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Biotechnological Contributions to Food Secrurity with Cassava and Rice

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Present world food production would provide ca. 2,400 kcal/day/person if it could be equally distributed amongst the world population. Food is, however, and will always be, unevenly distributed. Many of us are used to consume 3,400 kcal/day. However, 800 million are starving at 1,800 kcal/day, and 3.4 billion live at the minimal level of 2,200 kcal/day. Although food security to date may be mainly a poverty problem, it is increasingly becoming a production problem. The world population is growing by 90 million p.a. and will, probably, stabilise only when a total of 10–12 billion has been reached. At the same time, however, world-wide food production per capita is declining, as is the crop land and the water available for agricultural food production. The continuous increases in food productivity of the past decades are declining and two of the three major food systems—oceans and rangelands—are already exploited at their limits. The world population will continue to grow dramatically and most of this population growth will occur in Developing Countries, which will not be rich enough to compete on the world market for food surplus and which, therefore, will, have to increase their harvests from agricultural land dramatically. And this increase has to be achieved under sustained conditions, with reduced inputs in agrochemicals, energy, water and manpower. Yield per acre has at least to be doubled. The most direct approach to an increase in food production, without an additional increase in input of resources, would be via reduction of losses with the help of resistant crop varieties. As crop loss is still in the range of 50% for the major food security crops such as rice and cassava, the potential of such an approach is enormous. Genetic engineering could, therefore, substantially contribute to the rescue of lost harvests via production of resistant varieties. It also could contribute to a second facet of food security, the improvement of food quality with regard to vitamins, micronutrients and essential amino acids. From our work at the ETH Zürich we will present the state of the art of projects with the food-security crops rice and cassava on pest- and disease resistance, supply of provitamin A, iron and protein, and reduction of toxic compounds.

1. Introduction

1.1 Food security in developing countries is one of the major challenges for mankind

To date ca. 900 million people world-wide are starving [1]. This has economical, political, social, educational and further causes. One major cause is, however, the decrease in agricultural productivity per capita, especially in countries, where food security is already a problem now [1]. With 900 million already starving, the world population is increasing further by 85 million people every year [1]. Virtually all these additional people are born in the Developing Countries (**Table 1**). Since 10 years world food production per person is declining, e.g. rice and wheat by 12%, seafood by 9%, and beef and mutton by 13% [1]. Two of the three major world food systems—oceans and rangelands—are already exploited at their limits (**Table 2**).

The only remaining potential for increase in food productivity exists with the third food system, the crop land [1]. However, world grain harvest area also declines, e.g. world grainland per person from 0.24 hectares in 1950 to 0.12 in 1995 (**Fig. 1**), total grain harvest area of Japan, South Korea and Taiwan from 8 million hectares in 1950 to 4 million hectares in 1995 [1], total world harvest area from 740 million hectares in 1980 to 680 million hectares in 1995 [1]. World grain reserves suffice, to date, for less than 50 days only [1].

Therefore, grain available for Developing Countries is dramatically shrinking. The world grain market is shrinking as well and projections into the next decades predict, that it will soon be emptied, especially by countries with large populations and growing economy such as China (**Table 3**), where similar losses in grain harvest area are expected, as have recently occurred in highly industrialised Asian countries (see above).

The unprecedented increase in food production during the time period between 1950 into the 1980's, based on the development of high-yielding varieties, high external input, optimised production systems, expansion of crop land and political support—the time of the "Green Revolution"—is fading, even in optimised agricultural systems such as that of corn production in the U.S.A [1].

Instead of responding to all these problems by a substantial increase of the support for international agricultural research, financial support of international agricultural research (and for agricultural research in general) is declining [1]. (From where else should we expect support for the production of the necessary food?) Instead of taking responsibility for the solution of the problem, interest for the problem in the scientific community, at least that of the molecular biologists, is negligible. (Who else should develop new concepts and techniques?) And in-

Table 1 Population growth, 1950-90, with projections to 2030; increase for 1990-2030. Data from Brown and Kane 1994. (unit: million people).

Country	1950	1990	2030	Increase
Germany	68	80	81	1
France	42	57	62	5
Italy	47	58	56	−2
United Kingdom	50	58	60	2
Japan	84	124	123	−1
United States	152	250	345	95
China	563	1,134	1,624	490
India	369	853	1,443	590
Bangladesh	46	114	243	129
Pakistan	39	115	312	197
Ethiopla and Eritrea	21	51	157	106
Nigeria	32	87	278	191
Brazil	53	153	252	99
Indonesia	83	189	307	118

Table 2 World seafood catch and grain output (in million tons). Data from Brown and Kane 1994.

	1950	1990	2030	Change 1950–1990	Change 1990–2030
Seafood catch	22	100	100	+78	0
Grain output	631	1,780	2,149	+1,149	+369

stead of informing the public about the problem, the problem is widely ignored in the European media, by the European politicians, and, therefore, by the European public. (Who else should create the necessary pressure for financial support?)

Other agroeconomists, e.g. those of the International Food Policy Research Institute [2] do not judge the food security situation to be as dramatic, as the Worldwatch Institute. Unfortunately, to our understanding of the situation, the predictions of the Worldwatch Institute are probably more realistic, because they do not simply extrapolate from the unusually positive development of the 1960's to 1980's, but consider the obvious change in trend and potential, if the period from 1950 to 1990 is compared to the development from 1990 to 2030

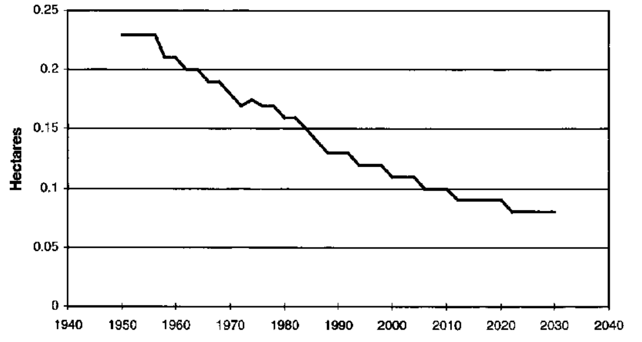


Fig. 1 World grainland per person, 1950-1993 (with projections to 2030). Data from [1].

