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PROGRESS REPORT NO. 3

SELECTION AND DEVELOPMENT OF VIRUS RESISTANT POTATO BY USING PROTOPLAST CULTURE AND FUSION TECHNIQUE.

A RESEARCH PROJECT

USAID/PSTC PROGRAM

GRANT NO. 936-5542-G-00-7021-00

SUBMITTED BY

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THAILAND

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Project Profile

Country	: Thailand
Grant No.	: 936-5542-G-00-7021-00
Program	: Program on Science and Technology Cooperation
Project Title	: Selection and Developing of Virus Resistant Potato by Using Protoplast Culture and Fusion Technique.
Project Leader	: Dr. Prasartporn Smitamana
Organization	: Faculty of Agriculture Chiang Mai University 50002 Chiang Mai, THAILAND.
Co-investigators	: Dr. Pajchima Smitamana Dr. Danai Boonyakiat Assoc. Prof. Kesinee Ramingwong
Research assistant	: Mr. Visnu Somsab
Project consultant	: Dr. Sherret S. Chase Dr. Stephen S. Sinden
Authorized Officer	: Prof. Avudh Srisukri
Total Project Budget	: 3,816,192 Baht (149,070 US $)
Project Duration	: 3 Year (August 1987 - July 1990)
Reporting Period	: August - January 1989
Budget Allocation for this Period	: 2,448,373.09 Baht (95,639.57 US $)

Content

1. Background/Introduction

Potato leafroll virus (PLRV) is one of the major viruses found in potato worldwide. The PLRV - infected plants generally produce smaller tubers. The depression in yield of secondary infected plants may be considerable : losses exceeding 20 - 87 % of the yield have been reported (Hunnius, 1977). Primary symptoms of the PLRV infected plants appear mainly in the young leaves at the top of the plants. The infected leaves are usually held upright with pale yellowish color. The leaflets of certain cultivars are often rolled upwards. If the primary infection take place late in the season, the symptoms and yield reduction might not occur. However, the progeny might be partially infected. The severe symptoms can be observed in the secondary infection, which are stunting, rolling of the older leaves and pale. For the basal leaves, stiff and leathery leaves signs are usually found. Tubers from the infected plants show clearly net necrosis, which can be seen when the tuber is cut. Phloem necrosis in stems and petioles can also be detected (Walker, 1952 ; Beemster and de Bokx, 1987). Since PLRV can not be transmitted by any mechanical means therefore, mechanical inoculation on the virus indicator or indexing plants is not possible. The best method for PLRV detection in the infected plants nowadays is by using the serological test. Enzyme-linked immunosorbent assay is one of the most sensitive serological

tests. After this method had been developed in 1976 (Voller et al., 1976) , it has been reviewed extensively (Clark, 1981 ; Torrance and Jones, 1981 ; Bar-Joseph and Garnsey, 1981) and a number of modifications and variations have been described (Clark and Adams, 1977) The principle of ELISA technique is that virus in the test sample is selectively trapped and immobilized by specific antibody adsorbed to the surface of the flat-bottomed 96 wells polystyrene microtitre plates. Retained virus is then reacted with further specific antibody to which an alkaline phosphatase enzyme has been linked. The reaction between the trapped virus and the enzyme linked antibody can be detected by adding a suitable enzyme substrate the p-nitrophenyl phosphate. Positive reactions are indicated by the substrate turning yellow which can easily be observed by the naked eyes. For the quantitative test, the plates can be further measured by 405 nm., the intensity of the yellow color is an indicator of the virus concentration in the test samples.

Testing for the virus resistant property in the parent materials is very important in any breeding program either by conventional pollination technique or somatic hybridization which the production of the haploid plants is significantly important as has been discussed in the previous reports. Besides that the morphology of the materials used in the program must also be considered. The growth and development of potatoes, like the other crops,

are affected by the environments that exist in the nature at different locations in the world. Though the genetic endowment of a plant (genotype) is essentially constant in comparison with changes in environment that a plant experiences, the expressions (phenotypes) of the genetic information are strongly influenced by environmental changes. Climates with regular and irregular shifts are the determinative components of a given genotype (Langridge, 1963). Morphology of one population is uniform when grown in their original environment, but showed great deviation in such characters as vigor, growth pattern, height and leaf size when transplanted to other altitudinal locations (Clausen and Hiessey, 1958). The performance of a clone at one transplanting location was no indication of its behavior at another, suggesting that different sets of genes were operating in dissimilar environments. Therefore a successful population in any climate region is one that has evolved its physiological mechanisms to be effective in the light -energy transformation, together with resistance to cold and drought (Cooper, 1963). In order to gain more informations on the adaptability of different potato cultivars to the Thai conditions which is important to the breeding program of the project, detail characterization and description of potatoes need to be studied.

2. Objective

2.1 To test for PLRV resistant property in both dihaploidy and tetraploidy potatoes.

2.2 To induce morphogenesis in the anther derived roots.

2.3 To study the characteristics of individual clones of *Solanum tuberosum* L. as well as other species e.g. *Solanum phureja*

2.4 To describe morphology of the potato clones.

3. Materials and methods

3.1 Testing for PLRV resistant in potatoes.

Both dihaploid and tetraploid potatoes were grafted with the PLRV infected potato cv. Sieglinde, by using Sieglinde as either scions or stocks. Since the stems of *Solanum acaule* were very small, so they were grafted on the infected Sieglinde stocks. After grafting the plants were kept in the high humidity incubator at 25 $\pm$ 2 ^{o} C. with 16 hr. light for 1 week, to enhance the survival of the grafted plants. Thereafter, the plants were transferred to the screen-house and grown under the normal conditions. In order to warranty the transmission of PLRV from the infected scions to the stocks, in the case of using virus infected Sieglinde as scions, the grafted scions were remained attach to the stocks for 1 month, and then they were cut off

