Adapting clonally propagated crops to climatic and commercial changes

Annual Report 2012

INEA:
The International Network for Edible Aroids

www.EdibleAroids.org
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1. Summary and context of the Action: INEA in 2012

The present Action (short name: INEA) brings together a global team of scientists, working directly with local growers, to develop a model for the adaptation of clonally propagated crops to climatic and commercial changes. To produce plants adapted to new environments - climate change, pest and disease outbreaks, market needs - it is necessary to broaden the genetic base. To do that successfully, requires cooperation between countries, the use of modern biotechnologies, and development of a network of scientists exchanging information and germplasm. INEA uses taro (Colocasia esculenta) and cocoyam (Xanthosoma sagittifolium) to construct a climatic adaptation model which can be transferable to other clonally propagated plant species. INEA main activities are:

- International network sharing resources and helping farmers with participatory breeding.
- International distribution of selected in vitro clones (virus indexed) and true taro seeds.
- Selection of genotypes from diverse crop gene pools and adapted to local conditions.
- National distribution of elite cultivars to smallholders.
- Participatory breeding for wide environmental and cropping systems adaptability.
- Characterization of physico-chemical properties and potential uses.

The work plan of INEA is broken down into eight work packages (WP). The main results obtained in 2012 are presented hereafter:

WP1: Project management and scientific coordination

The 2011 financial report of the project has been rejected by the EU because it is showing a large gap for fund justification due to the absence of financial reporting or un-reported co-financing. In 2012, the INEA partners have supported 100% of the project total costs and this had a major impact on their work. Up to now, the report has not been accepted and the second transfer of funds has not been implemented. The second annual meeting could not be organised in 2012. Many samples which were planned to be sent from the partners in the “South” to laboratories in the “North” could not be sent due to the absence of funds, and this is affecting the work programme of the project. It is hoped that the review of the project which will be conducted in February 2013, will find a solution to the financial management problem.

Scientific coordination was eased by the project web site where news, photos and reports are posted on a regular basis. A monitoring form has been devised for quarterly assessment of activities. INEA has received considerable international recognition since the programme began. In addition to requests to attend conferences and to submit reviews in refereed journals, several non-INEA countries have requested germplasm (Haiti, Gadeloupe, Cameroun, Mauritius, Bangladesh).

Most of the requests are from countries where Taro Leaf Blight has been devastating. Following a demand from Nigeria regarding the Phytophthora colocasiae epidemic in West Africa, Dr GVH Jackson, a plant pathologist, visited INEA partners in Umudike, and participated to the Symposium of the International Society for Tropical Root Crops (appendix 1). INEA was a co-organiser of the Global Conference on Aroids held in CTCRI, Bhubaneswar, India in January 2012. Six communications were presented by five INEA partners. The scientific coordinator of INEA (Dr V. Lebot) reviewed the PhD thesis of T. Mabhaudhi entitled “Drought tolerance and water use of selected South African landraces of taro (Colocasia esculenta) and bambara groundnut (Vigna subterranea)”, successfully defended at the University of Natal in South Africa in November 2012. A short list of morphological descriptors was developed and agreed among partners for Colocasia and Xanthosoma.

WP2: In vitro propagation of cultivars for international distribution

SPC developed a very efficient system for distribution of plants in plastic pouches and contamination rates were extremely low, and accordingly transfers to soil were highly successful. All transfers have been done according to the guidelines of the International Treaty on Plant Genetic Resources of the FAO using the Standard Mutual Transfer Agreement (SMTA). Demands from non participating countries came to INEA. SPC has propagated varieties in vitro for distribution. Plants have already...
been sent to Haiti and Cameroun. Batches are ready to be sent to Bangladesh, Mauritius and Guadeloupe and shipments will go as soon as the SMTAs are signed.

WP3: Breeding and on-farm participatory selection and evaluation
Breeding protocols have been agreed for Alocasia and Colocasia. The overall objective of the breeding programme is to produce different varieties tolerant to TLB, with drought resistance and good quality corms, to exchange them internationally and to distribute them to farmers to make adaptation to climatic changes possible. Vanuatu and Samoa are presently developing protocols for other countries to follow where there is breeding expertise. For those where expertise is not present a different strategy will be used. Seeds from the Vanuatu programme will be sent to partners. Seeds have already been sent to South Africa where a few local selections have been made. In Vanuatu, controlled crosses have been made in 2011 and 18 full-sib families have been produced and seedlings planted in February 2012 on VARTC research station in Santo. A total number of 2040 hybrids have been evaluated in their seminal generation. They have been replanted with head sets in Dec 2012 in their first clonal generation (C1). They will be harvested and replanted again in July 2013.

WP4: DNA fingerprinting of cultivars and heritability studies
Renan Traoré, a PhD candidate from Burkina Faso, visited CIRAD, Montpellier, France and tested 64 primer pairs of SSR. Among these, 58 are from Colocasia esculenta and six are from Amorphophallus paeoniifolius. Amorphophallus markers were tested to study their transferability on the Xanthosoma sp. DNA was extracted from 379 taro accessions and these were fingerprinted with 11 informative SSRs. All selected markers are from C. esculenta. Finally, statistical analyses were made on 354 accessions due to the poor quality DNA extracted from 25 of them. Unfortunately, the transferability test of the taro SSR markers on the Xanthosoma accessions did not give interesting results. Either there was no amplification or there was no polymorphism. These results are very useful because they reveal that many duplicates exist in some of INEA partners collections. It is recommended to avoid crossing parents presenting narrow genetic distances. A PhD candidate from the University of Montpellier, France (Laurent Soulard) arrived in Vanuatu in September 2012 and participated to the characterization and harvest of the family heritability trial established in VARTC, Santo. More than 2000 leaf samples have been prepared for DNA extraction. L. Soulard’s research will focus on QTL (Quantitative Trait Markers) and mapping analysis for traits related to corm quality.

WP5: Drought resistance of elite cultivars and seedlings
In Madeira, Portugal, the acclimation and growth of the varieties obtained from SPC and INEA partners has been successful. A preliminary assessment of drought tolerance was conducted, with the aim of modulate stress conditions, and test the suitability of several morpho-agronomic traits for stress evaluation. In this experiment, four varieties were used, in a total of 37 plants grown in pots. The obtained data are now being treated, using statistical tools. Preliminary data showed deficiencies in the irrigation model used, and with the methodology for parameters measurements. Data are being treated to see if it is possible to observe differences in the performance of the tested varieties.

WP6: Physico-chemical characterization of corms
A PhD candidate from the University of Slovenia (Andrej Mergudus) arrived in Vanuatu in November 2011 and established experiments to study the chemical variation between varieties, and following the age of the plant (from 4 to 14 months after plantation), in an attempt to study the corm quality variation. More than 400 freeze dried samples have been prepared in 2012 and sent to the University of Maribor for chemical analyses. The will be analysed for major compounds (starch, proteins, minerals, fibres, sugars) as well as for secondary metabolites (anthocyanins, carotenoids, tannins).

WP7: Virus detection and identification
Corms of taro obtained from CTCRI were collected and grown to mature taro plants at DSMZ. Dasheen mosaic virus was confirmed on a number of plants showing typical symptoms of DMV
infection. Partial sequence analysis showed considerable diversity among DMV isolates from taro which is significant for the development of virus diagnostic protocols and reagents. The complete genome of DsMV-T was sequenced and the genome assembled from sequence fragments obtained from RT-PCR. Leave homogenates from plants suspicious of CBDV infection (bobone and alomae) from the Solomon Islands were inoculated to a series of indicator plants for rhabdoviruses. DsRNA extraction and subsequent cloning of random PCR fragments generated revealed the presence of sequences of a putative tenuivirus.

**WP8: On-farm participatory selection of elite cultivars and hybrids**

In South-Africa, pre-sprouted corms of 22 lines were planted in the three evaluation trials and headsets of nine lines (Vanuatu seedlings and local lines) were planted in eight demonstration gardens. All demonstrations and trials were planted in a complete random design. The study was conducted with farmers groups to learn more about amadumbe (taro) production problems, perception of amadumbe cultivars and criteria in selection of cultivar. The work was done in five villages in KwaZulu Natal province of South Africa that differ in terms of ethnicity, geography and climate. There was no diversity in the villages in terms of uses and production but only change in status, as a result people felt that there were not that important anymore compare to other crops, it was for poor households. In all villages, data were collected with participatory methods. The techniques participatory rural appraisals (PRA) were used. Namely: Semi-structured interviews, Group interviews, Observation, Seasonal calendars, and Ranking. The results indicate that it is recommended to plant future trials in more “secure” localities. It was also decided to plant the future demonstration trials more concentrated geographically due to financial and time constraints. In other INEA countries, because of serious financial constraints, the work focused on field propagation of the planting material.
2. Activities carried out in 2012

WP 1: Project management activities

The foreseen deliverables of WP1 are:

1. Development of a website and platform to support INEA.
2. Fulfilment of financial, administrative and coordination obligations.
3. Production and circulation of annual reports.
4. Monitoring of project activities.

WP 1.1: Management activities

SPC is the contact person for the European Commission and is responsible for the day-to-day financial management of the project. SPC is supported by the Administrative and Financial Services of his institution (officer in charge: Mrs Sushil Narayan assisted by Nitesh Prasad and Romika Dayal). Dr Mary Taylor who was the lead officer on this project for SPC completed her contract in April 2012 and returned to the UK. In 2012, the project encountered serious financial reporting problems. The report for 2011 has been rejected on several occasions, delaying the second transfer of funds to all partners. Hereafter, is a brief recapitulation of the various attempts made by SPC during the year 2012 to produce a consolidated financial report for the first year of the project (2011).

In June 19th 2012, SPC (Mrs Sushil Narayan) informed the Senior Policy Officer, Agricultural Research for Development of the Europaid Development and Co-operation Directorate General (Dr David Radcliffe):

“... SPC is facing problems securing the funds for the 2nd year, as our financial reporting is not in line with the requirements put in by your office in Brussels. I understand from our finance department that they can only report on funds that SPC has received from the EU and funds that SPC has sent to partners. We cannot report or be responsible for the contribution to the project by third party. This seems to have reached a stalemate which is affecting the implementation of the project. Hope you can discuss this with your finance office and help us sort out the problem.”

The same day, the EU Office (Dr. David Radcliffe), replied that as a project coordinator SPC has an obligation to report on all the funds received for this project from the European Commission and asked to what contributions from third parties SPC was referring to. SPC (S. Narayan) responded on July 23rd with a clarification:

“... this project is for around 6 million Euro, of which EU provides 3m euro and the balance is contribution from the partners, like full time staff that is paid by the implementing organization but contributes to the project. When we prepared the accounts the partners have indicated they have 100% met their obligation and that is what we reported. We are not in a position to ask them to provide proof of their financial contribution because we did not give them that money. The technical report of the projects should be sufficient to give us an indication that the partners have kept their part of the bargain. What SPC is responsible is to provide financials for the funds that we received from EU and gave to the partners. The revised account is attached. The other issue is that the partners from the south are not acquitting on time and they are given very small sums of money. Because of their non acquittal the other partners suffer as well. Since the project has 19 partners, it is getting very difficult to get acquittal from all partners. Attached is the best we can do with the acquittals that have been received so far.”

The EU Office (Mr Philippe Thevenoux) explained to SPC on August 18th:

“1) SPC should report on the progress of expenses planned in their contract budget (5,991,186 EUR to be spent over a 5 years period, of which indicatively 1.374.157 planned for Year 1), including the co-funding of the partners contributing to the implementation of the project.
2) All expenses should be reported on a real cost basis (EU funds as well as co-fundings), using the format of their sub-contract regarding their component of the project, but original accounting documents should remain in each center. It is not possible to have different financial requirements for the South centers, nor any "lumpsum" calculation basis. It should be also noted that though the yearly EU-funded contribution to each center in the South is around 24 000 Euro, over the 5 years, this globally represents 1,5 million Euro for the 15 centers, or 3 million together with the co-financing of the centers, that is half of the total budget of the action.

I copy all possibly relevant persons in the Finance and Contract Unit, Head of Unit, Head of Sector and relevant members of staff, to allow them to react (upon their return from leave) in case any other interpretation would be acceptable. In case complying with the standard EU requirements for transparent financial reporting would prove to be impossible, we would like to hear your suggestions and discuss with you the possibilities to continue the project or to phase out our contribution.”

SPC (S. Narayan) explained (Aug 19th) to the EU that some partners were not responding and that was unfairly affecting everyone else. The EU (Ph. Thevenoux) replied (Aug 20th) that SPC should be in a position to report on more than 70% of the first year advance consumption. A new consolidated report was therefore prepared and sent to the EU on Aug 30th. This report was, unfortunately, rejected again on Aug 31st because it was still showing a large gap for fund justification due to the absence of financial reporting or un-reported co-financing. At that time, seven partners did not presented any acquittals, including two from the “North” and five from the “South”. Following a series of messages sent by INEA scientific coordinator (Dr Vincent Lebot) to the seven partners who failed to provide their acquittals, they all responded except two from the South. In Nov 11th, a new financial report was sent by SPC, including figures from all partners (except Kenya and Madagascar) and exceeding 70% expenditure rate. This new report was rejected again by the EU on Dec. 19th. A list of errors to correct in the report was provided by Mr. Ph Thevenoux and comments were made:

“We request you to correct all errors mentioned above and any other mistake you might identify from checking the financial records, supporting documentation and registers provided by all partners of the action. Please be aware that the above identification of errors might not be exhaustive, as only a (full scope) audit of the accounts of the action could pretend to be exhaustive. Should the analysis of your revised financial report give indication that some expenses are not properly reported or that the amount claimed might not correspond to the reality of the facts, we might decide to launch an independent verification of expenses, by a professional Audit firm.”

Finally, on January 1st 2013, the Head of Section (Dr JP Halkin) at EuropeAid wrote a letter to the Director General of SPC (Dr J. Rodgers), to explain the situation further:

“Considering the possible consequences of the project accounting and financial management weaknesses, we urge you to take all the necessary measures to put this scientifically and financially important project back on track, and inform us of your plans at your earliest convenience. We make use of this opportunity to inform you that an independent result oriented monitoring (ROM) assessment will be carried out in February 2013 on behalf of the European Commission. The EU will use this report to possibly decide on actions.”

In the mean time, CIRAD offered to SPC to produce a consolidated report on her behalf and requested all the acquittals to be sent to Montpellier for this purpose. These financial difficulties are very heavy for most partners as they have been supporting in 2012 100% of the total project costs. In many countries staff has been employed, four PhD scholarships have been funded and salaries have to be paid every month. Many partners are now disappointed and the project is loosing momentum. The ROM assessment will start in start on Feb 11th in Suva and will visit CIRAD in Port-Vila, VARTC in Santo, NARI in Lae, PNG, and CIRAD in Montpellier, France. It is hoped that solutions will be found.
WP 1.2. Scientific coordination

CIRAD is responsible for the day-to-day scientific coordination of the project, for the circulation of information, the collation of scientific annual reports from the partners and the production of reports detailing the successes of the project. Scientific progress is monitored regularly to avoid partners failing to deliver their project inputs. This is the role of CIRAD, the co-ordinator, and the lead contractors for each Work Package (WP). The project activities are structured in eight WPs with a lead contractor nominated for the detailed co-ordination, planning, monitoring and reporting of each activity. Regularly, each lead contractor submits to the scientific coordinator a short report on progress made, problems and solutions or alternatives:

WP 1: Dr. Vincent Lebot, CIRAD. lebot@cirad.fr
WP 2: Valérie Tuia, Val@spc.int
WP 3: Pr. Anton Ivancic, anton.ivancic@uni-mb.si
WP 4: Dr. Marie-France Duval, marie-france.duval@cirad.fr
WP 5: Pr. Miguel Carvalho, quercus@uma.pt
WP 6: Pr Janja Kristl, janja.kristl@uni-mb.si
WP 7: Dr. Stephan Winter, stephan.winter@jki.bund.de
WP 8: Dr. Danny Hunter, d.hunter@cgiar.org

Except for WP1 and WP2, all WPs will produce university degrees: two MScs and five PhDs. Information flows is both, top-down and bottom-up, aiming at ensuring maximum transparency for all partners involved and maximising synergies between them during the five years of the project. All information (i.e. meetings minutes, publications, reports. etc.) are communicated to the scientific coordinator who is responsible for dispatching this information to the various project stakeholders. Much of this information is placed on the website.

Communication with partners outside the partnership (i.e. local authorities, private sector...) is done via the web site (www.EdibleAroids.org). This website has being developed by a professional webmaster (Dr. GVH Jackson from PestNet) hired by SPC and based in Sydney, Australia. The visit of this website allows a follow up of project activities. The site contains a great deal of information about the network and its partners, the eight work packages (projects), reports, references, and photos illustrating activities. Alerts are given out via INEA News. In 2012, TaroPest, and interactive diagnostic “tool” for taro pests and diseases, produced by Queensland University of Technology, with ACIAR support has been added to the site. If partners contribute illustrated information on their pests and diseases TaroPest can be expanded. As it is, images of INEA’s activities can be found at the Photo Gallery.

Compared to the foreseen activities, a few delays have occurred due to financial difficulties described in WP.1.1. For example, the second annual meeting of the project was planned to be hosted during the “Global Conference on Aroids” held in Bhubaneswar, Orissa, India, in January 23-25th 2012. This conference was jointly organised by CTCRI (India), the Indian Society for Root Crops, INEA, the Indian Council of Agricultural Research and the State of Orissa. Unfortunately, because of the financial constraints, only five INEA partners were able to attend. The two papers presented by V. Lebot et al. are in appendix 2.

Dr Joseph Onyeka, Head, Plant Pathology & Microbial Biotechnology, National Root Crops Research Institute (NRCRI), Umudike, requested INEA to send a specialist on taro leaf blight to evaluate the situation in Nigeria, especially the reaction of the SPC varieties and to discuss the programme at Umudike to overcome the disease. Dr Graham Jackson visited on 26 September 2012 as part of a visit to attend the 16th symposium of the International Society of Tropical Root Crops at Abeokuta. Dr Jackson’s trip report is in Appendix 1. An exciting outcome of the visit was the identification of a
variety of *Xanthosoma* that appears to have resistance to root rot disease caused by the oomycete *Pythium*. This disease is a limiting factor on *Xanthosoma* cultivation worldwide. Nigeria has agreed to share the variety with INEA. It will be sent to SPC, pathogen indexed, and then distributed to Vanuatu, Costa Rica and other countries to assess its potential in breeding programmes to overcome root rot disease.

**WP 2: In vitro propagation of cultivars for international distribution**

The foreseen deliverables of WP2 are:

5. International distribution of 50 selected genotypes to all country partners.
6. Field propagation of introduced genotypes, evaluation, comparison with local ones.
7. Distribution of 30 selected genotypes to 10 villages per country (five farmers per village).

SPC maintains a collection of some 850 accessions of taro *in vitro* and has established an effective and efficient multiplication protocol to facilitate distribution of taro. For each partner, SPC has assembled a core sample composed of 50 genotypes representing high genetic diversity (genotypes from different geographic origins). These genotypes are from different Asian and Pacific countries and include some improved hybrids developed by SPC breeding programme in Western Samoa over the last ten years. These elite accessions are preserved *in vitro* in the *Centre for Pacific Crops and Trees* (CePaCT) in SPC, Suva (Fiji). They have already been DNA fingerprinted and their genetic distances are known.

