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Cross-compatibility between the Cultivated Tomato *Lycopersicon esculentum* and the Wild Species *L. peruvianum*, *L. chilense* Assessed by Ovule Culture *in vitro*

Lanzhuang CHEN and Shigeru IMANISHI

Faculty of Agriculture, Yamagata University, Tsuruoka 997

In this study the material of '*peruvianum*-complex' was extended to *L. peruvianum* to confirm the efficiency of ovule culture *in vitro* reported previously (IMANISHI *et al.*, 1985; IMANISHI, 1988) and cross-compatibility between the cultivated tomato and the '*peruvianum*-complex' was assessed by the *in vitro* ovule culture. Four cultivars as the female parents and *L. chilense* (2 lines) and *L. peruvianum* (1 line) as the male parents were used. No normal seeds were found in the ovules taken from the ripe crossed fruits. Visual observation, however, suggested that a few ovules had the potential for germination, based on the characteristics described by IMANISHI (1988). In the cross between Early Pink and *L. chilense* (PI 128652), 30 ovules out of the large number of ovules were selected by the visual observation to observe embryos contained. Dissection under a microscope revealed 23 incomplete embryos in the 30 ovules. Ovules with this potential for germination were selected visually in the crosses between Early Pink or Kyoryoku Toko and three male parents. They had high germination rates when plated on MS agar medium (MURASHIGE and SKOOG, 1962) without phytohormone. This result indicates that *in vitro* ovule culture based on the visual selection can be applied not only to *L. chilense* but also to *L. peruvianum*. The putative F_1 plants closely resembled the male parents in the morphology. Leaf culture also was used to compare the regeneration abilities of F_1 plants and both parents and to identify F_1 plants. This leaf culture revealed that the '*peruvianum*-complex' could show an incompletely dominant character against cultivated tomato as female parent with regard to regeneration ability. Most of the putative F_1 plants were considered to be true ones. The cross-compatibilities of the tomato cultivars and the '*peruvianum*-complex' were assessed by the *in vitro* ovule culture. There was a remarkable difference in cross-compatibility among the 4 cultivars tested. It was thought that Early Pink had general, high cross-compatibility for the '*peruvianum*-complex'. A difference in cross-compatibility also was found for the lines of the '*peruvianum*-complex' but it was small.

KEY WORDS: *Lycopersicon esculentum*, *L. peruvianum*, *L. chilense*, interspecific hybridization, cross-compatibility, ovule culture, shoot regeneration.

Introduction

In vitro ovule culture has been reported to be very effective for the hybridization of cultivars of tomato and the wild species *L. peruvianum* (IMANISHI *et al.*, 1985; IMANISHI, 1988). It is suggested that an ovule having the potential for germination in a ripe crossed fruit could be distinguished with high probability after the ovules are removed and sterilized with a low concentration of antiformin solution because its size is within a specific range and it has a characteristic color and shape. The effectiveness of ovule culture depended greatly on the cultivar used as the female parent because of the marked differences in the germination rates of the ovules of tomato cultivars.

IMANISHI (1988) used PI 128652 and PI 128644 as two lines of *L. peruvianum*. These lines should be designated *L. chilense* according to HOLLE *et al.* (1979). *L. chilense* is one of the

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two species that organize the '*peruvianum*-complex' (RICK, 1986). RICK and LAMM (1955) commented on the fact that *L. chilense* generally shows a higher degree of sexual compatibility with the cultivar than *L. peruvianum* which is the other species of the '*peruvianum*-complex'.

The present study extends the material of '*peruvianum*-complex' to *L. peruvianum* to confirm the efficiency of *in vitro* ovule culture and evaluate the cross-compatibilities between cultivars of tomato and male parents of '*peruvianum*-complex' by means of *in vitro* ovule culture.

