on a broader scale, e.g. through the regional information networks and/or a quarterly newsletter.

Other suggestions were aimed at improving communication and networking, such as discussion groups and an IMTP mailing list, as well as workshops and meetings. Many respondents recommended an annual global meeting, or regional ones, to help highlight difficulties and discuss with experts possible solutions.

Nearly three-quarters of the respondents said they were willing to share germplasm, but 40% of them specified that it could only be done under certain conditions, such as respecting national rules or getting approval from the management of the participating companies. A Material Transfer Agreement has to be in place. Sharing data and germplasm is conditional on everybody doing it and is an important factor in determining one's willingness to share. Quarantine issues should also be considered.

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A staple food with nutritious appeal

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Some of the earliest archaeological evidence of organized agriculture in humid tropical Africa is found in central Cameroon (Mbida *et al.* 2000). It suggests that farmers in this part of the world have been cultivating *Musa* for more than 2000 years, actively selecting varieties and generating the high levels of plantain diversity Cameroonians enjoy today. In the process, these early farmers created varieties that are now sought for their nutritional qualities.

Recently, Lois Englberger's work (Englberger 2003, Englberger *et al.* 2003) has highlighted the importance of orange-fleshed *Musa* as a source of provitamin A carotenoids (pVACs), plant-derived compounds that are converted to vitamin A in the human body. Vitamin A has a role in vision, as well as immunological, reproductive and embryo development functions. A deficiency of vitamin A in the diet represents one of the key challenges affecting the developing world. Up to half a million children are estimated to go blind every year from vitamin A deficiency and more than 50% of all deaths in any one year are associated with malnutrition (WHO 2003). These death rates would be higher if it wasn't for the costly and regular interventions by NGOs and governments distributing vitamin and mineral supplements.

In 2004, a number of CGIAR centres formed an alliance, coordinated by the International Food Policy Research Institute and the *Centro Internacional de Agricultura Tropical*, under the name HarvestPlus Challenge Programme. The concept behind HarvestPlus is that micronutrients can be conveyed to vulnerable populations more cheaply and effectively through biofortified staple food crops. The programme places a strong emphasis on increasing productivity and nutrient density through crop improvement. The first phase of the programme focussed on evaluating the genetic variability of maize, wheat, rice, cassava, sweet potato and beans for three key micronutrients, iron, zinc and pVACs.

In a second phase, additional crops are being investigated and both the International Institute for Tropical Agriculture and the International Network for the Improvement of Banana and Plantain (INIBAP) of Bioversity International have been commissioned to carry out research on *Musa*. INIBAP's work has involved bringing together a group of collaborators, the *Centre Africain de Recherches sur les Bananiers et Plantains* (CARBAP) in Cameroon, the Crop Research

Institute and Food Research Institute in Ghana and the *Katholieke Universiteit Leuven* (KULeuven) in Belgium, to evaluate, among other things, the plantain cultivars that are held in the CARBAP field collection. CARBAP manages one of the largest collections of *Musa,* including a broad representation of cultivars from traditional farming systems in sub-Saharan Africa and the Pacific. This paper provides a review of the issues surrounding the study of pVACs and the activities of the INIBAP-coordinated HarvestPlus project.

What are carotenoids?

There are some 600 types of carotenoids known, of which approximately 50 play a role in the human diet (Rodriguez-Amaya 1997). Beta-carotene has the highest level of vitamin A activity, hence the importance of determining which carotenoids are present when evaluating the nutritional value of foods. Depending on the method used, analyses of carotenoids may supply values for:

- total carotenoids (all carotenoids including those that have no vitamin A activity),
- pVACs (carotenoids that have vitamin A activity),
- beta-carotene equivalents (provitamin A carotenoids converted into equivalent beta-carotene units)
- individual carotenoids (pVACs plus lycopene, lutein, etc).

Table 1 shows the methods used to quantify carotenoid levels in the HarvestPlus project.