with the clean razor blades. In the opposite, if the healthy tested plants were grafted on the PLRV infected Sieglinde, only the axillary shoots from the stock part were removed to elaborate the scions growth rate (Fig. 1). Leaf samples were collected 8 weeks after grafting and were extracted in the sample buffer (Boehringer Mannheim GmbH, FRG). Polystyrene plates (Dynatech Laboratories, Inc., Alexandria, U.S.A. or Fa. Greiner, Nuertingen, FRG.) containing 8 x 12 wells were coated with 200 ul coating solution (Boehringer Mannheim GmbH, FRG), and then covered with the polyethylene film and incubated at 37 ^{o} C. for 2 hr. The coating solution was removed and washed 3 times with PBS-Tween by using washing machine (Miniwash, Dynatech Laboratories, Inc., Alexandria, U.S.A.). After washing, 200 ul of plant extracts were pipetted into the wells and incubated over night at 4 ^{o} C., as control standard healthy plant extracted and standard virus suspension were used. The solution was removed and washed 3 times as described, then the plates were filled with 200 ul of diluted 1:2,000 Antibody-AP conjugate solution (Boehringer Mannheim GmbH, FRG) and incubated for 4 hr. at 37 ^{o} C. The plates were subsequently washed 3 times with wash buffer and filled with 200 ul of substrate solution (Boehringer Mannheim GmbH, FRG) into each well, incubated for 1 hr. at room temperature. The resulting dephosphorylation was stopped by addition of 3 M NaOH, and the absorbance at 405 nm (A_{405}) was determined for the content of each well with ELISA-plate reader

(Minireader II, Dynatech Laboratories, Inc., Alexandria, U.S.A.).

3.2 Shoot formation on cultures roots

The starting material was obtained from the previous experiment. The roots derived from anther culture of various potato cultivars were maintained in modified Murashige and Skoog (1962) medium as aforementioned reports. The experimental media contained the inorganic salts (macro- and microelements) of Murashige and Skoog (1962) and organic constituents of Nitsch and Nitsch (1969) supplemented with 1 ppm Zeatin, 1 ppm IAA, glucose (24.3 g/l), inositol (0.1 g/l) and 0.7 % agar (w/v). The roots were culture under 25 $\pm$ 2 ^{o} C. with 16 hr. light period.

3.3 Characterization and description of some potato clones.

Twenty two clones of cultivated potato, as follows :

a. *Solanum tuberosum* : 21 clones

Atlantic	Atzymba	Br 63.76	Bzura
China 3	CIP 720088	CIP 800244	CIP 800944
Denali	DTO 28	H_3 703*	Jetta
Kennebec	Lemhi	Monza	Norchip
P-005-16	Pirola	V-2	Russet Burbank
Spunta			

*dihaploid clone

b. *Solanum phureja* : 1 clone

IVP

Every potato clone derived from cuttings which the mother - stocks were planted in the insect proof screen house. The transplanting materials were transferred in experimental plots during December, 1988.

Characterization

Characterization of the potato plants, which were settled in the field, were studied. The plants at the age of at least one month were observed and measured. Average of four plants from each clone were recorded for morphological structure. Characterization was done according to the "Descriptors for the Cultivated Potato" (Huaman et al, 1977) with some modifications. Standardized scoring system was usually used. The studied characteristics were growth habit, stems, leaves and flowers. Descriptors for the cultivated potato used is listed as the followings :

DESCRIPTORS FOR THE CULTIVATED POTATO

A. GROWTH HABIT

A1 **Growth Habit Type**

1 Erect
2 Semi-erect
3 Decumbent
4 Prostrate
5 Semi-rosette
6 Rosette

A2 Plant Type

3 Spreading
7 Compact

A3 Branching Habit

1 Single
2 Branched

A4 Number of Primary Stems

1 Single
2 Few (2.0-3.9)
5 Medium (4.0-7.0)
7 Many (>7)

A5 Plant Height at Flowering Stage

3 Short (<21.0 cm.)
5 Medium (21.0-30.0 cm.)
7 Tall (>30.0 cm.)

A6 Weeks to Flowering

Counted as the number of weeks from transplanting to flower bud emergence.

B. STEM CHARACTERS

B1 Stem Color

1 Green only
2 Green with few purple
3 Green with some purple
4 Purple with some green
5 Purple

B2 Stem Cross-section

1 Round
2 Angular

B3 **Stem Size** (stem diameter)

1 Thin (<5.00 mm.)
2 Medium (5.00-5.50 mm.)
3 Thick (>5.50 mm.)

B4 **Internode Length**

3 Short (<2.70 cm.)
5 Intermediate (2.70-5.0 cm.)
7 Long (>5.0 cm.)

B5 **Stem Wing**

0 Absent
1 Straight
2 Undulate
3 Dentate

B6 **Stem Pubescence**

0 Glabrous
1 Glabrescent
3 Pubescent
5 Strongly pubescent

LEAF CHARACTERS

C1 **Leaf Attitude**

3 Semi-erect
5 Horizontal
7 Drooping

C2 **Number of Leaves under First Inflorescence**

3 Few (<6)
5 Intermediate (6-8)
7 Many (>8)

C3 **Petiole Length**

1 Short (<2.0 cm.)
2 Intermediate (2.0-3.0 cm.)
3 Long (>3.0 cm.)

C4 **Leaf Color**

1 Yellowish green
2 Green
3 Dark green
4 Purplish green
5 Purple

C5 **Leaf Size**

1 Small
2 Medium
3 Large

C6 **Leaf Dissection**

1 Undissected
2 Pinnatilobed
3 Scarcely dissected
4 Weakly dissected
5 Medium dissected
6 Strongly dissected
7 Very strongly dissected

C7 **Adaxial Leaf Pubescence**

1 Glabrous
2 Glabrescent
3 Pubescent
4 Strongly pubescent

C8 **Abaxial Leaf Pubescence**

1 Glabrous
2 Glabrescent
3 Pubescent
4 Strongly pubescent

C9 Type of Hairs (trichomes)

1 Absent
2 Simple
3 Simple and glandular

C10 Terminal leaflet shape

1 Ovate (l/w = 1.0-1.5)
2 Ovate-lanceolate (l/w = 1.5-2.0)
3 Lanceolate (l/w = 2.0-3.0)

D. FLOWERS CHARACTERS

D1 Flower Size (flower diameter)

