esculentum* × year' was observed (Sacks *et al.* 1997). Furthermore, environmental factors may exert a large influence on the crossability between cultivated tomato and the *L. peruvianum* line. Kuriyama *et al.* (1970) reported that the crossing between cv. tomato and *L. peruvianum* during May, produced a larger number of F₁ progenies than the hybridization in September, suggesting that lower temperatures are favorable for the production of the F₁ population. Egashira *et al.* (1999) also reported a similar environmental effect. Owing to these efforts, hybridization between the two distantly related species was possible, and various useful, resistance genes of *L. peruvianum*, particularly for monogenic effects on tomato pests have been introgressed into the cultivated tomato (Kalloo 1991).

On the other hand, there are very few reports on the production of a BC₁F₁ population from the cross combination between *L. esculentum* and F₁ (Takashina *et al.* 1997,

Yamakawa *et al.* 1978, Doganlar *et al.* 1997, Fulton *et al.* 1997a,b). The production of many BC₁F₁ plants is essential for the successful introgression of useful traits from *L. peruvianum* into cultivated tomato. The strong breeding barrier of cross incompatibility between *L. esculentum* and *L. peruvianum* is also a constraint on the production of a BC₁F₁ population as well as on that of a F₁ population. Therefore, it is very difficult to produce many BC₁F₁ plants. The reason why many BC₁F₁ plants are required is, firstly, that the number of self-compatible plants suitable for practical breeding is much lower than that estimated from the ratio of 1 : 1 in the BC₁F₁ population, in spite of the fact that self-compatible plants have to be selected out of the BC₁F₁ population. Secondly, in general, a few plants with useful traits segregate in the BC₁F₁ population. Self-compatible segregants with useful traits are likely to be found in a much lower frequency in the BC₁F₁ population.

There were no reports on cross incompatibility in the cross combination of *L. esculentum* and F₁ (*L. esculentum* × *L. peruvianum*) hybrid for the production of a BC₁F₁ population. The first objective of this study was to determine the differences among *L. esculentum* genotypes from F₁ genotypes characterized by *L. peruvianum* lines, the differences among F₁ genotypes involving wild species lines from *L. esculentum* genotypes, the differences among years, and their interactions. If there is an interaction between the *L. esculentum* genotype and 'year' for the production of a BC₁F₁ population, as in case of the production of the F₁ hybrids described by Sacks *et al.* (1997), the use of many *L. esculentum* cultivars would have the advantage of reducing the risk of interaction. The second purpose of this study was to evaluate the use of many cultivars as pistillate parents for the production of a large BC₁F₁ population.

The third objective of this study was to evaluate the segregation of traits such as sweet aroma of leaf, morphological traits and self-incompatibility in the BC₁F₁ population. The *L. peruvianum* LA 1554 line is characterized by a distinct sweet aromatic fragrance. Interspecific hybridization between the cultivated tomato and *L. peruvianum* LA 1554 was performed and F₁ plants with a sweet aromatic fragrance were observed in the F₁ population (Takashina *et al.* 1997). Suzuki *et al.* (1996) performed a GC-MS analysis of the leaf extracts of the aromatic F₁ plants and identified 71 chemical compounds among 200 compounds detected in the F₁ leaf extracts. Several compounds such as limonene (5.43%), methyl jasmonate (0.33%), eugenol (0.12%) and vanillin (0.53%) contributed to the aroma which was not previously detected in the cultivated tomato. Biosynthetic pathways of these aromatic compounds depend on many genes. Thus, the typical aroma characteristic of F₁ plants is considered to be a quantitative trait. Therefore, a large BC₁F₁ population is required to recover all the genes responsible for quantitative traits such as sweet aromatic fragrance as well as morphological traits.

Materials and Methods

Plant materials

In order to evaluate the variations among cultivars, wild species lines involved in the F₁, years and their interactions for the production of a BC₁F₁ population, cvs. Kyoryoku Ogata Toko (KOT) and Early Pink (EP) were used as pistillate parents and F₁s from four different cross combinations (KOT and EP crossed with *L. peruvianum* var. *humifusum* accessions LA2153 and LA2334, *L. peruvianum* accession PI270435 and *L. chilense* accession PI128644) were used as pollen parents, according to the report of Takashina *et al.* (1997). This experiment was conducted for 2 years in 1994 and 1995. As for the seed parents, KOT is a hybrid variety and EP is a true bred variety.