Not less than 100 different genotypes have been propagated in vitro and in 2011, they were distributed to 15 INEA members. They represent a total of 3,180 tubes containing 5,647 plantlets. Overall, in 2011, countries received 50 varieties (150 clones per country). The detailed list can be found in 2011 annual report. Germany received genotypes for viruses studies and Portugal for drought stress tolerance experiments. Different countries received different genotypes but a set of improved hybrids and selected elite cultivars were sent to all 15 partners. In all cases, the aim was to distribute as much allelic diversity as possible to make sure that partners would broaden their germplasm genetic bases with the introduction of these varieties. The idea was also to introduce TLB tolerance/resistance and good quality (taste). The project is in a good position to offer resistant varieties to the African countries which have been struck by the TLB epidemic over the last five years.

In 2012, several partners provided photographs of their nurseries and/or propagation plots and these have been posted on the website ([www.EdibleAroids.org](http://www.EdibleAroids.org)). Most partners acknowledged the quality of the vitroplantlets received and the efficient professional service provided by SPC. Partners are now propagating these genotypes in the field and using rapid multiplication techniques to bulk the material on station. These introduced genotypes will be evaluated and compared to the local ones. After evaluation, 30 genotypes, corresponding to a combination of elite local and introduced genotypes will be selected and propagated for distribution to farmers. Hopefully, by month 24, the first selected genotypes (30 per country) will be planted in farmers’ fields. First results are expected by months 30-36 (in some countries, the maturity period is more than 8 months).

Unfortunately, in 2012, the financial constraints faced by all partners have severely delayed the field propagation work as many partners were not able to hire temporary labour or agricultural inputs (herbicides for ex).
**WP 3: Breeding and on-farm participatory selection and evaluation**

The foreseen deliverables of WP3 are:

8. Controlled crosses conducted in each participating country.
9. Hybrid seeds generated and F1 hybrids raised.
10. Hybrid clones distributed to five farmers in 10 villages (total of 50 farmers per country).
11. One PhD defended on taro breeding in South Africa (Mr WS Jansen van Rensburg).

In February 2012, an heritability trial has been established on VARTC station in Santo, to attempt to understand full sib families heritability of major agronomic traits. The ultimate objective is to identify important traits of high heritability. As taro is improved by crossing parents selected on their value per se, it is important to know how heritable are the traits of interest before conducting the controlled crosses. The field layout is composed of incomplete fully randomized blocks. The experimental unit is a family plot of 30 individuals and the spacing is 1x1m.

**Table 1.** Full sib families planted Feb. 15th at VARTC (Identity of the parents used in the cross, total no. of hybrids obtained per family, no planted per family, no of plots of 30 hybrids per plot).

<table>
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<tr>
<th>Crosses</th>
<th>Family no code</th>
<th>Total hybrids per family</th>
<th>No of plots with 30 plants</th>
<th>No of plots</th>
<th>Reps in Blocks (plots)</th>
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Experimental field design with incomplete randomized blocks

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<tr>
<td>C</td>
<td>21</td>
<td>31</td>
<td>32</td>
<td>33</td>
<td>34</td>
<td>35</td>
<td>36</td>
<td>37</td>
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<td>33</td>
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<td>D</td>
<td>31</td>
<td>32</td>
<td>33</td>
<td>34</td>
<td>35</td>
<td>36</td>
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<td>9</td>
<td>2</td>
<td>42</td>
<td>39</td>
<td>39</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>34</td>
<td>35</td>
<td>36</td>
<td>31</td>
<td>32</td>
<td>35</td>
<td>32</td>
<td>31</td>
<td>32</td>
<td>45</td>
<td>55</td>
<td>40</td>
<td>41</td>
</tr>
<tr>
<td>F</td>
<td>32</td>
<td>31</td>
<td>35</td>
<td>34</td>
<td>35</td>
<td>33</td>
<td>38</td>
<td>37</td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Within each plot, each plant is numbered to allow identification.
In F1, all plants have been morphologically characterised for **quantitative** and **qualitative** traits:

- No of stolons
- No of suckers
- Total height of the plant
- Length of longest lamina
- No of inflorescences
- Weight of the corm
- Lamina shape
- Edge of the lamina
- Colour of the lamina
- Colour of the joint of the nerves on the lamina
- Petiole colour
- Petiole variation
- Shape of the corm
- Colour of the corm
- Quality of the corm

The whole experiment has been harvested in November 2012 and the first clonal generation (C1) has been planted, following the same field design, using calibrated headsets, in December 2012.
Figure 1. For each family, the mean values of the total no of suckers (rejets) per plant, no of stolons (runners), no of inflorescences, length of longest lamina (longueur) and height of the plant (hauteur) are represented in different colours.

The corm yield of the taro plant is correlated to the weight of the propagule planted and genotype performance is therefore, difficult to assess at an early stage, especially in F1 when plants are seedlings. It is necessary to reach a propagule weight comparable to those used in traditional cultivation before an accurate assessment can be made. The screening of large populations for major traits is consequently, a laborious and expensive operation which leads taro breeders to develop visual tools to speed up the process. The development of a selection index which would take into consideration the vegetative attributes of a genotype and which could indicate the final yield potential of that particular genotype after years of vegetative propagation, would present useful practical implications. This type of index could allow breeders to eliminate rapidly numerous individuals with poor potential. A vegetative growth index (VGI), which takes into consideration the leaf area of the plant, was therefore computed. This area is estimated from the longest leaf length, knowing that there is no significant variation for the number of leaves per main stem (the one from which the corm yield is measured) between dasheen type varieties. This number varies from four to six, depending if the oldest leaf is senescing and/or if the younger leaf is fully open. All varieties were assumed to have at least five functional leaves per main stem with a lamina length on average equal to 1.4 of the lamina width. The following formula was used to compute the VGI: \[ VGI = \left( \frac{\text{leaf length}}{1.4} \times \text{leaf length} \right) \times 5 \times h / 100 \] – (suckers + stolons)². VGI was correlated with the mean corm yield.
**Figure 2.** The VGI is positively and highly correlated with corm yield. The best taro genotypes present a tall plant with large leaves, no stolon and few suckers per plant.

This architecture of the plant has the highest potential to produce a high corm yield in dasheen type varieties. The VGI appears to be a useful selection index to assess numerous genotypes in their early generations because it is based on the simple measurements of only four vegetative traits and is therefore easy to handle and compute: height of the plant, length of the longest lamina, no of suckers and no of stolons. In the future, it might be useful to determine if it is necessary to take into consideration the no of inflorescences.

**WP 4: DNA fingerprinting of cultivars and heritability studies**

The foreseen deliverables of WP4 are:
12. DNA fingerprints of elite cultivars used as parents in crosses.
13. Genetic distances determined between parents.
14. Segregation of molecular markers studied within and between seven full-sib progenies.
15. Markers associated to major genes, if any, indentified.
16. First heritability trial established and harvested (on major compounds related to quality).
17. Second heritability trial established and harvested.
18. Potential major genes controlling corm quality identified (e.g. amylose, starch, sugars).
19. One PhD defended on the genetic diversity of taro (Mr Renan Traoré from Burkina Faso)
20. One PhD defended on genetic studies between markers and chemical characteristics.
21. Four papers published in international journals.
The genetic diversity study represents part of the requested fulfilments for the PhD thesis programme of a Burkinabé scientist, Mr Renan Traoré (Université de Ouagadougou). In January 2012, Mr Renan Traoré started his work in CIRAD in Montpellier, France, and screened accessions received from partners in the “South” with a set of 11 SSRs markers to assess their genetic diversity. The aim of his work is to assist local breeding programmes and to determine if the cultivars selected locally exhibit enough genetic diversity to be used as different parents.

Figure 1. Dendrogram constructed for Indian accessions with 11 SSR markers indicating significant allelic diversity.

India hosts tremendous genetic diversity. It is hypothesised, based on the number of related wild *Colocasia* species growing there, that the area between West Bengal and Bangladesh is the area of origin of *C. esculenta*. Taro has over the centuries, been distributed very far from its area of origin, often under the form of clones. The subsequent selection of local morphotypes adapted to local conditions, has generated many landraces which often share a very narrow genetic base. This has already been well documented for the Pacific but data was still missing for other countries. The
dendrograms, hereafter, indicate that in some countries, many duplicates (at the SSR level) exist, although they may present different morphotypes.

Figure 2. The first dendrogram represents the genetic diversity revealed in South Africa with eleven SSR markers, the second dendrogram represents the diversity in Ghana. In both countries, there are many duplicates, probably landraces known under different vernacular names, or different morphotypes resulting from the selection of sports, somatic mutants.

Analysis of the full-sib progenies for segregation of quality traits started in February 2012 at VARTC (Vanuatu). Leaf samples of eight full-sib progenies sharing at least two parents have been sent to CIRAD. Laurent Soulard is now analysing all samples with available SSR markers in order to confirm the crosses. Corms of F₁ hybrids and full-sib progenies fingerprinted will be analysed for their
physico-chemical characteristics when mature (see WP 6). Overall, approx. 800 hybrids will be fingerprinted with markers.

**WP 5: Drought resistance of elite cultivars and seedlings**

The foreseen deliverables of WP5 are:

22. Field assays for drought tolerance studies established.
23. Morpho-agronomic traits associated with drought tolerance identified and evaluated.
24. Physiological and biochemical markers analysed and correlation studies completed.
25. Chemical analysis of corms from drought stressed plants or drought tolerant, analyzed.
26. Association of different traits and markers with drought tolerance studied.
27. One MSc defended on taro drought tolerance.
28. Two papers published in international journals.

The University of Madeira is evaluating drought tolerance of cultivars and their seedlings under controlled experimental conditions and field trials in Madeira (Portugal). The evaluation of drought tolerance is performed using morphological, physiological, biochemical and molecular features, biomass tests, yield and photosynthetic rates, and aims at identifying agronomic, physiological and molecular traits correlated with drought tolerance. The study is conducted in Europe with clones of elite cultivars received from all country partners through SPC, and compared with cultivars obtained among local (Madeira and Canaries) germplasm, to identify possible differences between elite cultivars and local taro gene pool. The University of Madeira is also advising all country partners on the development of suitable protocols for screening and characterising drought tolerance.

Pilot assays will be performed to screen different genotypes in relation to drought tolerance through the evaluation of major agronomic, physiological and biochemical traits. The results give a useful estimate of the variation in drought tolerance among taro cultivars. As result of this screening we expect to identify the genotypes showing improved drought tolerance, reducing the number of accessions to a smaller more manageable number. A second series of experiments will be performed on a smaller number of plants of cultivars and seedlings showing enhanced drought tolerance. It is expected that our knowledge about taro drought tolerance and its variation will improve, especially in ways to create a model of drought stress in taro and identification of useful traits, that using adequate protocols can assist farmers and stakeholders to select cultivars adapted this major treat imposed by climate changes.

During 2012, the primary goal of the ISOPlexis team, University of Madeira, was the acclimation and growth of the taro lines/varieties obtained from INEA partners, through SPC, the absence of a model of drought stress for taro determining the stress conditions determined also the need to realize a preliminary pilot assay. So steps were taken to do a preliminary assessment, with the aim to modulate stress conditions, and test the suitability of several morpho-agronomic parameters for stress screening. To achieve these goals, the pilot assay, a small scale experiment, was set up, during 180 days (6 months), using three local varieties, for which there were enough plants available, as well as one variety from the Canary Islands. Twelve morpho-agronomic parameters were selected and tested in this experiment.

In this experiment, four varieties were used, in a total of 37 plants initially (table 1). Plants were grown in pots, in a greenhouse, from February to August 2012. The soil used in the experiment was local basaltic soil, enriched with organic matter. Pot greenhouse distribution for all the experiment followed a random block design. Four control pots were used to determine soil field water capacity, water potential, and evaporation rates. Pot weight was measured constantly. During three months, plants were full irrigated providing enough water to allow normal plant growth and development.
After three months, plants from each variety were divided into three batches, and three different irrigation regimes were tested. In the first, pots were maintained near to field water capacity. A second batch was irrigated with two thirds the amount of water, and a third batch with a third amount of water. This irrigation scheme was maintained for another three months, until the end of the experiment.

Table 1. List of varieties and plant number used in preliminary drought stress assay.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Species</th>
<th>Variety</th>
<th>Origin</th>
<th>Number of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXOP 2059</td>
<td><em>C. esculenta</em></td>
<td>Blanco</td>
<td>Canary Islands</td>
<td>7</td>
</tr>
<tr>
<td>ISOP 2207</td>
<td><em>C. esculenta</em></td>
<td>Roxo</td>
<td>Madeira</td>
<td>10</td>
</tr>
<tr>
<td>ISOP 2216</td>
<td><em>C. esculenta</em></td>
<td>Branco</td>
<td>Madeira</td>
<td>10</td>
</tr>
<tr>
<td>ISOP 2220</td>
<td><em>C. esculenta</em></td>
<td>Vermelho</td>
<td>Madeira</td>
<td>10</td>
</tr>
</tbody>
</table>

During the experiment, periodic measurements were made of atmospheric parameters like temperature, humidity and PAR (Photossintetically Active Radiation). During and after the experiment, measurements were taken for twelve morpho-agronomic parameters; four were related to corm and eight to vegetative parts (table 2). Corm samples were collected for further biochemical study in the framework of MSc thesis project that is currently undertaken at University of Madeira.

Table 2. Morpho-agronomic parameters measured during the preliminary assessment of drought tolerance in taro (*C. esculenta*) varieties. The numbers (example 2059) are the accessions identification; C and S are the experiment control and stress variants, respectively.

<table>
<thead>
<tr>
<th>Trait</th>
<th>2059 (C)</th>
<th>2059 (S)</th>
<th>2227 (C)</th>
<th>2227 (S)</th>
<th>2216 (C)</th>
<th>2216 (S)</th>
<th>2220 (C)</th>
<th>2220 (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>69.73</td>
<td>40.83</td>
<td>72.77</td>
<td>44.83</td>
<td>71.40</td>
<td>42.14</td>
<td>70.48</td>
<td>44.30</td>
</tr>
<tr>
<td>Petiole insertion (cm)</td>
<td>7.53</td>
<td>6.50</td>
<td>9.78</td>
<td>5.45</td>
<td>8.23</td>
<td>4.80</td>
<td>8.70</td>
<td>5.45</td>
</tr>
<tr>
<td>Total plant weight (after harvest) (gr)</td>
<td>250</td>
<td>120</td>
<td>270</td>
<td>100</td>
<td>400</td>
<td>200</td>
<td>330</td>
<td>110</td>
</tr>
<tr>
<td>Leaf petiole coloration</td>
<td>Dark Green</td>
<td>Green</td>
<td>Purple</td>
<td>Purple</td>
<td>Rose</td>
<td>Green</td>
<td>Rose Green</td>
<td>Green</td>
</tr>
<tr>
<td>Chlorophyll content (CCI)</td>
<td>51.23</td>
<td>77.07</td>
<td>87.06</td>
<td>91.98</td>
<td>56.59</td>
<td>73.05</td>
<td>54.07</td>
<td>57.91</td>
</tr>
<tr>
<td>Corm length (cm)</td>
<td>5.10</td>
<td>6.27</td>
<td>4.57</td>
<td>4.45</td>
<td>5.34</td>
<td>6.48</td>
<td>5.20</td>
<td>5.14</td>
</tr>
<tr>
<td>Leaf number</td>
<td>3.25</td>
<td>1.67</td>
<td>2.50</td>
<td>2.00</td>
<td>2.8</td>
<td>1.40</td>
<td>2.5</td>
<td>1.20</td>
</tr>
<tr>
<td>Corm width (cm)</td>
<td>5.55</td>
<td>6.17</td>
<td>5.37</td>
<td>5.58</td>
<td>6.08</td>
<td>7.18</td>
<td>4.63</td>
<td>4.64</td>
</tr>
<tr>
<td>Leaf width (cm)</td>
<td>23.53</td>
<td>19.60</td>
<td>28.65</td>
<td>15.10</td>
<td>30.98</td>
<td>16.25</td>
<td>29.83</td>
<td>19.19</td>
</tr>
<tr>
<td>Flesh color</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Rose</td>
<td>Rose</td>
</tr>
<tr>
<td>Leaf length (cm)</td>
<td>26.43</td>
<td>22.30</td>
<td>34.18</td>
<td>20.08</td>
<td>35.20</td>
<td>18.18</td>
<td>34.27</td>
<td>27.04</td>
</tr>
<tr>
<td>Corm weight (gr)</td>
<td>50</td>
<td>70</td>
<td>50</td>
<td>40</td>
<td>80</td>
<td>120</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

The obtained data are now being treated, using statistical tools. Preliminary data allowed us to modulate the soil behavior under different irrigation regimes, used to determine the soil water capacity and identify the adequate water regimes (control and 2 water stress variants), showed the gaps in the irrigation model used. The figure 1 and 2 shows aspects of approach use in the assessment of methodology to be used in study of drought stress in taro aiming to and create a model of drought tolerance, namely in the determining soil water capacity and prediction of water percentage in soil (figure 1) and soil water evaporation (figure 2), during the assay. Both parameters are fundamental to determine the real water irrigation conditions (drought stress) and understand the plant behavior and growth, during field experiments.
Figure 1. Variation of water in soil control pots, without plant and irrigation, during stress assays. Pots have been saturated at beginning of the experiment and the line indicates the prediction of the percentage of soil water in the system.

The pilot assay also allowed tuning the methodology for parameters measurements. Data are being analyzed to see if it is possible to observe differences in the behavior of tested varieties for drought stress. A second experiment (pilot assay) is currently conducted under several determined irrigation regimes and procedures are being established to validate model and parameters measurements.

Figure 2. Soil water evaporation in soil control pots, without plant and irrigation, during stress assays. Pots have been saturated at the beginning of the experiment and the line indicates an estimation of the total water evaporated by the soil into the atmosphere.
WP 6: Physico-chemical characterisation of corms

The foreseen deliverables of WP6 are:

29. Intra-clonal variation of chemotypes studied and determined.
30. Physico-chemical variation of cultivars studied and correlated with molecular markers.
31. Physico-chemical variation between and within families correlated with mol. markers.
32. Assessment of physico-chemical variation due to drought, correlation with mol. markers.
33. One PhD thesis defended on the influence of stress on corm quality (Mr Andrej Mergedus).
34. Two papers published in international journals.

Various studies are being undertaken and aim at identifying cultivars with improved agronomic qualities and nutritional composition. Samples are sent to Maribor who studies the physico-chemical characteristics of genotypes grown. In Maribor analyses are done for residual moisture, starch, amylose, proteins, minerals and total sugars. The analyses are carried out using spectrophotometry, microwave digestion, and HPLC techniques. A PhD student has been recruited by the University of Maribor (Mr Andrej Mergedus) and arrived in Vanuatu November 23rd 2011 to start his field work. His research programme has been drafted by Prs J. Kristl and A. Ivancic. More than 400 hundred freeze dried samples have been prepared by A. Mergudus who will return to Slovenia on Feb 21st 2013.

Corms from selected cultivars grown in India, Costa Rica and Indonesia were sliced into chips, oven dried, packed and sent to the University of Maribor. In 2012, the mineral elements and total starch contents were studied in six cultivars from Costa Rica, ten cultivars from India and ten cultivars from Indonesia. In November 2012, we also obtained 80 different cultivars of taro corms from Ghana. These samples are current under investigation.