1 Small (<2.0 cm.)
2 Medium (2.0-2.9 cm.)
3 Large (=>3.0 cm.)

D2 Calyx Color

1 Green only
2 Red only
3 Purple only
4 Green with some red
5 Green with some purple
6 Red with some green
7 Purple with some green

D3 Calyx Symmetry

1 Irregular
9 Regular

D4 Corolla Shape

1 Very rotate
3 Rotate
5 Pentagonal
7 Semi-stellate
9 Stellate

D5 **Predominant Flower Color**

1 White
2 Yellow
3 Light purple
4 Purple
5 Dark purple
6 Light blue
7 Blue
8 Dark blue

D6 **Secondary Flower Color**

0 Absent
1 White
2 Light red
3 Intense red
4 Light blue
5 Intense blue
6 Light purple
7 Intense purple

D7 **Distribution of Secondary Flower Color**

0 Absent
1 White acumen - adaxial surface
2 White acumen - abaxial surface
3 White acumen on both surfaces
4 Star - adaxial surface
5 Stripe - adaxial surface
6 Stripe - abaxial surface
7 Stripe - both surfaces
8 Stripe (flecked)
9 Other

D8 **Anther Pigments**

0 Absent
1 Pigmented anther stripe (AS)
3 Pigmented anther tip (PA)
4 AS and PA
5 Other

D9 **Stamen Formation**

1 Normal
2 Shriveled
3 Partly fused column
4 Free and divergent column

D10 **Pollen Production**

0 None
3 Few
7 Abundant

D11 **Pollen Size**

1 Reduced
2 Normal

D12 **Pistil Morphology**

1 Normal
2 Irregular appearance
3 Fusion of pistil with anther

D13 **Pistil Pigments**

0 Absent
1 Pigmented stigma (PS)
2 Pigmented ovary (PO)
3 Pigmented ovary wall (OW)
4 PS and PO
5 PS and OW
6 PS and OW
7 PS, PS and OW
8 Pigmented stigma and style
9 Other

D14 **Stylar Length**

1 Shorter than anther and straight
2 Shorter than anther and S shaped
3 Equal to anther
4 Longer than anther
5 Much longer than anther

D15 **Stigma Shape**

1 Capitate
2 Clavate
3 Bilobed (or split apart)

D16 **Degree of Flowering**

0 No buds
1 Bud abortion
3 Flowering scarce
5 Flowering moderate
7 Flowering profuse

D17 **Premature Flower Abscission**

0 No
1 Yes

D18 **Duration of Flowering**

3 Short
5 Medium
7 Long

D19 **Number of Flower per Inflorescence**

1 Single
2 Few (2-5)
3 Intermediate number
4 Many (>20)

D20 **Pedicel Articulation Position**

1 Below the middle
2 At the middle
3 Above the middle

D21 **Pigment at Pedicel Articulation**

0 Absent
1 Present

D22 Self Compatibility

0 No
1 Yes
2 Undetermined

Descriptions

A descriptive information was organized and presented as the plant descriptions. Characters to be described were growth habit : growth habit type, plant type, branching habit, number of primary stems, weeks to flowering, and plant height at flowering stage ; stem characters : color, cross-section, size, internode length, wing, and pubescence ; leaf characters : leaf attitude, number of leaves under first inflorescence, petiole length, color, size, dissection, pubescence, type of hairs, and terminal leaflet shape and size ; flower characters : flower size, calyx color, calyx symmetry, collora shape, flower color, anther pigments, stamen formation, pollen production, pistil pigments and morphology, stylar length, stigma shape, degree of flowering, premature of flower abscission, duration of flowering, number of flowers per inflorescence, pedicel articulation position and pigment, and self compatibility.

4. Results and discussion

4.1 Testing for PLRV resistant.

All of the tested potatoes either dihaploidy or tetraploidy could support the multiplication of PLRV in the plants. This could be notified by the change of the substrate (p-nitrophenyl phosphate) from colorless to yellow. However, compared with the negative and positive controls all of the tested *Solanum acaule* were highly resistant to PLRV which would be remarked by the slightly yellowing of the substrate, almost non-detectable by the naked eyes, whereas the positive controls and the susceptible cultivars were dark yellow. The detectable virus in *S. acaule* might be the translocated virus from the Sieglinde stocks to the *S. acaule* scions as scions. This presumption was confirmed by the case of the dihaploidy H_3 703, where the H 3703 were used as stocks, they had only half O.D. value, compared with the same H_3 703 when used as scions grafted on the PLRV infected Sieglinde stocks.

Table 1 Comparison of the multiplication of PLRV in the infected potatoes by using ELISA technique.

Cultivar	O.D. value (A_{405})
Negative control	0.00
Positive control	0.92
Solanum acaule	
PI 7434	0.03
Japanese Clone	0.045
PI 3716	0.07
Dihaploid potatoes	
H_3 703 (stock)	0.155
K 4	0.305
H_3 703 (scion)	0.31
K 7	0.38
JH 71	0.40
JH 52	0.555
Tetraploid potatoes	
Altlantic	0.12
Lemhi	0.13
Russet Burbank (stock)	0.21
Russet Burbank (scion)	0.24
Sieglinde	0.255
Spunta	0.26
Kennebec	0.325

Among the commercial cultivars tested in this experiment Atlantic and Kennebec were the most resistant and susceptible cultivars respectively.

For the tested dihaploid potatoes, the most resistant and susceptible lines were H_3 703 and JH 52 respectively. In the case of the tested S. acaule clones all of them were resistant to PLRV infection. The virus found in the plants might due to the translocation of virus from the infected scions to the stocks. The summarization was supported by the results of the H_3 703 where used as stocks and scions grafted with virus infected plants. It was found that the virus could translocate from the infected stock to the H_3 703 scion easily. Anyhow, this phenomenon was not so obvious in the case of Russet Burbank potato where slightly difference in O.D. value of the stock and scion was detected (0.21 and 0.24 respectively). The virus multiplication in S. acaule clones might be further studied.