Another experiment was conducted in 1997 to evaluate 'within year variation' where a true bred variety cv. Sekaiichi (SK) and an F₁ plant (SK × *L. chilense* PI128652) were used as seed and pollen parents, respectively.

In order to evaluate the variations among cultivars and to develop a large BC₁F₁ population to introduce quantitative traits, mixed pollen from 5 selected aromatic F₁ plants (*L. esculentum* cv. KOT × *L. peruvianum* LA 1554) were used to pollinate 19 different Japanese commercial tomato cultivars as pistillate parents in 1998 (Table 1). Most of the commercial tomato varieties used in this experiment were derived from a hybrid variety. We used these varieties due to their high adaptability to cultivation in the field. Each accession of *L. peruvianum* and *L. chilense* used in this study is self-incompatible and heterogeneous in nature.

Production of a BC₁F₁ population through ovule selection and culture

The procedures for cross pollination, and ovule selection and culture adopted in the present study, were similar to those reported by Imanishi (1988). In the ovule selection and culture, about 20-30 fruits from the same cross combination were used at the same time of ovule collection. In 1994 and 1995, the number of GOFs (Germinated Ovules per Fruit) was used as key data to estimate the variation among cultivars (V), among the wild species lines involved in the production of F₁ (L), among years (Y) and their interactions (V × L, V × Y, L × Y and V × L × Y), according to the random effect model. Original data of GOFs were transformed into $\sqrt{\text{GOF} \times 100}$ for analysis of variance (ANOVA).

In 1997, within-year variation was estimated among the GOF (transformed into $\sqrt{\text{GOF} \times 100}$) values of ovule culture on different dates in the same year.

In 1998, in order to develop a large backcross population, we planned to obtain about 160 BC₁F₁ plants. Therefore, we needed to harvest approximately 970 fruits because an expected GOF value of 0.165 was estimated from the GOF values obtained by Takashina *et al.* (1997), namely 0.20 and 0.13 from KOT × (KOT × LA 1554) and EP × (EP × LA1554), respectively, for the production of the BC₁F₁ population. In general, as about 20% pollination may not

Table 1. Development of BC₁F₁ population through the ovule selection and culture method where F₁ (*L. esculentum* cv. KOT × *L. peruvianum* LA 1554) pollen was used for the crossing of 19 different tomato cultivars in 1998

Cultivar	No. of fruits harvested (A)	Weight per fruit (g)	No. of total ovules	No. of germinated ovules (B)	No. of germinated ovules per fruit {GOF = B/A}	$\sqrt{GOF \times 100}$
NATSU HIKARI	37	243	2187	16	0.43	6.5574
TOJU	25	210	977	9	0.36	6.0000
KIYOSU NO. 2 ¹⁾	19	203	534	6	0.32	5.6568
OGATA FUKUJU	52	215	1327	14	0.27	5.1961
OGATA TOKO	24	185	1201	6	0.25	5.0000
FUKUJU NO. 2	28	137	476	6	0.21	4.5825
OGATA ZUIKO	40	208	1299	8	0.20	4.4721
KYORYOKU NIKKO	49	200	1360	9	0.18	4.2426
HORYOKU	35	138	998	5	0.14	3.7416
TOKAI NO. 2	43	173	966	6	0.14	3.7416
KYORYOKU OGATA TOKO	256	225	11080	32	0.13	3.6055
KYORYOKU KYOKKO	106	169	5047	13	0.12	3.4641
MATSUDO PONDEROSA ¹⁾	27	234	593	3	0.11	3.3166
SATAN	50	194	2617	5	0.10	3.1622
EARLY PINK ¹⁾	81	154	1678	8	0.10	3.1622
KOCHI FAST	22	198	630	2	0.09	3.0000
KYORYOKU BEIJU	30	148	572	2	0.07	2.6457
MASTER NO. 2	36	190	1589	2	0.06	2.4494
TAKACHIHO	44	162	408	2	0.05	2.2360
Total	1004	—	35539	154	—	—
Average		188.74			0.175	4.0122

χ^2 -test of independence for the differences among GOFs (B/A) of 19 cultivars: $\chi^2 = 38.3246 > \chi^2_{0.001,18} = 34.81$.