Materials and Methods

Four cultivars of tomato: Early Pink, Kyoryoku Toko, Sekai-ichi and Giban No.1, were used as the female parents and 3 lines of the '*peruvianum*-complex': *L. chilense* (PI 128644), *L. chilense* (PI 128652) and *L. peruvianum* (PI 270435) were used as the male parents. Seeds of the tomato cultivars were sown in petri dishes in March 1988 then transferred to pots after germination. The potted plants were grown in a greenhouse until transplanted to the field in May. The male parent lines were grown in pots in the greenhouse during the experimental period. The second and third inflorescences of the female parents were used for cross-pollination which was done from 10 to 24 June. Pollination, harvest of ripe fruits and preparation and sterilization of ovules were performed as described by IMANISHI (1988). Cross-pollination was conducted in all cross-combinations between 4 cultivars and 3 lines of '*peruvianum*-complex'. About 10~20 fruits were harvested for ovule culture *in vitro* in each of the experiments of all cross-combinations. Fifteen to thirty sterilized ovules were selected and plated in each 5cm petri dish that contained MS agar medium (MURASHIGE and SKOOG, 1962) without phytohormone. In the selection and plating of ovules in the 5cm petri dishes, ovules that appeared to have better potentials for germination were selected visually on the basis of the characteristics described by IMANISHI (1988) which mean a lighter yellowish brown color and a slightly rounder shape than the others. At the first selection the better ovules were selected and were plated in dish 1. At the second selection, the ovules selected out of the remaining ones were plated in dish 2. At the third selection, the selected ovules were plated in dish 3 as they were done in dishes 1 and 2. In dishes 4 through 10, the remaining ovules were plated almost at random, because there were found few ovules having the potential for germination in the remaining ones. The dishes were incubated at 25°C under illumination of 300~500 lux for 16 hrs./day. Ovules which germinated were transplanted individually into test tubes containing MS agar medium without phytohormone. The experiment of ovule culture was conducted 1~2 times in each cross-combination.

When the young seedlings of the putative F_1 plants were grown 2 to 4cm long, they were transplanted individually to 300-mℓ Erlenmyer flasks containing the medium used for the germination of the ovules and maintained during the winter by repeated transplanting, approximately every month. True F_1 plants were identified by comparing the abilities of the putative F_1 plants with those of both parents to regenerate shoots (KOONNEEF *et al.*, 1987; IMANISHI and WATANABE, 1988) in addition to the comparison of morphology (THOMAS and PRATT, 1981; NAGATA and IMANISHI, 1984). For this experiment, 43 putative F_1 plants that showed good growth were chosen from all the plants derived from cross-combinations of Early Pink × male parents and Kyoryoku Toko × male parents. Leaves near the top of each

test plant were removed and cut in segments of 1.0 by 0.5cm that then were cultured on MS agar medium containing 1.0mg/ℓ 6-Benzylaminopurine (BA). Five leaf segments from each putative F_1 plant and from each of its parents were cultured. The test tubes with the leaf segments were incubated at 25°C and 2,500 to 3,000 lux for 16 hrs./day. The ability to regenerate shoots was determined 20 days after incubation started. The number of shoots per leaf segment was counted under a dissecting microscope, 3 leaf segments being used to calculate the average shoot number per plant after the segments with the most and least shoots had been discarded. In one experiment in the cross between Early Pink and PI 128652, 30 ovules out of the large number of ovules were selected visually as having the potential for germination. Embryos dissected from the ovules selected were observed under a dissecting microscope.

Results and Discussion

Visual selection of ovules with the potential for germination

No normal seeds were found in any fruits of all cross-combinations. However, it was inferred that some ovules that had the potential for germination could be recognized and selected visually in the crosses between Early Pink or Kyoryoku Toko and three male parents. The characteristics of the ovules that appeared to have better potentials for germination were a lighter yellowish brown color and a rounder shape than the others. No information has been obtained about the morphology of embryos in ovules having the potential for germination. Twenty-three embryos were dissected from 30 ovules visually selected as having the potential for germination (Table 1 and Fig. 1). These embryos were composed of one embryo with cotyledons and hypocotyl incompletely formed, 5 ones with small incomplete cotyledons, 9 rod-shaped ones with a twist in the middle and 8 short rod-shaped ones. Seven of the 30 ovules had no living embryos. Of the remaining ovules, none had living embryos. This result indicates the effectiveness of the visual selection of ovules.

