# Economic Botany

## A Comprehensive Study

5th edition

*Web Resource*

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# Green Revolution: An Overview

Although crop improvement is as old as agriculture itself, organised plant breeding dates back to not more than about 200 years ago. By the end of the eighteenth century, farmers, through the informal process of selection, created thousands of different varieties or landraces for each major species. These varieties have a broad genetic base and are highly heterozygous. Selection remained the principal method of crop improvement until only the beginning of the last century. Thousands of varieties were selected and cultivated for our major crops all over the world.

## History of Yield Advance

In the pre-hybrid days, the yield of the US corn never exceeded 37 bushels per acre. However, the introduction of hybrid corn varieties in the 1920s resulted in a fourfold increase in the yield between 1920 and 1990, and this was paralleled by the development of the corn breeding industry. Plant breeders associated with this industry (for details refer to pages 114-117) did most of the corn breeding work. The success of the hybrid crop was greatest in the first generation with yields diminishing in the succeeding generations. As a result, the farmers had to buy new hybrid seeds or grains every season. Later, the discovery of a gene responsible for male sterility and restoration of fertility led to a convenient method to produce hybrid corn. The new method was so convenient that within a very short span, all corn planted in the US had this particular gene.

## What Is Green Revolution?

The term 'green revolution' refers to the very substantial yield increases obtained by plant breeders, resulting from the development of new crop varieties under an intensive programme of fertilisers, water and pesticides management. The high yielding varieties (HYVs) of wheat and rice have been the key element in the green revolution.

Rice is the world's single most important food crop and a primary food source for more than a third of the world's population. More than 90 per cent of the world's rice is grown and consumed in Asia, where about 60 per cent of the earth's population live. It is planted on about 146 million hectares of land annually, that is, 11 per cent of the world's cultivated land. Wheat is grown on 240

million hectares, an area larger than that of any other crop. It contributes more calories and protein to the world's diet than any other food crop.

## Wheat Breeding–An Era of Vanishing Yield Barriers

The beginning of the green revolution* dates back to 1943 when the Rockefeller Foundation and the Mexican Ministry of Agriculture launched a joint research programme designed to increase the productivity of Mexico's basic crops, such as corn, beans, potatoes and wheat. In Mexico, the yield of wheat before the green revolution averaged 11 bushels per acre and it imported half of the wheat it consumed. In 1944, Dr Norman E. Borlaug (a geneticist by training with original interests in forestry and control of plant diseases) along with three agronomists joined the research programme with the general aim of improving the yield potentials of crops. Borlaug brought into Mexico high yielding wheat strains or varieties from all over the world. He then attempted to develop hybrids with the ultimate goal of producing a variety that would give high yields in the varied environments found in Mexico. The development of new wheats possessing rust resistance and improved cultural practices, such as the use of fertilisers and pesticides had made a substantial impact on the wheat yields by 1957–the year Mexico achieved self-sufficiency in wheat. Over a 10-year period, the average yield rose from 740 kg/ha in 1945–46 to 1440 kg/ha in 1957–58 and then the yield response began to level off. To increase the yields of wheat further, more fertiliser responsive varieties had to be bred.

In 1946, while all of this was going on, a USDA scientist, S.C. Salmon, who was then agricultural adviser to the US occupation army in Japan, brought back from Japan seeds of several dwarf wheat varieties (collectively called 'Norin' wheats) that remained erect under heavy application of fertilisers. He distributed the seeds to breeders in the United States. Working on these Japanese wheats, a group at Washington State University led by another USDA scientist, O.A. Vogel, succeeded in producing several semi-dwarf strains. One of these strains, known as 'Gaines' produced a world record of 216 bushels per acre in the US (Figure W1.1), but it was adapted to temperate zones, that is with long days of summer and short days of winter.

In 1953, Borlaug received seeds of Norin wheats and some advanced hybrid selections (from Norin 10 x Brevor) that Vogel's group had on hand. He crossed these with already developed disease resistant Mexican varieties to develop semi-dwarf spring wheats. The selected progenies not only received semi-dwarf stature from Norin 10 x Brevor lines but also inherited genes that increased the number of fertile florets per spikelet and the number of tillers per plant. Mexican farmers grew Pitic 62 and Penjamo 62 (both short-statured and lodging resistant spring wheat varieties) in 1962 (Figure W1.2). They were followed by the release of Sonora 63, Tobari 66, Jaral 66, Siete Ceross, Inia 66 and Norteno 67. In addition, some of the so-called miracle semi-dwarf wheats grew well in several climates. By the end of the 1960s, Mexican wheat yields had more than trebled to 39 bushels per acre, and Mexico became self-sufficient in wheat despite substantial population growth. In fact, by 1954 Mexico changed from a wheat importer to a wheat exporter. In 1963, the original Rockefeller

* It was in March 1968 that the director of the US Agency for International Development (US AID), William Gaud, first used the phrase "green revolution" to describe the great gains in yields of rice, wheat, maize, and other crops through the use of HYVs. Specificality the term "green revolution" refers to wheat and rice but some agricultural scientists even include in its compass maize, soybean and sugar cane, where spectacular gains in yield have occurred.

funded research programme in Mexico was reorganised and renamed the International Centre for the Improvement of Maize and Wheat (the Spanish acronym for which is CIMMYT).

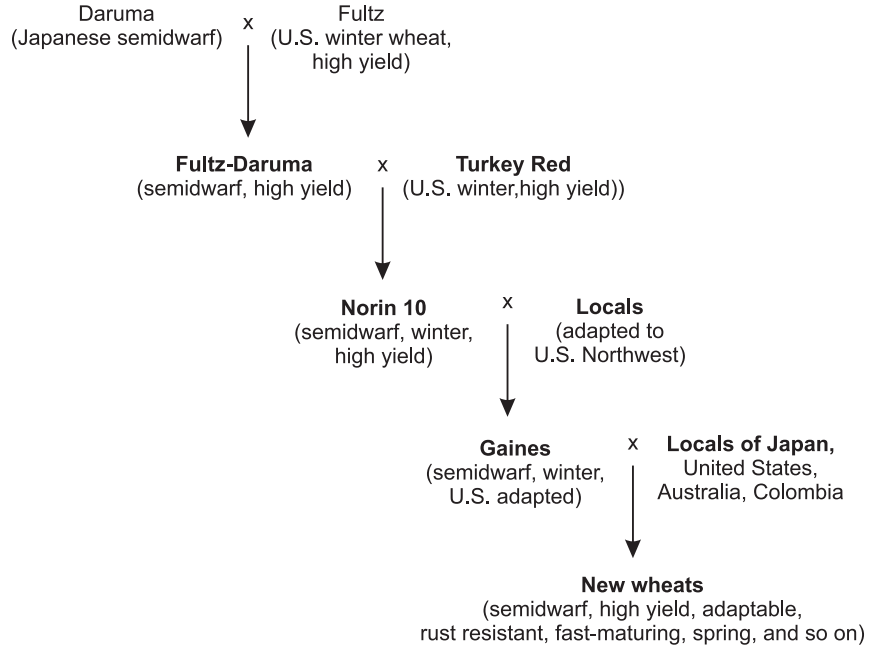



**Figure W1.1.** Origin of the semi-dwarf wheats. The first two sets of crosses, resulting in the production of Norin 10 (a high yielding, semi-dwarf winter wheat) were performed in Japan while the next cross producing Gaines was done by Orville Vogel in Washington, US. The last cross producing the new spring wheats was accomplished by Dr Norman E. Borlaug and his colleagues in Mexico.

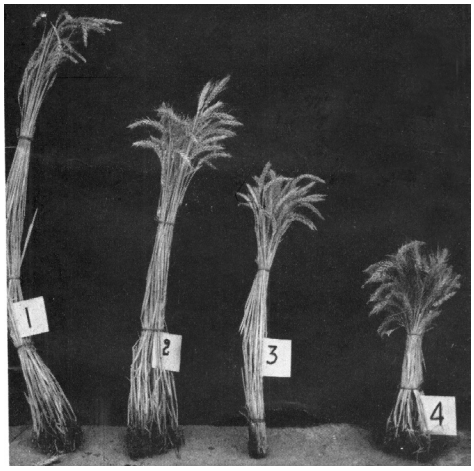



**Figure W1.2.** Wheat varieties with different stem lengths, (1) traditional tall wheat variety, (2) one-gene dwarf, (3) two-gene dwarf and (4) three-gene dwarf or triple dwarf. The Norin 10 dwarfing genes have a cumulative effect.

The Mexican varieties proved remarkably well adapted to India and Pakistan, both of whom were involved in the programme from an early date. New Mexican wheats, such as Sonora 63, Sonora 64, Lerma Rojo and Mayo were first grown in India in 1962. By 1965, India had placed an import order for 18 000 metric tonnes of Mexican wheats, and Pakistan for 42 000 metric tonnes.

The successful rapid transfer and diffusion of technology halfway around the globe was the 'break' that triggered off an era of vanishing yield barriers. Nearly 75 per cent of the wheat under high yielding varieties is concentrated in only three countries, that is, India, Pakistan and Argentina. In 1990, Indian farmers harvested over 50 million tonnes of wheat as compared to 12 million tonnes in 1964, that is, a 400 per cent increase in 25 years. Similar remarkable gains in the total wheat production have been reported elsewhere. The speed with which the Indian scientists, under the dynamic leadership of Dr M.S. Swaminathan, have achieved the success has hardly ever been witnessed anywhere else in the world. Even Mexico, where dwarf wheats were introduced earlier needed 17 years, whereas India had done it in five years (for details see the text from pages 81-83).

This dramatic change in the worldwide increase in productivity has come to be known as the 'green revolution'. Dr Borlaug won the Nobel Peace Prize in 1970, not so much for the technology that produced the HYVs, but more for his humanitarianism as he helped to feed the hungry world at a time when widespread famine was predicted (Figure W1.3).

**Figure W1.3.** Dr Norman E. Borlaug, the Father of green revolution, was awarded the Nobel Peace Prize in 1970 for his outstanding contribution to serve humanity. Standing towards his right is Dr M.S. Swaminathan, FRS–an outstanding geneticist, green revolutionist and former Director General of IRRI, Manila, Philippines.

**Box W1.1: The Wheat Revolution: Genesis and Growth**

**M.S. Swaminathan**

In a recent paper, Raghavan (1995) has analysed the improvement of wheat production and productivity in India during the period 1952–53 to 1992–93. He has concluded that the wheat crop has exhibited a robust growth trend for a considerably long period since the onset of the green revolution in 1968. In 1995, our farmers harvested nearly 63 million tonnes of wheat, while the wheat harvest at the time of our independence in 1947 was 6 million tonnes. Much of the increase in wheat production has resulted from productivity improvement. Had this not occurred, we would have needed nearly 65 million ha of area to produce the 63 million tonnes, in contrast to the current actual area of about 24 million ha. Such phenomenal progress has been possible because of the introduction of mutually reinforcing packages of technology, services and public policies through the High Yielding Varieties Programme introduced by the Government of India in 1966. Yield improvement in wheat is one of the most exciting adventures in the field of agricultural science not only in our country but also in the entire world. The proceedings of a Dialogue organised in Madras and New Delhi in 1990 helped to recapitulate the major events, which resulted in the Wheat Revolution (Swaminathan, 1993).

Wheat has been cultivated for several thousand years in India. Wheat kernels have been found in the Mohenjo-Daro excavations. The strain cultivated during the Mohenjo-Daro period was later described as *Triticum aestivum* sub-species *sphaerococcum*. As the name indicates, this sub-species has spherical grains and a dwarf plant stature. From the days of Mohenjo-Daro up to the dawn of India's independence in 1947, the country developed the capacity to produce about 6 million tonnes of wheat. In 1966, the country imported nearly 10 million tonnes of wheat, largely through the PL 480 programme of the United States to fill the gap between supply and demand.

Alarmed by the growing gap between the rate of growth of population and food production, India's first Prime Minister, Jawaharlal Nehru said in 1948, 'Everything else can wait but not agriculture.' Several measures to stimulate food production including land reform, irrigation, fertiliser production, strengthening of research and organisation of a national extension service were initiated in the fifties. Although, the production of wheat and rice went up, productivity per unit area of land remained practically stagnant. Enhanced production came from an increase in both total cropped area and irrigated area. The wheat production went up to 12 million tonnes in 1964, which from the point of view of monsoon behaviour, was a good agricultural year.

In order to enhance productivity in irrigated areas, the Government of India initiated the Intensive Agricultural District Programme (IADP) in 1961. The aim was to introduce good seeds and a package of agronomic practices, which could help optimise the benefit from irrigation water. Unfortunately, the early IADP experience was not encouraging. It was found that the promoted package of agronomic practices did not include one important ingredient, namely varieties which could respond well to good irrigation and soil fertility management. The missing ingredient was then provided in 1966 through the High Yielding Varieties Programme (HYVP) in wheat, rice, maize, sorghum (*Jowar*) and pearl millet (*Bajra*). Wheat production rose to nearly 17 million tonnes in 1968. To commemorate this quantum jump, Indira Gandhi, the then Prime Minister, released a special stamp titled 'Wheat Revolution' in July 1968. Soon, American agricultural expert Dr William Gaud, described the phenomenon as 'green revolution', since similar productivity improvement

was visible in rice. I had then described what was happening as 'land saving agriculture', since the pathway of improved production was higher productivity. 'Forest-saving agriculture' might have been a more appropriate term, since agricultural expansion is often at the expense of forest area. To produce 63 million tonnes of wheat at the 1964 yield level, we will need over 63 million ha . In contrast, the area under wheat cultivation is now about 24 million ha. After several thousand years, the stagnation in yield was broken in wheat, rice and other major food crops. How did this all happen?

Systematic breeding of crop varieties that can respond to higher levels of plant nutrition started in India in 1952, when at the instance of the late Dr K. Ramiah, a programme for incorporating genes for fertiliser response from temperate *japonica* rice varieties from Japan into *indica* strains was initiated at the Central Rice Research Institute, Cuttack under the sponsorship of FAO and ICAR. I worked on this project during 1954 under the guidance of the late Dr N. Parthasarathi. Our major aim was to select from segregating populations of *indica* x *japonica* crosses, lines that showed the ability to utilise effectively about 100 kg of N per hectare. With this quantity of nutrient supply, about 5 tonnes of rice per hectare can be produced. This programme led to varieties such as ADT-27 in Tamil Nadu and Mashuri in Malaysia. Several genetic problems arose, rendering the speedy selection of high yielding rice varieties from *indica* x *japonica* crosses difficult. With the advent in the early sixties of the semi-dwarf, non-lodging, relatively photo-insensitive *indica* varieties based on the Dee-gee-woo-gen dwarfing gene identified in China, interest in transferring genes for fertiliser response from *japonica* varieties waned. Semi-dwarf *indica* rices such as Taichung Native1, IR8 and Jaya provided the initial material for the High Yielding Varieties Programme. In later breeding experiments, tropical *japonicas* from Taiwan also proved useful as parents.

The late Dr B.P. Pal initiated a similar programme in wheat at the Indian Agricultural Research Institute (IARI), New Delhi. The emphasis of the wheat-breeding programme of IARI, carried out by eminent breeders such as Dr S.P. Kohli, Dr V.S. Mathur and Dr M.V. Rao in collaboration with plant pathologists such as Dr R. Prasad and Dr L.M. Joshi, was on both disease resistance and yield. The programme ultimately resulted in varieties like NP 809 possessing a broad spectrum of resistance to stem, stripe and leaf rusts, and NP 824 possessing the ability to respond to about 50 kg of nitrogen.

When I joined IARI in late 1954, I started, in collaboration with several colleagues and students, a research programme for developing non-lodging and fertiliser responsive varieties of wheat, on the lines of the work earlier initiated in rice. At that time, the research strategy adopted had three components. First, crosses were made between the semi-dwarf, stiff straw *compactum* and *sphaerococcum* (sub-species of *T. aestivum*) with cultivated bread wheat. Second, attempts were made to induce *erectoides* mutants in commercial wheat varieties using radiations and chemical mutagens. Third, studies on the potential for increasing straw stiffness through different chemical treatments were initiated. Unfortunately, in all these three approaches, short and stiff straw was always associated with short panicles with fewer grains (see Swaminathan 1964 and 1968). The reason why straw stiffness became such an essential prerequisite for a favourable response to water and fertiliser is the tendency to lodge or fall down among the then cultivated tall wheat varieties when fertiliser was applied. In addition, such lodging made it difficult to give irrigation during the grain development phase, when the crop would have benefited much from water availability. Thus, with the earlier tall varieties it was difficult to get an economic response to the application

of mineral fertilisers and adequate irrigation water. Average wheat yields stagnated at less than 1 tonne per hectare. Thus, the breeding of non-lodging varieties was accorded such high priority during the fifties, when the country had taken to the path of expanding the area under irrigation and manufacturing mineral fertilisers.

During the late fifties, scientific publications on the work done under the leadership of Dr Orville Vogel in Washington State on the transfer of dwarfing genes from the Norin-10 wheat variety to North American winter wheats started appearing (Figure W1.1). When requested, Dr Vogel was kind enough to send seeds of Gaines, a semi-dwarf winter wheat variety with red grains. He further suggested that Dr N.E. Borlaug in Mexico should be approached for seeds of semi-dwarf varieties in a spring wheat background (winter wheats need long hours of sunlight to flower and set seeds).

In March 1961, Dr M.V. Rao grew in the fields of IARI a few dwarf spring wheat strains, which were entered by Dr Borlaug in the International Wheat Rust Nursery sent by the US Department of Agriculture. Their phenotype was most impressive. They had reduced height and long panicles, unlike the earlier hybrids between *aestivum* and *compactum* and *sphaeroccum*, and the induced *erectoides* mutants in which short height was coupled with small panicles.

In May 1962, I wrote to Dr B.P. Pal, who was then Director of IARI, requesting him to arrange the visit of Dr Borlaug to India and for obtaining a wide range of dwarf wheat material possessing the Norin-10 dwarfing genes from Mexico. I explained in my letter why we should immediately take up a dynamic dwarf variety breeding programme based on genes which do not exert a pleiotropic effect on the panicle. Dr Pal forwarded my letter to the Ministry of Agriculture and the Ministry requested Dr R.W. Cummings of the Rockefeller Foundation to arrange for the visit of Dr Borlaug and the supply of dwarf wheat material. Dr Borlaug came to India in March 1963, and sent a wide range of material in September 1963. A detailed report on the trials conducted with the Mexican Wheat material was presented at the All India Wheat Research Workers' Conference held at Delhi in August 1964 (Swaminathan, 1965). In 1964, I also proposed that we should start a National Demonstration Programme in farmers' fields both to verify the results obtained in research plots and introduce farmers to the new opportunities opened up by semi-dwarf varieties for considerably improving the productivity of wheat (Swaminathan, 1966, 1971). When small farmers, with the help of scientists organised the National Demonstration Programme, they harvested over 5 tonnes of wheat per hectare; its impact on the minds of other farmers was electric. The clamour for seeds began and the area under high yielding varieties of wheat rose from 4 ha in 1963–64 to over 4 million ha in 1971–72. This was because of the bold decision taken in 1966 at the instance of Shri C. Subramaniam, the then Minister for Food and Agriculture, to import 18 000 tonnes of seed of the Mexican semi-dwarf varieties, Lerma Rojo 64A and Sonora 64. Shri C. Subramaniam (1995) recently chronicled the major policy decisions taken during 1964–67, leading to the green revolution. Shri C. Subramaniam was ably supported by the late Shri B. Sivaraman, the then Agriculture Secretary and by a panel of scientists headed by Dr K. Ramiah in designing and introducing the High Yielding Varieties Programme.

