

4. Research progress in potato propagation and breeding at Bogor Agricultural University

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Introduction

No one knows when or who introduced the potato to Indonesia. According to Koens (1948), in 1794 potatoes were cultivated around Cisarua, Bandung. In 1804, potatoes were cultivated in high elevations of West Java and Central Java. In 1811, the potato crops already covered the high mountains of Indonesia, namely: Bukit Tinggi (West Sumatra), Tanah Karo (North Sumatra), Takengon (Aceh), Curup and Kapahiang (Bengkulu), Pasemah (South Sumatra), Goa (South Sulawesi), Tomohon and Modinding (North Sulawesi), Bali, Timor, Manusela (Seram, Maluku) and Arfak (Irian Jaya). Potato is still cultivated in these areas but the main production centers are now in West Java, Central Java, East Java, and North Sumatra.

Since these early beginnings potato has become an important horticultural crop in the highland areas of Indonesia with annual production of around 1 million tons. To consumers, potato is considered as a vegetable rather than as main dish. Nevertheless, the Indonesian Diversification Food Program considered potato as one of the substitutes for rice as a source of carbohydrate. The booming fastfood industry, in which potato plays a big part, has changed the food habits of many youngsters in the middle- and high-income classes. To them, western restaurants such as Kentucky Fried Chicken, McDonald's, and Wendy's are more popular than the Indonesian restaurants. The potatoes processed as chips and fries are imported. The local processing plants can only supply 20 percent of the requirements of the domestic market for potato processing. The inability of the local processing plant to fulfill the domestic demand is mainly due to the lack of fresh potato tubers for processing. In addition to high domestic demand, production of fresh ware potatoes for export to Singapore and Malaysia is also increasing.

The increasing demand for potato becomes a challenge and an opportunity for increasing potato production in Indonesia. The main constraints on increasing potato production in Indonesia, however, are:

1. Lack of elite potato propagules in terms of true potato seeds (TPS), potato seed tubers, *in vitro*-derived seed tubers that are reasonably priced.
2. Lack of improved Indonesian potato cultivars that can:
 - a. adapt to specific environments such as moderate temperatures, problems soils, rainy season, dry season, high input, and low input;
 - b. fit special market requirements such as fresh consumption, french fries, chips, mashed potato, starch, and baby potatoes (small tubers);
 - c. resist or be tolerant to major pests and diseases in Indonesia;
3. Lack of appropriate postharvest and storage methods to secure minimum losses especially for seed tubers.
4. The application of the World Trade Organization's (WTO) regulation of IPR (plant breeders' rights and plant patent) will increase the burden of the potato farmers in Indonesia.

Potato farmers in Indonesia consider potato as a high-input, high-risk, and high-output crop. Crop failure is mostly due to low-grade seed tubers, pests, and diseases. Imported potato

seed tubers are too expensive and are not always available when needed. The major potato diseases and pests in Indonesia are: potato virus (PVX, PVY, PLRV), late blight (*Phytophthora infestans*), bacterial wilt (*Ralstonia solanacearum*), bacterial soft rot (*Erwinia caratovora*), root knot nematode (*Meloidogyne spp*), potato tuber moth (*Phthorimaea operculella*), and green peach aphids (*Myzus persicae*).

The long- and short-term potato research program must be able to change the potato crops from being high-input, high-output, high-risk to being high-input, high-output, but low-risk crops by producing elite potato propagules and improved potato cultivars resistant/tolerant to the major pests and diseases in Indonesia.

Research Strategy and Programs

Potato research at IPB started in 1985 as a continuation of the doctoral dissertation of Wattimena (1983) on *in vitro* microtuber research. Since then potato research has continued continuously, focusing especially on breeding and technologies for high-quality seed production.

Vision, mission, strategies, and objectives for IPB potato research program

1. Vision: Potato can be the among the most important future crops in Indonesia as:
 - a. Nutritive alternative to rice, and as raw material for food, feed, and textile industries.
 - b. Cash crop for the farmers and as export commodity.
 - c. Good model crop for the development of science and technology through its biological elasticity.
2. Mission
 - a. To develop and produce potato agrotechnology, elite propagules, nationally and globally improved potato cultivars.
 - b. To increase farmers' competitive capability to fulfill the need of the global market.
 - c. To find new facts to enrich the development of science and technology.
 - d. To disseminate the effects of R & D on the needy.
3. Strategies
 - a. Field observations and tests to find appropriate solutions/methods to solve problems.
 - b. Farmers' participation in R & D.
 - c. A good system for evaluating, disseminating, and applying R & D programs.
4. Objectives
 - a. To develop and produce elite potato propagules.
 - b. To develop improved potato cultivars.

Programs

The long-term umbrella program from the laboratory to the land are divided into two subprograms:

1. Producing elite potato propagules
2. Producing improved potato cultivars

Producing elite potato propagules

The diagram flow of R & D on elite potato propagules is shown in Figure 1 and consists of:

1. Elite seed tubers derived from selected hybrid seeds (true potato seed or TPS).
2. Transformation of selected potato cultivars with apomictic gene to produce clonal TPS.
3. Elite seed tubers derived from *in vitro* plants through several alternative pathways.

