Micronutrient bio-fortification and disease resistance in Banana A Unique Initiative of Public Sector Research for Public Good

a. Vegetatively propagated genetically complex crops like banana are difficult to improve by conventional breeding.

Food and nutritional security needs to be addressed in several ways, one of which could be biofortification of fruits. Being grown in tropical and sub-tropical regions of the world, bananas & plantains are among the most important crops. Yet, they are the least improved when compared to other major crops. The majority of banana production is based on the cultivars derived from wild collections. The genetic system of banana is complex and difficult to generate variability by crossbreeding and genetic recombination. Factors such as different genomic constitutions, heterozygosity, polyploidy and parthenocarpic fruit development make the application of conventional techniques for improvement even more difficult in banana. *It is important to mention that conventional breeding programs are not apt for bio-fortifications like enhancement of Vit. A and Iron and development of resistance against pests.* This complexity needs the development of innovative approaches to support conventional breeding programs, and a promising one would be to develop bio-fortified bananas.

Genetic improvement of banana is considered to hold great potential for the introduction of multiple useful characters like, disease resistance and enhanced nutritional value since through use of new technologies and approaches, such traits can be introduced relatively rapidly in elite cultivars without compromising their good native traits. Among the fungal diseases affecting banana, Black Sigatoka and Fusarium wilt are most threatening. Besides these, bacterial wilt and viral diseases such as banana bunchy top, banana streak and banana bract mosaic affect banana yield significantly. Due to these diseases the cultivation of some of the important elite Indian varieties such as Rasthali has been reduced considerably.

One of the major public health problems, ranking next to HIV/AIDS and malaria in Indian populations is micronutrient deficiencies. Vitamin A deficiency can result in blindness and night blindness while as iron deficiencies lead to anaemia, reduced immune competence which results in increased morbidity and mortality, usually as a consequence of increased severity of infectious diseases and less well defined retardation of growth. Bananas could be engineered to help overcome nutrient deficiencies.

b. Biofortification is an easily implementable solution to address malnutrition at population level.

An important science driven strategy that has been gaining acceptance for increasing micronutrient content in common food crops world over is to enhance the content of micronutrients in their natural form in farm produce, by an approach called as Biofortification. This involves selecting or developing cultivars of staple crops for high amount of specific micronutrients. This strategy has the potential to have a very significant impact on the reduction

of micronutrient deficiencies particularly in rural populations. Biofortification essentially improves the already grown and/or consumed and accepted crops and therefore does not require any significant change in eating behaviour, food habits, educating masses or food processing. Some of the important micronutrients whose deficiency is common in Indian population include vitamin A, iron, zinc, calcium, folic acid, vitamin D and iodine. Micronutrients can normally be taken in adequate quantity through a well-planned diet. For comparison, a number of Indian food crops with high content of iron and beta carotene (precursor of vitamin A) are listed in the tables below and compared with banana, taken as a model crop for improvement. In spite of these nutrients being present in diverse foods, serious deficiency of iron, iodine, zinc, calcium, vitamin A, vitamin D, Folic acid in Indian population warrants additional measures to enhance these in more commonly consumed but micronutrient poor sources of foods. These need to be increased, specially in staple sources of carbohydrate crops that are consumed in larger volumes by the young, old and poor and are often low in one or more of the essential micronutrients. As per International Health Standards, an average requirement of Iron by an individual is 15 mg on daily basis. However, the unfortified bananas have 0.4 mg/100 gm of banana while the fortified banana would supplement this to 2.6 mg/ 100grams. In other way, if an individual consumes a single banana-a-day, the contribution of daily intake of banana would be 13% in bio-fortified banana as against 4% in non-fortified banana (Table 1). Such a strategy would give all sections of community, a natural way to consume sufficient amount of micronutrients, without forcing them to change food habits or consume unusual amounts of specific foods.

c. Nutrient content alone does not address the problem of malnutrition.

