PLANT RESEARCH INTERNATIONAL

Final report BIOBREES II

Joint Research Co-operative Programme between Indonesia and the Netherlands on Biotechnology, Plant Breeding and Seed Technology Research for Horticulture

W.J. van der Burg (co-ordinator for the Netherlands)

CRIH-AARD

agriculture, nature management en fisheries

WAGENINGEN UR

PLANT RESEARCH INTERNATIONAL

Final report BIOBREES II

Joint Research Co-operative Programme between Indonesia and the Netherlands
on Biotechnology, Plant Breeding and Seed Technology Research for Horticulture

W.J. van der Burg (co-ordinator for the Netherlands)

Plant Research International
Droevendaalsesteeg 1
P.O.Box 16
6700 AA Wageningen
The Netherlands

Agency for Agricultural Research and Development
Central Research Institute for Horticulture
and Miscellaneous Crops (CRIH-AARD)
Jl. Ragunan 19
Pasar Minggu 12520
Jakarta, Indonesia

Plant Research International B.V., Wageningen
May 2003

WAGENINGEN UR

Plant Research International B.V.

Address	:	Droevendaalsesteeg 1, Wageningen, The Netherlands
	:	P.O. Box 16, 6700 AA Wageningen, The Netherlands
Tel.	:	+31 317 47 70 00
Fax	:	+31 317 41 80 94
E-mail	:	postkamer.pri@wur.nl
Internet	:	http://www.plant.wageningen-ur.nl

Table of contents

page

1. Summary 1

2. Background 3

3. Goal 5

4. Final reports per project 7

   4.1 Improvement of resistance to anthracnose in hot pepper 7
   4.2 Breeding for resistance to fungi in tropical shallots 8
   4.3 Breeding shallot for resistance to *Spodoptera* spp. 10
   4.4 Breeding Chrysanthemum for resistance to insects 11
   4.5 Breeding roses for ecological adaptation and vigour 13
   4.6 Breeding for Fusarium resistance in gladiolus 15
   4.7 Resistance of tomato to early blight 16
   4.8 Control of Tomato Spotted Wilt Virus 17
   4.9 Seed-borne diseases of vegetables 18
   4.10 Quality of vegetative planting material 20
   4.11 Plant Variety Protection and DUS testing 21

5. General observations 23

   5.1 Results 23
      5.1.1 Biotechnology 23
      5.1.2 Plant breeding 24
      5.1.3 Seed technology 26
   5.2 Training 27
   5.3 Participation of the private sector 28
      5.3.1 BIOPEP 28
      5.3.2 BIOTIC 29
      5.3.3 INDOROSA 29
      5.3.4 EARLYTOM 30
      5.3.5 TROPSHALLOT 30
      5.3.6 INDOSEED 30
      5.3.7 PROCULT 30
   5.4 Networking function 30
   5.5 Overall spin-off 32

6. Financial 33

Annex I. Publications and presentations 3 pp.

Annex II. Working visits 3 pp.

Annex III. Glossary 1 p.

1. Summary

BIOBREES II was a joint programme between the Indonesian and Dutch Ministries of Agriculture. It ran from 1 July 1998 to 31 December 2002. The programme continued and expanded on the collaboration under BIOBREES I (1994-1998). Formal partners were the Agency for Agricultural research and Development (AARD) in Indonesia and the Department of Science and Knowledge Dissemination (DWK) of the Ministry of Agriculture, Nature Management and Fisheries (LNV) in the Netherlands.
Executing agencies were the Central Research Institute for Horticulture and Miscellaneous Crops (CRIH), Indonesia and Plant Research International, the Netherlands.

BIOBREES II increased its scope and impact through many partnerships: it has become an ever increasing research and development network. Apart from the eleven projects of BIOBREES proper, the Indonesian and Dutch partners participated in seven projects of the private sector (co-sponsored through SENTER and the private sector partners). The BIOBREES partners also embarked on a training of the trainers programme (BIOTRAIN) with subsidy of the Ministry of Foreign Affairs, DGIS under the Asia Facility, five projects of BIORIN were granted under a programme of the Royal Netherlands Academy of Sciences (KNAW), and in 2001 a project to assist with the PVP implementation in Indonesia was granted under the PBSI-programme of DGIS, with additional support by LNV.

Training has been given special attention, for example through exchange of staff and training of trainers. A number of technical workshops have been organised (INDOSEED, BIORIN).

The PVP-project which started in February 2002, and will greatly benefit from the many links that have been established during BIOBREES I & II.

The programme was carried out with CRIH's institutes RIOP and RIV as main partners. They have been involved in all BIOBREES projects and wherever possible included in the SENTER proposals. This was to ensure that these institutes could contribute to the results and at the same time learn about the demands of modern science and how science can support agribusiness. Especially RIOP has taken up that message fervently. Staff engaged enthusiastically in all projects, and wrote proposals to the central Ministry in time, to ensure adequate funding for next year. BIOBREES has been a stimulus for RIOP staff to engage in true plant breeding and to bring out new cultivars. This has been done with considerable marketing and promotion, resulting in a much improved image of the institute. A spin-off of all this is the successful grant it received for centralising all RIOP's activities on one main campus. The new headquarters at Segunung are a clear example of how an institute can grow if it is successful in research and how this helps in securing funds.

The programme has been evaluated in 2003 and a recommendation was made for a new programme: HORTIN. This programme would be different from the BIOBREES programmes on a number of points: HORTIN will on the Dutch side be carried out by PRI and a new partner: PPO. This ensures a practical approach and will strengthen a shift towards more market-oriented activities. The programme will focus less on ornamentals and more on vegetables. It will contain more downstream activities, like a number of projects on protected cultivation, crop management, IPM and production of quality produce. On the other hand, upstream, emphasis will be on creating a stronger basis for breeding activities, such as new genetic resources for garlic, new technologies for hybrid seed production, more insight in the dangers of seed-transmitted diseases, and support to the management of a living ornamental genetic resources collection. These shifts in emphasis leave the midstream activities, like breeding and seed production, more to initiatives from the breeding institutes and the private sector.
A new element is the inclusion of a project on fruit flies: this pest which has a detrimental effect on

after harvest shelflife and transportability needed urgent attention. This means also that the institute for fruit research, RIF, has again become a partner. Finally, another attempt to improve the post-harvest situation will be tackled by a project on quality management in the vegetable chain.

HORTIN has started in January 2003, and had its first general meeting, the 'kick-off meeting', in Jakarta 4 February 2003. The Administrative Arrangement covering this co-operation was signed during the 8th meeting of the Working Group on Agriculture between Indonesia and the Netherlands in Jakarta, 3 April 2003.

We wish to thank the Dutch Agricultural Counsellor, Mr. Frans Claassen, for his continued and enthusiastic support.

2. Background

The recent Indonesian-Dutch research collaboration in horticulture started with the Lowlands Vegetables Project (1987-1992). BIOBREES started in 1994 as a collaboration between the Indonesian and Dutch Ministries of agriculture.
The programme included research on ornamentals (rose, chrysanthemum, gladiolus), vegetables (hot pepper, shallot) and one fruit project (mangistan). On the Dutch side a total of 0.9 mln Dutch Guilders was available per year for a period of four years. Indonesia would match this budget in manpower and facilities.

The programme was unique in its kind because it was formulated as a joint effort, and not as developing assistance. This meant that the funding would come from both sides, priorities were set with the interest of both sides in mind, and the outcome would be jointly owned.

By 1994 the projects were getting momentum and a continuation was advised: the partnerships were regarded very valuable on both sides and there was outlook at some interesting results. In the meanwhile the first steps were taken to include private partners with the financial assistance of SENTER, and additional PhD-projects were formulated for KNAW financing.

It was concluded that this collaboration merited a prolongation for another four years. This so-called BIOBREES II started formally 1 July 1998 and had a slightly different set-up: it consisted of 11 projects, excluding fruit trees but including seed technology and PVP. The budgets were similar, but were increased with 17,5% on the Dutch side because of the new VAT which became compulsory for the research partner in the Netherlands. This meant that the actual expenditures could remain at the same level.

3. Goal

The goal of the BIOBREES programme is bilateral collaboration on equal terms, to carry out fundamental and development oriented research of common interest in the fields of plant breeding, biotechnology and seed technology for horticulture.

The Administrative Arrangement of BIOBREES II makes this more specific:
1. Increase of knowledge on biotechnology as support for horticultural plant breeding and research on quality of planting material.
2. Production of breeding material with valuable characters, especially resistance against pests and diseases.
3. Training of junior researchers of Indonesia and the Netherlands in biotechnological research, advanced plant breeding and seed research, and promotion of bilateral co-operation between research institutes of both countries.
4. Stimulation of contacts between private sector enterprises (plant industry, seed companies) of both countries.

The 'Working Plan for BIOBREES II' mentions the recommendations of the evaluation committee, which needed to be taken into account during phase II. BIOBREES could continue only if a matching financing programme would again be formulated by Indonesia. Regarding the activities the following recommendations were made:
1. More emphasis on vegetable crops than on ornamental crops.
2. Research on ornamentals only if in collaboration and co-financed by the Dutch private sector.
3. Research on seed technology (seed and planting material) should be taken up.
4. The IPR of BIOBREES products should become well-protected, preferably within UPOV. A project on this subject should be formulated.
5. The exploitation of knowledge should be made explicit both in Indonesia and the Netherlands.
6. Linking of a number of projects with the KNAW programme.
7. An annual report shall be produced.

4. Final reports per project

4.1 Improvement of resistance to anthracnose in hot pepper

Objectives

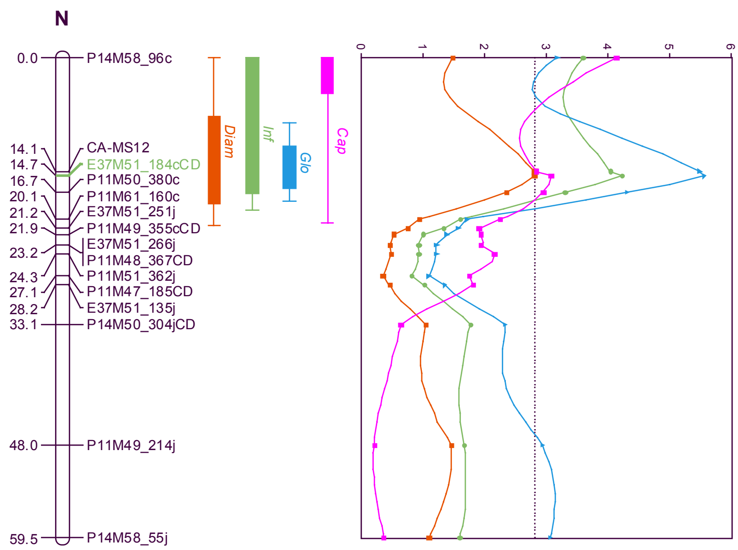
- Identification of sources of resistance to anthracnose
- Mapping QTLs (Quantitative Trait Loci) for resistance
- Characterization of resistance with respect to two pathogenic *Colletotrichum* species
- Development of pre-breeding plant material with resistance

Results

The goals of the project have been achieved: we developed a laboratory test method which gives a good evaluation of the resistance, with a high correlation with field results. Indonesian isolates have been analysed on their genetic relationship. A large collection ('core collection') of hot pepper and related Capsicum species has been made, which reflects most of the available genetic diversity. This collection was tested for resistance to anthracnose (both to *Colletotrichum capsici* and *C. gloeosporioides*). The most resistant progenies have been crossed with Indonesian cultivars. The crossing products have been handed over to the Indonesian partners for further use in their plant breeding programmes. Further, a genetic linkage map has been produced and a QTL-analysis of the resistance has been performed using two crossing populations. This resulted in the identification of molecular markers for anthracnose resistance.

Field test in Indonesia

The project has therefore been completed as planned. The plant material and molecular markers for resistance will prove very useful for future breeding work.



Main QTL for several resistance-related traits

Output

Sanjaya, L., 2002. Resistance to Anthracnose (*Colletotrichum* spp.) in Pepper (*Capsicum* spp.). PhD thesis, Bogor Agricultural University.

Sanjaya, L., Y. Nöllen & R.E. Voorrips. Screening *Capsicum* accessions for resistance to anthracnose (*Colletotrichum* spp.). Submitted to European Journal of Plant Pathology.

