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PROGRESS REPORT NO. 1

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

6.401

A RESEARCH PROJECT

USAID/PSTC PROGRAM

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

SUBMITTED BY

DR. PRASARTPORN SMITAMANA

PROJECT LEADER

Rec'd In SCI: JAN 26 1983

FACULTY OF AGRICULTURE

CHIANG MAI UNIVERSITY

CHIANG MAI 50002

THAILAND

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Dr. Prasartporn Smitamana

Project leader

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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 Development 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
Project Consultants	:	Dr. Sherret S. Chase Dr. Stephen L. Sinden
Authorized Officer	:	Prof. Avudh Srisukri
Total Project Budget	:	3,816,192 B (149,070 US$)
Project Duration	:	3 Years (August 1987 - July 1990)
Reporting Period	:	August - December 1987.
Budget Allocation for this Period	:	1,679,232 B (65,679.665 $)

Content

1. Background/Introduction :

The potato is one of the world's most important crop plants in which commercial propagation is asexual rather than through true seeds. This vegetative reproduction preserves essential varietal characteristics from one generation to the next, however vegetative propagation also assures the perpetuation of many infections such as viral, viroid, and mycoplasma diseases from one generation to the next. To over come this problem, breeding for disease resistance is necessary.

Protoplasts (plant cells without cell walls) isolated from different genotypes, can be cultured or fused together, then regenerated as hybrid plants. This approach promises to play an increasing role in crop improvement by overcoming some limitations of conventional breeding methods. Potato is one of the crops to which technology can be applied.

Since the rich sources of germplasm for disease resistant can be found in many tuber-bearing Solanum species and their close relatives, the problem becomes one of transferring or incorporating these resistance genes to commercial acceptable potato

cultivars. The potato is also amenable to tissue culture. The improvement of potato by plant breeding is also complicated by its heterozygous, tetraploid genotype. Therefore it takes more time and involves growing larger numbers of seedlings than for diploid species to derive valuable phenotypes. Further, if the protoplasts derived from the tetraploid potatoes are being used for fusion, then their chromosome numbers will be important, in order to maintain tetraploidy in the new cultivars.

Dihaploid potatoes (2n = 2x = 24) have been successfully obtained from the cultivated tetraploid S. tuberosum (2n = 4x = 48) by anther culture (Dunwell and Sunderland, 1973 ; Irikura, 1975; Marunenko and Kuchko, 1985), and also via parthenogenetic procedures (Hougas and Peloquin, 1957). However, it has proved difficult to obtain pollen plantlets from the anthers of the cultivated potato (Kohlenbach and Gier, 1972 ; Sinha et al., 1976), and this may be due to the genotype of the donor plants (Dunwell and Sunderland, 1973 ; Foroughi-Wehr et al., 1977 ; Jacobsen and Sopory, 1978 ; Sopory et al., 1978 ; Wenzel et al., 1979 ; Wenzel and Uhrig, 1981). In solving this difficulty many factors have been studied. The components of the culture medium, the stage of development of the microspores have been investigated and suggested (Fridborg and Eriksson, 1975 ; Dunwell and Sunderland, 1978 ; Sopory et al., 1978 ; Sopory, 1979).

2. Objective

To produce the dihaploid plants which can be used in the somatic hybridization and the breeding program as well.

3. Material and Methods :

In order to overcome the physiological problems of the donor plants in which the flowering can be retarded by the tuberization, the potato scions are grafted onto tomato stocks to guarantee vigorous flowering. All the grafted plants are kept in an aphid proof screen house. Flower buds of size 4 - 6 mm. with the anther length about 3 - 4 mm. are collected and kept in the dark for 48 h in dry test tubes at 6 - 10 ^{o}C. Surface sterilization of the buds is accomplished by using 70 % ethanol with 0.1 % Tween 20 added. Subsequently, the anthers are dissected in a clean lamina air flow , plated on the following medium Nitsch and Nitsch (1969), Linsmaier and Skoog (1965) , modified Murashige and Skoog (1962) and potato extract medium (Anonymous, 1976). Then the plated anthers are incubated at 25 ^{o} $\pm$ 3 C. with 16 h photoperiod (Sopory and Rogan, 1976 ; Weatherhead and Henshaw, 1979).