Macro- and microelements determination:

Samples were ground. For microwave digestion app. 0.4000 g of ground sample was weighted in PTFE vessel and treated with 5 ml HNO₃, 0.3 ml HF and 1 ml HCl and digested. After microwave digestion samples were cooled to room temperature and diluted to a total volume of 25 ml. The determinations of K, Ca, Mg, Fe, Mn and Zn were performed by aspirating the digested solutions into a FAA spectrometer using air-acetylene flame. Analyses of Cu and in some samples also analyses of Mn were carried out by ETAAS in the peak area mode. Argon of 99.999% purity at 3.0 L/min flow was used as the internal inert gas. Measurements were performed on pyrolytic graphite coated graphite tubes with L'vov platform. The contents of macro- and microelements are shown in Tables 1-3. Four commercial reference materials (NIST 8433, NIST 1575, NIST 1515 and NIST 1547) were used as the quality control samples. The accuracy was sufficient for all of the mineral elements. Each sample was analyzed in triplicate and the results expressed as mg kg⁻¹ of dry weight (DW) or as %.

The main minerals found in all the cultivars were K, P, Ca, and Mg. It was noticeable that the most abundant nutrient within all the corms studied was K. The K values for those corms grown in Indonesia ranged from 0.62 % to 1.43 %, in corms from India K values ranged from 1.44 % to 3.24 %, whilst in corms from Costa Rica the K values were from 1.00 % to 2.38 %. The results are reflecting that the cultivar could be defined as an important factor affecting mineral contents.

Total starch determination:

Starch contents were determined using total starch Megazyme procedure by applying a total starch assay kit based on the use of thermostable α-amylase and amyloglucosidase. The principle of determination is starch hydrolysis into maltodextrins with thermostable α-amylase. In the second step the amyloglucosidase is used which quantitatively hydrolyses maltodextrins into D-glucose.
The starch contents were in the range from 35% to 77% in cultivars from Indonesia, from 52% to 64% in cultivars from India, and from 58% to 72% in cultivars from Costa Rica (Tables 1-3).

**Table 1: Starch and mineral contents of taro samples from Indonesia**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Concentration (mg/kg)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn (mg/kg)</td>
<td>46±1</td>
<td>46±2.1</td>
<td>20.94±0.3</td>
<td>1059±3</td>
<td>1059±3</td>
<td></td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>8.68±0.47</td>
<td>29.94±1.2</td>
<td>8.13±0.5</td>
<td>497±34</td>
<td>497±34</td>
<td></td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>3.02±0.27</td>
<td>56.3±2.2</td>
<td>9.28±0.59</td>
<td>883±44</td>
<td>883±44</td>
<td></td>
</tr>
<tr>
<td>Ca (mg/kg)</td>
<td>3.02±0.27</td>
<td>56.3±2.2</td>
<td>9.28±0.59</td>
<td>883±44</td>
<td>883±44</td>
<td></td>
</tr>
<tr>
<td>P (%)</td>
<td>0.118±0.001</td>
<td>0.102±0.002</td>
<td>0.24±0.004</td>
<td>0.194±0.003</td>
<td>0.194±0.003</td>
<td></td>
</tr>
<tr>
<td>K (%)</td>
<td>0.79±0.01</td>
<td>0.84±0.03</td>
<td>1.38±0.05</td>
<td>0.99±0.01</td>
<td>0.99±0.01</td>
<td></td>
</tr>
<tr>
<td>Mg (mg/kg)</td>
<td>615±1</td>
<td>657±28</td>
<td>657±28</td>
<td>657±28</td>
<td>657±28</td>
<td></td>
</tr>
<tr>
<td>Starch (%)</td>
<td>61±1</td>
<td>72±1</td>
<td>63±2</td>
<td>63±2</td>
<td>63±2</td>
<td></td>
</tr>
</tbody>
</table>

*(mean values of three independent measurements ± standard deviation)*
<table>
<thead>
<tr>
<th>Sample</th>
<th>Starch (%)</th>
<th>Mg (mg/kg)</th>
<th>K (%)</th>
<th>P (%)</th>
<th>Ca (mg/kg)</th>
<th>Cu (mg/kg)</th>
<th>Zn (mg/kg)</th>
<th>Mn (mg/kg)</th>
<th>Fe (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE IC 264484</td>
<td>53±0.3</td>
<td>2227±1</td>
<td>3.24±0.01</td>
<td>0.472±0.006</td>
<td>3775±59</td>
<td>3.53±0.08</td>
<td>50±3</td>
<td>43±1</td>
<td>64±1</td>
</tr>
<tr>
<td>XS IC 57242</td>
<td>64±1</td>
<td>2100±50</td>
<td>1.59±0.01</td>
<td>0.473±0.013</td>
<td>936±30</td>
<td>16.3±0.7</td>
<td>84±4</td>
<td>100±1</td>
<td>63±1</td>
</tr>
<tr>
<td>CE IC 419621</td>
<td>62±0.7</td>
<td>1947±26</td>
<td>1.61±0.02</td>
<td>0.465±0.012</td>
<td>1327±19</td>
<td>9.66±0.45</td>
<td>94±5</td>
<td>30±1</td>
<td>67±1</td>
</tr>
<tr>
<td>CE IC 089560</td>
<td>55±0.7</td>
<td>1532±28</td>
<td>1.79±0.02</td>
<td>0.277±0.007</td>
<td>3134±15</td>
<td>13.1±0.2</td>
<td>101±1</td>
<td>90±1</td>
<td>38±1</td>
</tr>
<tr>
<td>CE IC 211584</td>
<td>64±1.5</td>
<td>1102±3</td>
<td>1.62±0.03</td>
<td>0.279±0.001</td>
<td>2357±30</td>
<td>11.3±0.8</td>
<td>73±1</td>
<td>24.0±0.1</td>
<td>46±1</td>
</tr>
<tr>
<td>CE IC 204239</td>
<td>52±1.0</td>
<td>2036±33</td>
<td>2.59±0.03</td>
<td>0.451±0.003</td>
<td>2230±49</td>
<td>11.5±0.6</td>
<td>131±8</td>
<td>49±1</td>
<td>74±1</td>
</tr>
<tr>
<td>CE IC 089624</td>
<td>62±1.4</td>
<td>1426±51</td>
<td>1.44±0.01</td>
<td>0.425±0.001</td>
<td>1366±32</td>
<td>9.14±0.78</td>
<td>63±4</td>
<td>20.5±0.7</td>
<td>41±1</td>
</tr>
<tr>
<td>CE IC 204336</td>
<td>55±0.3</td>
<td>1899±10</td>
<td>2.33±0.03</td>
<td>0.261±0.002</td>
<td>2976±44</td>
<td>12.5±±0.7</td>
<td>118±7</td>
<td>82±2</td>
<td>41±1</td>
</tr>
<tr>
<td>CE IC 12459</td>
<td>64±0.5</td>
<td>1100±18</td>
<td>2.04±0.01</td>
<td>0.362±0.004</td>
<td>1727±8</td>
<td>13.7±0.5</td>
<td>82±1</td>
<td>20.0±0.1</td>
<td>61±3</td>
</tr>
<tr>
<td>XS IC 440547</td>
<td>64±0.5</td>
<td>1986±7</td>
<td>1.81±0.03</td>
<td>0.447±0.004</td>
<td>1456±22</td>
<td>13.9±0.1</td>
<td>114±8</td>
<td>99±1</td>
<td>72±1</td>
</tr>
</tbody>
</table>

(mean values of three independent measurements ± standard deviation)

CE - Colocasia esculenta, XS - Xantosoma sagittifolium

Table 2: Starch and mineral contents in taro samples from India
Table 3: Starch and mineral contents in taro from Costa Rica

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fe (mg/kg)</th>
<th>Mn (mg/kg)</th>
<th>Zn (mg/kg)</th>
<th>Cu (mg/kg)</th>
<th>Ca (mg/kg)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Mg (mg/kg)</th>
<th>Starch (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-52</td>
<td>12.8±0.6</td>
<td>51±1</td>
<td>65±3</td>
<td>7.1±0.36</td>
<td>648±15</td>
<td>0.309±0.005</td>
<td>1.86±0.01</td>
<td>1.29±0.15</td>
<td>65±1</td>
</tr>
<tr>
<td>Co-53</td>
<td>13.2±0.5</td>
<td>30±1</td>
<td>65±3</td>
<td>6.84±0.44</td>
<td>727±10</td>
<td>0.13±0.001</td>
<td>1.00±0.01</td>
<td>791±20</td>
<td>63±1</td>
</tr>
<tr>
<td>Co-54</td>
<td>41±1</td>
<td>99±2</td>
<td>17.0±0.6</td>
<td>18.9±0.6</td>
<td>1882±27</td>
<td>0.17±0.003</td>
<td>2.38±0.03</td>
<td>1857±23</td>
<td>58±1</td>
</tr>
<tr>
<td>Co-55</td>
<td>13.2±0.5</td>
<td>30±1</td>
<td>17.0±0.6</td>
<td>8.48±0.12</td>
<td>646±38</td>
<td>0.103±0.004</td>
<td>1.04±0.02</td>
<td>983±24</td>
<td>72±1</td>
</tr>
<tr>
<td>Co-56</td>
<td>18.3±0.5</td>
<td>99±2</td>
<td>17.0±0.6</td>
<td>8.48±0.12</td>
<td>164±2</td>
<td>0.129±0.003</td>
<td>1.04±0.02</td>
<td>1488±16</td>
<td>62±1</td>
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<tr>
<td>Co-57</td>
<td>25.3±0.9</td>
<td>29±0</td>
<td>17.0±0.6</td>
<td>15.6±0.72</td>
<td>1662±26</td>
<td>0.177±0.006</td>
<td>1.41±0.02</td>
<td>855±3</td>
<td>66±1</td>
</tr>
</tbody>
</table>

Notes: The data for each mineral and starch content is presented as mean ± standard deviation.
**WP 7: Virus detection and identification**

The foreseen deliverables of WP7 are:

35. Virus diversity study conducted on viruses/strains in each participating countries.
36. Protocols for virus indexing seedling populations of taro fully optimised.
37. Parents and seedlings raised successfully.
38. Information on the rate of transmission in true seed of the important viruses or virus strains.
39. Guidelines for the safe movement of true taro seed between project partner countries.
40. One PhD defended on viruses identification/indexation studies (Ms Marion Liebrecht).
41. Two papers published in international journals.

DSMZ has reviewed the literature on viruses of taro and related aroids, and is now adapting and optimizing diagnostic tools for the viruses/virus strains present in INEA countries so that the tools can be used to detect viruses in seedling populations. All country partners have to send to DSMZ corms or leaf material of taro accessions for virus indexing in order to make an assessment of the diversity of viruses and strains present in each country. DSMZ is also studying the possibility of virus transmission with true botanical seeds. Initial indexing of parent plants is done using serological methods.

Partners will send to DSMZ batches of true botanical seeds that will be germinated in an insect-free glasshouse. Based on the results, guidelines will be developed for the virus-indexing of parental breeding lines and information on the potential for viruses to be disseminated in true seeds will be made available to the relevant authorities. Transfer of virus indexing techniques to all country partners is guaranteed, where necessary, DSMZ will provide training so that initial screening can be done in the country partner institutions. Guidelines for reliable virus indexing and safe movement of aroids germplasm will be developed. Training in advanced biotechnologies will be given and transfer of technologies will be done. Scientists from SPC will be trained by DSMZ in indexing procedures.

A protocol for leaf samples preparation has been developed and posted on the website. Plant materials to be tested for viruses were prepared for sending to Germany from Fiji, Papua New Guinea and Samoa. Corms of taro obtained from CTCRI were collected and grown to mature taro plants at DSMZ. Dasheen mosaic virus was confirmed on a number of plants showing typical symptoms of DMV infection. Partial sequence analysis showed considerable diversity among DMV isolates from taro which is significant for the development of virus diagnostic protocols and reagents. Currently antisera produced from recombinant DMV coat proteins expressed in bacteria are tested for serological detection of DMV in taro. Unfortunately, very few samples have been sent from country partners. SPC has however, tissue cultured a set of genotypes to be tested by DSMZ in Germany to assess the reliability of virus indexing techniques. A PhD student has been recruited (Ms Marion Liebrecht).

In the early part of 2012, samples with symptoms of virus infection were sent from Papua New Guinea. In Papua New Guinea staff from the National Agricultural Research Institute, Lae, were involved in making collections from farmers’ fields and sending the samples. However, although the plants from Papua New Guinea grew well in Germany, they did not show signs of disease. Because of this, samples were requested from Solomon Islands, where much work has been done on the etiology of taro virus diseases. Varieties are characterized by farmers on the island of Malaita as ‘male’ and ‘female’ depending on the types of disease symptoms they show, either Bobone or Alomae. Dr Grahame Jackson took time out from a visit to the country in April 2012 to work with staff from the Ministry of Agriculture and Livestock, and a local NGO, and sent plants by courier to
DSMZ. Before sending, the plants were carefully washed of soil, and roots were removed without damaging the corms and petioles, giving the plants maximum chance of survival when planted in screen houses at the DSMZ.

To date, Rhabdovirus-like particles and Badna sequences have been detected and also other infectious agents, including a tenuivirus, which has not been found in taro previously. This is a single stranded RNA virus, transmitted by members of the Delphacidae. Later in the year, another consignment was sent by Helen Tsatsia, Director of Research, Solomon Islands. These plants were collected from a garden in Honiara and were said to be from another islands, Choiseul. They showed symptoms of taro vein chlorosis virus and dasheen mosaic virus.

Work will continue to detect hitherto unknown viruses, and molecular sequences defined in order to develop sensitive tests. But as far as the aetiology of Bobone and Alomae are concerned, no progress has been made.

**Plant materials**

In the reporting period, Taro com materials were obtained from several countries, either collected or send by partners. A specific collection was made in Solomon islands (Grahame Jackson) to obtain Bobone/ Alomae suspected materials.

**Table 1.** Plant materials for virus studies at DSMZ

<table>
<thead>
<tr>
<th>Project partner (sender)</th>
<th>Country of origin</th>
<th>No. of plants sent</th>
<th>Findings (virus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTCRI¹</td>
<td>India</td>
<td>30</td>
<td>DoMV</td>
</tr>
<tr>
<td>SPEC²</td>
<td>Fiji</td>
<td>51</td>
<td>DoMV</td>
</tr>
<tr>
<td>NARI</td>
<td>Papua New Guinea</td>
<td>12</td>
<td>/</td>
</tr>
<tr>
<td>PostNet³</td>
<td>Solomon Islands</td>
<td>9</td>
<td>Unknown*, Rhabdovirus (CBDV)**, IstBv</td>
</tr>
<tr>
<td>ISOPlexis / Universidad do Madeira³</td>
<td>Portugal – Madeira</td>
<td>12 (leaf samples)</td>
<td>/</td>
</tr>
<tr>
<td>Helen Tsatsia⁴</td>
<td>Solomon Islands – Honiara</td>
<td>4</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

¹ total number of plant samples sent in 2011 and 2012  
² sent in 2011  
³ sent in 2012  
⁴ evidence of a newly discovered tentative tenuivirus  
** Rhabdovirus (CBDV) identity to be confirmed  
n.a. – virus studies pending

All Taro coms were maintained at DSMZ Plant Virus Department under tropical (>26°C glasshouse conditions) for symptom expression and subsequent virus studies.

All virus experiments and findings are summarized in sections under the virus species name. In 2013 it is expected that virus characterization and development ELISA and PCR systems is finalized. The tests will be made available for validation of sensitivity, inclusivity and exclusivity, repeatability and reproducibility. Ringtests are then conducted with laboratories to proof fitness for purpose. For virus characterization, detection of virus in seeds, fulfilment of Koch’s postulates etc. more samples need to be sent to and examined at DSMZ.
Dasheen mosaic virus (DsMV-T) genus Potyvirus

The complete genome of DsMV-T was sequenced and the genome assembled from sequence fragments obtained from RT-PCR. The genome of a previously sequenced isolate from Amorphophallus spp. (DsMV-A) was used as template for primer design. The coat protein gene of DsMV-T originating from plant sample C-I-14 (CTCRI, India) was cloned in an expression vector (pET 28a, Qiagen) and recombinant CP was expressed and purified from bacterial cells. Antibodies against recombinant proteins (native and denatured protein fractions) were raised and ELISA tests were established.

In “ISEM + decoration” test both decorated filamentous virus-like particles in DsMV-T samples from Fiji (Fi-I-03) and also virus-like particles from DsMV-A. Differences in decoration between both DsMV-T antisera were not found. In contrast DsMV-A antiserum against purified virions failed to decorate virus-like particles of DsMV-T and specifically decorated DsMV-A particles only.

The EM findings were confirmed by ELISA with samples from India and Fiji form Taro and Amorphophallus spp. Antisera raised against DsMV-T are found versatile to detect DsMV in Taro but also in Amorphophallus and other aroids.

Future developments: ELISA tests need to be validated for sensitivity, specificity, repeatability and reproducibility. In 2013, ELISA tests will be sent to partners for assessment.

DsMV indexing by RT-PCR: Primers recommended and generally used for detection of DsMV (3F/33R) are found not reliable in our hands. New primer series will be designed and RT-PCR established.

Taro bacilliform virus (TaBV) genus Badnavirus

The complete genome from a badnavirus isolated from Alomae suspected plants from Solomon islands was obtained by an inverse PCR with specifically designed abutting primers derived from initial PCR fragments obtained from generic PCR. This badavirus genome was consistent in size with previously reported full length genome sequences of TaBV Papua New Guinea and other known badnaviruses. All 4 ORFs were identified.

4kb fragment sequences derived from inverse PCR with taro sample from India were crippled and non functional circular artifact indicating for genome integrations of ancient badnavirus sequences.

Future developments: Antiserum production and establishment of ELISA tests: ORF 3 and ORF 2 will be cloned into bacterial expression vectors for recombinant protein expression and antibody productions.

Virus indexing via PCR: Primers recommended (TaBV 1/ 4) are found not reliable because of amplification of genome integrated sequences resulting in false positives. To detect episomal virus and discriminate integrated genome sequences several approaches will be followed including serological detection and PCR.

Etiology: Contribution of TaBV to Alomae disease.

Colocasia bobone disease virus (CBDV) genus Nucleorhabdovirus

Plants suspicious of CBDV infection (bobone and alomae) from the Solomon Islands showed symptoms suspicious for alomae only in one case. In those plants few rhabdovirus-like particle were demonstrated in EM.
All classical tests to tag rhabdovirus sequences (generic primers, antisera etc.) failed and all generic tests for virus sequences (dsRNA analysis) failed as well.

A next generation sequence approach was followed by combining illumine HISeq transcriptome sequences (mRNA) with small RNA sequencing of siRNA obtained from Alomae affected plants. Several thousand sequence contigs were generated and blasted to NCBI GenBank database for virus discovery. A 12 kb sequence contig showed similarities to rhabdovirus sequences and the order of open reading frames (ORFs) was that of rhabdoviruses. NGS data are to be confirmed by classical molecular approaches.

Future developments: The complete genome of the putative CBDV is to be reconstructed and confirmed as viral origin. An antiserum against nucleoprotein preparations will be tried and RT-PCR will be designed for specific detection.

Etiology: Contribution of CBDV to Alomae disease
Taro vein chlorosis virus (TaVCV) genus Nucleorhabdovirus

Plant samples from Honiara, Solomon Islands arrived in late December 2012 and are kept in the greenhouse under tropical conditions for symptom development.

Genome sequences of TaVCV are known and primers are described. Testing for presence of TaVCV will start as soon as the obtained plant material from Honiara reaches a state, which allows sampling. Future developments: The complete genome of the putative TaVCV will be reconstructed once this virus is confirmed in the samples. An antiserum against nucleoprotein preparations will be tried and RT-PCR will be designed for specific detection.