The table below shows estimated % relative contribution of individual food to total recommended daily requirement of iron and vitamin A. For micronutrients like iron, there is a serious problem of poor bioavailability, meaning that in spite of being present in food, the nutrient is not taken up by human body. For example, only about 2% of the iron present in spinach and cereal crops is available to the body. Amaranthus is significantly better than others, but cannot easily become a major part of favourite foods. In case of vitamin A, foods contain the precursor molecules like carotenoids but only a small fraction (a sixth or even lower) of these is converted in human body into vitamin A. Detailed data on the bioavailability and metabolic utilisation of nutrients present in food crops in future will hopefully aim at genetic designing of crops for higher bioavailability and bioconversion of nutrients. Thus the need for accelerating the search for feasible solutions, and opportunities in crop designing for nutritional wholesomeness requires inputs from the best of science to ensure inclusive human development.

Vegetables/Fruits/ cereals	Iron content	Assumed daily intake	Contribution to the daily iron intake ¹	Bioavailability ²
Iron fortified Banana	2.6 mg/ 100g banana	1 banana	17%	NA
Unfortified banana	0.4 mg/ 100g banana	1 banana	4%	NA

Table 1: List of food items with relatively high iron content and comparison with banana.

Apple	2 mg/ 100 gram apple	1 apple	13%	NA
Raisins	3 mg/100 gram	1 gram	3%	NA
Dried Coconut	3 mg/ 100 gram	NA	NA	NA
Lotus root/stem	0.9 mg/100 gram	NA	6%	NA
Spinach	3.5 mg/100 gram	100 gram	23%	2%
Amaranthus	7.6 mg/100 gram	50 gram	25%	11%
Chenopodium	1.2 mg/ 100 gram	100 gram	7%	NA
Moringa drumstick	5 mg/ 100 gram	50 gram	15%	NA
Olives	3 mg/ 100 gram	NA	-NA	NA
Potato	0.5 mg / 100 gram	200 gram	6%	NA
Sweet Potato	0.61 mg / 100 gram	100 gram	4%	NA
Cowpeas	3 mg/ 100 gram	100 gram	20%	1.7%
Oat	5 mg/ 100 gram	100 gram	32%	0.33%
Barley	2.5 mg/ 100 gram	100 gram	16%	5%
Soyabean	15.7 mg / 100 gram	100 gram	100%	7%
Lentil (sprout)	3 mg /100 gram	100 gram	20%	4%
Maize	0.5 mg/ 100 gram	100 gram	3%	1.8%
Rice	0.8 mg/ 100 gram	100 gram	5%	1.73%
Wheat	3 mg/ 100 gram	250 gram	40%	0.99%
Buckwheat	8 mg/ 100 gram	NA	NA	NA
Poppy seed	10 mg/ 100 gram	NA	NA	NA
Cumin seeds	66 mg/ 100 gram	NA	NA	NA
Tamarind	3 mg/ 100 gram	NA	NA	NA
Chickpeas	3 mg/ 100 gram	100 gram	18%	NA
Bajra	8 mg/ 100 gram	100 gram	53%	5%
Turmeric	40 mg/ 100 gram	1 gram	3%	NA
Amchur (dried green mango)	4.5 mg/100 gram	NA	NA	NA

• ¹% contribution based on 15 mg daily requirement of iron.

•² Iron bioavailability from non-heme food items varies from 1-15% of total iron present (Am J Clin Nutr 2003; 78(suppl):633S-9S)

• NA: Information not available.

• Values are from diverse published sources and therefore, may have technique based variations.

Table 2: β -Carotene content in foods known for high amount of carotenoids and comparison with banana.

Vegetables/Fruits/cereals	β-Carotene content	Assumed Daily intake in India	Contribution to the daily requirement ¹
-Carotene fortified Banana	2.4 mg / banana	1 banana/100g	67%
Unfortified banana	110 g/ banana	1 banana/100g	3%
Carrot	8.3 mg /100 gram	1 carrot/100g	100%
Raisins	NA	NA	-
Apple juice	0.0185 g/ ml	NA	-
Lotus root/stem	320 g /100 gram	NA	-
Spinach	5.6. mg /100 gram	100 gram	100%