4. Results/Discussion/Tables :

Callus induction is generally evidence 4 to 10 weeks after anther inoculation (Fig. 1). Though the pro - embryoid stage can be observed from the anther cultured in many kinds of media after 2 to 4 weeks culture (Fig. 2 and Table 1), but non of the true embryoid which normally can be observed rupturing the anther wall within 3 to 5 weeks after plating (Sopory et al., 1978 ; Wenzel and Foroughi-Wehr, 1984), can be detected by the time of this report. Some anthers have developed the green callus at the basal end of the anther (Fig. 3), anyhow , due to the limiting time, it is too early to test for the ploidy level of the callus. Even though the microspores at the uninucleate or late uninucleate stage have been selected in this experiment to enhance more chance of embryoid production (Dunwell and Sunderland, 1973 ; Sopory et all, 1978 ; Weatherhead and Henshaw 1979), still the true embryoid have not been observed yet. This may be caused by the influence of the genotype of the donor plant. Since it has long been noticed that some potato cultivars and dihaploid breeding lines respond more readily than the others (Dunwell and Sunderland, 1973 ; Foroughi-Wehr et al., 1977 ; Jacobsen and Sopory, 1978 ; Wenzel et al., 1979 ; Wenzel and Uhrig, 1981 ; Powell and Uhrig, 1987).

Table 1 Effect of media on callus development and pro-embryoid formation of the cultured anthers.

Media	Response		
	Browning[a]	Callus	Pro - embryoid
MS - PE*	14 - 21	+	+
N & N	3 - 5	-	-
N & N + 10% CW	14 - 28	+	+
L & S	7 - 12	-	+
P & U	7 - 10	-	-

* MS PE = Murashige & Skoog (1962) + 10 % potato extract
N & N = Nitsch & Nitsch (1969)
L & S = Linsmaier & Skoog (1965)
P & U = Powell & Uhrig (1987)
CW = Coconut water
a = days after inoculation
\+ = produce callus/pro-embryoid after 3 weeks inoculation
\- = not produce callus/pro-embryoid after 3 weeks inoculation.

The potato scions which have been grafted on the tomato stocks to avoid the physiological problem, due to the tuberization, which can hinder the flower formation, do form tubers at almost every node (Fig. 4). The tubers formed by this mean have the same characters as the normal ones, and the potato plantlets can be developed from such tubers (Fig. 6), however some of the aerial tubers are malformed (Fig. 5). This phenomenon has strong effect on the flowering, even 'nough can not be clearly explained the causes yet, but it could be probably the short photoperiod in the cool season under the northern Thailand's conditions.

5. Conclusion/Remarks :

By testing various media for anther culture experiment, almost every medium can induce the pro-embryoid formation. However the modified Nitsch & Nitsch medium with 10 % coconut water seem to be the best one, according to the result at this moment, because it can delay the browning of the anther and can also enhance the pro-embryoid formation simultaneously. Due to the limitation of time for this first progress report, the statistical analysis and the accurate data from the experiment can not be given here. Moreover the ploidy level of the developed callus can not be also determined by the time of this report.

The problem of tuberization of the grafted potatoes on tomato root stocks can be solved by increasing the photoperiod to 16 h by the artificial light source to stimulate more flower buds formation.

6. Workplan for the Next Period :

1. Continuing the anther culture experiment to get more information and the dihaploid plantlets.

2. Since the dihaploid potatoes can also be obtained by crossing with the *Solanum phureja* (Hougas and Peloquin, 1957 ; Hermsen and Verdenius, 1973 ; Van Breukelen et al., 1975), therefore the pollination of the desired potato cultivars will be done parallel with the anther culture experiment as soon as the *S. phureja* clones have been received from Dr. S.S. Chase.

3. Starting the protoplast isolation and culture work to find the suitable medium and method of cultivation to get high plating efficiency and regeneration rate of the isolated protoplasts from the tetraploidy and dihaploidy potato lines.

