Chapter V

Conclusion

In Chapter Ⅱ, the ovule selection culture mothod was evaluated in regard to the production of F_1 hybrids and BC_1 generations, which have not yet been observed between *L. esculentum* and the wide accessions of the "*peruvianum*-complex". Two cultivated tomatoes ('Kyoryoku Ogata Toko' and 'Early Pink') as seed parents and 9 lines of "*peruvianum*-complex" (2 lines of *L. peruvianum* var. *humifusum*, 5 lines of *L. peruvianum* and 2 lines of *L.chilense*) as pollen parents were used for obtaining F_1 hybrid and BC_1F_1 backcross generation by using the ovule selection culture method. In respect to the efficiency of obtaining F_1 and BC_1F_1 (which is evaluated by the number of germinated ovules per fruit) (GOF), three correlation coefficients were calculated, between the two cultivated tomatoes, between 1994 and 1995 for BC_1F_1 and between F_1 and BC_1F_1. Between the two cultivated tomatoes, a significant, combined correlation coefficient was obtained from the 3 correlation coefficients of F_1 in 1992, two BC_1F_1

in 1994 and 1995 (r=0.750**, d.f.=11). Between the two years, a significant, high, and combined correlation coefficient was obtained (r=0.907*, d.f.=3). The correlation coefficients between F_1 and BC_1F_1 and a combined correlation coefficient were not significant in either of the two cultivated tomatoes. One of the 9 lines used showed a different and unusual GOF for F_1 and BC_1F_1. After the one line is excluded, the correlation coefficient become significant (r=0.754*, d.f.=5). The analysis of the correlation coefficient indicates that the differences between the lines of "*peruvianum*-complex" as regards the cross-incompatibility with a cultivated tomato exist and that the differences are found similarly in both F_1 and BC_1F_1. The two lines of *L. peruvianum* var. *humifusum* belong to the highest cross-incompatibility group, most of the lines of *L. peruvianum* belong to the intermediate group, and the two lines of *L. chilense* belong to the least cross-incompatibility group. The alternative relationship between F_1 and BC_1F_1 was evaluated by linear regression : $Y\ (BC_1F_1) = 0.108X\ (F_1) + 0.336$ for KOT, $Y = 0.105X + 0.037$ for EP. From the result, it is shown that BC_1F_1 is not bigger than F_1 in terms of GOF. However, F_1 hybrids and BC_1F_1 generations could be obtained from almost the wild lines,

and the efficiency of the ovule selection culture was proved.

The purpose of Chapter Ⅲ was to identify molecular markers that can assist in the selection of high shoot regeneration capacity using BC_1F_1 and BC_2F_1 generations. The two generations between a tomato cultivar, KOT, and *L. chilense* PI128644 (together with both the parents and the F_1 hybrids) were used as materials. BC_2F_1 generation was developed by a cross between KOT and a self-compatible BC_1F_1 plant that had high shoot regeneration capacity. The root explant culture on the MS medium which was supplemented with 1 mg/l of zeatin riboside showed that KOT did not form any shoots, while those of the PI128644 and the F_1 hybrids had a shoot regeneration (SR) rate more than 80 %. The BC_1F_1 generation (which included 22 plants) indicated a two-peak distribution of frequency (less than 30 % and more than 80 %) in regards to the SR rate, but the BC_2F_1 generation (which included 113 plants) had a flat distribution of frequency more than 0 % to 100 % without a exceeding distribution of 0 %. The segregation of the RAPD markers and the acid invertase marker (inv^{chi}) which was specific to the PI128644, was evaluated for BC_1F_1 and BC_2F_1 to determine a possibility of linkage between these markers and

the shoot regeneration capacity. The Mann-Whitney test showed that the RAPD markers, (OPA02-1 and OPA20-3) and *inv*^{chi} were mostly present in the plants with the high shoot regeneration capacity but absent in those without any high shoot regeneration capacity. It was suggested that these three molecular markers, (OPA02-1, OPA20-3, and *inv*^{chi}) are closely linked to the high shoot regeneration capacity of *L. chilense* PI128644. However, the genetic model of the superior shoot regeneration capacity could not be evaluated, because of their huge skewed segregations.