Voorrips, R.E., Y. Nöllen, L. Sanjaya & A.P.M. den Nijs. Resistance to anthracnose (*Colletotrichum spp.*) in Capsicum. 25th International Horticultural Congress (IHC), Brussels, Belgium, 2-7 August 1998.

Voorrips, R.E., Y. Nöllen, L. Sanjaya & A.P.M. den Nijs. Resistance to anthracnose (*Colletotrichum spp.*) in Capsicum. X EUCARPIA Meeting on Genetics and Breeding of *Capsicum* and Eggplant, Avignon, France, 7-11 September 1998.

Voorrips, R.E., L. Sanjaya, R. Groenwold & H.J. Finkers, 2001. QTL mapping of anthracnose (*Colletotrichum spp.*) resistance in *Capsicum.* XI EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant, Antalya, Turkey.

Voorrips, R.E., L. Sanjaya, R. Groenwold, Chai Min & H.J. Finkers, 2002. QTLs for anthracnose resistance in *Capsicum.* First BIORIN Workshop, Bogor, Indonesia.

Voorrips, R.E., R. Finkers, L. Sanjaya & R. Groenwold. (In preparation): QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum and C. chinense.* (to be submitted to Theoretical and Applied Genetics)

Plant material

Interspecific and intraspecific crosses between resistant accessions and Indonesian cultivars, and backcrosses.

Conclusions in relation to objectives

The aims of the project have all been accomplished.

4.2 Breeding for resistance to fungi in tropical shallots

Objective

To develop a genetic marker map for the efficient selection of resistance to *Colletotrichum gloeosporioides* (anthracnose) and *Alternaria porri* (purple blotch) in tropical shallots.

Results

The two most important fungal diseases in Indonesia that can cause great damage to the shallot crop (*Allium cepa* group *Aggregatum*) are purple blotch (*Alternaria porri*) and anthracnose (*Colletotrichum gloeosporioides*). At the start of this project no sources of resistance to these diseases were known. Therefore we started with the search for these sources. To this end we first developed bio-assays for both pathogens. Resistances were found in wild relatives of shallot. For anthracnose these were found in *Allium fistulosum* and *A. galanthum,* while for purple blotch they were found in *Allium roylei* en *A. fistulosum*.

The next step was to investigate whether these genes could be introduced in the genome of shallot. With the help of differential chromosome painting we could demonstrate that the simultaneous introgression from *A. fistulosum* and *A. roylei* into the shallot was indeed possible.

> For the first time successful bridge crosses were made between shallot and two related *Allium* species

The crossing strategy used was with the so-called bridge cross or trihybrid cross. Using this type of crossing, it was also possible to circumvent the species barrier which exists between shallot and *A. fistulosum*. The bridge crosses proved to be fertile, which makes further breeding work possible. A molecular marker map was constructed based on a bridge cross between shallot and the interspecific hybrid between *A. roylei* and *A. fistulosum* (a so-called CC x RF cross). This map enables direct selection based on the properties of the genome. The next step in this research is to localise the genes that are responsible for the resistance to *Colletotrichum* and *Alternaria* on the marker map. This research will be carried out in the TROPSHALLOT project: a project which aims at a close co-operation between private breeding companies and research institutes in both Indonesia and the Netherlands.

This research has also led to a number of published papers in international scientific journals as well as in a prominent Indonesian journal and in a book. Furthermore an internal report was written and a seminar given.

Output

International scientific journals:

Galvan, G.A., W.A. Wietsma, S. Putrasemedja, A.H. Permadi & C. Kik, 1997. Screening for resistance to anthracnose (*Colletotrichum gloeosporioides*) in *Allium cepa* and its wild relatives. Euphytica 95, 173-178.

Khrustaleva, L.I. & C. Kik, 1998. Cytogenetical studies in the bridge-cross *Allium cepa* x (*A. fistulosum* x *A. roylei*). Theor. Appl. Genet. 96, 8-14.

Khrustaleva, L.I. & C. Kik, 2000. Introgression of *Allium fistulosum* into *A. cepa* mediated by *A. roylei*. Theor. Appl. Genet. 100, 17-26.

Indonesian journal:

Wietsma, W.A., G.H.J. Grubben, B. Henken, S.-J. Zheng, S. Putrasemedja, A.H. Permadi, E. Sofiari, F.A. Krens, E. Jacobsen & C. Kik, 1999. Development of pest and disease resistant shallot cultivars by means of breeding and biotechnology. Indonesian Agricultural Research and Development Journal 20, 78-82.

Book chapter:

Kik, C., 2002. Exploitation of wild relatives for the breeding of cultivated *Allium* species. In: H.D. Rabinowitch & L. Currah (Eds.). *Allium* Crop Science - Recent Advances. CABI International, Wallingford, UK. pp. 81-100.

Internal report:

Estiati, A., 2001. Further development of a high quality molecular marker for indirect selection for downy mildew (*Peronospora destructor*) resistance in onion. Internal report Plant Research International, 15 pp.

Seminar:

Kik, C., 1999. Breeding for resistance to *Colletotrichum* and *Alternaria* in tropical shallots. Seminar at RIFCB, April 1999.

Conclusions in relation to objectives

The end-objective has been partially fulfilled because a molecular markermap has not yet been constructed on which the resistance genes for *Colletotrichum* and *Alternaria* are mapped. However, considerable knowledge and progress has been gained concerning the use of bridge-crosses for introgression, the development of reliable bio-assays and the construction of a molecular marker map based on a bridge cross between shallot and its wild relatives.

4.3 Breeding shallot for resistance to *Spodoptera* spp.

Objective

To develop a reliable transformation system and to introduce resistance to the beet armyworm *(Spodoptera exigua)* for shallots.

Results

The most important pest insect that attacks shallots in Indonesia is the beet armyworm *(Spodoptera exigua)*. At the start of the project it was not clear whether good sources of resistance were present in shallot and its relatives, nor was a bio-assay available to test for this property.
After developing such an assay, we discovered that no good resistance was present in the accessions available. We therefore decided to follow a new path: try to transform shallot with constructs containing a Cry gene. From this gene it is known that it causes resistance to *Spodoptera*.
A first step was to develop a reliable transformation and regeneration protocol. This proved very successful and as one of the first groups in the world we are now able to transform shallots (and onions) with the use of *Agrobacterium tumefaciens* as vector.
The next step was the genetic analysis of the transformants. This was done with molecular biologic (Southern blotting, adaptor ligation PCR) and molecular genetic techniques (fluorescence *in situ* hybridisation: FISH). It was found that the insertions were located at the ends of the chromosomes and that often more than one copy of the construct were present.
The described procedure was developed with 'test constructs' in which no Cry sequences were present. In the last year of the project transformations were made using a Cry fusion gene and a number of plants were produced which were completely resistant to the beet armyworm.

Shallot foliage destroyed by the beet army worm

The developed transgenic shallot material resistant to beet armyworm is unique in the world and can used for the further breeding of new shallot cultivars.
The research was of high quality and resulted in 6 scientific papers, one in an Indonesian journal and five in renowned international journals. Moreover, a PhD-thesis appeared on the subject.

Output

International scientific journals:

Zheng, S.-J., 2000. Towards onions and shallots (*Allium cepa*) resistant to beet armyworm (*Spodoptera exigua*) by transgenesis and conventional breeding. PhD thesis Wageningen University and Research Centre, The Netherlands.

Resistance to beet army worm has successfully been introduced in shallot

Zheng, S.-J., B. Henken, E. Sofiari, E. Jacobsen, F.A. Krens & C. Kik, 1998. Factors influencing induction, propagation and regeneration of mature zygotic embryo-derived callus from *Allium cepa*. Plant Cell Tissue and Organ Culture 53, 99-105.

Zheng, S.-J., B. Henken, E. Sofiari, E. Jacobsen, F.A. Krens &. C. Kik, 2001. Molecular characterization of transgenic shallots (*Allium cepa* L.) by adaptor ligation PCR (AL-PCR) and sequencing of genomic DNA flanking T-DNA borders. Transgenic Research 10, 237-245.

Zheng, S.-J., B. Henken, E. Sofiari, P. Keizer, E. Jacobsen, C. Kik & F.A. Krens, 1999. Effects of cytokinins and lines on plant regeneration from long-term callus and suspension cultures of *Allium cepa*. Euphytica 108, 83-90.

Zheng, S.-J., B. Henken, E. Sofiari, W.A. Wietsma, E. Jacobsen, F.A. Krens & C. Kik, 2000. Screening for resistance to the beet armyworm (*Spodoptera exigua*) in *Allium cepa* and its wild relatives Euphytica 114, 77-85.

Zheng, S.-J., L.I. Khrustaleva, B. Henken, E. Sofiari, W.A. Wietsma, E. Jacobsen, C. Kik & F.A. Krens, 2001. *Agrobacterium tumefaciens* mediated transformation of *Allium cepa* L.: the production of transgenic onions and shallots. Molecular Breeding 7, 101-115

Indonesian journal

Wietsma, W.A., G.H.J. Grubben, B. Henken, S.-J. Zheng, S. Putrasemedja, A.H. Permadi, E. Sofiari, F.A. Krens, E. Jacobsen & C. Kik, 1999. Development of pest and disease resistant shallot cultivars by means of breeding and biotechnology. Indonesian Agricultural Research and Development Journal 20, 78-82.

PhD thesis:

Zheng, S.-J., 2000. Towards onions and shallots (*Allium cepa* L.) resistant to beet armyworm (*Spodoptera exigua* Hubner) by transgenesis and conventional breeding. PhD thesis Wageningen University, pp. 146.

Seminar:

Kik, C., 1999. *Spodoptera exigua* in tropical shallots: transformation and marker-assisted breeding. Seminar at RIV, April 1999.

Conclusions in relation to objectives

This project has been very successful, both from a scientific and a applied point of view. The end-objective has been met, namely the introduction of resistance to the beet armyworm in tropical shallots.

4.4 Breeding Chrysanthemum for resistance to insects

Objectives

- Selection of pest resistant cultivars in Indonesia
- Transformation of chrysanthemum with genes for resistance to insects
- Propagation of new varieties

Results

In this project, a combination of traditional and modern breeding is implemented to combat the most important pests in chrysanthemum cultures. Considerable progress has been made to adapt Dutch germplasm (from the BIOBREES I programme) to tropical culture conditions by classical breeding. Multilocational trials for yield, ornamental value, disease and pest resistance were carried out in 1998 and 1999. This breeding programme resulted in the release of 15 new varieties in total, of which two are already being grown commercially in Indonesia. Tissue culture is common practise at RIOP now; all new genotypes are maintained in vitro. In addition, several rapid screening methods have been developed, as for example for chrysanthemum virus B. At the Dutch side, a number of new insecticidal genes have been isolated. Emphasis was put on the use of protease inhibitors (PI genes) and their heterologous expression in different tissues (flower and leaf) of chrysanthemum. Transgenics were first used as model plants. The plants harbouring these genes have been generated and thrips bio-assays have been carried out. A clear reduction in the oviposition rate was observed, and could be correlated to the level of PI-activity. This is the first evidence that expression of PIs may lead to the control of thrips *in planta*. Transformation of the same constructs to chrysanthemum were less successful. No significant reduction in thrips damage was found. Further research indicated that the expression levels of the genes were probably not sufficient to provoke an insecticidal response in chrysanthemum. In 2002, interesting PIs have been transformed to chrysanthemum and assessed for thrips resistance, at the whole plant level instead of detached leaves. Bio-assays have been carried out with the several sets of transgenic plants also for leaf miner resistance. So far we have not observed thrips resistance in any of these lines.

Thrips on a leaf

In Indonesia emphasis has been on the selection of genetic materials resulting from the hybridisation programme 2000-2001. To this purpose, multilocational adaptation tests have been performed. The best selections will be prepared for introduction to the market.

Output

Annadana, S., Beekwilder, M.J., Kuiper, G., Visser, P.B., Outchkourov, N., Pereira, A., Udayakumar, M., De Jong, J. & Jongsma M.A., 2001b. Cloning of the chrysanthemum *UEP1* promoter and comparative expression in leaves and ray and disc florets (♀) of *Dendranthema grandiflora*. Transgenic Res 11, 437-445.