Producing improved potato cultivars

Potato has a very interesting biological elasticity ideal for breeding. Figure 2 shows the set-up for the research program at IPB for breeding improved potato cultivars. Potato's commercial cultivars and species have the same basic chromosome number ($X = 12$), easy to extract the haploid by using *S. phureja* as male parent, producing 2N gametes. It is easy to use for sexual and asexual hybridization, easy to transform, and easy to regenerate as *in vitro* plantlets.

The breeding program of potato starts with the *in vitro* disease-free collection of potato cultivars, clones, and species. From Figure 2 the breeding methods that are applied are:

1. Extraction of haploid via anther culture (androgenesis) and crossing with *S. phureja* (gynogenesis).
2. Sexual hybridization of $4x - 4x$, $4x - 2x$, and $2x - 2x$. Crossing of $4x - 2x$ or $2x - 2x$ only by parents that produce 2N gametes.
3. Somatic hybridization of $2x + 2x$ of intra- and inter-species somatic hybridization.
4. Transformation especially with plasmid-aided transformation.
5. Somaclonal variation especially for disease- and pest-resistant characters.

Research Progress

In vitro tubers (microtubers)

The research on microtubers started in 1985 and was temporarily suspended in 1992. In 2000, the research of *in vitro* tuberization resumed as a screening method in the potato breeding program. Some important data from the research program are (Wattimena 1995a,b):

1. The culture environments
The optimum temperature for *in vitro* tuberization is $15^{\circ} - 20^{\circ}\text{C}$, without light.
2. A sucrose concentration of 9 percent is the optimum concentration for *in vitro* tuberization medium.
3. The growth regulation of the medium
In vitro tuberization is induced by cytokinins and inhibited by gibberellin. The PGR used for *in vitro* tuberization consists of cytokinins (the inducer) and retardants or inhibitors for anti-gibberellin biosynthesis. The optimum concentration of cytokinins or cytokinin-like substances are as follows: benzyl adenine (BA) 5 mg/L, kinetin 10 mg/L, adenin sulphate 100 mg/L, benomyl 50 mg/L and coconut water 15 percent. The optimum concentration for retardants and inhibitors are: cycocel (CCC) 600 mg/L, ancymidol 10 mg/L, alar (B9) 10 mg/L, uniconazole 3 mg/L, paclobutrazol 10 mg/L, and coumarine 25 mg/L. Recommended concentrations are: BA 5 mg/L or coconut water 15 percent as cytokinins, cycocel 600 mg/L as retardant, or coumarine

25 mg/L as inhibitor. Using coumarine as inducer in the tuberization medium requires lower nitrogen concentration of 30 mM or 15 mM.

4. Tuberization methods

There are four methods of *in vitro* tuberization, namely:

- a. Direct solid medium
The explants are cultured directly in solid tuberization medium.
- b. Direct liquid medium
The explants are cultured in static liquid tuberization medium. The height of the liquid tuberization medium must be less than 1 cm.
- c. Solid-liquid medium
The explants are first cultured in solid shoot medium and after 3-4 weeks, the liquid tuberization is added.
- d. Static-shallow- liquid- liquid system (S2L2S)
The explants are cultured first in static shallow liquid shoot medium and after three weeks, the liquid tuberization shoot medium is added.

In vitro microtubers of potato are too expensive to use as elite propagules unless a cheaper method of producing microtubers is available, such as a bioreactor. The price of *in vitro* tubers is three times higher than that of microcuttings.

In vitro shoot culture (microshoot)

In vitro shoots are *in vitro* propagules that can go from the lab to the land because they are easy and cheap to produce.

The simple Murashige medium (MS), without adding any hormone to regulate growth is a fast and cheap method.

Single node explants are usually used for *in vitro* shoot multiplications. In one month, from one node alone, researchers were able to get eight nodes (2 nodes/weeks) or around 500 nodes in three months.

The only limitation with microshoots lies in the handling and transporting of the microshoots from the lab to the greenhouse. Therefore, further research must be focused on storage, handling, and transportation of microshoots. A light and temperature-controlled transport system will ease the handling of microshoots.

The alginate microshoot showed very low survival because of oxygen shortage. The researchers developed two methods of transportation, namely TAS and TIAS methods. In TAS the plantlets are planted in round plastic vessels on a growth medium of rice husk, charcoal, and enriched with foliage fertilizers. The TIAS method has two systems: transporting the microshoots in rolled tissue paper and watering with foliar fertilizer, and transplanting using the TAS method when they arrive at the greenhouse. The TAS method is good for short distances and the TIAS method for long distances. Temperatures above 30°C during transport, even for a short time, are harmful for the microshoots.

Further research is needed in microshoot handling and transportation, especially the container for controlling either temperature or light, or both.

Many researches have been done on producing minicuttings and *in vitro* shoots. Usually, with good planting medium and weekly application of foliar fertilizer, one microcutting can produce ten minicuttings with the same capacity as that of producing minitubers. The key factors of producing elite minitubers from minicuttings are the disease-free growth medium and disease-free irrigation water. The application of pesticides and

fertilizers through the overhead irrigation system is a good system to produce disease-free minitubers.

Potato seed tubers certification in Indonesia

From 1993 to 1996, IPB had a contract with PT Indofood Sukses Makmur to produce potato seed tubers from minicuttings of the cultivar Atlantic. At present, many Indonesian potato seed companies also have produced minitubers. Potato farmers are buying minitubers for seed at Rp. 350– Rp. 600/tuber, depending on size. Due to the uncontrolled system of producing minitubers, farmers sometimes buy small tubers not derived from minicuttings but from the ware potato crops.