Table 1. Morphological classification of 23 embryos dissected from 30 ovules that showed potential for germination

Characteristics	No. embryos	%
Incomplete cotyledon and hypocotyl formation	1	4.3
Small, incomplete cotyledons	5	21.7
Rod-shaped embryos with a twist in the middle	9	39.2
Short, rod-shaped embryos	8	34.8
Total	23	100.0

Note: The fruits of the cross between Early Pink × *L. chilense* (PI 128652), which were different from those of Table 2, were used for this experiment.

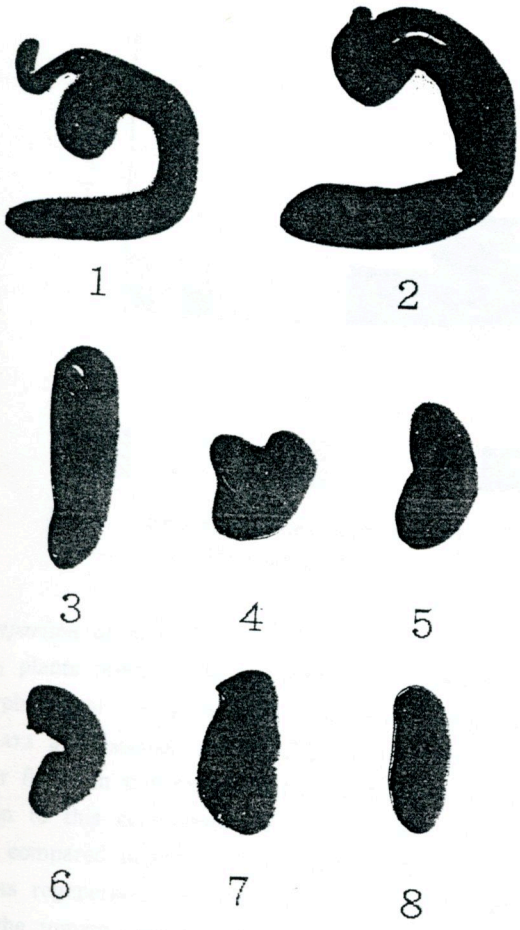

Fig. 1. Embryos dissected from ovules selected visually as having the potential for germination.

Note: 1: Normal embryo from normal seed. 2: embryo with incomplete cotyledons and hypocotyl. 3-4: embryos with small, incomplete cotyledons. 5-6: rod-shaped embryos with a twist in the middle. 7-8: short, rod-shaped embryos.

IMANISHI (1988) have described the criteria of ovules having the potential for germination, but have not showed the high germination rate of ovules selected visually. The present study was able to show the very high percent of germination of ovulees, as compared with the results of IMANISHI (1988). The results of selection for Early Pink and Kyoryoku Toko are shown in Table 2. Few ovules having the potential for germination were obtained from the crosses between Sekai-ichi or Giban No. 1 and three male parents. In the cross between Early Pink and PI 128644, 29 of 3,489 ovules were selected at the first time, being the most potentially viable. These were plated in dish 1 and 24 (82.8%) germinated. Secondly, 30 ovlues of the remaining ones were selected and plated in dish 2 and 14 (46.7%) germinated. On the following selection, ovules selected from the remaining ones were plated in dish 3 through 10. Of all the ovules selected and plated, 43 germinated, and 38 of these were from dishes 1 and 2 and corresponded to 66.4% of 59 ovules plated in dishes 1 and 2. Similar results were found for the cross between Early Pink and the other two lines of male parents. The percent of germination for the Kyoryoku Toko cross was very low, but most of the ovules that did germinate were from dishes 1 and 2. On a whole, ovules which were plated on dishes beyond dish 4 were picked up as having no potential for germination, because no ovules in the remaining ones were recognized as having potential for germination. The visual selection of ovules shown in Table 2 indicates that not only in *L. chilense* but also in *L. peruvianum*, even though ovules with the potential for germination were only a few in the crossed fruits in the cross-combination with Early Pink or Kyoryoku Toko, they could be selected with high probability after sterilization and could be grown to complete plants through *in vitro* culture.