Taro Tenuivirus genus Tenuivirus

Leave homogenates from plants suspicious of CBDV infection (bobone and alomae) from the Solomon Islands were inoculated to a series of indicator plants for rhabdoviruses. Three weeks after inoculation, Nicotiana benthamiana symptoms indicating for virus infections. Those comprised curling of leaf margins, chlorotic spots and mottling and malformation of leaves. DsRNA extraction and subsequent cloning of random PCR fragments generated revealed the presence of sequences of a putative tenuivirus.

The next generation sequencing approach of Alomae affected Taro plants (described above) revealed sequence contigs representing all RNA of this negative sense virus with a multipartite genome RNA 1-5. This was complemented with sequences of dsRNA fragments obtained from the random PCR approach of N. benthamiana covering all RNAs except RNA 3.

A reference isolate of the Taro tenuivirus was established and back transmission assays to proof Koch’s postulates were tried. The inoculated Taro plants are maintained in glasshouse conditions for symptom development.

Future developments: The 5’- and 3’-termini of all five RNA segments are to be determined to establish a full genome sequence of the tenuivirus. The relationship with rice stripe mosaic needs to be verified. From expressing the putative CP of this virus antisera will be raised for virus diagnosis. To fulfill Koch’s postulates vector insect populations might have to be established. Since two out of three verified vector species Tarophagus colocasiae and Tarophagus persephone are common on Solomon Islands those insects have to be included in further collections and in virus studies.
**Etiology: Contribution of Taro tenuivirus to Alomae disease**

**Table 2. Progress of virus characterization and detection**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Genomic data</th>
<th>Antisera</th>
<th>PCR assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS MV</td>
<td>Complete</td>
<td>Yes</td>
<td>New primers under development</td>
</tr>
<tr>
<td>TaBV</td>
<td>Complete</td>
<td>In progress</td>
<td>Immunoassay PCR for apical virus under development</td>
</tr>
<tr>
<td>CBDV</td>
<td>Terminals to complete</td>
<td>n.a.</td>
<td>RT-PCR with oligo-dT primed cDNA</td>
</tr>
<tr>
<td>TaVVCV</td>
<td>n.a.</td>
<td>n.a.</td>
<td>pending</td>
</tr>
<tr>
<td>Taro tenuivirus</td>
<td>3′ 5′- Terminis missing</td>
<td>n.a.</td>
<td>pending</td>
</tr>
</tbody>
</table>

**WP 8: On-farm participatory selection of elite cultivars and hybrids**

The foreseen deliverables of WP8 are:

42. Introduced elite genotypes distributed to farmers and propagated in their plots.
43. Introduced elite genotypes harvested and assessed by farmers.
44. C5 (first clonal generation) propagated and distributed to farmers.
45. On-farm trials harvested and participatory evaluation of C5 quality conducted.
46. On MSc defended in Papua New Guinea (Mr Jeffrey Waki).
47. Two papers published in international journals.

**Bioversity International** (Rome, Italy) collaborates with CIRAD and Maribor to assist all country partners. Taro focused participatory rural appraisals (PRAs) will be conducted with farmer groups (five per village in ten villages) to learn more about taro production problems, perception of taro cultivars and criteria in the selection of a cultivar.

One of the recommendations expressed by partners during the launching meeting in Kuala Lumpur in 2011 was that PRAs should be conducted just before the planting material distribution phase. It was therefore decided to postpone this exercise until there are sufficient propagules for distribution, probably at the end of 2012 or in 2013. Unfortunately, the financial problems of the project have seriously delayed the field propagation work and therefore the distribution of clonal materials to the farmers.

In order to assist partners with this work, two documents are being shared: 1) **“Guidelines for On Farm Breeding Trials”** which contains most of the information country representatives will need when organizing farmer groups to be part of a taro evaluation programme; and 2) **“Participatory Approaches for Exploring Farmer Conservation and Utilization of Taro Genetic Resources”**, a PowerPoint presentation which contains background information on the various participatory research tools and methods, approaches to documenting local knowledge of taro and effective ways of building farmer and community support for taro research. These guidelines, documents and background information have been posted on the INEA website.
3. Difficulties encountered and measures taken to overcome problems

The financial report of INEA has been rejected by the EU on several occasions. The second transfer of funds could not be made because the report was incomplete. Partners have supported 100% of the total project costs for the last 13 months. This difficult situation had a very serious impact on the technical and scientific work of the project.

The financial constraints and the complexity of the financial reporting are new for most partners. The EU was invited to the launching meeting of the project in Kuala Lumpur in April 2011. It was hoped that the EU staff could explain to the project partners the financial reporting procedures in order to ease this complex task. Unfortunately, no EU representative was able to participate to this important meeting. Partners have discovered the complexity of the financial reporting and are doing their best to satisfy the EU requirements.

The applicant, SPC, is however, facing serious problems collecting acquittals, receipts and financial reports in proper form and is encountering numerous difficulties for preparing a consolidated report for the whole project. CIRAD has offered to SPC to prepare the financial consolidated report on her behalf. In order to be able to do so, CIRAD has demanded to SPC to send copies of all acquittals and receipts received today (for the year 2011). As soon as these receipts will reach Montpellier, France, CIRAD staff will start working on a consolidated report.

A ROM assessment of the project is planned for the week of Feb 12-15\textsuperscript{th} and it is hoped that the ROM will be able to propose practical solutions. The situation deserves urgent solutions.

4. Changes introduced in implementation

The second annual meeting could not be organized due to the shortage of funds. Partners are exchanging via email but discussions are therefore, limited. So far there are no major changes planned. The project is constrained by the absence of funds to work with. Partners are doing their best to comply with the work plan.
5. Achievements and results using the indicators included in the Agreement

### Summary and time table of activities with their status:

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>1. Development of a web site and platform</td>
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<tr>
<td>2. Financial and administrative reporting</td>
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<tr>
<td>3. Production and circulation of annual reports</td>
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<td>4. Monitoring of project activities (annual meetings)</td>
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<tr>
<td><strong>WP2. In vitro distribution and field propagation:</strong></td>
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<td>5. In vitro propagation and distribution of 50 vars</td>
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<td>6. Field propagation</td>
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<td>7. Distribution of 30 selected varieties to farmers</td>
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<td><strong>WP3. Breeding &amp; on-farm participatory selection:</strong></td>
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<td>8. Conduct controlled crosses</td>
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<td>9. Raise F1 hybrids</td>
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<td>10. Distribute C1s to farmers</td>
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<tr>
<td>11. One PhD on breeding (South Africa)</td>
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<td><strong>WP4. DNA (SSRs &amp; SNP) fingerprinting:</strong></td>
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<td>12. Fingerprinting of elite cultivars</td>
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<td>13. Determine genetic distances between parents</td>
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<td>14. Study segregations of F1s</td>
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<td>15. New markers developed and mapped</td>
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<tr>
<td>16. Establish and harvest first heritability trial</td>
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<tr>
<td>17. Establish and harvest second heritability trial</td>
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<td>18. Identify potential major genes</td>
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<td>19. One PhD on genetic diversity (Burkina Faso)</td>
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<tr>
<td>20. One PhD on association studies (France)</td>
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<tr>
<td>21. Four papers published in international journals</td>
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<td><strong>WP5. Drought resistance studies:</strong></td>
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<td>22. Establishment of field experiments</td>
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<td>23. Evaluation of morpho-agronomic traits</td>
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<td>24. Physiological and biochemical marker analysis</td>
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<td>25. Corms from cultivars stressed analysed</td>
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<td>26. Traits and markers association with drought</td>
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<tr>
<td>27. One MSc on drought tolerance (Portugal)</td>
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<tr>
<td>28. Two papers published</td>
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<td><strong>WP6. Physico chemical characterisation:</strong></td>
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<td>29. Intra clonal variation</td>
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<tr>
<td>30. Variation between selected cultivars</td>
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<td>31. Variation within and between full-sibs families</td>
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<tr>
<td>32. Drought effect on corm characteristics</td>
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<tr>
<td>33. One PhD defended (Slovenia)</td>
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<tr>
<td>34. Two papers published in international journals</td>
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<tr>
<td><strong>WP7. Viruses detection and identification:</strong></td>
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<tr>
<td>35. Diversity of viruses and strains</td>
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<tr>
<td>36. Develop and optimize virus testing protocols</td>
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<tr>
<td>37. Raise parents and seedlings</td>
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<tr>
<td>38. Detect viruses in parents and seedlings</td>
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<tr>
<td>39. One PhD defended (Germany)</td>
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<td>40. Formulate guidelines for exchange of seeds</td>
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<td>41. Two papers published in international journals</td>
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<tr>
<td><strong>WP8. On-farm trials and participatory activities:</strong></td>
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<tr>
<td>42. Selected genotypes distributed to farmers</td>
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<tr>
<td>43. Selected genotypes evaluated by farmers</td>
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<tr>
<td>44. C1s propagated and distributed to farmers</td>
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<tr>
<td>45. On-farm trial harvested and quality tests done</td>
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<tr>
<td>46. One MSc defended (Papua New Guinea)</td>
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<tr>
<td>47. Two papers published in international journals</td>
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</table>
6. Work plan for 2013 with objectives and indicators of achievement

Assuming that the serious financial constraints and problems being presently faced by the project could be solved rapidly, the tentative work plan of the project for 2013 is presented here below and remains the same as the one originally planned.

<table>
<thead>
<tr>
<th>Work Package:</th>
<th>Semesters:</th>
<th>2013</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td>WP1. Project coordination and management:</td>
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<td>2</td>
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<tr>
<td>2. Financial and administrative reporting</td>
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<tr>
<td>3. Production and circulation of annual reports</td>
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<tr>
<td>4. Monitoring of project activities (annual meetings)</td>
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<tr>
<td>WP2. In vitro distribution and field propagation:</td>
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<tr>
<td>6. Field propagation</td>
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<td>7. Distribution of 30 selected varieties to farmers</td>
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<tr>
<td>WP3. Breeding &amp; on-farm participatory selection:</td>
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<tr>
<td>8. Conduct controlled crosses</td>
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<td>9. Raise F1 hybrids</td>
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<tr>
<td>10. Distribute C1s to farmers</td>
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<tr>
<td>11. One PhD on breeding (South Africa)</td>
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<tr>
<td>WP4. DNA (SSRs &amp; SNP) fingerprinting:</td>
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<td>14. Study segregations of F1s</td>
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<tr>
<td>15. New markers developed and mapped</td>
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<tr>
<td>16. Establish and harvest first heritability trial</td>
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<tr>
<td>17. Establish and harvest second heritability trial</td>
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<tr>
<td>18. Identify potential major genes</td>
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<tr>
<td>19. One PhD on genetic diversity (Burkina Faso)</td>
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<td></td>
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<tr>
<td>20. One PhD on association studies (France)</td>
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<tr>
<td>21. Four papers published in international journals</td>
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<tr>
<td>WP5. Drought resistance studies:</td>
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<td>22. Establishment of field experiments</td>
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<tr>
<td>23. Evaluation of morpho-agronomic traits</td>
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<td>24. Physiological and biochemical marker analysis</td>
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<td>25. Corms from cultivars stressed analysed</td>
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<td>26. Traits and markers association with drought</td>
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<tr>
<td>27. One MSc on drought tolerance (Portugal)</td>
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<tr>
<td>28. Two papers published</td>
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<tr>
<td>WP6. Physico chemical characterisation:</td>
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<td>29. Intra clonal variation</td>
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<td>30. Variation between selected cultivars</td>
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<tr>
<td>31. Variation within and between full-sibs families</td>
<td></td>
<td></td>
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<tr>
<td>32. Drought effect on corm characteristics</td>
<td></td>
<td></td>
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<tr>
<td>WP7. Viruses detection and identification:</td>
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<tr>
<td>38. Detect viruses in parents and seedlings</td>
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<td>WP8. On-farm trials and participatory activities:</td>
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<td>42. Selected genotypes distributed to farmers</td>
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<tr>
<td>43. Selected genotypes evaluated by farmers</td>
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<tr>
<td>44. C1s propagated and distributed to farmers</td>
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Appendix 1: Taro Leaf Blight in Nigeria

Trip report: National Root Crops Research Institute, Umudike, Nigeria
by Dr GVH Jackson

1. Background

Nigeria is a member of INEA, the International Network for Edible Aroids, and has received two lots of *Colocasia* taro germplasm as part of distribution of varieties from the Secretariat of the Pacific Community to partners worldwide. Many of the 50 varieties sent are from the TANSAO collection and breeding programmes in Hawaii, Papua New Guinea and Samoa, varieties that have been bred for resistance to taro leaf blight. Taro leaf blight spread to West Africa in 2009, and has done considerable damage. All local varieties appear susceptible.

Dr Joseph Onyeka, Head, Plant Pathology & Microbial Biotechnology, National Root Crops Research Institute, Umudike, requested INEA to send a specialist on taro leaf blight to evaluate the situation in Nigeria, especially the reaction of the SPC varieties and to discuss the programme at Umudike to overcome the disease. Grahame Jackson visited on 26 September 2012 as part of a visit to attend the 16th symposium of the International Society of Tropical Root Crops at Abeokuta. A longer visit was not possible due to logistical problems.

2. Research on taro and cocoyam in Africa: ISTRC symposium

Of the 343 abstracts presented to the Symposium only 20 involved cocoyam, a generic term for both *Xanthosoma* and *Colocasia*. Of these, *Xanthosoma* was the subject of papers on eight occasions, *Colocasia* on three, both crops on six, and in three papers the crops were unspecified “cocoyams”.

Interestingly, 17 of the papers came from Nigeria, two from Ghana and one from Benin.

Reports on aspects of agronomy were relatively scarce: there were two papers on varietal evaluation, one on rapid multiplication, four on pests (millipedes and aphids), and single papers on diseases (taro leaf blight), weeds (herbicides, intercropping with pumpkins), and the influence of leaf harvests on corm yield. One paper made projections of cocoyam production in the 21st century in relation to forecasts of population increase, area of land under cultivation and productivity; the paper then considered policy options. The remaining papers (11) were on the analysis of corms and cormels and physicochemical properties of flours and starches.

Apart from routine chemical analyses of corms and cormels, studies were also reported on different drying techniques on oxalate content, on impurities – particularly dangerous levels of arsenic - of street-sold chips, and on carotenoid, polyphenol and antioxidant values.

Analysis of both flours and starches was a subject of several papers. A common theme was the physicochemical composition of cocoyam flours and how substitution for wheat flour affected loaf volume, protein, mineral and moisture content, as well as consumer acceptability. There was also interest in steeping times and drying temperatures of fermented flour, and the use of these flours in the production of confectionaries.

By contrast, two reports on the physicochemical properties of starches assessed their potential in food and for industrial uses. The studies looked at granule morphology, viscosities, amylose content, pasting temperatures, gelatinisation, solubility and swelling.
2.1 Severity of taro leaf blight in Nigeria

The severity of the taro leaf blight epidemic was outlined in a paper given by Dr Onyeka at the ISTRC meeting, entitled: *Distribution and severity of taro leaf blight in different agroecological zones of Nigeria*. Interestingly, a survey across the country showed that the disease was severe in all states, even in those considered “dry”. Only 3% of the farmers’ fields inspected were without the disease and none of the varieties had useful resistance to the blight.

2.2 The Cocoyam Rebirth Initiative, NRCRI, Umudike

The Cocoyam Rebirth Initiative to increase production and processing of both *Colocasia* and *Xanthosoma* has attracted considerable interest from schools and non-government organisations, ably led by Dr Godwin Chukwu. The Initiative has come at a critical time following the introduction of taro leaf blight in 2009. For instance, letters from community organisations in Enugu and Anambra States, near Umudike, tell of the severity of the disease. One letter reads:

*The disease has spread like wild fire and has forced the crop into extinction. It kills the plant completely leaving nothing behind for the farmer to harvest. The situation at present is that we no longer have planting materials within the community, which has put the whole community into turmoil.* (Cocoyam Producers Association of Nigeria, Enugu State).

The reports mention that food security has been compromised, as taro is a staple of the States. Planting material and assistance with control using pesticides have been requested.

2.4 Recommendations

- Management of taro leaf blight by cultural practices – leaf pruning, spacing, etc – is not an effective strategy;
- Fungicides are effective (a trial is in progress at Umudike), using metalaxyl or phosphorus acid, but fungicides have to be applied frequently, and in most countries are too expensive for smallholder farmers;
- As breeding for resistance has been shown to be successful in Hawaii, Papua New Guinea and Samoa, NRCRI, Umudike, should rapidly evaluate germplasm from Asia and the Pacific, and if selections fail to meet farmers’ needs, use the best in crosses with local varieties, with guidance from INEA;

3. Reaction of local *Colocasia* varieties to taro leaf blight

The collection of varieties at Umudike shows that all are very susceptible to taro leaf blight. The varieties were planted 5 months ago and have been sprayed three times with Ridomil, the last time was 3 weeks previously; nevertheless, the leaves show multiple lesions. Some, such as NCe005, have lesions on the petioles and this is a good indicator of extreme susceptibility to taro leaf blight. It is reminiscent of the reaction of the commercial variety grown in Samoa - Niue - which was annihilated by the introduction of the disease in June 1993 along with all other varieties.

It is not clear whether all the varieties in the country are in the collection at Umudike. Therefore, those present should be described using an internationally accepted (shortened\(^1\)) version of the Descriptors for taro (*Colocasia esculenta*), and photographed. The photographs of the varieties in the collections should be distributed to agriculture centres throughout the country and comparisons made with local germplasm. Additional varieties should be sent to Umudike, and if the differences

\(^1\) [http://www.ediblearoids.org/PROJECTS/WP2Germplasm/Descriptors%28Colocasia%29.aspx](http://www.ediblearoids.org/PROJECTS/WP2Germplasm/Descriptors%28Colocasia%29.aspx)
are confirmed they should be placed in tissue culture and conserved, with a backup collection in tissue culture at IITA and/or SPC.

### 3.1 Quarantine considerations

There are $A^0$, $A^1$ and $A^2$ strains of taro leaf blight. It would be of interest to determine the mating type of the isolate(s) now in Nigeria. Recent work of Tyson and Fullerton\(^2\) has confirmed that the $A^2$ mating type is the most common with the $A^1$ mating type restricted to Hainan Island, China and northern India. If a determination of the mating type is required, it is suggested that Dr Fullerton is contacted by email (Bob.Fullerton@plantandfood.co.nz).

Additionally, it is worth noting what the Tyson & Fullerton paper says about further spread of strains:  

> ... the likelihood of its (the $A^1$ mating type) introduction to the Pacific region is considered to be relatively low. Nevertheless, its introduction would constitute a risk to the region. Not only would the resultant oospores create a new, longer-lived form of inoculum, but the potential for new, more virulent genotypes is increased both by the new incursions and by their genetic recombination with existing locally adapted strains.

Quarantine authorities should be alerted to the fact that other mating types of *P. colocasiae* exist and that the introduction of edible aroid planting material should be controlled.

### 3.2 Recommendations

- Use shortened descriptor list to describe local taro varieties at Umudike;
- Take photos of leaves, petioles and corms of local varieties and laminate;
- Send photos to regional agricultural centres to check against local varieties;
- Request centres to send varieties of those not in national collection to Umudike;
- Plant and compare varieties with those in the germplasm collection;
- Ensure all varieties are in tissue culture for safe conservation;
- Back up *Colocasia* collection at eg IITA and/or SPC;
- Determine mating types of the isolates of *P. colocasia* in Nigeria;
- Maintain strict control on edible aroid introductions to prevent entry of other mating types.

### 4. Reaction of SPC varieties to taro leaf blight

During the visit, the varieties were assessed for reaction to taro leaf blight; those that appeared to be tolerant (a few lesions only) or susceptible (many lesions) to the disease are recorded below, together with their reaction in the countries of origin (Table 1).