4.2 Inducing shoot formation from the root cultures.

Green calli formation on the cultured roots was first observed after 7 days of sub-culturing. The developed calli were normally compact. After this phase ca. 20 - 25 days, adventitious shoots were induced from the Russet Burbank cultured roots (Fig. 2). The other clones were in

stage of shoot development with the expectation of the true leaf formation within the next reporting period. Anyhow, the regenerated plantlets have to be tested for the chromosome number and other characteristics. Since there was the callus phase involved in the induction process, therefore some variations of the plants could be expected. This was not the same as the result form Espinoza and Dodds (1985) where the root cultures of the diploidy and tetraploidy potatoes were used and no callus stage had been associated. Moreover, these variations are eventually useful for the breeding program in this experiment.

4.3 Characterization and description of potato clones.

4.3.1 Characterization

Characterization of growth habit, stems, leaves and flowers are shown in Table 2, 3 and 4 respectively. The title of each column in every table referred in the descriptor listed in 3.3.

4.2 Descriptions

Each clones were described for the important morphological characters observed from the field.

China 3 : Plant decumbent, moderately compact, branched with 3.75 primary stems; flowering at short height, 19 25 cm. and in the 6 th week after transplanting. Stems green, angular, 5.15 mm. in diameter, internode 2.60 cm. long, wing straight, pubescent. Leaves horizontal, 12.0 in number; petioles 3.0 cm. long; lamina green in color, 10.25 x 12.50 cm., weakly dissected; glabrescent on both surfaces, hairs simple; terminal leaflet shape ovate, 5.20 x 6.70 cm.

Table 2 Characterization of growth habit

No.	Clone	A1	A2	A3	A4	A5	A6
1	China 3	3	4	2	3	3	3
2	H 703 3	3	7	2	5	4	1
3	Kennebec	3	4	2	4	5	1
4	Lemhi	3	6	2	4	5	2
5	Monza	3	5	2	5	6	2
6	Norchip	3	4	2	3	3	3
7	Atlantic	3	4	2	4	4	1
8	P 005-16	3	5	2	2	4	3
9	Atzymba	3	6	2	5	6	2
10	Br 63.76	3	5	2	3	7	2
11	Bzura	3	6	2	4	3	2
12	Denali	3	6	2	4	4	2
13	DTO 28	3	7	2	3	3	1
14	Jetta	3	5	2	4	2	3
15	Pirola	3	6	2	5	5	2
16	V-2	3	4	2	2	4	3
17	CIP 800944	3	3	2	2	5	3
18	CIP 720088	3	6	2	4	6	2
19	CIP 800244	3	6	2	6	3	2
20	Russet Burbank	3	5	2	4	4	2
21	Spunta	3	6	2	4	5	2
22	IVP	3	6	2	5	2	1

Table 3 Characterization of stems

No.	Clone	B1	B2	B3	B4	B5	B6
1	China 3	1	2	2	2	1	2
2	H 703 3	3	2	3	3	2	3
3	Kennebec	1	2	3	2	2	5
4	Lemhi	1	2	3	1	2	3
5	Monza	2	3	3	7	2	3
6	Norchip	1	2	2	2	2	5
7	Atlantic	2	2	2	3	2	3
8	P 005-16	2	2	1	2	2	3
9	Atzymba	2	2	3	3	1	2
10	Br 63.76	3	2	1	4	1	3
11	Bzura	1	2	2	3	2	1
12	Denali	3	2	2	4	1	3
13	DTO 28	2	2	3	5	1	1
14	Jetta	1	2	1	1	1	5
15	Pirola	1	2	2	6	2	3
16	V-2	3	2	1	2	1	2
17	CIP 800944	2	2	1	2	2	3
18	CIP 720088	2	2	1	2	1	2
19	CIP 800244	2	2	3	4	1	1
20	Russet Burbank	2	2	1	3	1	3
21	Spunta	4	2	2	4	1	3
22	IVP	4	2	2	7	1	1

Table 4 Characterization of leaves

No.	Clone	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
1	China 3	5	1	3	2	3	4	1	1	1	1
2	H 703 3	5	2	2	3	2	4	1	2	1	1
3	Kennebec	4	4	2	3	3	3	1	1	1	2
4	Lemhi	5	5	3	3	2	4	2	2	1	1
5	Monza	4	1	2	2	2	5	1	2	1	2
6	Norchip	5	4	3	2	2	4	2	2	1	1
7	Atlantic	4	6	2	3	2	4	1	2	1	1
8	P 005-16	3	3	2	3	1	3	1	1	1	1
9	Atzymba	3	7	3	2	2	4	2	2	1	1
10	Br 63.76	3	1	1	2	2	3	1	2	1	1
11	Bzura	3	6	2	3	2	5	1	2	1	2
12	Denali	4	6	3	2	3	4	2	2	1	2
13	DTO 28	3	6	3	2	2	3	1	2	1	1
14	Jetta	3	3	3	2	2	4	1	2	1	2
15	Pirola	3	5	3	3	2	4	2	2	1	1
16	V-2	5	1	3	2	2	4	2	2	1	1
17	CIP 800944	5	4	1	2	1	4	2	2	1	1
18	CIP 720088	4	6	3	2	3	4	1	1	1	1
19	CIP 800244	7	4	3	2	2	4	2	2	1	1
20	Russet Burbank	7	1	1	2	2	4	2	1	1	2
21	Spunta	4	1	2	3	2	4	1	2	1	1
22	IVP	3	3	3	4	1	4	1	1	1	2

Table 5 Characterization of flowers

No.	Clone	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
1	China 3											
2	H 703 3											
3	Kennebec											
4	Lemhi											
5	Monza											
6	Norchip											
7	Atlantic	2	5	6	5	1	0	0	2	1	2	2
8	P 005-16											
9	Atzymba	2	1	3	3	1	0	0	3	1	5	2
10	Br 63.76	2	5	8	5	3	1	7	2	1	6	2
11	Bzura											
12	Denali	3	5	6	5	6	1	3	3	1	2	2
13	DTO 28											
14	Jetta											
15	Pirola											
16	V-2											
17	CIP 800944											
18	CIP 720088	2	5	7	7	3	1	7	3	1	2	2
19	CIP 800244	2	5	6	5	4	1	7	2	1	4	2
20	Russet Burbank											
21	Spunta											
22	IVP	1	7	9	5	5	1	4	3	1	5	1

Table 5 Characterization of flowers (cont.)