After several meetings in Jakarta and Lembang on the potato seed certification system, IPB proposed a seed certification system (Figure 3) that was different from the system applied for the cultivar, Granola. The system's steps are (Figure 3):

1. Seeds from the breeder are propagated *in vitro*. The *in vitro* plantlets are tested twice for diseases.
2. The disease-free plants are genotyped using molecular markers (RAPD'S, SSR, STMS, etc). Each potato cultivar has its specific molecular markers as its passport.
3. The disease-free cultivars with their genotype passports are kept in *in vitro* collection or mass propagated for seed production.
4. The farmers have a choice to buy any quality of certified seed tubers they want. Before certification, screenhouses, fields, plants, and tubers must be inspected.
5. The government only regulates and controls. Production and distribution are left to the private sector, farmers' cooperatives, and other agencies.

If the system is approved, Indonesia may no longer import potato seed tubers. The proposed system of potato seed certification (Figure 3) has been sent to the directorate of the Seed Development Department of Agriculture at Pasar Minggu, Jakarta and is under consideration.

In vitro collection

In vitro collection with good recordkeeping is very essential in potato breeding. It needs a special room separated from other tissue culture activities and a person specifically assigned to take care of the collections. Poor collection and recordkeeping have led to losses of some good accessions. The collection at IPB comes from the Plant International-Wagenningen University, Inter-Regional Potato Introduction Station, NRSP-6 Sturgeon Bay, Wisconsin, CIP Peru, and its own breeding clones. At present, it has around 200 accessions consisting of important commercial cultivars, *Solanum* sp., differential R genes, and breeding clones. *Solanum stenotomum* IP234013 ($2n = 2x$) has many good characteristics. It is resistant to PLRV, PSTV, PVM, Fusarium wilt, Verticillium wilt, Erwinia soft root, Colorado potato beetle, and potato aphids. It has good vigor, good flowering, fertility, high percentage of tuber dry matter, mid-early maturity, is easy to regenerate from protoplast, and is compatible for interspecies protoplast fusion. Still being evaluated are other pest and disease characters as well as agronomic and tissue culture characters for *S. stenotomum* IP 234013 and other clones.

Somaclonal variation

Somaclonal variation has been done by regenerating plantlets from *in vitro* internodes and leaves with and without irradiation with gamma rays. The cultivars that have been induced by somaclonal variation were Atlantic, MS 42.3, Eba, Russet, Burbank, Desiree, and Red Pontiac.

Sexual and asexual hybridization

The only sexual crossing done was between Astarte (4x) and DTO 28 (4x). Some clones derived from this crossing were moderately resistant to bacterial wilt and had high tuber dry matter contents. These clones were: AD4, AD6, AD9, AD12, AD25, and AD36.

Purwito (1999) did the asexual hybridization or protoplast fusion between diploid species and dihaploid cultivars (interspecies fusion) and between two dihaploid cultivars. Interspecies hybridization was between BF15 (2x) and one of the following species: *S. berthaultii* (2x), *S. stenotomum* (2x), *S. vernei* (2x), *S. phureja* (2x), and *S. stoloniferum* (4x). All the protoplast interspecies fusion regenerated to callus but not all callus regenerated into shoots. The callus fusants of BF15 + *S. berthaultii* and BF15 + *S. stoloniferum* were not able to produce plantlets. The fusion between BF15 + *S. vernei* produced viable plantlets but the genome of *S. vernei* was eliminated in the plantlets.

The intraspecies or intercultivar fusion consisted of SVP10 (4x) + Cardinal (2x), BF15 (2x) + Nicola (2x), BF15 (2x) + Nicola (2x), BF15 (2x) + Cardinal (2x), Aminca (2x) + Cardinal (2x). All the intercultivar fusants were able to regenerate into calli and plantlets except the fusants of SVP10 (4x) + Cardinal. These fusants' vigorous calli were very difficult to regenerate as plantlet. It seemed that the hexaploid calli were also very difficult to regenerate as plantlets. The same holds true for the calli from the protoplast of BF15 (2x) + *S. stoloniferum* (4x).

The heterofusion of interspecies fusion produced very vigorous plants but the tubers did not look good to consumers. The reverse was true for intraspecies or intercultivar fusion. Some clones of interspecies fusion between BF15 (2x) + *S. Stenotomum* (2x) were resistant to *Ralstonia solanacearum*. The clones were BS-23, BS-43, BS-73, and BS-75 (Samanhudi 2001).

Genetic transformations

Tan (1997) transformed cultivar Atlantic with coat protein of PVY (Cp-PVY) while Wiendi (2002) constructed the chi-gene and transformed the gene into the cultivar Desiree. The seven transgenic plants of CP-PVY of Atlantic did not show any resistance to potato virus Y (PVY). The reason for the unexpression of the CP-PVY gene in the transgenic plant of the cultivar Atlantic is not known. One of the transgenic clones, B-2, showed a shorter and compact plant vigor.

IPB staff and students isolated chi-gene, constructed the plasmid carrying the chi-gene, transformed the cultivar Desiree, detected the gene, and expressed it. Wenungenan (1996) isolated the chi-gene from *Aeromonas caviae* and constructed it into plasmid pWS506 (6.0 kb) that carried the chi-gene of 3.0 kb. Wiendi (2002, in progress) did the sense construction into plasmid pARS57(15.9 kb) and reversed the sense construction into plasmid pARS52 (15.9 kb).