In general, there was good agreement with the previously recorded reaction of varieties to taro leaf blight in Asia and the Pacific with the same varieties growing in Nigeria. Comparisons between the varieties in Samoa and Nigeria are the most important as their reaction to taro leaf blight has been checked over a number of years by researchers and farmers.

As can be seen from Table 1, the reactions of all 10 are the same in both Samoa and Nigeria. This was to be expected.

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Table 1. Reactions to taro leaf blight of some of the Asia/Pacific taro introduced to Nigeria comparison with their reported reaction in their countries of origin (in brackets).

<table>
<thead>
<tr>
<th>TLB tolerant</th>
<th>Reaction in Asia/Pacific</th>
<th>TLB susceptible</th>
<th>Reaction in Asia/Pacific</th>
</tr>
</thead>
<tbody>
<tr>
<td>*BL/SM/125 (check)</td>
<td>Tolerant</td>
<td>Ce/IND/12 (Indonesia)</td>
<td>Susceptible</td>
</tr>
<tr>
<td>*BL/SM/80 (Samoa)</td>
<td>Tolerant</td>
<td>BL/PNG/11 (PNG)</td>
<td>Resistant</td>
</tr>
<tr>
<td>BL/SM/151 (Samoa)</td>
<td>Tolerant</td>
<td>Ce/Tha/05 (Thailand)</td>
<td>Susceptible</td>
</tr>
<tr>
<td>*BL/SM/152 (Samoa)</td>
<td>Tolerant</td>
<td>Ce/IND/10 (Indonesia)</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Ce/IND/24 (Indonesia)</td>
<td>Susceptible</td>
<td>Ce/Tha/24 (Thailand)</td>
<td>Susceptible</td>
</tr>
<tr>
<td>BL/SM/83 (Samoa)</td>
<td>Tolerant</td>
<td>Ce/IND/06 (Indonesia)</td>
<td>Susceptible</td>
</tr>
<tr>
<td>*BL/SM/120 (Samoa)</td>
<td>Tolerant</td>
<td>Ce/IND/19 (Indonesia)</td>
<td>Tolerant</td>
</tr>
<tr>
<td>*BL/SM/116 (Samoa)</td>
<td>Tolerant</td>
<td>BL/HW/26 (Hawaii)</td>
<td>Tolerant</td>
</tr>
<tr>
<td>*Ce/Tha/08 (Thailand)</td>
<td>Susceptible</td>
<td></td>
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<tr>
<td>BL/SM/136 (Samoa)</td>
<td>Tolerant</td>
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<tr>
<td>BL/SM/13 (Samoa)</td>
<td>Tolerant</td>
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<tr>
<td>*BL/SM/158 (Samoa)</td>
<td>Tolerant</td>
<td></td>
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<tr>
<td>*Ce/IND/32 (Indonesia)</td>
<td>Immune</td>
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<tr>
<td>Ce/IND/14 (Indonesia)</td>
<td>Susceptible</td>
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<tr>
<td>Ce/Tha/09 (Thailand)</td>
<td>Susceptible</td>
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<tr>
<td>BL/SM/148 (Samoa)</td>
<td>Tolerant</td>
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<tr>
<td>Ce/Tha/07 (Thailand)</td>
<td>Susceptible</td>
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<tr>
<td>*Ce/JP/01 (Japan)</td>
<td>No information</td>
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</table>

Three varieties had stolons: BL/SM/151; Ce/IND/24 and Ce/IND/06. BL/SM/116 is flowering and Ce/Tha/08 is small. Ce/IND/10 has petiole infections. There is need to check BL/SM/125 as there is no SPC variety with that number. *indicates that the plants had shiny, waxy, leaves presumably a consequence of Malaysian parentage.

Taro in the Pacific has been bred for horizontal - minor gene - resistance, which is not pathotype specific. In other words, the varieties are likely to show similar reactions to different strains of the oomycete, *P. colocasiae*. However, the disease level will differ according to the weather, ie local rainfall and temperature.

The varieties listed above (Table 1) have been transplanted to another field and planted at wider spacings; others from the second batch sent by SPC have been planted adjacent to them, and both groups are awaiting infection from taro leaf blight. This is not likely until the major rainy season beginning around April next year.

4.1 Recommendations

- Recheck varieties in Table 1 for correct numbers and reaction to taro leaf blight;
- Assess varieties of the second planting for taro leaf blight resistance, comparing reactions with those given in annex table of the Annual Report, see http://www.ediblearoids.org/REPORTS/AnnualReports.aspx

5. Xanthosoma with resistance to root rot disease

Root rot disease is severe among the collection of *Xanthosoma* varieties at Umudike. However, one variety, NXs003, appears resistant to the disease, a variety with yellow flesched acrid corms. It is the
least preferred of all those in the collection. This variety is said to be in tissue culture at NRCTI. *Colocasia* varieties are unaffected by root rot.

Root rot disease of *Xanthosoma* is severe in many parts of the world, including Central America and West Africa. As far as the writer is aware resistance has not been well recorded, although there is some anecdotal evidence. Dr Chokwu writes about NXs003 as follows:

“The cocoyam variety NXs 003 is edible, yellow fleshed and for now it is the only variety that is resistant to root rot disease. However, it is no longer popular as food as it was in the 1970s because of improvement in socio-economic status. So it grows wild and constitutes a weed. It grows more luxuriantly than the popular edible varieties (NXs001 and NXs002). Unlike the other varieties NXs 003 is highly acrid and requires prolonged cooking. Currently, it is processed into cocoyam fufu flour as a substitute for semovita and wheat flour. We are trying to popularize this as a way to encourage people to eat it. It is perennial and the corm can weigh up to 50 kg after many years.” (Dr Godwin Chukwu, Coordinator Cocoyam Research Programme, National Root Crops Research Institute, Umudike, Abia State, Nigeria.)

If indeed sharing is possible, cormels or tissue cultures should be sent to SPC where pathogen testing can be carried out, before distribution to countries interested in breeding for resistance, for example, Costa Rica, Nicaragua and Vanuatu.

5.1 Recommendations

- INEA to request tissue cultures of *Xanthosoma* variety NXs003 under the germplasm sharing provisions of the project;
- Transfers should use the Standard Material Transfer Agreement used by the International Treaty on Plant Genetic Resources for Food and Agriculture, see http://www.planttreaty.org/content/what-smta;
- NXs003 to be indexed at SPC and then sent to *Xanthosoma* breeding programmes in eg Vanuatu and Costa Rica, again using the SMTA;
- Replant NXs003 in plots were other varieties are showing severe symptoms of root rot at Umudike to confirm its resistance to this oomycete disease.

6. Acknowledgements

The writer would like to thank Dr Okonkwo, Executive Director; Dr Godwin Asumugha, Head of Extension; the Senior Director Revenue Mobilisation (standing in for the Executive Director); Dr (Mrs) Egbichi Mbanaso, Director of Biotechnology; Dr Joe Onyeka, Head, Plant Pathology and Microbial Biotechnology; Dr Godwin Chukwu, Coordinator Cocoyam Research Program; and Ms Blessing Elijah, Lab Technologist, for hosting the visit to NRCRI, Umudike, to see the work on edible aroids in the field and lab.
ABSTRACT:

Aroids corms and cormels are chemically very variable and there is significant variation of their major constituents (starch, sugars, cellulose, proteins, minerals) between genotypes. A fairly common difficulty for breeding programmes is to assess precisely these compounds as chemical analyses are too expensive for routine screening. These programmes are often based on mass recurrent selection and great numbers of hybrids have to be screened to achieve some progress. However, the wrong selection of a parent can cause a serious constraint to the development of new varieties for processing purposes. Likewise, if the table quality is not acceptable for consumers, years of intense and expensive breeding efforts can lead to complete failure. Low-cost methods for rapid evaluation of numerous hybrids are urgently needed. The present paper, assess the potential of NIRS (Near Infrared Reflectance Spectroscopy) as an alternative method for predicting these major constituents and evaluating corms and cormels quality. Models have been developed using data from 642 root crops accessions and their predictive potential has been tested on 100 varieties and hybrid lines of taro, *Colocasia esculenta*. The NIRS calibration results for major constituents, and their practical applications for aroids breeding and genetic improvement of quality traits, are discussed.

Keywords: Aroids, breeding, corms, chemical composition, major constituents, NIRS, quality

Introduction

Aroids are foods of great cultural and economic importance throughout the humid tropics. In many countries, there is a desire to strengthen production by breeding for improved nutritional quality and agronomic performance. All species (*Alocasia macrorrhizos*, *Amorphophallus campanulatus*, *Colocasia esculenta*, *Cyrtosperma chamissonis*, *Xanthosoma sagittifolium*) are vegetatively propagated and are highly polymorphic. Morphological characterisation of germplasm collections is usually conducted on hundreds of accessions, each represented by few plants. Once the promising genotypes are identified, agronomic evaluation involves randomized field designs which allow the study of only a limited number of clones and genotypes. Their results are often biased by the heterogeneity of the clonal material (headsets, corms, cormels, suckers) needed to compose a plot treatment.

Breeding programmes start with the evaluation of germplasm followed by crossing the selected accessions. Crosses can be done in polycross plots where natural open pollination is used to produced half-sib progenies and when bulked together, populations for recurrent selection. Crosses are then conducted between parents chosen on their performance *per se* and presenting complementary traits. Controlled crosses done and aroid inflorescences can produce numerous full-sib progenies, often with several hundreds individuals per family. Controlled pollination allows the production of numerous seedlings but seedling growth is very slow and it takes about four years for a new genotype to be properly evaluated because of the time needed to produce enough homogenous clonal propagules for accurate agronomic comparison (Ivancic and Lebot, 2000). Once seedlings are
obtained, they must be clonally propagated and multiplied through clonal generations before accurate assessment of their performance can be conducted. The corm yield of the taro plant, for example, is correlated to the weight of the propagule planted and genotype performance is therefore, difficult to assess in F1s (seminal generation) or even the first (C1) or second (C2) clonal generations. It is necessary to reach a propagule weight comparable to those used in traditional cultivation before an accurate assessment can be made. The screening of large populations for major traits is consequently, a laborious and expensive operation which leads taro breeders to develop visual tools to speed up the process (Ivancic et al., 2003) and these traits are efficient for most agro-morphological traits. The significant genotype x environment interactions are limiting the relevance of complex field layouts and adding complexity to the evaluation process. Simple methodological alternatives are therefore needed for rapid and efficient evaluation and screening of numerous genotypes, prior to clonal distribution or use as parents in breeding programmes.

Depending on financial means, there is some variation between the existing programmes but the rationale is the same. Heavy selection pressure is applied at the seedling stage for resistance to diseases. This selection process is visual without any data recording in order to minimize the costs and maximize the number of genotypes assessed. The selected clones are then released as new varieties. A new selection cycle can begin, in which the new selected varieties are used as parents. Unfortunately, chemotypes with very attractive properties can be eliminated at an early stage because of the high selection pressure on other traits (Figure 1).

Each selected offspring individual can represent a new potential cultivar. However, the chances of getting a high yielding hybrid with excellent eating quality are very low and they become much lower when the selection procedure includes resistance against diseases. Corm yield and corm quality appear to be negatively correlated. Soft corms, with high water content, generally characterize high yielding early maturing hybrids. Unfortunately, the physico-chemical characteristics determining the quality of the corms are very expensive and laborious to assess. Low-cost methods for evaluation of numerous accessions need to be developed.

NIRS (Near Infrared Reflectance Spectroscopy) has been used to predict major constituent contents in maize (Berardo et al. 2009), rapeseed (Amar et al. 2009), sorghum (De Alencar Figueiredo et al. 2006), sugar beet (Roggo et al. 2004), malt (Marte et al. 2009), wheat (Rakszegi et al. 2008), potato (Haase, 2006) and tropical root and tuber crops (Lebot et al. 2009). NIRS has been intensively used for quality testing of hybrid lines from cereal breeding programmes around the World since the late 1970s with great success (Osborne 2006). More recently, Lebot et al. (2011) developed models based on 245 accessions of taro to predict concentrations in a 58 varieties with encouraging results. The \( r^2_{\text{pred}} \) values were 0.76 for starch, 0.74 for sugars, 0.85 for both proteins and minerals but amylase and cellulose could not be predicted.

These various studies indicate that NIRS could be a useful tool for aroids breeding, selection and quality control. However, the limiting factor for predictive models development remains the cost of chemical analyses (approx 100 euros per sample for five major components) which limit the number of accessions that can be used for comparison between chemical and spectral data. In the present study, we attempt to investigate the potential of new models developed on samples originating from different root crop species (cassava, sweet potato, yam, taro) and to test their predicating values on a set of 100 randomly chosen taro varieties. The results and their practical applications for breeding and improving the quality of Aroids corm and cormels are discussed.

**Materials and Methods**

**Chemical analyses.** Overall, 742 accessions representing varieties as well as hybrid lines from four different species and various geographical origins, were chemically analyzed (Table 1). One full corm, root or tuber was peeled and cut. Approximately 0.5-1 kg of fresh weight, corresponding to the central part of the underground organ, were manually sliced into chips and oven dried at 60°C for 48 h. Dry matter samples were split into two sub-samples: one sub-sample was used for chemical analysis and the other for NIRS. Samples of 200g were sent to Laboratoire d'Analyses Agricoles
Teyssier, Bourdeaux, France, for chemical analyses. Samples of approximately 50g of dried chips were milled into flour just after oven drying and dried chips were ground in a stainless kitchen steel mill (SEB, France) prior to NIRS analysis in Vanuatu.

Major constituents (starch, sugars, cellulose, total N and ash) were analyzed according to AFNOR (Association Française, the French standards association) and EEC methods (AFNOR, 2011). Following NF (Norme Française) V 18-109 for dry matter (DM) determination, samples were dried again to remove residual moisture (measured as % of total dry weight) and the powder was analyzed on an oven-dried air basis. Moisture was therefore expressed as a measurement of the sample prior to drying. All measurements were then expressed in %DM and the data were adjusted by the residual moisture following oven drying.

Starch was quantified using Ewers protocol (NF ISO 10-520) corresponding to hydrolysis in HCl, filtration and polarimetric measurement (specific rotation: 185.7°). Total sugars were quantified through the colorimetric method of Luff Schoorl (CEE 98\54\CE). Crude cellulose (total fibers) was measured by Weende method (NF V 03-040) which corresponds to non soluble organic residue obtained by sulfuric acid and alkaline treatments. Total N content (considered as equivalent total proteins) was calculated using the Kjeldahl method (NF V 18-100). Estimation of total minerals content was obtained from ashes produced at 550°C (NF V 18-101). All analyses were performed in duplicate with accepted mean coefficient of variation (SEL) of ± 3% for starch, sugars, cellulose, and residual moisture and ± 2% for proteins (equivalent N), and ashes (minerals).

**NIRS measurements and data pre-treatment.** Dry matter samples were milled into flour and granules size was homogenized using four sieves with decreasing diameters until granules passed through the 106 μm sieve. An ASD LabSpecPro spectrophotometer from Analytical Spectral Devices Inc. (ASD Inc., Boulder, Colorado, USA) fitted with a “muglight” or High Intensity Source Probe (HISP) (ASD Inc.) was used for the measurement of all spectra over the wavelength range of 350–2500 nm (Figure 2). On average, six grams of homogenized taro flour were placed in an individual cell and compacted with a tea spoon to eliminate air voids within the sample. Each spectrum was obtained by averaging three different cells (repetitions) per sample with 25 scans for each. A reference reading (baseline) was taken when starting a session and another every 30 min. All of the spectra were recorded in diffuse reflectance as \( \log(1/R) \) with respect to a Labsphere’s Spectralon material reflectance standard (Labsphere Inc., North Sutton, New Hampshire) which is a Lambertian reflective PTFE (thermoplastic resin) with high overall reflectance. For each sample, corresponding to individual accession, three sub-samples were scanned 25 times each and then averaged. The resulting averaged spectrum was recorded for the accession. Overall 742 spectra were recorded and converted to absorbance using the Indico software (ASD Inc.). In order to assess the performance of the calibration, samples were separated in two sets: the calibration and the prediction sets. The prediction set was created by randomly selecting 100 taro (Colocasia esculenta) accession numbers (approx. 13% of total 742 acc.) and the calibration set contained 642 samples.

**Data analysis.** Major constituents chemical data were subjected to multivariate analysis using XLSTAT (version 6.02, 2009). Multivariate analysis (Principal Component) of the spectra was conducted with GRAMS/AI (version 8.0). The spectra and reference data were mathematically modeled using with PLSPlus/IQ spectroscopy software (Thermo Electron Corporation, Ohio, US). Using the values obtained with chemical analyses as the analyte value, a separate calibration was made for each of the six major constituents. Calibration of residual moisture was not attempted because spectra were recorded in Vanuatu, just after oven drying the samples, while residual moisture was measured in France on hygroscopic dry raw material. Partial least-squares (PLS) regression technique was used to develop a predictive model of the near-infrared part of the spectra. The optimum number of PLS factors used for prediction was determined by full cross-validation and PRESS (Prediction Residual Error Sum of Squares). Additionally, light scattering effects due to particle size differences were corrected by multiplicative scatter correction (MSC). The data was mean-
centered and the average spectrum calculated from all of the calibration spectra and then subtracted from every calibration spectrum.

As part of the model process a Principal Component Analysis (PCA) was used to check for gross spectroscopic outliers. The Mahalanobis distance of each spectrum to the mean spectrum of the group was calculated and the removal of outliers was based on distance \( H > 3 \) from the average spectrum of the file. Spectra and concentration outliers were removed and PLS was run again until the highest \( r^2_{cv} \) (determination coefficient for cross validation) corresponding to the smallest SECV (Standard Error of Cross Validation) were obtained. At that point, factor loadings were used to determine which wavelengths were important to correlate with concentrations in order to narrow down the spectroscopic region. The loading plots showed which wavelengths were important to correlate with concentrations. The loading weights showed how much each wavelength point contributed to explaining the response along each model component. For starch, proteins and minerals, the regions used were 800-2400nm, while for sugars, and cellulose the region was 1200-2400nm. The PLS analysis was then conducted again on these new regions in order to obtain for each constituent, equations with higher explanation of the total variability in the calibration values without increasing the number of PLS factors used.

Statistical parameters used to evaluate models performances included the standard error of calibration (SEC), the determination coefficient for cross validation \( (r^2_{cv}) \), the standard error of cross-validation (SECV), the determination coefficient for prediction \( (r^2_{pred}) \), and the standard error of prediction (SEP). SEC and SEP were calculated using Excel spreadsheet by squaring the differences of the actual minus the predicted concentrations for each sample in the calibration (SEC) and test (SEP) sets. These values were then summed and the sum was divided by the number of samples \( (n) \). The square root of this value was used for SEC and SEP. SEC describes the calibration set (642 acc.) and SEP describes the test set composed of 100 taro samples not included in the calibration set. The ratio of performance to deviation (RPD= SD/SECV) was also used to evaluate performances of the models (with SD as the standard deviation of the original chemical data in the calibration set (Williams, 2003).

**Results**

Overall, 742 accessions were analyzed for the chemical variation of their major constituents. Results of the chemical analyses are presented in Table 1. Significant variation was observed for all major constituents. The least variable constituent was starch (CV\%= 10.50) and the most variable was total sugars (CV\%= 83.25). Correlation coefficients calculated between major constituents indicate that starch content is positively correlated with %DM but negatively correlated with sugars, cellulose, proteins and minerals contents (Table 2).