Variance ($\hat{\sigma}^2_v$) in the number of GOFs calculated from $\sqrt{GOF \times 100}$: 1.5072.

¹⁾ True bred variety, the others are hybrid cultivars.

enable to develop fruits, 1213 pollinations were necessary for this experiment.

Confirmation of hybridity, evaluation of aromatic plants and characterization of the BC₁F₁ progenies

Several morphological characteristics of the putative BC₁F₁ plants were compared with those of the parents as described by Nagata and Imanishi (1984) and Doganlar *et al.* (1997) to identify the hybridity. Morphological resemblance such as growth habit, stem color and stem girth, leaf and flower morphology and aroma of the leaves were considered to be the traits required to identify the hybrid nature of the BC₁F₁ plants. Furthermore, in the 1998 experiment, a polymerase chain reaction (PCR) assay was used to confirm the hybridity of the BC₁F₁ plants. Total DNA was extracted from the fresh leaves of the BC₁F₁ plants along with their parents by using the modified CTAB method (Böhm *et al.* 1993). Eight RAPD primers from Operon 10-mer kits (OPD 1, 4, 5, 7, 9, 10, 14 and OPB 7) were used to analyze the BC₁F₁ plant-DNA. The primers that generated only clear bands specific to the wild parent were used to identify the hybridity of the BC₁F₁ plants. The details of the method applied in this experiment were described by Takashina *et al.* (1998). A BC₁F₁ plant with at least one band specific to the wild parent was considered to be a true hybrid.

All of the BC₁F₁ plants developed in 1998 were grown

in the green house in soil pots (15 cm in diameter). Sensory evaluation for the aroma was performed by a panel for this purpose in late June when the plants started flowering. The leaf odor of 100 BC₁F₁ plants was tested by a panel of 8 members that was composed of university students. A ballot paper was provided to the panel and each plant was evaluated based on the following question: "Does the sample plant (leaf) have the same leaf smell as the F₁ ?" The panelists performed the test separately and had no chance to discuss the experiment among themselves. Each member of the panel smelt the leaves of the F₁ plant and compared the smell with that of the sample plant. According to the panel opinion, the sample plants were divided into 9 classes. The evaluation of the panel was statistically determined by a non-parametric test (Sign test: Yonezawa 1988). A survey on morphology was performed and the data were recorded based on a comparison with the parental lines, e.g. *L. peruvianum* LA1554 and cv. tomato KOT. Stem color, stem girth and texture of stem; leaf shape, ratio of length and width of the leaf blade; calyx, corolla and position of the stigma in the flower were compared with those of the parental lines for this purpose. A preliminary survey was performed also for the self-compatibility of the BC₁F₁ progenies depending on their fruit setting ability.

Results

Development of BC₁F₁ population

The results from 1994 and 1995 are shown in Table 2 (cited partially from Table 3 in the report of Takashina *et al.* 1997) and ANOVA of the results according to the random effect model is shown in Tables 2 and 3. The interaction between the cultivar and wild species line ($V \times L$) was significant ($F = 12.6852$) at 1% level while other interactions such as $V \times Y$ and $L \times Y$ were not significant. The variations among 2 cultivars (V), 4 wild species lines involved in the production of F₁ hybrids as pollen parents (L) and 2 years (Y) were also non-significant.

Within-year variation was significant within the range of GOF values of 0.11–0.44 ($\chi^2 = 13.044$, $P < 0.025$; Shinjo

1986) (Table 4). Within-year variance ($\hat{\sigma}_{WY}^2$) was 2.4017, showing a variation among culture dates in 1997. The data also revealed that a higher GOF value could be obtained by collecting and culturing the ovules in early summer. The decrease in the fruit weight at later harvest adversely affected the production of ovules with a germination ability.