No.	Clone	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22
1	China 3											
2	H 703 3											
3	Kennebec											
4	Lemhi											
5	Monza											
6	Norchip											
7	Atlantic	1	0	4	1	3	-	-	2	3	0	
8	P 005-16											
9	Atzymba	1	8	4	1	3	-	-	3	3	0	
10	Br 63.76	1	9	4	1	4	-	-	3	3	1	
11	Bzura											
12	Denali	1	1	4	1	5	-	-	2	1	1	
13	DTO 28											
14	Jetta											
15	Pirola											
16	V-2											
17	CIP 800944											
18	CIP 720088	1	1	4	1	3	-	-	2	1	1	
19	CIP 800244	1	1	4	1	3	-	-	2	3	1	
20	Russet Burbank											
21	Spunta											
22	IVP	1	1	4	1	6	-	-	3	1	1	

H_3 703 : Plant decumbent, compact, branched with 5.75 primary stems; flowering at medium height, 21.62 cm. and in the 3.5 th week after transplanting. Stems green with some purple, angular, 5.65 mm. in diameter, internode 3.05 cm. long, wing undulate, pubescent. Leaves horizontal, 13.50 in number; petioles 2.75 cm. long; lamina dark green in color, 9.55 x 10.25 cm., weakly dissected ; adaxial leaf glabrescent but pubescent underneath, hairs simple; terminal leaflet shape ovate, 4.60 x 6.20 cm.

Kennebec : Plant decumbent, moderately compact, branched with 4.00 primary stems; flowering at medium height, 25.62 cm. and in the 4 th week after transplanting. Stems green, angular, 6.00 mm. in diameter, internode 2.35 cm. long, wing undulate, strongly pubescent. Leaves semi-erect, 15.0 in number; petioles 2.85 cm. long; lamina dark green in color, 10.25 x 14.65 cm., scarcely dissected; glabrescent on both surfaces, hairs simple; terminal leaflet shape ovate-lanceolate, 5.55 x 8.50 cm.

Lemhi : Plant decumbent, almost compact, branched with 4.50 primary stems; flowering at medium height, 24.87 cm. and in the 5.5 th week after transplanting. Stems green, angular, 5.75 mm. in diameter, internode 2.00 cm. long, wing undulate, pubescent. Leaves horizontal, 16.0 in number; petioles 3.55 cm. long; lamina dark green in color, 9.00 x 10.65 cm., weakly dissected; pubescent on both surfaces, hairs simple; terminal leaflet shape ovate, 4.15 x 5.45 cm.

Monza : Plant decumbent, moderately compact, branched with 5.75 primary stems; flowering at almost tall height, 29.00 cm. and in the 5 th week after transplanting. Stems green with few purple, angular, 5.70 mm. in diameter, internode 5.80 cm. long, wing undulate, pubescent. Leaves semi-erect, 12.0 in number; petioles 2.25 cm. long; lamina green in color, 8.75 x 11.75 cm., medium dissected; adaxial leaf glabrescent but pubescent underneath, hairs simple; terminal leaflet shape ovate-lanceolate, 3.50 x 5.75 cm.

Norchip : Plant decumbent, moderately compact, branched with 3.625 primary stems; flowering at short height, 18.62 cm. and in the 6 th week after transplanting. Stems green, angular, 5.30 mm. in diameter, internode 2.50 cm. long, wing undulate, strongly pubescent. Leaves horizontal, 15.0 in number; petioles 3.10 cm. long; lamina green in color, 8.10 x 11.60 cm., weakly dissected; pubescent on both surfaces, hairs simple; terminal leaflet shape ovate, 4.90 x 7.10 cm.

Atlantic : Plant decumbent, moderately compact, branched with 4.625 primary stems; flowering at medium height, 21.62 cm. and in the 4 th week after transplanting. Stems green with few purple, angular, 5.45 mm. in diameter, internode 2.80 cm. long, wing undulate, pubescent. Leaves semi-erect, 17.0 in number; petioles 2.50 cm. long; lamina dark green in color, 9.60 x 11.75 cm., weakly dissected; adaxial leaf glabrescent but pubescent underneath, hairs simple;

terminal leaflet shape ovate, 4.80 x 6.30 cm. Flowers medium, 2.45 cm. in diameter; calyx green with some purple, moderately regular; corolla pentagonal, white in color; anther tip pigmented, stamen normal, pollen production few, pollen size normal; pistil normal and without pigmentation, style longer than anther, stigma capitate; flowering scarce; 1.50 flowers per inflorescence; pedicel articulation above the middle, and without pigmentation.

P 005-16 : Plant decumbent, moderately compact, branched with 2.50 primary stems; flowering at medium height, 21.50 cm. and in the 6 th week after transplanting. Stems green with few purple, angular, 4.80 mm. in diameter, internode 2.60 cm. long, wing undulate, pubescent. Leaves semi-erect, 14.0 in number; petioles 2.00 cm. long; lamina dark green in color, 4.75 x 6.60 cm., scarcely dissected; glabrescent on both surfaces, hairs simple; terminal leaflet shape ovate, 3.40 x 4.40 cm.

Atzymba : Plant decumbent, almost compact, branched with 5.25 primary stems; flowering at almost tall height, 28.75 cm. and in the 5 th week after transplanting. Stems green with few purple, angular, 5.85 mm. in diameter, internode 2.70 cm. long, wing straight, pubescent. Leaves semi-erect, 19.0 in number; petioles 3.30 cm. long; lamina green in color, 8.80 x 11.50 cm., weakly dissected; pubescent on both surfaces, hairs simple; terminal leaflet

shape ovate, 3.50 x 5.05 cm. Flowers medium, 2.50 cm. in diameter; calyx green, almost irregular; corolla rotate, white in color; anther stripe and tip pigmented, stamen normal, pollen production intermediate, pollen size normal; pistil normal with pigmented style; style longer than anther, stigma capitate; flowering scarce; 5.75 flowers per inflorescence; pedicel articulation above the middle, and without pigmentation.

