Wide Cross Hybridization of the Genus *Lycopersicon* and Genetic Analysis of a Superior Shoot Regeneration Capacity

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WIDE CROSS HYBRIDIZATION OF THE GENUS *LYCOPERSICON* AND GENETIC ANALYSIS OF A SUPERIOR SHOOT REGENERATION CAPACITY

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要約

トマト栽培種と '*peruvianum*-complex' の間には強い交雑障害が存在し，雑種獲得が非常に困難である．また，その戻し交雑世代の獲得においても交雑障害が存在する．本研究では，まず最初に '*peruvianum*-complex' に属する種々の系統について，トマト栽培種との交雑不親和性を評価するために胚珠選抜培養法を用いて F_1 世代および BC_1F_1 世代の獲得を試みた．

トマト野生種群 '*peruvianum*-complex' に属する *Lycopersicon peruvianum* 5 系統，*L. peruvianum* var. *humifusum* 2 系統，*L. chilense* 2 系統を花粉親に，栽培種 2 品種を種子親に用いた．F_1 雑種および F_1 雑種を花粉親とする BC_1F_1 戻し交雑種は胚珠選抜法によって育成した．F_1 雑種および BC_1F_1 の獲得効率は GOF(果実あたり発芽数)により評価した．F_1 雑種および 1994 年と 1995 年の BC_1F_1 について GOF の栽培品種間の相関係数を求め，さらに，それらを組合わせた相関係数を求めたところ，正の有意な値となった（r=0.750**,d.f.=11）．年次間においても組合わせた相関係数は有意な正の高い値となった（r=0.907*,d.f.=3）．F_1 雑種と BC_1F_1 間相関係数は，供試系統の中の 1 系統(LA2575)を除くと正の有意な相関係数が得られた(強力大型東光: r=0.754*, d.f.=5; Early Pink: r=0.924*, d.f.=3).

得られた相関係数の結果は，栽培種に対する野生種の交雑不親和性は，野生種系統間で差があり，さらに BC_1F_1 の獲得において野生種の各系統の交雑不親和性が F_1 雑種の場合と同じように現れることを示している．供試した系統の交雑不親和性を 3 グループに分けると次のようになった．最も交雑不親和性の高いグループに *L. peruvianum* var. *humifusum* の 2 系統が入っており，中間のグループは全て *L. peruvianum* であった．最も交雑不親和性の低いグループは *L. chilense* の 2 系統であった．一方，F_1 雑種と BC_1F_1 間の GOF の回帰直線は，$Y\ (BC_1F_1) = 0.108X\ (F_1) + 0.336$: 強力大型東光，$Y = 0.105\ X + 0.037$: Early Pink となった．この結果から，予想に反して BC_1F_1 の獲得効率が F_1 雑種よりも小さいことが推察された．しかしながら，全ての組合せから F_1 雑種が得られ，ほとんどの BC_1F_1 も得られたことから，胚珠選抜法が

'*peruvianum*-complex' の雑種獲得に広く有効であることが示された.

トマト野生種 '*peruvianum*-complex' は，高再分化能を持つ．本研究では，つぎに '*peruvianum* complex' を構成する野生種の 1 つ *L. chilense* の高再分化能に注目し，先に得られた戻し交雑世代を用い，高再分化能を持たない栽培種にこの形質を導入し，遺伝的メカニズムを解析するため，高再分化能に連鎖する分子マーカーの探索を行ない，連鎖地図の作成を試みた.

まず，BC_1F_1，BC_2F_1 世代を用いて，この形質に連鎖する分子マーカーの探索を行なった．再分化能の判定は，根切片を 1 mg/l zeatin riboside, 2 % sucrose を含む MS 培地で 4 週間培養して行なった．BC_1F_1 世代における再分化能の分離は 2 頂分布を示したが，BC_2F_1 世代では全体の 70%を超える 86 個体が栽培種と同じ 0%を示し，残りの 27 個体が 100%まで連続的な分布を示した．BC_1F_1 および BC_2F_1 世代における野生種特異的 RAPD マーカーと酸性インベルターゼ遺伝子(*inv*)の分離を調査した結果，高い再分化率を示した個体中には高頻度で存在し，再分化率 0 %の個体中にはほとんど存在しない 3 つのマーカー（OPA02-1，OPA20-3，inv^{chi})を見出した．Mann-Whitney の U 検定の結果，これら 3 つのマーカーと再分化率の関係が 1 %水準で有意であることが示され，再分化能遺伝子とこれらのマーカーが連鎖する可能性が示唆された．しかしながら，これらのマーカーには分離の歪みが観察され，再分化率の分離もどの理論比にも適合しなかったため遺伝様式については検討できなかった.