As one of the purposes of this study was to evaluate the segregation of quantitative traits in the BC₁F₁ population, a large BC₁F₁ population was required. Therefore, we pollinated approximately 1400 flowers from 19 different tomato cultivars in 1998 and about 83% of all the pollinated flowers set fruits. A total of 1004 fruits were harvested, from which 833 ovules were selected and cultured on MS (Murashige and Skoog 1962) medium without the use of any growth regulators. A total of 154 ovules germinated but

Table 2. Ovule selection and culture in 1994 and 1995 from the crosses of cvs. KOT and Early Pink with the F₁s from 4 different cross combinations

Seed parents	Pollen parents	No. of fruits	No. of germinated ovules	GOF ¹⁾ in 1994	GOF ²⁾ in 1995
KOT	F ₁ (KOT $\times$ LA2153)	106	10	0.11	0.08
	F ₁ (KOT $\times$ LA2334)	92	8	0.06	0.10
	F ₁ (KOT $\times$ PI270435)	88	14	0.19	0.15
	F ₁ (KOT $\times$ PI128644)	89	61	0.80	0.63
Early Pink	F ₁ (EP $\times$ LA2153)	100	4	0.07	0.03
	F ₁ (EP $\times$ LA2334)	92	7	0.07	0.08
	F ₁ (EP $\times$ PI270435)	100	7	0.08	0.07
	F ₁ (EP $\times$ PI128644)	75	15	0.14	0.22

KOT: Kyoryoku Ogata Toko, EP: Early Pink, LA 2153: *Lycopersicon peruvianum* var. *humifusum* accession LA 2153,

LA2334: *L. peruvianum* var. *humifusum* accession LA 2334, PI270435: *L. peruvianum* accession PI270435,

PI128644: *L. chilense* accession PI128644.

¹⁾ and ²⁾: Germinated Ovules per Fruit.

Note) The data were cited partially from Table 3 in the report of Takashina *et al.* (1997).

Table 3. Analysis of variance (ANOVA) in the number of GOFs for 3 factors (cultivars, wild species lines and years; data from Table 2) performed under the condition of random effect model

Sources of variation	df	Sum of square (S.S)	Mean of square (M.S)	F value	
Cultivars (V)	1	10.7256	10.7256	3.2823	$MS_V/MS_{V \times L}$
Wild sp. lines (L)	3	35.7817	11.9272	3.6500	$MS_L/MS_{V \times L}$
Years (Y)	1	0.0961	0.0961		
$V \times L$	3	9.8031	3.2677	9.9473*	$MS_{V \times L}/MS_R$
$V \times Y$	1	0.1057	0.1057		
$L \times Y$	3	0.7118	0.2373		
Residue	3	0.9854	0.3285		MS_R
E (M.S.):		$V: \sigma_{VLY}^2 + 4\sigma_{VY}^2 + 2\sigma_{VL}^2 + 8\sigma_V^2$ $L: \sigma_{VLY}^2 + 2\sigma_{VL}^2 + 2\sigma_{YL}^2 + 4\sigma_L^2$ $Y: \sigma_{VLY}^2 + 4\sigma_{VY}^2 + 2\sigma_{YL}^2 + 8\sigma_Y^2$ $V \times L: \sigma_{VLY}^2 + 2\sigma_{VL}^2$ $V \times Y: \sigma_{VLY}^2 + 4\sigma_{VY}^2$ $L \times Y: \sigma_{VLY}^2 + 2\sigma_{YL}^2$ $V \times L \times Y: \sigma_{VLY}^2$			

*): Significant at 5% level.

a few died during the cultural and acclimatization processes (Table 1). A few ovules, after germination, stopped growing and failed to differentiate into true plants. Finally, 127 backcross (BC₁F₁) plants were obtained from this experiment. A χ^2 -test of independence showed significant differences in the number of GOFs among different cultivars ($\chi^2 = 38.32$, $df = 18$, $P < 0.001$; Shinjo 1986). We observed a GOF value of 0.115 in the two cross combinations {(KOT $\times$ F₁ (KOT $\times$ LA 1554) and {EP $\times$ F₁ (KOT $\times$ LA1554)} in 1998. But the average GOF value of 19 cultivars was 0.175 in 1998 with a range of 0.43~0.05 (Table 1). The average of $\sqrt{GOF \times 100}$ was 4.0122 with a variance ($\hat{\sigma}^2_v$) of 1.5072 among 19 culti-

vars. The two tailed confidence limits of the population mean (μ) at 1 % level were 3.2017 and 4.8215. Based on this calculation, 5 cultivars, e.g. Natsuhikari (6.55), Toju (6.00), Kiyosu No.2 (5.65), Ogata Fukuju (5.19) and Ogata Toko (5.00) were beyond the upper confidence limit and could be selected as pistillate parents with a higher cross-compatibility for the production of the BC₁F₁ population.

