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FINAL

TISSUE CULTURE FOR VIRUS-FREE POTATO PROPAGATION PROJECT

FINAL REPORT OF COOPERATION PROJECT

INDONESIA - USAID

(AID/W : 936 - 5542)

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FINAL REPORT OF

TISSUE CULTURE FOR VIRUS FREE POTATO PROPAGATION PROJECT

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I. INTRODUCTION

The potato has been given the highest priority in the vegetable production due to the nutritional need and expanding local and export markets for this crop in Indonesia. It is nutritious and important vegetable which is very much needed in this country.

The present production is approximately 10 tons per hectare which is considered very low and generally it is due to the use of low quality potato seed. Imported seed of some varieties has been used as foundation seed but this seed degenerates rapidly and can only be used for about three years. Imported seed is very expensive to the growers. A 30 kg lot of this seed (tubers) costs approximately Rp 45,000.- or about two million rupiahs per hectare. Since Indonesia has approximately 35,000 hectares of potato growing areas at least 70,000 tons of potato seed are needed every year. An estimate of $ 70,000,000.- of devisa would have been spent yearly if all of good potato clean seed would be imported.

The utilization of tissue culture as a mechanism to clean-up potato seed has long been practised in temperate

countries where the potato is a staple food. But, it has not been done in tropical countries such as Indonesia. The introduction of this feasible and timely as both research facilities and trained man power to facilitate the adoption of this technique to assure and available supply of quality seed will enhance potato production and the nutritional status of the Indonesian people. With the insurance of a continuous supply of quality seed, the current potato production can be increased markedly. It is estimated by experiences that the average of 10 tons per hectare can easily be doubled and possible tripled by the introduction of clean virus-free seed coupled with good production practices.

The use of tissue culture as a mechanism to "clean-up" potato seed has been a general practice in temperate climates where the potato is a staple and major crop. Techniques and procedures to produce clean seed had been developed by several research workers (Kassanis, 1957, Kassanis, 1965; Thompson, 1956) and subsequently by Westcott et. all (1977). The scientist developed a technique for purifying virus infected potato clones by treating the infected plants with gibberellic acid to stimulate apical elongation. The elongated tissue culture which was free of virus was then multiplied by culturing on solid medium in-vitro. More recently, the International Potato Center (CIP) has used meristem

culture as a means of transferring germplasm from the International Institute to its cooperating national program (Roca et. all, 1979).

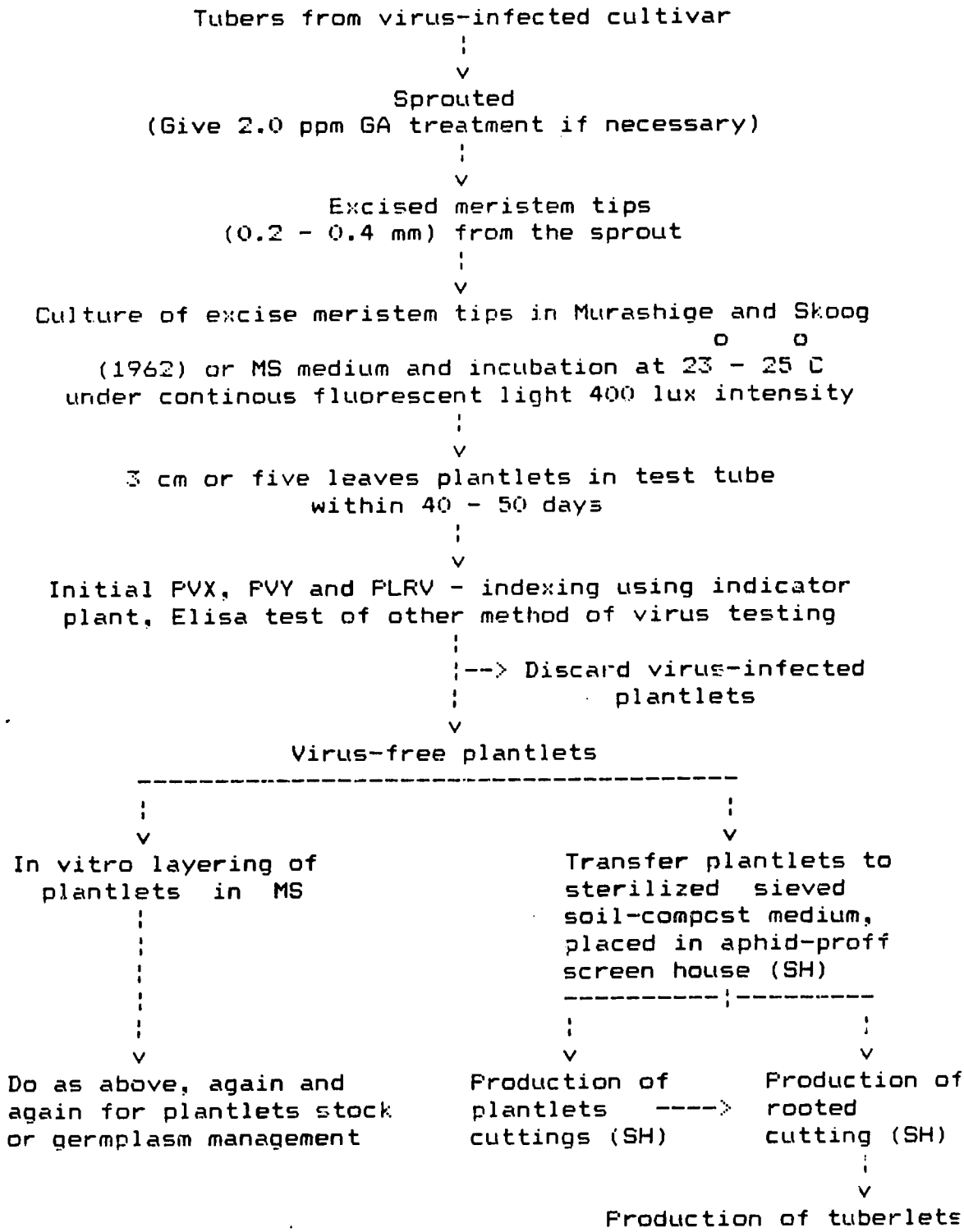
There are two objectives of the projects, first is to develop the capability to produce and maintain virus-free nuclear stock of widely grown commercial cultivars via meristem culture and enhance the effectiveness of the Lembang Research Station in receiving introduced genetic from abroad. Second, is to utilize these virus-free nuclear stocks in the production of virus-free foundation seed and ultimately healthy certified seed for distribution to farmers as an integral part of the national potato production program.

II. WORK PLAN

1. Technical aspect

The meristem tip culture techniques will be used to both "clean-up" the virus infected but widely grown local cultivars such as Katela, Rapan 106, Thung 151, Cipanas, Granola, MH 8025, Segunung and TD 84-152. And to hasten the multiplication of promising introduction such as Cosima, Desiree, DTO-28, LT-1, and others. While the other cultivars or clones that have valuable in breeding program are kept in-vitro to be a working collection.