Principal Component Analysis conducted on the data matrix (742 acc. x 5 major constituents) reveals the respective contribution of the five variables to the projection, with axes 1 and 2 totalizing 72.49% of the total variance (Figure 3).

The comparison of the NIRS spectra and the chemical values allowed the establishment of equations of calibration for the prediction of starch, sugars, proteins (equivalent N) and minerals. The results are presented in Table 3. For starch, the SECV (2.11%) and SEC (2.11%) values are identical indicating robust fitting. The SEP (2.34%) is not too distant and the \( r^2_{pred} \) of 0.79 indicates an acceptable estimation of the equation accuracy on the 100 validation samples. Deviations of single samples are visualized in a scatter plot between measured and predicted starch values of the 58 acc. in the test set (Figure 5A). In terms of predictive performance, the equations for starch could be considered as good with RPD parameters close to 4. Some authors claim that a RPD value of at least 3 is necessary for efficient NIR reflectance predictions with values above 3.5 indicating a very good predictive model (Williams, 2003).

The total sugars model also presents similar SECV (1.30) and SEC (1.32) values but the SEP is not too far (1.40) and the \( r^2_{pred} \) is of 0.80. The RPD value of 4.11 indicates a good predictive potential for this equation. Deviations of single samples are visualized in a scatter plot between measured and
predicted sugars values (Figure 5B). Cellulose could not be satisfactorily predicted and a poor $r_{cv}^2$ (0.31) was obtained, with very low $r_{pred}^2$ (0.13) and RPD (1.91). Proteins, (measured as total N equivalent) produce similar SECV, SEC and SEP values (respectively 0.51, 0.53 and 0.63) and a $r_{pred}^2$ of 0.78 indicating good and robust prediction with 78% of confidence. However, the RPD value above 6.0 confirms a very good potential of prediction for this model. If the $r_{pred}^2$ value is not higher than 0.78, this might be due to the fact that the samples selected for the validation test were first of all chosen on the decreasing value of their starch contents, not on proteins, and that therefore they might not represent properly the extent of variation found in proteins. Deviations of single samples are visualized in a scatter plot between measured and predicted proteins values (Figure 5C). Minerals are known to have a poor relationship with NIRS but they presented similar SECV, SEC and SEP values (respectively 0.46, 0.51, 0.38) and could be predicted with 87% of confidence with a good RPD value of 2.52. Deviations of single samples are visualized in a scatter plot between measured and predicted minerals values (Figure 5D).

The $r_{pred}^2$ values of starch, sugars, proteins and minerals are high enough to allow good estimates of their contents. RPD between 3.90 and 4.11 for the starch and sugars models, also allow good quantitative predictions to be made. Values above 2.5 for proteins are considered to be good models (Williams, 2003) and the value here is above 6.0. The number of terms is also relatively low if we consider a general recommendation of 1 factor for every 10 samples in a model (Table 3).

Models for starch, sugars, proteins and minerals present good potential but will need to be further tested on independent samples. When SECV and SEP values differ significantly, this could be an indication that too many samples (HT >3 = 21) were removed during the modeling process.

The models developed in the present study show good accuracy but it remains to be seen whether larger sample sets will improve them to enable more precise prediction. When comparing the performance of the new calibration models (with n=642 from different species), with the values reported by Lebot et al. (2011) for taro with 245 accessions in the calibration set and only 58 accessions in the validation set, high $r_{cv}^2$ and RPD values were confirmed. Determination coefficients ($r_{pred}^2$) generally improve as the working range increases. Consequently, if more range is added in the same model then it could improve coefficient values. Additionally, when different samples are added, a larger spectroscopic diversity is described and, therefore, some samples might actually be better spectrally described as the number of samples in the calibration set increases. However, determination coefficients for the prediction set ($r_{pred}^2$) cannot reflect the whole situation because the range of the 100 accessions values affects the coefficient values. These values change according to the type and number of validation samples and it is necessary to consider the long term effects. Errors of prediction values have been shown to have uncertainties and it is therefore recommended to be cautious while reporting prediction errors because they may change according to the validation set used (Sileoni et al., 2011). SEP is, therefore, a better overall indicator. A better sample selection might be helpful by selecting, for example, on constituent concentration rather than a random selection of numbers. Further work should concentrate on validating the results over different years.

The models for starch, sugars, proteins and minerals present potential for improvement if more samples could be added. The protein content calibration is particularly interesting as it can be further improved. Proteins content is usually estimated by multiplying the total N content by a standard conversion factor of 6.25. However, the nitrogen to protein ratio does vary according to the species considered and change with amino acid content and mineral nitrogen and non-protein nitrogen. For the present study, we decided to present our results measured as total N as proteins. In the future, it would of interest to improve the calibration models on the real protein content of taro which vary according to amino acids. Once known, the values obtained by the Kjeldahl method could be converted into more accurate measurements for NIRS calibrations on taro.

In taro breeding programs, mass selection results in the rapid accumulation of suitable genes but has to be complemented with efficient screening techniques of hundreds of hybrids generated in controlled crosses. Correlation coefficients between major constituents indicate that breeding for increased DM and starch contents will reduce sugars, proteins and minerals. These correlations do not present practical problems as “poor” quality varieties have been shown to present low DM and
starch and high sugars, cellulose, proteins and minerals (Lebot et al., 2011). Obviously, NIRS could assist taro breeders in their choice and selection of the best genotypes, based on the chemical composition requested by consumers by predicting simultaneously starch, sugars, proteins and minerals on a single sample. As starch is significantly negatively correlated with the other three major constituents, the simultaneous prediction of all four constituents allow for rapid estimation of the variety chemotype and therefore its quality.

Taro is a diploid species but nothing is known on the segregation of these major constituents. The problem is rather complex as these constituents are most likely controlled by many sets of different genes and molecular tools can hardly be used for markers assisted selection and conventional selection of parents for breeding or selection. NIRS offer interesting perspectives for spectra assisted selection.

Abbreviations Used

SEC: standard error of calibration,
SECV: the standard error of cross-validation,
SEP: the standard error of prediction,
SEL: the standard error of the laboratory analysis,
$\hat{r}_c^2$: the determination coefficient for cross validation,
$\hat{r}_p^2$: the determination coefficient for prediction,
RPD: the ratio of performance to deviation.
H: Mahalanobis distance limit.
HT: number of outliers removed.
%DM: percentage of dry matter.
CV%: coefficient of variation.

Acknowledgements

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References


### Table 1. Descriptive statistics on 742 accessions

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### Table 2. Correlation coefficients (Pearson (n-1)) for 742 accessions:

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<td>- 0.252</td>
<td>- 0.147</td>
<td>+ 0.010</td>
<td>+0.336</td>
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### Table 3. Statistical Parameters of the New Calibration and Validation Sets.

<table>
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<th>Constituents*</th>
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<th>Validation</th>
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</table>

*starch, proteins, minerals in 800-2400, sugars in 1200-2400
Figure 1:

Figure 2
Figure 3

Observations (axes F1 et F2 : 72.49 %)

Figure 4

Proteins on Independent Test Set: R² = 0.78
Y1 = 0.6830 + 0.8096 * X1
Starch on Independent Test Set: $R^2 = 0.79$

$Y_1 = 13.0743 + 0.83477 \times X_1$

Sugars on Independent Test Set: $R^2 = 0.80$

$Y_1 = 0.5325 + 0.8744$

Minerals on Independent Test Set: $R^2 = 0.87$

$Y_1 = 0.1339 + 0.9762 \times X_1$
Biofortification of taro (*Colocasia esculenta*) through breeding for increased contents in carotenoids and anthocyanins

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**Abstract:**

Biofortification of taro (*Colocasia esculenta*) has never been studied. The aim of the present study is to compare the chemical compositions and individual constituent contents for major compounds (starch, sugars, cellulose, proteins, minerals), carotenoids and anthocyanins between parents and hybrids selected first for their agronomic performance and second for their corm characteristics (flesh colour, quality and taste). For major compounds, 45 selected hybrids were compared to 66 cultivars and for carotenoids and anthocyanins, 34 selected hybrids were compared to 79 cultivars. All plants were planted the same day within the same plot and harvested together to avoid environmental factors effects. Total sugars, cellulose and minerals contents presented moderate increases in hybrids. Carotenoids and anthocyanins contents were correlated with corm flesh colours. Anthocyanins contents could not be increased in the selected hybrids. However, total carotenoids contents were increased by more than four folds in the hybrids. The results of this study indicate that carotenoids contents can be rapidly improved by selecting plants of good agronomic performance and corm shape with increased density of yellow and orange colours. Potential applications to taro breeding programmes are discussed.

**Keywords:** anthocyanins, biofortification, carotenoids, primary compounds, taro

**Introduction**

Taro (*Colocasia esculenta*) is an important staple in many developing countries, particularly in the Pacific and in Melanesia where the annual consumption per inhabitant is among the highest in the world. Many traditional varieties exist and there is now a need to improve production and corm quality. In spite of its importance, taro is under-researched with insufficient knowledge on its micronutrients, especially carotenoids and anthocyanins.

A deficiency in vitamin A can lead to blindness and is a serious problem in developing countries. In the Pacific, vitamin A deficiency has been diagnosed since lifestyle changed and there is now a need to identify sources of provitamin A among locally-grown staple foods (Englberger et al., 2008). Human health benefits have also been shown to be related to non-provitamin A carotenoids. Lutein and zeaxanthin, for example, are found in the macula area of the human retina and are associated with a reduced risk of age-related retina degeneration (Mares et al., 2006). More generally, natural carotenoids protect the skin from damaging solar radiations and reduce the risk of developing certain cancers (Stahl and Sies, 2005). Carotenoids also exhibit antioxidant properties and their regular consumption from fresh product has been proven to bring numerous health benefits (Miller et al., 1996; Sies and Stahl, 1995; Stanner et al., 2004). Since carotenoids have such a beneficial impact on human health, biofortification of taro, and other aroids, has the potential to play a role in health improvement programmes.
Phenolic compounds including flavonoids (e.g., anthocyanins, flavonols or flavanols) and phenylpropanoids (e.g., cinnamic acids, or cinnamic acid esters) are metabolites considered as valuable natural products. They exhibit different functional properties. The human health benefits of anthocyanins intake have been thoroughly investigated. Evidence suggests that phenolic compounds consumption may protect from some disorders including cancers (Ding et al., 2006; Yang, et al., 2001), cardiovascular diseases (Bell and Gochenaur, 2006; Ross and Kasum, 2002) and age-related neurodegenerative declines (Lau et al., 2005).

Carotenoids and phenolic compounds composition and content of taro have been studied and remarkable variation was observed among varieties (Champagne et al., 2010, 2011). Taro genetic improvement is based on the selection of genotypes on their per se value. The identification of parents with complementary traits, their recombination and the subsequent screening of large full-sib families are required to identify improved hybrids. Breeders need well-designed technologies and reliable data to select the most suitable parents and progenies. The limited knowledge available on taro secondary metabolites is a serious constraint to its biofortification through breeding. Major compounds (starch, sugars, cellulose, proteins, minerals) and secondary metabolites are involved in the palatability of traditional varieties. Colours are linked to the presence of secondary metabolites such as carotenoids and flavonoids and are essential traits of different cultivars. Moreover, the traditional selection system practiced by local farmers for attractive and palatable chemotypes has generated tremendous diversity which deserves to be studied. Because of socio-cultural requirements and traditional exchanges of germplasm, smallholders pay a particular attention to morphological variation, such as corm flesh colours which are directly determined by their physico-chemical composition.

Plant biofortification is the nutritional enhancement of crops through breeding. Various breeding programmes are now improving major compounds such as proteins and sugars, but also secondary metabolites such as carotenoids and anthocyanins. These programmes start with the evaluation of germplasm followed by crossing the selected accessions. Crosses can be done in polycross plots where natural open pollination is used to produce half-sib progenies and, when bulked together, populations for recurrent selection. Once seedlings are obtained, they must be clonally propagated and multiplied through clonal generations before accurate assessment of their performance can be conducted. Depending on their scope, programmes may differ slightly but their rationale remains the same. Heavy selection pressure is applied at the seedling stage for vigour and resistance to diseases. This selection process is visual without any data recording in order to minimize the costs and maximize the number of genotypes assessed. The selected clones are then released as new varieties. A new selection cycle can begin, in which the new selected varieties are used as parents. Unfortunately, chemotypes with interesting properties can be eliminated because of high selection pressure on other traits. The selection process is equivalent to mass recurrent selection since great numbers have to be screened to achieve some progress. Each individual offspring can be selected to yield a new cultivar. Most current biofortification programs use this method (Bradshaw, 2010).

Previous studies have unveiled opportunities for improvement as a result of high variation and particular relationships between compounds (Champagne et al., 2009). Starch content in taro is negatively correlated to other major compounds (Lebot et al., 2011). Local consumers are also favouring certain chemical compositions. A chemical composition suitable for preparing a good traditional dish laplap in Vanuatu (a pudding-like dish) is more complex to fulfill than for daily consumption through simple boiling. The same is observed with the West African fufu prepared from taro corms. Varietal characteristics are of utmost importance. The objectives of the present study were to i) assess progresses achieved within current breeding lines in order to confirm biofortification potentials, ii) evaluate conventional breeding method for biofortification in major compounds (starch, sugars, proteins, minerals and cellulose) and secondary metabolites (carotenoids and anthocyanins). The selection process and its perspectives are then discussed.
Materials and Methods

Origin of genotypes
Most cultivars used as parents were from Vanuatu and were selected for corm quality (Bourrieau, 2000). Others were from South-East Asia (Lebot et al., 2005). Taros from both genetic pools were cross-pollinated via controlled crosses to produce highly variable progenies. Seeds were mixed before planting to generate a highly variable population (Lebot et al., 2004). Cultivars and hybrids were grown together on the same plot at Téouma (17°45'S/168°18'E, on Efate island in Vanuatu) to prevent variation due to environmental factors. Accessions were planted at the same time and harvested at optimum maturity, to avoid ontogenic variation. Taro hybrids were selected for major agronomical traits through four consecutive generations (the seminal, F₁ and three clonal ones, C₁, C₂ and C₃):

- F₁ or seminal generation (originally from seeds): hybrids were mostly selected for agronomic performance (vigour), the major taro pathogen (*Phytophthora colocasiae*) being absent from Vanuatu.
- C₁ or first clonal generation: hybrids were selected again for the same trait and for yield which was estimated from individual weights. Low yielding hybrids (< one kilo per plant) were discarded.
- C₂ or second clonal generation: 5 to 10 plants per hybrid were evaluated for the same previous traits with an additional care to quality characteristics. Shape of the corms should be smooth and oblong. White flesh was not favoured because local consumers prefer coloured flesh. However, all white fleshed genotypes were not eliminated because some produced nice corm shapes and high yields. Corms hardness (ease of hand-cutting the central section) was also evaluated because it is related to dry matter content. Watery corms were rejected.
- C₃ or third clonal generation: 10 to 20 plants per hybrid were evaluated for all of the previous traits plus for tasting quality through consumption preference by local staff. These assessments were mainly qualitative and sensorial and were conducted on thousands of hybrids. About 90% of the hybrids were eliminated during these three successive selection steps. Selected hybrids were compared to the parent cultivars used for breeding. Quantitative evaluation is still in progress to reduce hybrids number.

Samples preparation
Accessions analysed for major compounds involved 66 cultivars (parents) and 45 C₃ hybrids. Accessions analysed for carotenoids and anthocyanins included 79 cultivars (parents) and 34 C₃ hybrids. For major compounds, corms were peeled, washed, dried with a towel and cut into 2 mm thick transversal slices. Slices were dried in a ventilated oven at 60°C until constant weight. The dried flours were enclosed in paper bags and kept in a dry place until analysis. For carotenoids and anthocyanins, the preparation procedure was very same that previously described (Champagne et al., 2010, 2011)

Primary compounds analysis
Analyses of major compounds were performed as previously described (Champagne et al., 2009). Briefly, about 150 g of dry matter prepared in Efate (Vanuatu) were sent to France, where residual moisture, starch, sugars, proteins, minerals and cellulose were quantified. For dry matter determination, samples of flours were dried again in an air oven to remove residual moisture and then analyzed on a dry matter basis according to NF (Norme Française) V 18-109. Mineral contents were estimated from ash produced at 550°C (NF V 18-101). Crude cellulose was measured using Weende’s method (NF V 03-040). Protein content was measured using the Kjeldahl method (NF V 18-100) for quantification of total nitrogen (N x 6.25). After starch extraction, reducing sugars were estimated using the standard iodometric method of Luff Schoorl (CEE 98\54\CE). Starch was quantified using the Ewers protocol (NF ISO10-520). All measurements are expressed in percentages of dry matter (DM).
Reagents and standards
Acetone, methanol and chlorhydric acid were purchased from VWR Int. (Fontenay-sous-Bois, France). Tert-butyl-methyl-ether, ammonium acetate and ethyl acetate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Acetic acid was purchased from J.T. Baker (Phillipsburg, USA) and ethylic ether from Cooper (Melun, France). All-trans-β-carotene and all-trans-lutein were pure standards purchased from Carotenature GmbH (Lupsingen, Switzerland) and lycopene from Sigma-Aldrich Co. (St. Louis, USA). Phytoene and zeaxanthin were obtained from Escherichia coli harbouring the plasmids pAC-PHYT and pAC-ZEAX kindly provided by Dr F.X. Cunningham Jr. (University of Maryland, USA). Carotenoids were extracted from bacteria cultures using ethyl ether. Cyanidin-3-glucoside was purchased from Extrasynthese (Genay, France).

Carotenoids extraction and analysis
The extraction method was performed as previously described (Champagne et al., 2010). Briefly, a 2-4 g freeze-dried powder sample was homogenized in 10 mL acetone using a polytron Biotrona 6403 (Küssnacht, Switzerland). To ensure full recovery of analytes, knifes were rinsed with 5 mL of acetone and the 5 mL was then pooled with the first 10 mL. The sedimentation of the powder was achieved by centrifugation at 4°C at 3000 g for 10 min. The supernatant was recovered with a Pasteur pipette and the extraction process was repeated on the pellets. In order to guarantee optimal extraction conditions, the process optimization was followed using High-Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD). The DAD was set at 460 and 290 nm for coloured and non-coloured carotenoids. Generally two to four extractions were needed for optimal results. Each extract was evaporated to dryness under a nitrogen stream. The extracts were then dissolved in an adequate volume of ethyl acetate and filtered on a 0.45 μm PTFE filter (C.I.L., Sainte-Foy-La-Grande, France) prior to injection.

Carotenoids were analyzed by HPLC-DAD using the method described by Fraser et al. (2000) with modifications. Extracts were separated on a Spectra system (Thermo Finnigan) equipped with a reverse-phase C30 column (YMC Inc. Europe GmbH, Germany), 5 μm, 4.6 x 250 mm. The mobile phases were methanol as eluent A, methanol/ammonium acetate 1% in water (5:1, v:v) as eluent B, and tert-butyl-methyl ether as eluent C. The injection volume was 50 μL, the flow rate was set at 1 mL·min⁻¹ and the column temperature was set at 25°C. The gradient program was performed as follows: initial conditions 0-12 min, 95%A /5%B; 12-12.1 min, to 80%A /5%B/15%C; 12.1-40 min, to 30%A /5%B /65%C; 40-43 min, to 5%B /95%C; 43-46 min, 5%B /95%C; 46-49 min, to 95%A /5%B; and back to the initial conditions for re-equilibration. Carotenoids were monitored between 200 and 800 nm with an UV-visible DAD (UV-6000, Thermo Finnigan). Data were collected and processed using Chromelone software v.6.60 (Dionex Co., Sunnyvale, USA). When sample were shown to be over-concentrated via HPLC run, it was re-analyze with higher dilution. An external astaxanthin standard was injected daily to monitor repeatability of the HPLC analytical separation through changes in retention time, and repeatability of the detection through peak area. All compounds eluted in 45 min.