The variation was estimated among the F₁ lines (derived from different pollen parents) crossed with cvs. KOT or EP as shown in Table 5. The variances among the wild species lines involved in the F₁ as a pollen parent were 13.3151 ($df = 7$) for KOT and 2.5893 ($df = 5$) for EP, respectively. The

Table 4. Within-year variation estimated from the data for the production of BC₁F₁ population through backcrosses between SK $\times$ F₁ (SK $\times$ PI128652) in 1997

Date of culture	No. of fruits (A)	Fruit weight (g)	No. of germinated ovules (B)	GOF ¹⁾	$\sqrt{GOF \times 100}$
1-Aug	50	124	22	0.44	6.633
6-Aug	42	147	16	0.38	6.164
7-Aug	42	116	10	0.24	4.899
19-Aug	44	64	5	0.11	3.317
24-Aug	46	85	5	0.11	3.317
Total	224		58		
Average		107.20		0.259	

¹⁾: Germinated Ovules per Fruit.

Within-year variance (calculated from $\sqrt{GOF \times 100}$): $\hat{\sigma}^2_{wY} = 2.4017$.

χ^2 (calculated from A and B) = 13.0447 > $\chi^2_{0.025,4} = 11.14$ (Shinjo 1986).

SK: cv. Sekaiichi, PI128652; *L. chilense* accession PI128652

Table 5. Variation among the wild species lines involved in the F₁ for the production of BC₁F₁ population in 1995 from the crosses between KOT $\times$ F₁s from 'KOT $\times$ 8 wild species lines' and EP $\times$ F₁s from 'EP $\times$ 6 wild species lines'

Seed parent	Pollen parent	GOF	$\sqrt{GOF \times 100}$	df	F-value
KOT	F ₁ (KOT $\times$ LA2153)	0.08	2.8284		
	F ₁ (KOT $\times$ LA2334)	0.10	3.1622		
	F ₁ (KOT $\times$ LA1554)	0.20	4.4721		
	F ₁ (KOT $\times$ LA2575)	1.36	11.6619		
	F ₁ (KOT $\times$ LA1722)	0.19	4.3589		
	F ₁ (KOT $\times$ PI270435)	0.15	3.8729		
	F ₁ (KOT $\times$ PI128644)	0.63	7.9372		
	F ₁ (KOT $\times$ PI128652)	1.32	11.4891		
		Variance: 13.3151		7	40.5330**
		MS _R ¹⁾ : 0.3285		3	
Early Pink	F ₁ (EP $\times$ LA2153)	0.03	1.7320		
	F ₁ (EP $\times$ LA2334)	0.08	2.8284		
	F ₁ (EP $\times$ LA1554)	0.13	3.6055		
	F ₁ (EP $\times$ LA2575)	0.00	0.0000		
	F ₁ (EP $\times$ PI270435)	0.07	2.6457		
	F ₁ (EP $\times$ PI128644)	0.22	4.6904		
		Variance: 2.5893		5	7.8822
		MS _R ¹⁾ : 0.3285		3	0.05 < p < 0.10

¹⁾: MS_R of Table 3.

**): Significant at 1 % level; KOT: cv. Kyoryoku Ogata Toko; EP: cv. Early Pink.

GOF: Germinated Ovules per Fruit.

Note: The data were cited partially from Table 3 in the report of Takashina *et al.* (1997).

variances for the wild species lines involved in the cross combinations in 1995 were treated in an F-test using the residue (MS_R , $df = 3$) in Table 3 that were highly significant for KOT at 1% level ($F = 40.5330$, $df = 7$), but not significant for EP ($F = 7.8822$, $df = 5$).

ANOVA between the years of the production of the BC_1F_1 population (1995: Table 5, 1998: Table 1) in the cases of cvs. KOT and EP crossed with KOT $\times$ LA1554, revealed that considerable variations occurred among the cultivars ($F = 9.664$, $df = 1$) and years ($F = 9.664$, $df = 1$) even though these were statistically non-significant and their interaction ($V \times Y$) was very small ($MS = 0.0444$, $df = 1$).