Table 2. Efficiency of visual selection of ovules with potential for germination from cross-combinations between Early Pink or Kyoryoku Toko and 3 lines of '*peruvianum*-complex'

Cross-combination	Total number of ovules	Dish 1			Dish 2			Total No. of ovules germinated in Dishes 1 and 2	Total No. of ovules germinated in dish 1 through dish 10
		No. ovules plated (A)	No. ovules germinated (B)	B/A (%)	No. ovules plated (A)	No. ovules germinated (B)	B/A (%)		
EP×PI128644	3489(19)	29	24	82.8	30	14	46.7	38	43
EP×PI128652	4304(25)	30	23	76.7	30	2	6.7	25	28
EP×PI270435	6935(26)	30	26	51.0	30	1	2.0	27	27
KT×PI128644	6422(39)	45	6	13.3	55	2	3.6	8	12
KT×PI128652	4422(31)	40	4	10.0	40	0	0.0	4	4
KT×PI270435	4306(26)	45	0	0.0	45	0	0.0	0	0

Note 1: Numerals in parentheses show the number of fruits.
Note 2: EP; Early Pink, KT; Kyoryoku Toko, PI128644 and PI128652; *L. chilense*, PI270435; *L. peruvianum*
Note 3: Ovules were selected visually from total ovules in the order of better potential for germination and plated in dish 1>dish 2>—>dish 10 with this order.

Table 3. Cross-compatibility of cultivars of tomato and '*peruvianum*-complex', expressed as percent of germination of ovules selected visually

'*per.*-complex'	PI 128644			PI 128652			PI 270435			Average percentage of germination
Cultivar	No. ovules (A)	No. ovules germinated (B)	B/A (%)	No. ovules (A)	No. ovules germinated (B)	B/A (%)	No. ovules (A)	No. ovules germinated (B)	B/A (%)	
Early Pink	3489(19)	43	1.23	4304(25)	28	0.65	6935(26)	27	0.39	0.76
Kyoryoku Toko	6422(39)	12	0.19	4422(31)	4	0.09	4306(26)	0	0.00	0.09
Sekai-ichi	3434(26)	1	0.03	2204(30)	3	0.14	5598(41)	3	0.05	0.07
Giban No. 1	935(22)	0	0.00	417(12)	0	0.00	580(12)	0	0.00	0.00
Average percentage of germination			0.36			0.22			0.11	
Friedmann test	Among cultivars			$\chi^2=7.71>7.4=5\%$ level, a=4, b=3						

Note 1: PI 128644 and PI 128652; *L. chilense*, PI 270435; *L. peruvianum*
Note 2: Numerals in parentheses show the number of fruits.