In 1967, I observed a tendency among farmers with relatively large holdings in North-West India to use large quantities of fertilisers and grow in large and contiguous areas a single genetic strain. In my Presidential Address to the Agricultural Sciences Section of the Indian Science Congress held at Varanasi in January 1968, I therefore dealt with the need for adding the dimension of

ecological sustainability in efforts to improve yield and for greater support for agricultural research and extension (Swaminathan 1968). I wish to quote what I said then:

> Exploitive agriculture offers great possibilities if carried out in a scientific way, but poses great dangers if carried out with only an immediate profit motive. The emerging exploitive farming community in India should become aware of this intensive cultivation of land without conservation of soil fertility and soil structure would lead, ultimately, to the springing up of deserts. Irrigation without arrangements for drainage would result in soils getting alkaline or saline. Indiscriminate use of pesticides, fungicides and herbicides could cause adverse changes in biological balance as well as lead to an increase in the incidence of cancer and other diseases, through the toxic residues present in the grains or other edible parts. Unscientific tapping of underground water will lead to the rapid exhaustion of this wonderful capital resource left to us through ages of natural farming. The rapid replacement of numerous locally adapted varieties with one or two high yielding strains in large contiguous areas would result in the spread of serious diseases capable of wiping out entire crops. Therefore the initiation of exploitive agriculture without a proper understanding of the various consequences of every one of the changes introduced into traditional agriculture, and without first building up a proper scientific and training base to sustain it, may only lead us, in the long run, into an era of agricultural disaster rather than one of agricultural prosperity.

Even in the very first year of the semi-dwarf wheat breeding programme involving the material sent from Mexico by Dr N.E. Borlaug, namely in 1963, we initiated the following five pronged strategy for breeding a wide range of high yielding varieties possessing a broad spectrum of resistance to major biotic and abiotic stresses:

- Direct introduction of promising Mexican wheats; this led to the release of Lerma Rojo 64 A and Sonora 64.
- Selection from the advanced generation material received from Mexico; this led to the release of KalyanSona and Sonalika.
- Hybridisation between Mexican strains and Indian varieties; this resulted in many high yielding and rust-resistant strains in different parts of the country.
- Mutation breeding for changing the red grain colour of Lerma Roja 64 A and Sonora 64; this led to the release of Pusa Lerma and Sharbati Sonora.
- Crossing the semi-dwarf *T. aestivum* material with *T. durum* varieties, in order to produce semi-dwarf *T. durum*; *Durum* varieties like Malavika resulted from such crosses.

In all cases, attention was paid to disease resistance and grain quality from the point of view of *chapatti* making. Above all, the yield potential of dwarf wheats would never have been realised without the adoption of appropriate agronomic practices, such as shallow seeding and doing the first irrigation at the crown root initiation stage (see *Five Years of Research on Dwarf Wheats*, Indian Agricultural Research Institute New Delhi 1968, 52 pp).

Anticipatory research to avoid potential environmental problems was strengthened and a wide variety of high yielding strains possessing resistance or tolerance to the principal disease causing organisms were developed. I am mentioning this only to underline the fact that agricultural scientists were fully alive to the need for conducting an action-reaction analysis while introducing new technologies. Such awareness led to intensified efforts in varietal diversification and to the pyramiding of genes for tolerance to biotic and abiotic stresses. This is why wheat production had continued to show an upward trend during the last 25 years.

The remarkable speed with which the high yielding varieties were identified from the initial Mexican material and later developed within the country was the result of the multi-location testing and interdisciplinary research organised under the All India Coordinated Wheat Research Project of the Indian Council of Agricultural Research. The country owes a deep debt of gratitude to the first coordinator of this programme, Dr A.B. Joshi, for his vision and dynamism and to his successors Drs S.P. Kohli, M.V. Rao, A. Austin, J.P. Tandon and Dr S. Nagarajan. The coordinated wheat project is an outstanding exercise in meaningful cooperation. We must salute the late Dr B.P. Pal who initiated organised wheat breeding and coordinated varietal testing programmes in the country. The invaluable services rendered by the late Dr R.G. Anderson, who served as Joint Coordinator of the All India Wheat Research Project between 1964 and 1970 should also be recalled with gratitude. Dr Ralph W. Cummings, Field Director of the Rockefeller Foundation in New Delhi between 1958 and 1966, responded to our requests with speed and our thanks go to him. Above all, numerous young research workers and scholars participated with enthusiasm in the different aspects of the wheat programme, including the organization of a Seed Village in the Jounti Village of the Khanjawala Block of Delhi State (Swaminathan, 1968). Dr Norman E. Borlaug remained and continues to remain a pillar of strength to the wheat research and development programme of our country. Another great blessing of the wheat programme has been the continued leadership provided by the outstanding breeders such as the late V.S. Mathur, Dr Khem Singh Gill, Dr Y.M. Upadhya, Dr S.M. Gandhi, Dr Y.R. Mehta, Dr Dhani Ram Vasudeva and many others. Dr D.S. Athwal of the Punjab Agricultural University provided dynamic leadership to the programme during 1963–65. Breeding efforts alone would not have borne fruit but for the outstanding support given by plant pathologists, agronomists, soil chemists and specialists in other disciplines. In short, the participants in the wheat research programme functioned like members of a symphony orchestra. Such harmony and cooperation led to path-breaking results.

In the ultimate analysis, it is only farm men and women who produce food. Scientists, administrators and political leaders can only stimulate and support them. The hero of the wheat revolution is the hard working farmer. Hence, I would like to conclude with an extract from an article I wrote in the *Illustrated Weekly of India* in 1969.

> Brimming with enthusiasm, hardworking, skilled, and determined, the Punjab farmer has been the backbone of the wheat revolution. Revolutions are usually associated with the young, but in this revolution, age has been no obstacle to participation. Farmers, young and old, educated and uneducated, have easily taken to the new agronomy. It has been heart-warming to see young college graduates, retired officials, ex-army men, illiterate peasants and small farmers queuing to get the new seeds. At least in the Punjab, the divorce between intellect and labour, which has been the bane of our agriculture, is vanishing.

## Rice: A Case History in Accelerated Evolution

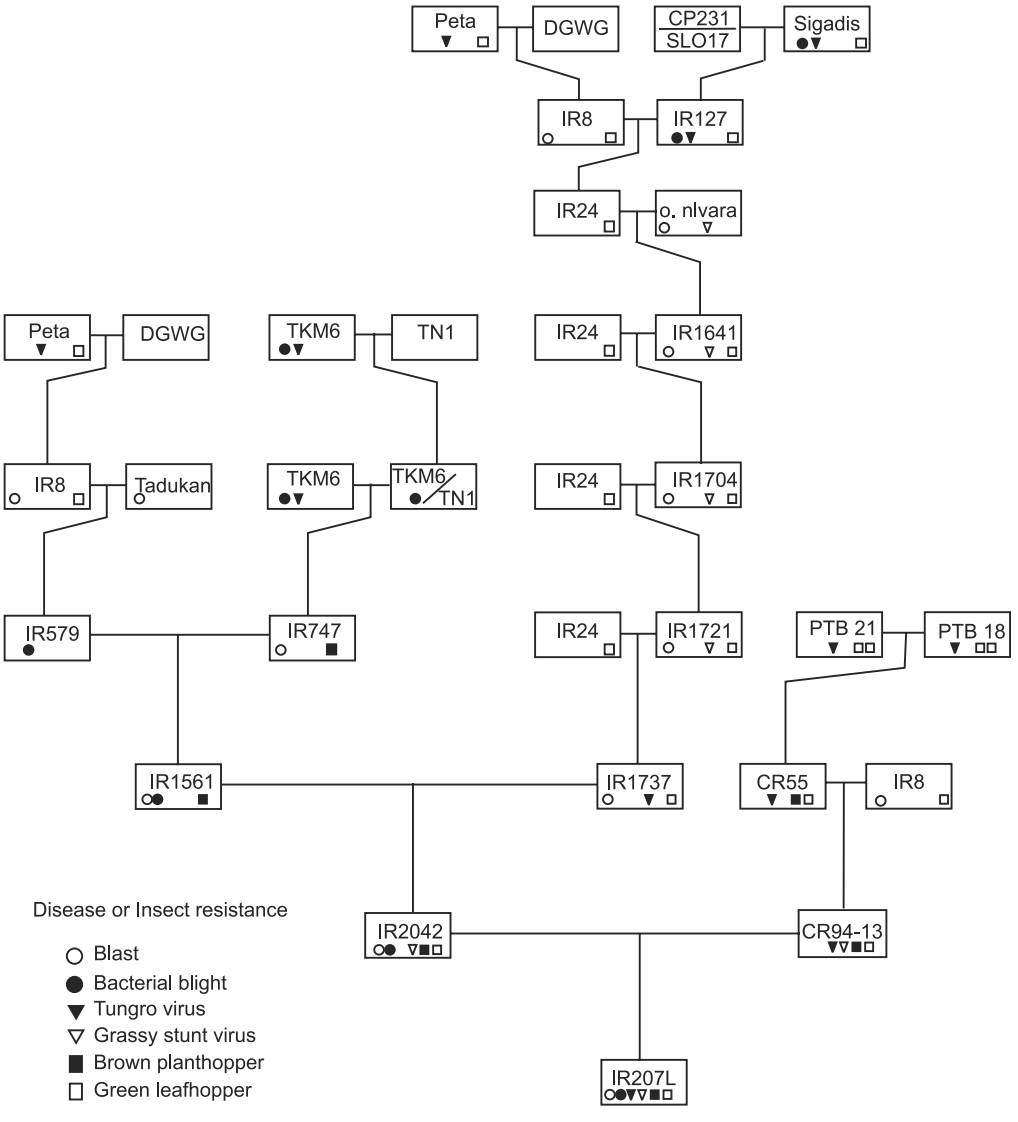
A parallel effort in the Philippines resulted in the breeding of new high yielding varieties of rice, adapted like wheat to intensive agriculture, that is, performing well in areas where irrigation water is available and require extensive applications of fertilisers. The HYVs of rice have a history very similar to that of wheat. The discovery of spontaneous dwarf mutant Dee-geo-woo-gen was a great landmark

in the history of rice breeding. Using these dwarfing genes, scientists at Taichung District Agricultural Improvement Station produced the first semi-dwarf, fertiliser responsive variety Taichung Native 1 in 1956 (for details refer to pages 99-100), which gave yields in excess of eight metric tonnes per hectare. The vast yield potentials and adaptability of dwarf strains such as Dee-geo-woo-gen, 1-geo-tze (very similar to Dee-gee-woo-gen) and Taichung Native 1, however, remained unexploited until the establishment of International Rice Research Institute (IRRI) in 1962 at Los Baños, founded by the Ford Foundation and the Rockefeller Foundation in collaboration with the Government of the Philippines.

Their first major success, with which the green revolution began, was the variety IR8 released in 1966, which also earned the soubriquet 'miracle rice'. The miracle rice did well in a number of countries, giving a yield of over 10 tonnes per hectare under ideal conditions. For this breakthrough, H.M. Beachell, Peter Jenning and T.T. Chang shared the John Scott Award of Philadelphia. The rice variety IR8 was a short-statured, sturdy-stemmed, photoperiod insensitive, high yielding and fertiliser responsive variety adapted to a wide range of environments. IR8 dominated rice production in tropical Asia during the 1960s, giving record yields in experimental plots of up to 11 tonnes per hectare unlike 2.0 to 3.5 tonnes per hectare of the tropical rice varieties (Figure W1.4). Surprisingly its cultivation spread with great speed, displacing a larger number of landraces and old cultivars. However, soon serious constraints on IR8's potentials became apparent, as it was susceptible to seven* serious diseases and pests.


**Figure W1.4.** The first semi-dwarf variety IR8 that earned the soubriquet 'miracle rice'. It is the result of a cross between Peta (has in its parentage the genes from Latisail –an *indica* variety from Bengal, India) and Dee-geo-woo-gen. IR8 is a short-statured variety with a combination of many desirable features, such as profuse tillering, dark green and erect leaves and sturdy stem. It responds well to fertiliser inputs, much better than the parent tall variety Peta. It has a harvest index of 0.5 unlike 0.3 of the pre-green revolution varieties (ratio of dry grain weight to the total dry matter) and double the yield potential of traditional tall varieties. In addition, being photoperiod insensitive, it could be planted all the year round in the tropics.

* Grassy stunt virus, tungro virus, blast (a fungal disease), bacterial blight and among the insects, stem borer, green leafhopper and three types of brown planthopper.




Pedigree of IR207L

**Figure W1.5.** The pedigree of IR36–a variety evolved under the leadership of Dr Gurdev S. Khush. IR2071 was the cross from which IR36 was selected (IR 2071-625-1-252). This is the most widely planted rice variety in history, growing on about 11 million hectares of the rice land. The pedigree illustrates the crucial role that landraces or primitive varieties from all over the world played in imparting resistance to pests and pathogens. IR36 is an early-maturing (107-110 days) and high yielding variety (producing between 4-6 t/ha in farmer's field and up to 9 t/ha in experimental trials). The grains are long, slender and translucent with good cooking and milling qualities. It resists many of the major rice pests and diseases, including green leafhopper, brown planthopper, stem borer, gall midge, blast, bacterial blight, tungro and grassy stunt. Furthermore, IR36 tolerates moderate drought, soil salinity, alkalinity, iron and boron toxicity and zinc deficiency in wetlands. In addition, it tolerates iron deficiency and aluminium toxicity in drier regions.

IR8 was followed by a series of IR varieties (IR20, IR24, IR26, IR36, IR54 and IR72 to mention a few). These varieties progressively overcame the deficiencies of IR8 and spread throughout Asia as fast as had CIMMYT-bred wheats. IR36 alone was grown on 11 million hectares, the most widely planted rice variety in history (Figure W1.5). Several countries of South and South-East Asia increased their annual rice production by a factor greater than that achieved in the last 5000 years. Its use has now spread to some of the rice growing areas of Africa too. A team of IRRI scientists (led by Dr Gurdev S. Khush) in collaboration with developing countries and industrial nations produced the variety IR36. Rice lines from six countries have contributed genes in the IR36's genealogy. This variety provides a good example of the trend in agricultural research towards a global enterprise and illustrates the importance of gene banks and international cooperation in germplasm conservation and breeding.

The pedigree of the recently developed IR72 illustrates the genetic complexity involved in producing a new variety. Some two dozen 'landraces' from all over the world contributed to the development of IR72. This variety, for example, has bred-in genetic resistance to a dozen pests and environmental stresses.

Building on the momentum of green revolution success stories in Mexico and Asia, the Consultative Group on International Agricultural Research (CGIAR), the umbrella organisation of the International Agricultural Research Centres (IARCs) was founded in 1971, with its headquarters in Rome to facilitate raising funds for expansion of the system. Currently there are 13 IARCs with a total annual base operating budget in excess of USD 180 billion. This group now coordinates the research for all the research institutes, each with responsibilities for particular crops (see Appendix III).

**Box W1.2: Gurdev S. Khush**

Dr Gurdev S. Khush was born in 1935 in Rurkee, Punjab (Figure W1.6). He graduated from the Government Agricultural College with a major in plant breeding and obtained a PhD in genetics from the University of California, Davis. He then worked with Professor Charles Rick, a renowned tomato cytogeneticist, for seven years. While at Davis, he wrote his first book, *Cytogenetics of Aneuploids*, published by Academic Press, New York, in 1973. He later joined the International Rice Research Institute, Manila, as a rice breeder, where he was the Principal Plant Breeder and Head, Division of Plant Breeding, Genetics and Biochemistry.

Dr Khush has had a distinguished career, having won many International awards, including the Borlaug Award for Achievements in Plant Breeding in 1977, the Japan Prize (given by the Science and Technology Foundation of Japan) in 1987 and World Food Prize. He has also served as a consultant to the rice improvement programmes of Australia, Bangladesh, China, India, Indonesia, Sri Lanka and many other countries.

Dr Gurdev S. Khush is now one of the world's premier rice breeder and has led the rice varietal improvement programme of the International Rice Research Institute (IRRI) for the last 28 years. More than 300 breeding lines of rice developed under his leadership have been released as varieties by the national programmes throughout the world. Numerous other IRRI breeding lines have been used in national hybridisation programmes. IRRI developed breeding materials and their progeny are now planted in about 60 per cent of the world's rice land. One of these varieties, IR36, was planted on 11 million hectares of the rice land in the 1980s. No other variety of rice or any other food crop has been planted so widely before. The only other grain crop variety that came close to matching IR36 in farmer acceptance was an aggregation of semi-dwarf wheat varieties selected from CIMMYT cross 8156.

Farmers harvest five to seven tonnes of unmilled rice from modern varieties, compared to 1.0 to 3.0 tonnes with traditional varieties. Since 1966, when the first modern rice variety was released, rice harvested area has increased only marginally from 126 to 146 million hectares (16 per cent), and the average rice yield from 2.1 to 3.6 tonnes per hectare (71 per cent). The world rice production has doubled in a 25-year period, that is, from 256 million tonnes in 1966 to 518 million tonnes in 1990. This increased production feeds 700 million more people than earlier varieties would have been able to do. Most of the rice-growing countries in Asia, where 92 per cent of the world's rice is produced, became self-sufficient in rice and several have exportable surpluses.

In many rice-growing countries, the growth in rice production has been faster than the increase in population, leading to a substantial increase in cereal consumption and per capita calorie intake. During 1965–90, the daily calorie supply (in relation to requirement) improved from 81 to 120 per cent for Indonesia, 86 to 111 per cent for China, 82 to 99 per cent for the Philippines and 89 to 94 per cent for India. The increase in per capita availability of rice and a decline in the cost of production per tonne of output contributed to a decline in the real price of the rice, in both the international and the domestic market. The unit cost of production is 20 to 30 per cent lower for modern varieties than the traditional varieties and the price of rice adjusted for inflation was 40 per cent lower in 1992, compared to the level in the mid-1960s. The decline in food prices has benefitted the urban poor and rural landless who spent more than half of their income on food grains.

**Figure W1.6.** Dr Gudev S. Khush FRS–one of the world's premier rice breeder of IRRI.

The diffusion of modern rice varieties has also contributed to the growth of income of the rural landless workers. Modern varieties require more labour per unit of land because of more intensive care in agricultural operations and harvesting of larger output. Labour requirement has also increased because of higher intensity of cropping, made possible through a reduction of time in crop growth. Marketing of larger volume of rice and increased demand of non-farm goods and services on account of larger farm incomes has generated additional employment in rural trade, transport and construction activities. Rapid development of the non-farm sector and the economic miracle under way in many Asian countries was triggered by the growth in agricultural incomes and its equitable distribution, which helped expansion of the domestic market for non-farm goods and services.