Major constraints affect the interpretation and presentation of carotenoid analyses:

- Carotenoid content is highly variable within a plant and between plants and varieties. It also varies with fruit ripeness. This presents a substantial sampling challenge. The most appropriate sampling time and methods have to be established for comparative work.
- Carotenoids oxidize easily. Exposure to light, air and physical damage affect the rate of carotenoid loss once the sample is removed from the plant. Again this presents a challenge in terms of storing and transporting samples.
- Methods vary in their accuracy and precision. Results are often based on different analytical protocols, and are sometimes published without reference to what has been measured (total carotenoids or beta-carotene, fresh or dry weight, etc), and which methodologies were used. Processed materials may be directly compared with raw. Consequently, little standardized information exists to compare different foods or crops.

Once a value for beta-carotene equivalents has been determined, the nutritional value of the food (consumed in the form in which it was analysed) can be estimated using conversion factors for the absorption and metabolism of carotenoids in the body. The UN Food and Agriculture Organization uses a 1:6 ratio of Retinol Equivalents (RE) to beta-carotene and a 1:12 ratio for the other provitamin A carotenoids, based on the estimated absorption of 30% of the beta-carotene. The US Institute of Medicine more recently advised a 1:12 ratio of Retinol Activity Equivalents (RAE) to beta-carotene equivalents—the conversion rate used by HarvestPlus.

Table 1. Methods used in the INIBAP-coordinated HarvestPlus project for quantifying the carotenoid content in *Musa* fruit.

Method	Tools	Type of results	Comments
Colour assessment	Colour fans, charts and colorimeters	Ranking by colour as a proxy for total carotenoids	In *Musa*, there is a correlation between colour and carotenoid content. This method is cheap and quick for ranking varieties of the same species according to potential carotene content.
Spectrophotometry	Spectrophotometer	Total carotenoids	Effective method of quantifying total carotenoids. However, it is not possible to distinguish the range of individual carotenoids.
High performance liquid chromatography (HPLC)	HPLC system and diode array detector	Total provitamin A carotenoids, beta-carotene equivalents, individual carotenoids, cis and trans isomers.	Costly technique but the preferred means of quantifying specific carotenoids (e.g. beta and alpha-carotenoids, lutein, etc.) and their geometric isomers.

Screening plantain

Plantain samples were flown fresh from the field to the laboratory in Leuven, where they were immediately prepared and frozen for later analysis using High Performance Liquid Chromatography (HPLC) and spectrophotometric methods. A standardized protocol for the HPLC screening of large numbers of *Musa* samples developed by Davey *et al.* (*in press*), resulted in increased throughput and reduced analysis time and costs.

Preliminary results suggest that the orange-fleshed plantain cultivars, which are locally popular in Cameroon, are significant sources of provitamin A carotenoids, although none so far is as rich as the Fe'i bananas studied in Micronesia (Table 2). The pVACs consist of roughly equal amounts of alpha- and beta-carotene (44-48% of total carotenoids). Sweet potatoes and Fe'i bananas have higher proportions of beta-carotene (60-90%) (Englberger *et al.* 2006). Using the 1:12 bioconversion ratio, a regular meal of 200 g of the plantain 'Batard' would appear to provide around a third of the daily vitamin A requirement for an average adult (500-900 µg/day), assuming that these pVACs are retained during processing.

Not only does the quantity and type of carotenoid influence the nutritional quality of foods, but other factors also have an effect:

- State of the food upon preparation (time in storage, ripeness, physical state),
- Age and physiological state of the consumer,
- The retention of pVACs in the food matrix (this relates to the digestibility of the food),
- The cooking or processing method,
- The other foods consumed at the same time.