In the Chapter Ⅳ, the purpose was to identify the genes controlling the shoot regeneration capacity and to construct the linkage map. BC_1F_2-44-15 generation that was obtained by selfing BC_1F_1-44-15 plant was used as material. Contrary to the BC_2F_1 generation, BC_1F_2 generation did not indicate any skewed segregation in regard to the SR rate and the markers which linked with the shoot regeneration capacity. The SR rate of the BC_1F_2 plants indicated the distinct two-peak distribution : one has more than 40 % of SR rate and the other has less than 20 % of SR rate. The segregation ratio, 31 : 11, fitted the expected ratio, 3 : 1, that is the ratio of a dominant gene of F_2, suggesting that a major

dominant gene, designated *Rg*, would control this trait. Three new RAPD markers linked with this trait were detected. All 6 PCR-based markers (including 2 RAPD and *inv* that are described in Chapter Ⅲ) were mapped on the same linkage group. And the *Rg* locus was mapped between OPA02-900 and OPB12-480. As the result of RFLP analysis, TG102 that is located on the chromosome 3 was mapped on the same linkage group between OPC02-450 and OPC19-680. This indicates that this linkage group is on the chromosome 3 and suggestes that the *Rg* locus of *L. chilense* PI128644 is the same locus of the *Rg*-1 that controls the superior shoot regeneration capacity of *L. peruvianum* (Koornneef *et al.* 1993).

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Appendix 1

Site information of the wild species, "*peruvianum*-complex"

Species	Accession number	Nation	Latitude and longitude	Collected site
L. peruvianum var. *humifusum*	LA2153	Peru	S15° W75°	San Juan, Cajamarca
	LA2334	Peru	S15° W75°	San Juan, Cajamarca
L. peruvianum	LA1554	Peru	S11° W77°	Rio Huaura, Lima
	LA1722	Peru	S13° W75°	Ticrapo Viejo, Huancavelice
	LA2575	Peru	S11° W79°	Valle de Casma, Ancash
	PI126944	nc	nc	nc
	PI270435	nc	nc	nc
L. chilense	PI128644	Peru	S17° W70°	Moquegua
	PI128652	Chile	S19° W70°	Azapa valley