(Tables 2 and 3). However, above all the differences in the interpretation of details, there is general agreement, that the dramatically increasing world population will require substantially more food than is available to date. It is relatively easy to conceive, that twice as many people will need twice as much food. As the arable land can not be further extended this means, that agriculture will have to increase the present yield by at least 100%. This is an enormous task.

1.2 How can molecular plant biology contribute to food security?

The key problem, of course is population growth. Sustainable food production can not indefinitely keep up with the present increase, and L. Brown and H. Kane ask the question of the "Earth's population carrying capacity" [1]. Family planning has to receive highest priority and has already been successful in some countries. The same holds true for education and economic development, as well as reforestation, soil conservation, water management, environmental protection measures etc. [1]. However, even with the totally unrealistic scenario of immediately being able to reduce fertility rate world-wide down to the replacement level of 2.12, the world population would still grow to 9 billion [3]. A more realistic scenario is, however, that the world population will grow to 12–16 billion before it will stabilise or decline. How then can molecular plant biologists contribute to the production of more food, where ever possible in those countries, where the food is needed? The increasing knowledge of the molecular basis of plant biology opens numerous long-term, medium-

Table 3 Grain production, consumption and net trade. Data from Brown and Kane 1994. (unit: million metric tons cereal grain).

	Grain production			Grain consumption			Net trade		
	1950	1990	2030	1950	1990	2030	1950	1990	2030
USA	133	290	377	121	214	295	+12	+76	+82
China	109	329	263	109	335	479	0	−6	−216
India	57	158	222	55	158	267	+2	0	−45
Pakistan	6	19	28	6	20	54	0	−1	−26
Indonesia	12	34	48	12	37	60	0	−3	−12
Egypt	4	11	18	5	19	39	−1	−8	−21

term, and some short-term opportunities—mostly with non-food plants. The task is, to transfer these opportunities to the important crop plants. As there is not much time ahead of us, our group at the ETH Zürich is focusing on the most important crop plants—rice, wheat, cassava, and sorghum—and on the most effective short-term strategy: to rescue food which, so far, is lost to pests. Despite the enormous success in crop protection based on development of efficient pesticides, breeding of resistant crop varieties, and development of novel production schemes, loss of harvests to pests and weeds is, to date, still in the range of 40–60% (**Table 4**) [4] and this situation is especially true for the crops in the tropical countries. Besides other options, plant molecular biology and genetic engineering could help to prevent at least part of these losses. This would make a substantial contribution to food security.

1.3 Biotechnologists and molecular biologists are expected to take this responsibility

A.L. Hammond et al. [5] state:

"Over the next 25 years, food production in the developing world will have to double just to keep up with population growth. While there is still room to elevate food production through traditional breeding programs and extended use of fertilisers and irrigation, experts caution that these means alone cannot double the food supply."

"Thus, if developing nations are to have enough food in the years to come, harnessing the potential of biotechnology is not just an option, it is a critical necessity."

"Conditioning crops to absorb environmental stresses could allow marginal lands to be pressed into service as well as to reduce the wide fluctuations in food supply that plague developing nations."

"Likewise, improving the pest and disease resistance of staple crops might gradually increase yields and help to protect the health of wildlife and farmers by reducing pesticide use. Enhancing the nutritional value of staples would mean squeezing more from every bushel."

"However, significant barriers stand in the way of biotechnology's application in the developing world."

These barriers include (according to [5])

* control of tools and products of biotechnology in the industrialised world;
* research predominantly in large transnational chemical, seed, pesticide and food processing companies;
* widespread patent and proprietary research;
* research priorities set by lucrative markets of the industrialised world;
* problems and special needs of the South ignored;
* important subsistence crops such as rice, millet and cassava have received little attention from the private sector;
* even developing nations focus their biotechnology projects on export crops at the expense of subsistence crops;

We have some understanding for the fact, that these problems enhanced the development of a radical opposition of NGO's, grassroot organisations and other humanitarian organisations against biotechnology. However, we can not agree with their view, that because of these borders, gene technological solutions for problems have to be prevented. In contrast we conclude, that the best response to these barriers is not to abandon biotechnology, but to strengthen government- and foundation-financed public research in biotechnology towards the benefits of biotechnology with subsistence crops for sustained food security in developing countries. And we believe, that our ETH-, Rockefeller Foundation-, and SDC-supported research on rice, cassava, wheat and sorghum at the Institute of Plant Sciences, exemplifies, what important contributions can come from dedicated work of a single research team. In the following presentation we focus on selected examples from cassava and rice only.

2. Genetic engineering of rice (*Oryza sativa* L.)

Together with wheat and maize, rice belongs to the three most important food crops world-wide, occupying one tenth of all the arable land. Rice provides the major calorie source for more than two billion people in humid and subhumid Asia, and is also an important crop in several countries of Africa, Latin America and the Middle East [6].

Thanks to the Green revolution, world-wide rice production was doubled from 250 million tons in the early sixties to more than 500 million tons in 1989. In 1997, 569 million tons of rice were produced on 149 million hectares with an average yield of 3.8 t/ha [7]. Seventy percent of the production growth came from increased yields and increased cropping intensity, 30% resulted from new land brought under rice cultivation [6].