Effect of ripening

In plantain, evidence suggests that the yellowing of the fruit pulp during ripening is caused by the breakdown of the chlorophyll, a process which reveals the carotenoids, rather than by carotenoid biosynthesis, as occurs in other fruits such as apricot, mango, papaya (Rodriguez-Amaya 1997). Giami and Alu (1994) found that total carotenoids in plantain almost halve during ripening. Similar trends were observed by Ngoh Newilah (2005), one of the collaborators in the HarvestPlus project, suggesting that the loss of beta-carotene in some micronutrient-rich varieties can be as high as 75%.

Table 2. Available estimates of provitamin A carotenoid content and retinol activity in a selection of staple foods.

	Beta-carotene equivalents (µg/g)	Retinol activity equivalents (µg/g)
Orange-fleshed sweet potato	194[1]	16
Utin lap (Fe'ii type banana)	85[2]	7.1
New strain 'golden rice'	37[3]	
Batard (plantain, AAB)	14[4]	1.2
Cassava	7.7[1]	0.64
Cavendish dessert banana	1.4[4]	0.12
White rice	0[5]	0

[1] Rodriguez-Amaya D.B. & M. Kimura. 2004. HarvestPlus Handbook for Carotenoid Analysis.
[2] Englberger L. *et al.* 2006.
[3] Coghlan A. New Scientist 27 March 2005. (reported as 37 mg of provitamin A - presumed to be beta-carotene equivalents)
[4] Davey, M. Unpublished. 2005 technical report on the INIBAP-coordinated HarvestPlus project.
[5] USDA National Nutrient Database for standard reference. Release 18.

The present project is attempting to determine the point in fruit development at which carotenoid biosynthesis stops, which types of carotenoids are affected, the impact of letting the fruit ripen on the plant as opposed to ripening in storage, and how these vary according to the variety.

Plantains are cooked (e.g. fried, boiled, roasted, pureed) at various stages of ripeness depending on the maturity of the available fruit. For example, a surplus production may mean overripe plantain for breakfast, lunch and dinner. There is, however, evidence that the ripeness of plantain in processed meals is associated with the preferences of the consumer (Dury *et al.* 2002). If carotenoid content declines during ripening in many plantain varieties, then a change in storage and eating habits could deliver more micronutrients to the consumer.

Effect of cooking and processing

Cooking has contradictory effects on carotenoid levels. Processed foods may have higher levels of bioavailable carotenoids because of the loosening of the food matrix, allowing them to be more easily absorbed (Englberger *et al.* 2003, Van den Berg *et al.* 2000). On the other hand, cooking, especially under high heat and for a long time, destroys carotenoids, and converts *trans* isomers into *cis* isomers, which have lower vitamin A activity (Booth *et al.* 1992). A report suggests a large percentage of carotenoids are retained in frying plantain (Rojas-Gonzalez *et al.* 2006).

Furthermore, the levels of anti-nutrients in foods eaten at the same time, as well as their digestibility, influence the degree to which micronutrients are absorbed and converted in the body. For instance, carotenoids are fat-soluble and evidence indicates that dietary fats in the meal facilitate the absorption of carotenoids (Yeum and Russell 2002).

In terms of micronutrient content, an exemplary meal might be plantain fried in red palm oil, one of the richest sources of carotenoids (Ngoh Newilah *et al.* 2005). The HarvestPlus project will examine more closely the effects on micronutrients of different traditional processing methods and practices, and the bioavailability of micronutrients to consumers.

Rather than focusing on the micronutrients in one variety, we could explore plantain- and banana-based subsistence systems as a whole. How do they function in terms of providing the full complement of nutrients required for a healthy diet? Which minerals or micronutrients are lacking or brought in from elsewhere?

Delivering micronutrients to those who need them

Tackling micronutrient deficiencies through improving the diet is clearly not just a question of identifying nutritious foods, but also of making such foods available in the quantities necessary to have an impact on health. The key question is whether the populations suffering from malnutrition have access to the micronutrient-rich foods intended for them.