nc: not clear

Appendix 2-1

Plant posture and floral type of "*peruvianum*-complex" wild accessions

LA2153 : *L. peruvianum* var *humifusum*

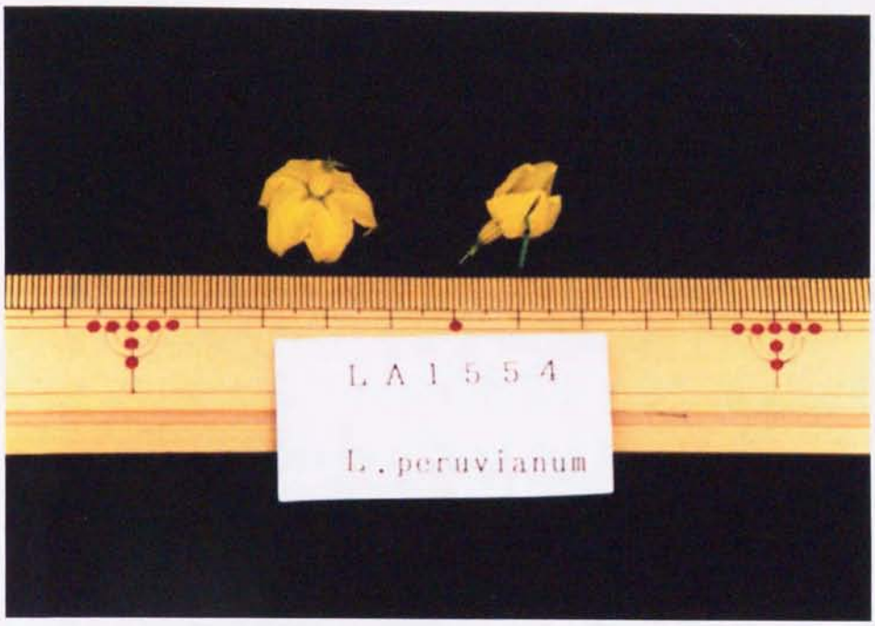
Appendix 2-2

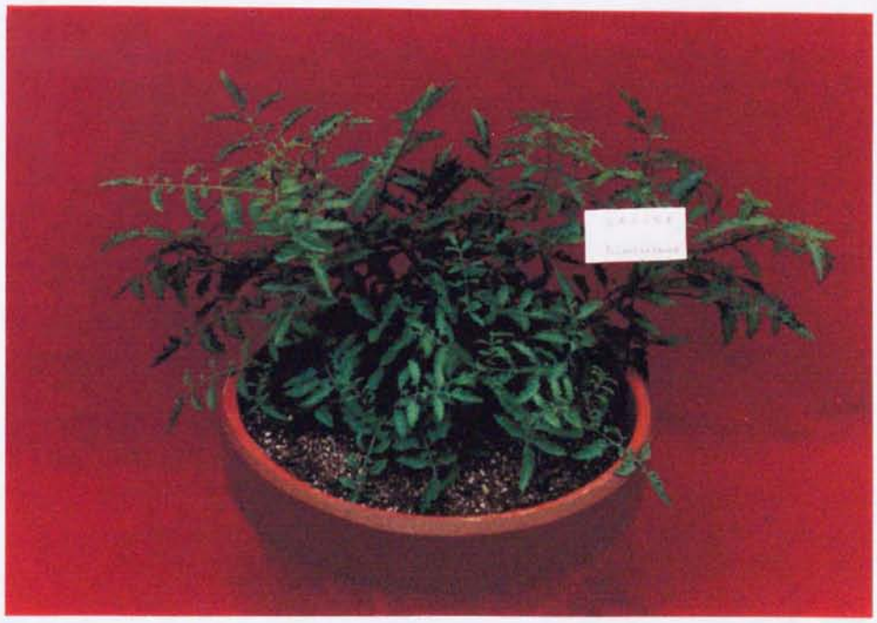
Plant posture and floral type of "*peruvianum*-complex" wild accessions