したがってつぎに，BC_1F_2 世代を用い同様の解析を行った．任意に培養した 42 個体の BC_1F_2 世代は，3 : 1 に分離し(再分化率 20%以下と 40%以上で)，この形質が優性の主働遺伝子（*Rg*）によって支配されていることを示唆した．また新たに見つかった 3 つの RAPD マーカーを加え計 6 個の分子マーカーが，全て同一連鎖群に属することが示され，再分化遺伝子 *Rg* は RAPD マーカーOPA02-900 と OPB12-480 の中間ほぼ 5 cM の位置にあることが示された．RFLP 分析の結果，この連鎖群が第 3 染色体上に座乗することが示されたことから，*L. chilense* PI128644 の *Rg* 遺伝子は *L. peruvianum* の再分化能を支配する *Rg*-1(Koornneef *et al.* 1993)と同じ遺伝子座である可能性が得られた.

Chapter I

Introduction

1. Tomato

1.1 Economic Importance

Tomatoes are an important source of minerals and vitamins (Hobson and Davies 1971). Globally it is one of the most intensively produced vegetables. World tomato yield was more than 78,282 thousand tones ; the largest producer was the USA (11,000 kt) followed by China (8,928 kt), Turkey (7,150 kt), Egypt (5,050 kt) and Italy (4,860 kt) (Japan 760 kt)(FAO 1995). The yield of tomato production per 1000 m^2 was the highest in Denmark (26.2 t) followed by the Netherlands (24.5 t) and Finland (20.1 t). The yield per 1000 m^2 in Japan was 5.2 t (FAO 1986). Northern European countries have many horticultural facilities so that tomatoes can be produced in all seasons as they are in Japan. Most tomatoes that are produced in Japan are eaten fresh. Japanese tomato consumption was 7.4 kg per person that was about one eighth in Greek. Tomato consumption is increasing in Japan because of modern eating habits.

1.2 Taxonomy of tomato

The cultivated tomato originated in the New World. The Andean area is thought to be the origin of wild tomato species. However, the domestication of tomatoes occurred in Mexico. The most likely ancestor of the cultivated tomato is the cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*). *Lycopersicon* is a relatively small genus within the extremely large and diverse family *Solanaceae*. The genus *Lycopersicon* consists of 9 species that are *Lycopersicon esculentum* Miller, *L. pimpinellifolium* Mill., *L. cheesmanii* Riley, *L. hirsutum* Humb. and Bonpl., *L. chmielewskii* Rick, Keaicki, Fobes and Holle. and *L. parviflorum* Rick, Fob. and Holle (which are included in the "*esculentum*-complex") and *L. peruvianum* (L.) Mill., *L. chilense* Dun, and *L. pennellii* D'Arcy, which are included in the "*peruvianum*-complex" (Rick *et al.* 1990).

The wild species of the geus *Lycopersicon* are rich genetic resources that can assist in the improvement of tomato cultivars (Rick *et al.* 1987 ; Daunay *et al.* 1991 ; Kalloo 1991) . Many useful and commercial traits have been introduced to tomatoes from wild species such as *Fusarium* wilt (Bohn and Tucker 1940) and

Pseudomonas tomato (Pilowsky 1982) from *L. pimpinellifolium*, Jointless from *L. cheesmanii* (Rick 1967), higher sugar and high vitamin C contents from *L. chmielewskii* (Rick 1973, Chmielewski *et al.* 1964).

1.3 "*peruvianum*-complex"

The "*peruvianum*-complex" is the most variable genetic resources for the cultivated tomato (Rick 1979a). Researchers have observed resistance in this species to costly tomato diseases such as bacterial canker, *Corynebacterium michiganensis* (Laterrot *et al.* 1978) ; collar rot, *Alternaria solani* (Hodosy and Kiss 1975) ; Verticillium wilt, *Verticillium dahliae* (Saccardo *et al.* 1981) ; Cucumber mosaic, cucumber mosaic virus (CMV) (Jacquemond and Latterot 1981) ; Curly top, Beet curly top virus (Martin 1970) ; Tomato yellow leaf curl, Tomato yellow leaf curl virus (Pilowsky and Cohen 1990) ; Tobacco mosaic, tobacco mosaic virus (TMV) (Alexander 1963) ; Tomato spotted wilt virus (TSWV) (Stevens *et al.* 1992) ; and Root-knot nematode, *Meloidogyne incognita* (Gilbert and McGuire 1956 , Doganlar *et al.* 1997), *Meloidogyne javanica* (Veremis and Roberts 1996) ; Fusarium wilt, *Fusarium oxysporum*