Plantains and cooking bananas are subsistence crops in large parts of tropical Africa, including in areas where micronutrient deficiency has been identified as a problem. In Cameroon, for example, plantains form the major part of the diet almost everywhere. They are consumed in a multitude of ways, roasted, baked, fried, boiled, steamed, dried, pureed, or eaten raw (Ngoh Newilah *et al.* 2005). Few staple crops offer such versatility. In cities, however, plantain and cooking bananas are relatively expensive foods that are often out of reach for the urban poor. Reducing the price of plantain would require a substantial increase in year-round yields.

Yields of plantain and cooking banana in sub-Saharan Africa are notoriously low even though the technologies that could improve yields are tantalizingly simple; using clean planting material and encouraging denser planting are proving effective ways to increase production in trials. Any micronutrient-rich cultivars will need to be promoted together with production technologies that boost yields. For this reason, the HarvestPlus project is also carrying out on-farm trials on high-density production in Ghana and Cameroon.

The World Bank recently placed nutrition at the centre of the development agenda (World Bank, 2006). Agriculture and crop diversity clearly has an important role to play here. The HarvestPlus project, perhaps, represents yet another harbinger of a new era that demands research to consider its impact not just in terms of yields but in terms of health and well-being delivered.

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Interactions between plant parasitic nematodes and plant secondary metabolism, with emphasis on phenylpropanoids in roots

Thesis

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Plant parasitic nematodes impose a serious threat on agricultural production worldwide. Nematode resistant crops are generally considered the most favourable management option, as opposed to the much disputed use of chemical nematicides. For most crops, including banana, naturally resistant varieties are scarce or do not meet production or cultural standards. Knowledge on resistance mechanisms is still poor for many plant-nematode interactions, so breeding or genetic improvement techniques are not applied to their full capacity.

Plants produce a wide range of biologically active chemicals, secondary metabolites, which are involved in plant defence against pests and diseases. The major classes of secondary metabolites include alkaloids, terpenoids and phenylpropanoids. The biosynthetic pathway of the phenylpropanoids, the so-called phenolic compounds, is well-characterized and constitutes a potential target for the improvement of resistance against nematodes.

The objective of the present study was to gain a better understanding of the interaction between plant parasitic nematodes and plant secondary metabolites, in particular phenylpropanoids, in order to increase knowledge on plant defence against nematodes. The study was focused on the interaction between banana and its major nematode species *Radopholus similis*. Better knowledge of resistance mechanisms in banana and of the characteristic features of resistant varieties may facilitate breeding and screening of germplasm and hybrids, or provide a rationale for genetic improvement.

In vitro bioessays showed that secondary metabolites affect the behaviour of *Musa* nematodes, including *R. similis* and *Meloidogyne incognita*. Metabolites act as attractants or repellents, induce paralysis, reduce hatch or even cause death.

Five banana varieties with well-characterized host statuses for *R. similis*, including the susceptible ‘Grande naine’ (AAA, Cavendish subgroup) and ‘Obino l’ewai’ (AAB, plantain) and the resistant ‘Yangambi km 5’ (AAA, Ibota subgroup), ‘Pisang jari buaya’ (AA, Pisang jari buaya subgroup) and ‘Calcutta 4’ (*Musa acuminata* ssp. *burmannicoides*) were selected for the identification of potential physical and chemical barriers to nematode infection in banana roots. Methods included a quantitative lignin assay, liquid chromatography and mass spectrometry. Through histochemical staining phenylpropanoids were localized in root tissue.

Resistant banana varieties had more phenylpropanoids than susceptible ones. Cell walls of resistant roots contained significantly higher levels of lignin and ferulic acid esters. Lignin appeared to take part mainly in the protection of the vascular bundle both constitutively and upon infection. Ferulic acid esters in cortical cell walls act as substrates for peroxidase-catalysed dimerization and cross-linking of cell wall components and as initiation sites for lignification. Higher levels of these compounds in resistant varieties means that their cell walls are better equipped