Annadana, S., Mlynarova L., Udayakumar M., de Jong J. & Nap J.P.H., 2001a. The potato *Lhca3.St.1* promoter confers high and stable transgene expression in chrysanthemum, in contrast to CaMV35S-based promoters. Mol Breed (in press).

Annadana, S., Outchkourov, N., Schipper, A., Beekwilder, M.J., Udayakumar, M. & Jongsma, M.A., 2001c. Cysteine proteins inhibitors reduce fecundity in western flower thrips; Frankliniella occidentalis'. J. Insect Physiol. (in press).

Annadana, S., Rademaker, W., Ramanna, M., Udayakumar, M. & de Jong, J., 2000. Response of stem explants to screening and explant source as a basis for methodical advancing of regeneration protocols for chrysanthemum. Plant Cell Tissue Organ Cult, 62: 47-55.

Annadana, S. Towards protease inhibitor mediated resistance to western flower thrips in chrysanthemum. Thesis Wageningen University and Research Centre. ISBN 90-5808-550-3. 126 pp.

Marwoto, B., Darliah, Badriah, D.S. & Effendie, K. (2001) Monograf Varietas Ungull; Krisan-Mawar-Anyelir-Gladiol. 20 pp. ISBN 979-8842-13-8, RIOP, Pacet Cianjur, Indonesia.
Seetharam Annadana, Kuiper G., Visser P.B., De Kogel, W.J., Udayakumar M. & Jongsma, M.A. , 2002. Expression of potato multicystatin in florets of chrysanthemum and assessment of resistance to western flower thrips, *Frankliniella occidentalis*. Acta Horticulturae 572: 121-129.
Visser, P.B., Annadana, S., Kuiper, G., Kuipers, A., Outchkourov, N., De Kogel, W.J. & Jongsma, M.A., 2001. Application of cysteine protease inhibitors to protect chrysanthemum against thrips. Eucarpia Congress section Ornamentals July 3-6, 2001, Melle, Belgium.
Visser, P.B., Seetharam Annadana, Kuiper, G., Kuipers, A., Outchkourov, N., De Kogel, W.J. & Jongsma, M.A., 2002. Application of cysteine protease inhibitors to protect chrysanthemum against thrips. 10th IAPTC&B Congress. Coronado Springs Resort, Orlando, USA. June 23-28, 2002 In: In vitro cellular and developmental biology, Plant, Vol. 38(6): P-2043.

Conclusions in relation to objectives

> The introduction of new technologies in chrysanthemum breeding in Indonesia has led to the release of superior and commercially interesting varieties

The expected outcome at the end of the project was a series of high performing Indonesian bred chrysanthemum cultivars that give equal or superior quality products than leading Dutch cultivars, when grown in Indonesia. The project has harvested approximately 15 new Indonesian-bred varieties that were introduced to the market. An easy-to perform bio-assays to screen germplasm for virus, thrips, leaf miner and white rust resistance has been brought into practise in Indonesia. Tissue culture of chrysanthemum has become common practise.
In the Netherlands, although no thrips resistant chrysanthemums have been delivered, large progress has been made in understanding -transgenic- gene control in this species. Bio-assays for several pests have been worked out further and exciting new genes and promoters have been isolated.

4.5 Breeding roses for ecological adaptation and vigour

Objectives

Selection of roses with improved adaptation to Indonesian conditions
Transformation of rose with the ipt gene for improved vigour

Results

Efforts have been made to adapt well-performing Western cut rose varieties to the Indonesian climate. To this purpose new crosses have been made between the best selections of the BIOBREES I programme and traditional Indonesian varieties. Multilocational trials in the tropical highlands resulted in selected materials. To date, 8 new rose varieties have been released. First attempts have been made to identify the new varieties with the use of molecular markers. This will eventually help to protect the intellectual property rights of the plant material (see paragraph 4.10). It was found that roses are best grown on 'Multic' rootstocks. Serious problems occurred with virus infection in the rootstock. It was found that heat-treatment (40^{0}C during two weeks) could fully eliminate the infection from the propagation stocks.
In the Netherlands, two approaches to transform roses have been studied in detail; direct transformation and transformation of somatic embryos. A small number of transgenics containing the vigour-inducing gene *ipt* (isopentyltransferase) via somatic embryogenesis were generated, but the numbers are still unsatisfactory. These plants show the expected phenotype of extra branching. Time within this project was too limited to evaluate the plants in the greenhouse. Transgenic plants harbouring the antifungal *Hth* (hordothione) gene have been tested for mildew resistance. This gene, however, showed no effect on the growth of this pathogen.

RIOP rose breeder Darliah explains

At the end of the project the deliverables will be: A series of commercially attractive Indonesian rose cultivars, which can be distinguished from other varieties with molecular markers; Improved culture methods to grow roses in the tropical highlands of Indonesia and improved communication lines with the growers; Continued efforts in breeding new varieties and build-up of a variety collection at Segunung research station; An improved and standardised transformation protocol for rose, which can be applied to both Dutch and Indonesian varieties to introgress elite traits such as vigour and mildew resistance.

The quality of newly gained knowledge is high and very useful in practice. New varieties have been released, a method to eliminate virus infection was found, and efforts are being made to improve the quality of rose by easily-applicable cultural methods. In the Netherlands, in-depth knowledge has been generated to eventually overcome the transformation problems of rose. Now that we succeed in developing an efficient protocol, a world of possibilities lies open to introduce new useful traits into rose germplasm.

An efficient protocol for the transformation and regeneration of rose is now available

In order to increase the impact of this breeding programme it would be necessary to 1) develop the market structure for ornamentals, 2) to improve the physical quality of rose by cultural methods, and 3) the generation of disease-free planting materials. A successful breeding programme necessitates a continuous flow of innovations by means of continued crossing programmes and biotechnological interventions. The programme should be market-driven.

Output

Condliffe, P.C., Koehorst-van Putten, H.J.J., Davey, M.R. & Visser, P.B., 2003. An optimised protocol for rose transformation applicable to a number of different cultivars. Submitted to Eucarpia symposium section Ornamentals 'Classical versus Molecular Breeding'. August 25-29, 2003, Freising-Weihenstephan, Germany.

Marwoto, B., Darliah, Badriah, D.S. & Effendie, K., 2001. Monograf Varietas Ungull; Krisan-Mawar-Anyelir-Gladiol. 20 pp. ISBN 979-8842-13-8, RIOP, Pacet Cianjur, Indonesia

Visser, P.B., Keizer, L.C.P., Van der Salm, Th.P.M. & De Jong, J., 2000. Rootstocks transformed with rol a, b, c genes enhance rose flower production. Rose Conference 2000, 28 May-1 June, 2000 Kazanlak, Bulgaria.

Visser, P.B., Keizer, L.C.P., Van der Salm, Th.P.M. & De Jong, J., 2000. Introduction of rol A,B,C genes in rootstocks enhances rose production. 3rd Int. Congress on Adventitious root formation, 27 June-1 July, 2000, Veldhoven The Netherlands.

Visser, P.B., Annadana, S., Kuiper, G., Kuipers, A., Outchkourov, N., De Kogel, W.J. & Jongsma, M.A., 2001. Application of cysteine protease inhibitors to protect chrysanthemum against thrips. Eucarpia Congress section Ornamentals July 3-6, 2001, Melle, Belgium

Visser, P.B., Keizer, L.C.P., Van der Salm, Th.P.M. & De Jong, J., 2000. Introduction of rol A,B,C genes in rootstocks enhances rose production. 3rd Int. Congress on Adventitious root formation, Eucarpia Congress section Ornamentals July 3-6, 2001, Melle, Belgium.

Visser, P.B., 2002. Biotechnology in the flower industry in The Netherlands. SDCMA Symposium 'New genetic approaches to flowering in ornamentals and crops', San Diego Center for Molecular Agriculture, Oct. 17-18, 2002, Salk Institute, San Diego CA, USA.

Conclusions in relation to objectives

In the Netherlands two types of transgenic roses have been produced. The Hth plants have been evaluated in full detail, but unfortunately did not show enhanced mildew resistance, the ipt plants showed the expected phenotype, but time was too limited for full evaluation. The project has allowed us to improve the transformation protocol for rose considerably, both in speed and efficiency.
In Indonesia, objectives were met related to the production of new varieties, which can compete with traditional varieties on the Indonesian market. New diagnostic tools for variety identification were introduced and a way to control virus infection in rose rootstock has been implemented.

4.6 Breeding for Fusarium resistance in gladiolus

Objectives

- Selection of *Fusarium*-resistant gladioli adapted to the Indonesian conditions.
- Transformation of gladioli with *Fusarium* resistance genes
- Propagation of *Fusarium*-resistant new varieties

Results

Part A. Selection of Fusarium resistant gladioli adapted to the Indonesian conditions.

Several batches of gladiolus material have been prepared and sent to Indonesia for testing on *Fusarium* resistance combined with Indonesian consumer demands on phenotype. This material consisted of an F1 population obtained after crossing Dutch varieties, a selection of 10 Dutch varieties and clones obtained from crosses between Dutch and Indonesian cultivars. The last batch was sent in November 2001. Several accessions or varieties were found to be resistant to *Fusarium* and to meet consumer demands for traits such as stem length, flower diameter and flower number.

Promising new BIOBREES material

Part B. Transformation of gladiolus.

Twenty cultivars were taken to test the transformation protocol. Thirteen of them proved amenable for genetic modification by particle bombardment of suspension cultures followed by subsequent regeneration of transgenic plants in six of these cultivars. Unfortunately, the cultivars that are best suited for further breeding were found to be recalcitrant to this protocol. In the most recent period new cell biological and tissue culture approaches were tested for these cultivars and successful transformation was obtained using explants derived from gladiolus beads; also successful regeneration was obtained from fresh suspension cultures generated in a new and different manner.

> New cultivars suitable for the Indonesian market have been developed

We expect at the end of the project to have available in Indonesia new accessions and cultivars that are resistant to *Fusarium* and that meet the demands of Indonesian consumers. We also expect to have transgenic gladiolus plants of most of the important gladiolus cultivars.
A follow-up of this project may be aimed at optimising gene transfer protocols to allow a larger number of transgenic plants to be produced per inoculation and at producing the appropriate gene-constructs for transferring *Fusarium* resistance by genetic modification and to test the newly identified plant accessions that are adapted to the Indonesian market.

Output

The output of this project is concrete gladiolus plant material prepared in The Netherlands and transferred to Indonesia, where it was tested for resistance and meeting Indonesian consumer demands.

Conclusions in relation to objectives

- Selection of *Fusarium*-resistant gladioli adapted to the Indonesian conditions. This objective has been achieved; Indonesia can take this further.
- Transformation of gladioli with *Fusarium* resistance genes. This objective has been achieved partly; a successful transformation protocol has been established, but *Fusarium* resistance genes still need to be identified, cloned and introduced.
- Propagation of *Fusarium*-resistant new varieties. As will be clear from the first objective, interesting material has been identified and awaits further processing in Indonesia.

4.7 Resistance of tomato to early blight

Objectives

1. Identification of sources of resistance to early blight
2. Mapping QTLs (Quantitative Trait Loci) for resistance
3. Characterization of QTLs with respect to physio-specific resistance and resistance mechanism
4. Development of pre-breeding plant material with resistance

Results

This project was intended to run parallel with a PhD-study in the same field (funded through BIORIN). Since this programme started much later than planned due to administrative reasons at the Dutch side, the researcher also arrived much later. For this reason, the project period has been extended and the budget will be taken out of the BIOBREES programme.

This project will be reported on separately.

4.8 Control of Tomato Spotted Wilt Virus

Objectives

In order to be able to decrease the constraints of tomato productivity due to viral diseases, in specific due to Tomato spotted wilt virus (TSWV), sensitive virus diagnostic technologies have to be developed and implemented in Indonesia to score for the presence and incidence of this virus.