Flowchart of activities leading to development of high quality certified seed through virus-free nuclear stock and foundation seed is shown in the figure below :



In order to effectively reduce the dependency of the potato growers on imported seed to further save the devisa and to increase the efficiency of the production of virus-free potato seed, a good seed program needs to be started as soon as possible. A three steps propossed to solve the potato seed problem is as follows :

a. Production pathogen free seed (tissue culture technology) at Research Institute.
b. Production of clean stock of foundation seed of recommended varieties at seed farms.
c. Production of clean or certified seed by farmers, cooperatives and other seed agencies.

This program requires coordinated efforts of a multidisciplinary team from institution of research, development and extension. There are many problems to be considered and solved in the production and distribution of the potato seeds to the farmers such as the initial meristem culture technique to clean and produce the quantity and quality of seed needed, management and production. Physiological and other storage problem, degeneration rate of seed, incidence of pests and disease including seed-borne disease, propagations procedure, and rate of increase and handling and transportation of seed.

2. Development and Strengthening Potato Research Program

As indicated earlier, Indonesia is attempting to diversify its food sources in order to maintain self-sufficiency in food. The white potato has a great potential to meet this objective. The production of the white potato, including provision of good seed supply, is a top priority in the national vegetable research program of Indonesia.

The addition of expertise in tissue culture will not only enhance production of virus-free seed stocks but also provide techniques for and then cultivar in breeding programs. It is worthy of note that tissue culture is also being utilized in the improvement of other vegetable, fruit and ornamental cropps such as, garlic, citrus and orchid.

3. Collaborative program with International and National Agencies

<u>International.</u> Indonesia is an active participant in the southeast Asian Regional program of the International Potato Center (CIP), The Southeast Asian Program for Potato Research and Development (SAPPRAD) linking Indonesia with Papua New Guinea, the Philippines, Sri Lanka and Thailand in potato improvement programs. Within SAPPRAD Project Indonesia has responsibility to

develop production techniques for potatoes at medium elevation in tropical areas. Cooperation has also been established with Victoria Seed Producer's Association where tissue culture techniques are utilized for producing virus free seed sources.

National. The headquarters of national vegetable research program is in Lembang, West Java (Lembang Horticultural Research Institute). This institute is one of the two research institute managed by Central Research Institute for Horticulture Crops in Pasarminggu Jakarta, Agency for Agricultural Research and Development (AARD) in the Ministry of Agriculture.

The national collaboration activity was expanded with other institution like Agriculture Extension Service and Bank of Indonesia (BI) Yogyakarta especially in the colaborative project between LEHRI and BI. Other than technical colaboration, there were also education and training program activities, which have been done in collaboration with Agricultural Education and Training Institute, Agency for Agricultural Education, Training and Extention.

III. DETAILED BUDGET

Detailed budget covering items of technical assistance, commodities, training, travel, operation and

maintenance either by USAID or Government of Indonesia is shown in the following figures (in US $) :

No.	Item	Budget USAID	Budget G O I
1.	Technical Assistance		
	a. Expatriate Consultants	13,500	-
	b. AARD Staff	-	44,100
2.	Commodities		
	a. Capital Items/Equipment	66,200	70,150
	b. Supplies	18,940	21,000
3.	Training		
	a. Abroad	10,000	-
	b. Local	20,000	10,500
4.	Travel		
	a. International	24,000	18,000
	b. Local	-	15,750
5.	Operation and Maintenance	-	46,200
	T o t a l	152,640	207,650

IV. PROJECT IMPLEMENTATION

1. Training, workshop and comparative study of the AARD staffs in relating countries

During the project activities, a number of AARD

staffs had been sent abroad to attend meeting, workshop, training and comparatived study. In addition to the staffs, two extension specialists and two potato growers visited Philippines to study on potato seed production in this country, especially in the seed farm managed by CIP.

1.1. Technical training of moleculair plant virology (Ati Srie Duriat, 1983, American Culture Collection, Rockville, Maryland, USA).

a. Multiplication of virus on the potato crop. Xanthi nc tobacco that grown in growth chamber had been used for virus multiplication. Relation between virus-satelite and host plant was studied through serial passage on Xanthi nc, then viruses had been obtained were isolated.

b. Purification of viruses. CMV purification followed Lot and Kaper procedure by using of polyethelene glycol and differential ultracentrifugation technique.

c. Determination of virus strain. Virus strain determination was established through virion gel, where each of virus strain would migrate to a certain distance.

d. Extraction of RNA virus and analyzed of the present of CARNA 5.

- RNA of CMV can be isolated through phenol extraction followed by alcohol precipitation.

- Characteristic of RNA virus can be obtained by using of electrophoresis of RNA of virus at 2 % of polycrylamideagarose compocite or survey gel. This slub gel will determine RNA component and in the mean time CARNA 5 satelite.
- Variant of the CARNA 5 can be observed through testing at 9 % gel and comparing with the markers.
- Isolation or purification of CARNA 5 can be done by top fractionation at the gradient sucrose had been centrifuged at 36,000 rpm, 20 hours. CARNA 5 can be separated from the RNA viruses.
- Purification of RNA virus, i.e. RNA 123 was determined through bottom fractionation at the gradient sucrose had centrifuged at 36,000 rpm, 20 hours. This method was the development of top fractionation.
- Purification of CARNA 5 variant was established by gel elusion. Top fractionation can only separate CARNA 5. Nine percent of polyacrylamide will do of each CARNA 5 variant.

e. Biological assay of the satelite had been obtained. The satelites obtained was mixed with

helper virus or other strain, and noted how it work in tomato variety Rutger. The combination between virus and satelite give different effect, i.e. necrotic, mozaic, mild or no symptom at all in the host plant.

1.2. First Annual Conference of the International Plant Biotechnology Network (Azis Azirin Asandhi, October 1985, Colorado State University, USA).

A paper about tissue culture in Indonesia with specific on rapid clonal propagation and its role in the development of agriculture had been sent to the organizing committee. It summarized the works on tissue culture research in Indonesia. Some research result were present as well.

a. Of the kopyor coconut research result, White's basal medium modified by De Gusman was the most effective for growth promoting of the embryo. In stimulating development of lateral root and promoting a balanced shoot as well as root growth of seedlings, a solid medium Murashige and Skoog's formulation supplemented with 10 mg/l IAA, 0.5 mg/l IBA, 6 % dextrose and 0.5 % activated charcoal are the best culture medium.

b. At potato crop, solid medium composition containing Murashige and Skoog's basal media

supplemented with 0.25 mg/l BAP, 0.5 mg/l GA, 2 mg/l Ca-Panthotenate or Murashige and Skoog medium supplemented with 0.5 mg/l BAP, 0.5 mg/l GA, 0.02 mg/l NAA, 2 mg/l Ca-Panthotenate are the best for potato meristem culture.

c. A modification of Vacin and Went medium supplemented with coconut water, Benzyl Adenine and NAA, stimulated formation of somaclone (protocorm like bodies) of Dendrobium Youppa Dewan and Aranthera James Store orchids. Apical bud culture is better than lateral one.