Today, world population is growing with 85 million people every year. An increase of 13 million tons of rough

Table 4 Estimates of the potential (potl.) and actual (actl.) losses and of attainable yield in rice in Asia from 1988 to 1990. Data from Oerke et al. 1994.

Region	Rice crop losses (%) due to							Yield (kg/ha)	
	Diseases		Animal pests		Weeds		total		
	potl.	actl.	potl.	actl.	potl.	actl.		actl.	potl.
South Asia	27–32	21	45–50	32	54–59	23	76	2478	6014
Southeast A.	28–33	20	45–50	31	54–60	23	74	2616	5954
East Asia	36–41	10	38–43	13	48–53	11	34	6558	9377
Near East	30–35	20	30–35	25	65–70	25	70	3066	6814

rice each year will be needed to meet the projected rice requirements by the year 2025 [6]. Rice production can be increased in two ways: By an increase of the yield potential of cultivars growing under favourable conditions and by a reduction of the losses of cultivars growing under unfavourable environmental conditions like adverse climate, adverse soils, pests and diseases.

Next to an increased yield, improved rice nutritional quality is an important issue in developing countries for people depending mainly on rice. Rice is rich in energy and is a good source of protein. Compared with milled rice, brown rice has a higher content of protein, minerals and vitamins but also higher levels of antinutrition factors like phytin and protease inhibitors. Diets based mainly on milled rice lead to malnutrition with deficiencies most severely in lysine, vitamin A, iron, iodine and zinc [8].

Our group employs the tools of genetic engineering in several projects that aim at a reduction of the damage caused by pests and diseases. In addition, two projects try to improve the nutritional value of rice endosperm.

2.1 Resistance to fungal diseases

Around 40 fungal rice diseases are known worldwide; the most devastating among them are rice blast (*Pyricularia oryzae*) and sheath blight (*Rhizoctonia solani*). In response to pathogen infection, many plants synthesise proteins which presumably are involved in defence mechanisms. First attempts with transgenic rice (cultivar Chinshura Boro II) constitutively over-expressing a rice chitinase gene under control of the CaMV 35S promoter led to reduced disease symptoms after infection with *R. solani* [9]. Several more genes coding for such proteins have been transformed to rice. They include: tobacco β1,3-glucanase, bean endochitinase, tobacco osmotin-like protein AP24, and an antimicrobial protein from onion. These genes are under control of the CaMV 35S or the maize ubiquitin promoter and contain signals for targeting the proteins to the vacuole or the apoplast, respectively. With all the different genes, several transgenic lines have been generated which produce the expected RNAs. Bioassays are underway to determine the resistance of these transgenic lines to *R. solani* and *P. oryzae*.

2.2 Resistance to stemborers

Hundreds of insect species attack standing and stored rice world-wide. The lepidopterous stemborers are chronic pests in many rice growing regions. Important stemborers of Asia include the striped stem borer (*Chilo suppressalis*) and the yellow stemborer (*Scirpophaga incertulas*). The adult moths lay eggs on rice leaves and the larvae bore into the stem. Feeding in the stem leads to the characteristic stemborer symptoms "deadheart" and "whitehead". Heavy infestations can locally cause losses of up to 60% [10]. The crystal δ-endodoxins from the soil bacterium *Bacillus thuringiensis* (Bt) are highly specific against a narrow spectrum of insect pests, including lepidopterae. Several Bt-genes have been isolated and their insecticidal activity has been demonstrated in many transgenic crops [11]. With the transfer of a synthetic cryIA(b) gene to IR58, a stemborer resistance trait was introduced into the germplasm of an elite indica rice cultivar [12]. Molecular analyses demonstrated the stable integration of the transgene in a single locus in the genome of IR58 and the production of a Bt-protein with the expected size of 65 kDa. Insect feeding assays in petri dishes demonstrated the insecticidal activity of the transgenic leaf material with mortalities of up to 100% with larvae of yellow stem borer and striped stem borer. Feeding assays with whole transgenic plants showed efficient protection against stem borer attack. Whitehead damage and alive larvae were only found in non-transgenic control plants. In addition, with transgenic leaves, feeding inhibition was observed with the two Lepidoptera defoliator species *Cnaphalocrocis medinalis* and *Marasmia patnalis* [12]. The potential evolution of pest resistance to genetically engineered insect resistant crops has to be considered a serious problem. Insect resistance to Bt-toxins has already been reported earlier [13]. One way to retard the evolution of pest resistance to Bt toxins is to combine several independently acting insecticidal proteins in the same plant. Therefore, rice was transformed with another Bt gene, cryIA(c), under control of the maize ubiquitin promoter. Since cross-resistance between CryIA(b) and CryIA(c) has been observed with lepidopterae before [14], additional independently acting genes like proteinase inhibitors or lectins will be needed for a more durable resistance in rice to stemborers.

2.3 Resistance to rice tungro bacilliform virus

Fifteen viruses are known to occur in rice. Many of these viruses have become serious problems since rice cultivation has been intensified. Especially planthopper- and leafhopper-borne viruses have reached epidemic proportions in many countries and have caused serious damage in rice [15]. The most devastating virus disease in Southeast Asia is rice tungro disease (RTD) which occurs epidemically and which is caused by a combined interaction of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Both viruses are transmitted in a semipersistent manner by the green leafhopper, *Nephotettix virescens*. Genetic engineering offers strategies to confer resistance to viral diseases. Sanford and Johnston [16] postulated the concept of pathogen-derived resistance (PDR) which aims at the disruption of the viral life cycle by the expression of viral sequences within the host plant. The concept has successfully been exploited in different crops. Many virus-resistant plants have been obtained after expression of viral coat proteins (reviewed by e.g. [17]). Also the transfer of mutated forms of viral replicase genes (reviewed by [18]) and genes for viral RNA polymerases has led to strong viral resistance. The mechanism involved in these latter resistances is probably related to gene silencing. Müller et al. [19] proposed homology-dependent gene silencing with post-transcriptional degradation of viral RNA. Twenty-two different constructs for the production of RTBV proteins and four constructs for the production of RTBV antisense RNA were transferred to rice. Resistance tests showed that none of these constructs led to virus resistance. However, some transgenic lines containing sequences from the promoter and the untranslated leader of RTBV, silenced the transgene driven by the RTBV promoter. Moreover, these transcriptionally silenced loci were able to silence other transgenes in trans after crossing to other transgenic lines, containing normally expressing transgenes driven by the same promoter. In resistance tests with one of these lines, 20% of the tested plants did not show disease symptoms up to 30 days post infection. However, later all the plants developed disease symptoms and failed to set seeds (Klöti et al. submitted). Further efforts with new transgenic plants may be more successful.