- identification and isolation of Indonesian strain(s) of TSWV occurring in tomato
- production of polyclonal antiserum to Indonesian TSWV strain(s)
- identification of thrips species present in field crops
- development of sensitive diagnostic technologies, i.e. RT-PCR, for detection of tomato spotted wilt virus (TSWV) in tomato crops in Indonesia
- extension of ELISA and RT-PCR detection to other potentially occurring tospoviruses in tomato and/or other crops in Indonesia
- RT-PCR cloning of the nucleoprotein encoding (N) genes from TSWV and other tospoviruses occurring in tomato and other crops

Results

This project, which has no funding through BIOBREES is carried out by Wageningen University on the Dutch side (Dr. R.J.M. Kormelink and Prof.Dr. R.W. Goldbach, Laboratory for Virology, Wageningen UR) and Dr. A.S. Duriat (RIV). For reasons of efficiency the Dutch Ministry of Agriculture has requested to include this project in the BIOBREES reports.

At the Laboratory of Virology of Wageningen University efforts have been made to develop RT-PCR protocols that will allow RT-PCR detection of tospoviral N genes from more than 3 different Tospovirus species, which is the detection limit of the currently available systems. For these RT-PCR methods, primers have been designed homologous to conserved sequences from the terminal ends of all Tospoviral RNA segments, and homologous to conserved sequences from internal regions within the S RNA. Throughout the last years a RT-PCR approach based on these primers has been developed and shown the capacity to detect more than three different species, including new (and uncharacterised) ones, i.e. one new tospovirus species from Holland denoted *Iris yellow spot virus* (IYSV), and another one originally collected from *Physalis* in Thailand and meanwhile denoted *Melon yellow spot virus* (MYSV). Moreover, a modified version of this RT-PCR approach has allowed quick and feasible cloning of the respective N genes.

> A sensitive RT-PCR detection system has been developed that so far has allowed the identification and cloning of at least 5 different tospovirus species

Iris yellow spot virus (IYSV)

The modified approach has meanwhile also been tested independently and with success, by tospovirus researchers in Brazil to whom the Wageningen group closely collaborates with. These tests, furthermore, demonstrated that the modified RTR-PCR approach additionally enabled cloning of other parts of the tospoviral genome.

The results altogether emphasise that the both these RT-PCR approaches have become a very useful tool to identify new tospovirus species and to enable quick cloning of the N genes of different Indonesian TSWV isolates to be used for transformation of tomato for resistance.

Although a potentially new tospovirus species has recently been identified that needs to be further characterised using the developed RT-PCR approaches, no researcher/PhD-student is currently being assigned in Wageningen on the collaborative project to proceed on the work.

Output

Bezerra, I.C., Resende, R. de O., Pozzer, L., Nagata, T., Kormelink, R. & De Ávila, A.C., 1999. Increase of tospoviral diversity in Brazil, with the identification of two new tospovirus species, one from Chrysanthemum and another from Zucchini. Phytopathology, 89, 823-830.

Cortez, I., Livieratos, I.C., Derks, A., Peters, D. & Kormelink, R., 1998. Molecular and serological characterization of *Iris* yellow spot virus, a new and distinct tospovirus species. Phytopathology 88, 1276-1282.

Cortez, I., Aires, A., Pereira, A.-M., Goldbach, R., Peters, D. & Kormelink, R., 2001. Characterization and analysis of *Iris* yellow spot virus M RNA: Indications for functional homology between the tospoviral G(c) glycoprotein and those of the animal-infecting bunyaviruses. Manuscript in preparation.

Cortez, I., Saaijer, J., Wongjkaew, K.S., Pereira, A.-M., Goldbach., R., Peters, D. & Kormelink, R., 2001. Identification and characterization of a novel tospovirus species using a new RT-PCR approach. Archives of Virology 146, 265-278.

Pozzer, L., Bezerra, I., Kormelink, R., Prins, M., Peters, D., Resende, R. de O. & de Ávila, A.C., 1999. Characterization of a distinct tospovirus isolate of *Iris* yellow spot virus associated with a disease in onion fields in Brazil. Plant Disease 83, 345-350.

Conclusions in relation to objectives

This project has stopped for the last few years because it got no funding through BIOBREES or by any other means.

4.9 Seed-borne diseases of vegetables

Objectives

- Making a survey of existing disease problems of seed-borne nature
- Setting up and testing indexing methods for the most important seed-borne diseases of vegetable crops (based on 1)
- Determining seed infection levels for seed lots from various origins
- Developing and testing seed treatments for control of priority seed-borne diseases
- Establishment of interactions between seed health and other seed quality parameters (germination, maturity)

Results

This project is run in close conjunction with INDOSEED: the latter has been prepared under BIOBREES to accelerate the activities. RIV has limited experience with seed technology, and even less with seed health testing. For this reason two staff from RIV have been trained in seed pathology during the first year. In the second year, when the capabilities and facilities of RIV to perform seed health tests were to be further developed, no activities were carried out at RIV because of lack of funding.
In that year the Senter-subsidised INDOSEED project was jointly formulated with the Indonesian seed company PT Ewindo, the Dutch seed company ENZA, and with IPB, Laboratory for Seed Technology. With these active partners, many activities are now ongoing. RIV agreed to carry out a number of essential tasks as well. The BIOBREES-funding is used by Plant Research International to actively participate in this project.

Significant progress has been made in all work packages. Methods for seed health control have been evaluated and knowledge on detection and identification procedures have been transferred during a two workshops in Indonesia (August 2001 and October 2002) which were evaluated very positively by the participants and the gained knowledge was considered as directly applicable. The workshops were focussed on seed-borne bacterial and fungal pathogens in cabbage, tomato and pepper. The role of seed transmission in the epidemiology of an important viral pathogen (pepino mosaic virus) in cucumber has been elucidated. Plant extracts (essential oils) have been selected, which show a high activity against important seed-borne bacteria when applied in combination with synergistic compounds. These oils have a great potential in sustainable agriculture. An excellent correlation has been found between chlorophyll measurements in seed and seed germination. Contacts were strengthened between the different partners in the project, which has resulted in the applications of an AARD grant for an PAATP-fellowship and IAC fellowships for a post-doc and a PhD student from Indonesia. The work within the INDOSEED-BIOBREES project is strongly linked to a project within DWK programme 'Biological plant propagation material' and results from both projects will be of mutual benefit.

Seed technology is gaining momentum: many subjects are being investigated and the collaboration network is expanding

At the end of the project, all partners are trained in seed health test methods for several economically important viral, bacterial and fungal seed transmissible diseases. A better understanding will be gained in the role of seed transmission in the epidemiology of *Didymella bryoniae* and pepino mosaic virus. Environmentally-friendly seed treatments have been fine-tuned and evaluated for control of seed-borne diseases. The effect of seed maturity on the tolerance for seed- and soil-borne diseases has been determined.

In 2003 procedures for decontamination of seed with 'green chemicals' will be evaluated at ENZA and PT Ewindo. The relationships between seed health and seed maturity will be tested to understand possible effects on seed processing and seed treatment.

Contacts with Indonesia were hampered several times due to technical problems, such as a block in E-mail transfer. In spite of that, a smooth collaboration with ENZA and Ewindo has been established. The role of RIV so far was restricted to participation in the workshop, and writing a proposal for PAATP. Recently they have started with the production of naturally infected pepper seeds.

Output

Anwar, A., Van der Zouwen, P.S. & Van der Wolf, J.M. Bacterial canker (Clavibacter michiganensis subsp. michiganensis) of tomato in commercial seed produced in Indonesia. Plant Disease, submitted.

Belfiore, B. & Van der Wolf, J.M., 2002. *Didymella bryoniae*, causal organism of stem and fruit rot in cucurbits. A literature study. 9 pages .

Langerak, C.J. (Editor), 2001. Management of seed health of important vegetable crops, 1st INDOSEED-BIOBREES workshop. Bogor, Indonesia, 20-24 August 2001. Workshop Manual. 71 pages.

Van der Wolf, J.M. (Editor), 2002. Management of seed health of important vegetable crops, 2nd INDOSEED-BIOBREES workshop, Van der Wolf, J.M. (editor). Bogor, Indonesia, 7-11 October 2002. Workshop Manual. 93 pages.

Conclusions in relation to objectives

The project has largely extended the knowledge on seed-borne diseases and strategies for management of seed borne diseases at all project partners. The identification of an unknown disease problem in Indonesia (bacterial canker in tomato) in Indonesia in an early stage, can avoid serious epidemics in the future. During two workshops, the expertise on seed health management has been extended and exchanged in a meaningful way to many participants working in the field of seed production and technology. During experimental work, plant extracts have been selected which can be efficiently and cost-effectively applied for seed treatments. By establishing a relationship between seed germination and seed infection, the importance of the time for seed harvest is emphasised.

4.10 Quality of vegetative planting material

Objectives

- Establishment of a method for identification of rose varieties
- Evaluation of identification methods with Indonesian material

Results

Now that varieties are being released on basis of the BIOBREES collaboration, the subject of protection becomes pertinent. It is important for the institutes involved that they are able to claim royalties on their products. These royalties will then for instance enable further research and marketing activities.
In this respect it is important to show that the varieties can be identified on the bases of a unique set of characters. Molecular techniques can provide such markers. They can help us to quickly trace companies producing our varieties without any licensing contract.

In order to investigate the possibilities to distinguish between varieties on DNA level, 70 BIOBREES and related varieties were fingerprinted using 9 microsatellite markers. However, quality and quantity of the DNA obtained from Indonesia were not sufficient to accurately genotype all varieties. Only 37 DNA samples could be characterised successfully. The molecular information has been stored into a database.
The main conclusion is that most roses can be distinguished with a very high degree of accuracy. Three clusters were found, of two varieties each, which are not distinguishable from each other, like for example 97.7 and 97.9. These samples are most likely clones of the same variety or mutants thereof. The data indicate that only three primers instead of the nine used will be necessary to distinguish between all Indonesian varieties tested, when mutants are excluded. The existing database at PRI contains the microsatellite profiles of 485 varieties and is based on 9 different STMS markers from at least 6 different linkage groups. Six of these, including the most informative markers were also present in the set used for genotyping the BIOBREES varieties. On the basis of these six markers the BIOBREES varieties were compared to the Dutch database. All BIOBREES varieties, except for the mutants, were identified as a unique variety and were not clustered together but partitioned among the Dutch varieties. This is consistent with the fact that progenitors of the tested BIOBREES varieties originate from the same genepool as most of the modern hybrid tea varieties.

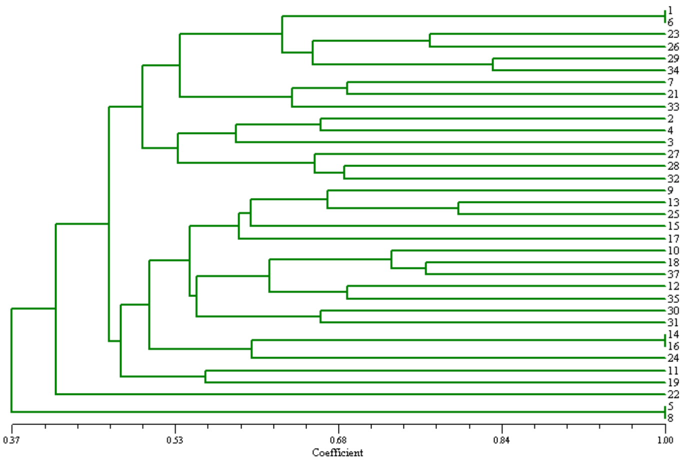
> Most Indonesian roses that were investigated can be distinguished to a high degree of accuracy using microsatellite DNA-analysis

Output

Esselink, G.D., Smulders, M.J.M. & B. Vosman, 2003. Identification of cut rose (Rosa hybrida) and rootstock varieties using robust Sequence Tagged Microsatellite Site markers. Theor. Appl. Genet. 106: 277-286.

Conclusions in relation to objectives

The microsatellite markers developed by Plant Research International are a very powerful tool for characterization of rose varieties, including the BIOBREES varieties. To fully benefit from them it is suggested that in future all varieties released by RIOP for the Indonesian market will be fingerprinted. In this way an Indonesian reference database is build which can be used for tracing infringements on Plant Breeders Rights and tracing illegal propagation. Also, mutants of existing varieties are easily recognised It must be remarked here however, that until present morphological distinctions between varieties remain the sole criterion for granting Plant Breeders' Rights. Molecular analysis can be used as additional evidence against forgeries and infringements.