Identification of hybridity, evaluation of aromatic plants and characterization of the BC_1F_1 progenies

The morphological characteristics of the BC_1F_1 plants such as growth habit, stem color, stem girth and petal size of the flowers resembled those of the parental lines and were intermediate between those of the two parents. The aroma of the leaves, deep serration in the leaf margins of the BC_1F_1 plants resembled those of the wild parent *L. peruvianum* LA 1554, and confirmed the hybrid nature in most of the BC_1F_1 plants. However, morphological characteristics of some of the BC_1F_1 plants were not sufficiently distinct to confirm their hybridity. A PCR based analysis was then used for further confirmation. Twelve RAPD bands specific to the wild parent were generated using 8 primers. Among the 100 BC_1F_1 plants subjected to the RAPD analysis, a total of 89 plants showed a minimum of one (Fig. 1) to a maximum of 6 bands specific to the wild parent (Table 6-a). Five BC_1F_1 plants showed 6 bands specific to the wild parent. Eleven plants failed to show any parent-specific band with the primers used in this experiment, but the morphological

characteristics were prominent enough to confirm the hybrid nature of these plants.

A total of 100 BC_1F_1 plants were evaluated by the sensory panel compared with the F_1 plant and the scores are summarized in Table 6-b. The data revealed a considerable variation in the panel opinion. Agreement from all of the 8 members was obtained for 2 BC_1F_1 plants (class A). Based on a non parametric test (sign test; Yonezawa 1988), this result was statistically significant at 1% level ($P = 0.008$). In contrast, all the members of the panel disagreed about 24 BC_1F_1 plants (class I). Although the typical aromatic fragrance was observed in the other plants of the population, but the panel opinion differed.

Lycopersicon peruvianum is strictly self-incompatible and is characterized by a prostrate habit of the stem and comparatively smaller leaf blades with pointed tips. The style of the flower is relatively longer and the stigma is exerted and lies outside of the pollen sacs, which is a typical characteristic of a self-incompatible wild line. In contrast, self-compatible commercial hybrid cultivars, used as seed parents for producing a BC_1F_1 population, have a strong, erect and vigorous stem; the leaf blade is wider and the leaf apex is more or less oval in shape, deep green in color; flowers are also vigorous and petals are thicker than those of *L. peruvianum*, the style is comparatively shorter and the stigma stays inside and provides the physiological basis for the self-compatibility of cultivated tomato. In the BC_1F_1 population, the stems of 79 plants were the same or similar to the *L. peruvianum* stem, 8 plants were found to be the same as or similar to the cultivated tomato whereas, 13 plants were intermediate between the two. The morphology of most of the leaves and flowers of the population was intermediate between the two (74%) but that of others was

Fig. 1. Polymorphic RAPD bands specific to the wild and cultivated parents generated by a random primer OPD-7. BC_1F_1 plants no. 2, 3, 5, 6, 7, 8 and 10 showed wild parent specific bands (b, c) and all of the plants showed cultivated parent specific band (a). M: pHY marker, W: wild parent, CV: cultivated tomato parent.

Table 6. Characterization of BC₁F₁ population: a) BC₁F₁ population was divided into six classes depending on the number of parent-specific RAPD bands generated by 8 primers.
 b) Segregation of aroma in 100 BC₁F₁ plants (leaves) was revealed by sensory evaluation.
 c) Morphology of stem (S), leaf (L) and flowers (F) of BC₁F₁ plants was compared with those of the *L. peruvianum* (P) and cv. tomato. d) Morphology of self-compatible (SC) and self-incompatible (SI) progenies in the BC₁F₁ plants

a)										
No. of bands	0	1	2	3	4	5	6	Total		
No. of plants	11	16	38	17	8	5	5	100		
b)										
Class ¹⁾	A	B	C	D	E	F	G	H	I	Total
No. of plants	2*	2	14	13	9	11	8	17	24	100
c)										
Overall type ²⁾	S.P.	S.I.	S.T.	L.P.	L.I.	L.T.	F.P.	F.I.	F.T.	
No. of plants	79	13	8	19	74	7	14	74	12	
Total	100			100			100			
d)										
Type ³⁾	SC.P.	SC.I.	SC.T.	SI.P.	SI.I.	SI.T.	Total			
No. of plants	4	21	11	29	33	2				
Total SC and SI	36			64			100			

¹⁾: A, B, C, D, E, F, G, H and I are classes of BC₁F₁ plants depending on panel opinion where the 8 panelists were asked whether the BC₁F₁ plant sample had the same smell as the F₁. Class A: agreement from 8 panelists, class B: agreement from 7 panelists, class C: 6; class D: 5, class E: 4, class F: 3, class G: 2, class H: agreement from 1 panelist and class I: 8 panelists disagreed.