2.4 Engineered provitamin A biosynthesis in rice endosperm

Unlike photosynthetic tissues, rice endosperm contains neither β-carotene (provitamin A) nor its C40 carotenoid precursors [20]. Insufficient dietary (pro)vitamin A leads to severe clinical symptoms like the eye disease xerophthalmia and several childhood diseases [21]. Improved (pro)vitamin A nutrition for people depending mainly on rice could be expected to prevent one to two million deaths annually among children aged one to four years, and an additional 250,000 to 500,000 deaths during later childhood [22].

β-Carotene is synthesised in plastids from the general C20 isoprenoid precursor geranyl geranyl diphosphate (GGPP) by four specific plant enzymes: phytoene synthase, phytoene desaturase, ζ-carotene desaturase and lycopene cyclase. Since GGPP is abundant in rice endosperm, expression of the four genes should lead to engineered β-carotene production in rice endosperm. 2 mg β-carotene per gram dry seed weight would cover the daily provitamin A needs of children in 300 grams of rice. The first gene, a phytoene synthase gene from daffodil (*Narcissus pseudonarcissus*) with its own plastid localisation signal and under control of the glutelin 1 promoter has been transferred to rice. The highest phytoene content measured was 0.74 mg phytoene per gram dry seed weight in a heterozygous seed population [20]. This result shows that it is possible to engineer an isoprenoid pathway in rice endosperm. However, it remains to be demonstrated that the phytoene level can be increased and that it is possible to engineer the remaining three steps on the way to β-carotene production.

2.5 Increased iron content in rice endosperm

Iron deficiency is the most prevalent micronutrient deficiency world-wide and it has been estimated that more than one billion people suffer from iron deficiency anaemia [8]. Milled rice is characterised by a very low content of iron between 0.2 and 2.8 mg/100 grams. In addition, absorption of non-hem iron in the intestinal lumen is affected by factors like phytin, impairing iron availability and leading to reduced iron absorption of as little as one percent only [23]. None of the current intervention strategies like iron supplementation in the form of tablets or food fortification with different iron compounds was successful so far in developing countries. Genetic engineering of rice may improve the situation in three ways: (i) Increase of the total iron content of rice by expression of a ferritin gene from *Phaseolus vulgaris*. Iron from ferritin can be a dietary source as shown recently by Beard et al. [24]. (ii) Decrease of the level of phytin by expression of phytase genes from *Aspergillus niger* or from *Aspergillus fumigatus*. For a significant increase in iron absorption, phytic acid level in the endosperm has to be reduced below 0.01% [25]. (iii) Addition of absorption-enhancing cysteine by expression of a gene for a cysteine-rich, methallothionein-like protein from rice. Cysteine increases the absorption of dietary non-haem iron about two-fold as shown for cysteine-containing peptides from meat [26]. Experiments are under way to express all these genes in rice under control of the rice glutelin 1 promoter. The effect of the transgenes on iron-uptake in the human intestine and a putative additive effect of the genes remains to be demonstrated.

3. Genetic engineering of cassava (*Manihot esculenta* Crantz)

Cassava (*Manihot esculenta* Crantz), although fairly unknown in the northern hemisphere, is one of the most important food crops in the tropics. It is a perennial shrub grown in over 60 countries for its tuberous roots that contain starch up to 85% of their dry weight and provide food for over 500 million people [27]. The annual production of cassava is presently about 162 million metric tonnes from 16 million hectares, 60% of this in Africa [28, 29].

In developing countries cassava is the fourth most important and the cheapest calorie source, and in one third of the low-income food deficient countries it the most important locally produced crop (for a review see [30]). In certain regions cassava leaves are used as a major component of the diet to provide supplementary protein, minerals and vitamins to complement the carbohydrate rich staple. Cassava is mainly grown by small scale and subsistence farmers in the poorer regions, as it is well adapted to poor soils, allowing acceptable harvests even on acidic aluminium-rich marginal and eroded soils unable to support any other crop without costly external inputs. It also has the unique advantage over e.g. cereals, that its harvesting time is highly flexible. This makes it an excellent famine reserve because the plants can be partially harvested and left growing in the ground until the roots are needed. Cassava is propagated vegetatively from stem cuttings, which means that none of the yield, roots, need be set aside to secure next harvest. Besides being a major basic staple crop, cassava also provides rural jobs and income, thus being irreplaceable for food security in developing countries and especially in Africa, where the food security problems are the most severe in the world.