The widespread adoption of modern varieties has helped rice-growing countries meet their growing food needs from irrigated and favourable rain-fed lowland areas, and thereby has reduced the pressure on them to open up fragile uplands and tidal wetlands for rice cultivation. Availability

of rice varieties with multiple resistance to diseases and insects has reduced the need for application of insecticides and facilitated the adoption of integrated pest management practices. For example, the Indonesian government recently banned 56 types of insecticide, yet the upward trend in rice yield continues. Reduced insecticide use helps enhance environmental quality and improve human health in rice-farming communities.

The population of rice consumers is increasing at the rate of 2 per cent annually. The number of rice eaters will probably increase by 57 per cent and requirement by 70 per cent during the next 25 years. To feed these people, an additional 380 million tonnes of rice will have to be produced by 2020 from the shrinking land resources. To meet this challenge, a team led by Dr Khush has developed a new rice plant type with 25 per cent higher yield potential than that of the existing modern varieties. *Time* magazine selected IRRI's new plant type, identified as 'Super Rice', as one of the five best environmental stories of 1994. The selection appeared in *Time's* annual year-end special issue (26 December–2 January ). When introduced for on-farm production by the turn of the century, new plant type varieties will help feed 400 million additional rice consumers.

A few of the many contributions of Dr Gurdev S. Khush are as follows:

- Investigated the cytotaxonomic relationships of cultivated rye *Secale cereale* and the wild species of the genus *Secale*.
- Established secondary, tertiary and compensating trisomics of tomato, and employed them in locating genes to specific chromosome arms and determined the orientation of linkage maps.
- Delimited the position of marker genes to the small chromosome segments of tomato through induced deficiency technique.
- Established primary trisomics of rice and employed them to associate linkage groups of rice with cytologically identifiable chromosomes for the first time.
- Identified numerous genes for disease and insect resistance in rice.
- Located the known isozyme loci of rice to respective chromosomes through the use of primary trisomics.
- Produced several interspecific hybrids between cultivated rice and wild species through embryo rescue, and transferred useful genes from wild species to cultivated rice.
- Established alien addition lines having complete chromosome complement of rice and a single alien chromosome of the wild species.

## The Pros and Cons of the Green Revolution

The results of the green revolution are mixed, but its merits outweigh the negative aspects. At a time when famine seemed imminent, new varieties of wheat and rice introduced to Asia and Latin America along with fertilisers, pesticides and mechanised farm equipment, dramatically increased the harvest yields. Thus, this agricultural strategy, which transformed the lives and prospects of hundreds of millions of people, is considered the most successful achievement in the international development and no doubt has helped the poor countries of the world feed their expanding population. As discussed, the worldwide rice production doubled during the 25-year period from 1965 to 1990, from 254 million tonnes to 516 million tonnes. It has turned pessimism into optimism in our dramatic race between

the population explosion and production of food. One of the greatest contributions of the green revolution has been the protection of agricultural land, which would have been otherwise needed to feed the growing population; earlier in many countries the production gains at such scale came largely through area expansion (Tables W1.1 and W1.2). More importantly, the green revolution has generated self-confidence among farmers, extension workers, scientists and political leaders in their ability to bring about rapid increases in food production. As self-confidence is a basic requisite for success in any area of human endeavour, this is a great gain. Secondly, as agriculture moved from a subsistence level to a market-oriented one, rural communication, rural electrification and other areas of infrastructure development became an economic and social necessity, thereby improving the quality of life of the rural people. Thirdly, since agriculture was earlier regarded as a profession that required only brawn and no brain, the educated and intellectual classes did not consider farming as a profession but sought employment in urban areas. Similar was the situation in agricultural colleges and universities that could not attract the brighter students who preferred fields such as medicine, engineering and commerce. The success of the green revolution in the countryside led to greater social prestige and recognition for the farm sector. Now young students find agriculture not only remunerative but also intellectually satisfying. Finally, an important outcome of increased productivity was the accumulation of grain reserves. For example, Indonesia, once the world's leading rice importer, became a rice exporter. Countries like India, which once faced chronic food shortages, today have substantial grain reserves that provide insurance against famine. This has enabled the economically weaker sections of our society to increase their calorie intake, thereby preventing a further growth in the number of undernourished people.

**Table W1.1.** Profile of wheat production in India and land saved through productivity gains

| *Year* | *Area* (*1000 ha*) | *Yield* (*kg/ha*) | *Production* (*1000 mt*) | *Land saved* (*1000 ha*) |
|---|---|---|---|---|
| 1961–66 | 13 191 | 830 | 10 950 | _ |
| 1970 | 16 626 | 1 209 | 20 093 | 7 582 |
| 1975 | 18 111 | 1 338 | 24 235 | 11 087 |
| 1980 | 21 962 | 1 437 | 31 560 | 16 061 |
| 1985 | 23 100 | 1 909 | 44 100 | 30 032 |
| 1990 | 23 500 | 2 120 | 49 850 | 36 560 |
| 1995 | 25 490 | 2 560 | 65 240 | 53 112 |

*Source*: FAO Production Yearbooks.

Dr Borlaug's monumental contribution and that of his fellow scientists will go in vain until we are able to control the population growth that continues to increase at a staggering rate. Even the optimists who praise the green revolution and Borlaug's work admit that it is not the ultimate solution to feeding a hungry world, where one out of four people is still undernourished. The green revolution has won a temporary success in man's war against hunger and deprivation. It has given man a breathing time, perhaps 15 years or so, during which we can evolve new strategies to remove the disparities between food production and population growth by concrete acts, and soon by striking a balance between population and food resources.

Although the benefits of modern agriculture have been substantial, there are legitimate concerns and doubts about the sustainability and social impacts of this pathway of agricultural advance. There are debates with questions like 'is this Green Revolution really 'green' or it has a 'brown' side as well'. In the next section, we will discuss the 'brown side' of the green revolution.

**Table W1.2.** Profile of rice production in India and land saved through productivity gains

| *Year* | *Area* (*1000 ha*) | *Yield* (*kg/ha*) | *Production* (*1000 mt*) | *Land saved* (*1000 ha*) |
|---|---|---|---|---|
| 1966–70 | 36 360 | 981 | 35 770 | _ |
| 1975 | 37 890 | 1 045 | 39 580 | 2 487 |
| 1980 | 38 970 | 1 082 | 42 180 | 4 027 |
| 1985 | 41 100 | 1 418 | 58 300 | 18 329 |
| 1990 | 42 170 | 1 756 | 74 053 | 33 827 |
| 1995 | 41 640 | 1 952 | 81 260 | 41 194 |

*Source*: FAO Production Yearbooks.

## The Brown Side of the Green Revolution

The green revolution is essentially a result of Western technology to increase food production, and as such is dependent on high-energy inputs in the form of fertilisers, pesticides, herbicides, farm machines and irrigation systems, all requiring fossil fuels for their manufacture and use. The green revolution technologies have been criticised and many reviews have appeared, particularly on its socio-economic and, to a lesser extent, its environmental impacts. We will briefly examine some of these as follows:

- ***Increasing land concentration and landlessness:***

High-yielding varieties made agriculture more profitable. Many large property owners who had tenant farmers living on their land, for generations, often evicted these people and began to operate the farm themselves, thereby were increasing the extent of landlessness. In addition, large farmers could get credit for fertilisers and pesticides, and could as well avail themselves of governmental subsidies to buy farm machinery easily, whereas smallholders or small neighbours were forced out of business.

- ***Disruption of social system:***

Mechanised planting and harvesting operations resulted in greatly reduced farm employments, displacing the small farmers and farm workers from their jobs. Thus, the poor got even poorer because no alternative jobs were available. As cultural practices and jobs changed, there were alterations in family life and role too. Whole communities were displaced and the people started moving to cities, for example, reflected in the mass exodus of the rural people to Mexico (a country where green revolution first began)–the largest urban area in the world.

However, the protagonists of the green revolution feel that it has not accelerated labour displacement in rural areas, but has created more work and hence a greater demand for labour. Although, some categories of the labour declined, such as planting, threshing, etc., many ancillary job opportunities opened up in sectors such as farm machinery operations, inputs supply, grain marketing and other

agronomic services. Thus, the net effect on rural employment has been positive. In addition, as farmers became prosperous, a new demand was created for consumer goods such as cycles, sewing machines, transistors, radios and television sets.

- ***Marginalisation of women:***

In many agricultural societies in the developing countries, women provided most of the labour for crop production. Studies have shown that women work more hours and expend more energy on food production than men expend. Activities, such as planting, weeding, transporting, storing and processing were mainly the work of women. The green revolution technology displaces labourers, of which women were often the first to lose their jobs. For example, in India and elsewhere in South-East Asia, most weeding has traditionally been done by hired women. As the use of herbicides increased so has these women's unemployment. The green revolution type of agriculture needs access to capital and only men have access to the money. Thus, women in much of the Third World have lost control over food production, leading to their increasing marginalisation, although they remain responsible for the nutrition of the family. The decline in the status of women is well documented in the UN's 'Report of the Committee on the Status of Women in India'.

- ***Increased crop vulnerability created by genetic uniformity:***

The practice of using HYVs on a vast stretch of land year after year, called monoculturing, has displaced the primitive varieties or landraces that grew together with the wild relatives in the marginal lands around the farm and often interbred with the crops. The loss of these plants is potentially dangerous because they represent the repository of valuable genes, which will be needed for plant breeding in the future. The dangers of a narrow genetic base in modern HYVs is well illustrated by the southern corn leaf blight in 1970, that lowered the US corn production by 15 per cent and by more than 50 per cent in some southern states. This man-made catastrophe mainly occurred because the gene that conferred male sterility was also responsible for the susceptibility to corn leaf blight. Similar disease epidemics (caused by tungro virus and plant hoppers) have occurred in successive varieties bred by the IRRI. The green revolution has accelerated the process of genetic erosion, especially in Turkey, Iraq, Afghanistan, Persia and India. Thus, landraces[*] have disappeared and the genetic base has narrowed down because of the green revolution.

- ***Environmental impacts:***

Numerous critics have questioned the sustainability of the green revolution on ecological grounds (Perelman, 1977; Ophuls, 1977; Mooney, 1979). A few of the notable grounds are (a). the tendency of HYV technology to intensify monocultural cropping; (b). the decrease in fallow periods (a period when the soil is kept free of plants so that moisture can be stored and nutrient level can be built-up),

[*] A primitive plant variety of unknown pedigree, sometimes called farmer's variety because the origin is unknown. These landraces perform poorly under heavy application of fertilisers, water and intensive cultivation, because they are the product of a long evolutionary history lacking these factors. However, they have a wide range of ability, such as to withstand cold temperatures, drought, diseases, insect damage and other variables. Landraces have a very broad genetic base. In genetic terms, they are highly heterozygous, being used by plant breeders as the raw material.

which lead to waterlogging, salinisation and other problems associated with expansion of irrigation; (c). increased chemical runoff and soil erosion; and (d). the loss of genetic diversity inherent in traditional varieties and landraces as well in wild relatives. In order to sustain yields, the HYVs needed fertilisers and irrigation–the former promoted 'weed growth' and irrigation stimulated 'insect development'. These twin developments encouraged the use of herbicides and pesticides–both polluted streams and rice paddies, ultimately leading to the death of a large numbers of fishes. This in turn has wiped out a major source of income and animal proteins for Asian farmers.

Thus, the techniques of monoculture and continuous cultivation increase the damage caused by the destructive unholy triple alliance of pests, pathogens and weeds.

- ***Maldistribution of food:***

Mexican wheats can be grown only on about one-fourth of the India's agricultural land with three-quarters lacking irrigation and the technological hardware needed for the new HYVs. Even today, India faces a peculiar situation with local areas of great productivity and warehouses overflowing with surplus wheat, while most of our people remain poorly fed. Poor people do not have access to the available food. In other words, hunger is caused not by the failure of the agricultural system, but by the failure of the socio-economic system. Three-quarters of the world's hungry people are found in the countries of Asia (India, Pakistan, Bangladesh, Indonesia, Cambodia and the Philippines), South America (Brazil) and Africa (Zaire and Ethiopia). Many of these countries actually export food and therein lies the paradox.

- ***Uneven spread of HYVs:***

The success of the green revolution has been highly unequal spatially, that is, the benefits are not being felt everywhere. This is because largely the HYVs are suitable only to a few favoured agro-ecological zones, where farmers could afford fertilisers, pesticides, irrigation machinery and small tractors–the inputs required to reap most of the benefits from the green revolution strains. Africa gained the least in part, because it has large areas with sufficient rainfall but poor soils, and other areas with reasonable soil but insufficient rains. Additionally, its population cannot afford the necessary inputs. The green revolution emphasised wheat and rice, but the staples of Africa are maize, sorghum, millets, cassava and yams–all crops that do well on marginal land.

- ***Reduced arable land for grain legumes cropping:***

The high-yielding varieties of cereals, being more remunerative, have pushed aside the cultivation of pulses. Ironically, this has led to a decline in the availability of pulses and the average consumption has now fallen down in India and elsewhere. Even the current level of 50 g a day in India seems difficult to maintain because of the increasing population, as the production of pulses has remained stagnant.

## Beyond the Green Revolution

The green revolution of the sixties witnessed impressive gains in food grain production due to the release of HYVs, adapted to intensive agriculture. At that time, the world did not anticipate the energy crisis that has now become a harsh reality, for example, the price of oil has increased many times

over the current lower trend is not going to last longer. Indications are that the world's oil supply will run out within a century or less and until then, the prices will continue to skyrocket. The OPEC oil embargo of 1973, coinciding with a quadrupling of crude oil prices, initiated general economic disorientation. The agricultural areas of the world discovered that the increased costs (involved in producing fertilisers, pesticides, herbicides, farm machines and building irrigation systems) suddenly converted that had been profits into deficits. Apart from this, it has taken all of us so far afield from sound ecological management to serious environmental degradation. Important gains in yields were countered by surface erosion and build-up of pesticides in the soil and atmosphere. The vast majority of farmers in the world cannot now afford to grow the demanding genotypes produced by the green revolution, and are now returning to traditional older varieties. Although lower yielding, these produce reliable harvests with little or no fertilisers or irrigation and show some resistance to pests and pathogens without pesticides.

Both international and national organisations have belatedly recognised and addressed these social and ecological impacts of new technology. For example, today's modern varieties are more fertiliser efficient (than fertiliser responsive) and more resistant to insects and diseases than earlier modern or traditional varieties. Integrated pest and nutrient management have further reduced our dependence to the energy requirements. Germplasm collection and preservation have become an integral part of different international and national institutes spread all over the world. Developed and developing countries alike are trying to address this problem with *in situ* conservation (in natural or managed ecosystems, including on farms) or *ex situ* conservation (in field collection, seed stores and gene banks, or as tissue culture samples).

Raising the yield of food crops will continue to be the main goal of agricultural research. At the same time, people must shift from a high-input agriculture to sustainable traditional farming coupled with good agronomic practices, such as crop rotation, intercropping, hoeing, increasing crop plant density and sanitation (by open field burning of crop residues after harvest-it is now becoming a grave pollution concern). By manipulating planting and harvesting dates, we can keep the pest and weed population down. This will further help in enhancing crop productivity without degrading the environment.

At present, many approaches are being pursued to solve the food production problems. A few of them are as follows:

- To extend the green revolution technology to other crops–precisely what CGIAR institutes are doing currently.
- Crop rotation and diversified agriculture to replace existing practice of monoculture.
- Multiple cropping, that is, growing two or sometimes three different crops in succession on the same land within a year.
- Agroforestry, that is, planting a crop underneath a canopy of trees, usually nitrogen fixing.
- Creating new crop varieties adapted to subsistence agriculture system rather than modifying the environment to suit crop varieties.
- To tap new crops among the many naturally occurring plants[*].
- Aquaculture (the culturing of certain aquatic animals and plants in farms on land and sea) may offer, at the very least, a partial solution to the problem of shrinking supply.

[*] The readers are referred to an article 'Plant Resources for AD 2001' appearing in *Plants & Society*, pp. 629, edited by M.S. Swaminathan and S.L. Kochhar, 1989. Basingstoke. London: Macmillan Publishers (UK) Ltd.

- Biological control, relying on the use of predators, parasites and pathogens of pests–an alternative to the use of pesticides. The principles of Integrated Pest Management (IPM) have also led to a reduction in the use of pesticides.
- To reduce post-harvest losses of food grains.
- Agricultural biotechnology has the potential to produce new crop varieties more quickly than the conventional breeding, as researchers have to manipulate single cells rather than entire plants. This saves space and time.
- The crops may be tailored to use water and nutrients more efficiently, and to perform well in mixed cropping practices that many poor farmers employ.

However, for the most part, agricultural biotechnology (involving the use of genetic engineering or recombinant DNA technology, plant tissue culture techniques, etc.) today is focused on attempts to multiply existing yields of crops by altering their genetic architecture. With conventional breeding and agricultural practices having reached their saturation points as far as increasing productivity go, much has to be gained from bringing about a 'gene revolution' in the twenty-first century, which is poised for a big leap from cloistered labs into our lives. Agricultural biotechnology is being hailed as the 'second green revolution' that can be used to create high-yielding crop varieties that are (a). herbicide tolerant, (b). insect resistant, (c). resistant to viruses, (d). resistant to fungi and bacteria, and (e). have a better nutritional value.

The use of transgenic crop varieties (refer to the web supplement) will provide an alternative to the widespread use of agrochemicals, such as herbicides and pesticides to control weeds and pests that have wrought havoc in our environment.

An integration of the biological and the genetic approaches (traditional breeding and genetic engineering) would provide the key basis for building an ecologically sound agricultural system without any hazard for many agricultural workers as well as any threat to the environment we live in.

In the words of Dr M.S. Swaminathan, the father of the green revolution in India and the former Director-General of IRRI (Founder Chairman, M.S. Swaminathan Reserch Foundation, Chennai, India), "The greening of agriculture requires the greening of both technology and public policy. Producing more food and agricultural commodities from less land, water, and energy is a task that will call for the integration of the best in modern technology, with the ecological strengths of traditional farming practices". National and international research programmes are turning to a new challenge, that is, to develop crops and technologies for farmers who do not irrigate their fields and who lack the resources to purchase fertilisers and pesticides.

The second green revolution, which is in the offing, would be greener, equitable and sustainable. Government policies rather than scientific work will have to provide the tools for enabling all farmers (irrespective of the size of their holding, input purchasing, risk-taking capacity and social status) to derive economic benefits from the new technology.