Anthocyanins extraction and analysis
The phenolic compounds were extracted from the corm using a method previously described (Champagne et al., 2011). Briefly, the procedure was optimized using HPLC-DAD monitoring. A 2 g freeze-dried powder was homogenized in 15 mL of 7% acetic acid in milliQ water (v/v) using a polytron Biotrona 6403 (Küssnacht, Switzerland), knifes were rinsed with 5 mL of 7% acetic acid in milliQ water then the 5 mL were pooled with the first 15 mL. Extraction took place in a capped opaque tube at room temperature overnight and under stirring. As water used as an extraction solvent yields an extract with a high content of impurities, solid phase extraction (SPE) was used to eliminate non-phenolic compounds including organic acids, sugars, and soluble proteins, among others. Samples were purified and concentrated with a SPE system from Grace-Alltech, GracePure SPE C18-Max, 500 mg/3 mL (Columbia, USA). For each step of the purification procedure, samples were kept away from light and when possible, were maintained at 4°C. SPE columns were pre-
treated with 3 mL of 0.1% HCl in methanol (v/v) and were balanced with 6 mL of 7% acetic acid in milliQ water (v/v). Residues contained in the extraction solution separated by centrifugation at 4°C, 4800 g, for 10 min. Supernatants were applied to the SPE column, and then bounded phenolics were washed with 6 mL of 7% acetic acid in milliQ water. In order to keep anthocyanins in the red flavylium form which confers higher stability, they were eluted with 1 mL of 0.1% HCl (v/v) in methanol, prior to injection. The recovery rate of the SPE procedure was calculated as the percent ratio between the quantity found after the purification step and the amount added as standard. The rate was 95.2% for cyanidin-3-glucoside (expressed as the mean of triplicates). This rate was used to calculate anthocyanin contents.

A volume of 20 μL of the anthocyanins extract solution was injected by an automated sample injector ASI-100 and gradient was performed with a HPLC pump P680, both from Dionex Co. (Sunnyvale, USA). Separation was performed on a reversed-phase Ultrasphere column 250 x 4.6 mm, 5 μm particle sizes fitted with an Ultrasphere C18 guard column 45 x 4.6 mm purchased from Beckman Coulter Inc. (Fullerton, USA). The column and guard column temperature was maintained at 25°C with an external oven Igloo-CIL Peltier C.I.L. (Sainte-Foy-La-Grande, France). Solvent A was composed of water and formic acid (90/10, v/v), and solvent B of methanol and formic acid (90/10, v/v). A binary gradient at 0.6 mL.min⁻¹ flow rate was used. The initial conditions were 90%A/10%B; 0-40 min, to 60%A/40%B; 40-44 min, to 100%B; 44-48 min, back to the initial conditions, and finally 48-70 min for re-equilibration. Anthocyanins were monitored at 520 nm with an UV - Visible DAD 340U Dionex Co. (Sunnyvale, USA). Data were collected and processed using Chromeleon software v.6.60 (Dionex Co., Sunnyvale, USA). For anthocyanins seen on HPLC chromatograms of extracts, detection and quantification were based on their maximum absorption wavelengths (λmax), therefore at 520 nm. All compounds eluted in 42 min.

**Determination of peak purity and identification**

Carotenoids quantification was achieved by external calibration curves made of five carotenoids standard concentrations injected in triplicate. Concentrations were calculated using molar extinction coefficients. Correlation coefficients were linear (r ≥ 0.98). When available, contents were calculated with HPLC-quality standards. Unidentified compounds were labelled as follows: a figure representing characteristic retention time (in minutes), preceded by C for carotenoids and A for anthocyanins (i.e. C24.9: a carotenoid exhibiting a retention time of 24.9 min).

The purity of carotenoids standards was determined by HPLC-DAD and checked to be over 90% for each standard (Rodriguez-Amaya and Kimura, 2004). Each peak was checked to make sure it displayed the same characteristic spectrum on the ascending and descending slopes and at maximum. When standards were not commercially available, contents were expressed as all-trans-β-carotene equivalent (βCeq). For instance, cis-β-carotene isomers were quantified as all-trans-β-carotene. For anthocyanins, standard and peak purity were checked as previously mentioned for carotenoids. However, all contents were expressed as cyanidin-3-glucoside equivalent (CGeq). Total contents were estimated on basis of total peaks area at 460 and 520 nm and therefore expressed in βCeq and CGeq, respectively for carotenoids and anthocyanins. For carotenoids, phytoene contents determined at 290 nm were added.

**Colours assessment**

Colour codes ranging from 1 to 7 were attributed to each storage organ at harvest. Colours were assessed visually. The corm flesh colour code was attributed as follow: 1 = white, 2 = yellow, 3 = orange, 4 = pink, 5 = red, 6 = light-purple, 7 = dark-purple, several numbers imply several colours, e.g.: 3-6 = orange-light purple.

**Statistical analyses**

Coefficients of variation of the mean were calculated to provide an estimate of the dispersion of a probability distribution of specific datasets and are reported as percentages (CV%). Linear correlation analysis was conducted to establish the relationships between corm flesh colour and carotenoids and
anthocyanins contents with the free open source software environment for data analysis and graphics, R version v2.9.0 (9) and the “RcmdrPlugin. FactoMineR” packages with mean-centred data scaled to unit variance. Relationships between major compounds, visually determined corm flesh colour codes, and quality were estimated by calculating Pearson’s product-moment correlations. Significance was determined using Student’s t test. Analyses of variance were carried out using transformed data if required. Significance of differences among least square means was determined by Turkey’s test ($P < 0.05$).

**Results and discussion**

**Impact of selection method on major compounds**

Taro is a staple across the Pacific and local farmers practice traditional selection by mainly focusing on starch contents. This could explain the very low CV% for starch in the 66 cultivars (Table 1). Variability of other major compounds is large and congruent with previous results. The average starch content (and maximum and minimum values) was higher in the group of cultivars than in hybrids in opposite to average total sugar contents and in agreement with previous reports on the inverse relationship between the accumulations of these two substances in taro (Champagne *et al.*, 2009, Lebot *et al.*, 2011). The hybrids exhibited lower CV%. Not intentionally, our selection method may have, therefore, favoured hybrids with high total sugars content and this could reduce starch levels. Variability in total proteins contents was significantly enhanced in hybrids compared to cultivars, with respective CVs of 48% and 25% respectively (Table 1).

Our selection method decreased the variability in minerals content (lower CV%). Though the mean value was virtually unchanged, maximum values for mineral content were severely curbed. Taste quality of hybrids was frequently evaluated by the local technical staff of the VARTC research station. It appeared that minerals content plays a crucial role in consumers’ acceptance. These results confirmed preliminary results reporting that a "good" variety must exhibit high dry matter and starch contents coupled with low total minerals content (Bourrieau, 2000, Lebot *et al.*, 2004, Lebot *et al.*, 2011).

It has previously been also shown that mid-range starch content is preferred for varieties to be boiled and roasted rather than transformed into *laplap* (Champagne *et al.*, 2009). Biofortification of sugars, proteins and minerals therefore seems achievable for “table” type of varieties. However, as these are negatively correlated with starch, it is expected that the starch content will be reduced. Genetic improvement for higher starch content has to be done within an independent set of hybrids where starch and dry matter contents can be jointly improved as the two are positively correlated (Lebot *et al.*, 2011).

**Impact of selection method on carotenoids**

Contents of total and major carotenoids varied greatly among cultivars and hybrids (Table 2). Coupled with previous results based on comparative evaluation of different clones of the same genotype to double check the robustness of the quantification, this indicates again that individual secondary metabolites contents are genetically controlled (Champagne *et al.*, 2010). A previous study has shown that satisfaction of local consumers’ preferences is not compromised by an enhancement of flesh colour (*i.e.* by increasing carotenoids content) (Champagne *et al.*, 2009). Maximum content of each individual carotenoid was higher in hybrids compared to cultivars, except for lutein (Table 2). More particularly, an increase from 0.17 to 2.04 µgβCeq/gDM of P25.5 content was observed (unidentified compound, putative cis-isomer of β-carotene). All-trans-β-carotene maximum value increased by a factor of 3.5. The average content of all carotenoids increased in the hybrid group. For instance, all-trans-β-carotene and zeaxanthin were respectively increased by a factor of 7 and 36. These results were in opposition to CV% variations that exhibited remarkable decreases in hybrids, most likely due to lesser sample size. Surprisingly, phytoene was detected in small amount in 4 hybrids, while it was not detectable in the parent cultivars. The same was observed with lycopene which was found in only one hybrid. Since phytoene and lycopene are non-cyclic carotenoids found in earlier steps of the carotenoid biosynthetic pathway, their detection may
result from either a disturbance of downstream enzymatic steps or from a global enhancement of the whole biosynthetic pathway in the hybrids.

**Impact of selection method on anthocyanins**

The largest peak found in *C. esculenta* purple-fleshed genotypes corresponded to compound A22.1 and represented 57-74.5% of the total peak area. Only two previous studies identified anthocyanins in *C. esculenta*, the first as pelargonidin-3-glucoside, cyanidin-3-rahmnoside and cyanidin-3-glucoside (Chan and Kao-Jao, 1977), and the second as cyanidine-3-rutinoside (Terasawa et al., 2007). However, our analyses could not reveal the presence of pelargonidin-3-glucoside in *C. esculenta*. Cultivars exhibited mid-range anthocyanins contents. No anthocyanins were found in the widely consumed local Fijian cultivars of taro (Lako et al., 2007), indicating that Vanuatu cultivars are richer in anthocyanins. As expected, the white-fleshed cultivars displayed no detectable anthocyanins.

A significantly lower content was observed for most anthocyanins in hybrids than in cultivars (Table 3). This was particularly clear for maxima of both individual and total anthocyanins contents. The only compound which maximum and CV% were almost unaffected was A28.5. Nevertheless, the major anthocyanin (A22.1) content correlated to total anthocyanins content and showed higher CV% and lower means. A broader distribution of anthocyanins contents was thus revealed in the hybrid group, coupled with decreased maxima. The maximum hybrid value was clearly lower than for cultivars which maximum value of 243.3 µgCGeq/gDM was close to a previously reported value of 265.6 µgCGeq/gDM despite our wider sampling of 113 taros against 33 (Champagne et al., 2011).

After grouping accessions into classes corresponding to flesh colour, classes were sorted by ascending order of total carotenoids (Table 4) or total anthocyanins means (Table 5). This revealed that yellow flesh taros exhibited the smallest anthocyanins contents, while dark-purple taros exhibited the highest. In addition, higher total anthocyanins contents were found in orange and white flesh taros than in yellow ones though they were very small compared to the values observed in the pink-purple varieties. Orange cultivars had the highest total carotenoids (mostly made of isomers of β-carotene) contents. The red varieties contained surprisingly high levels of carotenoids. Because red carotenoids were not detected and these varieties contained high anthocyanins content, it is very likely that the anthocyanins masked the carotenoids to the human eye. Tough not apparent from these tables, a colour gradient was obvious when observing sliced-open corms of light-purple fleshed taro (Fig. 1). The younger part (the proximal, upper end) was more coloured (darker). This colour gradient was not observed in yellow fleshed corms but existed in orange fleshed ones where it went from orange at the proximal end to yellow at the distal end.

**Chemotypes promoted by the selection method**

Variations in major compounds have previously been studied within a larger sample size and are therefore not discussed here (Champagne et al., 2009, Lebot et al., 2011). However, taro carotenoids and anthocyanins compositions and contents have never been studied on such a broad sample scale. The extent of variation unveiled by the present study indicates that biofortification opportunities exist.

Selection for corm characteristics was applied during the second and third clonal generations only, not in F1 and C1 (Fig. 2). In addition, selection based on corm shape was also combined with a selection for hybrids with coloured corm fleshes. Therefore, the white fleshed corms were usually discarded because they were not attractive to local consumers in Vanuatu. Also, the export markets to New Zealand and Australia are looking for pink flesh taros. Our selection also discarded accessions with low dry matter content. The present study indeed revealed that our selection process promoted high soluble sugars and carotenoids, and low minerals and anthocyanins, contents simultaneously. In addition, lower CV% among hybrids compared to cultivars suggested that the selection pressure reduced the range of variation of secondary metabolites content. Taken together, these findings reveal the impact of the selection method and indicate that potential maxima are not reached yet. Consequently, biofortification opportunities still exist for improving taro corm quality.
In the Pacific, taro is an ancient crop. It is a diploid species and genetic analyses have revealed that a narrow genetic base was introduced in Vanuatu (Kreike et al., 2004; Caillon et al., 2006; Quero-García et al., 2004, 2006). Among the 79 parents (cultivars) used to produce hybrids, only eight had a non-Vanuatu origin. Since this crop is traditionally very important in Vanuatu, morphotypes corresponding to local preferences were prioritised by the method. The principal use of taro is evidently related to starch and its content appeared to be relatively stable (Table 1).

Conclusions
This study represents a first attempt to assess the biofortification potential of taro through breeding for increased carotenoids and anthocyanins contents. Previous studies have shown that variation in major compounds is genetically controlled and that there is tremendous potential for genetic improvement by crossing parents with suitable chemotypes. Dry matter and starch contents are positively correlated to each other but negatively correlated to other compounds (sugars, cellulose, proteins and minerals). As quality of taro corm has been shown to be closely related to starch and dry matter contents and negatively related to proteins, cellulose, sugars and minerals content, the focus on starch is likely to improve quality if it does not go beyond a certain point (approx. 78-82%DM) to avoid dryness in the mouth.

The present study has shown that it is fairly easy to improve total carotenoids content through this simple breeding process. The situation for anthocyanins is more complex. Though orange taros had the highest carotenoids contents and deep-purple taros the highest anthocyanins contents, the simple screening of a germplasm by eye or with a chromameter may not be the best option. Chromameter measurements may be biased by the existence of orange and red pigments gradients within tubers. Red corms were also shown to contain significant quantities of both anthocyanins and carotenoids. Though this demonstrates that simultaneous biofortification for both classes of compounds is possible, it reveals that the human eye may be made blind to some pigments due to colour co-suppression effects.

Acknowledgements
This research was financially supported by the Food Security Thematic Programme of the EU (grant no DCI-FOOD/2010/230-267) and the International Network for Edible Aroids (INEA, www.EdibleAroids.org).

References


Table 1. Variation of major compounds for 66 parents (cvs) and 45 hybrids (hyb) of taro (expressed as percentage of DM).

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<td>6.58</td>
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<td>7.8</td>
<td>66.0</td>
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</tbody>
</table>

Table 2. Variation in major and total carotenoid content for 79 cvs and 34 hyb of taro from polycross (mg/gDM).

<table>
<thead>
<tr>
<th></th>
<th>lutein</th>
<th>zeaxanthin</th>
<th>phytoene</th>
<th>C25,5</th>
<th>13-cis-β-carotene</th>
<th>all-trans-β-carotene</th>
<th>9-cis-β-carotene</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>cvs</td>
<td>hyb</td>
<td>cvs</td>
<td>hyb</td>
<td>cvs</td>
<td>hyb</td>
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<tr>
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<td>0.14</td>
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<tr>
<td>Max</td>
<td>5.58</td>
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<td>104.23</td>
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<tr>
<td>Mean</td>
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<td>0.28</td>
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<td>0.01</td>
<td>0.37</td>
<td>0.50</td>
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<td>0.28</td>
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<tr>
<td>CV%</td>
<td>164</td>
<td>328.3</td>
<td>400.1</td>
<td>305.7</td>
<td>571.5</td>
<td>343.6</td>
<td>528.3</td>
<td>221.7</td>
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<tr>
<td>Median</td>
<td>0.06</td>
<td>0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.16</td>
<td>0.02</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* in all-trans-β-carotene equivalent

Table 3. Variation in major and total anthocyanins content for 79 cvs and 34 hyb of taro from polycross (mg/gDM).

<table>
<thead>
<tr>
<th></th>
<th>A21.0*</th>
<th>A22.1*</th>
<th>A24.2*</th>
<th>A26.6*</th>
<th>A28.5*</th>
<th>Total*</th>
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<tbody>
<tr>
<td></td>
<td>cvs</td>
<td>hyb</td>
<td>cvs</td>
<td>hyb</td>
<td>cvs</td>
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<td>Max</td>
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</table>

* in cyanidine-3-glucoside equivalent
Table 4. Variation in major and total carotenoids content for 113 accessions of taro grouped by flesh colour.

<table>
<thead>
<tr>
<th>Flesh color</th>
<th>Nb. Acc.</th>
<th>Means expressed in mg/gDM</th>
<th>Lutein</th>
<th>Zeaxanthine</th>
<th>Phytoene</th>
<th>C25,5*</th>
<th>13-cis-β-carotene</th>
<th>all-trans-β-carotene</th>
<th>9-cis-β-carotene</th>
<th>Total*</th>
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<tbody>
<tr>
<td>4</td>
<td>16</td>
<td></td>
<td>0.05^a</td>
<td>0.01^a</td>
<td>0.01^a</td>
<td>0.06^a</td>
<td>0.15^a</td>
<td>0.20^a</td>
<td>0.13^a</td>
<td>0.65^a</td>
</tr>
<tr>
<td>1</td>
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<td></td>
<td>0.10^b</td>
<td>0.03^b</td>
<td>0.00^a</td>
<td>0.06^a</td>
<td>0.16^ab</td>
<td>0.22^b</td>
<td>0.15^b</td>
<td>0.77^bc</td>
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<tr>
<td>7</td>
<td>3</td>
<td></td>
<td>0.14^b</td>
<td>0.04^b</td>
<td>0.00^a</td>
<td>0.05^a</td>
<td>0.16^b</td>
<td>0.26^c</td>
<td>0.11^ab</td>
<td>0.82^c</td>
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<tr>
<td>6</td>
<td>15</td>
<td></td>
<td>0.10^b</td>
<td>0.00^e</td>
<td>0.00^a</td>
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<td>0.35^d</td>
<td>0.17^bc</td>
<td>0.90^cd</td>
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<td>0.00^a</td>
<td>0.04^b</td>
<td>0.24^d</td>
<td>0.40^e</td>
<td>0.18^c</td>
<td>1.04^e</td>
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<tr>
<td>5</td>
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<td></td>
<td>0.19^c</td>
<td>0.09^f</td>
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<td>0.23^c</td>
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<tr>
<td>3</td>
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<td>0.13^d</td>
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<td>5.31^g</td>
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<td>3-6</td>
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<td>0.00^a</td>
<td>1.51^e</td>
<td>4.77^g</td>
<td>15.83^h</td>
<td>2.30^f</td>
<td>25.27^h</td>
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</table>

113 acc min. n.d. n.d. n.d. n.d. n.d. n.d. 0.087
max. 1.21 1.36 0.19 2.04 4.77 15.83 2.30 25.57
mean 0.20 0.04 0.00 0.18 0.69 1.44 0.43 3.074
CV% 136.4 423.5 589.6 247.7 173.0 214.2 148.4 176.9
Median 0.08 0.00 0.00 0.00 0.16 0.22 0.16 0.89

* in all-trans-β-carotene equivalent

Mean values with the same letter in a column are not significantly different at P ≤ 0.05 (Turkey’s test).
Table 5. Variation in major and total anthocyanins content for 113 accessions of taro grouped by flesh colour.