In Africa food security has been steadily deteriorating, and the marginalisation of this region will continue in the future [1, 31–34]. In Sub-Saharan Africa cereal production per capita has constantly declined since 1970 [34], and most countries are dependent on net cereal imports and direct food aid. The food imports and food aid notwithstanding, the daily calorie gap per person has increased by 670 calories between 1961 and 1993, and the number of chronically undernourished people has more than doubled between 1970 and 1991 [34]. The population in Africa continues to increase at an annual rate of 3.1% [35], with sub-Saharan Africa having the highest population growth rate in the world. At present 40% of people in Sub-Saharan Africa suffer from malnutrition, and according to FAO [7] this number is expected to increase to 50% by year 2000. It has been estimated that food production has to be doubled during the next 25 years, in order to keep up with the population growth. To eliminate hunger and qualitative malnutrition, the amount of plant-based calorie production has to be increased by sevenfold [7]. The small scale and subsistence farmers in Africa have little opportunity to invest in expensive inputs, e.g. fertilisers or pesticides needed to increase their yields, and thus have little possibilities to meet the increasing demand for food. Crops, which can be produced with limited inputs offer a potential of increasing food security in these countries. Cassava has been grown as subsistence and famine reserve crop in Africa for centuries, and in the past years its importance as a reliable crop giving acceptable yields with limited inputs has also been recognised internationally. Cassava is the main staple in sub-Saharan Africa providing food for over 200 million people, many of them among the poorest in the world. In 15 sub-Saharan countries, 30 million people get up to 60% of their daily calorie intake from cassava [27, 36, 37].

In contrast to other major food crops, and despite its key role in food security in the tropics, cassava was long neglected, and often considered as a hardy crop with little problems. Up to 80 t/ha roots can be produced under optimal conditions in a 12 month growing season [38], but the actual yields are only 2–27 t/ha [29]. Various pests and diseases alone are estimated to cause 20–50% yield losses world-wide, and locally they can lead to total crop failures. On another level, the low protein content of the roots and the poor storability of freshly harvested roots constitute a problem. In addition, all parts of the plant contain toxic levels of cyanogenic glucosides, which have to be removed by laborious processing before cassava can be safely consumed.

Traditional breeding is severely limited by the low fertility and highly heterozygous and outcrossing nature of the plants, which is linked to strong inbreeding depression; it is further restricted by limited gene availability in the sexually compatible germplasm. Many of the available resistance traits are in addition polygenic and/or recessive, which makes breeding for such characteristics complicated. One way of improving food security, without any external inputs directed towards higher yields, would be to secure the harvest of a larger portion of the roots already being produced in the field via reducing the losses caused by pests and diseases by producing resistant varieties, and on the other hand by improving the quality of cassava roots. Genetic improvement of cassava via biotechnology has, however, been constrained by the lack of routine, efficient and genotype independent transformation methods.

Our aim is to develop methods for applying biotechnology for cassava improvement. Hence, our first goal was to develop a method allowing production of transgenic cassava plants [30, 39, 40] (**Fig. 2**). Current projects aimed at engineering agriculturally important traits, running parallel to further method development, include insect resistance, ACMV resistance, improving root quality and prolongation of leaf life.

3.1 Prolongation of leaf life

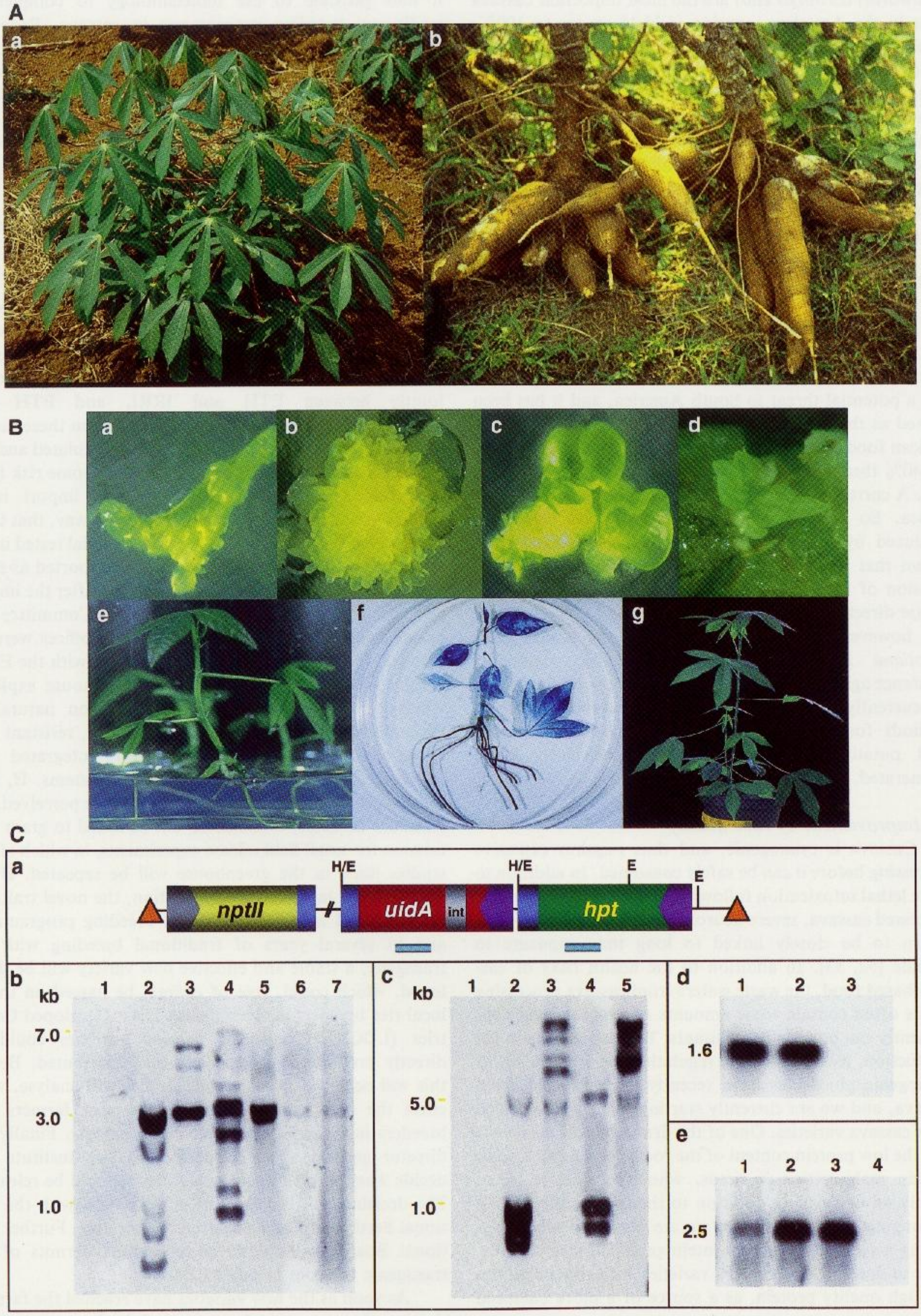
This project addresses the possibility of prolonging the life span of individual cassava leaves by use of physiologically regulated phytohormone production. In most cassava cultivars the leaf life is below the optimum, leading to suboptimal leaf area index (LAI), which in turn causes reduced root yields. Premature shedding of leaves during short drought periods in semiarid regions also influences the root quality negatively (C. Iglesias, pers. comm). On the other hand, in certain areas cassava leaves constitute a major dietary protein source [41]. To prevent root yield losses, leaves can only be harvested every two months. Should it be possible to prolong the leaf life, more frequent harvesting could follow, as the photosynthetically active older leaves would still supply the plants with an acceptably high LAI to ensure good root yields. As the market value of leaves is often higher than that of the roots [41], this could also contribute to household economies.