Web Resource 2

# Transgenic Crops: Objectives, Achievements and Concerns

**A. Grover[*], S.C. Maheshwari[**] and S.L. Kochhar**

Gene transfers through natural hybridisation have occurred throughout the course of plant evolution, and they are mostly restricted to individuals belonging to the same species. However, gene transfer between very different organisms also occurs in nature. Plant breeders, over the centuries and more intensively in the last few decades, have been able to produce hybrids (or strains) with the desired combination of traits. This has substantially contributed to increased production as well as productivity in different crops. In conventional plant breeding, which employs sexual hybridisation, new gene combinations are obtained by bringing together the whole nuclear genome (tens of thousands of genes) from selected parents or by introducing random mutations through various mutagenesis techniques. Its success depends upon the ability of the breeder to recognise and select desirable gene combinations. However, this type of breeding approach has certain inherent limitations. First, only sexually compatible individuals of a species can be interbred and thus the breeders have to work with a limited gene pool. Secondly, specific gene alterations are difficult to achieve while keeping other characters constant. During such crosses hundreds of other genes from the wild or species closely related to the cultivated counterpart are transferred at the same time along with the genes that the breeder intends to introduce.

More recently, researchers have been able to crossbreed sexually incompatible species and have succeeded in introducing specific and useful gene segments known to encode the desired trait from unrelated organisms, which was never possible before. By using recombinant DNA technology, scientists can direct the movement of genes of interest between totally unrelated organisms. Such plants wherein transfer and expression of 'foreign' or 'alien' genes in whole plants have been accomplished are often referred to as 'transgenic' plants. The transgenic plants begin as tiny plantlets that are initially grown in a glasshouse and then transplanted to the fields.

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Although genetic engineering is more complex than conventional breeding, it offers several advantages. First, it is far more precise and a single trait encoded by a gene or group of genes can be introduced into the plant without change or loss of any of the desirable original features. Secondly, the desired trait is incorporated much faster, taking a season or two instead of a decade or more, as required in conventional breeding. Thus, it is a time and space-saving approach. Thirdly, sexual incompatibility is not a barrier, and it is remarkable that genes from organisms ranging from bacteria to mammals can now be transferred to plants–considered one of the real power of biotechnology. Although, plant genetic engineering has certainly emerged as a powerful adjunct to conventional plant breeding for bringing in specific alterations, one must not carry the impression that it will replace, in any way, the plant breeding efforts.

The emergence of biotechnology itself is a consequence of the fusion of the two fields, tissue culture and molecular biology. Because of the constraint of space, we shall not go into much detail in this chapter, but it must be stated that plant biotechnology owes its spectacular success to the totipotency of the plant cells. Indeed, it is now possible to not only regenerate plants from leaves, roots, stem segments involving large masses of cells, but even from single cells such as pollen grains. In such regeneration, one employs the aseptic culture technique and advances in our knowledge of hormones has made it possible for us to take plant cells either towards a callusing phase (in which cells remain in an undifferentiated state) or a phase in which formation of roots or shoots or both can be induced at will. Happily, while botanists perfected the media and tissue culture method, biochemists and molecular biologists unravelled the structure and function of various nucleic acids and enzymes such as restriction nucleases and ligases. Thus, the eighties saw the development of the first transgenic.

In this chapter, we will have an introductory overview of the changing scenario and to some extent a detailed discussion of the methodology and examples of transformed crops.

## Methods of Transformation

For the introduction of the desired gene in a specific crop species, it is obligatory that the method of genetic transformation of the given species be well standardised. Several techniques have been optimised to deliver the desired genes to plant cells. Admittedly, *Agrobacterium tumefaciens* (Smith and Town) Conn. has been the workhorse in the hands of plant scientists for transferring genes in an enormous range of dicotyledonous species.

### (I) *AGROBACTERIUM*-MEDIATED GENE TRANSFER

The development of a crown gall represents a clever strategy of the bacterium to perpetuate itself, subverting the host's basic capacity of assimilating carbon and nitrogen especially to synthesise compounds required for its own survival. The bacterium brings in the host cells additional genes for synthesising hormones, namely auxin and cytokinin, as well as opines–a special class of nitrogenous compounds that are metabolised by the infecting bacteria for their nutrient supply but are of no special significance to the plant. Auxins and cytokinins lead to a proliferation of the infected host cell where the bacterium lives and multiplies.

The most promising natural plant-cloning vector (DNA carrier) of all those investigated so far is the pathogenic soil bacterium, *A. tumefaciens* that cause tumours (called crown galls) in many plants. When these bacteria infect a wound site, usually on the stem close to the ground, a portion of its DNA (called T-DNA), encoding the enzymes for auxin, cytokinin and opine synthesis, is transferred

and becomes integrated into the chromosome of the host cells which begin to proliferate, leading to the formation of tumour-like growths or galls.

The genes transferred from the bacterium to the plant are carried on the extrachromosomal circular DNA molecules called tumour-inducing (Ti) **plasmids** (see Box W2.1) within the bacterial cells. *Agrobacterium rhizogenes*, a related species of *A. tumefaciens*, is known to cause more extensive root growth in the recipient plant. The corresponding plasmid molecules in the case of *A. rhizogenes* are termed as Ri plasmids.

The transfer of T-DNA occurs after the bacterium has attached itself to the cell walls of the host's wound cell. Specific chemicals, leaching out of the wound site activate the bacterial genes that code for restriction endonucleases needed to splice the T-DNA region from the Ti plasmid and re-integrate it into the host DNA. This natural gene transfer mechanism of the bacterium has been used to produce a wholly transformed or transgenic plant.

For the *Agrobacterium* to be an effective vehicle for DNA transfer, the tumour causing genes of the Ti plasmids are removed (a modification which in biotechnological terms is called *disarming*) and replaced by the gene(s) of interest to be inserted into the plant cells (after disarming, the bacterium is unable to cause tumours without impairing its ability to transfer to the plant cell's genome whatever foreign gene(s) has been attached). In practice, the gene to be transferred contains the coding sequences controlling the trait as well as (a). promoter sequences (typically towards the 5' end) that regulate the gene expression with respect to specific tissues, conditions or the developmental stages and (b). termination sequences (towards the 3' end of the gene) which ensure that the mRNA transcript is correctly terminated. Most often, *selectable marker* or reporter genes (such as those that govern resistance of plant cells against antibiotics and herbicides) are co-transformed along with the gene of interest in order to enable selection of transformed cells amidst millions of cells that are untransformed.

**Box W2.1: In addition to the main genome, bacterial cells contain an extrachromosomal DNA molecules, called plasmids**

Plasmids are double-stranded rings of DNA molecule that frequently occur in bacteria, in addition to the main genome. These are known to carry genes for sexuality, antibiotic resistance and degradation of toxic substances. However, it may be noted that the bacterial cells can survive even without them and hence plasmids are dispensable genetic determinants. They replicate independently of the main genome and, being small in size, can come out or get into a cell with relative ease. Besides plasmids, restriction endonucleases also hold great importance in the genetic engineering programme. They are special enzymes that are used to cut or 'splice' the plasmid as well as the foreign DNA molecules at specific sites in such a manner so as to leave 'sticky' or complementary ends in both the molecules (largely, foreign and plasmid DNA are receptive to each other only when they are cut by the same restriction endonuclease enzyme). In addition, the restriction enzymes provide protection to the host bacterial cells from invasions by pathogenic phages.

Different bacteria produce hundreds of restriction enzymes which are highly specific, each of which recognising a specific DNA sequence of 4-8 nucleotides. For example in Figure W2.1, Eco RI (harvested commercially from *E. coli*) recognises the nucleotide sequence GAATTC and then makes a staggered cut across the two strands of DNA (its antiparallel complement is CTTAAG) between the guanine and the adenine, producing sticky ends. The single-stranded end of one fragment can thus recognise and bind to the end of any other fragment produced by the 'same' enzyme. Another enzyme, DNA ligase, can ligate the sticky ends together again. This process can occur whether the DNA is from the same organism or different organisms.

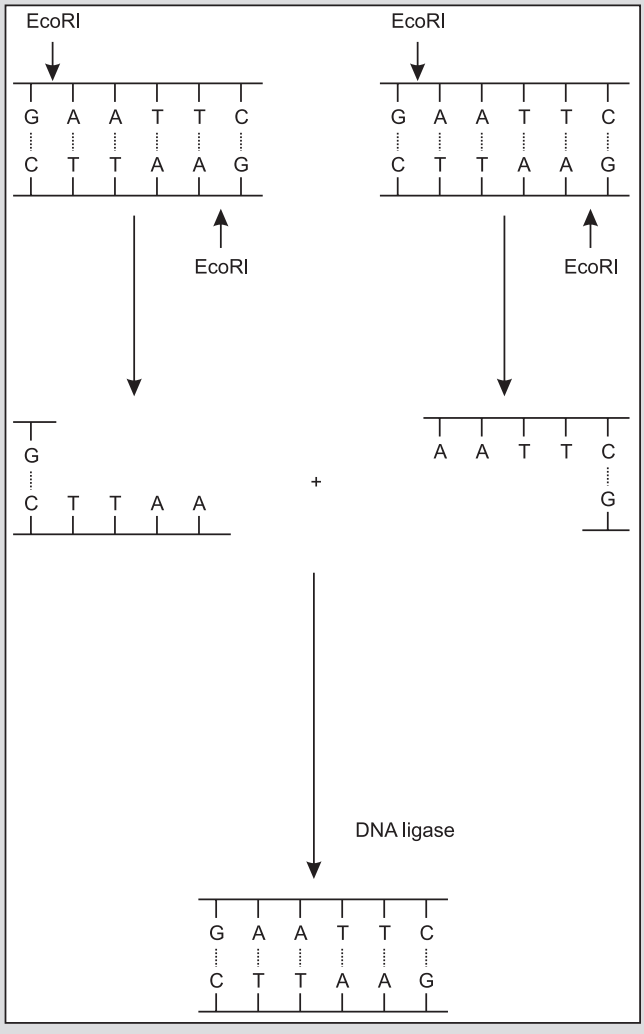


**Figure W2.1.** Diagram showing the restriction enzyme Eco RI recognising a specific nucleotide sequence GAATTC.

The steps involved in the transfer of the foreign gene to *E. coli* plasmid are as follows (Figure W2.2):

- Plasmid (a ring of extrachromosomal DNA) is isolated from *E. coli*, and so is the plant DNA whose desired genes are to be integrated.
- The plasmid and the foreign plant DNA are then treated with the same restriction endonuclease (RE), thereby exposing the same sticky ends on both (foreign DNA and plasmid DNA are receptive to each other when cut by the same RE.).
- When the two kinds of DNA are mixed, they anneal because their sticky ends are complementary. The small 'nicks' are then sealed by DNA ligase, which makes sugar-phosphate bonds between the two molecules of DNA.

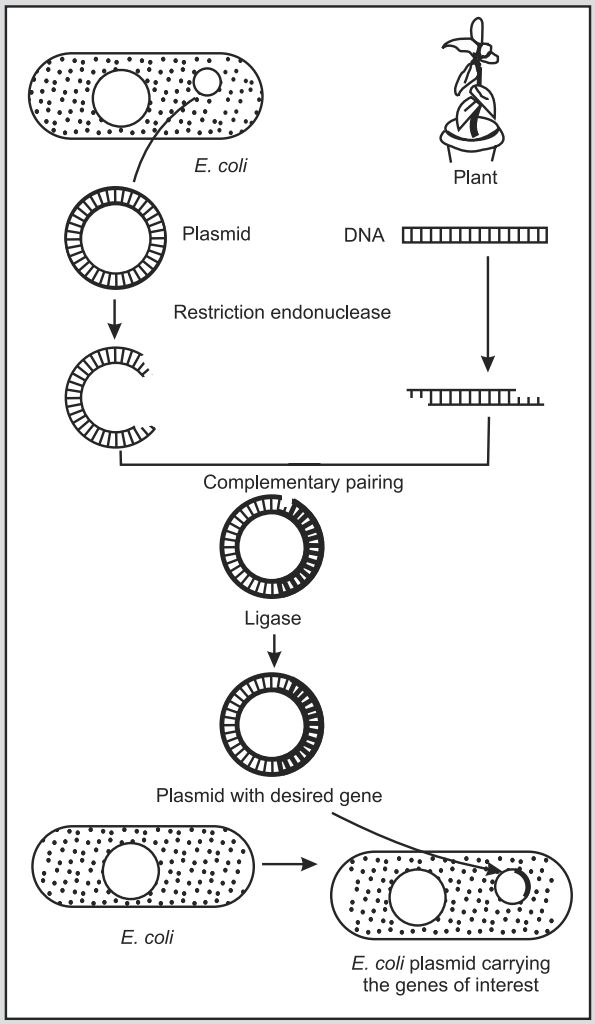


**Figure W2.2.** Diagrammatic representation of the steps involved in the transfer of foreign gene in *E. coli* plasmid.

- After the foreign DNA is inserted into isolated plasmids, they are transferred back into the bacterium where it makes many copies of the foreign gene by duplicating the plasmid during reproduction.

Plant transformation with *Agrobacterium tumefaciens* also relies on plasmids.

Today, a large number of restriction endonuclease enzymes have been identified, isolated and are commercially available, each cutting DNA at specific nucleotide sequences. The free sticky ends of plasmid and foreign DNA serve as convenient points for complementary pairing and the resulting gaps are then sealed (or annealed) by another enzyme called DNA ligase, thus forming a new circular DNA which contains the plasmid genes as well as a piece of foreign DNA. Such a recombinant

(chimeric) DNA molecule can be introduced into a bacterial cell as well as into a host plant cell where it can replicate and express.

> In any transformation study, it is possible that the gene(s) of interest will not have an easily detectable product. In such regulatory investigations, it is necessary to use a gene whose product can be easily assayed. Any gene that is well characterized, both genetically and biochemically, and whose expression leads to a phenotypic effect that can be easily noticed is termed as 'reporter' or 'marker' gene. Thus, a reporter gene can be used to identify individual transformants or their progeny. The most commonly used reporter genes are for enzymes such as β-glucuronidase (*gus*), chloramphenicol acetyltransferase (*cat*) and firefly luciferase (*luc*).
>
> The use of a reporter gene can be further explained by taking the example of *gus*. The gene, originally isolated from *E. coli*, can be cloned into the *Agrobacterium* plasmid and then transferred into the host genome where it degrades certain organic substrates into a coloured product, which can be taken as the measure of gene expression. The gene, perhaps the most widely used, was introduced (for the first time) into the plant system by Richard Jefferson (UK), now heading an organisation called GAMBIA, Canberra (Australia). GAMBIA has the mandate to support the development of molecular biology and biotechnological sciences in developing countries.

The task of employing *A. tumefaciens* for transferring plant genes was accomplished by Jozef Schell (Max Planck Institute for Plant Breeding in Cologne, Germany) and Marc van Montagu (State University of Ghent, Belgium). The two scientists, employing traditional DNA recombination techniques, made it possible to delete the tumour-inducing genes from the T-DNA region of the Ti plasmids. In 1984, these researchers introduced the disarmed Ti plasmid into tobacco plant cells to produce the first transgenic plant expressing foreign genes. The technique has successfully worked with several other plants of the Solanaceae family such as *Petunia*, tomato and potato, and for this reason, commercial development of crop varieties in these crops has proceeded rather quickly.

Robert B. Horsch (Monsanto Company, US) popularised the use of a common paper hole punch to cut disks from leaves of plants for *Agrobacterium*-mediated transformation. In this method, sterile leaf disks bearing incisions (to increase the area of infection) are co-cultured for two to three days in a liquid medium containing *A. tumefaciens* (or *A. rhizogenes*) carrying genes that the molecular biologist wishes to incorporate into the plant. The wounded cells at the edge of the disk release 'factors' that induce the agrobacteria to infect the cells. After immersing in the liquid medium, containing an antibiotic such as kanamycin for 1-2 hours to kill off the bacteria sticking to the outside of the plant cells and not needed any more and also the plant cells that did not receive the T-DNA.

The leaf discs are then placed on sterile blotting paper to wipe away excess bacteria and then subsequently shifted to a solid medium which induces the formation of shoots within a few weeks. The regenerated shoots are later transferred to a nutrient medium that induces root development. The whole process, from cutting out leaf discs to obtaining rooted plants, takes between four to seven weeks only (Figure W2.3). This technique is applicable to a wide variety of dicotyledonous crop species, and *Agrobacterium*-mediated gene transfer is now routinely used in hundreds of industrial and academic laboratories around the world. At Monsanto Company (US) alone, more than 45 000 independent transgenic plant lines have been produced in this way.

Although the *A. tumefaciens*-mediated genetic transformation technique is simple and precise, many plant species belonging to monocots, especially grain crops, such as rice, corn and wheat are by and large not susceptible to *Agrobacterium* infection. That is why new methods of transforming cells had to be developed to insert foreign DNA directly into plant cells.

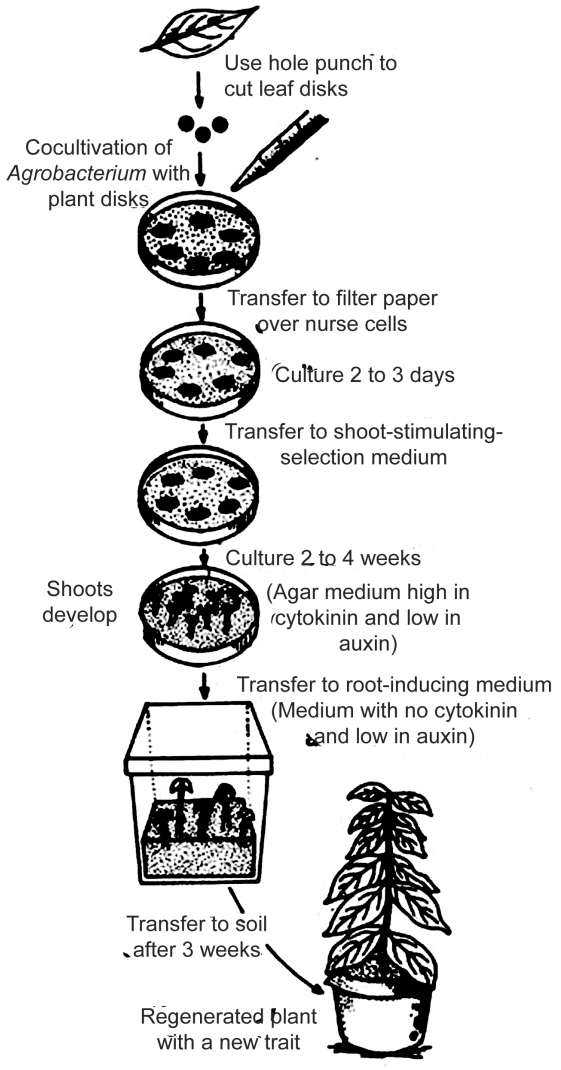


**Figure W2.3.** A diagram showing regeneration of leaf disks infected by *Agrobacterium*. Leaf disks are cut out and after surface sterilisation placed in a shallow dish, to which an inoculum of agrobacteria is added. The infected leaf disks are transferred for several days onto a nurse cell medium that stimulates shoot development. Cells carrying the plasmid are selected by culturing in shoot stimulating medium with an appropriate antibiotic, such as kanamycin (regeneration/selection medium). Shoots develop from transformed cells within a few weeks and these shoots are then transferred to a medium that induces root formation. This whole process is extraordinary fast, taking only few weeks (between 3-6 weeks) as compared to the protoplast culture.