LA2334 : *L. peruvianum* var. *humifusum*

Appendix 2-3

Plant posture and floral type of "*peruvianum*-complex" wild accessions

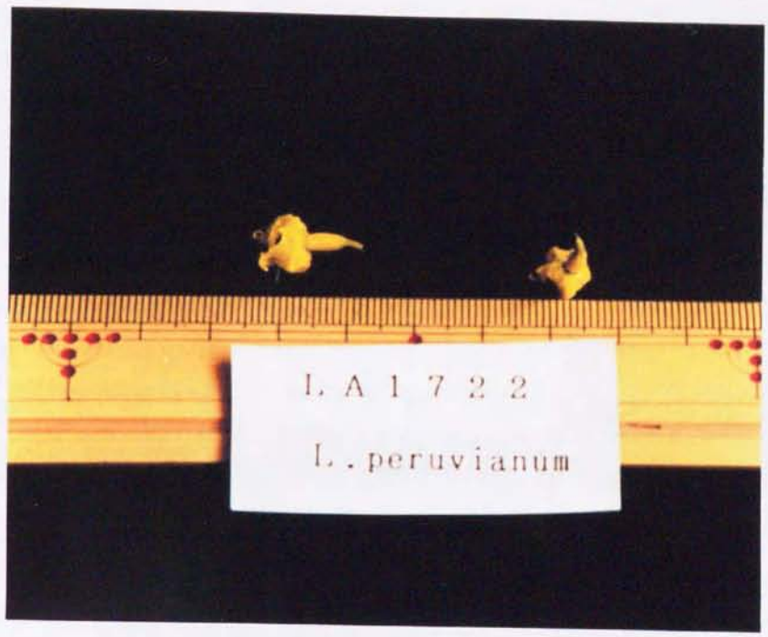




LA1554 : *L. peruvianum*

Appendix 2-4

Plant posture and floral type of "*peruvianum*-complex" wild accessions



LA1722 : *L. peruvianum*

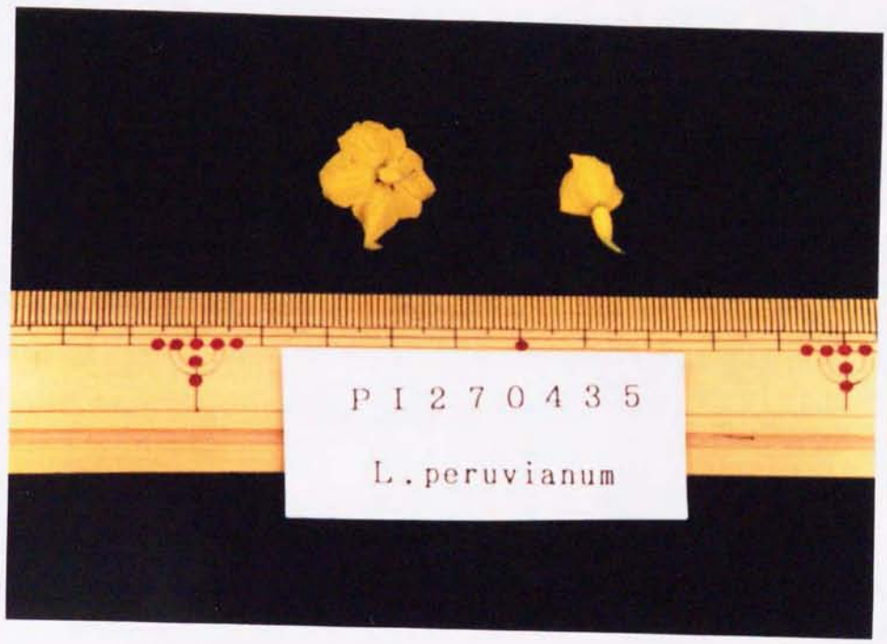
Appendix 2-5

Plant posture and floral type of "*peruvianum*-complex" wild accessions

LA2575 : *L. peruvianum*

Appendix 2-6

Plant posture and floral type of "*peruvianum*-complex" wild accessions



PI270435 : *L. peruvianum*

Appendix 2-7

Plant posture and floral type of "*peruvianum*-complex" wild accessions

PI128644 : *L. chilense*

Appendix 2-8

Plant posture and floral type of "*peruvianum*-complex" wild accessions

PI128652 : *L. chilense*

Appendix 3

Fruits of F_1 hybrids between *L. esculentum* and "*peruvianum*-complex" wild accessions