A construct, containing a gene encoding cytokinin biosynthesis (*ipt*) driven by a senescence specific promoter (*sag* from Arabidopsis) [42] was transformed into cassava. The system is tightly autoregulated, as starting senescence activates the SAG12 promoter, leading to cytokinin production, which in it terms prevents leaf senescence and inhibits SAG12 activity. Thus no abnormalities encountered in plants with consitutively expressed *ipt* genes should be expected. Molecular analysis have confirmed the transgenic nature of the regenerated plants and the next step will be to examine the phenotype of the transgenic plants under natural growth conditions.

3.2 Insect resistance

Due to its long growth period, 8–24 months, cassava is susceptible to repeated and prolonged attacks from several insect pests [43]. Cassava is mainly grown by subsistence farmers, for whom use of pesticides is economically prohibitive, in addition to being environmentally

Fig. 2 Panel A. (a) young cassava plant (b) cassava roots. Panel B. (a) induction of cassava somatic embryos (b) cycling somatic embryos (c) germination of somatic embryos (d) induction of organogeneis on somatic cotyledon (e) regeneration of cassava via organogenesis (f) GUS staining of transgenic cassava shoot (g) transgenic cassava plant in the greenhouse. Panel C. (a) transformation construct used for production of transgenic cassava indicating restriction sites and probes used (b) Southern blot of transgenic cassava; lanes 3, 5–7, *Hin*dIII digest from plants 1, 2, 3 and 4 respectively; lanes 2, 4, *Eco*RI digest from plants 1 and 2, respectively; lane 1, negative control; hybridisation to *uidA* probe (c) Southern blot of transgenic cassava plants, hybridised to *hpt* probe; lane 1, negative control, lanes 2, 4, *Eco*RI digests; lanes 3, 5, *Hin*dIII digests from plants 1 and 2, respectively; (d) Northern blot, *hpt* probe (e) Northern blot, *uidA* probe.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unsound. Cassava stem borers (*Chilomina clarkii*) and hornworms (*Erinnyis ello*) are the most important cassava pests in the Americas, causing yield losses up to 100%. *Bacillus thuringiensis* has been shown to be efficient in biological control of cassava hornworm [44] (Bellotti, pers. comm.), but the stem borer larvae are inaccessible to sprayed Bt toxin. An attractive alternative to spraying Bt would be the production of the toxin in the plants themselves, thus complementing the available methods for pest control in an environmentally and economically sustainable way. The first putative transgenic cassava shoots carrying Bt genes have been regenerated in our laboratory, and the material is currently being multiplied for molecular analysis.

3.3 ACMV resistance

African cassava mosaic disease (ACMD) is the most serious threat to cassava production in Africa at present, and a potential threat to South America, and it has been ranked as the most important vector-borne disease in all African food crops [45]. The yield losses caused by ACMD are 40% throughout the continent and locally up to 80% [46]. A current pandemic is devastating large areas in East Africa. So far no totally resistant cassava has been produced by traditional breeding. It has already been shown that the most commonly used strategies like expression of the virus coat protein in either sense or antisense direction is not efficient against ACMV [47]. There are, however, recent indications from the model plant *Nicotiana benthamiana* that genetically engineered resistance against ACMV can also be achieved [48–51]. We are currently developing and evaluating several different methods for engineering ACMV resistance in cassava. First putative transgenic shoots are currently being regenerated.

3.4 Improvement of root quality

Cassava is cyanogenic, and thus requires extensive processing before it can be safely consumed. In addition to acute lethal intoxication following ingestion of improperly processed cassava, severe neurological disorders have been shown to be closely linked to long time exposure to cyanide [52, 53]. In addition to the health risks of cassava-based food, the waste waters from cassava processing plants often contain toxic amounts of cyanide, and consequently can be serious pollutants. The key enzyme in the production, as well as those regulating the breakdown of cyanogenic glucosides have recently been isolated from cassava, and we are currently starting a project to design safer cassava varieties. One of the drawbacks of cassava is also the low protein content of the roots, which can lead to protein malnutrition in areas, where the diet is based mainly on cassava. In addition to the project directed to prolonging cassava leaf life, we are also currently transferring a synthetic storage protein gene [54] into cassava, so as to develop new cassava varieties with roots containing high quality protein, as a source of a more balanced diet.