### (II) DIRECT DNA UPTAKE INTO PROTOPLASTS

Before a plant cell can take up a plasmid DNA molecule (containing the gene of interest) from the medium, its cell wall must be removed with enzymes to produce the protoplasts. Uptake of plasmid

DNA molecules by the isolated protoplasts from the medium has been shown to take place under the following three sets of conditions:

- *PEG-mediated DNA uptake*: Polyethylene glycol (PEG), a thick organic polymer, is the most commonly used chemical agent that reportedly acts on both the DNA structure and the plasma membrane of the host cell. The addition of PEG in combination with calcium chloride in the medium has been found to stimulate the process of DNA uptake as well as integration in the isolated protoplasts. The PEG/$CaCl_2$ stimulated DNA uptake has been reported in monocots, such as *Triticum monococcum* L. and *Oryza sativa* L. and dicot crops, such as oilseed rape and tobacco.
- *Electroporation*: In this technique, the isolated protoplasts are given a shock treatment electrically (by giving a short, high voltage pulse) to make transient pores in the cell membranes. This enables the plasmid DNA molecules from the culture medium to pass through the pores in some of the cells. The cell wall then grows back within a day or so and cell can again divide. Depending upon the plant species used, the efficacy of the procedure varies and, in general, is quite low; at times only one in ten thousand or one in a hundred thousand protoplasts subsequently divide, and most of these cells do not form plants.

  Recently, however, several scientists both abroad (for example working at Plant Genetic Systems, PGS Belgium) as also in India (University of Delhi) have shown that electric shock treatment to an organised meristem or an embryo can also work. Happily, the cell wall does not seem to pose a barrier to the entry of DNA. Thus, the problem of having to regenerate plants from protoplasts has been completely circumvented. This method holds great promise for transforming recalcitrant crop species, such as wheat, rice and maize–our staple foods.
- *Liposome-mediated uptake*: In this method, a suspension of positively charged liposomes (vesicles of oil or fat) is mixed with the negatively charged DNA molecules. The general principle is to wrap-up genes into liposomes, and later mix these with the protoplasts. Alternatively, specific genes for a trait are inserted into a 'plasmid' that in turn is packed into liposomes, which are then mixed with the isolated protoplasts. The cells that pick up these liposomes can be easily identified since they can be stained and visualised under the microscope.

The first two of the methods outlined are the ones most commonly employed to stimulate uptake of DNA. The major advantages of direct DNA uptake are its simplicity and applicability to many organisms and cell types. Hundreds of thousands of cells may be simultaneously treated. This method has been extremely useful for basic studies of gene expression, such as efficacy of promoter or entry of DNA in a protoplast where results can be seen in only a few hours, and complete plants do not have to be necessarily regenerated. However, regeneration of plants from isolated protoplasts has proven to be problematic in some of the crop species, especially the cereals (such as corn and wheat) which respond poorly to regeneration and yield mostly the infertile plants.

### (III) MICROPROJECTILE BOMBARDMENT

Microprojectile bombardment or biolistics, compared to the other methods of plant transformation, is a relatively recent innovation. The credit for biolistics mainly goes to J.C. Sanford and T.M.

Klein (Cornell University, US) who in 1987 constructed a microprojectile gun or DNA particle gun (marketed by Dupont). This microprojectile gun is used to deliver DNA directly into plant cells by shooting it through the cell wall and cell membranes. The resulting holes in the membranes rapidly close by themselves.

In this technique, microscopic particles of tungsten or gold are coated with plasmid DNA molecules and then fired into the target cells. In a proportion of the cells, the DNA is transferred to the nucleus, providing an opportunity for integration into the plant genome and which is then passed on to the cell's progeny. The tungsten or the gold particles are sufficiently small (4 μm in diameter) to penetrate individual cells without destroying their integrity and viability.

One of the earliest and probably the most commonly used DNA particle gun is the Biolistic™ particle accelerator. It initially employed a standard 0.22-calibre gun cartridge for firing the microprojectile, which then travels down the barrel and hits the back of stopper plate. The DNA-coated pellets then pass through a central hole in the stopper plate into the plant tissue placed below (Figure W2.4).

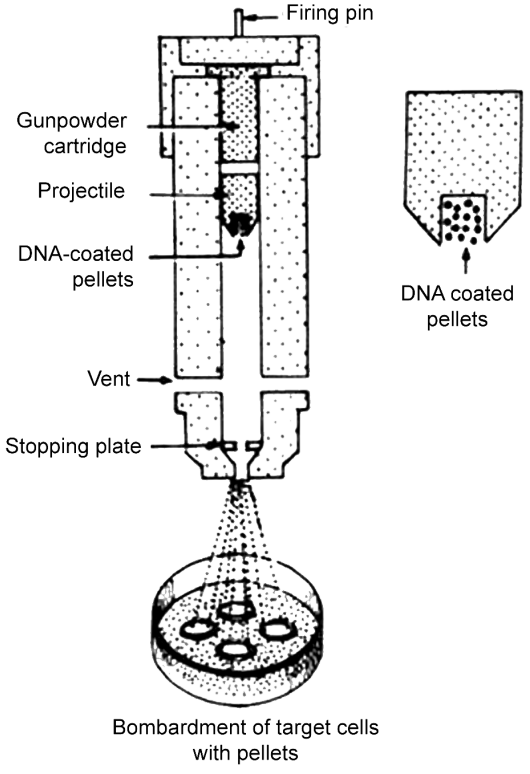


**Figure W2.4.** Diagram of a DNA particle gun. A gene gun is a device that shoots or fires tungsten pellets coated with DNA directly into cells. The pellets are held by a plastic microprojectile, which is accelerated by a gunpowder charge. Although the projectile is held at the stopping plate, the momentum sends the DNA coated pellets into the target. The vents permit air in front of the projectile to escape. In the particle gun, the advantage is that it introduces DNA into differentiated tissues, such as leaves or meristems.

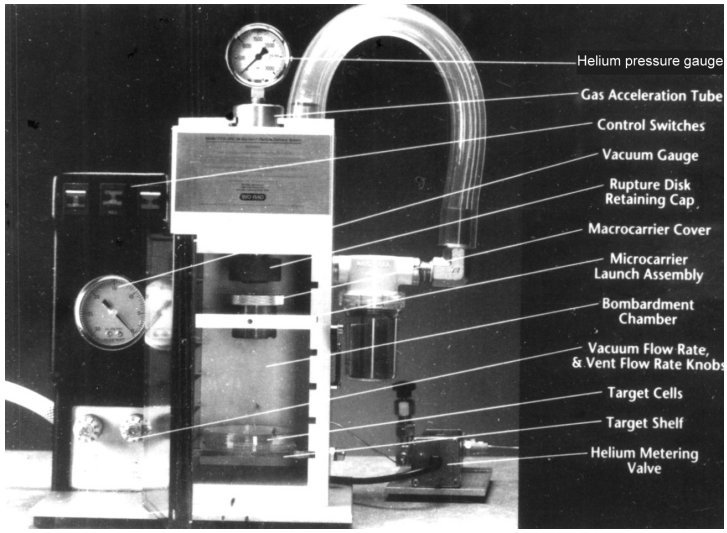


**Figure W2.5.** A photograph of DNA particle gun, developed by John C. Sanford and T.M. Klein of Cornell University, US.

The earliest device has now been modified to use compressed helium for propulsion, since using it results in a more uniform strike and avoids burnout (Figure W2.5). Another approach is to use a high-voltage electrical discharge through a water droplet to create a shock wave and accelerate a thin sheet carrying the DNA-coated particles. Overall, however, microprojectile bombardment has so far proved to be the most versatile method, especially for cereal transformation.

The biggest advantage of the microprojectile bombardment technique is that it introduces DNA into the differentiated tissue, such as leaves or meristems, so that there is no need for cells to be grown in an undifferentiated state, and that it eliminates the need for troublesome protoplasts. However, as with most other methods for transferring genes, blasting genes into tissue is a random process. Scientists cannot predict where, how, or even whether the foreign DNA will be integrated into the plant genome. Consequently, the successful recovery of transformed culture depends on an efficient and discriminating means of selection for the proliferation of those cells that carry the introduced DNA. As with other methods of transformation, selection in bombardment technique has been achieved by using herbicides or antibiotics. Among the widely used selectable marker genes in cereals, is the *bar* gene from *Streptomyces hygroscopicus*, which codes for phosphinothricin N-acetyl transferase (PAT) that confers resistance to phosphinothricin-based herbicides ,such as Bialaphos and Basta. Effective selection of transformed monocot tissues has also been achieved using antibiotics, such as kanamycin and hygromycin B.

### (IV) MICROINJECTING THE DNA

Another method of inserting DNA into intact plant cells is to inject directly the foreign DNA molecules into individual cells. In this technique, an elaborate apparatus consisting of a microscope and delicate micromanipulators is employed to view cells, hold them steady and then inject into them a solution containing DNA with fine glass hollow needles. However, the microinjection method has not been popular for several reasons, the main problem being that transforming cells one at a time

is a highly laborious and time-consuming process, demanding great skill, and thus unsuitable for commercial manipulation. At times, one might have to inject DNA into at least 10 000 cells or so, just to ensure that one of them will carry the new gene.

Another recent approach is to use silicon carbide fibres with an average diameter of less than one μm. The method involves vortexing a mixture of DNA, silicon carbide fibres and plant cells. The basic principle is that the fibres pierce the plant cell wall to allow the entry of the DNA. This method has been used to produce fertile, transgenic maize plants following the treatment of embryonic suspension cultures. Another development has been the use of fine laser beams to puncture holes in the cell wall and plasma membrane, and this technique has recently been applied to rice transformation.

Several other innovative approaches requiring no sophisticated, expensive laboratory facilities have been attempted to produce transgenic plants. These include techniques such as (a). delivering foreign DNA molecules using pollen tube as a conduit and (b). uptake of plasmid DNA by embryos during seed imbibition. Such methods, if optimised to produce stable transformation, offer hope in developing countries with access to limited resources.

In fact, using the mentioned innovations in techniques, successful genetic transformations have been achieved for a large range of agricultural and ornamental crops. Table W2.1 shows a chronological account of the achievements in the transformation of diverse crops.

**Table W2.1.** The history of plant transformation

| *Year* | *Plant* |
|---|---|
| 1983 | Tobacco |
| 1984 | Carrot, *Lotus* |
| 1985 | Oilseed rape, *Petunia* |
| 1986 | Alfalfa, *Arabidopsis*, cucumber, tomato |
| 1987 | *Asparagus*, cotton, flax, horseradish, lettuce, poplar, potato, rye, sunflower |
| 1988 | Cauliflower, celery, eggplant, corn, orchard grass, rice, soybean, walnut |
| 1989 | Apple |
| 1990 | Buckwheat, birch, chrysanthemum, citrus, clover, grapevines, mustard, papaya, strawberry |
| 1991 | Carnation, cowpea, kiwi, melon, plum |
| 1992 | Sugar beet, wheat |
| 1993 | Pea, barley |

## Goals of Recombinant DNA Technology in Agriculture

In the past decade, the tools and techniques of plant genetic engineering technology have been fruitfully employed for the improvement of several agronomic traits. Some of the important goals of recombinant DNA technology, either achieved or well-conceived but yet in the experimental stage, are listed in Table W2.2. Of the various applications mentioned therein, raising of transgenic crops that are resistant to insect and viral diseases and herbicides has been very successful. In this section, we will provide selective information on these developments.

**Table W2.2.** Goals of recombinant DNA technology in agriculture

| | |
|---|---|
| 1. | Crop resistance to herbicides |
| 2. | Crop resistance to insects and diseases |
| 3. | Reduction of photorespiration in $C_3$ plants |
| 4. | Atmospheric nitrogen fixation by crop plants |
| 5. | Tolerance to high-salt soils and to flooding in crops |
| 6. | Drought resistance in crops |
| 7. | Enhancing the nutritional qualities of crops |
| 8. | Cold-tolerance in tropical and subtropical crops |
| 9. | Prolonging the storage or shelf-life of fruits and vegetables |
| 10. | Enhanced production of plant chemicals and enzymes used in pharmaceutical and food processing industries |
| 11. | Increased productivity of food crops, including ornamentals |
| 12. | Induction of male sterility in agricultural crops |

## GENETIC ENGINEERING FOR INSECT RESISTANCE

Worldwide, insects cause nearly 15-20 per cent reduction in crop yield. Traditionally, chemicals have been used for the control of insects; though the introduction of chemical pesticides has revolutionised pest management, their large-scale use at the same time has increased the cost of inputs and also led to degradation of the environment. Further, insects tend to develop resistance to such insecticides over a period of time. The discovery of insecticidal proteins in the soil bacterium *Bacillus thuringiensis* Berliner (BT toxin), stimulated an enormous interest in the development of biopesticides. The endotoxin BT binds to the cell membrane lining the gastrointestinal tract of the feeding insect, causing disorganisation of the membrane and killing off the insect larvae. BT-endotoxin is a relatively environmentally benign pesticide because of a number of attributes, such as (a). it is biodegradable and does not accumulate in tissues and (b). it is non-toxic to vertebrates. Scientists have been successful in isolating the gene that encodes for the BT toxin (Bt gene). This gene was initially expressed in tobacco plants at high levels, and such transgenic plants showed resistance to the attack by insects. The cotton plants have been genetically engineered using Bt gene to protect them completely from the ravages of insects, especially the bollworm (*Heliothis armigera*). The use of Bt gene can save billions of dollars that are spent every year for control of cotton against the bollworm (as a matter of fact, cotton is among the most heavily pesticide-treated crops in the world). Thus, transgenic cotton would reduce our dependence on chemical pesticides with no impending danger to the environment and also make for decreased input costs to the farmers. Likewise, transgenic corn with a BT toxin that makes it resistant to European corn borer has been produced. Several biotechnology companies are conducting field tests on different transgenic crops with BT toxin genes, and these are expected to be in the market within the next few years (Table W2.3).

A team of IRRI scientists led by Joachim Wünn has successfully transferred Bt genes from *B. thuringiensis* into IR 56 variety of the rice. This variety of transgenic rice resists attack from at least half a dozen of the common insect pests.

**Table W2.3.** Biotechnology companies that have conducted field tests or have had field test approved by the Unites States Department of Agriculture (USDA) for transgenic plants with in-built resistance derived from *Bacillus thuringiensis* (Bt gene).

| *Company* | *Crops* |
|---|---|
| Monsanto | Cotton, Potato, Tomato, Corn |
| Calgene | Cotton, Tobacco, Potato |
| Ciba-Geigy | Tobacco, Corn |
| Agrigenetics | Canola (Rapeseed) |
| Dekalb | Corn |
| Northrup King | Corn |
| Rogers NK Seed | Tomato |

Apart from BT toxin, several other proteins with insecticidal properties have been discovered in recent years. These include lectins, α-amylase inhibitors and protease inhibitors. All of these proteins retard metabolism and growth of insects when ingested in high dosages. Using genes for these proteins, transgenic crop plants have been raised in several laboratories and, in specific instances, it has been shown that insects feeding on such transgenic plants cannot survive for long because of the damage to their digestive tract.

## GENETIC ENGINEERING FOR INCREASED VIRUS RESISTANCE

Diseases caused by viruses result in considerable losses to crop plants. A simple and widely used approach for protecting plants from a viral infection is through the application of a mild strain of the same virus to the plant. This causes the development of resistance against increased levels of virus infection. This approach is called cross-protection. However, the cross-protection approach is cost-ineffective, laborious and unsatisfactory. At times, the mild strains of the applied viruses act synergistically to enhance infections caused by more severe viral strains that ensue. Use of chemicals for the control of viruses has the same drawbacks as enumerated above for the control of insect attack. It is thus of great interest that Roger Beachy and his colleagues (US) discovered that cross-protection induced by the mild strain of a virus is due to coat protein moiety of the virus particle. Subsequently, this group succeeded in isolating and introducing gene coding for the viral protein coat of tobacco mosaic virus (TMV) into tobacco plants. Transgenic tobacco plants were shown to synthesise viral coat proteins in high amounts and, more importantly, the plant showed considerable resistance to infection by TMV. A single coat protein gene can protect the host plant against many viruses. Subsequent to these initial findings, a large number of crop plants have been genetically engineered through the strategy of over-expressing viral coat protein genes in the host plant using strong constitutive promoters. (Figures W2.6, W2.7 and W2.8).

For instance, potato virus X, potato virus Y and potato leaf roll virus cause considerable damage to potato crop. Potatoes are vegetatively propagated, and the 'seeding' material must be certified virus-free. By introducing the gene of coat protein of potato virus X into two commercial cultivars, most susceptible to viruses, scientists at Mogen International (Netherlands) have been able to produce a transgenic potato line resistant to viral infections.

**Figure W2.6.** Transgenic rice plants of cultivar Taipei 309 in the greenhouse. These plants are derived from different transformation experiments that involved the bombardment of embryogenic callus or embryogenic suspensions with DNA-coated microparticles. The DNA contained sequences coding for the coat proteins of Rice Tungro Bacilliform Virus (RTBV) and Rice Tungro Spherical Virus (RTSV). Preliminary experiences in field trials conducted at the Malaysian Agricultural Research and Development Institute (MARDI, Kuala Lumpur, Malaysia) resulted in protection against RTSV infection in plants transformed with RTSV coat protein genes.

**Figure W2.7.** The first transgenic cassava plant. The plant is derived from microbombarded embryogenic suspension cultures of the African cassava cultivar TMS 60444. The genes used for transformation were an antibiotic resistance gene and a gene coding for the enzyme β-Glucuronidase (GUS). Work is in progress at ILTAB with the aim to transfer genes that confer resistance to viruses affecting cassava, that is, African cassava mosaic virus (ACMV) and cassava common mosaic virus (CasCMV).

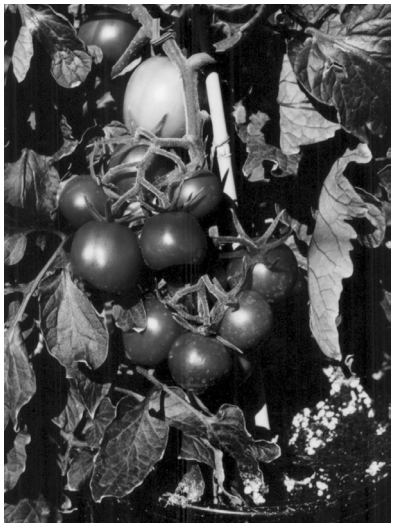

**Figure W2.8.** A transgenic tomato plant that resulted from the transformation of tomato leaf disks with *Agrobacterium tumefaciens*. Field tests with plants into which genes of tomato yellow leaf curl virus (TYLCV) have been introduced are being tested for resistance to the virus at the Agricultural Genetic Engineering Research Institute (AGERI, Cairo, Egypt).