*: 2 BC₁F₁ plants were determined to have the same sweet smell as the F₁ at 1% level of significance based on non-parametric sign test (Yonezawa 1988).

²⁾: S.P. = Stem resembles that of *L. peruvianum*, S.I. = Intermediate type of stem, S.T. = stem resembles that of cv. tomato; L.P. = Leaf resembles that of *L. peruvianum*, L.I. = Intermediate type of leaf, L.T. = Leaf resembles that of cv. tomato; F.P. = Flower resembles that of *L. peruvianum*, F.I. = Intermediate type of flower, F.T. = Flower resembles that of cv. tomato.

³⁾: SC.P. = Self-compatible but resembles *L. peruvianum*, SC.I. = Self-compatible and intermediate, SC.T. = Self-compatible but resembles cv. tomato; SI.P. = Self-incompatible but resembles *L. peruvianum*, SI.I. = Self-incompatible and intermediate type, SI.T. = Self-incompatible but resembles cv. tomato; SC = Self-compatible, SI = Self-incompatible.

the same as or similar to that of their parental lines (Table 6-c). Most of the 'self-compatible' BC₁F₁ plants belonged to the intermediate group (21) or cultivated tomato type (11), whereas, only 4 BC₁F₁ plants were found to be self-incompatible as they morphologically resembled *L. peruvianum*. On the other hand, self-incompatible BC₁F₁ plants belonged mainly to the other two groups, intermediate (33) and the '*peruvianum*' type (29) morphologically (Table 6-d).

Another survey revealed that the number of self-compatible BC₁F₁ plants was fewer than the expected number. A total of 36 percent of the BC₁F₁ plants was self-compatible, yielded fruits and produced viable seeds. The remaining plants (64%) were self-incompatible and failed to produce fruits following self- and even open pollination (Table 6-d).

Discussion

Takashina *et al.* (1997) observed a significant correlation between the number of GOFs for the production of F₁ and BC₁F₁ in their study. They pointed out the difficulty in producing BC₁F₁ plants as well as F₁. But variations among

cultivars, among wild species lines, among years and their interactions were not studied. In order to develop a large BC₁F₁ population, a breeding strategy to enhance production efficiency is required. In the first step of this study, 3 factors — cultivars as seed parents (V), wild species line involved in the F₁ (L), year (Y) and interactions among these 3 factors were investigated including within-year variation. ANOVA of the results of 1994 and 1995 showed a statistically significant interaction between the cultivars and wild species lines (V × L). This is the first study in which significant interaction between cultivars and wild species is reported. Based on the results obtained, it is difficult to identify common cultivars with a 'high cross compatibility' as seed parents because the interaction was significant and the cross compatibility of a certain cultivar(s) was specific to a specific wild species line for the production of the BC₁F₁ population. It remains to be determined whether some of the cultivars those are high cross compatible to a specific wild species line will show the similar cross compatibility with the other wild species lines.

The significant differences among the GOF values of

the cultivars to obtain BC₁F₁ progenies in the 1998 experiment suggest that the crossability to the specific F₁ line derived from the crosses between cultivated tomato and the 'peruvianum-complex' may vary depending on the cultivars. The cultivars Natsuhikari, Toju, Kiyosu No. 2, Ogata Fukuju and Ogata Toko with higher GOF values showed a higher cross compatibility with *L. peruvianum* LA1554. As a result, those cvs. could be selected as seed parents for efficient production of a large population of interspecific hybrids and advanced generations intercrossed with the *peruvianum* line LA1554. Intercrossing with other wild species lines, may result in differences depending on the significant interaction between the cultivar and wild species line (V × L), as revealed in the present study.

The variation among the wild species lines was much larger than the variation observed among the cultivars. These results suggest that wild species lines exert significant effects on the efficient production of a BC₁F₁ or F₁ population. The efficiency of the production of progenies could be increased by using wild species lines with a higher cross compatibility.