Cluster analysis of BIOBREES rose varieties using 9 microsatellite markers

4.11 Plant Variety Protection and DUS testing

Objectives

Support in developing an effective and efficient Plant Variety Protection system

Results

The lack of legal protection of intellectual property rights and enforcement of such legislation in Indonesia blocks the access to novel technologies, such as varieties and gene constructs by both public and private partners in Indonesia. This particularly affects the private sector and public-private collaboration in research. As a result, BIOBREES has included a project halfway the programme that would support the Indonesian Government to introduce an internationally harmonised system for Plant Variety Protection (PVP).

This project has made an important start through a mission by a technical and a legal specialist in this field to Indonesia. Subsequently, detailed advice has been given on a draft PVP-law. Actual work on this field depended however on the political decisions which led to the formal acceptance of the law in 2001. Furthermore, the PVP-project suffered from a lack of mandate of the BIOBREES partners (RIV, RIOP) in Indonesia to be involved in the development of PVP-policies (task of Parliamentary commissions), and uncertainties about the tasks of these institutions in the future implementation of this law.

BIOBREES has played an important role in the identification of PVP as an important vehicle for future collaboration in aspects of breeding and seeds

The project has, however, created a platform for the preparation of a project on the implementation of PVP in Indonesia, which will be funded by the Netherlands Ministry of Foreign Affairs (with an essential contribution in staff and funds from LNV) in the framework of the Government-to-Government support programme of PBSI. With this project on PVP, this very important topic will get impetus and will hopefully result in a successful start-up of the implementation phase.
The project has started with a formulation mission of a number of experts from the Netherlands to discuss the project objectives and project plan with the Indonesian authorities. It was consolidated in a workshop on PVP on 27 February 2002 and a first series of DUS-test trials has been carried out during that year.

Output

A national workshop on PVP legislation and implementation was held
The first DUS-trials on ornamentals and vegetables were carried out

Conclusions in relation to objectives

The BIOBREES activities on PVP have successfully initiated the contacts and provided the basis for a joint grant application for a Government-to-Government programme on PVP implementation.

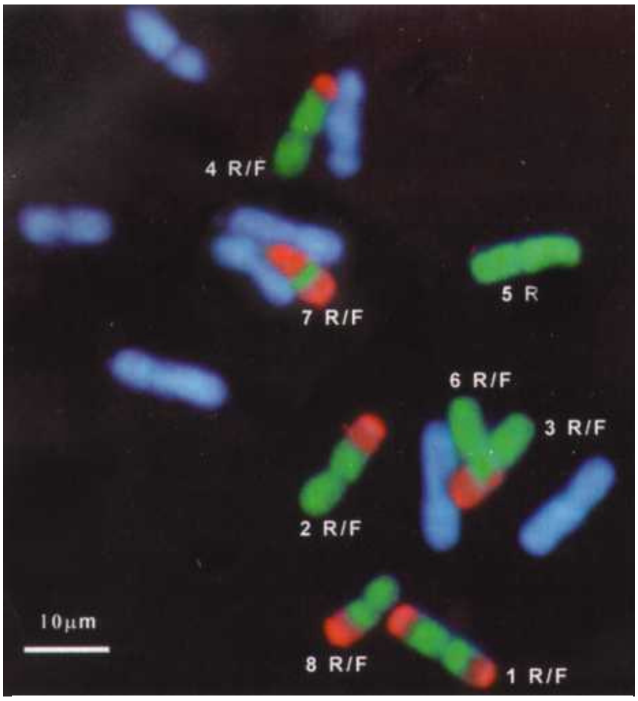
5. General observations

5.1 Results

5.1.1 Biotechnology

BIOBREES II has been marked by the successful utilisation of many sophisticated technologies. The work on tissue culture, transformation and regeneration has shown many breakthroughs. Transformation can now be performed in all crops under the programme.

Tissue culture work has been one of the essential tools in about all projects: mass propagation, making material disease free, providing a substrate for transformation, regeneration of transformed cells, etc. Both RIOP and RIV already had extensive experience in tissue culture, but they greatly benefited from the Dutch expertise. New protocols were tested and others were improved.



Example of FISH analysis: blue chromosomes are of A. cepa, red parts are of A. fistulosum, green parts are of A. roylei

Significant progress has been made in the field of genetic mapping, characterization of genes, and the development of markers. For shallot, hot pepper, rose genetic markers are now available which can be used to speed up the breeding process considerably.

The development of double haploid plants from a crossing population between a susceptible and a resistant plant, enables the preparation of genetic marker maps. The plants can also be used very profitably in further crossings: the genes present in the double haploid plant will also for 100% be present in the progeny.

Mapping of genetic properties such as many resistances, which are controlled by more than one locus. So-called QTL-analyses have been carried out resulting in a number of markers for resistance.

Until now the genetic transformations were performed in the Netherlands for a number of reasons: the wide experience and equipment available, which made it more efficient to perform these tasks in the Netherlands, although in many cases performed by Indonesian visiting scientists. Another important reason were the facilities for testing gmo material: also here, the facilities and expertise were available in the Netherlands, and only very recently they became available in Indonesia. A third and most important factor was the legislation needed to protect intellectual property. Also here much progress has been made recently: patent laws and laws which regulate the testing and introduction of transgenic material have now been adopted, and very recently legislation has been adopted which regulates plant variety protection. Other highlights were the development of a bio-assay for testing shallot for resistance to *Spodoptera exigua*, the development of a reliable transformation protocol for shallot and onion, and the development of transgenic shallots (and onions). The researchers used many different techniques for genetic analysis, of which FISH is a good example: with this technique the insertion of genes from another species in the host genome can be made visible. Other techniques used were molecular techniques such as Southern blotting and AL-PCR.

In the ornamentals the production of virus-free mother stock and propagation in vitro of rose is now operational, and a transformation protocol for rose has been worked out, which allows the generation of transgenic plants within 1 year. The first transgenic roses have been tested in The Netherlands. For chrysanthemum these technologies were already developed during BIOBREES I. In this crop new constructs are now available to combat leaf miners.

Also in rose, a crop which develops much slower than chrysanthemum, the release of varieties is starting up.

Regeneration and transformation protocols have been developed for several gladiolus cultivars. Now, a cultivar-independent method for genetic modification and the production of transgenic gladiolus plants has become available, albeit at low productivity.

On the introduction of plant biotechnology in Indonesia

Introduction of the above mentioned biotechnologies in Indonesia has sometimes been difficult. This has a number of reasons:
The facilities at the AARD-institutes are not adequate, many pieces of equipment are still needed.
The number of staff capable of using such technologies is very limited. Especially the absence of junior staff with a molecular background blocks the transfer of technology.
The protection of intellectual property in Indonesia is uncertain: legislation has been put in place but not yet into practice.
There was not enough budget for Indonesian visiting scientists.

It is hoped that after the refurbishing of RIOP also funds will be allocated for equipment, training and the appointment of well-trained young staff. At present the scientific staff of RIOP is relatively small and senior. Future successors will have to be trained now, to prepare the institute for the future.

5.1.2 Plant breeding

Most projects have gone very well and have produced results as planned. Large steps forward have been made in the breeding of shallots, where the bridge cross concept was developed and put to work. This now enables introducing important resistance genes hitherto unavailable in any shallot genetic stock. This very positive result has created new opportunities in shallot breeding. The private sector could be interested to engage in a long term project together with the BIOBREES partners to develop true seed shallots (see TROPSHALLOT below).

In hot pepper much has been achieved: elaborate collections were made, which have all been tested for resistance to anthracnose. In this material new sources of the desired resistance were identified. These have been used in crossing programmes with popular local varieties. Also a doubled haploid population has been produced through the Senter-subsidised project BIOPEP, which has allowed to extend the QTL mapping results. QTL for resistance have been identified, which can be selected for through the use of linked markers. This material will soon give rise to new anthracnose resistant hot pepper varieties.

The work on early blight in tomato also started with the collection of plant material and testing for the presence of resistance. A test method for resistance, although still in development, is already a considerable improvement over existing methods. This project has been delayed in order to run in parallel with a BIORIN project, and will therefore continue past the end of BIOBREES-II.

The work on gladiolus has concentrated on the generation of much crossing material. This was done by crossing Dutch with Indonesian varieties. The populations obtained were tested extensively in a rigid screening programme for characters such as resistance (to *Fusarium oxysporum*), several flower properties and the time of harvesting. All are traits considered important for the Indonesian market. Several accessions or varieties were found to be resistant to *Fusarium* as well as to meet consumer demands.

Since the inception of BIOBREES a total of 15 new chrysanthemum cultivars has been released. They were presented at fairs and other occasions, and attracted a lot of publicity. A method for rapid detection of chrysanthemum virus B has been developed.

Also in rose, a crop which develops much slower than chrysanthemum, the release of varieties is starting up.

Professional breeding

FTissue culture at RIOP, Cipanas

An important element in the collaboration was to demonstrate to Indonesian scientists how professional breeding in horticulture is carried out. Essential in the ornamental projects was to demonstrate that breeding a different colour alone is not enough. There are dozens of characteristics that new varieties must fulfil, in order to be attractive for consumers and farmers alike. For this, very extensive crossing and testing programmes have to be developed, with constant search for new genitors and many new crossings every year. Among breeders one often encounters the misconception that it is sufficient to make a few crossings at one time and that one than has sufficient material to select in for years. Of the thousands of gladiolus, chrysanthemum and rose plants produced during the past BIOBREES collaboration, now less than 20 of each are still under investigation. The rest had to be discarded. This means that new crossings are badly needed to cater for the market needs of the future. Of course such programmes can only be successful if the Government of Indonesia decides to allocate sufficient resources for such a programme during many years. This is true for both vegetables and ornamentals. The research must become of a more strategic nature: choices will have to be made as to crops and as to how far the institutional breeding work should go: or in other words, what are the essential research actions needed (e.g. introduction of a new resistance into a crop), and when can the private sector take over?

Marketing strategy

Crucial for the future will be the development of a good marketing strategy for the intellectual properties generated by the AARD institutes. The Indonesian authorities have taken this up energetically, and even formed the technology transfer agency KIAT. Contracts are now being established with the private sector via the intervention of this agency. Royalties for the institute and the individual breeder have been defined. RIOP has acquired a facility for the production of mother stock and intends to serve the market with disease-free planting material. A weak point in this set-up is the fact that researchers are by definition no good producers. A slightly different task division between the market partners and the institute may prove more practical. We estimate that advice from the Netherlands' experience may be of help.

5.1.3 Seed technology

Protocols and procedures were estimated for isolation and detection of the following pathogens *Xanthomonas campestris* pv. *vesicatoria*, *Clavibacter michiganensis* subsp. *michiganensis*, *Colletotrichum capsici*, tobamoviruses and pepino mosaic virus. Transfer of this knowledge has resulted in the first step of the establishment of new laboratory tests at Ewindo.

Reference panels have been compiled of strains of *X. c.* pv. *vesicatoria, X. c.* pv. *campestris* and *C. m.* subsp. *michiganensis* from international collections supplemented with strains isolated from fields in Indonesia. Protocols were evaluated for isolation of *Xanthomonas campestris* pv. *vesicatoria* and a bioassay has been developed.

Seed transmission of pepino mosaic virus was investigated. A low rate of seed transmission could be established, but this had no significance in the epidemiology of this viral pathogen.

To find safe and effective alternatives for chemical seed treatments, the antimicrobial activity of 16 essential oils were tested in a micro-assay for *Clavibacter michiganensis* subsp. *michiganensis*, a Gram-positive bacterium causing bacterial canker in tomato and *Xanthomonas campestris* pv. *campestris*, a Gram-negative bacterium causing black rot of crucifers. Strong synergistic effects were found when essential oils were combined with a chelator and a natural detergent.

A relationship was established between cucumber seed maturity and seed quality, to find the optimal time for harvesting seeds. Seed lots of different maturity were analysed and sorted by chlorophyll measurements. An obvious relationship between chlorophyll content and ripeness was found.

During the 1st and 2nd INDOSEED BIOBREES Workshops on 'Management of seed health of important vegetable crops', organised in Bogor (Indonesia) in 2001 and 2002, groups of seed technologists and seed pathologists from different universities, institutes and seed companies in Indonesia were trained in several aspects of seed health. The practical courses were complemented by lectures given by Indonesian and Dutch specialists on management of seed borne diseases and other seed quality aspects. Workshop manuals are available on request.