These plasmids were carried into *Agrobacterium tumefaciens* GV3010 through triparental mating before transforming the cultivar Desiree. About a hundred transformed clones had been screened as transgenic clones, but only 49 clones were identified for the copy genes. Twenty-four clones have a sense orientation with one to five copies and 25 clones with

reverse orientation carrying one to two copies. The chi-gene of these clones was tested on *Fusarium* spp. and it showed different degrees of resistance, depending on the number of copies and orientation. This was the first plant transformation research in Indonesia, in which work was carried out from the isolation of the gene until its expression.

Most of the transformation researches in Indonesia at present are dependent on patented genes from foreign researchers and institutions. Working with these genes is under strict Material Transfer Agreements (MTA) and may create problems later on regarding the release of transgenic cultivars especially for the Indonesian main food crops: rice, corn and soybean.

Research budget and human development

There is a difference between the government research agencies and the universities concerning research budget and human development. The research budget for government research agencies are allocated yearly by the government. The research budget for universities, however, must be raised from national competitive grants and international sources. The research program in the university must also accommodate student researchers.

Since 1985, IPB has never run out of research funds. It has been supported by the following: (1) PSTC – IAC/SCI, USA (2) Hibah Bersaing (National Competitive Grant), (3) Hibah Tim (National Competitive Team Grant), (4) RUT (National Integrated Merit Grant), (5) RUK (National Partnership Merit Grant), and (6) National Graduate Student Sandwich Program.

Students doing their undergraduate, master's, and doctoral theses carry out most of IPB's research programs. The IPB staff advise, coordinate, and write the research report. Many students from all levels (S1, S2, S3) like to be involved in IPB's umbrella potato research program because of the availability of funds and the continuous research program.

The following are IPB's PhD students and their researches on potato:

1. Adisarwanto, T. W. (1990). University of Brawijaya. The effect of high temperature on potato tuberization in the lowland.
2. Fransisca Tan (1997) Catholic University, Medan. Transformation of potato cultivar Atlantic with cp-PVY and clone cp-PVX.
3. Lukman Hakim (1999) University of Syah Kuala, Aceh. Analyzing the IPM component for bacterial wilt *Ralstonia (Pseudomonas) solanacearum* Yabuchi et al. in potato.
4. Irfan Suliansyah (1999) University of Andalas, Padang. Virus degeneration in non-transgenic cultivars and in transgenic cp-PVY of the cultivar Atlantic.
5. Agus Purwito (1999) Bogor Agriculture University, Bogor. Inter and intra protoplast of potato.
6. Sudirman Numba (2000) Moslem University, Ujung Pandang. Analyzing the genetic variability and election of special primers to identify somatic hybridization of several potato cultivars (*Solanum tuberosum* L.) and their wild species.
7. Wiendi, N. N. A. (in Progress) Bogor Agriculture University, Bogor. Construction of transformation plasmid containing chi-gene derived from *Aeromonas caviae*, transformation and their expression in potato cultivar Desiree.
8. Johan Marthen Tutupary (in Progress) University of Pattimura, Ambon. Evaluation of late blight resistance of potato, genetic analysis of field resistance to late blight of the population derived from crossing *S. microdontum* x *S. tuberosum* using CAP`S marker.

9. Rosmayati (in Progress) University of North Sumatera Medan. Analyzing the effect of potato genotype maturity and their resistance to late blight (*Phytophthora infestant*).
10. Warnita (in Progress) University of Andalas, Padang. Prediction of potato field maturity by tuberization and modified heat unit using potato cultivars of known maturity class.

Other master's and undergraduate theses on IPB's potato research program are available at the Faculty and University Libraries.

Tissue Culture Method to Speed Up Potato Breeding in Indonesia

Tissue culture is a breeding method in itself. Based on this assumption, people try to group the breeding methods into: molecular methods, tissue culture methods, and conventional methods. Nowadays, breeders use combinations of these methods to speed up their breeding program.

Figure 4 shows the gene flow from the donor to receiver plant genotypes. The transgression of genes can be molecular or by conventional crossing. Further, there are gene interactions in three different environments, namely:

1. The cell environment
The cell environment regulates how the gene is transcribed, translated, and metabolized. This is mainly the work of molecular geneticists and biochemists.
2. The culture medium environment
The culture medium determines how genes express in the cell, organ, and plantlets. The very important steps in the tissue culture environment are methods of regenerating and screening the plantlets carrying the gene. This is the work of tissue culturists.
3. The edaphic, climatic and biological environments
The interaction of genotypes and environments create a phenotypic performance. A phenotype can be evaluated if it carries a specific gene that has a high or low heritability. Low heritability means specific cultivars only fit specific conditions or specific locations. The phenotypic performance is very important because phenotype and not genotype is what determines consumption and use preferences (e.g., taste, shape, color). This is the main work of conventional breeders

Figure 4 shows how the tissue culturist can help the molecular biologist and the conventional breeder.

1. To the molecular biologist/breeder: The tissue culturist regenerates the transformed cells or explants into plants by manipulating the medium composition and culture environment, and efficiently screening plantlets regarding their transformation.
2. To the conventional breeder: The tissue culturist keeps the breeding collection, searches for *in vitro* methods for screening plantlets carrying specific characters and helps the conventional breeders to save wide-crossing embryos or produce a homozygous line (double haploid) for producing F1 hybrids. (???)