f. *radicis lycopersici* (Yamakawa *et al.* 1987) ; Leaf mold, *Cladosporium fulvum* (Yamakawa *et al.* 1988) ; Corky root, *Pyrenochaeta lycopersici* (Hogenboom 1970). *L. peruvianum* is potentially the richest source for the development of high ascorbic acid levels, (Rick 1979b) and salt tolerance (Tal 1971) and shoot regeneration capacity (Koornneef *et al.* 1989, 1993) in cultivated tomatoes. Saccardo *et al.* (1981) found several useful traits in the progeny between *L. esculentum* and *L. peruvianum*, including dwarf plant habit, large numbers of flowers per inflorescence, male sterility, high soluble solid content of fruit, uniform ripening, and brown seeds which is a useful marker for F_1 hybrid seed production.

1.4 Sexual hybridization in the genus *Lycopersicon*

The wild species of the genus *Lycopersicon* have the same number of chromosomes (2n=2x=24) as the cultivated tomato, *L. esculentum*. Recently, Tanksley *et al.* (1992) developed a high density molecular map from the cross *L. esculentum* × *L. pennellii* LA716. The order of the molecular markers on genetic maps (which was based on inter- and intra- specific crosses) is

coliner, which suggests a strong synteny within the genus *Lycopersicon* (Grandillo and Tanksley 1996, Lindhout *et al.* 1994, Maliepaard *et al.* 1995, Paterson *et al.* 1990, Paterson *et al.* 1991, Van Heusden *et al.* 1995, Van Ooijen *et al.* 1994, Zamir *et al.* 1994). This suggests that the fertile hybrids between *L. esculentum* and other wild species and their progeny that can improve the tomato cultivars are not always difficult to develop.

If a tomato is used as the female parent, F_1 hybrids can be easily obtained by crossing *L. esculentum* and wild species *L. pimpinellifolium*, *L. cheesmanii*, *L. parviflorum*, *L. chmielewskii* and *L. pennellii* (Rick 1979c). F_1 hybrids between *L. esculentum* as a female parent and *L. hirsutum* are relatively easy to produce ; the F_1 hybrid plants are often sterile but could be backcrossed with *L. esculentum* as female parent (Hogenboom 1972).

On the other hand, it is extremely difficult to obtain an interspecific hybrid between *L. esculentum* and *L. chilense* or *L. esculentum* and *L. peruvianum* because the embryos abort during fruit development (Hogenboom 1972, Barbano and Topoleski 1984). Here, the pollen-tube growth to fertilization is normal, but post-zygotic congruity appears to be rare (Choudhury 1959).

Unilateral incompatibility (UI) occurs in the reciprocal crossing. Many studies have attempted to overcome the breeding barriers between *L. esculentum* and *L. peruvianum* or *L. chilense*. Bohn (1948) found that *L. esculentum* and *L. peruvianum* were inter-crossable if 4n *L. esculentum* was used as the female and 2n *L. peruvianum* as the male. Different techniques have been used to obtain hybrids between *L. esculentum* and *L. peruvianum* or *L. chilense* as zygote rescue after fertilization : embryo culture (Smith 1944), embryo callus culture (Thomas and Pratt 1981), ovule selection culture as ovule culture (Imanishi 1988, Chen and Imanishi 1991) ; improving crossability efficiency : stigma complementation of bud pollination (Gradzil and Robinson 1991), use of a bridge line (Poysa 1990), gamma radiation to pollen (Yamakawa 1971) , use of a self-compatible mutant line (Rick 1982). However, the progeny of the interspecific hybrids between *L. esculentum* and *L. peruvianum* or *L. chilense* still face the following breeding barriers : F_1 hybrids are often sterile (Lesley 1950, Rick 1963) and embryo abortion occurs in the backcrossing with *L. esculentum*. In a similar fashion, various researchers have observed significant variations between wild accessions of

"*peruvianum*-complex" in regard to the efficiency of obtaining the hybrids (Valkova-Achkova and Sotirova 1981, Rick 1983, Poysa 1990, Imanishi *et al.* 1993). Therefore, a method of production for interspecific hybrids that can be used for *L. esculentum* and the wide accessions of "*peruvianum*-complex" in order to utilize the valuable genetic resources of the "*peruvianum*-complex" that have not yet been completely utilized in order to improve tomato cultivars is desirable.