Br 63.76 : Plant decumbent, moderately compact, branched with 3.50 primary stems; flowering at very tall height, 32.00 cm. and in the 5 th week after transplanting. Stems green with some purple, angular, 4.65 mm. in diameter, internode 3.60 cm. long, wing straight, pubescent. Leaves semi-erect, 12.0 in number; petioles 1.65 cm. long; lamina green in color, 6.15 x 7.95 cm., scarcely dissected; adaxial leaf glabrescent but pubescent underneath, hairs simple; terminal leaflet shape ovate, 4.45 x 4.90 cm. Flowers medium, 2.32 cm. in diameter; calyx green with some purple, regular; corolla pentagonal, light purple with white stripe on both surfaces; anther tip pigmented, stamen normal, pollen production abundant, pollen size normal; pistil normal with pigmented stigma and style, style longer than anther, stigma capitate; flowering scarce; 5.50 flowers per inflorescence; pedicel articulation above the middle, and with pigmentation.

Bzura : Plant decumbent, almost compact, branched with 4.375 primary stems; flowering at short height, 20.87 cm. and in the 5.5 th week after transplanting. Stems almost green only, angular, 5.15 mm. in diameter, internode 2.90 cm. long, wing undulate, glabrescent. Leaves semi-erect, 17.5 in number; petioles 2.40 cm. long; lamina dark green in color, 6.85 x 10.80 cm., medium dissected; adaxial leaf glabrescent but pubescent underneath, hairs simple; terminal leaflet shape ovate-lanceolate, 3.00 x 4.65 cm.

Denali : Plant decumbent, almost compact, branched with 4.625 primary stems; flowering at medium height, 22.87 cm. and in the 5.5 th week after transplanting. Stems green with some purple, angular, 5.40 mm. in diameter, internode 3.70 cm. long, wing straight, pubescent. Leaves semi-erect, 17.0 in number; petioles 3.70 cm. long; lamina green in color, 11.00 x 12.20 cm., weakly dissected; pubescent on both surfaces, hairs simple; terminal leaflet shape ovate-lanceolate, 5.15 x 7.85 cm. Flowers large, 3.00 cm. in diameter; calyx green with some purple, moderately regular; corolla pentagonal, light blue with white acumen on both surfaces; anther stripe and tip pigmented, stamen normal, pollen production few, pollen size normal; pistil normal with pigmented stigma, style longer than anther, stigma capitate; flowering moderate, 3.16 flowers per inflorescence; pedicel articulation below the middle, and with pigmentation.

DTO 28 : Plant decumbent, compact, branched with 3.50 primary stems; flowering at short height, 18.50 cm. and in the 3 th week after transplanting. Stems green with few purple, angular, 5.65 mm. in diameter, internode 4.25 cm. long, wing straight, glabrescent. Leaves semi-erect, 17.5 in number; petioles 3.65 cm. long; lamina green in color, 8.85 x 11.15 cm., scarcely dissected; adaxial leaf glabrescent but pubescent underneath, hairs simple; terminal leaflet shape ovate, 4.95 x 6.40 cm.

Jetta : Plant decumbent, moderately compact, branched with 4.50 primary stems; flowering at very short height, 17.50 cm. and in the 6 th week after transplanting. Stems green, angular, 4.60 mm. in diameter, internode 1.90 cm. long, wing straight, strongly pubescent. Leaves semi-erect, 14.0 in number; petioles 3.85 cm. long; lamina green in color, 9.00 x 11.95 cm., weakly dissected; adaxial leaf glabrescent but pubescent underneath, hairs simple; terminal leaflet shape ovate-lanceolate, 4.95 x 7.60 cm.

P 005-16 : Plant decumbent, almost compact, branched with 5.00 primary stems; flowering at medium height, 26.50 cm. and in the 6 th week after transplanting. Stems green, angular, 5.55 mm. in diameter, internode 4.40 cm. long, wing straight, pubescent. Leaves horizontal, 13.5 in number; petioles 3.35 cm. long; lamina green in color, 9.60 x 11.65 cm., medium dissected; adaxial leaf pubescent but glabrescent underneath, hairs simple; terminal leaflet shape ovate, 4.15 x 5.90 cm. Flowers large, 3.02 cm. in

diameter; calyx green with some purple, moderately regular; corolla pentagonal, very pale yellow in color; anther stripe and tip pigmented, stamen normal, pollen production abundant, pollen size normal; pistil normal, without pigmentation, style longer than anther, stigma capitate; flowering profuse; 4.66 flowers per inflorescence; pedicel articulation above the middle, and without pigmentation.

Pirola : Plant decumbent, moderately compact, branched with 5.875 primary stems; flowering at medium height, 25.75 cm. and in the 5 th week after transplanting. Stems green, angular, 5.35 mm. in diameter, internode 4.50 cm. long, wing undulate, pubescent. Leaves semi-erect, 16.0 in number; petioles 3.20 cm. long; lamina dark green in color, 7.50 x 11.50 cm., weakly dissected; pubescent on both surfaces, hairs simple; terminal leaflet shape ovate, 4.20 x 5.85 cm.

V-2 : Plant decumbent, moderately compact, branched with 2.50 primary stems; flowering at medium height, 23.00 cm. and in the 6 th week after transplanting. Stems green with some purple, angular, 4.90 mm. in diameter, internode 2.55 cm. long, wing straight, pubescent. Leaves horizontal, 12.5 in number; petioles 3:00 cm. long; lamina green in color, 6.15 x 7.20 cm., weakly dissected; pubescent on both surfaces, hairs simple; terminal leaflet shape ovate, 2.70 x 3.55 cm.

CIP 800944 : Plant decumbent, spreading, branched with 2.00 primary stems; flowering at medium height, 25.00 cm. and in the 7 th week after transplanting. Stems green with few purple, angular, 4.20 mm. in diameter, internode 2.15 cm. long, wing undulate, pubescent. Leaves horizontal, 15.0 in number; petioles 1.70 cm. long; lamina green in color, 5.30 x 7.80 cm.. weakly dissected; pubescent on both surfaces, hairs simple; terminal leaflet shape ovate, 3.50 x 4.65 cm.