d. On citrus plant, a micro grafting technique has been introduced. This shoot tip grafting comprises of four step, i.e. preparation of root stocks, preparation of scion, grafting and maintaining the grafted plants and transfer to soil. Root stock obtained from sterilized peeled seeds are sown in 25 ml medium of Murashige and Skoog solidified with 1 % Bacto Agar and placed in the dark at 27 ^{o}C for two weeks. Shoot tip for grafting obtained from actively growing flushes of green house-grown plant. Grafted plants cultured in the liquid medium of Murashige and Skoog riched with 0.2 mg/l thianube-HCl, 1.0 mg/l pyridoxin-HCl, 1.0 mg/l nicotinic acid, 100 mg/l inositol and 75 mg/l sucrose. At 4 - 6

weeks old, grafted plants are ready to be transplanted to pots containing a steamed soil mixture.

e. Experiments on sugarcane are almost similar to that of Heinz and Mee methode. Callus formation was induced by 3 ppm 2,4-D, 0 - 0.3 ppm kinetin and added with 10 % of coconut water. Differentiation of plants were induced by transfering the callus segments to a non 2,4-D medium and plantlets could be grown in soil and developed into a normal plant.

f. Experiment on rice crop had been conducted for drought tolerant varieties, i.e. IR-28, IR-30, IR-36 and IR-40. These materials were germinated on Lismaier and Skoog medium. Callus was formed at concentration of 1.5 ppm 2, 4-D and 7.5 ppm IAA. Callus formation also appeared on root segments with 6.5 ppm 2, 4-D medium while plantlets were formed with 0.65 ppm 2, 4-D medium.

g. Clonal propagation of ginger was achieved on medium Murashige and Skoog in combination with that of Hildebrant for the minor elements and vitamins. Four weeks old bud of rhizomes were used and callus formation was induced with 1 - 2.5 ppm BAP, shoot and roots formation were

produced on medium with 0.2 - 1 ppm NAA or 0.05 ppm 2, 4-D and 1 - 2 ppm BAP. Additional adventious shoots was induced by 1 - 2.5 ppm NAA and 0 - 0.3 ppm BAP.

1.3. Southeast Asia and Pacific Potato Seed Production Workshop (Ati Srie Duriat and Asih Kartasih Karjadi, June 1986, Baguio City, Philippines).

The objectives of the workshop were : status of seed production in Sotheast Asian Countries, new technology of the potato rapid multiplication technique and formulate the seed potato production program for each country by using of rapid multiplication technique.

Workshop was attended by 33 scientist from 7 countries (Indonesia, Philippines, Thailand, Sri Lanka, Papua New Guinea, Vietnam and People Republic of China). In this occasion Dr. Batugal (SAPPRAD Coordinator) was asking about information of virus disease status in country member. This information will be a great value of seed potato production program.

At the last days of the seminar, each country must present proposal of seed production and related experiments. Indonesia gave proposal of "Improvement of Seed Potato Production in Indonesia" for the year of 1987 - 1992. This proposal paid attention on

research field rather than in seed production it self. It consisted of three elements, i.e. how to obtain high quality of seed, virus free seed; aplication of rapid multiplication technique; economic analysis of rapid multiplication technique.

1.4. Comparative study of Potato Production Transfer Technology. (Azis Azirin Asandhi, LEHRI; Indra Mardi and Suradi, Extension Specialist; Mashud and Joko Lesmono, Potato Growers;February 1987, Philippines).

The objective of this mission was to study about transfer of technology in the Philippines that had been established one year ahead than that of in Indonesia. There were three steps of transfer technology activities that had been reached by the Philippines, i.e. :

a. Development of available seed potato production scheme using rapid multiplication technique (RMT) and diffuse light storage (DLS). Establishment of potato seed growers in Philippines was done in highland of Baguio. Six farmers have been provided with screen house. Potato seeds produced from them are sources of potato seeds of the lowland farmers. Price of seed was much

cheaper compared with imported one (P 0.16/rooted cutting or P 16.0/kg, compare P 45/kg for imported seed). Varieties developed for lowland were also favorable for the highland farmers, so seed marketing is not the problem. In Indonesia LEHRI just provided two farmers with screen house, i.g. at Wonosobo for Central Java and at Sumber Brantas for East Java and Bali. Seeds produced were sold only for local farmers, not spread out yet to mid elevation. This is because there is no variety that suitable both for high and mid elevation. Variety Granola was the only variety produced by cooperating farmer was not suitable for mid elevation.

b. Refinement and packaging of technologies from research results and viable farmer practices to make them technically feasible, economically viable, socially acceptable and environmentally safe in specific growing area. In this case Philippines has advantages to adopt the research result from CIP Region VII, either for lowland (Canlubang) or highland (Baguio).

c. Establishment of Pilot Potato Communities, which can serve as focal points for expanded potato production preferably near big consumption centers and the training of technicians and farmers from these communities on the potato

production. Because of good flow of seed from seed growers to the potato farmers at lowland area, planting time can be done at the right time (November 15 - December 15). From the farmer's field visited around big cities it was seen that the growth of potato crop was very good (7 - 20 m above see level). Based on the sample observed (500 g/plant) it was estimated that the yield would be 30 t/ha. In Indonesia potato seed supply was always late, so the planting time was delayed from June - July to August - September. As the result, from a number of tests (Malang, Blitar and Karanganyar) the yield was low, while in Bali, Yogyakarta, Tegal and Magelang the yield was about 10 - 31 ton/ha.

2. Research facilities

The project was initiated by setting up the tissue culture laboratory. Two rooms (3 x 7 m2) and (4,5 x 7 m2) were modified to be incubation room and preparation room. Inside of this preparation room, another small room for placing laminar cabinet had been built. The later is isolated one, where the explants was cutted (see figure 1 and 2). Equipment and chemicals either locally available or imported were ordered and purchased. For imported items, it had taken more than one year, however by now the are being used.

Figur 1 : Incubation room.

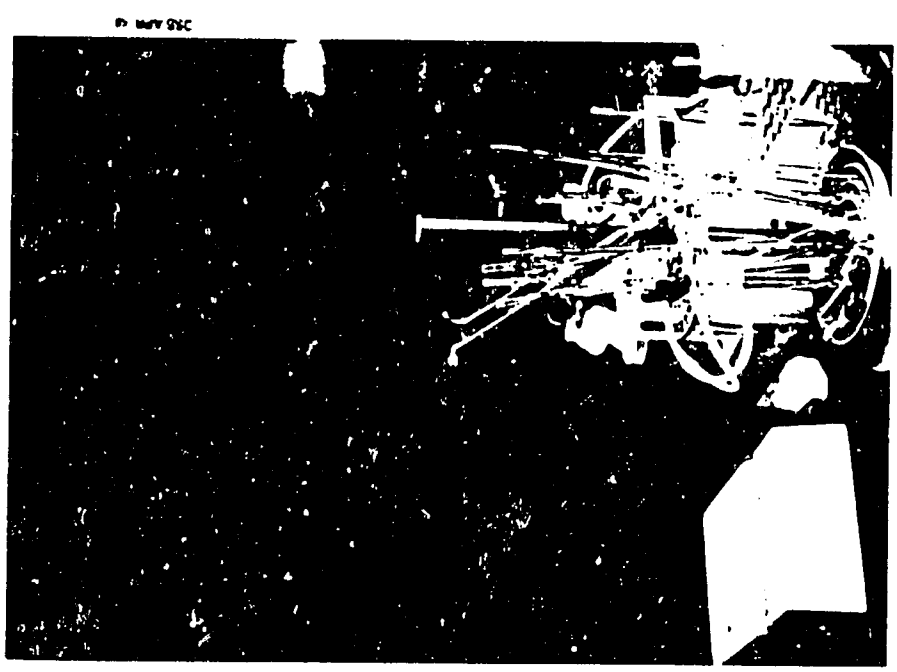


Figure 2 : Preparation room and isolated room.