In conclusion, the development of a transformation method allowing regeneration of transgenic plants makes it now possible to use biotechnology to complement traditional breeding programmes in cassava. Problems that could be efficiently solved by biotechnology include for example disease resistance, pest resistance, improved stay-green index and improved nutritional quality of cassava roots. These projects as part of integrated crop management programmes will offer a considerable contribution to food security and sustainable agriculture.

4. Careful biological and socio-economic risk assessment ensures that the release of transgenic rice and cassava varieties will not have negative effects

Every single transgenic variety has to pass an extensive series of rigorous technology assessment studies before it will be released to the farmers. As there is no financial return expected from the varieties developed jointly between ETH and IRRI, and ETH and IITA/CIAT, there is no external pressure on these assessments. Work in the laboratory is strictly regulated and not even opponents to gene technology expect some risk from this phase. For export, transport and import international regulations are designed in such a way, that there is not much concern either. The ETH material tested in the containment greenhouse at IRRI was transported as seeds in a double-walled, unbreakable container after the import permission from the Philippine Biosafety Committee was granted and the Swiss national biosafety offices were informed. At IRRI, in further collaboration with the ETH, careful studies in the containment greenhouse explored whether there was any negative effect on natural or agricultural ecosystems and how the novel, resistant varieties could best be used to support integrated pest management and integrated production systems. If, as a result of these studies, no special risk can be perceived, the National Biosafety Committee will be asked to grant permission for small field release experiments, in which all the studies done in the greenhouse will be repeated. If the results speak in favour of application, the novel trait will be integrated into the traditional breeding programmes, and in several years of traditional breeding with the transgene, a stable and effective new variety will be developed, which could, free of charge, be passed on to the local rice breeders in the different Least Developed Countries (LDC's). Transgenic cassava varieties could be directly propagated vegetatively and distributed. Before this will occur, socio-economic studies will analyse, what effect the new variety may have on poor farmers and breeders in different economic environments. Finally the director general of the respective CGIAR-Institute will decide whether or not the new variety should be released. The decision will be taken after discussion with the National Seed Board and Biosafety Committee. Further National Boards will decide about import permits of the transgenic varieties to other LDC's.

As soon as the new varieties have reached the farmer, they are the unrestricted property of the farmer who can, if he wishes, continue to produce his own seed or vegetative propagule without any limitations. There is no "patenting" involved, no "new dependencies" of Third World countries from industrialised countries, no "danger for biodiversity", no "genetic contamination of the rice or cassava gene pool", etc. The CGIAR-institute will, together with the material, pass on to the farmer the information on how the new varieties are used in integrated pest management in the different rice ecosystems.

The entire procedure is so sophisticated not because it is expected, that these transgenic varieties pose any special risk, but because all involved want to ensure, that at the beginning of the application of a new technology, every risk which could be imagined using all present knowledge and experience of biology (including ecology and molecular biology), agronomy and social sciences, should be avoided.

Besides all the concerns mentioned above, it should not be neglected, that genetic engineering, with the cases described above, can save food for hundreds of millions of poor people, food which is required for their survival, and which, to date, can not be recovered by any other means; food which is the more difficult to gain, the closer we get to the limits of the "earth's carrying capacity". It would, therefore, be irresponsible and unethical, not to take every possible effort now, to apply genetic engineering for food security in the Least Developed Countries.

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References

[1] Brown, L.R., Kane, H., 1994. The Worldwatch Environmental Alert Series, (ed. Stark, L.), W.W. Norton and Company, New York, London.

[2] Islam, N., 1995. Population and food in the early twenty-first century: meeting future food demand of an increasing population. International Food Policy Research Institute.

[3] Birg, H., 1996. Die Eigendynamik des Weltbevölkerungswachstums, In "Spektrum der Wissenschaft. Dossier Dritte Welt," pp. 34–42, VDO, Heidelberg.

[4] Oerke, C.E., Dehne, W., Schönbeck, F., Weber, A., 1994. Crop production and crop protection. Elsevier, Amsterdam.

[5] Hammond, A.L. et al., 1994. World Resources 1994–95. The World Resource Institute, Oxford, University Press.

[6] IRRI, 1993. IRRI Rice Almanac. IRRI, Manila.

[7] FAO, 1998. FAOSTAT Agriculture Statistics Database, <http://www.fao.org/WAICENT/Agricul.htm>; Background documents for the Food Summit, <http://www.fao.org/wfs/resource/resource.htm>.

[8] FAO, 1993. Rice in human nutrition. FAO, Rome.

[9] Lin, W., Anuratha, C.S., Datta, K., Potrykus, I., Muthukrishnan, S., Datta, S.K., 1995. Bio/Technology, **13**: 686-691.

[10] Pathak, M.D., Khan, Z.R., 1994. IRRI, Los Banos, Philippines.

[11] Krattiger, A.F., 1997. ISAAA Briefs No. 2. ISAAA: Ithaka, NY.

[12] Wünn, J., Klöti, A., Burkhardt, P.K., Ghosh Biswas, G.C., Launis, K., Iglesias, V.A., Potrykus, I., 1996. Bio/Technology, **14**: 171-176.

[13] Whalon, M.E., McGaughey, W.H., 1993. In "Advanced engineered pesticides" (ed. Kim, L.), p. 215–232, Marcel Dekker, Inc., New York.

[14] Gould, F., Anderson, A., Reynolds, A., Bumgarner, L., Moar, W., 1995. J. Econ. Entomol., **88**: 1545–1559.

[15] Hibino, H., 1996. Annu. Rev. Phytopathol., **34**: 249–274.