The Asgrow Seed Company, at Kalamazoo (Michigan, US), has developed a crookneck squash (a cucurbit) resistant to the virus. In this case, the genes coding for the viral coat protein of two viruses have been introduced into the plant genome. As a result, the yield of Asgrow squash was found to be five times more than from the standard seeds.

Among the other approaches to inhibit viral infection are through the expression of satellite RNA and the antisense approach. Satellite RNA represents small RNA molecules present in some RNA viruses. The satellite RNA is not a constituent part of their genomic RNA. Expression of the satellite RNA molecules in high amounts in transgenic plants has been found to provide protection against further viral infections in limited instances. In the antisense approach, various portions of the viral genome have been tested with respect to their expression using strong plant promoters for making virus resistant plants (see later for a detailed discussion on antisense approach). However, as it stands at present, coat-protein-mediated protection has emerged as the most powerful technique for combating virus attack.

## GENETIC ENGINEERING FOR ENHANCED PROTECTION AGAINST FUNGAL AND BACTERIAL PATHOGENS

A large number of fungal and bacterial species infest crop plants and cause significant losses in crop yields. The common approaches for combating these microbes have been to breed resistance against these pathogens by conventional breeding methods and control the pathogenic forms through the application of chemicals. Both of these approaches, however, provided a marginal success only. Apart from being expensive, the use of fungicides and bactericides is a threat to the environment. Therefore, for the control of fungal diseases, genes for chitinase and glucanase enzymes, which degrade the major constituents of the fungal cell wall (namely chitin and β-1, 3-glucans, respectively) have been

introduced in the test crops, killing the pathogen and thus preventing the onset of disease. Transgenic tobacco plants in which chitinase and glucanase genes were independently overexpressed have shown high levels of resistance to fungal pathogens. More recently, the constitutive coexpression of chitinase and glucanase has been achieved in tobacco. The coexpression has conferred considerably higher levels of resistance to fungal pathogens than either gene alone, indicating a synergistic interaction of these two enzymes in combating fungal infection. Apart from these, expression of barley ribosome inactivating or inhibiting protein (RIP) in transgenic tobacco has also been shown to confer resistance to fungal pathogens.

Bacterial cell walls are made up of unusual polymers called peptidoglycans (containing proteinaceous and carbohydrate components). These chemicals have been shown to be sensitive to digestion by lysozyme, which is found in abundance in the stomachs of cows and chickens. Scientists have inserted the gene for lysozyme into yeast which, when grown in large fermenters, yields lysozyme on a commercial scale. This technology has enabled low-cost lysozyme production with potent properties.

## GENETIC ENGINEERING FOR RESISTANCE AGAINST HERBICIDES

Weeds compete with crop plants for water, light and nutrients. Manual weeding is the most common practice, but for obvious reasons cannot be applied when the size of the farm is large. Herbicides provide a cost-effective means of controlling weeds in the farmer's field. A good herbicide must be selective in its action, that is, it should eliminate the weeds without having any ill effects on the crop species and the animals depending on those species. In recent times, wide-spectrum herbicides that are effective against a whole range of plant species have been developed. Glyphosate marketed by Monsanto [Roundup®], phosphinothricin[*] marketed by Hoechst AG [Basta®] and sulfonylurea marketed by Dupont are considered versatile herbicides since they are required in low doses, are readily biodegradable and do not seep into ground water. However, because of their non-selective nature, it has become necessary to engineer crop plants to tolerate such herbicides.

Glyphosate mostly kills the broad-leaved plants and grasses by inhibiting the action of enol pyruvyl shikimate phosphate (EPSP) synthase–an enzyme needed for the synthesis of aromatic amino acids in the chloroplast; failure of plants to synthesise essential amino acids leads to a halt in their metabolism and they perish. Resistance against glyphosate has been engineered in plants employing EPSP synthase from a mutant strain of *E. coli* that is resistant to glyphosate. The mutant gene was isolated from the bacterium, and was then modified and inserted in tobacco to raise transgenic tobacco which showed increased resistance to glyphosate (Stephen Rogers and Ganesh Kishore at Monsanto; Luca Comai and David Stalker at Calgene). Recently genetically engineered soybeans have been produced that contain the glyphosate-insensitive EPSP synthase gene.

In another approach, resistance to herbicides has been engineered by overexpression of the target protein, which is specifically affected by the given herbicide application. This approach is practised with a viewpoint that even if most of the target protein were inactivated by the application of the herbicide, there would be some molecules left to carry on essential metabolism, and hence the plant would survive. The over-expression of the gene for the target protein is achieved by using strong plant promoters, which include cauliflower mosaic virus (CaMV) 35S promoter for the dicot plants and actin and ubiquitin promoters for the monocot plant species. Using this principle, herbicide-resistant transgenic tomato, soybean, cotton, oilseed rape and a number of other crops have been produced in recent years.

[*] Also known as Glufosinate with an emperical formula $C_5H_{15}N_2O_4P$.

Yet another strategy to engineer herbicide-resistant plants is through the expression of an enzyme that can detoxify or degrade the herbicide inside the plant. This approach can be exemplified by phosphinothricin. It is an effective non-selective herbicide as it blocks glutamine synthetase, an enzyme necessary for assimilation of ammonia. As a result, ammonia accumulates within the plants to such toxic levels that the plants are unable to survive. Scientists at PGS (Belgium) and Hoechst (GDR) have introduced PAT gene (coding for phosphinothricin acetyltransferase) that inactivates the herbicide Basta in many crop plants; the transgenic plants detoxified phosphinothricin in the presence of this gene and thus survived the herbicide treatment, whereas the weeds died.

## GENETIC ENGINEERING FOR IMPROVING POST-HARVEST QUALITIES

One of the classical examples of successful application of plant biotechnology research is the genetic engineering for delayed ripening in tomatoes. The plant hormone ethylene is primarily involved in the process of fruit ripening. Through biochemical experiments, it is established that aminocyclopropane carboxylic acid (ACC) synthase and ACC oxidase enzymes, as shown in Figure W2.9, control the biosynthesis of ethylene.

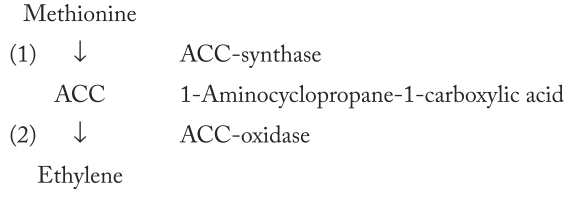


**Figure W2.9.** Steps in the biosynthesis of ethylene.

G. Kishore and H. Klee (Monsanto, US) introduced in tomatoes a bacterial gene that encodes an enzyme which degrades ACC as soon as it is formed. Because of this genetic manipulation, transgenic tomato plants failed to accumulate ethylene and consequently, there was a significant delay in fruit ripening. This accomplishment may enable the farmers to leave the fruits on the plant until the time they manage to market them.

Further, softening of tomatoes involves a cell wall degrading polygalacturonase enzyme. Scientists from Calgene, US have been successful in suppressing production of this enzyme by using antisense approach (see Box W2.2). Such transgenic tomatoes can be left on the vine longer than the normal ones. Calgene has marketed these tomatoes as Flavr Savr$^{(R)}$ tomatoes that resist softening. Such tomatoes can be mechanically harvested and, further, need not be refrigerated during shipping. In *Petunia*, ethylene inhibition with antisense RNA approach has helped in extending the vase-life.

## MALE STERILITY THROUGH GENETIC ENGINEERING

Hybrid varieties out-yield self-bred lines with respect to growth and productivity. Hybrid seeds, although necessarily purchased annually, produce more uniform stands, and offer greater profit margin than the non-hybrid seeds. Hybrid seed production in normally self-pollinated crops is possible only if the pollen development system from such plants is efficiently eliminated. The commonest method of achieving this is to do emasculation. However, the approach is labour intensive, costly, time-consuming and difficult. The production of hybrids in corn and other such self-fertilising agriculturally important crops has been possible by banking on naturally occurring male sterile and fertility restorer breeding lines.

**Box W2.2: Antisense RNA technology to help slowing down the fruit ripening process or extending the vase-life**

Normally in plant genetic engineering, one or more new genes are added to the plant genome to allow the novel protein/enzyme to be formed in the transgenic plant. The antisense strategy, on the other hand, is an important tool in the hands of the biotechnologists to suppress the synthesis of an existing cellular protein. In the usual course, genes present on the sense strand of DNA are transcribed as mRNA, which is translated to form an active protein. In the antisense approach, genes present on the untranscribed or antisense strand of DNA are made to make mRNA, which is the antisense message. Due to complementary base pairing, however, the sense RNA binds with its antisense RNA to form a double-stranded structure incapable of being translated on ribosomes–thereby preventing the synthesis of proteins. An antisense genetic construct consists of a suitable promoter sequence and the protein-coding region of a gene already present in the plant, in reverse orientation.

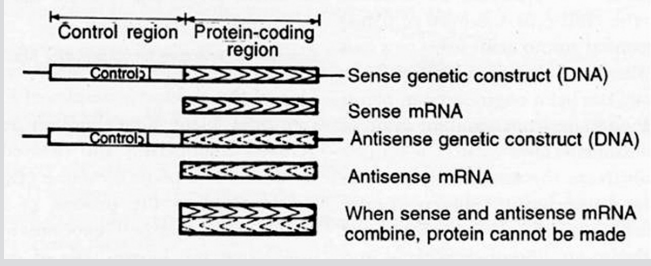


**Figure W2.10.** Schematic representation of sense and antisense genetic constructs.

By using antisense technology, biotechnologists have been able to knock out the enzyme that catalyses biosynthesis of ethylene in tomatoes, thereby slowing down the ripening process (Figure W2.10).

In a significant development, R. Goldberg (University of California, US) together with the scientists of PGS (Belgium), have shown that male sterility can be genetically engineered in tobacco plants. This group has been able to introduce and express genes coding for a particular ribonuclease (RNase) specifically in the tapetal cells for causing an uncontrolled breakdown of RNA in these cells (tapetal cells provide nutrition to the developing pollen grain mother cells), thus aborting normal pollen formation. This new technique has been described as a 'genetic laser' because of the precision with which it emasculates plants without affecting them in any other way. In addition, the technique has been tested to work satisfactorily in oilseed rape as well. Scientists at PGS (Belgium) are developing this technique commercially with a hope to have the first hybrid oilseed rape in the next few years. Guaranteed male sterility is now an almost priceless boon to seed companies engaged in hybrid seed production in crops such as sunflower, tomato, brassicas, etc. In principle, male sterility can now be genetically engineered into any crop species.

## GENETIC ENGINEERING FOR IMPROVED RESISTANCE TO ABIOTIC STRESSES

Abiotic stresses such as drought, salinity, flooding, submergence, UV radiations and high and low temperatures limit the productivity of crop plants to a great extent in all parts of the world. It is

realised that dealing with abiotic stresses is a complicated endeavour as tolerance to these edaphic and atmospheric factors is often a multigenic phenomenon whereas the present-day plant genetic techniques can manipulate only single or limited genes. In recent years, there is some success for genetically engineering plants against abiotic stress factors too. For instance, it is commonly observed that in response to osmotic stresses (salt, drought, to an extent low and high temperature) plants accumulate osmoprotective solutes, which presumably provide protection to plants against such stress conditions. It is thus considered worthwhile to increase constitutive and stress-induced levels of these compounds in cells to improve their stress tolerance. The most commonly observed osmoprotectants include proline, glycine betaine, sugars and sugar alcohol. Recently, H.J. Bohnert and associates (University of Arizona, US) have been able to genetically engineer tobacco plants that synthesise and accumulate high levels of mannitol through overexpression of a bacterial gene that codes for the mannitol-l-phosphate dehydrogenase. Such transgenic plants performed better under simulated salt (NaCI) stress than the untransformed (control) plants. Genetic tolerance against salt stress has also been engineered by the overexpression of proline in tobacco plants by D.P.S. Verma and associates (Ohio State University, US).

The degree of unsaturation of membrane lipids has been shown to play a crucial role in providing protection against cold stress in some lower plant systems. In tobacco, sensitivity to cold stress has been altered by a Japanese group through changing the composition of fatty acids in chloroplast membranes. The alteration was achieved by introducing a gene that encodes glycerol-3-phosphate acyltransferase. Furthermore, it has been found that cold damage to crop plants can also be minimised by introducing genes for antifreeze proteins (AFPs), which are found in the blood of arctic fishes. Frost-resistant tomatoes have been produced recently by introducing the gene for AFPs isolated from polar fishes (*Pseudopleuronectes americanus*) living in ice waters. Such tomatoes have the potential of withstanding frost and retaining flavour while being stored in a refrigerator. The AFPs inhibit the freezing of water in the cells.

Genetic engineering experiments to impart plants tolerance to other stresses, such as high temperature (through a high-level constitutive expression of heat shock proteins) and desiccation (through overexpression of fructans) have been conducted and showed positive results.

## SOME OTHER EXAMPLES OF PLANT BIOTECHNOLOGY RESEARCH

There is a big market for potato chips and French fries internationally. A small increase in starch content has been achieved by introducing a bacterial gene into potato plants; thus, potato chips with less cooking fat will soon be marketed. Tomatoes form the basis of a multibillion-dollar industry that produces ketchup, soup, paste and canned products. Scientists are attempting to introduce a gene that codes for the enzyme ADP-glucose phosphorylase in tomato fruits with the objective of increasing the viscosity of tomato juice, thereby requiring less water evaporation (during ketchup manufacture). Scientists at the Carnegie Institute of Washington (US) have genetically engineered a small weed plant (*Arabidopsis thaliana* (L.) Heynh.) to produce polyhydroxybutyrate (PHB), a biodegradable plastic.

Oilseed rape plants producing primarily long-chain fatty acid ($C_{18}$) have been bioengineered to produce short-chain (C12) fatty acid by incorporating the genes for ($C_{12}$) thioesterase enzyme with seed-specific promoter sequences (there is a unique enzyme for the synthesis of each carbon chain length of fatty acid). Canola, soybean, flax, cotton, sunflower and castor have all been transformed with 'alien' genes.

Plants offer a potential resource for the production of foreign proteins, which can be used in health care systems. It has been shown that neuropeptides, blood factors, antibodies and growth hormones could potentially be produced in transgenic plants in a cost-effective manner.

## Transgenic Crops and Environment

From the discussion in the last section, it is amply clear that recombinant DNA technology has opened up opportunities for moving genes across sexual barriers. Therefore, the possibilities of genetically engineered crops are immense and virtually any cloned gene can now be transferred to plants. Selected examples of these possibilities are presented in this chapter. In future, plant biotechnologists are likely to genetically engineer crop plants with nitrogen-fixing ability, thus lessening our dependence on expensive nitrogenous fertilisers. A great deal of effort is being made to reduce photorespiration in $C_3$ plants, which if achieved, is going to have a tremendous impact on food production. The global human population is increasing at an alarming rate and it appears that plant biotechnology research has the potential to achieve a balance to the increased demand. However, while counting on these benefits, one has to take into consideration the likely fallout of this technology. It is well realised that no technological advancement is fool proof. The possibility of large-scale usage of the transgenic crop species has raised a few ecological, economical and ethical issues.

On the ecological front, it is to be appreciated that nature has provided us a fine balance with respect to fauna and flora that has come through millions of years of natural selection. To what extent is man going to change this balance by using transgenic materials, and what would be the consequences? What if the transferred gene is introduced into non-target species such as wild relatives of the cultivated species? If this happens, it may cause a real threat in altering the gene pool of the crop plants. If herbicide-resistance genes find a way of getting incorporated in weedy species, this may result in a lot of problems in controlling weeds (apart from the fact that industries making herbicides would have to close, which may be an economic disaster for some countries). What are the safety measures and how far are they practised? A lot of discussion on these and related issues is now engaging the attention of biologists and environmentalists. Nonetheless, a recent report of the United States National Academy of Sciences has concluded that (a). crops modified by molecular and cellular methods should pose risks no different from those modified by classical genetic methods for similar trait, (b). transgenic plants engineered with the newer techniques are likely to be at least as safe as those produced through traditional breeding and (c). genetic changes incorporated with modern techniques are generally better circumscribed and characterised.

However, the transfer of 'moth' genes into potatoes or 'fish' genes in tomatoes is an important risk that merits consideration. Similarly, while pollen transfer or gene flow into the wild species may not be such a problem in the United States (since none of the world's 20 most important food crops are indigenous to North America), it may be a serious risk in the centres of origin where wild relatives of crop species are found in abundance. Thus, in most cases, strict guidelines have been suggested for the glasshouse and field trials with transgenic materials. For every new application, there can be high, medium or low levels of environmental hazard. It is extremely important that all guidelines framed by the national regulatory bodies be adhered to in the release of transgenic materials. In addition, the federal agencies should be able to take up remedial measures, including the right to deal with plants that inadvertently become weeds by their impounding, uprooting or destruction. Finally, all biotechnologists should make sure that transgenics are taken to field under strong vigilance and without bias, and that all possible risk factors are calculated and brought to consumers. In short, before releasing transgenic crops, we must take all safeguards and strengthen

our ongoing efforts *in situ* and in *ex situ* conservation of the biodiversity present in their original homelands.

The economic risks of transgenic crops can be illustrated by taking the example of vanilla (*Vanilla planifolia* Andrew), an important cash crop of Madagascar involving about 70 000 small land owners. Vanilla accounts for 10 per cent of the export earnings of the nation. The production of high-value vanillin as a secondary metabolite in the tissue culture of a genetically engineered crop (if it becomes more cost-effective) may have a negative impact on the economy of the exporting country. Similar dislocation of economies may occur from the production of pyrethrins in tissue culture raised from a transgenic plant instead of pyrethrum [*Chrysanthemum cinerariifolium* (Trev.) Schultz Bip.] plantations, which form an important agriculture base of Myanmar (formerly Burma) and Papua New Guinea. Such potential economically damaging effects must be identified and monitored, and required remedial strategies should be developed. Another well-founded apprehension is that the technology for such gene transfers is largely available in the laboratories of advanced countries, which might lead to economic exploitation of the developing nations. To allay such fears, the patent rights should be made more just and equitable, free from possible economic exploitation.

Another problem is the unacceptability of the transgenic materials by consumers, as they want to eat 'natural products', not products that have been 'tempered' in the laboratory. At the moment, however, there is no reason to believe that transgenic plants will not be safe to consume. According to an estimate, we eat about 10 000 different genes daily, and this DNA is efficiently broken down into simpler components in our digestive tract. Investigations have shown that the genes introduced in transgenic plants are equally efficiently digested in our body. The fact remains that all our food crops that sustain us have been modified through gene transfer either natural or manmade over the last 10 000 years or so.