Sacks *et al.* (1997) observed a significant interaction between the cultivar and year (V × Y) by using 41 cultivars for the production of F₁ hybrids. However the interaction of V × Y was found to be non-significant by Egashira *et al.* (1999) for the production of the F₁ population. Our results are in agreement with the findings of Egashira *et al.* (1999), even though those were related to the production of the BC₁F₁ population. The interaction might vary depending on the years. Comparison between the years 1994-1995 and 1995-1998 did not show a significant difference, although there may be a significant difference among years, as Sacks *et al.* (1997) and Egashira *et al.* (1999) pointed out for the production of F₁ hybrids. Since the interaction V × Y varies with the years, the existence of V × Y can not be confirmed unless experiments are carried out. We obtained a comparatively higher within-year variation which was statistically significant. Within-year variation is considered to affect the interaction of V × Y. Our strategy of 'using many cultivars as seed parents' may enable to reduce the risk of interaction of V × Y by decreasing the variation among years.

The expected average GOF value of 0.165 for the production of BC₁F₁ plants, was adopted based on 2 GOF values of 0.20 and 0.13, obtained in 1995 by Takashina *et al.* (1997) in two cultivars — KOT and EP, respectively. The individual GOF values of the two cultivars (0.13 and 0.10 respectively) decreased in 1998 compared with those reported by Takashina *et al.* (1997). In contrast, the average GOF value (0.175) was higher than the expected GOF value (0.165). This comparison suggests that the effect of a year may adversely affected the production of progenies in 1998, though the values were not statistically significant in the ANOVA between 1995 and 1998 in the production of the BC₁F₁ population from the cvs. KOT and EP crossed with LA 1554. By using many cultivars of unknown crossability for the production of a BC₁F₁ population, 8 cultivars were

found to show a higher GOF value than the expected GOF value of 0.165. Therefore, the average GOF value was not adversely affected in spite of the adverse effect of the year. The use of many cultivars could enhance the probability to obtain a stable and consistent average GOF value.

The self-incompatibility in *L. peruvianum* is very strict and occurs generally (McGuire and Rick 1954, Nettancourt *et al.* 1974). The self-incompatibility of *Lycopersicon* is considered to be controlled by a single self-incompatibility (S) – locus gene. In the production of BC₁F₁ fruits, 36 percent of the plants were found to be self-compatible, a value lower than the expected ratio of 1 : 1. Several researchers have reported that the self-incompatibility in the progenies of the interspecific hybrids between cultivated tomato and some wild species may be controlled by two or more genes (Nettancourt *et al.* 1973, 1974, Hogenboom 1972). In addition, the dual incompatibility – unilateral stylar incompatibility along with the self-incompatibility, and genetic rearrangement may account for the large number of undetectable segregations and all these factors may affect the reduction in the number of self-compatible plants in the BC₁F₁ population (Nettancourt *et al.* 1973, 1974, Hogenboom 1972). Environmental factors may also account for the sensitivity of the factors associated with self-incompatibility (Nettancourt *et al.* 1971).

Five BC₁F₁ plants showed 6 RAPD bands specific to the wild parent species, suggesting that a quantitative trait involving 6 loci could be successfully introduced into a population of 100 BC₁F₁ plants derived from the cross between a cultivated tomato and an accession of the 'peruvianum-complex'. The sensory panel evaluation suggested that the number of BC₁F₁ plants having the same aromatic fragrance as the F₁ plant is very low. However, the results also indicated that the segregation of aroma occurred clearly in the BC₁F₁ population. Other BC₁F₁ plants may have a similar aroma to that of the F₁ and contain some of the QTLs of the aroma with various combinations of genes. Chemical analyses such as GC-MS are required for further confirmation of the aromatic constituents in the BC₁F₁ population, as in case of the F₁ plants (Suzuki *et al.* 1996).

The objective of the research reported here was to evaluate the variation among cultivars, wild species lines, between and within years, interactions, and to obtain a large BC₁F₁ population to evaluate the segregation of some characteristics such as sweet aroma in the BC₁F₁ population produced by using many cultivars. In conclusion, we obtained a large BC₁F₁ population with an expected number of plants. Sensory attributes of some of the BC₁F₁ progenies suggested that the expected quantitative trait had been successfully introduced into the BC₁F₁ population.

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