Amaranthus	20 mg /100 gram dw	-NA	-
Chenopodium	0.19-5.91 mg / 100 gram fresh weight	NA	100%
Moringa drumstick	23 mg / 100 gram dw	10 gram	100%
Cowpeas	240 µg/ 100 gram	100 gram	7.0 %
Oat	NA	-	-
Maize	52 g /100 gram	100 gram	1.40%
Rice	ND	-	0
Barley	ND	-	0
Potato	1 g /100 gram	200 gram	-
Cassava	8 g /100 gram	NA	-
Sweet Potato	8.5 mg / 100 gram	100 gram	100%
Soyabean	ND	-	0
GM corn	5.9 mg /100 gram	100 gram	100%
Golden rice	3.6 mg /100 gram	100 gram	100%
Lentil (sprout)	NA	-	-
Bajra	NA	-	-
Wheat	ND	-	0
Turmeric	ND	-	0
Turmeric (leaf)	7.6 mg /100 gram dw	-	0
Amchur (dried green mango)	6 mg /100 gram	-	-
Chickpeas	ND	-	0
Buckwheat	NA	-	-
Olives	20 g /100 gram	NA	-
Poppy seed	NA	-	-
Cumin seeds	64 µg / 100 gram	NA	-
Tamarind	ND	-	0

• ¹% contribution, based on 3600 μ g daily requirement for β carotene.

• NA: Information not available.

• ND: Below the detection limit.

• The values are from diverse published sources, and may have technique based variation.

d. Why Banana is a good system to work on for Biofortification .

Bananas are an ideal crop for micronutrient biofortification in India. They are very widely and commonly grown and consumed and are traditionally important dietary component. As most of the edible bananas are vegetatively propagated, concerns regarding transgene outflow are minimal in banana and therefore genetically modified bananas can be grown alongside non-GM bananas in the same field. Also since the GM bananas are sterile, the existing diversity of bananas in India will not be affected and there won't be any heritable mixing of GM and non-GM cultivars in nature.

e. Varietal diversification and improvement in banana are important to guard against monoculture.

The cultivation of a single high yielding disease and pest resistant Cavendish variety in large areas has resulted in the reduced cultivation of native landraces, which are generally susceptible to various diseases and pests. Generation of genetically modified plants of the native landraces like Rasthali will help in conservation of these important elite varieties. Further, because most of the commercial banana cultivation in India is done as large monocultures of Cavendish banana of same genetic constitution, a single crippling pathogen can wipe out the whole plantation rapidly. Keeping in mind this scenario, India needs to be ready with the state of art technologies like the GM approaches, for rapidly introducing multiple disease resistance and multiple micronutrients in Indian banana varieties.

Dessert bananas are consumed fresh and the flesh of the dessert bananas is not exposed to light prior to consumption; therefore carotenoid degradation is minimal in banana. This is particularly advantageous as compared to increasing carotenoids, like vitamin A in seeds where carotenoids degrade during storage. Successful development of micronutrient fortified and disease resistant banana lines will therefore be most suited to our larger national objective of increased food productivity and availability of healthy food leading to eradication of micronutrient related malnutrition in the country.

With the background, and strength of the technology developed by an eminent Scientist Prof. James Dale from Queensland University of Technology, Brisbane, Australia, it was felt appropriate to start initially a collaborative Technology Development Program for bringing in the benefits of this technology to our Public Sector Researchers.

In order to facilitate the development of disease resistant Indian cultivars and increase the micronutrient content in Indian bananas, the Biotechnology Industry Research Assistance Council (BIRAC), Govt. of India has initiated a network project with five collaborating Indian partners National Agri-Food Biotechnology Institute (NABI), Mohali; National Research Centre on Banana (NRCB), Trichy; Bhabha Atomic Research Centre (BARC), Mumbai; Indian Institute of Horticulture Research (IIHR), Bangalore and Tamil Nadu Agriculture University (TNAU), Coimbatore for the fortification of Indian banana fruits with iron, pro-vitamin-A and the development of resistance to Fusarium wilt and Banana Bunchy Top Virus disease. This has been initiated in collaboration with Prof. James Dale's group placed in Queensland University of Technology, Australia so that several leads already available with them can be applied and improved further. This technology when developed by the Public Sector Scientist would be an excellent example of Public Sector Research for Public good

Besides getting bio fortified banana for the Indian varieties like Rasthali and Grand Naine, these technology transfer project includes **capacity enhancement of Indian scientists and students, at QUT on transformation, regeneration methodology and studies on** Safety, Compliance, Containment and confinement of the improved product.