Fruits of a hybrid between KOT and *L. chilense* PI128644

Fruits of a hybrid between KOT and *L. peruvianum* PI270435

Fruits of a hybrid between EP and *L. peruvianum* LA1554

Appendix 4

Selected ovules of a F_1 hybrid between EP and *L. peruvianum* LA1554

Germinated ovules of a F_1 hybrid between KOT and *L. chilense* PI128644

Appendix 5

Stock solution using to isolate DNA for PCR analysis	
2-Mercaptoethanol	
1.0 M Tris-HCl (pH7.5)	
1.0 M Sorbitol	
1.0 M $Na_2S_2O_5$	
0.5 M EDTA	
TE (10 : 1) buffer (pH8.0)	
Na-N-Lauryl sarcosine	5 % (w / v)
Isopropanol	store at -20 ℃
80 % ethanol	store at -20 ℃
Chloroform-isoamylalcohol	Chloroform : isoamylalcohol = 20 : 1 (v / v)
Extraction buffer	0.35 M Sorbitol, 0.1M Tris-HCl (pH 7.5), 0.005M EDTA, 0.02M $Na_2S_2O_5$
Lysis buffer	0.2M Tris-HCl (pH 7.5), 0.005M EDTA, 2M $NaCl_2$, 2% CTAB (w / v)

Appenndix 6

Stock solution using to isolate DNA for Southern hybridazation	
2-Mercaptoethanol	
1.0 M	Tris-HCl (pH 8.0, 7.5)
1.0 M	EDTA pH8.0
3.0 M	Sodium acetate adjusted by acetic acid (pH 5.2)
TNE buffer	0.05 M Tris-HCl, 0.2 M EDTA (pH8.0), 0.1 M NaCl
Proteinase K	20mg / ml in TE buffer
RNase A	10mg / ml, 10 mM Tris-HCl (pH 7.5), 15 mM NaCl

Extraction buffer for 10ml	
7.6 ml	TNE buffer
0.6 ml	10% (w/v) SDS
0.06 ml	20mg ml-1 Proteinase K
1.74 ml	H_2O
40 mg	Sodium bisulphite ($Na_2S_2O_5$)
40 mg	Sodium diethldithiocarbamate ($C_5H_{10}NS_2Na$)

CTAB buffer	
0.2 M	Tris-HCl pH 7.5
0.05 M	EDTA pH 8.0
2.0 M	NaCl
2 % (w/v)	hexadecyltrimethil ammonium bromide (CTAB)

CIA	chloroform : isoamylalcohol = 24 : 1
Phenol (pH. 7.5)	store at 4 ℃
Chloroform	store at room temprature
100 % ethanol	store at -20 ℃
80 % ethanol	store at -20 ℃
Isopropanol	store at -20 ℃

Appendix 7

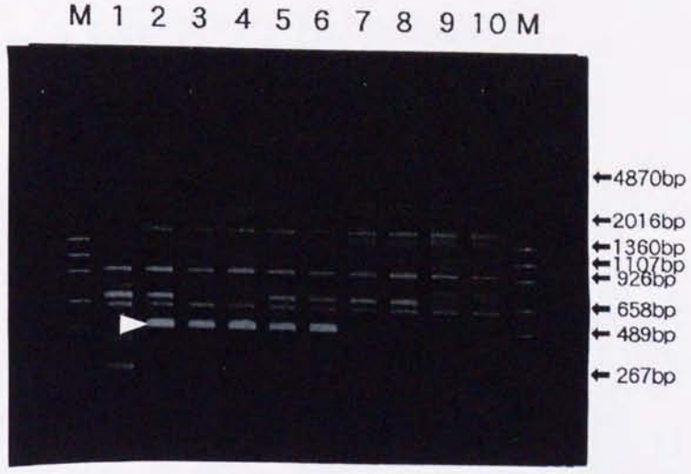
Stock solution using for Southern hybridization	
Denaturation solution	0.5 M NaOH, 1.5 M NaCl
Neutralization solution	1 M Tris-HCl (pH 7.5), 1.5 M NaCl
Transfer solution (20×SSC)	3 M Trisodium citrate dihydrate, 3 M NaCl
Hybridization solution	1 % w/v Blocking reagent 0.1 % w/v N-laurylsarcosine SDS (0.02 % w/v), 5 ×SSC

For washing	
Washing buffer 1	2×SSC, 0.1 % SDS (w/v)
Washing buffer 2	0.1×SSC, 0.1 % SDS (w/v)