The 200 m2 aphid-proof screen house construction had taken more than one year for obtaining an approval of the architectural drawing. It was constructed in May 1984 and finished on November 10, 1984. Some reparation work was done during the maintenance period. It has consumed 41 percent of USAID grant. This screen house is needed for maintenance and multiplication of clean plant materials (see figure 3 and 4) through rapid multiplication technique (stem cutting).

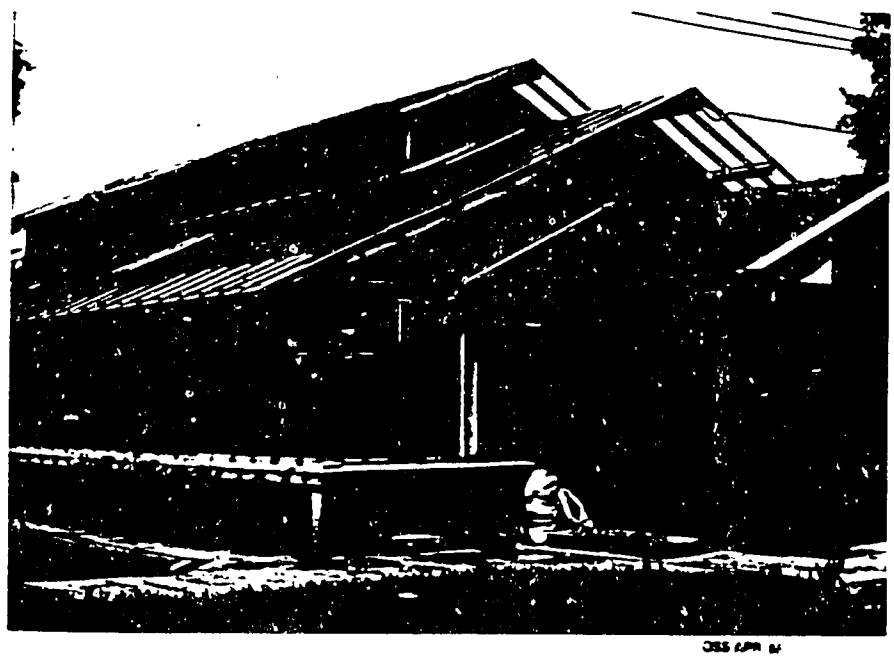


Figure 3 : Aphid-proof screen house.

During this time one simple propagation house and three simple net houses were built. These net houses are used for tuberlets propagation (see figure 5 and 6 and 7).

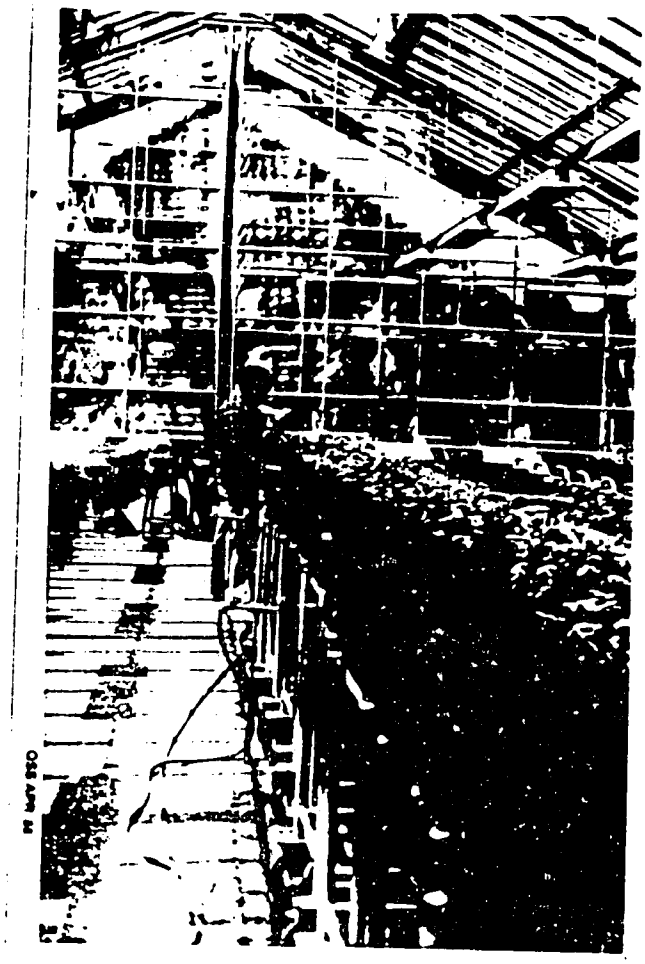


Figure 4 : Inside of the screen house (notice the car-trays inwhich the mother plants were propagated).

Figure 5 : Simple propagation house.

Figure 6 : Simple net house with iron framed.

Figure 7 : Inside of the net house (notice the square boxes, inwhich rooted cutting are propagated for tuberlets production.

3. Research Development

3.1. Meristem Culture

Preliminary studies on screening of the meristem culture media with cultivars Cipanas, Katela, Desiree and Cosima had been done as follows :

a. Comparing solid and liquid media with or without water for meristem culture.
b. Screening 10 media composition for meristem culture with four cultivars Cosima, Cipanas, Late;a amd Desoree.
c. Testing of selected media for multishoot culture.
d. Preliminary study on using locally available materials for propagation in vitro media.
e. Preliminary study on storage media.
f. Virus indexing.

3.2. Rapid multiplication techniques

The rapid multiplication techniques by stem cuttings, single node cuttings and leaf bud cuttings has been initiated in November 1978. The techniques can be used to clean mother plants grown from tuber. The techniques for rapid multiplication of plant materials coming from in vitro are being studied in order to prepare the technology to be adapted and applied by the

3.4. Clean seed supply

Lembang Horticultural Research Institute should produce at least 60,000 tuberlets of desired varieties per year in order to provide clean seed production program. It can be done with a cooperation with Directorate of Horticulture Crops Production. Information regarding desired varieties, time of planting, quantity of seed from recipients should be given by this Directorate. In 1986 it was produced 27,000 mother plants at Lembang Screen House and then sent to one farmer at Wonosobo for further multiplication. The farmer was able to produce more than 100,000 cuttings for 1 ha of land.

4. In Country Training and Workshop

Originally only one research-workers was in charge for the work and then two field workers were recruited and were trained to be a technician in the laboratory and in the screen house. Research worker on tissue culture and seed production were trained on tissue culture and rapid multiplication techniques for potato at St. Lucia, Philippines and also attending training and workshop on the same subject in Vietnam. These training were sponsored by the International Potato Center (CIP). In 1984 one more research worker and two technicians joined the project.