CIP 720088 : Plant decumbent, almost compact, branched with 4.50 primary stems; flowering at medium height, 27.00 cm. and in the 5 th week after transplanting. Stems green with few purple, angular, 4.60 mm. in diameter, internode 2.45 cm. long, wing straight, pubescent. Leaves semi-erect, 17.5 in number; petioles 3.45 cm. long; lamina green in color, 10.55 x 12.80 cm., weakly dissected; glabrescent on both surfaces, hairs simple; terminal leaflet shape ovate, 5.50 x 8.00 cm. Flowers medium, 2.60 cm. in diameter; calyx green with some purple, almost regular; corolla semi-stellate, light purple with white stripe on both surfaces; anther stripe and tip pigmented, stamen normal, pollen production few, pollen size normal; pistil normal with pigmented stigma, style longer than anther, stigma capitate; flowering scarce; 3.75 flowers per inflorescence; pedicel articulation below the middle, and with pigmentation.

CIP 800244 : Plant decumbent, almost compact, branched with 6.25 primary stems; flowering at short height, 18.12 cm. and in the 4.5 th week after transplanting. Stems green with few purple, angular, 5.75 mm. in diameter, internode 3.65 cm. long, wing straight, glabrescent. Leaves drooping, 15.0 in number; petioles 3.30 cm. long; lamina green in color, 9.90 x 11.30 cm., weakly dissected; pubescent on both surfaces, hairs simple; terminal leaflet shape ovate, 5.55 x 7.35 cm. Flowers medium, 2.30 cm. in diameter; calyx green with some purple, moderately regular; corolla pentagonal, purple with white stripe on both surfaces; anther tip pigmented, stamen normal, pollen production intermediate, pollen size normal; pistil normal, with pigmented stigma, style longer than anther, stigma capitate; flowering scarce; 2.00 flowers per inflorescence; pedicel articulation above the middle, and with pigmentation.

Russet Burbank : Plant decumbent, moderately compact, branched with 4.75 primary stems; flowering at medium height, 22.12 cm. and in the 5.5 th week after transplanting. Stems green with few purple, angular, 4.85 mm. in diameter, internode 2.90 cm. long, wing straight, pubescent. Leaves drooping, 11.5 in number; petioles 1.90 cm. long; lamina green in color, 7.80 x 9.40 cm., weakly dissected; adaxial leaf pubescent but glabrescent underneath, hairs simple; terminal leaflet shape ovate-lanceolate, 3.70 x 5.65 cm.

Spunta : Plant decumbent, almost compact, branched with 4.50 primary stems; flowering at medium height, 24.12 cm. and in the 5 th week after transplanting. Stems purple with some green, angular, 5.40 mm. in diameter, internode 3.50 cm. long, wing straight, pubescent. Leaves semi-erect, 12.0 in number; petioles 2.60 cm. long; lamina dark green in color, 7.00 x 8.45 cm., weakly dissected; adaxial leaf glabrescent but pubescent underneath, hairs simple; terminal leaflet shape ovate, 4.45 x 5.00 cm.

IVP : Plant decumbent, almost compact, branched with 5.75 primary stems; flowering at short height, 17.75 cm. and in the 4 th week after transplanting. Stems purple with some green, angular, 5.35 mm. in diameter, internode 5.10 cm. long, wing straight, glabrescent. Leaves semi-erect, 14.0 in number; petioles 3.30 cm. long; lamina purplish green in color, 5.45 x 8.25 cm., weakly dissected; glabrescent on both surfaces, hairs simple; terminal leaflet shape ovate-lanceolate, 2.20 x 3.40 cm. Flowers small, 1.60 cm. in diameter; calyx green with some purple, regular; corolla pentagonal, dark purple with white star on adaxial surface; anther stripe and tip pigmented, stamen normal, pollen production intermediate, pollen size reduced; pistil normal with pigmented stigma, style longer than anther, stigma capitate; flowering profuse, 6.25 flowers per inflorescence; pedicel articulation below the middle, and with pigmentation.

The overviews of the characteristics and descriptions of potatoes used in this studies could be concluded as the followings :

1. Growth habit : It was found that every clone of the cultivated potato appeared as decumbent. This meant that the stems trailed on the ground but raised at the apex. Branching habit was also recorded at variable number of primary stems, from 2.50 to 6.25. All plots showed the termination of growth by a number of weeks to flowering, as from 3.0 to 7.0 weeks after transplanting. The plant height at flowering was also varied from 17.50 to 32.C0 cm.

2. Stem characters : Color of the stems were predominantly as green only or with some degree of purple to greenish purple. The stem diameters and the internode length varied from 4.20 to 6.00 mm. and 1.90 to 5.80 cm., respectively. It was found that every clone bore angular stem cross-section with straight or undulate stem wing. Presence of simple trichomes on stems appeared in all plants.

3. Leaf characters : The attitude of the leaves was found to be more or less semi-erect or horizontal as well as drooping. Number of leaves on the main stem was recorded to be 11.5 to 19.0. Petiole length was also varied from 1.65 to 3.85 cm. The leaves were green with some purple pigmentation in some clones. The

leaves were odd-pinnate with scarcely to moderately dissected. Simple trichomes appeared on both surfaces. Terminal leaflets was ovate with leaf ratio of 1.0-1.5 or ovate-lanceolate with leaf ratio of 1.5-2.0.

4. **Flower characters** : As every potato clone showed some degree of flowering, some data could be presented. It was unfortunate that many clones produced flower primordia but the primordia could not further develop into flower buds. It was likely that the flower buds were dormant which could be the influence of the abnormal high temperature of this year and no flower characters could be recorded. The flower buds still did not abscise, therefore, no data on premature flower abscission exist.

5. Conclusion/Remarks

According to the previous reports, the roots could be obtained from the anther culture, but non of the true shoot formation could be achieved. Within this reporting period the adventitious shoot formation could be induced from the Russet Burbank 's anthers derived roots. Therefore, even though the direct embryogenesis from the cultured anthers were not occurred, the other possible way to obtain the intact plants is via the root culture.