2. Regeneration ability

2.1 Plant regeneration

Plant regeneration from plant tissue cultures have two ways that are via adventitious shoot and root regenerations and somatic embryogenesis. They are different in regard to how they form shoot and root apexes. Somatic embryogenesis is necessary to form shoot and root apexes on a regular position (polarity) with a close connection at the same time, however, adventitious shoot and root regenerations are not necessary. Adventitious shoots are induced mainly from epidermal cells or the surface of callus ones.

The technical basis of plant biotechnology is plant tissue culture. The primary purpose of plant tissue culture is plant regeneration. Plant regeneration from plant cells, tissue and organs *in vitro* is an important phenomenon in regard to its practical use in agriculture, such as the micro propagation of seedlings, the production of virus free plants, the production of inter -specific or -generic hybridization and the production of transformed plants. In addition, it is a very important topic in biology in regard to plant differentiation mechanisms such as the growing point, adventitious shoot and root regeneration, somatic embryogenesis, and sporophytic embryogenesis.

2.2 Genetic analysis of regeneration ability

The genetic analysis of plant regeneration began in the 1970's. High regeneration ability was introduced to a variety with lower regeneration ability in alfalfa (Bingham *et al.* 1975) by backcrossing and selection. Since the late 1980's, various researchers used statistical genetic analysis with diallel analysis in regard to regeneration ability of many plants (Keyes *et al.* 1980, Frankenberger *et al.* 1981, Beckert and Qing 1984, Tomes and

Smith 1985, Willman *et al.* 1989, Chu and Croughan 1990). They found that there were some genes which controlled regeneration ability and these genes indicated additive effects (summarized by Henry *et al.* 1994).

There are two strategies most often used to approach the redifferentiation mechanism : one is to directly isolate the genes from the specific molecular products which are related to redifferentiation, and the other is to identify the location of the genes on the chomosomes which are related to redifferentiation by genetic analysis. Utilizing the former, some cDNA clones were isolated that preferentially accumulate in a young embryo : *Daucus carota* L. (Sato *et al.* 1995), *Solanum melongena* L. (Momiyama *et al.* 1995), *Brassica nupus* L. (Heck *et al.* 1995), *Hordeum vulgare* L. (Heck *et al.* 1993). In the tomato, Torelli *et al.* (1996) isolated specifically expressed mRNA (G36) during shoot regeneration using a differential display method ; the function of this gene, however was unknown.

On the other hand, using the latter strategy, some genes were located on the high density genetic map of advanced analyzed crops as a single gene or QTL (Quantitative Trait Loci) : immature

barley embryo, Komatsuda *et al.* 1993, 1995, Mano *et al.* 1996 ; immature corn embryo, Armstrong *et al.* 1992 ; corn anther, Cowen *et al.* 1992, Murigneux *et al.* 1994, Beaumont *et al.* 1995 ; rice seed, Taguchi-Shiobara *et al.* 1997 ; tomato roots, Koornneef *et al.* 1993; alfalfa petioles, Yu and Pauls 1993. Sugiyama (1997) proposed a genetic model by mapping the genes (*SRD*1, 2 and 3) that control the callus formations and adventitious shoot and root formation in *Arabidopsis thaliana* on the chromosome 1 by using temperature-sensitive mutants.

2.3 Genetic study of regeneration in Tomato

Tomatoes are also one of the plants in which the highest density genetic map was constructed. Koornneef *et al.* (1993) reported that, using the backcrossing progeny of the hybrids between *L. esculentum* and *L. peruvianum*, the superior regeneration capacity that was derived from the *L. peruvianum* of a self-compatible mutant could be controlled by a combination between a major dominant gene (*Rg*-1) that was located on chromosome 3 and other 1 or 2 genes that originated from *L. peruvianum* or *L. esculentum*. However, the genes in neither of

these plants have been isolated.

2.4 Problems of genetic study

To date, the plant regeneration mechanism has not been elucidated. Therefore, in order to understand it clearly, it is necessary to make a higher density genetic map of regeneration genes for map-based cloning and/or to integrate the isolated genes that were related to plant regeneration. Genetic research has only shown allelic differences between two genotypes, which does not exclude the possibility that more genes are involved in other genotypes. Therefore, it is crucial when chosing the experimental materials to select parents that have 1) genotypes with winder genetic distance in regard to regeneration capacity , 2) many molecular markers which indicate polymorphism, 3) germ-fertility and also in their hybrid progeny.

3. Content of the present study

Therefore, in Chapter Ⅱ, we evaluated ovule selection culture (Imanishi 1988) (which is relatively easy to obtain their F_1 hybrids) in regard to producing F_1 hybrids and BC_1 generations,

which have not yet been observed between *L. esculentum* and the wide accessions of the "*peruvianum*-complex".