The training on rapid multiplication techniques was held at Lembang Horticultural Research Institute on November 4 to December 4, 1984. Previous knowledge plus experience from St. Lucia Philippines were given in the training in which 37 participants from extension workers, seed growers and farmer from West Java, Central Java, East Java, Bengkulu, Jambi, Bali, Sumatera, Jakarta and Yogyakarta were trained. One consultant from St. Lucia Philippines had been invited to join this training in addition to the lecturers and instructors from Lembang Horticultural Research Institute. The second training was held at Sidomulyo Extension Service Farm on 12 - 15 March 1985 and was attended by 10 farmers and 2 extension workers. The third training was held at Ketindan Agricultural Education and Training Center on May 27 - June 10, 1985 and was attended by 30 agricultural workers of extension service and seed farm. The fourth training was held at LEHRI on October 27 - November 8, 1986 and attended by 26 agricultural staffs.

By the Project Implementation Letter No. 8 approval, one researcher from LEHRI, two Extension Specialist from Central Java and two Key Farmers from Central and East Java were sent to Philippines. The purpose of the trip is to gain additional knowledge regarding tissue culture and rapid multiplication

techniques. The trip was taken place from February 22 to March 1, 1987.

Some of the training activities can be observed at Figure 8, 9 and 10, below :

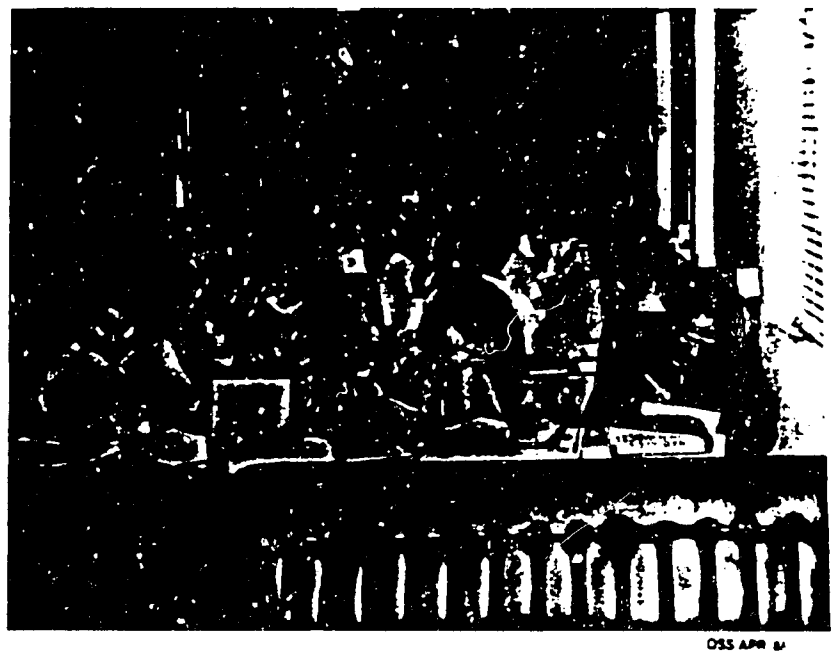


Figure 8 : Participants were receiving lecture in the class room.

Figure 9 : Participants were doing practical subjects in the nursery.

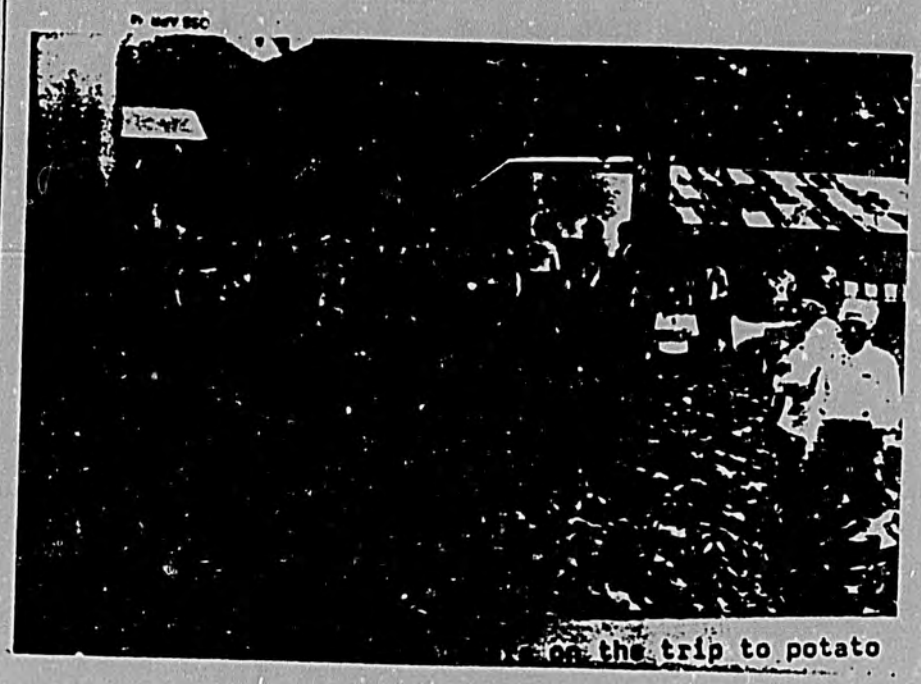


Figure 10 : Participant were on the trip to potato growing area, Wonosobo, Central Java.

5. On Farm Research

The technology of rapid multiplication of seed potato production and diffuse light seed storage must be disseminated to the farmers. For this purpose on farm research had been initiated at two provinces, Central and East Java. This activities involved 4 farmers at Central Java and 11 farmers in East Java. Two of these farmers were provided with net-houses to propagate clean materials. From 15 farmers, it seemed only two farmers who were provided with screen house improved very much. One of them, now is continously doing rapid multiplication to provide high quality of potato seeds. Figure 11, shows the activity of rooting the stem

cutting done by one of the participant in which more than 100,000 cuttings were produced for 1 ha of land.

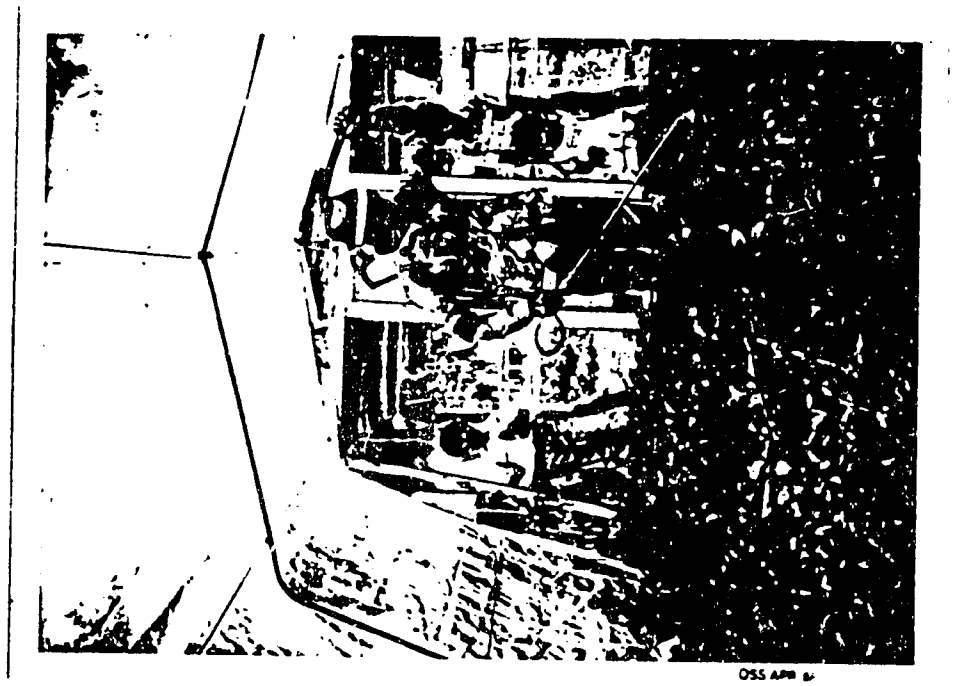


Figure 11 : Farmer activities in the net house, at Wonosobo, Central Java.