Seed technology in Indonesia

After an initial delay, the seed technology collaboration is now going very fast. This is especially thanks to the enthusiastic participation of the IPB Laboratory for Seed technology and PT Ewindo. The last is the first professional breeder of vegetables in Indonesia, which uses foreign and local material to produce well-adapted new varieties. The many disease problems encountered in Indonesian horticulture proved very challenging. These diseases are a limiting factor in the production of high quality horticultural products. In order to improve the situation a lot of knowledge about the occurrence, the way of transmission, and possibilities for control must be generated.
It would be good to define an Indonesian strategy for the management of diseases in horticulture: the tasks and responsibilities of the various institutes and control agencies as well as of the stakeholders in the production chain need to be defined. An action plan as to how to approach the issue should be made. Advice from the Dutch experience could be of assistance here.

5.2 Training

All projects have an inherent training component: the very fact that researchers have professional contacts, that they exchange materials and technologies, results in increased knowledge and proficiency. In many cases this effect was enhanced by exchange visits: quite a number of Indonesian scientist took the opportunity to visit the Netherlands, Wageningen UR and Plant Research International in particular to increase their skills (see Annex II: Working visits).

BIOTRAIN participants

A number of PhD-students (BIORIN programme) are performing research or are now writing their thesis based on sandwich grants. By using this methodology, the young scientists are exposed to high-level research and at the same time are being forced to think of how to apply these technologies in Indonesia. The applicability of the results is enhanced by this complementarity of the different approaches.

A third means to transfer knowledge is by training courses. We were very glad to get the grant from the SENTER Asia Facility for the BIOTRAIN project.

BIOTRAIN ran from March 2000 to March 2002 and was subsidised through the Asia Facility programme of the Netherlands. It concentrated on the improvement of biotechnological skills of staff of Indonesian companies and to improve the ties between institutes and companies in the field of plant biotechnology.

To achieve this, a great number of candidates from a number of research institutes have been interviewed and finally eight candidates were selected to follow a training of one month in the Netherlands. From these eight candidates, three stayed behind to follow training during an additional period of five months.

After return of the trainees to Indonesia a number of Dutch consultants went to Indonesia to make a start with the development of a number of modules, together with the trainees. These modules were based on an inventory of needs of the private sector. To map these needs better, a *training needs analysis* (TNA) has been performed at a number of companies at the start of the project. The eight trainees developed eight modules in total. Each trainee was responsible for one of the modules, but all participated in the realisation of each module.

Eight BIOTRAIN course modules have been developed

All modules including the extensive course material are now available in Bahasa Indonesia.

Finally, workshops are a very effective and efficient tool to get technology transferred: the combination of scientific lectures and hands on exercises warrants a large learning effect. In August 2001 the first Seed Health Workshop was held at IPB and co-organised by seed technologists of Plant Research International. It created awareness of the many disease problems that occur in Indonesia and provided insight in how to combat these. Staff of RIV took part in this workshop and assisted with the organisation: their initial training in Wageningen proved very helpful. A second workshop has been organised by the same groups in October 2002. A third one is scheduled at RIV for 2003.

In the framework of BIORIN meetings and workshops are also held annually in which all project leaders concerned are participating. Participation of staff in these meetings is in part made possible through the activities and funds of BIOBREES.

5.3 Participation of the private sector

All projects mentioned below are being supported by Senter, through the former INDONESTEC and BIT programmes (currently replaced by the TIS programme). The subsidy is usually maximised at 37.5% however, which means that the remaining budget must come from the private sector or institutional partners. Thanks to the LNV subsidy for BIOBREES, it was possible for Plant Research International to participate in these projects. This subsidy has been fully used for counter-financing purposes and as such enabled additional projects with a total of over Dfl 8 million (see chapter 6. Financial). These initiatives would not have been possible without BIOBREES. This means that BIOBREES was very successful in attracting additional financing, its budget was tripled. On top of that, also the BIOTRAIN, PVP and five BIORIN projects were made possible (budgets of approx. 0.7, 0.9 and 2.5 Dfl mln respectively).

Purpose of all of these projects is to ensure the utilisation of the BIOBREES results through a joint commercialisation with the private sector: private companies from the Netherlands and from Indonesia form partnerships, and wherever possible Indonesian BIOBREES partner institutes are being involved. Participation of the institutes is regarded important because one can profit from their extensive knowledge of Indonesian agriculture and their facilities for screening under local conditions, but also to demonstrate to the Indonesian scientists how companies work, and to get an idea of what role the institutes can play in supplying agribusiness with desired knowledge and products.

The direct impact of these projects will become apparent during the coming years, when completed varieties or developed technologies become available on the market.

5.3.1 BIOPEP

The BIOPEP project, which focused on the introduction of resistance to anthracnose in hot pepper, ran from 1-9-1997 to 31-12-2001. It is essentially a collaboration between ENZA Seeds, EWINDO, and Plant Research International. RIV was also included, especially for the very important field work under local conditions, but failed to give an input. Fortunately, Ewindo filled this gap very successfully. Also, the link with ENZA Seeds gave access to the valuable doubled-haploid plant material.
The plant material and knowledge developed in the BIOPEP project are available to all project partners. Through the link with EWINDO and RIV, both of which are involved in breeding varieties for the commercial market, Indonesian horticulture will benefit from the availability of resistant varieties.

5.3.2 BIOTIC

Goal of this project is the development and production of locally adapted, heat tolerant cultivars with high ornamental value and resistances to pests, by integrating classical and molecular breeding technologies. After resignation of Dutch chrysanthemum breeding company Deliflor during the initial project period 1999-2002, another company Agriom took over the tasks and the new term for execution of the project is envisaged for 2002-2005.

In the two past years, Plant Research International successfully carried out all the proposed work in the field of biotechnology. Main goal in this first phase to develop a prototype transgenic chrysanthemum with (partial) resistance against thrips. A number of transgenic lines were produced carrying different resistance genes (type protease inhibitors) with proven (vitro) activity against thrips. Bio-assays were carried out to study vivo-activity of those genes against thrips. Thus far, no significant increase of resistance was found with this set of plants. We have adapted the prototype by using new genes and promoter-elements. These plants are to be tested in the year 2003.
Unfortunately, Deliflor has not carried out her tasks in the first phase of the project. Contacts between Deliflor and the Indonesian partner Pt. Saung Mirwan have been neglected. During a mission to Indonesia (November 2001) , Agriom and Plant Research International have re-established contacts with Pt. Saung Mirwan and immediately after, Agriom sent selected plants to Saung Mirwan for assessment under Indonesian conditions (the first phase of the project). Although with delay, we are now back to the original workplan.
Saung Mirwan and the Research Institute for Ornamental Plants are currently carrying out joint research. Envisaged in the BIOTIC-project was a collaboration for the development of new Indonesian chrysanthemum varieties. Materials from Agriom, but also modern Dutch varieties will be brought to RIOP. These materials will be used for new crossings. Progeny will be assessed by both the research institute and the commercial company. It is expected that this type of collaboration in breeding (so-called shuttle breeding) will boost the Indonesian breeding activities, resulting in real Indonesian varieties which could be commercialised by Indonesian companies (with Dutch participation).
Newly selected material could be used for crossing with transgenic thrips resistant lines. This is not part of the current project, due to uncertainties regarding consumer acceptance of transgenic plants.
Thus, the project deliverables will be new, adapted chrysanthemum cultivars.
Future products: transgenic chrysanthemum with improved resistance to thrips (and other insects).

5.3.3 INDOROSA

Goal of the project is to redesign the rose plant and make it fit for production of high quality flowers under tropical conditions. The methods being used are a blend of classical and biotechnological procedures. The proposed project period was from June 1999- June 2003. Due to the disappointing results so far in the generation of transformed plants, as well as the sub-optimal communication between partners Terra Nigra and Pt. Saung Mirwan. The envisaged goals will not be met. In consultation with Senter, the research goals have been adjusted. Plant Research International will focus in this last phase of the project on making the transformation protocol more efficient and Terra Nigra will test the year 2002 seedlings in an Indonesian company of choice, preferably Pt. Ciputri.
The INDOROSA and BIOTIC projects are quite similar in set up. Difference is that within this project we would have tested transgenics in Indonesia (field tests), if current regulation would permit us to do so.
Thus, the project deliverables will be new, adapted rose cultivars. Future products will be transgenic rose with improved growth habit.
Dr. P.B. Visser joined the ‘match-making event’ in Jakarta, organised by Senter, to establish new contacts with the Indonesian private sector and to maintain old contacts related to BIOBREES and SENTER projects.

5.3.4 EARLYTOM

This project started 1-1-2001 and will end 31-12-2004. It concerns the introduction of resistance to early blight in tomato. Because of this very recent start, not many results can be reported here. One deliverable has already been produced however: a collection of possible sources of resistance, which has been field tested. This is important for the selection of the parents.
The role of RIV and EWINDO, the Indonesian partners in EARLYTOM, is mainly the testing of source material and cross progenies under local field conditions, and the collection and characterization of pathogen isolates. ENZA will perform interspecific crosses that require special techniques such as embryo culture.
The plant material and knowledge developed in the EARLYTOM project are available to all project partners. Through the link with EWINDO and RIV, both of which are involved in breeding varieties for the commercial market, Indonesian horticulture will benefit from the availability of resistant varieties.

5.3.5 TROPSHALLOT

This project aims at introducing resistance genes of the two most important fungal diseases of shallots: purple blotch and anthracnose as well as to develop a system using true shallot seeds for the production of high quality planting material. It is believed that this combination will prove a breakthrough in the battle against the excessive fungicide use in shallots.

5.3.6 INDOSEED

This project started in 2001 and is scheduled until June 2004.
The INDOSEED project was jointly formulated with the Indonesian seed company PT Ewindo, the Dutch seed company ENZA, and with IPB, Laboratory for Seed Technology. With these active partners, many activities are now ongoing. RIV agreed to carry out a number of essential tasks as well. For a combined report see 4.9 Seed-borne diseases of vegetables.

5.3.7 PROCULT

This project is anticipated to start January 2002. It concerns the development of plastic tunnel greenhouses for the tropical lowland. An important technology to be introduced is the use of plastic foils with selective filters, which would enable keeping the climate in the tunnel relatively cool. Advantages of using tunnels are the possibility to create a more ideal climatological environment (lower humidity, no rainfall or storm damage, no inflying insects, no cross pollination, less accessible for theft, etc.). It also offers the possibilities for integrated crop management: the use of natural enemies of insects is much more effective in greenhouses than in the open, and if artificial media are being used, recycling of nutrients becomes an option as well.
Until now the absence of counter-financing for the institutional partners has been problematic.

5.4 Networking function

BIOBREES has developed into an extended network: apart from the direct project partners under BIOBREES proper, many others have joined through Senter or KNAW-projects. A list of the major partners is given in the text box.
BIOBREES has been instrumental in bringing these partners together: through a central research function, with its core activities in plant breeding and seed technology it formed the backbone of an increasing number of activities.
It is this continuity which made it possible to establish common goals, prepare proposals, start negotiations and make a start with additional projects. Many of the additional projects have their roots

in BIOBREES I, but only very few have started at that time (f.i. BIOPEP). The majority became realities during BIOBREES II, some have only just started (INDOSEED, TROPSHALLOT) or are about to start (PROCULT, PVP). It was in the confidence that support for these activities would continue in one form or another that these commitments were made.

The quality of the network is outstanding: during the past years this partnership has developed into a basis for mutual trust and understanding. For instance with the initiation of both BIOTRAIN and INDOSEED, one email was enough to get the necessary co-operation.