The following help speed up the conventional potato breeding program in Indonesia by tissue culture:

1. Indonesia has many tissue culture laboratories and abundant manpower. So far, most of the tissue culture facilities and manpower are for micropropagation and a few are for helping the conventional breeders. The Research Institute of Vegetable (RIV) in

- Lembang has advanced tissue culture laboratories and facilities suitable for tissue culture methods.
2. CIP has selected good male parents (TPS-13, TPS-67) and female parents (Atzimba, Achirana, Lt-8, MF-I, MF II, TPS-7, TPS-25, and Serrana) for producing true potato seeds especially for Southeast Asia (Upadhya 2000). The Indonesian conventional breeders can use any combination of these male and female parents for breeding.
 3. The new potato cultivars must be different, uniform, and stable (DUS). The DUS for self-pollinated crops propagated by seed is achieved through pure line selection. The DUS for cross-pollinated crops propagated by seed is achieved by using F1 hybrids of homozygous parents. For cross-pollinated plants propagated clonally such as potato, their DUS can be achieved by single seed clonal descents. Last year, the researchers (Wattimena et al. 2001) proposed methods that combine conventional breeding and tissue culture methods. The combined methods were called single seed *in vitro* clonal descent (SSICD). It consists of:
 - a. Germination of selected seeds *in vitro*
 - b. Selection of vigorous seedling and propagated *in vitro* with single nodal explants. The *in vitro*-propagated clonal generation of each seed is an *in vitro* clone.
 - c. *In vitro* selection for the disease character and maturity includes:
 - i. *In vitro* tuberization for maturity and tuber characters
 - ii. *In vitro* disease screening for bacterial wilt (*Ralstonia solanacearum*), soft rot (*Erwinia* spp.), Fusarium dry rot (*Fusarium* spp.), root knot nematode (*Meloidogyne* spp.), and late blight (*Phytophthora infestans*).

The clones that passed the *in vitro* screening will be evaluated further in the field for their agronomic characters.
 4. Research on these *in vitro* methods is still in progress. The tissue culture method for bacterial wilt and bacterial soft rot was highly correlated with the test in the greenhouse. The maturity test with *in vitro* tuberization methods did not correlate with the field test. This *in vitro* tuberization will be further modified by keeping the light duration in the lab similar to that in the field (12 hour-light duration).
 5. Resistance to *Erwinia* spp. was tested using the drip method and for *Ralstonia solanacearum*, the dipping method was used. For the drip method, 1 ml bacterial inoculate of 10^9 cell/ml drips into the culture bottle containing *in vitro* plantlets. For the dipping method, a pair of scissors is dipped into the bacterial suspension (10^9 cell/ml) before each cutting is made. Table 1 shows the comparison of different methods used to evaluate the resistance of potato clones against *Ralstonia solanacearum*.
 6. The SSICD method of potato breeding that combines tissue culture and conventional breeding methods will speed up the potato breeding program in Indonesia. The SSICD method can be used not only for potato but also for orchids, fruit crops, and other clonally propagated crops.
 7. By using CIP male parents and female parents in combination with SSICD, Indonesia will be able to produce new potato cultivars in three years.

Conclusions

1. Microtubers as elite potato propagules are too expensive for the farmers unless a mass production system such as a bioreactor is available.

2. The research on *in vitro* plantlets aims to create a system of producing elite seed tubers according to the proposed scheme for potato seed certification.
3. Continuous research is still needed for: (1) handling and transporting *in vitro* plantlets from the laboratory to the nursery, and (2) better and efficient production of microcuttings and minitubers.
4. All available potato breeding methods (somaclonal variation, haploidization, sexual hybridization, somatic hybridization, and transformation) have been successfully applied and some potential clones need further evaluation.
5. The first molecular breeding in Indonesia successfully isolated the chi-gene from the bacteria *Aromonas caviae*, constructed the plasmid carrying the chi-gene for its transformation into the potato cultivar Desiree, and expressed the chi-gene in transgenic Desiree.
6. The tissue culture methods have been explored for evaluating maturity, tuber characters, and disease resistance to *Ralstonia solanacearum*, *Erwinia* spp., *Fusarium* spp., *Meloidogyne* spp. and *Phytophthora infestans*. The tissue culture methods for screening potato clones resistant to *Ralstonia solanacearum* were well-correlated with the field tests.
7. A quick method of potato breeding in Indonesia can be achieved by using CIP-selected male and female parents in combination with SSICD.
8. The potato research was not able to produce clonal seed propagules by introgression of apomictic gene and micro-protoplast fusion.

References

- Koens, A.T. 1948. Knolgewassen. *In*: C. J. J. Hall and C. Van de Koppel (eds), De Landbouw in de Indische Archipel IIA, W. Van Hoeve, Den Haag. pp 162-240.
- Purwito, A. 1999. Fusi protoplas intra dan interspesifik pada tanaman kentang, Disertasi Doktor, Program Pascasarjana IPB.
- Samanhudi. 2001. Identifikasi ketahanan klon kentang hasil fusi protoplast BF15 dengan *Solanum stenotomum* terhadap Penyakit Layu Bakteri (*Ralstonia solanacearum*), Tesis Magister Program Pascasarjana IPB.
- Samanhudi, G.A. Wattimena, M. Machmud and A. Purwito. 2001. Evaluasi ketahanan klon kentang hasil fusi protoplast BF15 dengan *Solanum stenotomum* terhadap *Ralstonia solanacearum*, Hayati 8(4):102-106.
- Tan, F. 1997. Transformasi kentang kultivar Atlantic dengan gen protein selubung Potato Virus Y dan kloning gen selubung Potato Virus X, Disertasi Doktor Program Pascasarjana IPB.
- Upadhyha, M. D. 2000. Present and future research for true potato seed technology. *In*: K.O. Fuglie (ed) Performance and prospects of hybrids true potato seed in South and South East Asia , Proc CIP-ADP, Symp. Bogor. pp9-34.