[16] Sanford, J.C., Johnston, S.A., 1985. Theor. Biol., **113**: 395–405.

[17] Wilson, T.M.A., 1993. Proc. Natl. Acad. Sci. USA, **90**: 3134–3141.

[18] Carr, J.P., Zaitlin, M., 1993. Sem. Virol., **4**: 339–347.

[19] Müller, E., Gilbert, J., Davenport, G., et al., 1995. Plant J., **7**: 1001-1013.

[20] Burkhardt, P.K., Beyer, P., Wünn, J., Klöti, A., Armstrong, G.A., Schledz, M., von Lintig, J., Potrykus, I., 1997. Plant J., **11**: 1071–1078.

[21] Sommer, A., 1988. J. Nutr., **119**: 96–100.

[22] Humphrey, J.H., West, Jr, K.P., Sommer, A., 1992. WHO Bulletin, **70**: 225–232.

[23] FAO, 1988. FAO food and nutrition series, **23**: 17–32. FAO, Rome.

[24] Beard, J.L., Burton, J.W., Theil, E.C., 1996. J. Nutr., **126**: 154–160.

[25] Hurrell, R.F., Juillerat, M.A., Reddy, M.B., Lynch, S.R., Dassenko, S.A., Cook, J.D., 1992. Am. J. Clin. Nutr., **56**: 573–578.

[26] Layrisse, M., Martinez-Torres, C., Leets, I., Taylor, P.G., Ramirez, J., 1984. J. Nutr., **114**: 217–223.

[27] Cock, J.H., 1985. Cassava: new potential for a neglected crop. Westview Press, Boulder, Colorado.

[28] CIAT, 1996. Cassava: the latest facts about an ancient crop. CIAT, Cali, Colombia.

[29] FAO, 1997. Faostat Database Agriculture Statistics Database, <http://apps.fao.org>.

[30] Puonti-Kaerlas, J., 1998. In "Biotechnology and Genetic Engineering Reviews" (Tombs, M.P. ed), Intercept Ltd, Andover, Hampshire, UK **15**: 329–364.

[31] Dresrüsse, G., 1996. Perspective of World food supply and demand—challenges and new focuses. Proceedings of Intl. Symposium Food Security and Innovations: Successes and Lessons Learned.

[32] FAO, 1996. Food outlook, <http://www.fao.org/waicent/faoinfo/economic/gviews/english/fo9604/sect2toc.htm>.

[33] IFPRI, 1995. A 2020 vision for food, agriculture and the environment.

[34] Singer, H.W., 1996. A global view of food security. Proceedings of Intl. Symposium Food Security and Innovations: Successes and Lessons Learned.

[35] World Bank, 1995. Development Indicators 1995.

[36] CIAT, 1994. Facts and Figures on cassava. CIAT, Cali, Colombia.

[37] Koch, B.M., Sibbessen, O., Swain, E., Kahn, R.A., Liangcheng, D., Bak, S., Halkier, B.A., Lindberg Moeller, B., 1994. Acta Hortic., **375**: 45-60.

[38] Hersey, C.H., 1993. In "Genetic improvement of vegetable crops" (eds. Kalloo, G., Bergh, B.O.), pp. 669-691. Pergamon Press, Oxford, New York.

[39] Li, H.-Q., Sautter, C., Potrykus, I., Puonti-Kaerlas, J.,

1996. Nature Biotechnology, **14**: 736–740.

[40] Puonti-Kaerlas, J., Li, H.-Q., Sautter, C., Potrykus, I., 1997. African J. Root Tuber Crops, **2**: 181–186.

[41] Bokanga, M., 1994. Acta Hortic., **375**: 203–207.

[42] Gan, S., Amasino, R.M., 1995. Science, **270**: 1986–1988.

[43] Bellotti, A.C., 1979. In "Proceedings, Cassava Protection Workshop, CIAT, Cali, Colombia 1977" (eds. Brekelbaum, T., Bellotti, A., Lozano, J.C.), pp. 17–28. CIAT, Cali, Colombia.

[44] Bellotti, A., Arias, B., 1979. In "Proceedings, Cassava Protection Workshop, CIAT, Cali, Colombia 1977" (eds. Brekelbaum, T., Bellotti, A., Lozano, J.C.), pp. 227–232. CIAT, Cali, Colombia.

[45] Geddes, A.M.V., 1990. The relative importance of crop pests in sub-Saharan Africa. Natural Resources Institute Bulletin 36, Chatham, UK.

[46] Thresh, J.M., Otim-Nape, G.W., Fargette, D., 1977. African J. Root Tuber Crops, **2**: 13–19.

[47] Bejarano, E.R., Lichtenstein, C.P., 1994. Plant Mol. Biol., **24**: 241–248.

[48] Stanley, J., Frischmuth, T., Ellwood, S., 1990. Proc. Natl. Acad. Sci. USA, **87**: 6291–6295.

[49] Frischmuth, T., Stanley, J., 1991. Virology, **183**: 539–544.

[50] Hong, Y., Saunders, K., Hartley, M.R., Stanley, J., 1996. Virology, **220**: 119–127.

[51] Hong, Y.G., Stanley, J., 1996. Molecular Plant-Microbe Interactions, **4**: 219–225.

[52] Rosling, H., 1988. Cassava toxicity and food security. Unicef, African Household Security Programme. Tryck Kontakt Uppsala, Sweden.

[53] Akintowa, A., Tunwashe, O., Onifade, A., 1994. Acta Hortic., **375**: 285–288.

[54] Yang, M.S., Espinoza, N.O., Nagpala, P.G., Dodds, J.H., White, F.F., Schnorr, K.L., Jaynes J.M., 1989 Plant Science, **64**: 99–111.