In Chapters Ⅲ and Ⅳ, we focused on one of the useful traits of the wild species *L. chilense* PI128644 which has superior shoot regeneration capacity. *L. chilense* PI128644 indicated that the highest level of shoot regeneration capacity was contained in the wild accessions of the "*peruvianum*-complex" that were used in Chapter Ⅱ. This superior shoot regeneration capacity was designed to introduce from *L. chilense* into *L. esculentum*. *L. chilense* PI128644 is considered to be a valuable resource that can improve the regeneration ability of tomatoes. The regeneration mechanism in the genus *Lycopersicon* is not yet clearly undrestood, and the regeneration capacity of *L. chilense* has not yet been analyzed genetically.

In Chapter Ⅲ, we evaluated the segregation of PCR-based markers and shoot regeneration capacity and we detected the molecular markers that are linked with the shoot regeneration capacity of *L. chilense* PI128644 using BC_2F_1-44-15 generation which was obtained by the ovule selection method, which was described in Chapter Ⅱ.

In Chapter Ⅳ, we proposed a genetic regeneration capacity model and we made the linkage map of the genes that are related to regeneration capacity using BC_1F_2-44-15 generation. We characterized a BC_1F_1-44-15 with self-compatibility and the superior shoot regeneration capacity that was derived from *L. chilense* PI128644.

Chapter II

Evaluation of the Cross-incompatibility of "*peruvianum*-complex" Lines with *Lycopersicon esculentum* Mill. by the Ovule Selection Method

Key Words : ovule selection method, *Lycopersicon esculentum*, *L. peruvianum*, *L. chilense*, *L. peruvianum* var. *humifusum*, F_1 hybrid, BC_1F_1, cross-incompatibility.

1. Introduction

The green fruited wild species group, "*peruvianum*-complex" is very rich in genetic resources (Allen and Rick 1986). It has many useful characteristics in regards to disease resistance as well as other agronomically important traits in regards to tomato breeding (Rick *et al.* 1987, Rick and Yoder 1988). An analysis of these characteristics and their incorporation into a cultivated tomato (*L. esculentum*) have been hampered by the strict cross-incompatibility barriers that exist between the cultivated tomato and the "*peruvianum*-complex" (Hogenboom 1972). There are

differences among the lines of the "*peruvianum*-complex" in regards to cross-incompatibility, which is assessed by the ratio of the hybrids that are obtained by using the ovule selection method (Imanishi 1991, Imanishi *et al.* 1993). The "*peruvianum*-complex" is a large and genetically diverse population which contains, on one side, a species, *L. pennellii*, which has a high cross-compatibility with *L. esculentum* and produces a hybrid with *L. esculentum* with no help from either the embryo or the ovule culture (Rick 1960, Hardon 1967) and, on the other side, a species (*L. peruvianum* var. *humifusum*) which has a strict cross-incompatibility barrier with *L. esculentum* due to disruption in the earlier stage of a hybrid embryo (Taylor 1986). Not only the hybrid embryo but also the first backcross hybrid embryo to *L. esculentum* are unable to develop into normal seeds (Thomas and Pratt 1981). In the Chapter Ⅱ, we discusses the cross-incompatibility relationship between F_1 and BC_1F_1 when they are obtained by the ovule selection method.

2. Materials and Methods

L. esculentum c.v. 'Kyoryoku Ogata Toko' and 'Early Pink' were used as seed parents. The 'Early Pink' that was used in the present study was provided by the Department of Genetic Resources (NIAR MAFF), Tsukuba, in 1992. Nine lines which belong to a "*peruvianum*-complex" were used as pollen parents as follows: *L. peruvianum* var. *humifusum* (LA2153, LA2334), *L. peruvianum* (LA1554, LA1722, LA2575, PI270435, PI126944), *L. chilense* (PI128644, PI128652). Wild species with an LA number were kindly provided by Dr. C. M. Rick (The C. M. Rick Tomato Genetic Resource Center, University of California). The PI numbers were provided by the Institute of Radiation Breeding (NIAR MAFF) in 1977.