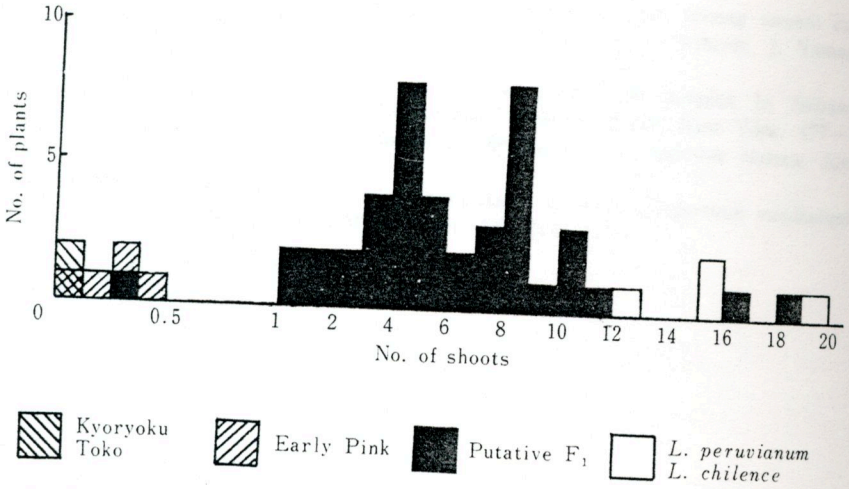

Fig. 2. Comparison of the abilities of putative F_1 plants and both parents to regenerate shoots in leaf segment culture

Comparison of morphology and culture of leaf segments in putative F_1 plants

F_1 plants produced by crossing *L. esculentum* and *L. peruvianum* are identifiable by their morphological resemblance to the male parent *L. peruvianum* (THOMAS and PRATT, 1981; NAGATA and IMANISHI, 1984). All the putative F_1 plants that were grown in 300-mℓ Erlenmyer flasks in this experiment closely resembled the male parents in the morphology. In addition to this comparison, the regeneration ability of putative F_1 plants and both parents was compared in order to identify the true F_1 plants. Except in one case the numbers of shoots regenerated per leaf segment from 42 putative F_1 plants were clearly greater than for the tomato cultivars and less than for male parents (Fig. 2). From these facts, the 42 putative F_1 plants should be true F_1 plants. Therefore, most of the F_1 plants obtained in this study are considered to be true F_1 plants. The ability of *L. peruvianum* to regenerate shoots has been reported to be completely dominant against the character for cultivated tomato (KOORNNEEF *et al.*, 1987; IMANISHI and WATANABE, 1988). In our study, however, it is thought that the ability of the '*peruvianum*-complex' to regenerate shoots can be incompletely dominant against that of cultivated tomato, depending on the conditions of plant growth to take leaves for leaf culture.

*Difference in cross-compatibilities of tomato cultivars and lines of the '*peruvianum*-complex*

The cross-compatibilities between *L. esculentum* and the '*peruvianum*-complex' were analyzed from the results of ovule culture for all the combinations between the 4 cultivars of tomato and 3 lines of male parents. The percentages of the total numbers of ovules that germinated are shown in Table 3. The percentages were 1.23% for PI 128644, 0.65% for PI 128652 and 0.39% for PI 270435 when Early Pink was the female parent. The values of the other female parent cultivars were very low. The average percent of ovules that germinated was 0.76% for Early Pink, 0.09% for Kyoryoku Toko, 0.07% for Sekai-ichi and

0.00% for Giban No. 1, if the lines of male parents are taken as blocks (replications). These values indicate a general cross-compatibility of each cultivar. There was a significant difference at the 5% level in the Friedmann test for the 4 cultivars. This result suggests that a particular cultivar which has high cross-compatibility with a particular line of the '*peruvianum*-complex' also has high cross-compatibility with other lines. Early Pink showed the highest cross-compatibility of the 4 cultivars when it was evaluated for the 3 lines of '*peruvianum*-complex'. It also had the high cross-compatibility evaluated for the F_1 of Kyoryoku Toko × PI 128644 (IMANISHI, 1988) and for *L. per.* var. *humifusum* (LA 2153) (IMANISHI, 1991). It is thought that Early Pink has general, high cross-compatibility for the '*peruvianum*-complex'. In contrast, the percent of ovules that germinated in male parent was 0.36% for PI 128644, 0.22% for PI 128652 and 0.11% for PI 270435, if the cultivars are taken as blocks (replications). There was no significant difference in the Friedmann test among 3 lines of male parents. The order of the percentages of 3 male parents, however, clearly were PI 128644, PI 128652 and PI 270435 for both cultivars of tomato, Early Pink and Kyoryoku Toko. Although the difference was much smaller than that for the cultivars of tomato, it is seggested that *L. peruvianum* (PI 270435) has lower cross-compatibility than two lines of *L. chilense* as the suggestion by RICK and LAMM (1955).