## Summary and Concluding Remarks

The power of biotechnology is no longer a fantasy. It is now offering tremendous potentials for improving crop production, animal husbandry and bioprocessing, and has been hailed, rightly, as the second Green Revolution (or more precisely Gene Revolution). This technology ought to supplement our traditional breeding efforts to fight against hunger. Through the tools and techniques of plant genetic engineering, a lot of efforts has been made to raise transgenic crops with desired agronomic traits and to date, there has been a reasonable success in producing insect, viral, fungal and herbicide resistant plants. However, a lot remains to be done in this endeavour. The foremost problem that remains so far is that many useful genes controlling different agronomic traits have not been precisely identified and isolated.[*] The second major bottleneck is that transformation methods for all crop species are not yet optimised to work at high efficiency. Furthermore, the present-day transformation methods cannot transfer larger stretches or chunks of DNA, which is desirable if we want to bring alterations in quantitative traits. It is most likely that during the coming decade or so of this century, the transgenic crops will increasingly replace our traditional crops and this second green revolution will be greener, equitable and sustainable with a successful management of resources such as land, water, environment and genetic diversity, to meet changing human needs.

[*] The National Research Centre for DNA Fingerprinting, established in 1995, has a mandate to characterise specific gene(s) of all the Indian released and notified varieties and landraces of plants by using recent techniques such as polyacrylamide gel electrophoresis (PAGE), restricted fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD).

**Box W2.3. Terminator Technology**
**(Professor Ashwani Pareek, School of Life Sciences, JNU, New Delhi-110067)**

As the name indicates, terminator technology allows the termination of seed viability upon treatment with a special chemical. Technically, it is a kind of 'technology protection system (TPS)', which relies on a trait that kills developing plant embryos and hence seeds cannot be saved and replanted in subsequent years. This technology has also been referred to as 'genetic use restriction technology' or GURT and the seeds as 'suicide seeds' (Figure W2.11).

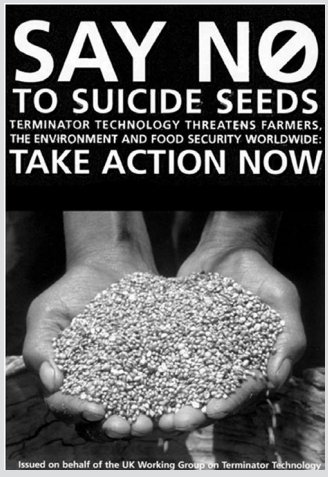


**Figure W2.11.** Poster depicting 'Say No to Suicide Seeds'.

The multinational seed companies (MNCs) would like the farmers, in developing as well as developed countries, to buy seeds from them for each sowing to ensure a fair return for the heavy investments they make in developing improved crop varieties. Although, farmers in the developed countries or well-off farmers can afford to pay for this purchase to the seed companies, farmers in the developing countries would like to use their own harvested seeds for replantation. The provisions of Article 27.3(b) of the Trade-Related Aspects of Intellectual Property Rights (TRIPS), Chapter of World Trade Agreement and those of the 1991 Act of Union International Pour la Protection des Obtentions Vegetables (UPOV), allows the seed companies to develop technologies to prevent such reuse of the seeds. Thus, terminator technology has been conceptualised by the United State Department of Agriculture (USDA-ARS) and Delta and Pine Land Company in the year 1998, and they were granted a joint patent (US patent number 5,723,765) entitled Control of Plant Gene Expression. Although, several variations of the gene components used in this technology can be proposed, but in general, it involves the following three essential steps:

- Firstly, a set of genes known as terminator genes, arranged in a special sequence, are engineered into the crop plants.
- Secondly, the owner seed company would add an inducer to these seeds to initiate the termination process before selling the seeds to farmers.
- Thirdly, the farmers buy these seeds and plant them. These plants would grow like normal ones but bear sterile seeds.

Essentially speaking, there are two variants of GURT technology. They are as follows:

- *V-GURT*: The technology allows farmers to grow the treated seeds, but next generation seeds are killed by the action of the terminator genes present in them. This allows the restriction of the technology at the plant variety level and hence known as V-GURT.
- *T-GURT*: A modification of V-GURT, which does not kill the seeds but will not exhibit the superior or enhanced trait for which the seed company has engineered it until the seeds are treated with a specific chemical, which is sold only by the company. Thus, it allows the seeds to germinate in contrast to the V-GURT, but these seeds would not exhibit the enhanced or superior trait and hence called trait-GURT or T-GURT.

The terminator technology has been seen by the farmers as seed companies with contrasting viewpoints . It is believed that the technology would encourage the seed companies to develop more improved crop varieties, even in those crops where the farmers generally save the seeds for the next season (such as rice and wheat) without any fear of not having the return of investments. It is also believed that the transgenic crops with this technology may not spread their seeds to surrounding plants if TPS pollen is used to pollinate other plants. This would also allow the scientists to address one of the major criticisms of GM technology, that is, to be able to prevent accidental gene flow from transgenic plants to wild populations. On the other hand, it is also true that pollen from the TPS plants may fertilise the non-TPS crops in surroundings, resulting in the unintentional killing of seeds in the non-TPS plants. From farmers' viewpoint, this technology may not be affordable to all, especially the poor ones. The technology would not allow the farmers to save seeds for the next sowing and they will be forced to invest heavily in the purchase of expensive seeds every year. Keeping these issues in mind, in the year 2000, the United Nations Convention on Biological Diversity (CBD) has recommended a *de facto* moratorium on the field-testing and commercial sale of the terminator seeds, which was again reaffirmed in 2006. Several countries, including India, have their own legal restrictions on the use of this technology (Figure W2.12).

Essentially, in the terminator technology, a set of three special genes are arranged in a unique order in a vector and then inserted into the genome of the plant through routine transformation methods. The genes are as follows:

- *Toxin gene*: A gene coding for a toxin protein under the control of a late promoter (LP) is the key to this technology. An example of the toxin could be ribosome inhibitor protein (RIP) from the plant *Saponaria officinalis* L. This protein is capable of stopping the synthesis of all proteins, which in turn results in death of the cells quickly. In addition, the toxin is non-toxic to any other organism other than plants. A late promoter that is active only during the late stage of seed development when the embryo is developing (for example LEA promoter) controls the toxin gene. A piece of DNA called a *blocker* is placed between the late promoter and the toxin gene, which interferes with the ability of the promoter to turn on the gene. The whole setup does not allow the toxin gene from being active until long after the farmers raise their crops.
- *Gene encoding a repressor protein*: A gene coding a repressor protein, under the control of a promoter that is active all the time.

- *Gene encoding a recombinase protein*: A gene coding for a special protein called recombinase, an enzyme that snips out pieces of DNA, controlled by a promoter that is active all the times. This protein is also regulated by the repressor protein, which in turn can be overridden by an inducer chemical such as tetracycline (an antibiotic).
  - *A. Terminator genes in the absence of the inducer*: In the absence of the inducer, the repressor binds to a specific site in the DNA near the promoter of the recombinase gene and the plant cannot produce the recombinase protein. In the absence of the recombinase (which is capable of snipping out the blocker), no toxin is produced. Thus, under these circumstances viable seeds are produced.
  - *B. Terminator genes in the presence of the inducer*: The seed companies treat the seeds with a chemical called 'inducer' just before it is sold to the farmers. The presence of the inducer interferes with the repressor attachment to the binding site, thus allowing the production of the enzyme recombinase. Recombinase, in turn, snips out the blocker and allows the late promoter to turn 'on' the production of the toxin gene late in the season. During seed germination, several enzymes such as amylase are required to break down starch into simple sugars, which are required for the growth of the germinating embryo. In the absence of the protein synthesis, the seeds are not capable of germination, thus resulting in the terminator seeds.

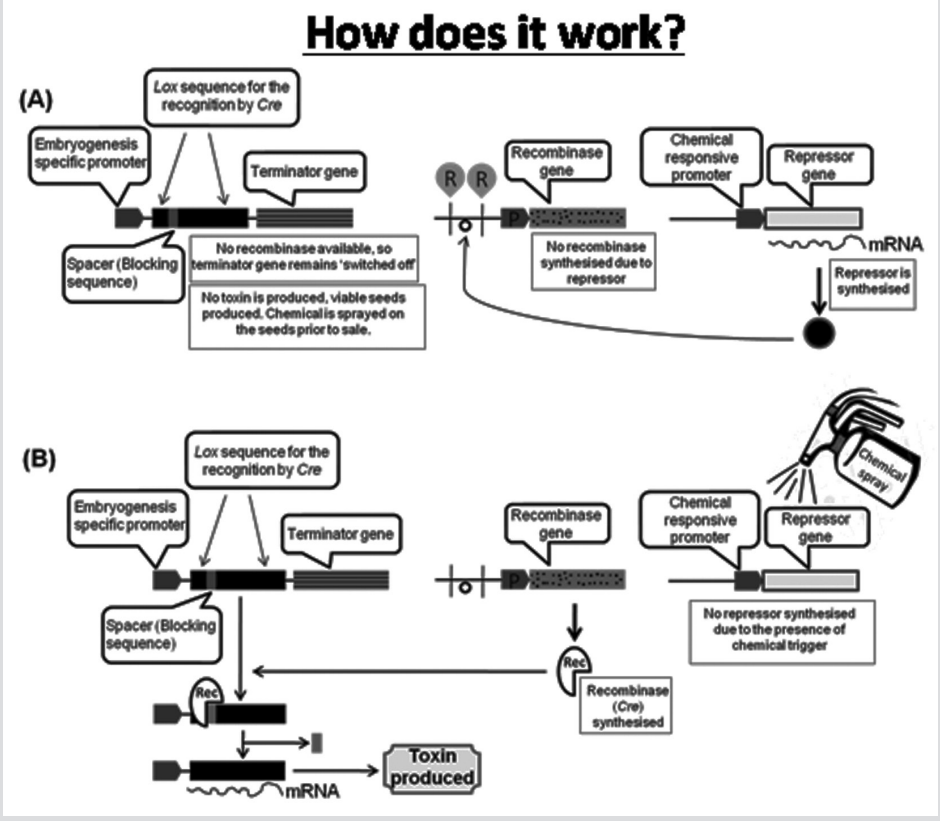


**Figure W2.12.** Terminator technology: How does it work?

Web Resource 3

# Plant Biotechnology is Essential for an Economically Rewarding and Environmentally Sustainable Agriculture

**Professor J. Schell**

Professor Jeff Schell, born in Belgium, 1935, received his early university education in Gent . As Professor and Director of the Laboratory of General Genetics, State University, Gent, he centred his attention on the molecular mechanism of crown gall formation on plants by *Agrobacterium tumefaciens*. His work was instrumental in demonstrating that crown gall formation resulted from the transfer of a specific piece of DNA, the T-DNA from the bacteria to the plant cell. These results helped in the development of vectors for use in the transformation of plants. In 1978, he became the Director of the Max-Planck-Institut für Züchtungsforschung in Cologne and concentrated his interest in gene transfer techniques for crop improvement and as a tool to understand the control of plant growth and development. In 1995, he became the Professor of Plant Molecular Biology at the Collége de France, Paris.

**Figure W3.1.** Professor Jozef S. (Jeff) Schell (20 July 1935–17 April 2003) was a Belgian molecular biologist. Together with his colleague, Marc Van Montagu, he discovered the gene transfer mechanism between *Agrobacterium* and plants.

Jeff Schell was elected member of many academies, for example, the National Academy of Sciences (US), as well as a member of many national and international scientific advisory boards, councils and boards of scientific institutions. He was a member of the EMBO Council and played an active role in organising scientific research programmes within the European Community. Through the years, he received many prizes and distinctions; most recently, he was awarded the 'Sir Hans Krebs Medal' by the Federation of European Biochemical Societies in Barcelona.

In the world of dwindling resources and ever-increasing demands, agriculture is bound to play an ever more important role. Agricultural productivity is essential in the face of increasing world population and limited cultivable land. Plant breeding is one of the few methods available, and one of the most effective, to maintain and improve productivity without destroying the environment. Gene technology is an essential element in modern plant breeding. Examples of transgenic plants resistant to viruses, herbicides, insects and fungi, and applications related to food quality and the production of valuable chemicals are available. Because plant genetic engineering is based on the transfer of defined genes, the risks are predictable and can therefore be controlled. Several thousand tests of transgenic plants have already been carried out in field worldwide without indication of dangers or unpredicted consequences. Therefore, appropriate regulations should be able to ensure both the safe and efficient use of this essential technology.

A new and rapidly developing technology, gene technology, had its beginning in the late 1970s. In the near future, agriculture – whether global or regional, intensive or extensive, industrial or familial – will no longer be optimally productive without an important contribution of new scientific knowledge and without responsible application of the best and most effective technologies. This is particularly true for countries in the developing world. Regulations should therefore primarily focus on making the use of this essential technology safe and efficient.

To evaluate the possibilities of plant biotechnology one should keep in mind the following:

- Agriculture, as it is practised currently, is one of the biggest sources of environmental pollution. Continuation of these practices can lead to rapid and possibly irreversible deterioration of the environment, thus putting the sustainability of agriculture in question.
- Agriculture must be productive in order to be commercially viable, and socially and environmentally acceptable. If agriculture is to remain an attractive occupation, it must be economically rewarding. If one wants to diminish the negative impact of agriculture on the environment, one should optimise productivity, that is, maximum quality and yield for a given input, such that one can reduce input and at the same time conserve or even improve quality and yield.
- Plant breeding is one of the few, and one of the most effective methods, to improve agricultural productivity without simultaneously destroying the environment. This is true for the industrialised world, perhaps even more true for the developing world, and holds for both intensive and extensive agriculture.
- If plant breeding is to contribute to the solution of the enormous problems that we will face in the next decades, then the best techniques must be used, including genetic engineering. The resulting plants should be compared to already available crops for their effect on health and the environment.

In relation to gene technology, in particular, one must remember the following:

- Transgenic plants and microorganisms can help to diminish the negative environmental effects of intensive agriculture.
- Several thousand tests in the field have already been carried out around the world and have given no indication of real dangers or consequences that had not been predicted.
- Transfer of genes occurs in nature (as in the case of *Agrobacterium tumefaciens*).

## State of the Art

The basic underlying fact is that the genetic code is universal. Therefore, it is possible to express any genetic trait of any organism in plants. In practice, this allows us to make plants expressing a defined property of other types of organisms, be it bacteria, yeast or animal cells. This vastly increases the potential for plant breeding.

Two scientific breakthroughs underlie genetic engineering in plants. The first was the development of recombinant DNA technology, which made it possible to isolate individual genes from any organism. The second was in the 1970s, the discovery indicating there are bacteria in the soil that transfer genes into plants, for example, *Agrobacterium tumefaciens.* In nature, this bacterium is a pathogen that causes tumours in plants. It was discovered that these tumours are the consequence of the transfer of a sequence of DNA, called transferred DNA (T-DNA), from the bacterium into the nuclear genome of the plant cell. The transformed plant cells grow as tumours because the T-DNA contains oncogenes. If one removes the oncogenes from the T-DNA, the system is still capable of transferring DNA into the plant cell. Therefore, one can use the T-DNA based gene vectors to introduce foreign genes into plants and obtain the so-called transgenic plants.

If one wants to express a foreign gene in plants, it is usually not effective to transfer the whole DNA sequence, the whole gene. Indeed, although the coding sequence is universal, and it should work in every cellular environment, it was soon found that a coding sequence must be surrounded by plant-specific sequences (promoters) in order to be functional in plants. If, therefore, one wants to express a bacterial gene in plants, one has to make a 'chimeric gene' combining the coding sequence of the bacterial gene with the promoter sequence from a known plant gene. It is possible to predetermine in which organs of the plant a chimeric gene will work by using a promoter from a plant gene that is specifically expressed in the desired organ. In other words, it is now possible to tailor-make genes of any origin so that they function in a predetermined organ of the plant.

The first transgenic plants were developed in 1983. These plants expressed chimeric genes that protected them against toxic chemicals such as antibiotics. They were resistant to kanamycin because they expressed a bacterial gene producing an enzyme (neomycin phosphotransferase), which can detoxify kanamycin. This gene has proven useful in the isolation of transgenic plants. Many plant cells have the remarkable property of 'totipotency'; vegetative tissues in tissue culture can be induced to regenerate fully fertile plants. If such a regeneration, for example, from a leaf-derived tissue, is performed in the presence of a toxic chemical like kanamycin, only cells that can resist kanamycin will be able to produce plantlets. Therefore, transgenic plants can be selected using this method, and any foreign gene can be introduced when linked to such a selectable marker gene. In tobacco, the whole process, from leaf to fertile transgenic plants, takes only about three months.

Thus far, most transgenic plants have been obtained using the natural *Agrobacterium* Ti-plasmid T-DNA vectors. The list of transformed plants is getting longer every day and includes crops such as rice and corn. It can be safely predicted that provided sufficient work is invested, any type of plant, every crop or vegetable can be transformed with one or another of these methods. Concerning tropical crops, with larger regional value, it will not be possible to leave it to the multinational industries to provide the technical know-how. Publicly funded, national and international agencies will have to assume this responsibility.

Historically, the first genes to be developed for molecular plant breeding were herbicide resistance genes. A bacterial enzyme (acetyltransferase) can modify the herbicide phosphinothricin (with the

commercial name 'Basta') into an acetylated form that is no longer toxic. By introducing this bacterial phosphinothricin acetyltransferase into various plants, the resulting transgenic plants were made selectively resistant to the herbicide. Such transgenic tobacco plants were used in the first ever field experiments in 1987 in France.

The ideal modern-day herbicide would be a one that is nontoxic to animals and humans, not harmful to the environment and selectively toxic to weeds as opposed to agriculturally important crops. Bialaphos and phosphinothricin are nontoxic to animals and humans, and easily degrade in the soil so that they disappear after their usefulness in controlling weeds is over. Thus, they approach the above ideal. However, their weakness is that they are not selective, that is, they have not been developed to be used in conjunction with agriculturally important crops and, therefore, they kill the crops as well as the weeds.

Consequently, 'selective' herbicides have now been designed. This was possible because some crop plants have genetically determined mechanisms to detoxify these particular 'selective' herbicides. The important question was whether one can find genes that produce enzymes capable of detoxifying 'non-selective' herbicides.

Let us again take the example of phosphinothricin. Phosphinothricin, an analogue of glutamic acid, is a potent competitive inhibitor of the enzyme glutamine synthase. Its use leads to the accumulation of ammonia in plant cells, which is toxic and kills the weeds but also most crops. The natural product bialaphos is very similar to phosphinothricin (identical with the exception that it is a tripeptide rather than a single amino acid) and is produced by some soil microorganisms. Microorganisms use such products to compete with other microorganisms. The bacterium *Streptomyces hygroscopicus*, which produces this tripeptide, protects itself by detoxifying the product via acetylation. The acetylated product is no longer a competitive inhibitor because it cannot bind to the enzyme. Thus, in order to protect plants specifically against phosphinothricin, the strategy was very straightforward. From the *Streptomyces* strain that produces the detoxifying acetylase enzyme, one can isolate the corresponding gene and then can modify it so that it works in plants. The strategy turned out to be relatively simple and has been done by several groups. The results were as expected. The growth of transgenic plants expressing the transacetylase gene cannot be distinguished, even in the presence of the herbicide, from the control normal plants grown in the absence of the herbicide. Thus, in this case, the goal of breeding by the addition of a single defined gene was achieved, that is, genetic information originating not from another plant but from soil microorganisms was used in order to contribute to an important agricultural goal.