The interspecific hybrids were obtained in 1992 and the first backcrossing into a recurrent parent (a cultivated tomato) was carried out in 1994 and 1995. The seeds of both parents were sown in late March and grown in pots in a glasshouse. The cultivated tomatoes of the seed parents were transplanted into an experimental field after the flowering of the first inflorescence and were grown in a commonly used cultivation. The wild species of the

pollen parents were grown in pots in the glasshouse. The F_1 hybrid plants for the first backcrossing were maintained *in vitro* during the winter and were grown in pots after acclimatization in March. Five of the flowers in the second, third, and fourth inflorescences of the cultivated tomatoes were pollinated with collected pollen from approximately 5 plants in each wild species, according to Imanishi (1988). Reddish fruit that was just maturing was harvested and immature ovules that were capable of germination were selected and cultured in a MS medium (Murashige and Skoog 1962) without any phytohormone according to the ovule selection method (Imanishi 1988, Chen and Imanishi 1991). About 100 to 200 ovules were selected to be cultured in a cross combination in which there were many estimated ovules capable of germination. However, no more than 50 ovules were selected in a cross combination which had less viable ovules.

3. Results and Discussion

In each of the cross combinations, approximately 20% of the putative F_1 plants (shown below as F_1 and BC_1F_1) failed to grow during *in vitro* cutting maintenance and subsequent

acclimatization in pots. The F_1 plants which flowered in pots were able to identify their hybridity because the F_1 plants between *L. esculentum* and a "*peruvianum*-complex" resembled a wild species as a pollen parent (Nagata and Imanishi 1984). All the F_1 plants were hybrids between the cultivated tomatoes and the "*peruvianum*-complex" that was used for this study. As shown in Table 2-1, the F_1 plants in each combination were classified as follows : (LA2153, LA2334), (LA1554), (LA2575), (LA1722), (PI126944, PI270435, PI128644, PI128652). This indicates possible classification among the parentheses. Both the F_1 plants of LA2153 and LA2334 were distinguished from the others by the scent of Japanese pepper. Distinguishing between LA2153 and LA2334 or among PI126944, PI270435, PI128644, and PI128652 was found to be difficult unless a molecular marker was used. For example, all the F_1 plants of LA1554 or LA2575 were found to be true hybrids in regards to physiological and morphological identification because they had a distinguishing trait (Table 2-1). From this, it can be inferred that no pollen contamination occurred among LA2153 and LA2334 plants or among those of PI126944, PI270435, PI128644, and PI128652. The identification of

segregated BC_1F_1 plants among different cross combinations was more difficult than was that of F_1 plants. The backcrossing of F_1 plants into cultivated tomatoes requires stricter pollen control in order to prevent pollen contamination than the development of a F_1 hybrid does.

Tables 2-2 and 2-3 show the results of interspecific hybridization and backcrossing as the number of germinated ovules in a fruit (GOF). Table 2-4 shows 3 correlation coefficients between 'Kyoryoku Ogata Toko' and 'Early Pink' in which the correlation coefficients in F_1 in 1992 and BC_1F_1 in 1995 were not significant but that in BC_1F_1 in 1994 were highly significant. A significant, combined correlation coefficient was then obtained from the 3 coefficients according to the statistical method (Snedecor 1956). These results indicated that both of the cultivated tomatoes that were used as seed parents were generally similar in regards to cross-incompatibility expression with the lines of the "*peruvianum*-complex". However, 'Kyoryoku Ogata Toko' showed a relatively high GOF and 'Early Pink' showed a low GOF when LA1722 and PI126944 were used as pollen parents in order to obtain a F_1 hybrid, as shown in Table 2-2. Similarly, when LA2575

was used as pollen parents, 'Kyoryoku Ogata Toko' showed a relatively high GOF and 'Early Pink' a low GOF in BC_1F_1, as shown in Table 2-3. This suggests that a given line as a pollen parent may have different cross-incompatibilities with different cultivated tomatoes that are serving as seed parents.

There was a highly significant, combined correlation (r=0.907*,d.f.=3) between the two years (1994 and 1995) in the GOF of BC_1F_1 (Table 2-4). Two high correlation coefficients between 1994 and 1995 in both 'Kyoryoku Ogata Toko' and 'Early Pink' were obtained from the development of BC_1F_1 and then the combined correlation coefficient was highly significant. This suggests that there was less environmental effect on the cross-incompatibility of a line belonging to "*peruvianum*-complex".

The correlation coefficients between F_1 and BC_1F_1 in Table 2-4 are 0.324 in 'Kyoryoku Ogata Toko', 0.587 in 'Early Pink', and 0.433 in a combined one. These results by themselves are not significant. It is believed that LA2575 and LA1722 differed from each other as regards their cross-incompatibility with the seed parent, 'Kyoryoku Ogata Toko', as shown in Tables 2-2 and 2-3. In addition, LA2575 showed a different GOF between F_1 and BC_1F_1

in 'Early Pink'. Those correlation coefficients between F_1 and BC_1F_1 that excluded LA2575 are highly significant: r=0.754* (d.f.=5) in 'Kyoryoku Ogata Toko' and 0.924*(d.f.=3) in 'Early Pink'.