BIOBREES network participants	
Indonesia	*Netherlands*
AARD	ENZA
Agriom	Horizon
Asbindo	IMAG
BRUEC	Keygene
CRIH	KNAW
Ewindo	Leiden University
IBC (KBI)	Plant Research International
IPB – Agricultural University Bogor	Plasthill
Joro	Rovero Systems
Kebun Ciputri	SENTER
LIPI	Snoek
RIFCB (Balitbio)	STOAS
RIOP (Balithi)	Terra Nigra
RIV (Balitsa)	Wageningen University
Saung Mirwan	
VEDCA (PPPG-P)	
	(Alphabetical order)

The programme has brought many institutions together: public and private partners are now closely collaborating, private sector partners found counterparts in Indonesia or the Netherlands, and institutions in the Netherlands and Indonesia have established or renewed their contacts, also within their own country. A striking example of this is RIOP, the Research Institute for Ornamental Plants. When BIOBREES started in 1994, there were very few contacts between the Indonesian private sector and the breeding department of this institute; the institute did not develop varieties that were interesting for the market, they lacked the basic facilities and know-how for crop growing, and the plant breeding programme was very limited. During the various projects Dutch colleagues advised on setting up of better research facilities and a planned breeding schedule. The researchers were brought into contact with Indonesian and Dutch companies and saw the needs of that sector and learnt a lot about crop growing.
The programme has been very successful and when the first BIOBREES varieties started to appear, it became clear that the institute was right on track. Researchers learned to submit grant proposals and got them rewarded, including a grant to refurbish the Segunung site and to create new headquarters there. Now RIOP is being approached by companies which try to establish contacts. RIOP is in an upward spiral now, it is part of an important and growing agribusiness sector in Indonesia. This is to a large extent thanks to the excellent researchers who grabbed the chance that this collaboration offered.

The possibilities for expansion are endless. The restricting factor has always been the availability of funds; funds for counter-financing the Dutch contribution in private-private Senter projects, which was exhausted at a certain moment, or the financing on the Indonesian side, which limited the contributions of RIV, restricted the mobility of the researchers and resulted in a lack of good facilities in Indonesian labs. Any funding on that side, would help a great deal. A good exception to be mentioned in this context are the new labs of the Seed Laboratory of IPB, where a workshop could be held on very short notice thanks to the very good new facilities at the Darmaga campus (and the enthusiasm of its staff).

5.5 Overall spin-off

The overall impact of BIOBREES is a better relation between the two countries, more specifically between the two Ministries of Agriculture. It has undoubtedly kept the relations between the countries alive after the diplomatic incident of 1992. Since then, the Netherlands, and Wageningen have gained recognition as a place where a lot of expertise is available and which could be made useful for Indonesia.
The first and even more so the second phase have demonstrated that if one engages in real collaboration, that much can be achieved with a minimal amount of resources. No development project in the classical sense could have had such impact as BIOBREES in relation to the money involved.

6. Financial

BIOBREES II

The project spending has largely followed the agreed financing rhythm. There was one large exception, however. Because of the late start of the KNAW-funded BIORIN programme, EARLYTOM and hence the BIOBREES project which depends on the first (and vice versa), the funds have not been exhausted yet: the remaining funds have been removed from the BIOBREES budget and will remain still available for this project.

Public-private projects

Special target of BIOBREES II was to link up intensively with the private sector in order to make sure that the research results would become utilised in everyday Indonesian horticulture. The budget of BIOBREES was utilised to a maximum to engage in such partnerships. This helped stimulate the private sector to invest more than double the BIOBREES budget, and Plant Research International acquired an additional subsidy of about one-third its total budget. It must be mentioned here that the subsidy received from SENTER in the framework of TROPSHALLOT is not given here, because Plant Research International has made a special arrangement with the private sector partners on its contribution. BIOPEP started already during BIOBREES I, so part of this amount should be deducted. On the other hand, the subsidies given here have not all been received yet: they are allocated by SENTER, and will be made available depending on the continued execution of these projects. Nevertheless we can conclude that BIOBREES has been very successful in generating public-private projects and generating additional funding. The total budget has tripled (Dfl 4,2 mln became Dfl 13 mln) with the SENTER projects, BIOTRAIN (total budget Dfl 0.9 mln) and PVP/PBSI (Dfl 0.75 mln). The almost Dfl 2.5 mln of BIORIN (KNAW) comes on top of this.

Project	*Total project costs (Dfl)*	*Total subsidy (37,5%, Dfl)*	*Subsidy of DWK (BIOBREES) (Dfl)*
BIOPEP	739,811.00	277,429.00	170,634.00
BIOTIC	1,870,916.00	701,594.00	303,168.00
INDOROSA	1,978,810.00	742,054.00	364,800.00
EARLYTOM	460,697.00	172,761.00	128,736.00
TROPSHALLOT	813,625.00	305,109.00	0.00*
INDOSEED	1,595,949.00	598,481.00	278,782.00
PROCULT	879,526.00	329,822.00	110,236.00
Totaal	**8,339,334.00**	**3,127,250.00**	**1,356,356.00**

* Plant Research International is not a partner but a subcontractor in this project.
Source: SENTER, Dec. 2001

Annex I.
Publications and presentations

Annadana, S., M.J. Beekwilder, G. Kuiper, P.B. Visser, N. Outchkourov, A. Pereira, M. Udayakumar, J. de Jong & M.A. Jongsma, 2001b.
Cloning of the chrysanthemum *UEP1* promoter and comparative expression in leaves and ray and disc florets (♀) of *Dendranthema grandiflora*. Transgenic Res (in press).

Annadana, S., L. Mlynarova, M. Udayakumar, J. de Jong & J.P.H. Nap, 2001a.
The potato *Lhca3.St.1* promoter confers high and stable transgene expression in chrysanthemum, in contrast to CaMV35S-based promoters. Mol Breed (in press).

Annadana, S., N. Outchkourov, A. Schipper, M.J. Beekwilder, M. Udayakumar & M.A. Jongsma, 2001c.
Cysteine proteins inhibitors reduce fecundity in western flower thrips; Frankliniella occidentalis'. J. Insect Physiol. (in press).

Annadana, S., W. Rademaker, M. Ramanna, M. Udayakumar & J. de Jong, 2000.
Response of stem explants to screening and explant source as a basis for methodical advancing of regeneration protocols for chrysanthemum. Plant Cell Tissue Organ Cult, 62: 47-55.

Annadana, S.
Towards protease inhibitor mediated resistance to western flower thrips in chrysanthemum. Thesis Wageningen University and Research Centre. ISBN 90-5808-550-3. 126 pp.

Anwar, A., P.S. van der Zouwen & J.M. van der Wolf.
Bacterial canker (Clavibacter michiganensis subsp. michiganensis) of tomato in commercial seed produced in Indonesia. Plant Disease, submitted.

Bezerra, I.C., R. de O. Resende,. L. Pozzer, T. Nagata, R. Kormelink & A.C. de Ávila, 1999.
Increase of tospoviral diversity in Brazil, with the identification of two new tospovirus species, one from *Chrysanthemum* and another from Zucchini. Phytopathology, 89, 823-830.

Belfiore, B. & J.M. van der Wolf, 2002.
Didymella bryoniae, causal organism of stem and fruit rot in cucurbits. A literature study. 9 pages.

Chaerani, R & R.E. Voorrips.
A new test method for screening tomato accessions for resistance to early blight (*Alternaria solani*). Will be submitted to European Journal of Plant Pathology before the end of the project period.

Cortez, I., I.C. Livieratos, A. Derks, D. Peters & R. Kormelink, 1998.
Molecular and serological characterization of Iris yellow spot virus, a new and distinct tospovirus species. Phytopathology 88, 1276-1282.

Cortez, I., A. Aires, A.-M. Pereira, R. Goldbach, D. Peters & R. Kormelink, 2001.
Characterization and analysis of Iris yellow spot virus M RNA: Indications for functional homology between the tospoviral G(c) glycoprotein and those of the animal-infecting bunyaviruses. Manuscript in preparation.

Cortez, I., J. Saaijer, K.S. Wongjkaew, A.-M. Pereira, R. Goldbach, D. Peters & R. Kormelink, 2001.
Identification and characterization of a novel tospovirus species using a new RT-PCR approach. Archives of Virology 146, 265-278.

De Vries, D.P., L.A.M. Dubois, Darliah, A. Muharam & T. Sutater, 2000.
Breeding cut roses for the tropical highland. Biotechnol. & Biotechnol. Eq. 14/2.

Estiati, A., 2001.
Further development of a high quality molecular marker for indirect selection for downy mildew (*Peronospora destructor*) resistance in onion. Internal report Plant Research International, 15 pp.

Esselink, G.D., M.J.M. Smulders & B. Vosman, 2003.
Identification of cut rose (*Rosa hybrida*) and rootstock varieties using robust Sequence Tagged Microsatellite Site markers. Theor. Appl. Genet. 106: 277-286.

Galvan, G.A., W.A. Wietsma, S. Putrasemedja, A.H. Permadi & C. Kik, 1997.
Screening for resistance to anthracnose (*Colletotrichum gloeosporioides*) in Allium cepa and its wild relatives. Euphytica 95, 173-178.

Langerak, C.J. (Editor), 2001.
Management of seed health of important vegetable crops, 1st INDOSEED-BIOBREES workshop. Bogor, Indonesia, 20-24 August 2001. Workshop Manual. 71 pages.

Khrustaleva, L.I. & C. Kik, 1998.
Cytogenetical studies in the bridge-cross *Allium cepa* x (*A. fistulosum* x *A. roylei*). Theor. Appl. Genet. 96, 8-14.

Khrustaleva, L.I. & C. Kik, 2000.
Introgression of *Allium fistulosum* into *A. cepa* mediated by *A. roylei*. Theor. Appl. Genet. 100, 17-26.

Kik, C., 1999.
Breeding for resistance to *Colletotrichum* and *Alternaria* in tropical shallots. Seminar at RIFCB, April 1999.

Kik, C., 1999.
Spodoptera exigua in tropical shallots: transformation and marker-assisted breeding. Seminar at RIV, April 1999.

Kik, C., 2002.
Exploitation of wild relatives for the breeding of cultivated *Allium* species. In: H.D. Rabinowitch & L. Currah (Eds.). *Allium* Crop Science - Recent Advances. CABI International, Wallingford, UK. pp. 81-100.

Marwoto, B., Darliah, D.S. Badriah & K. Effendie, 2001.
Monograf Varietas Ungull; Krisan-Mawar-Anyelir-Gladiol. 20 pp. ISBN 979-8842-13-8, RIOP, Pacet Cianjur, Indonesia.

Pozzer, L., I. Bezerra, R. Kormelink, M. Prins, D. Peters, R. de O. Resende & A.C. de Ávila, 1999.
Characterization of a distinct tospovirus isolate of iris yellow spot virus associated with a disease in onion fields in Brazil. Plant Disease 83, 345-350.

Purwito, A., 2001.
Agrobacterium tumefaciens-mediated transformation in shallot (Allium cepa L.) and garlic (*Allium sativum* L.). Internal report Plant Research International, 15 pp.

Sanjaya, L., Y. Nöllen & R.E. Voorrips.
Screening *Capsicum* accessions for resistance to anthracnose (*Colletotrichum* spp.). Submitted to European Journal of Plant Pathology.

Sanjaya, L., 2002.
Resistance to Anthracnose (*Colletotrichum* spp.) in Pepper (*Capsicum* spp.). PhD thesis, Bogor Agricultural University.

Silva, M.S., C.R.F. Martins, I.C. Bezerra, T. Nagata, A.C. de Ávila & R. de O. Resende, 2001.
Sequence diversity of NSM movement protein of tospoviruses. Archives of Virology 146, 1267-1281.

Van der Wolf, J.M. (Editor), 2002.
Management of seed health of important vegetable crops, 2nd INDOSEED-BIOBREES workshop, Van der Wolf, J.M. (editor). Bogor, Indonesia, 7-11 October 2002. Workshop Manual. 93 pages.

Visser, P.B., L.C.P. Keizer, Th.P.M. van der Salm & J. de Jong, 2000.
Rootstocks transformed with rol a, b, c genes enhance rose flower production. Rose Conference 2000, 28 May-1 June, 2000, Kazanlak, Bulgaria.

Visser, P.B., L.C.P. Keizer, Th.P.M. van der Salm & J. de Jong, 2000.
Introduction of rol A,B,C genes in rootstocks enhances rose production. 3rd Int. Congress on Adventitious root formation, 27 june-1 July, 2000, Veldhoven, The Netherlands.

Visser, P.B., S. Annadana, G. Kuiper, A. Kuipers, N. Outchkourov, W.J. de Kogel, W.J. & M.A. Jongsma, 2001.
Application of cysteine protease inhibitors to protect chrysanthemum against thrips. Eucarpia Congress section Ornamentals July 3-6, 2001, Melle, Belgium.

Visser, P.B., L.C.P. Keizer, Th.P.M. van der Salm & J. de Jong, 2000.
Introduction of rol A,B,C genes in rootstocks enhances rose production. 3rd Int. Congress on Adventitious root formation, Eucarpia Congress section Ornamentals July 3-6, 2001, Melle, Belgium.