4. Because of the late coming of the needed equipment from the U.S.A. (refrigerated centrifuge and the gel electrophoresis set) the isoenzyme determination has to be shifted until April 1988.

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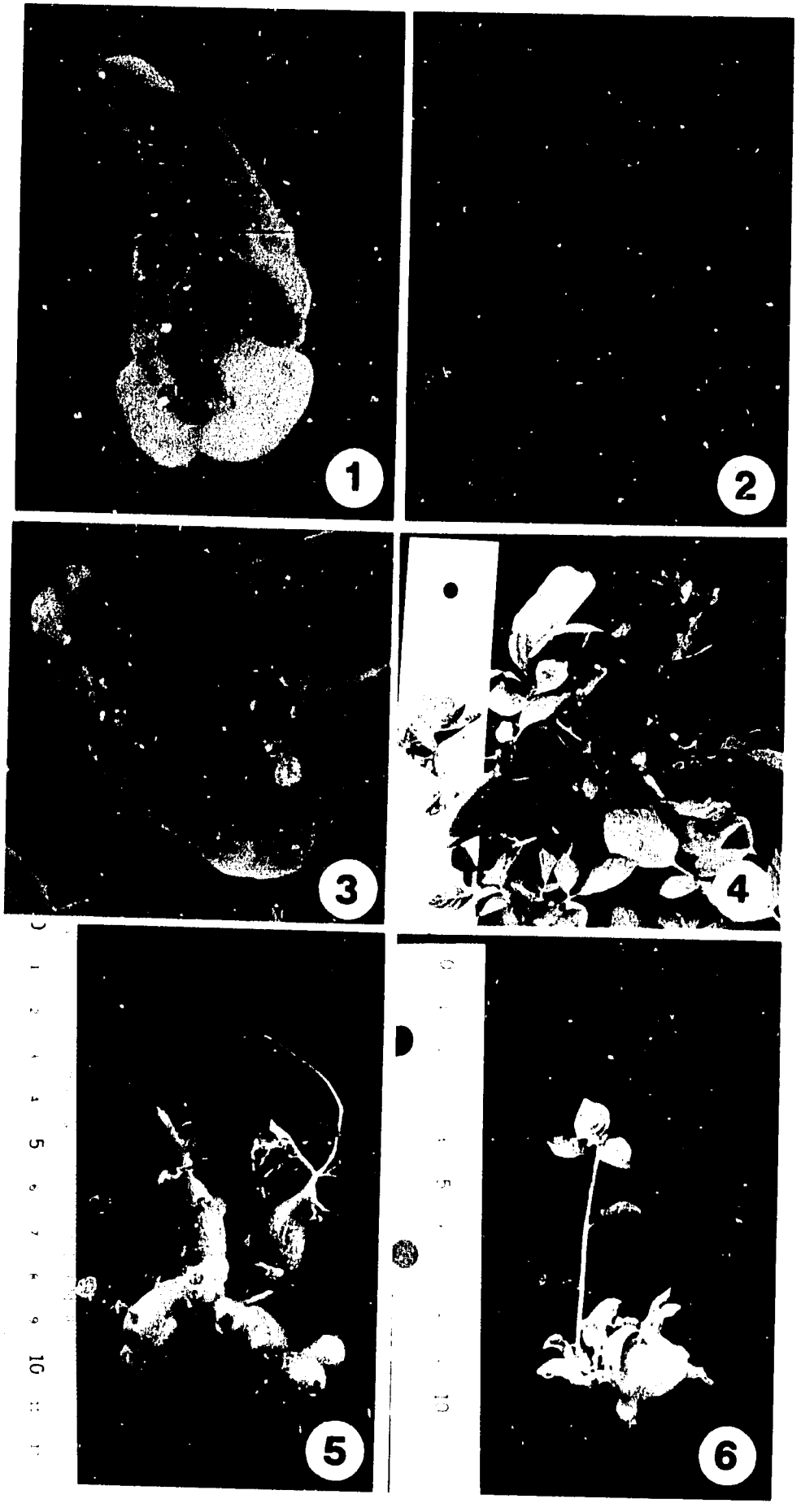
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