The authors investigated the GOF of the F_1 hybrids between a cultivated tomato ('Early Pink') and four lines: LA2153, PI128644, PI128652 and PI270435 (Imanishi 1991), out of those used in the present study. GOF was assessed by the ovule selection method. The resulting GOF was 0.34, 2.00, 1.00 and 1.04 for LA2153, PI128644, PI128652 and PI270435, respectively. These results were almost similar to those in the present study. The correlation coefficient between them was high (r=0.9216, d.f.=2), although it was not significant. The GOFs of BC_1F_1 which the authors obtained in the previous experiment were 0.15, 0.76 and 1.55 for LA2153, PI128644 and PI128652, respectively (Imanishi 1991). These relative values paralleled the results of the present study.

The significant correlation coefficients between the two years in BC_1F_1, between 'Kyoryoku Ogata Toko' and 'Early Pink', and between F_1 and BC_1F_1 (excluding LA2575) as well as the comparison of the present results with our previous ones indicate

the differences among the lines of "*peruvianum*-complex" as regards the cross-incompatibility with a cultivated tomato. The lines of the "*peruvianum*-complex" that were used in the present study could be classified by the differences in cross-incompatibility (as assessed by the GOF) as follows : the highest cross-incompatibility group is LA2153, LA2334 and PI270435, the next group is LA1554, LA1722, LA2575 and PI126944 and the least cross-incompatibility group is PI128644 and PI128652.

The alternative relationship between F_1 and BC_1F_1 in regards to GOF is shown as a linear regression in (F_1 : X, BC_1F_1 : Y). The linear regressions are $Y=0.108X + 0.336$ and $Y=0.105X + 0.037$ for 'Kyoryoku Ogata Toko', and 'Early Pink', respectively. These regressions suggest a much larger GOF for F_1 than for BC_1F_1.

There are very few papers that describe the efficiency of obtaining BC_1F_1 plants in the first backcrossing of F_1 plants between "*peruvianum*-complex" and cultivated tomato to recurrent parent. Yamakawa *et al.* (1978) obtained 84 BC_1F_1 plants from 3301 fruits in the first backcrossing of 3 F_1 plants between a cultivated tomato ('Syugyoku') and *L. peruvianum* PI126944 into a male sterile line of 'Syugyoku'. The GOF of the BC_1F_1 plants is

0.025, which is lower than the GOF of the F_1 plants (Yamakawa 1971). Imanishi (1988, 1991) obtained a higher GOF for BC_1F_1 and BC_2F_1 plants than F_1 plants in a cross combination of a cultivated tomato and the *L. chilense* lines. However, a BC_1F_1 from a cross combination of a cultivated tomato and *L. peruvianum* var. *humifusum* was more difficult to obtain than was F_1 (Imanishi *et al.* 1996). In summary, the GOF of BC_1F_1 is not always larger than that of F_1. This suggests that the development of BC_1F_1 in a cross combination of a cultivated tomato and a "*peruvianum*-complex" is as difficult as it is in F_1. The F_2 population that is derived from the cross between F_1 plants is as self-incompatible as F_1 and the wild species populations are. The F_2 plants are usually cross-incompatible with a cultivated tomato. The incorporation of a useful trait of the "*peruvianum*-complex" into a cultivated tomato requires many BC_1F_1 plants due to the segregating generation of BC_1F_1 while a minimum number of F_1 plants are required. The ovule selection method is effective for interspecific hybridization between a cultivated tomato and a "*peruvianum*-complex" because of the feasibility of many hybrid fruits.

Table 2-1
Character and morphology of the F_1 plants

F_1 plant line		Prostrate type	Leaf margin	Lobation of the leaf margin	Scent	Pubescence
L.esc.[1)]	×LA2153	creeping	wavy	slight	Japanese pepper	normal
L.esc.	×LA2334	creeping	wavy	slight	Japanese pepper	normal
L.esc.	×LA1554	intermediate	wavy	deep	sweet aroma	thick
L.esc.	×LA2575	standing	serrate	deep	weak	thin
L.esc.	×LA1722	standing	wavy	intermediate	green smell	normal
L.esc.	×PI126944	intermediate	wavy	intermediate	green smell	normal
L.esc.	×PI270435	intermediate	wavy	intermediate	green smell	normal
L.esc.	×PI128644	intermediate	wavy	intermediate	green smell	normal
L.esc.	×PI128652	intermediate	wavy	intermediate	green smell	normal