In vitro ovule culture is a very useful way to obtain hybrids between cultivars of tomato and the '*peruvianum*-complex', but, ovules that have potential for germination must be selected visually by the method of IMANISHI (1988) after they have been removed from the fruits and have been washed and sterilized. It is very important that a cultivar with high cross-compatibility be used. Early Pink proved to have the highest cross-compatibility of the 4 cultivars when it was evaluated for the 3 lines of '*peruvianum*-complex'. There was a small difference of cross-compatibility among the 3 lines of '*peruvianum*-complex'.

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In vitro 胚珠培養法によって評価されるトマト栽培種 *Lycopersicon esculentum* および野生種 *L. peruvianum*, *L. chilense* における交雑親和性

陳 蘭莊・今西 茂
（山形大学農学部，鶴岡市若葉町，997）

本研究は，前報（IMANISHI *et al*. 1985 : IMANISHI, 1988）で報告した栽培種と *L. chilense* 間の種間交雑における *in vitro* 胚珠培養法を *L. peruvianum* との種間交雑に適用し，その有効性を確かめ，交雑親和性の栽培種間差異や野生種間差異を明確にするために行った．そのため，種子親として4栽培品種，花粉親として *'peruvianum*-complex' の3系統，すなわち，*L. chilense* 2系統と *L. peruvianum* 1系統を用い，全組合せの交雑を行った．

成熟した交雑果から取り出した胚珠には正常な種子は全く含まれていなかった．しかし，IMANISHI (1988) に従って，水洗した胚珠を消毒後肉眼観察すると，やや大きく，狐色をし，やや丸みを帯びた，発芽力を有するように見える胚珠がわずかに含まれていて，それらを明瞭に識別できた．発芽力をもつ胚珠中の胚の形態を知り，それによって胚珠の肉眼選抜の有効性を示すため，Early Pink と *L. chilense* (PI 128652) の組合せについて，非常に多数の胚珠の中から発芽力を有すると推定される30個の胚珠を肉眼選抜し，それらを実体顕微鏡下で解剖した．そして，不完全な胚ではあるが23個の生きた胚を取り出すことができた．それらは1個を除いて胚の発育初期に相当する，少し異常な形態をした胚であった．さらに，発芽力をもつ胚珠を高い確率で選抜できることを実際の発芽率で示すため，全組合せの各々において，肉眼観察でより発芽力を有すると思われる胚珠を順番に選抜し，その順に植物ホルモン抜きのMS寒天培地に置床して培養した．その結果，発芽力を有する胚珠を高い確率で選抜できることが確かめられ，この肉眼選抜法が *L. peruvianum* にも適用できることが分かった．

F_1 の判定は葉や茎などの形態の比較によって行うと共に葉片培養による苗条再分化能の比較によっても行った．葉片培養によって *'peruvianum*-complex' の系統が有する高い苗条再分化能は，本研究のように栽培種に対して不完全優性を示す場合のあることが分かった．この方法によって F_1 個体を栽培種と野生種から区別できる可能性が示された．これらの結果から，胚珠培養によって獲得できた個体の大部分が F_1 個体であると推定された．