6. Budget Allocation

On January 6, 1986, Project Amandement that proposed by LEHRI had been approved by USAID. This budget mus be amended to allow LEHRI to produce more commodities and construct four simple screen house which are still needed to strengthen project implementation. Commodities had been procured to support research activities in Laboratory including heating and drying oven, glasswares, plastic pots, chemicals, text books and type writter. Four simples screen house had been used to

isolate clean tuberlet production in four separate location. The budget amendment that had been agreed are as follows :

Items	Original ($)	Amendment ($)	Change ($)
Technical Assistance	13,500	6,000	7,500 (-)
Commodities	85,140	122,640	37,500 (+)
Training :			
- Abroad	10,000	-	10,000 (-)
- Local	20,000	10,000	10,000 (-)
Travel	24,000	4,000	20,000 (-)
Contingency	-	10,000	10,000 (+)
T o t a l	152,640	152,640	

Total budget that had been allocated to LEHRI from the project and GOI at the end of the project are as follow :

Item	USAID (Rp)	G O I (Rp)
AARD Staff	-	106,178,202.90
Commodities	86,700,500.00	24,760,041.80
Training	10,208,080.00	25,096,600.00
Travel	2,304,940.00	16,202,900.00
Operation/Maintenance	600,000.00	24,736,059.05
Contingency	2,930,330.00	8,560,493.70
T o t a l	102,743,850.00	205,434,288.45

7. Research Result

7.1. Tissue Culture and Rapid Multiplication Techniques for Tuberlets production studies

The potato has been given the highest priority in the vegetable production program due to expanding local and export markets, and its nutritive value. Its yield at average of 10 tons per hectare is lower than the potential yield due to several factors including quality.

One of the main constrains in growing potatoes is the availability of healthy seeds. The only source of healthy seed is imported seed which will usually degenerating after three to four generations. To overcome this situation tissue culture especially meristem culture and rapid multiplication techniques have been initiated by the project to produce first generation tubers.

Traditional method of potato tuber seed production is based on low multiplication ratio that varies from 1 : 3, to 1 : 15 (1 tuber yields 3 to 15 tubers). The ratio is influenced by variety, agronomic practices and manipulation of the physiological age of the seed tuber (Bryan, 1981).

A rapid multiplication scheme for seed production using cutting, single or in combination with tissue culture. Recently ten propagation methods have been published, four using cutting in glass house style while

the rest by tissue culture techniques (Goodwin, 1980). A healthy plant (free from disease) can be poliferated into multiple shoots that can be further multiplication through cuttings. This can potentially yield thousands of plantlets in a short time with a minimum space required. Another advantage is the maintenance of clean stock for planting. Unlike tubers cutting they have no risk of degeneration and infection of viral or bacterial disease. It is a clone propagation, and there is no segregation. Uniform plantlets can also be produced in a specific time frame.

Lembang Horticultural Research Institute has been doing experiment in RMT for tuberlets production since 1983. The experiment consist of tissue culture, cuttings and tuberlets production.

The purpose of the experiments in RMT is to increase the quality and quantity seed potato production via tissue culture and rapid multiplication. And the techniques are being studied in order to provide technology which can be easily adapted by farmers or seed growers.

7.1.1. Tissue Culture

a. Preliminary study on screening media for potato meristem culture - fifteen media composition.

The experiment was done at tissue culture

laboratory of LEHRI from June 1983 to March 1984. They were ten solid media and five liquid media were screened for potato culture media. And four potato varieties were used : Cosima, Desiree, Katela and Cipanas.

The media composition (Tabel 1), basic media used were Murashige and Skoog (1962), Morel and Muller; Kmudson C media.

Meristem were exised under stereo microscope in aseptic condition and then planted either into solid media in test tuber 10 x 75 mm or liquid media in 50 ml flash or test tube 10 x 75 mm with paper bridges. They were incubated in the room with temperature 25 ^{o}C - 27 ^{o}C, relative humidity 80 %, and continous light from 15 watt TL tuber (4 tubes/m2).

b. Effect of two concentration of Murashige and Skoog media on the in vitro growth of three potato cultivars

The experiment was conducted at the tissue culture Laboratory of LEHRI from May to July 1986 and to see the effect of the concentration of Murashige and Skoog (1962) media on growth of three potato cultivars using meristem. The cultivars used were Rapan 106, Thung 151, and TD 84 - 152. Two concentration : recommended by MS (M_1) and 1,25 times recommended concentration (M_2) were evaluated.

c. Mass propagation in-vitro for five potato cultivars

The experiment was conducted at Tissue Culture Laboratory of LEHRI and the purpose of the experiment was to make shoot node growth factor for five potato cultivars.

The media used for the experiment was a liquid media of Murashige and Skoog (1962) with suplement 20 g/l sucrose; 2 mg/l CaP; 0.04 mg/l GA_3 0.05 mg/l BAP and 0.01 mg/l NAA. The five cultivars used were Desiree, I.931 PSF 30.10, MS 1C-2, N>565.1 (they were introduced from CIP Peru).

In vitro plantlets were cut in pieces with three to four nodes and the large leaves were removed. These nodal segments were placed in liquid media and the flasks were shaken continously (60 rpm).

Table 1. The media composition.

Basic Media (1)	Suplement mg/l GA3 (2)	NAA (3)	BAP (4)	Kin (5)	CaP (6)	CW (7)	Inositol (8)
a. Solid Media							
MS / MC 1	0.25	-	-	-	-	-	100
MS / MC 2	0.50	-	0.25	-	2.00	-	100
MS / MC 3	0.20	-	-	-	2.00	-	100
MS / MC 4	0.50	0.02	0.50	-	2.00	-	100
MS / MC 5	-	1.00	-	2.00	-	-	100
MS / MC 6	0.10	-	-	-	-	-	100
MM / MC 7	0.10	-	-	-	-	-	-
MM / MC 8	0.10	-	-	-	1.00	-	100
K / MC 9	-	-	-	-	-	-	-
BM / MC10	-	5.00	-	-	-	150 cc	-
b. Liquid Media							
MS / MC 1	0.40	0.01	0.50	-	2.0	-	100
K / MC 2	-	-	-	-	-	-	-
MS / MC 3	0.10	-	-	-	-	-	100
MS / MC 4	0.20	-	0.25	-	2.0	-	100
MS / MC 5	0.50	-	0.25	-	2.0	-	100