Wattimena, G. A. 1983. Micropropagation as an alternative technology for potato production in Indonesia. PH.D. Thesis, University of Wisconsin, Madison, USA.

Wattimena, G. A. 1995a. In vitro micropropagation as an alternative technology for potato production. Final Report PSTC-USAID. Department of Agronomy, Bogor Agricultural University.

Wattimena, G. A. 1995b. Produksi propagul unggul bermutu, Laporan Akhir Hibah Bersaing I, Jurusan Budidaya Pertanian, Fakultas Pertanian IPB.

Wattimena, G. A., A. Purwito, H.M. Machmud dan Samanhudi. 2001. Perakitan kultivar kentang unggul Indonesia secara cepat dengan metode turunan klonal biji tunggal dan pra evaluasi secara in vitro. Simp. Pemuliaan dan Seminar Hasil Penelitian Jurusan Budidaya Pertanian, Bogor 24-25 April.

Wenungenan. 1996. Pengklonan gen kitinase bakterial menggunakan teknik mutagenesis transposon dan DNA pelacak heterologus. Tesis IPB, Bogor.

Wiendi, N. N. A. 1999. Ekspresi Gen Chitinase Asal; Bakteri *A. caviae* pada Tanaman Kentang (*Solanum tuberosum* L) CV Desiree, Penelitian Disertasi Doktor, Pascasarjana IPB, Bogor.

Wiendi, N. N. A. 2002 (in progress). Construction of transformation plasmid containing chi-gene derived from *Aeromonas caviae*, transformation and their expression in potato cultivar Desiree. Penelitian Disertasi Doktor, Pascasarjana IPB, Bogor.

Table 1. The correlation between several *in vitro* testing methods and the greenhouse testing for resistance to *Ralstonia solanacearum* of clones derived from somatic hybrids between *S. stenotomum* (2x) and BF15 (2x) (Samanhudi et al. 2001)

Potato Clones	Disease Incidence %			Level of Ristance		
	DRM	DIM	PBM	DRM	DIM	PBM
<i>S. stenotomum</i>	0.00	0.00	16.67	R	R	R
Nooksack	0.00	0.00	16.67	R	R	R
BS-23	6.67	0.00	10.00	R	R	R
BS-43	10.00	0.00	13.33	R	R	R
BS-73	10.00	0.00	20.00	R	R	R
BS-75	10.00	0.00	20.00	R	R	R
BS-53	23.33	36.67	33.33	MR	MR	MR
BS-54	26.67	33.33	36.67	MR	MR	MR
BS-38	33.33	43.33	56.67	MR	MS	MS
BS-49	36.67	46.67	60.00	MR	MS	MS
BS-55	36.67	73.33	70.00	MR	S	S
BS-51	76.67	63.33	76.67	S	S	S
BS-34	66.67	83.33	83.33	S	S	S
BF15 (2x)	76.67	93.33	90.00	S	S	S
BS-21	80.00	100.00	96.67	S	S	S
Atlantic	100.00	100.00	100.00	S	S	S

DRM : dripping methods, DIM : dipping methods, PBM: polybag methods, *S. stenotomum*, Nooksack: resistant parents and resistant control, BF15 (2x), Atlantic : susceptible parents, and susceptible control, BS = somatic hybrid clones, R : Resistance, MR: Moderate Resistant, MS: Moderate, Susceptible, S: Susceptible

Figure 1. The conceptual research program for the production of elite potato propagules