The results from the ELISA testing for the PLRV multiplication in various commercial potato cultivars, diploidy potatoes and *S. acaule* revealed that the *S. acaule* clones used in the experiment were resistant to PLRV. Though the virus could be detected from the *S. acaule* plants, it might due to the translocation of the virus from the infected stocks. However, this need to be confirmed by using *S. acaule* as stocks and grafted with infected scions. The scions have to be excised after the complete transmission, then the virus concentration could be measured. In the other hand, the PLRV infected *S. acaule* from this experiment can be further propagated by the single node cutting technique to get more planting materials and the concentration of the virus will be then tested. If the concentration of the virus drops, no multiplication of PLRV in the *S. acaule* could be confirmed. Besides, other

Thai conditions as the results from this experiment and has PLRV resistant property e.g. Monza (Boulton et al., 1987) and the other clones, are needed to evaluate for their PLRV resistant property.

Characterization and description of potatoes though not fully completed, because no data for the flower characters could be observed, due to the abnormal high temperature of this year which has tremendous effect worldwide even in the U. S. A. It is expected to repeat in the next planting period. Moreover, the tuber characters as well as the solid and starch contents were not mentioned in this paper, these will be described in the next report.

Due to the late coming of the essential equipment for the isozyme pattern analysis, the work on this part and the protoplast fusion will be reported in the next progress report.

6. Workplan for the Next Period :

1. Regenerate the plants from the cultured anthers and calli derived protoplasts.

2. Working on the isozyme pattern analysis of various potato clones and the plants derived from the anther culture. Emphasis will be put on the *S. acaule* clones

3. Continuing the work on selection of the PLRV resistant in the potato clones.

4. Detail study on the characterization of tubers obtained from the last planting period. Solid and starch contents will be measured.

5. Continue working on the chromosome number analysis of the plants derived from the anther culture and the commercial potatoes.

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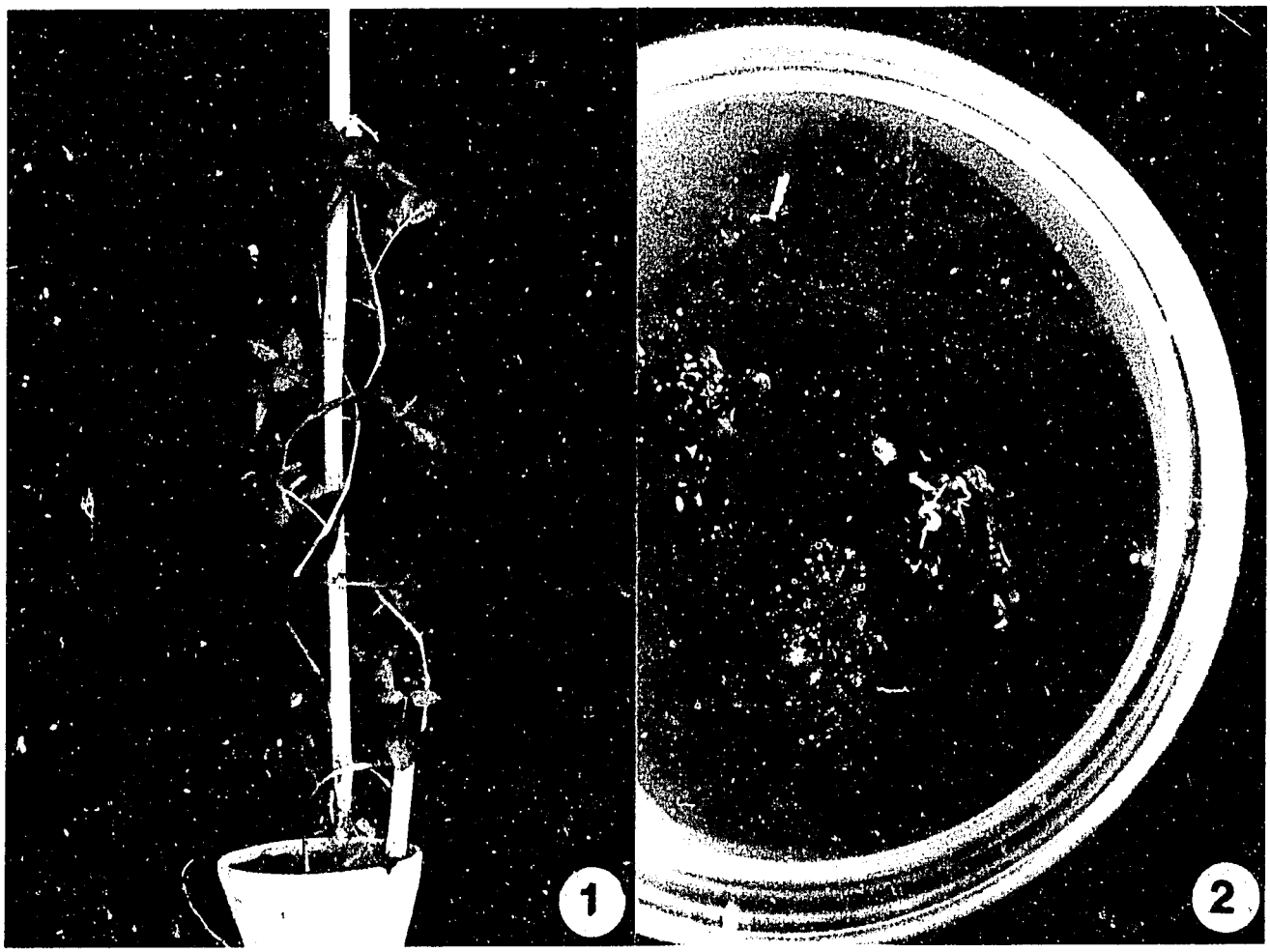
Fig. 1 Growth of the potato cv. Kennebec grafted with infected Sieglinde scion. After 4 weeks of grafting the scion was removed and the leaf sample was collected for the PLRV test by using ELISA technique.

Fig. 2 Adventitious shoot formation on the cultured potato roots which derived from the anther culture. Small compact green calli could be first observed before the real morphogenesis evolved.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