	Trypton (9)	AS Formula (10)	Socrose (11)	Agar (12)
a. Solid Media				
MS / MC 1	-	-	30,000	5,000
MS / MC 2	-	-	30,000	5,000
MS / MC 3	-	-	30,000	8,000
MS / MC 4	-	-	30,000	8,000
MS / MC 5	1000	-	30,000	6,000
MS / MC 6	-	-	25,000	6,000
MM / MC 7	-	-	20,000	6,000
MM / MC 8	-	-	20,000	6,000
K / MC 9	-	-	20,000	7,000
BM / MC10	-	2000	20,000	7,000
b. Liquid Media				
MS / MC 1	-	-	30,000	-
K / MC 2	-	-	20,000	-
MS / MC 3	-	-	25,000	-
MS / MC 4	-	-	30,000	-
MS / MC 5	-	-	30,000	-

MS : Murashige and Skoog; BM : Balittan Malang;
MM : Muller and Martin

7.1.2. Cuttings

a. Study on medium and density of mother plants to produce stem cuttings on potato cultivars DTO - 28

The experiment was conducted at LEHRI (1,250 m above sea level) in January to May 1985. The experiment was aimed to find out the best medium and density to produce cuttings for cultivar DTO-28. The medium used were M_1 (sterilized subsoil with sterilized moss 3 : 1), M_2 (sterilized sub soil stable manure 3 : 1), M_3 (sterilized subsoil with sterilized compost 3 : 1). The density used were K_1 = 1,000 cuttings/m2, K_2 = 700 cuttings/m2, K_3 = 400 cuttings/m2. The experiment was harvested one month after planting.

b. Study on N fertilization and varieties for cuttings production

The experiment was conducted at screen house LEHRI (1,250 m adpl) to see the effect of N fertilization and three potato varieties on cuttings production. The varieties used were AVRDC 1287.12, LT-1 and I.931.

Three treatments for fertilization were T_1 (N = 2 g/tray as control), T_2 (N = 2 g/tray, was applied every week) and T_3 (N = 2 g/tray was

applied every week). The density was 48 cuttings per tray (the tray size 35 x 40 x 15 cm). All cuttings in the experiment was also fertilized with TSP 17,5 g/tray. The first harvest was done one month after planting and every 10 - 15 days.

7.1.3. Tuberlets Production

a. Study on media and density of cuttings to produce tuberlets of Desiree

The experiment was conducted at LEHRI (1,250 m asl) in December 1984 to April 1985. The purpose of the experiment was to see the effect of media and cuttings density on the production of tuberlet of Desiree.

The treatments were cutting density of 100 cuttings/m2, 36 cuttings/m2 and composition of media mixture of subsoil + stable manure 3 : 1, sobsoil + stable manure 3 : 0. During the growing period subsoil was added twice to make the beds 15 cm depth. Nitrogen as Urea 50 g/m2 and NPK (15-15-15) 33 g/m2 were applied before rooted cuttings transplanted.

b. The effect of cuttings age of rooting to produce tuberlet of Desiree

The experiment was conducted at LEHRI (1,250 m asl) in December 1984 to April 1985 and aimed to

determine the proper age of rooted cuttings for tuberlet production of Desiree.

The treatment were potato cutting age on rooting A (2 weeks), B (3 weeks), C (1 week). And the composition media mixture of subsoil + stable manure 3 : 1. During the growing period subsoil was added twice to make the beds 15 cm depth. Nitrogen as Urea 33 g/m2 and TSP 36 g/m2, was applied before rooted cutting transplanting.

c. The effect of media, density and variety on potato tuberlets production

The experiment was conducted at LEHRI (1,250 m asl), and the purpose of the experiment was to find out the best composition of media and density for potato tuberlet production.

The media treatments were M_1 (mixture of subsoil + stable manure 1 to 1, M_2 (mixture of subsoil + stable manure 3 to 1), M_3 (mixture of subsoil + stable manure 5 to 1). And density treatments were D_1 = 36 plants/m2, D_2 = 50 plants/m2, D_3 = 100 plants/m2. The variety used were V_1 = Desiree and V_2 = MS 1C-2. Nitrogen as Urea 33 g/m2 and TSP 100 g/m2 were applied before rooted cuttings transplanted. During the growing period subsoil was added twice to make beds 15 cm depth and the plot size was 1 x 1 m2.

7.2. Result and Discussion

The result of experiment is shown in Table 2, i.e. number of grown, contaminated and dead meristem of four varieties of potato.

Table 2. Number of grown, contaminated and dead meristem of four varieties of potato.

Basic Media	Desiree			Cosima			Cipanas			Katela		
	*1	2	3	1	2	3	1	2	3	1	2	3
Solid Media												
MC 1	1	3	1	1	2	2	2	-	1	-	1	4
2	3	2	-	2	1	2	2	1	2	2	1	2
3	1	2	2	-	2	3	-	1	4	-	1	4
4	3	-	2	-	2	2	1	-	4	1	2	2
5	3	1	1	-	1	4	1	3	-	-	2	3
6	2	1	2	-	3	2	-	2	3	-	3	2
7	1	2	2	-	2	3	-	-	5	-	2	3
8	1	2	2	-	2	3	-	-	5	-	1	4
9	1	2	2	-	2	3	-	1	4	2	1	2
10	1	1	3	-	1	4	-	1	4	-	3	2
Liquid Media												
MC 1	-	4	1	-	2	3	-	2	3	1	2	2
2	1	2	2	-	3	2	-	1	4	-	-	5
3	1	2	2	1	2	2	1	2	2	1	2	2
4	-	3	3	1	4	1	-	3	2	-	3	2
5	-	2	3	-	3	2	-	3	2	-	3	2

*1. Grown meristem
2. Contaminated meristem
3. Dead meristem

Based on meristem proliferation and plantlets produced solid media composition containing Murashige and Skoog (1962) basal media with 0.25 mg/l BAP, 0.5 mg/l GA_3, 0.02 mg/l NAA, 2 mg/l CaP, were the best.

After three months, proliferated meristem were sub cultured into fresh media containing MS basal media with 0.25 mg/l BAP, 0.5 mg/l GA_3, 2 mg/l CaP, 30 mg/l sucrose and 8 g/l agar. Then propagated by single node cuttings and transplanted to fresh media MS. Then they treated with the same for meristem culture.

The result of experiment on meristem culture in MS medium, showed that the three potato varieties were growing equally well in both media. Plant regeneration in this experiment was more than 80 % at 6 weeks after inoculation (Table 3).

Table 3. Percentage of shoot formation at 6 weeks after establishment in vitro *

Variety	Shoot formation (Ratio/%)	
	Medium I	Medium II
Rapan 106	9/10 = 90	11/18 = 94
Thung 151	13/15 = 87	11/11 = 100
TD 84-152	13/14 = 93	16/16 = 100

$$* \% \text{ Shoot formation} = \frac{\text{Total Shoot}}{\text{Total Growth}} \times 100 \%$$

Increasing the medium concentration as Medium II shoot formation by 4 - 13 percents. In general the growth was slow. Plantlets shoot and leaf growth ranged 20 to 40 percents with no clear varietal and media differences (Table 4).