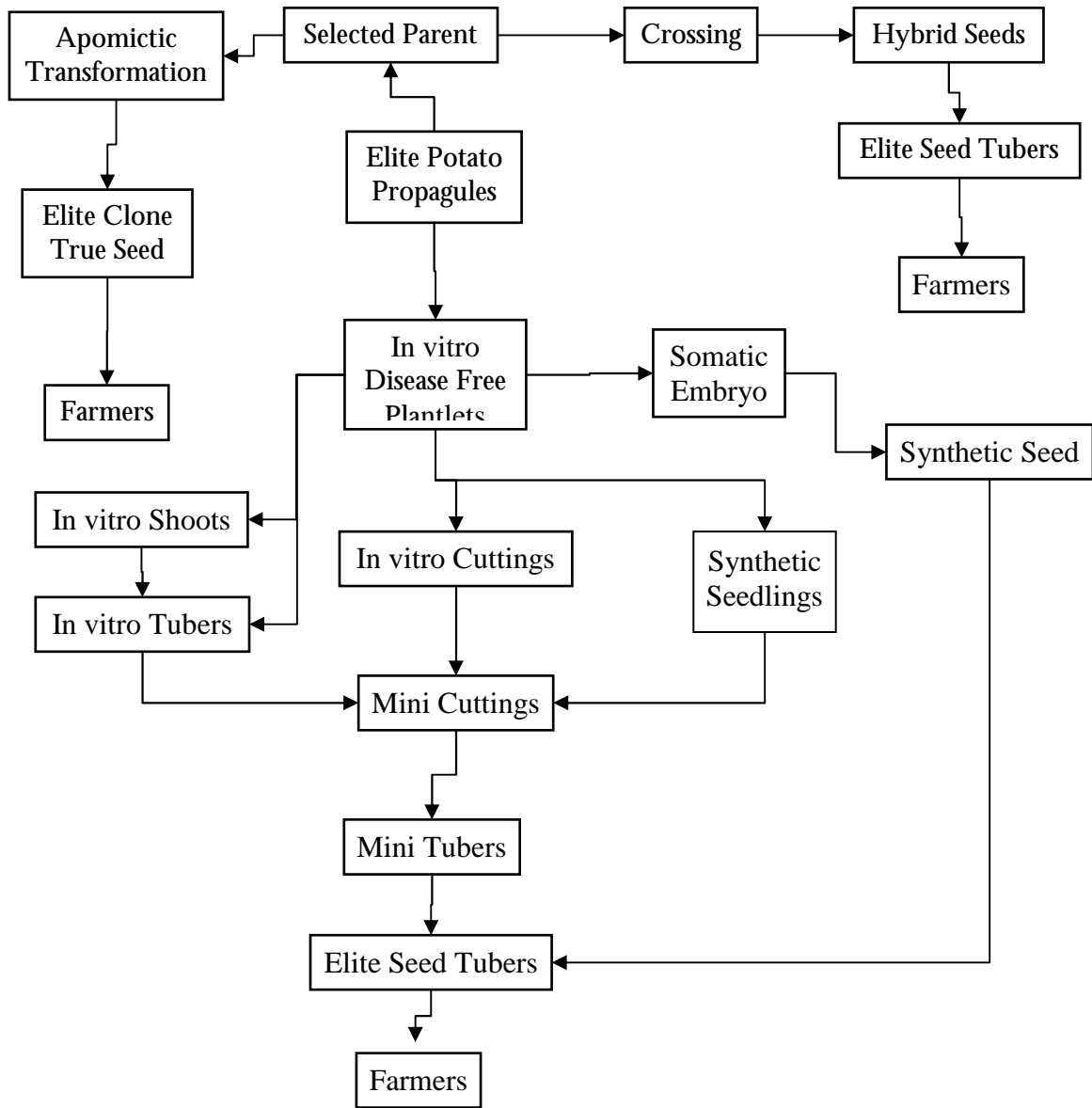


Figure 2. The conceptual research program for breeding improved potato cultivars

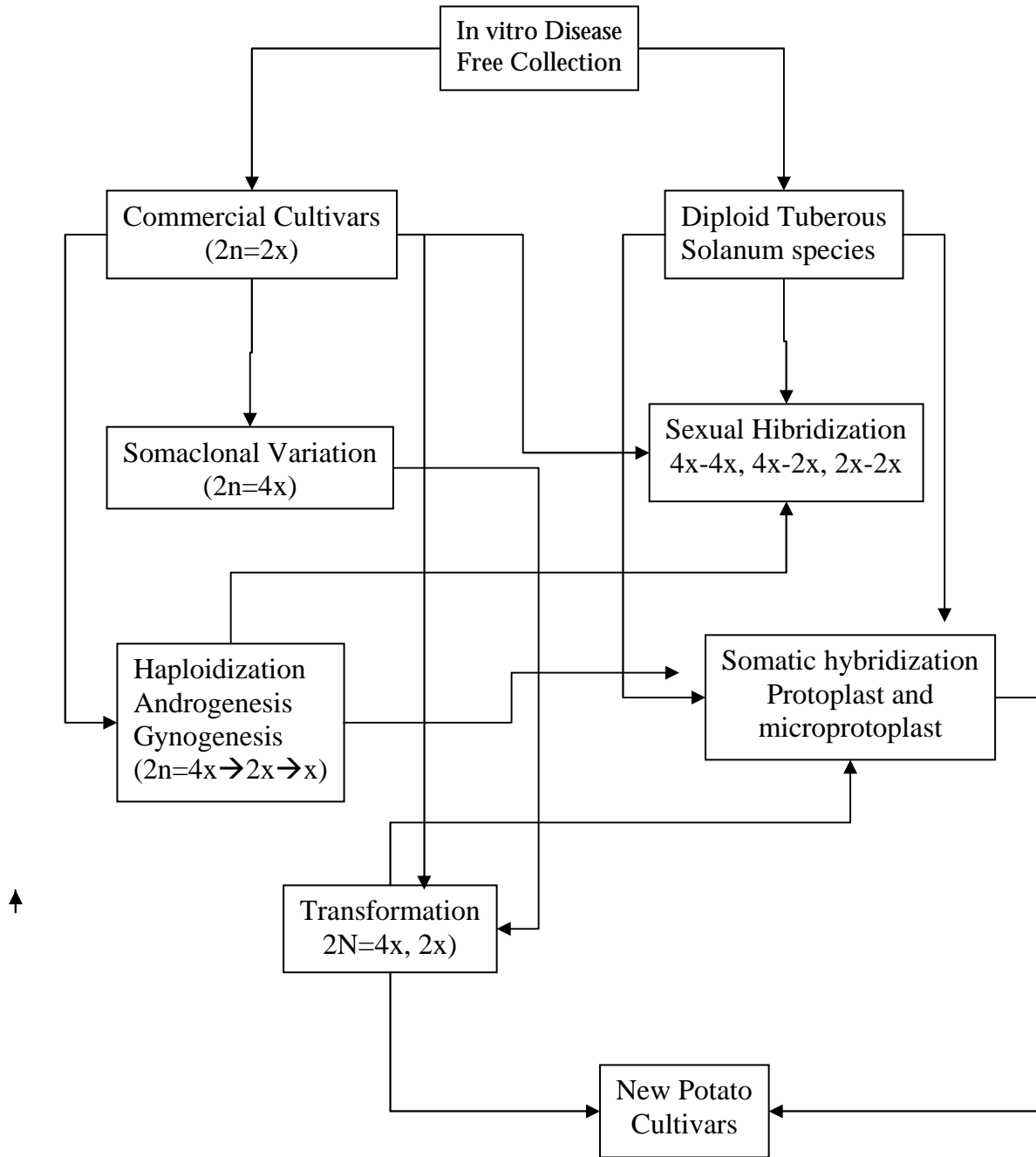


Figure 3. Proposed potato seed certification system in Indonesia