For detection	
Buffer 1	0.1 M Malic acid, 0.15 M NaCl
Buffer 2	Buffer 1 + 1 % w/v Blocking reagent
Buffer 3	0.1 M Tris-HCl (pH 9.5), 0.1 M NaCl, 0.05 M $MgCl_2$

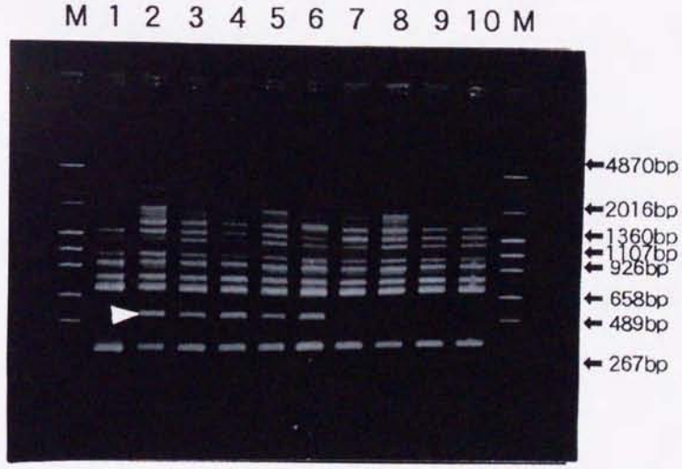
DIG Nucleic Acid Detection Kit (BOEHRINGER MANNHEIM)

Appendix 8



8-1. Segregation of the RAPD marker OPB12

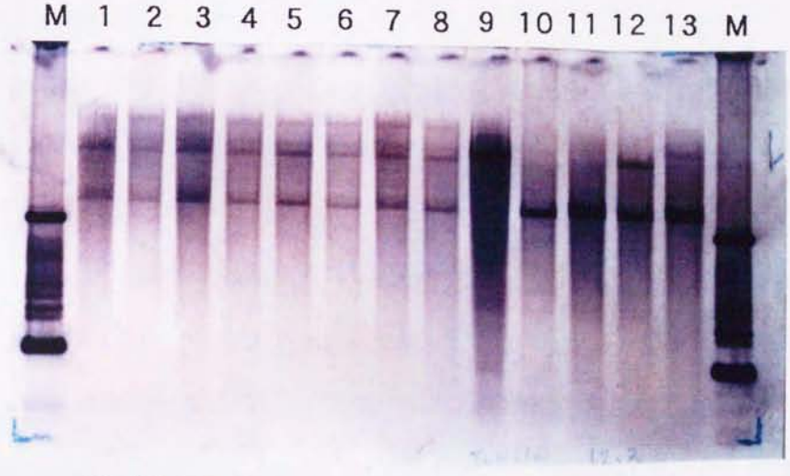
Lane number M; pHY marker, 1; *L. esculentum* cv. 'Kyoryoku Ogata Toko' , 2; BC_1F_1-44-15, 3～6; BC_1F_2-44-15-2, -3, -4, -5, respectively., 7～10; BC_1F_2-44-15-16, -18, -19, -21, respectively. The arrow head indicates the RAPD marker OPB12-480.



8-2. Segregation of the RAPD marker OPC02

Lane number M; pHY marker, 1; *L. esculentum* cv. 'Kyoryoku Ogata Toko' , 2; BC_1F_1-44-15, 3～6; BC_1F_2-44-15-2, -3, -4, -5, respectively., 7～10; BC_1F_2-44-15-16, -18, -19, -21, respectively. The arrow head indicates the RAPD marker OPC02-450.

Appendix 9



Segregation of the RFLP marker TG102 in the BC_1F_2-44-15 plants

Lane number : M; marker, 1～13; BC_1F_2-44-15 -52, -53, -56, -59, -61, -62, -66, -67, -69, -70, -75, -78, -79, respectively.

1～8; Heterozygote, 9; Wild type, 10～11; Tomato type, 12～13; Heterozygote

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The author