Voorrips, R.E., Y. Nöllen, L. Sanjaya & A.P.M. den Nijs.
Resistance to anthracnose (*Colletotrichum spp.*) in Capsicum. 25th International Horticultural Congress (IHC), Brussels, Belgium, 2-7 August 1998.

Voorrips, R.E., Y. Nöllen, L. Sanjaya & A.P.M. den Nijs.
Resistance to anthracnose (*Colletotrichum spp.*) in Capsicum. X EUCARPIA Meeting on Genetics and Breeding of *Capsicum* and Eggplant, Avignon, France, 7-11 September 1998.

Voorrips, R.E., L. Sanjaya, R. Groenwold & H.J. Finkers, 2001.
QTL mapping of anthracnose (*Colletotrichum spp.*) resistance in *Capsicum.* XI EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant, Antalya, Turkey.

Voorrips, R.E., L. Sanjaya, R. Groenwold, Chai Min & H.J. Finkers, 2002.
QTLs for anthracnose resistance in *Capsicum.* First BIORIN Workshop, Bogor, Indonesia.

Voorrips, R.E., R. Finkers, L. Sanjaya & R. Groenwold.
(In preparation): QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense.* (to be submitted to Theoretical and Applied Genetics).

Wietsma, W.A., G.H.J. Grubben, B. Henken, S.-J. Zheng, S. Putrasemedja, A.H. Permadi, E. Sofiari, F.A. Krens, E. Jacobsen & C. Kik, 1999.
Development of pest and disease resistant shallot cultivars by means of breeding and biotechnology. Indonesian Agricultural Research and Development Journal 20, 78-82.

Zheng, S.-J., 2000.
Towards onions and shallots (*Allium cepa*) resistant to beet armyworm (*Spodoptera exigua*) by transgenesis and conventional breeding. PhD thesis Wageningen University and Research Centre, The Netherlands.

Zheng, S.-J., B. Henken, E. Sofiari, E. Jacobsen, F.A. Krens & C. Kik, 1998.
Factors influencing induction, propagation and regeneration of mature zygotic embryo-derived callus from *Allium cepa*. Plant Cell Tissue and Organ Culture 53, 99-105.

Zheng, S.-J., B. Henken, E. Sofiari, E. Jacobsen, F.A. Krens &. C. Kik, 2001.
Molecular characterization of transgenic shallots (*Allium cepa* L.) by adaptor ligation PCR (AL-PCR) and sequencing of genomic DNA flanking T-DNA borders. Transgenic Research 10, 237-245.

Zheng, S.-J., B. Henken, E. Sofiari, P. Keizer, E. Jacobsen, C. Kik & F.A. Krens, 1999.
Effects of cytokinins and lines on plant regeneration from long-term callus and suspension cultures of *Allium cepa*. Euphytica 108, 83-90.

Zheng, S.-J., B. Henken, E. Sofiari, W.A. Wietsma, E. Jacobsen, F.A. Krens & C. Kik, 2000.
Screening for resistance to the beet armyworm (*Spodoptera exigua*) in *Allium cepa* and its wild relatives Euphytica 114, 77-85.

Zheng, S.-J., L.I. Khrustaleva, B. Henken, E. Sofiari, W.A. Wietsma, E. Jacobsen, C. Kik & F.A. Krens, 2001.
Agrobacterium tumefaciens mediated transformation of *Allium cepa* L.: the production of transgenic onions and shallots. Molecular Breeding 7, 101-115.

Annex II. Working visits

Dr. Prakosa, former Indonesian Minister of Agriculture, visited RIV on May 1, 2000. He was accompanied by Mr. W. Steemers, former agricultural counsellor, Netherlands Embassy, Jakarta, and Dr. W. de Wit, former Director of the Directorate Industry and Trade of the Dutch Ministry of Agriculture, Nature Management and Fisheries.

Dr. Muhammad A.S. Hikam, Indonesian Minister of State for Research and Technology, visited Plant Research International on May 26, 2000 with a large delegation.

Prof. Aman Wirakartakusumah, rector of IPB visited Wageningen UR and Plant Research International, 11 June 2001.

Prof.Dr.Ir. Bungaran Saragih, Indonesian Minister of Agriculture, visited Plant Research International 1 November 2001. He was accompanied by Dr. Sumarno, Director General of Horticultural Production, Dr.Ir. Rachmat Pambudy, Expert Staff of the Minister, Mr. Andriyono Kilat Adhi, Agricultural Counsellor in Brussels, and Mr. Baginda, Secretary to the Minister.

Staff exchange

1998

Dr. A.S. Duriat (director RIV) and Dr. Anggoro Hadi Permadi (head Breeding Dept.) visited Wageningen (Plant Research International) on 10 August 1998 for discussion of the vegetable research.

Dr. A. den Nijs (head Department Vegetable Crops) and Dr. G. Grubben (co-ordinator) visited Indonesia 12-23 September 1998 for discussion of the Working Plan of BIOBREES II and co-operation with the Indonesian Biotechnology Consortium (IBC).

Dr. R. Bino (head Dept. Reproduction Technology; acting head Dept. Ornamental Crops) and Dr. G. Grubben (co-ordinator) visited Indonesia 8-18 December 1998 for discussion of the Working Plan of BIOBREES II, for participation at a Broker's Event organised by SENTER in Jakarta, and co-operation with the Indonesian Biotechnology Consortium (IBC).

1999

Dr. Sugiono, deputy director of Research Institute for Food Crops Biotechnology RIFCB at Bogor visited Plant Research International in 1999 for co-operation in biotechnology projects e.g. Allium and tomato breeding.

Dr. Toto Sutater, director of RIOP, was at IAC in Wageningen 10-22 May 1999 as participant of the International Course on Variety Protection, and visited CPRO for discussion of the research projects on ornamental plants.

Dr. Satriyas Ilyas of IPB Bogor visited CPRO from 9 to 15 June 1999, to study possibilities for joint research.

Mrs. Ineu Sulastrini and Mrs. Lussi Ratnawati, RIV researchers, visited Plant Research International in the period 1 May – 1 August 1999 to obtain training in detection of seed-borne diseases.

Dr. C. Kik visited RIV (Lembang) and RIFCB (Bogor) for lectures and visit to the Tegal/Brebes area for shallot breeding and cultivation research. April 1999.

Dr. H. Löffler visited CRIH and RIOP from 1 to 9 May 1999 to discuss the research projects for chrysanthemum, roses and gladiolus, and general affairs. He had a meeting with the Indonesian Biotechnology Consortium to discuss the proposals for co-operative research to be funded by KNAW.

Dr. R.W. van den Bulk and Mr. W.J. van der Burg visited Indonesia from 13 to 21 November 1999, for co-ordination and discussions about seed technology.

2000

Mrs. Ridho Kurniati, junior scientist at RIOP, visited Plant Research International for a 6 month training period (January-June 2000) in the regeneration and transformation of roses.

Mrs. Lia Sanjaya (RIV, Lembang) visited Plant Research International four times during the last years, 24 months in total for her PhD-thesis research on anthracnose-resistance in hot pepper.

Ir. C.J. Barendrecht and Mr. H. Pluim-Mentz, PVP experts, visited Indonesia from 11 to 21 April 2000.

Dr. A.W. van Heusden, Mr. H. Blankestijn (STOAS) and Mrs. Dr. M. de Jeu (Wageningen University) visited IPB 18-25 June 2000 to discuss the BIOTRAIN project and to select the candidates for the TOT-part of the project.

Dr. G.J.H. Grubben and Mr. W.J. van der Burg visited Indonesia from 11 to 21 April 2000, for co-ordination and discussions with target groups.

Dr. P.B. Visser and Mr. W.J. van der Burg visited Jakarta, Bogor, Segunung and Lembang from 2-12 October 2000, for co-ordination and discussions about the ornamental and seed projects.

Dr. C.J. Langerak, Dr. J.M. van der Wolf visited Indonesia 1-12 October 2000 to collaborate with IPB, RIV and EWINDO in the framework of starting up BIOBREES seed technology and the Senter INDOSEED project.

2001

Mrs. Amy Estiati (LIPI) carried out a three month research at Plant Research International, 2001.

Mr. Agus Purwito (University of Bogor) carried out a three month research at Plant Research International, 2001.

Dr. Budi Marwoto attended the international PVP training course at IAC, Wageningen June 2001.

Mrs. Reni Chaerani (Research Institute of Food Crop Biotechnology) visits Plant Research International from May 2001 to February 2002; this is the first of four visits for her PhD-thesis research on early blight-resistance in tomato.

Dr. Budi Marwoto attended the International Course on Plant Variety protection at IAC Wageningen; funding was done though the Dutch side of the Agreement.

Dr. C.J. Langerak, Dr. J.M. van der Wolf and Mrs. P.S. van der Zouwen visited IPB to co-organise the workshop 'Management of Seed Health of some important vegetable crops', 20-24 August 2001. They also visited RIV and EWINDO at that occasion.

Dr. P. Visser and a director of a Dutch flower breeding company visited Indonesia to prepare a restart of BIOTIC.

2002

Dr. R.E. Voorrips visited Indonesia 16 Feb – 1 March 2002. He participated in the first BIORIN workshop at IPB Bogor, and in the 'Growing Together' workshop at Bandung. He also visited RIV for discussions on their co-operations on hot pepper and tomato.

Dr. A.J.P. Everaarts and Mr. W.J. van der Burg visited Indonesia for the Growing Together matchmaking & workshop organised by the Agricultural Bureau in Bandung, 28 & 29 February 2002. This meeting was also attended by many other Indonesian and Dutch officials and representatives from private companies.

Ir. C.M.M. van Winden, Mr. K. Fikkert and Dr. A.J. van Wijk visited Indonesia for a kick-off meeting of the PVP-project, 27 February 2002. The meeting was attended by many other Indonesian and Dutch officials and representatives from private companies.

Ir. N.P.A. van Marrewijk visited the PVP-trials at RIV in July 2002.

Dr. J.M. van der Wolf and Ms. Ing. P.S. van der Zouwen. 4 - 14 October 2003. '2nd INDOSEED-BIOBREES Workshop on Management of Seed Health of Important vegetable Crops', Bogor, Indonesia.

Ir. W.J. van der Burg visited Indonesia for co-ordination purposes from 21 - 25 October 2002.

Ir. C.J. Barendrecht visited the PVP-trials at RIOP in November 2002.

Mr. Aswaldi Anwar (MSc student of IPB in Bogor) worked in the labs of PRI during 27 June 2002 - 23 December 2002.

Annex III. Glossary

Acronyms and abbreviations used

AARD[1]	Agency for Agricultural Research and Development (Badan Penelitian dan Pengembangan Pertanian)
BIOBREES	Biotechnology, plant breeding and seed technology collaboration Indonesia - Netherlands
BIORIN	Academic research collaboration Indonesia - Netherlands
BIOTRAIN	Developing biotechnology skills
BIT	A subsidy provided by Senter
BPTP	Agricultural Technology Assessment Institutes (Balai Pengkajian Teknologi Pertanian)
BRUEC	Biotechnology Research Unit for Estate crops
CRIH	Central Research Institute for Horticulture and Miscellaneous Crops
GoI	Government of Indonesia
IPB	Agricultural University Bogor (Institute Pertanian Bogor)
KIAT	Intellectual Property and Technology Transfer Office (Kekayaan Intelektuel dan Ahli Teknologi)
KNAW	Royal Dutch Academy of Sciences
LIPI	Indonesian Institute of Sciences
MoA	Ministry of Agriculture
PRI	Plant Research International
PVP	Plant Variety Protection (*i.e.* Plant Breeders' Rights)
RIF	Research Institute for Fruits (Balitbu and now called IFRURI)
RIFCB	Research Institute for Food Crop Biotechnology (Balitbio)
RIFFC	Research Institute for Food Crops
RIOP	Research Institute for Ornamental Crops (Balithi and now called IOPRI)
RIV	Research Institute for Vegetables (Balitsa and now called IVEGRI)
Senter	Funding agency of the Netherlands Ministry of Economic Affairs
UPOV	International union for plant variety protection

1 The names of the institutes of AARD are given als valid in 2002; they have changed names in 2003.