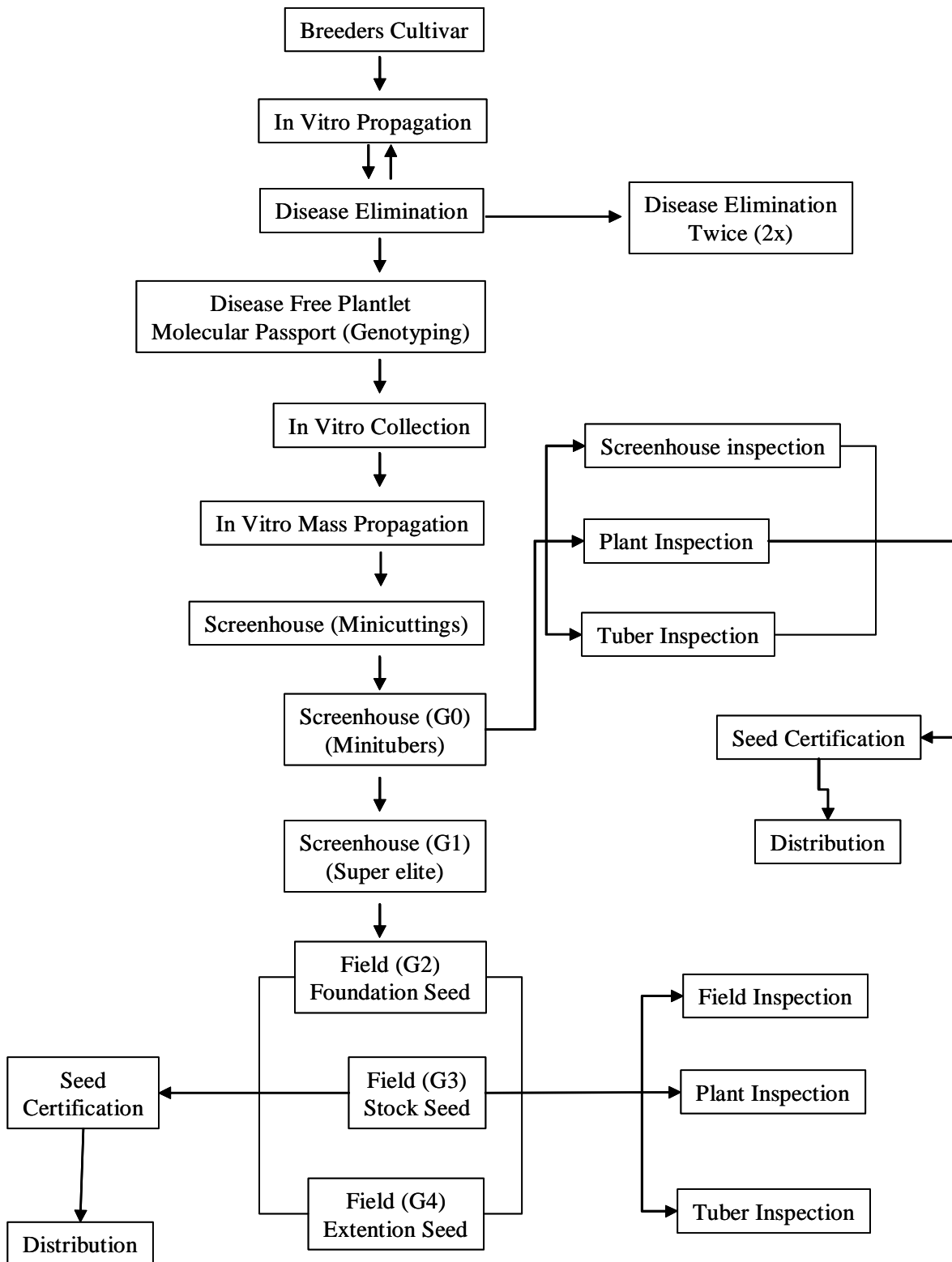


Figure 4. Diagrams of gene flows from donor to the receiver plant genotype and the role of molecular biologist, plant tissue culturist, and conventional plant breeder