<table>
<thead>
<tr>
<th>Flesh color acc.</th>
<th>Nb. acc.</th>
<th>A21.0</th>
<th>A22.1</th>
<th>A24.2</th>
<th>A26.6</th>
<th>A28.5</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>2</td>
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<td>0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.49&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>4</td>
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<td>2.58&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.08&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.16&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>1.61&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>20.41&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>129.45&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>13.38&lt;sup&gt;g&lt;/sup&gt;</td>
<td>145.81&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

113 acc

| max. 5.58 | 243.26 | 12.74 | 32.86 | 22.86 | 306.79 |
| mean 0.11 | 9.10 | 0.21 | 0.71 | 1.23 | 11.36 |
| CV% 505.4 | 390.0 | 668.2 | 459.1 | 308.1 | 369.4 |
| Median 0.00 | 3.29 | 0.00 | 0.76 | 0.00 | 7.00 |

* in cyanidine-3-glucoside equivalent

Mean values with the same letter in a column are not significantly different at P ≤ 0.05 (Turkey’s test).

Figure 1: Color gradient along longitudinal section of orange-fleshed corm of taro
Figure 2: Flow chart diagram of the selection process
Appendix 3: Participatory evaluation in South Africa

Evaluation of agronomic traits of Amadumbi:

Investigating methods to develop amadumbi as a commercial crop

This report was compiled by

Mr WS Jansen van Rensburg, ARC-Roodeplaat, Private bag X293, Pretoria, 0001
wjvrensburg@arc.agric.za

1. INTRODUCTION

Amadumbi (Colocasia esculenta) is a tuber crop that is used by some communities as a staple starch source. In South Africa, Amadumbi is a relatively unknown vegetable crop and areas of cultivation include coastal arias of KwaZulu-Natal and the Eastern Cape Province and the eastern areas of Maphumalanga. Little information is available on propagation methodology and cultivation practices, emphasizing the need to develop cultivation practices to produce the crop efficiently.

Its tubers are processed into various types of food and the leaves as well as the stems, are eaten as spinach and provide a supplement to maize. Traditionally Amadumbi is grown as a staple food, but during the past few years Amadumbi has been used for other food products. These include Amadumbi base (an indigenous tuber and spinach pizza base), Amadumbi crisps, Amadumbi curry, and fresh Amadumbi, known as madumbi, sold in the chain store Woolworth. The inclusion of Amadumbi by Woolworth among its products significantly increased the status of this traditional vegetable and staple food.

ARC-VOPI has built up an amadumbi germplasm collection of material originating form Maphumalanga, KwaZulu-Natal, Eastern Cape and Nigeria. This material is in the early stages of multiplication for evaluation. An in vitro multiplication protocol was established but alternative more cost effective (and more appropriate for farmers) methods of rapid multiplication will be investigated. Agronomic research will be done on superior lines identified by the evaluation.

The initial agronomic trials show that the size of the corm planted does not influence plant height, fresh and dry root weight, or number of side shoots but have an influence corm weight, thus larger corms are more suitable sources of plant material. Both tissue culture material and corms are suitable as propagation material. An in-row spacing of 60 cm seems to be the optimum spacing under the trial conditions.

2. MATERIALS AND METHODS

2.1: Demonstration trials

Pre-sprouted corms of 22 lines were planted in the three evaluation trials and headsets of nine lines (Vanuatu seedlings and local lines) were planted in the eight demonstration gardens. All demonstrations and trials were planted in a complete random design. The layout of the design depends on the space available in the garden. Furrows were prepared manually by the community members with hand hoes. The spacing between the furrows was one meter. The amadumbi corms were planted in the furrows with spacing of 60 cm between the plants depending on the space available in the garden. Well decomposed manure was added in the furrows at planting at a rate of 250ml per plant. The plants were then covered manually. Planting was done during the week of 28 November 2011 to 2 December 2011.
2.2 Baseline Study:

Study was conducted with farmers groups to learn more about amadumbe production problems, perception of amadumbe cultivars and criteria in selection of cultivar. The work was done in five villages in KwaZulu Natal province of South Africa that differ in terms of ethnicity, geography and climate. There was no diversity in the villages in terms of uses and production but only change in status, as a result people felt that there were not that important anymore compare to other crops, it was for poor households. The villages each represented a geographical area.

The villages were:
- Zimiselelni, Elandskop:
- Ifalesizwe project, Elandskop:
- Embali 13, Pietermaritzburg:
- Masizethembe project, Richmond
- Ezemvelo farmers, Mbumbulu

Data in all the areas were collected with participatory methods. The techniques participatory rural appraisals (PRA) were used. Namely:

- Semi-structured interviews
- Group interviews
- Observation
- Seasonal calendars
- Ranking

The type of information needed determined the tools used. As appropriate with collecting information a number of specific questions were developed beforehand to ensure that information was relevant.

3. RESULTS

3.1 Demonstration trials

3.1.1 The eight demonstration gardens:

<table>
<thead>
<tr>
<th>Garden</th>
<th>Zimiselelni</th>
</tr>
</thead>
<tbody>
<tr>
<td>District</td>
<td>Msunduzi (Elandskop, Pietermaritzburg)</td>
</tr>
<tr>
<td>GPS coordinates:</td>
<td>29°40.457’ S 30°05.914’ E</td>
</tr>
<tr>
<td>Altitude</td>
<td>±1325m</td>
</tr>
<tr>
<td>Planted</td>
<td>28th November 2011</td>
</tr>
</tbody>
</table>

Zimiselelni is a community garden about 40km south of Pietermaritzburg. Six women are benefiting from the garden project. The women also produce other vegetables. There is a high degree of commitment from the woman, but they prefer cash crops like tomatoes, cabbage and onions. The garden is cultivated communally.

The Zimiselelni garden members were very keen on growing amadumbe, but they lack experience. There was only one member who was growing it. They show interest in the foreign material.
Figure 1: Photos taken during planting. Headsets of local and foreign material were planted.

Figure 2: Photos during follow up visit 14-17 February 2012. The plants are growing well.
Thuthukani is a very well maintained garden. Twenty four garden members benefit from the garden. One gentleman said he knows purple fleshed amadumbi and that a farmer in the Inanda area, close to Durban, produces it. The garden members show a great amount of interest and some said they would prefer to plant at their homesteads.
Figure 4: The Thuthukani garden can only reach by foot. The individual members have their own plots that they cultivate independently.

Figure 5: Photos taken during planting. Headsets were planted.

Figure 6: All the garden members help with planting the amadumbi headsets.
Figure 7: Photos during follow up visit 14-17 February 2012. Weed control is starting to be a problem. This became even more serious later in the season.

Figure 8: Local line with very round leaves. No material was collected as the owner of the plot was not available

Garden: Masizethembe
District: Richmond
GPS coordinates: 29°54.985’ S 30°13.327’ E
Altitude: ±682m
Planted: 29th November 2011
Masizethembe is a community garden about 20 km outside Richmond. Seven women are benefiting from the garden project. The women also produce vegetables and broilers. The women are very enthusiastic and would like to try new things. They feel, however, that amadumbe will not be profitable for them.

Figure 9: Photos taken during planting. Sprouted corms in black planting bags were used as planting material.

Figure 10: Weed control is a major problem in all communal gardens. It can be seen that the weeds are rampant in the areas that is not being used.
Figure 11: Photos during follow up visit 14-17 February 2012. Trail was well maintained. Weeds only became problem late in the season.

Garden: Emgwenyeni
District: Vulamehlo (Amamzintoti)
GPS coordinates: 30°08.126’ S 30°42.514’ E
Altitude: ±260m
Planted: 30th November 2011

Emgwenyeni is a rural community garden about 50km form Amamzintoti. The garden members produce various vegetables. The garden is a mixture of personal and communal plots. The demonstration was well cared for but unfortunately plants were removed during March to make place for a container to store garden equipment.

Figure 12: Garden is situated on a steep slope but is well managed and erosion is not a problem.
Figure 12: Photos taken during planting

Figure 13: 14-17 Feb 2012. The plants grow well and foreign lines started to flower naturally.

Figure 14: A amadumbe planting of one of the other garden members.
Figure 15: Heavy aphid infestation was observed in the farmers planting

Garden: Lethithemba
District: Pongola
GPS coordinates: 27°20.657’S 31°34.683’E
Altitude: ±310m
Planted: 5th December 2011

Lethithemba is a peri-urban garden in the outskirts of Pongola. The members produce vegetables and broilers. The land is managed communally. The trail was cared for in the beginning, but was totally overgrown at the end of the season. They feel that amadumbe will not be profitable.

Figure 16: Photos taken during planting

Figure 17: Photos during follow up visit 14-17 February 2012. Plants was ridged just before the visit.
Garden: Siyeza  
District: Big 5 False Bay (Hluhluwe)  
Altitude: ±35m  
Planted: 2\textsuperscript{nd} December 2011  
Extension: Tuli 082 945 3736

Siyeza is a community garden about 18km outside Hluhluwe. The garden is next to the road and the members report that theft is a problem. The demonstration trial was planted here. The trail was harvested by the community members before any yield data was taken.

\textbf{Figure 18:} Photo taken during planting

\textbf{Figure 19:} Photo taken during follow up visit 14-17 February 2012
Garden: Vukujule Mama
District: Umhlabuyalingana (Manguzi)
GPS coordinates: 27°02.025’ S  32°40.767’ E
Altitude: ±76m
Planted: 2nd December 2011 Extension: 083 551 7667

Vukujule Mama was chosen originally to plant the demonstration trial in the North but the farmers said that they were having a problem with the availability of water and would not have enough to sustain a large number of plants. The garden members are very commuted but the burden of manual watering was just too much and the trail failed. Crops like sweet potato will be more appropriate for them.

Figure 21: The Vukujule Mama garden is situated on the coastal plans close to Kosi Bay. The soil is pure sand and water is a constraint. All water is pump by hand from a borehole with a capacity of about 5 litre per hour.

Figure 21: Headsets were used as planting material. Organic material was placed in the furrows and mulch was used to retain moisture in the sandy soils
Figure 22: Irrigation was done by hand with a watering can.

Figure 23: Photos taken during follow up visit 14-17 February 2012. Additional mulch was added around the plants.

Figure 24: “Eco-circles” is an alternative way of irrigation. A 2 l plastic bottle with holes at the bottom is buried in the sand between the plants. The water slowly seep out of holes at the bottom.
Thandinhlabathi is a community garden outside Paul Pietersburg. It is very well maintained and the members take great pride in their garden.
Figure 25: Photos during follow up visit 14-17 February 2012

Garden: Roodeplaat
District: Tshwane (Pretoria)
GPS coordinates: 25° 36.281S 28° 21.197E
Altitude: ±1163m
Planted: 6th December 2011

A trail was also planted at Roodeplaat.
Figure 26: Photos taken on 29th February 2012

Figure 27: Leaf abnormalities observed in Thandinhlabathi
2 Baseline study
The information gathered is presented in full for each locality

2.1 Zimiseleni, Elandskop:

Most of Zimiseleni farmers know about amadumbe but it’s not something they prefer compare to other vegetables as a result there were no cultural activities linked to them and usually they are not successful with their yields. Those who cultivate amadumbe do it in small area for family consumption. They don’t have any training or access to information on cultivation of amadumbe; they plant in any soil once a year. Two types of amadumbe identified were idumbe elibomvu and idumbe elimhlophe. During planting they prepare ridges that are about 1m apart from each other with intra-row spacing of about two (human) feet. They plant seeds in a small hole of about 30-40cm
on moist soil mixed with manure. Most problems they experienced with amadumbe were millepede and moles, which ate both leaves and corms of the plants. They used nothing to treat their problems. They don’t process them into any other product and there are also not marketed in any way. Most people preferred boiled corms called injalo.

Table 2.1: Seasonal calendar of Amadumbe activities as identified by Zimiseleni project

<table>
<thead>
<tr>
<th>Name of activity</th>
<th>Jan</th>
<th>Feb</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
</tr>
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<tbody>
<tr>
<td>Land preparation</td>
<td></td>
<td></td>
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<td></td>
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Table 2.2: Amadumbe constraints as identified by Zimiseleni project

<table>
<thead>
<tr>
<th>Rank</th>
<th>Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>After harvesting corms were too small</td>
</tr>
<tr>
<td>2</td>
<td>Moles and millepede</td>
</tr>
<tr>
<td>3</td>
<td>Idumbe elimhlophe is fully of water and sticky inside</td>
</tr>
</tbody>
</table>

Figure 29: During interviews with Zimiseleni group, Elandskop
2.2 Ifalesizwe project, Elandskop:

The Community of this area is also not used to planting amadumbe, they once planted but have since stopped, due to problems with erosion, and their area is situated in a wet land. Yet, they only know two types, the wet and dry ones but they don’t have much information about them.

During planting they used a tractor and kraal manure. They bought seed from someone who used to plant amadumbe and there was no criteria used in choosing the cultivar to be planted, they planted whatever they could get. Their plants were not irrigated, but only rain fed and covered with soil twice after planting. They also preferred planting them in a loamy soil once a year. Weeding was only done twice. They didn’t have any problems with pest and disease.

2.3. Embali 13, Pietermaritzburg

This is the small community project that donates to sick individuals. They get their seeds from department of Agriculture. We could not do most of PRA exercises in this community because only one project member was available. Through interviews we succeeded to understand the situation of amadumbe in Pietermaritzburg. They planted amadumbe once in very small area using kraal manure and there were not successful due to the fact that they planted very late as a result their corms were very small. They didn’t irrigate and no experience of any problems with pest or diseases was reported. They eat boiled corms and leaves.

2.4. Masizethembe project, Richmond

Masizethembe project members learned about amadumbe from their elders and there are usually used during weddings in a community. Almost every household in the community cultivate amadumbe once a year, in an area which is about half a hectare for selling and for home consumption.

During planting they prefer loamy soil for easy germination and big corms which are good in producing smaller to medium corms called injalo that grow off the sides of the primary corm. Two types of cultivars identified were idumbe elimhlophe and idumbe eLibomvu. Idumbe eLibomvu is most favoured one compare to idumbe elimhlophe which is normally used as a border to control moles because it’s having an unpleasant taste. They boil corms and mix them with meat. They sell from home and their customers prefer injalo. However their major problem was identifying the markets, and since competition was too high locally, the result was that they get a lot of left overs.

Table 2.3: Seasonal calendar of amadumbe activities as identified by Masizethembe project

<table>
<thead>
<tr>
<th>Name of crop</th>
<th>Jan</th>
<th>Feb</th>
<th>March</th>
<th>April</th>
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<th>June</th>
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<tbody>
<tr>
<td>Land preparation</td>
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### Table 2.4: Amadumbe constraints as identified by Masizethembe project

<table>
<thead>
<tr>
<th>Rank</th>
<th>Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>To get tractor on time for land preparation</td>
</tr>
<tr>
<td>2</td>
<td>Goats and cows eating amadumbe</td>
</tr>
<tr>
<td>3</td>
<td>To identify market</td>
</tr>
<tr>
<td>4</td>
<td>Moles</td>
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<tr>
<td>5</td>
<td>Aphids</td>
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<td>6</td>
<td>They keep their seed underground but it spoils</td>
</tr>
<tr>
<td>7</td>
<td>Heavy rains making it difficult for amadumbe to germinate</td>
</tr>
<tr>
<td>8</td>
<td>Too sunny weather as the result tubers stop to grow and rot under ground</td>
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</tbody>
</table>

### Table 2.5: Amadumbe cultivation

<table>
<thead>
<tr>
<th>Step</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Land preparation with tractor</td>
</tr>
<tr>
<td>2</td>
<td>Use tractor to make ridges</td>
</tr>
<tr>
<td>3</td>
<td>Mix soil with kraal manure</td>
</tr>
<tr>
<td>4</td>
<td>Dig 30cm deep for seed then cover with soil</td>
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<td>5</td>
<td>Weed and cover the plant with soil at least three-four times after planting</td>
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<tr>
<td>6</td>
<td>Harvesting by hand after 6 months</td>
</tr>
<tr>
<td>7</td>
<td>Irrigate if you have water</td>
</tr>
</tbody>
</table>

**Future needs:**
- To identify market
- Training on processing and storage
- Tractor
- Fence
2.5 Ezemvelo farmers, Mbumbulu

Amadumbe are well known and grown by lot of farmers in Mbumbulu. One of the groups is Ezemvelo farmers who are specializing in organic farming. They sell amadumbe to Woolworths and to community members. They normally plant an average area of 1-5 hectares per season. They get moles, millipedes and unidentified disease which in most cases are left untreated since they don’t have any training on how to treat such. During planting they only plant idumbe elibomvu because it’s always in demand, even Woolworths only buys it. Its corms are dry and hard. They plant on a loamy soil mixed with kraal manure and grass, no irrigation only rain fed. Harvesting begins after 6 months and they keep their own seeds underground in about 30 cm deep holes covered with soil. They keep them until next season and it’s also helps with preventing them from spoiling. Small and medium sizes corms sell faster so they keep bigger sizes for planting. They eat both leaves and corms.

Only 3 cultivars were identified namely:

- Idumbe elibomvu-Most preferred cultivar with reddish stem, big leaves and dry corms.
- Idumbe elimhlophe -Leaves are small with light green/whitish and thin stem. Most people don’t like it because of its bitter taste and it’s not dry like idumbe elimhlophe
- Upitsi- Its plant and corms are too small. Cultural it is only served to grannies
Table 2.6: Seasonal calendar of amadumbe activities as identified by Ezemvelo farmers

<table>
<thead>
<tr>
<th>Name of crop</th>
<th>Jan</th>
<th>Feb</th>
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<th>July</th>
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Table 2.7: Amadumbe cultivation constraints as identified by Ezemvelo farmers.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Constraints</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>During harvesting some of amadumbe will be too small and even damaged</td>
</tr>
<tr>
<td>2</td>
<td>Wild pigs eating corms</td>
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<tr>
<td>3</td>
<td>Moles</td>
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<tr>
<td>4</td>
<td>Porcupine</td>
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<tr>
<td>5</td>
<td>Millipede</td>
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<td>6</td>
<td>Fence</td>
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Important characteristics they look for when they select amadumbe cultivar for planting in their order of importance:

- They preferred corms with red stem because of its good taste compared to the ones with white stem
- Big ones are also good for planting but not good for markets
Figure 30: Ezemvelo farmers while we were waiting for other group members

Figure 31: The Mbumbulu area
4. CONCLUSIONS AND RECOMMENDATIONS

Due to the various problems in the communal gardens with trial it will be better to plant in future all trials that data are taken form in more “secure” localities.

It is also decided to plant the future demonstration trials more concentrated in the due to financial and time constraints.

5. ACKNOWLEDGEMENTS

• European Union for funding

6. OUTPUTS LINKED TO ARC KEY PERFORMANCE INDICATORS

<table>
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<tr>
<td>Number Methods developed/adapted</td>
<td>0</td>
</tr>
<tr>
<td>Number of services provided to clients</td>
<td>0</td>
</tr>
<tr>
<td>Number Germplasm developed/maintained</td>
<td>99 + 25 new accessions</td>
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<tr>
<td>Number of products developed</td>
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<td>Information Dissemination</td>
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7. SHORT TERM OBJECTIVES FOR FOLLOWING YEAR

• Conservation of amadumbi germplasm in glasshouse collection. The feasibility for in vitro conservation will be investigated

• Broadening of the genetic base of amadumbi
  o Build up a representative amadumbi germplasm collection
  o Establish the genetic base and variation of South African amadumbi germplasm
    ▪ Molecular techniques
    ▪ Morphological techniques
  o Hybridization

• Rapid multiplication techniques