1) 'Kyoryoku Ogata Toko' and 'Early Pink'
LA2153, LA2334 : *L. peruvianum* var. *humifusum*
LA1554, LA2575, LA1722, PI126944, PI270435 : *L. peruvianum*
PI128644, PI128652 : *L. chilense*

Table 2-2
Germinated ovules from cross-combinations between *L. esculentum* and the "*peruvianum*-complex" (1992)

Pollen parents[1]	Seed parents[2]							
	'Kyoryoku Ogata Toko'				'Early Pink'			
	No. of fruits	No. of germinated ovules	No. of plantlets recovered	GOF[3]	No. of fruits	No. of germinated ovules	No. of plantlets recovered	GOF
LA2153	15	3	2	0.20	16	1	1	0.06
LA2334	22	7	2	0.32	27	3	3	0.11
LA1554	24	6	6	0.25	28	13	11	0.46
LA2575	44	14	10	0.32	20	13	7	0.65
LA1722	9	17	10	1.89	13	1	1	0.08
PI126944	25	46	33	1.84	7	1	0	0.14
PI270435	14	17	4	1.21	16	7	4	0.44
PI128644	10	44	27	4.40	8	9	8	1.13
PI128652	14	40	25	2.86	–	–	–	–

[1] *L. peruvianum* var. *humifusum* ; LA2153, LA2334, *L. peruvianum* ; LA1554, LA2575, LA1722, PI126944, PI270435, *L. chilense* ; PI128644, PI128652

[2] 'Kyoryoku Ogata Toko', 'Early Pink'; *L. esculentum*

[3] Germinated ovules in a fruit

Table 2-3
Germinated ovules from cross-combination between *L. esculentum* and F_1 plants (1994 and 1995)

Seed parents[1]	Pollen parents[2]	No. of fruits	No. of germinated ovules	No. of plantlets recovered	GOF[3] 1994	1995	2 years[4]
KOT	KOT×LA2153	106	10	4	0.11	0.08	0.09
	KOT×LA2334	92	8	5	0.06	0.10	0.09
	KOT×LA1554	40	8	4	--	0.20	0.20
	KOT×LA2575	42	57	48	--	1.36	1.36
	KOT×LA1722	27	5	2	--	0.19	0.19
	KOT×PI270435	88	14	7	0.19	0.15	0.16
	KOT×PI128644	89	61	19	0.80	0.63	0.69
	KOT×PI128652	114	131	72	0.77	1.32	1.15
EP	EP×LA2153	100	4	1	0.07	0.03	0.04
	EP×LA2334	92	7	5	0.07	0.08	0.08
	EP×LA1554	61	8	4	--	0.13	0.13
	EP×LA2575	27	0	0	--	0.00	0.00
	EP×PI270435	100	7	4	0.08	0.07	0.07
	EP×PI128644	75	15	8	0.14	0.22	0.20
	KOT×PI128652	39	37	29	--	0.95	0.95

[1] KOT ('Kyoryoku Ogata Toko'), EP ('Early Pink') ; *L. esculentum*

[2] F_1 hybrids between *L. esculentum* (KOT, EP) and wild species

[3] Germinated ovules in a fruit

[4] Weighted means of the two years. No. of fruits, no. of germinated ovules and no. of plantlets recovered show a total of the two years.

Table 2-4
Correlation coefficients between two cultivated tomatoes, between two years, and between F_1 and BC_1F_1 in no. of germinated ovules in a fruit (GOF) in cross of *L. esculentum* and "*peruvianum*-complex"

Correlation	Condition	n	r	Combined correlation
KOT[1]-EP[2]	F_1(1992)	8	0.6089	
	BC_1F_1(1994)	4	0.9982 **	χ^2= 4.16(n.s.)(d.f.=2)
	BC_1F_1(1995)	7	0.5458	Combined r[3] = 0.750**(d.f.=11)
1994-1995	KOT(BC_1F_1)	5	0.8721	χ^2= 0.169(n.s.)(d.f.=1)
	EP(BC_1F_1)	4	0.9515 *	Combined r = 0.907*(d.f.=3)
F_1[4]-BC_1[5]	KOT	5	0.3244	χ^2= 0.212(n.s.)(d.f.=1)
	EP	4	0.5868	Combined r = 0.433(n.s.)(d.f.=3)

[1] 'Kyoryoku OgataToko'

[2] 'Early Pink'

[3] estimated according to Statistical Methods ; Snedecor (1956)

[4] *L.esculentum* × "*peruvianum*-complex"

[5] BC_1F_1 (*L. esculentum* × F_1)

n.s. : not significant, * : significant at 5% level, ** : significant at 1% level

d.f. : Degree of freedom, n : number of pairs