Table 4. Percentage of grown explant at 6 weeks after establishment in vitro *

Growth Stage	Medium I			Medium II		
	Rapan 106	Thung 151	TD 84-152	Rapan 106	Thung 151	TD 84-152
	..... %					
Growth and leaf formation	25	35	25	30	5	25
Growth and short multiplication	5	0	10	25	10	25
Shoot and leaf growth	20	40	35	35	40	30
Contaminated	0	10	25	0	0	0
Dead	50	15	5	10	45	20

$$* \% \text{ dead} = \frac{\text{Total died}}{\text{Total cultured}} \times 100 \%$$

$$\% \text{ contaminated} = \frac{\text{Total contaminated}}{\text{Total cultured}} \times 100 \%$$

Growth and shoot multiplication was more pronounced with medium II. Rapan 106 and TD 84-152 tended to produce more shoot than Thung 151.

Fifteen days after inoculation there was no significant difference in number of shoot, but at 30 days after inoculation there was significant difference in number of shoot on variety N 565. 1 and PSP 30.10 (Table 5). In 15 days and 30 days after inoculation there was no significant difference in average number of nodes (Table 6).

Table 5. Average number of shoots at 15 days and 30 days after establishment in vitro.

Variety	15 days	30 days
Desiree	4.50 a	11.0 b
I - 931	4.75 a	11.25 b
PSP 30.10	4.50 a	7.0 c
MS 1C - 2	5.67 a	10.0 b
N 565.1	5.0 a	5.67 d

Noted : Means followed by the same letters are not significantly different according to DMRT at 5 %.

Table 6. Average number of nodes per stem at 15 days and 30 days after establishment in vitro.

Variety	15 days	30 days
Desiree	3.38 a	4.75 b
I - 931	2.50 a	4.0 b
PSP 30.10	3.50 a	3.5 b
MS 1C-2	3.33 a	4.0 b
N 565.1	2.67 a	4.0 b

Noted : Means followed by the same letters are not significantly different according to DMRT at 5 % level.

Study on cutting production of DTO-28 showed that the density of 1,000 cuttings/m2 gave higher production of cuttings, although the percentage of mother plant died was more than the others (Table 7 and 8). Composition of media mixture of subsoil + compost and subsoil + stable manure were better than subsoil + moss.

Table 7. Average of stem cuttings production per plot.

Density	Media			Average
	M1	M2	M3	
1000 plants/m2	280.03	293.67	315.67	296.56 (x)
700 plants/m2	192.67	240.33	251.67	288.22 (y)
400 plants/m2	135.67	182.00	174.33	164.67 (z)

Means followed by the same letters are not significantly different according to DMRT at 5 % level.

Tabel 8. Average of mother plant died (%).

Density	Media			Average
	M1	M2	M3	
1000 plants/m2	24.15	35.54	32.96	30.88 (x)
700 plants/m2	31.40	27.16	30.11	29.56 (x)
400 plants/m2	16.67	20.19	25.11	20.66 (y)

Means followed by the saame letters are not significantly different according to DMRT at 5 % level.

Studv on the effect of N fertilization and varieties on cutting production showed that threre was no effect of fertilizer on number of cuttings per plant and number of mother plants survive. The difference was obtained on the variety. LT-1 produced cuttings higher that the others, but not significantly different with I.931. No interaction effect of fertilization and variety (Table 9).

Table 9. Average of stem cutting production per plant.

Varieties	T1	T2	T3	Average
AVRDC 1287.12	11.770	12.530	12.480	12.637 a
LT - 1	25.320	25.387	32.633	27.780 b
I. 931	27.583	27.823	27.613	27.613 b
Average	21.648 (A)	21.853 (A)	24.409 (A)	

Means followed by the same letters are not significantly different according to DMRT at 5 % level.

Study on tuberlet production of variety Desiree showed that there was no significant effect of density and media composition on number of tubers per plot and tuber weight per plant. But there was significant difference on tuber weight per m2 caused by different density and media composition. Composition of media with stable manure could increase tuberlet production (Table 10).

Table 10. Influence media and density of stem cuttings potatoes to produce tuberlet variety Desiree.

Treatment	Weight tuber per plant (g)	Weight tuber per m2 (kg)	Number of tuber/plant (knol)
3 : 1/100	160.47 a	5.47 c	7.83 b
3 : 0/100	156.83 a	5.27 c	7.67 b
3 : 1/36	192.73 a	3.63 cd	7.63 b
3 : 0/36	136.65 a	2.0 d	6.65 b

Means followed by the same letters are not significantly different to DMRT at 5 % level.

The result of the study on the age of rooted cuttings showed that the highest percentage of plant survive was obtained from one week old rooted cuttings at seed beds. However, rooted cuttings of 3 weeks old in seed beds had the highest number and weight of tubers. And the tuber size reached 11.7 to 27.7 per tubers.

The experiment on density, media and variety showed that population of 36 plants/m2 gave higher number and tuber weight per plant compared to those of 50 and 100 plants/m2.

Variety MS 1C-2 produced more tubers per m2 than Desiree. No interaction effect of variety, media and density on tuber weight was formed (Table 11 and 12).

Table 11. Tuber weight per plant (g).

Treatments	M 1 1 : 1	M 2 3 : 1	M 3 5 : 1	Average
D1 = 36 plants/m2	253.97	201.37	227.50	227.61 a
D2 = 50 plants/m2	156.0	222.93	173.10	184.01 b
D3 = 100 plants/m2	188.97	161.23	149.83	166.68 a

Means followed by the same letters are not significantly different according to DMRT at 5 % level.

Table 12. Tuber weight per m2 (kg)

Varieties	D1 36 plants/m2	D2 50 plants/m2	D3 100 plant/m2	Average
Desiree	1.50	0.81	1.29	1.20 a
MS 1C-2	2.99	2.96	4.43	3.13 b

Means followed by the same letters are not significantly different according to DMRT at 5 % level.

V. CONCLUSION

Looking at the implementation, the result facilities, equipment and trained people and the practice being done by LEHRI and the cooperating farmers and the seed production program, the tissue culture for virus-free potato propagation project was very successful. The available facilities equipment and trained people would be very valuable as he starting point in the effort of improving the national potato seed production system.

V. FOLLOW - UP

Another project is being discussed to develop and to implement the result of tissue culture for virus free potato propagation project in the bigger scale which involve private sector. The project might be funded by JICA and it hopefully will be started in 1989.

VII. SUMMARY OF THE PROJECT

The main constraint in the effort of increasing potato production in Indonesia is the availability of good quality seed. Two techniques which are used in the production of good quality seed (disease-free potato seed) are meristem culture and rapid multiplication

techniques and they have been adopted by several countries. In Indonesia, those two techniques were used to produce disease-free potato since 1984 supported by USAID to construct the facilities, to purchase the equipment and to finance the training and the travels.

One permanent screen house of 200 sqm was established at LEHRI. Two rooms were modified to be used as incubation and preparation rooms. Net houses were also built to produce tuberlets.

Training, workshop and comparative study to other countries have been done not only by LEHRI staffs but by the extension workers and the farmers as well. In the country training were held at Lembang (2x), Sidomulyo (1x) and Malang (1x) and attended by 105 participants from extension workers, seed farms and farmers.

Some studies on meristem culture media mass propagation, stem cuttings productions and tuberlet production were carried out and the result were put forward in the report.

The project was successful, however, it must be followed up by other project which will involve the private sector to engage in seed business.

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