胚珠培養による F_1 個体の獲得率を用い，栽培品種や *'peruvianum*-complex' の系統の交雑親和性を検討した結果，栽培種には交雑親和性の顕著な品種間差異が存在し，一方，*'peruvianum*-complex' の系統間にも顕著ではないが系統間差異が認められた．すなわち，Early Pink は一般的な高い交雑親和性をもち，逆にギ番1号はきわめて低い交雑親和性をもつと推定された．*'peruvianum*-complex' の3系統の中では，*L. chilense* の PI 128644 が最も高い交雑親和性をもっていて，*L. peruvianum* の PI 270435 は，少なくとも Early Pink と強力東光との交雑において *L. chilense* の2系統よりも低かった．

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Effect of Absence of Seed α-Amylase Inhibitor on the Growth Inhibitory Activity to Azuki Bean Weevil (*Callosobruchus chinensis*) in Common Bean (*Phaseolus vulgaris* L.)

Masao ISHIMOTO and Keisuke KITAMURA

National Agriculture Research Center, Tsukuba, Ibaraki, 305

Individual seeds of 212 common bean cultivars were analyzed by Ouchterlony immunodiffusion method using the antiserum raised against common bean α-amylase inhibitor (CBAI). Two cultivars, Ofuku-5 and Ofuku-8, gave no reaction with the anti-CBAI serum and no inhibitory activity against porcine pancreatic α-amylase. Furthermore, these CBAI-lacking cultivars contained no lectin. Analysis of F_2 seeds from the cross between Ofuku-5 and Taishou-kintoki (CBAI-containing) indicated that the absence of CBAI was recessive to the presence of CBAI and tightly linked to the absence of lectin. In a previous report, we showed that CBAI strongly inhibited the larval growth of *Callosobruchus chinensis*. Thus, effects of the absence of CBAI on the larval growth of *C. chinensis* were examined using artificial beans containing seed components of the two CBAI-lacking cultivars and two CABI-containing cultivars (Taishou-kintoki and Ofuku-1). The flour of the CBAI-lacking cultivars showed weak inhibitory activity on the larval growth compared with that of the CBAI-containing cultivars, although the larvae could not grow in the intact seeds of the CBAI-lacking cultivars. The role of lectin, which was also absent in the seeds of Ofuku-5 and Ofuku-8, has been implicated with resistance to insects, especially bean weevils. CBAI and the lectin in the seeds of the CBAI-containing cultivars were separated well by extraction with 20mM sodium phosphate buffer solution and subsequent ammonium sulfate fractionation. The feeding tests using the protein fractions separated from the CBAI-lacking cultivars and the CBAI-containing cultivars demonstrated that the absence of CBAI is considerably responsible for the lowering of the growth inhibitory activity of the flour of CBAI-lacking cultivars.

KEY WORDS: *Phaseolus vulgaris*, α-amylase inhibitor, lectin, *Callosobruchus chinensis*, *Callosobruchus maculatus*, insect resistance.

Introduction

Seeds of the common bean (*Phaseolus vulgaris* L.) contain a proteinous α-amylase inhibitor (common bean α-amylase inhibitor, CBAI), which inhibits the α-amylase activities from mammals and insects (MARSHALL and LAUDA 1975, POWERS and WHITAKER 1977). However, CBAI does not inhibit endogenous plant α-amylases (JAFFE *et al.* 1973).

Recently, ISHIMOTO and KITAMURA (1989) confirmed that CBAI completely suppressed the larval growth of the azuki bean weevil (*Callosobruchus chinensis* L.) and the cowpea weevil (*C. maculatus* F.), major storage pests of cowpea (*Vigna unguiculata* (L.) Walp.), mungbean (*V. radiata* L.) and azuki bean (V. angularis (Willd.) Ohwi and Ohashi), and markedly inhibited the midgut α-amylase activity in the larvae of these two bean weevils. However, both the growth and the α-amylase activity of the Mexican bean weevil (*Zabrotes subfasciatus* Boheman), a well known pest of common bean, were hardly affected by CBAI. If the resistance of common bean to attack by *C. chinensis* and *C. maculatus* is mainly due to the presence of CBAI, the larvae of these two bean weevil species might be expected to grow in the

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