Another transgenic crop that is ready for commercialisation by a US company is insect tolerant cotton. Let us take this example to illustrate pest control by plant biotechnology. The flowers of cotton are attacked by the bollworm (*Heliothis armiigera*) which results in dramatic yield losses. Some bacteria (*Bacillus thuringiensis*) produce peptide toxins, which bind to specific receptors in the intestine of certain insects and are thus specifically toxic to them. By taking the genes that code for these peptide toxins and expressing them in plants, several groups have been able to produce transgenic tobacco, tomatoes, potatoes, cotton, corn, etc.. These transgenic crops express the bacterial toxin in their leaves, flowers or other organs and, thereby, are effectively protected against attack by some insects. Insect larvae feed on these plants, but because of the presence of the toxin, their digestion is severely impaired, and they stop feeding and ultimately die.

The results of various field experiments indicate that a significant measure of additional crop protection can be achieved by these genetic means. The combination of growing plants harbouring different genes, which reduce their sensitivity to pests, with responsible use of environmentally

acceptable pesticides, can be expected to provide great advantages both in making agriculture more efficient and economical and in reducing its negative impact on the environment. Indeed, by combining several different methods to achieve pest control, one will drastically reduce the probability that any of these methods will become obsolete because of adaptation by the pest.

Thus, useful pesticides would not rapidly become inefficient and could be used at lower concentrations, and the pests would not readily overcome the biological control achieved by the products of a number of specific genes. In this respect, it may be of importance to mention that various other strategies, aimed at the genetic containment of deleterious insect populations are also being investigated. The goal would be to combine several different insect controlling properties for the same crop. Hence, one could keep insect populations in check and by appropriate regulations and agronomical procedures, one could prevent the total eradication of certain insect populations. The aim cannot be and does not have to be insect eradication but instead a control over the size of insect populations. It is to be expected that adequate regulations will require an integrated pest management approach and companies involved in the marketing of insect tolerant crops are developing effective integrated pest management schemes. Virus tolerance is another example of successful plant biotechnology.

Fungi produce some of the major diseases in plants. Some, but not all, fungi have a layer of chitin at the surface of their growing tips (at the growing end of hyphae). At the top of the hyphae, the chitin layer is exposed since a covering layer of polyglucans and mannans does not protect it. With the help of a scanning electron microscope, Israelian researchers showed that the growing tip of such fungi could be damaged by chitinases (that is, enzymes that degrade the chitin polymer). By reducing the growth rate of the fungal hyphae in plants, the normal mechanisms by which plants protect themselves against fungal attack could possibly control the fungus. One could hope to reduce the rate of growth of fungal hyphae by expressing a bacterial chitinase gene in plants, and thus allow the plants to protect themselves against these pathogens. This approach was tried in the laboratory of Professor J. Schell in collaboration with a group in Israel (Professor I. Chet) who isolated a chitinase gene from the bacteria capable of stopping the growth of some of the fungi. For trial, a chitinase gene (ChiA) was expressed in tobacco seedlings, and whereas in a control many of the seedlings could not grow in soil heavily infected with spores from the Basidiomycete, *Rhizoctonia solani*, the transgenic seedlings appeared to be largely protected against the pathogen.

The search for other genes producing fungicidal proteins led to the study of the RIP gene from barley. This gene, which is normally expressed in barley seeds, codes for a ribosome inhibiting protein (RIP). The protein inactivates ribosomes by N-glucosidase modification of some of the nucleotides in the 28S subunit RNA of the ribosomes. Interestingly, this ribosome inhibiting protein does not inactivate the ribosomes of plants, but does inactivate the ribosomes of a number of fungi. In barley, the protein is only present in the endosperm of the seeds and apparently protects seeds against fungal attack. The barley RIP gene was cloned by a group at the Carlsberg Laboratory in Denmark. The gene was modified in our laboratory and introduced into tobacco. The RIP transgenic tobacco showed resistance against the attack by *R. solani*. In the next step, RIP and chitinase gene was combined in the same plant, leading to even better protection. Other research groups around the world developed other 'antifungal' genes, and are currently testing the effectiveness of various combinations of these genes.

Genetic engineering has thus far not only produced crops with improved protection against various biotic stresses but also some with improved food quality. The most advanced example of this type of application concerns tomatoes with an improved ripening control. It is well known that ripening in

tomato and other vegetables and also in fruits, such as papaya is controlled by a simple chemical signal, that is, ethylene. The direct precursor of ethylene is aminocyclopropane-1-carboxylic acid (ACC).

There are two ways to slow down the formation of ethylene, which leads to slowing down of the ripening process. One way, which was successfully used by D. Grierson in the UK and developed by I.C.I., is the expression of a so-called 'antisense gene' in transgenic tomatoes. An antisense gene of the ethylene synthase was thus used successfully. Another technology was developed at Calgene, leading to the production and commercialisation of the 'Flavr Savr' tomato.

By slowing down and controlling ripening, it ought to be possible not only to increase the 'shelf life' of some vegetables and fruits but also to improve their taste because taste develops relatively late in development. The FDA agency in the US has proposed rules to regulate the commercial release of a product which will go into the food chain. The rule sensibly stipulates that it is not the way the food product was produced that is of importance. What one has to test are the new properties of the food product.

In the same line of thinking, significant progress was recently made in attempts to use plants for the production, not of food or ornamentals, but of valuable renewable chemical feedstocks. Two of the most important sources are carbohydrates and lipids. Results obtained thus far show that it will be possible to use plants to produce tailor-made starches or lipids with fatty acids with predetermined chain lengths. Work along these lines has progressed considerably not only in the US but also in Europe. What one would like to do, for instance, is to increase the level of starch in tubers of potato from 20 to 23 per cent, for example, by expressing bacterial genes involved in starch biosynthesis. What is also of commercial importance and has been realised is to change the ratio of amylose to amylopectin, to make potatoes producing either only amylose or only amylopectin.

These examples show that present day biotechnology has the potential to improve agricultural productivity (via better disease and pest control) while reducing its negative impact on the environment (for example, by the drastically reduced use of environmentally safer agrochemicals). However, this is only the beginning. The main benefit to be expected from the use of gene biotechnology is a very rapid and indeed dramatic increase in our knowledge of the mechanisms that control plant growth and yield in reaction to various biotic and abiotic (climate, soil composition etc.) stresses. Biotechnology also has the potential to make a number of plants, which presently have no agricultural value, into important 'cash crops'. Indeed, many plants are known to produce highly valuable chemicals (for example, pharmaceuticals). However, the plant genes responsible for the control of the rate of synthesis of these valuable chemicals are usually active at a low level and only under some circumstances. By the technique of 'gene activation' via the random insertion of so-called promoter elements derived from very active plant genes, it is possible to activate such 'lazy' genes and thereby significantly increase the production of valuable chemicals from a variety of plants that presently have no agricultural significance.

## Safety Considerations

The risks of the application of gene technology to plant breeding are relatively small. Most importantly, they are usually predictable and therefore controllable. This is because one is dealing with defined genetic changes and can avoid any risky changes from the start. In addition, the genetically modified plant will often, although not always, be replanted in its original environment.

Genetically modified plants or their seeds should be given to the breeder or farmer only when they have been thoroughly tested under exactly defined conditions, which will vary from case to case.

The design of these tests should be based on the following:

- The nature of the transformed gene.
- The surroundings of the planted field.
- The existence of related plants in the surroundings.

Herbicide-resistant plants should be tested together with non-resistant plants under the same selective pressures. In the case of insect resistance, it is important to measure possible effects on other insects. All field tests must include determination of environmental impact.

In conventional breeding, one crosses plants that are related. As a consequence, two different total genomes are mixed, literally thousands of genes and enormous masses of DNA. Breeders then carefully select new combinations from the offspring that provide the plant with new characteristics, interesting for agriculture, human health and the enjoyment of food. Thus, in conventional breeding the amount of new DNA added can be considerable. The less DNA one adds, but even more importantly, the better one understands the function of the DNA that is being introduced, the better one will be able to predict the consequences. The introduction of a new gene does not necessarily change the basic biological and physiological properties of the crop in which it occurs.

This being said, however, if a metabolic gene is introduced, as in the case of an herbicide resistance gene, one should certainly worry about the metabolic products. For example, in the case of the acetylated herbicide accumulating in a plant, one should worry about that which chemicals are made by the degradation of the acetylated form and what will be the physiological properties of these new products. However, if the function of the introduced genes is not known, as is often the case in conventional breeding, one cannot predict the consequences. What breeders have done is to test new varieties over many years before commercialisation. Thus, it is not correct that the technology per *se* is the danger. It is important to realise that molecular breeding will also be carried out by breeders with all their knowledge and experience, not by molecular biologists in their laboratories. Therefore, the high predictability of recombinant DNA will be combined with a high level of experience. All the experience from conventional breeding is relevant to plants made with recombinant DNA.

## Conclusion

Plant biotechnology can significantly help to make intensive agriculture less damaging to the environment and make low-input (organic) agriculture more productive. It should be possible to improve major crops, for example, rice, wheat, corn, soybean, potato, as well as more regional crops.

If used wisely and in a responsible manner, there is no inherent danger in this new technology. It would be short-sighted and irresponsible not to make optimal use of these methods. They can relieve at least some of the tensions that already exist and which will dramatically increase in the near future because of population growth and the intensive or unproductive use of land for agricultural production. These technologies are also very likely to help increase the economic value of agriculture both in the developed and in the developing world.

Web Resource 4

# Nikolai Ivanovich Vavilov: His Life and Accomplishments

**Monica Manchanda[*], H.Y. Mohan Ram[**] and S.L. Kochhar**

'I would not hesitate to give my life if only for the sake of a small advance in science...'

**NIKOLAI IVANOVICH VAVILOV**

*Speaking at a national conference on combating droughts (1931).*

## Introduction

The man has been on this planet for some two million years and during most of this long history, he has been a hunter of animals and gatherer of plants. Man evolved and increased his knowledge about plants until about 10 000 years ago when he began to cultivate selected plants and thus became an agriculturist. How valuable and exciting it is to know about agricultural beginnings! We now have a fair idea about how, where and when agriculture originated. In this chapter, we will make an endeavour to collate information about Nikolai Ivanovich Vavilov, an outstanding Russian geneticist and crop geographer, well-known for his ideas on centres of plant diversity and crop domestication. Vavilov's life and his achievements are an inspiration and challenge to generations of scientists and students alike, who are engaged in the field of genetic conservation and breeding. His name and legacy will live forever in the history of crop improvement. He will long be remembered as one of the world's outstanding contributors to scientific thoughts in genetics, plant breeding, systematics and evolution.

## The Life and Work of N.I. Vavilov

N.I. Vavilov was born in Moscow on 25 November 1887. He was the first child of a very wealthy merchant who later became the director of a famous textile company. Vavilov's wealthy parents provided

[*] A University gold medallist in BSc Honours Botany (1991), she did her post-graduation in Botany from Sri Guru Tegh Bahadur Khalsa College, a constituent college of the University of Delhi.

[**] Formerly Professor and Head, Department of Botany, University of Delhi.

substantial facilities for the education of their children, two sons and two daughters. They built up a large library of books on botany, physics, archaeology, chemistry, history and geography. The family also took an interest in the arts, the sisters were music lovers and attended sittings of the philharmonic society, while the brothers attended concerts, were regular visitors to museums and collected reproductions of famous paintings. The family gave USSR two Academicians (both the brothers became President of the Academies), and one of the daughters became a bacteriologist and the other a microbiologist.

However, their father Ivan Ilyich was a man of short temper and often flogged his sons for their mischief. Sergei (who later became a physicist) would endure his punishment without flinching, but Nikolai behaved differently. In fact, once when his father was about to flog him, Nikolai then only 13 years old, clambered onto a windowsill and shouted, 'Stay where you are or otherwise I will jump out'. The episode worked, and, indeed, reflects his intelligence.

By all accounts, Nikolai matured into a kind gentleman–a man of immense talents driven by his passion for science. He was endowed with astounding energy and was unbelievably efficient. Vavilov's memory was legendary; he could recite books by Pushkin, word for word, from memory. He knew English, German, Latin, French, Spanish, Persian and Turkish. He found a little time for rest.

## SCHOOLING AND HIGHER EDUCATION

Although a business person, Ivan Ilyich enrolled Nikolai in a gymnasium (a Continental School) rather than a commercial school. The curriculum laid stress on modern European languages and natural sciences, and distinguished scholars taught physics, chemistry and mathematics. Regional ethnographic excursions were often arranged to train students in the techniques of research and experimentation. Frequently, students were taken on trips to Moscow Polytechnic Museum to encourage them to attend lectures and establish contacts with famous scientists of those times. All this made a great impression on young Nikolai and fostered his passion for natural sciences.

In 1905, Nikolai joined the Petrovsko-Razumouskoe Academy in Moscow and chose to specialise in biology and agriculture. At the Department of Zoology and Entomology, he did his first independent research on 'a study of snails, the pests of winter crops', and won the 'Polytechnical Museum Prize' for the same. In 1908, Nikolai joined the student expedition to the Caucasus and in 1909, at the age of 22, he wrote his first scientific report entitled 'Darwinism and experimental morphology'. He graduated from the Moscow Agricultural Institute (now the Timiryazev Agricultural Academy) in 1910 and started his postgraduate research at the Department of Crops headed by Dmitry Nikolayevich Pryanishnikov. He led research expeditions to Northern Caucasus and Transcaucasia, collected plants and studied ancient farming techniques. Vavilov kept contacts with Dionissii Leopoldovich Rudzinsky, founder of the selection and breeding work in Russia. In 1911, he joined the Bureau of Applied Botany at St. Petersburg (known in the communist era as Leningrad) and worked under the guidance of R.E. Regel and A.A. Yachevsky–two prominent scientists.

During 1913–14, Nikolai worked in the best laboratories of Great Britain, one headed by W. Bateson (who had an unflinching interest in conserving the diversity of perennial fruit crops), one of the founders of genetics, and then in France under the foremost seed specialist, Roger P.V. de Vilmorin; later he went to Germany. In England, he worked at several research centres with Dr John Innes, Sir John Percival and Sir Rowland Biffen (Figures W4.1, W4.2 and W4.3).

In 1914, at the start of World War I, the young Vavilov returned to his country and married Elena Ivanovna Barulina–a research scientist at the All-Union Institute of Plant Industry. They had two sons, Oleg and Yuri–both became physicists. Yuri is still alive.

**Figure W4.1.** Nikolai Ivanovich Vavilov holding herbarium sheet of different wheats.

**Figure W4.2.** Nikolai Ivanovich Vavilov with William Bateson, an English biologist who coined the name 'the science of genetics' and later he became the first Director of John Innes Horticultural Institution, England.

**Figure W4.3.** Nikolai Ivanovich Vavilov at his work place.

## Expeditions and Missions

Vavilov organised scientific expeditions to more than 50 countries, visiting all continents except Antarctica and Australia, and explored the major agricultural regions of the world. In 1916, he undertook his first expedition to Persia* (now Iran) and also visited Turkestan and Pamir where he discovered a great many varieties of soft wheat, barley and flax, previously unrecorded anywhere else. While there, he faced a number of difficulties, such as was abandoned by his Kirghiz caravan, attacked by footpads, mobbed, deserted by his guides and charged as a German spy.

The most productive years of Vavilov's life were between 1920 and 1936, during which he organised worldwide exploration studies to examine the plant resources of the Mediterranean area (Greece, Italy, Portugal, Spain, Algeria, Tunisia, Morocco, Egypt, Palestine, Syria and Transjordan), Ethiopia, Afghanistan, Japan, Western China, Korea and the countries of North, Central and South America. In fact, he was involved personally in directing many of the expeditions. Table W4.1 lists the numerous expeditions and field assignments (along with specific countries) undertaken by Vavilov.

**Table W4.1.** Expeditions and Missions

| | |
|---|---|
| 1916 | Central Asia: Persia (now known as Iran) and Pamir |
| 1923–24 | Afghanistan, Bukhara and Khorzm in Central Asia |
| 1926 | Mediterranean area: Syria, Palestine, Jordan, Cyprus, Tunisia, Algeria, Morocco, Crete, Greece and Italy |
| 1927 | Italy, Ethiopia and Erithrea |
| 1929 | Western China, Japan and Taiwan |
| 1930 | Mexico and Central America |
| 1933 | Peru, Chile, Argentina, Uruguay, Trinidad and Cuba |

* He was commissioned in the military service on 26 February 1916 but was directed by the Ministry of Agriculture to undertake an expedition to Persia (now Iran). Meanwhile, the Russian regiments had invaded the northern parts of Persia where the Russian soldiers had begun to develop a peculiar disease that 'intoxicated' them and, as a result, they committed acts of folly. The military authorities requisitioned the services of an experienced agricultural specialist and for this purpose, the Ministry of Agriculture chose Vavilov. After detailed investigations, Vavilov's simple recommendation was to supply bakeries with flour brought from Russia, rather than using local flour infested with poisonous bearded darnel. After this 'unusual assignment', Vavilov did not return home immediately but equipped a tiny caravan that contained only three horses. He set off hunting the cherished wheats along dusty roads skirting the weedy and squalid fields. One horse carried Vavilov, the other the interpreter and the third the packs. He had no guards. The military authorities became suspicious of Vavilov's movements. He was detained and frisked; all his efforts to establish his identity were of no avail. The bellicose Cossacks ransacked his collections, mixing up all the wheat spikes he had collected. They put him in a wattle and daub shack, which was as hot as an oven. In the meantime, the soldiers sent a message asking their Chief whether the most dangerous criminal was to be deported to Russia or shot dead on the spot. Fortunately, at the intervention of the top officials, he was released along with his collections that were muddled up to such an extent that it took him a long time to sort them out.

In addition to the expeditions mentioned in Table W4.1, Vavilov travelled widely in the United States, in most of the countries of Western Europe and attended many international scientific conferences.

In 1924, Vavilov carried out comprehensive studies in Afghanistan, visiting almost inaccessible and little investigated western parts of Kafirstan (present day Nuristan). The results of this expedition are summarised in *Agricultural Afghanistan* (1926), covering elements of geography, ethnology, etc. The expedition of Ethiopia (earlier known as Abyssinia) in 1926–27 was of particular interest as Vavilov found many hard wheats that had originated there.

The places Vavilov was unable to cover and investigate were Australia (original home for *Eucalyptus* and macadamia nut), North America[*] (centre of origin for sunflower, *Helianthus annuus* L. and Jerusalem artichoke, *H. tuberosus* L.) and West Africa (original home for sorghum, yams and African rice). It is a great pity that he never visited the Indian subcontinent.

His expeditions brought him encounter with malaria in Syria, typhus and bandits in Ethiopia, a landslide in the Caucasus mountains and a plane crash followed by a sleepless night next to a lion's den in the Sahara. These travels also brought him in contact with crop diversity that no one else had ever seen before.

## STAY AT SERATOV

At the age of 30, in 1917, Vavilov was elected Professor and became Head of the Department of Genetics, Selection and Applied Agriculture at Seratov University on the Volga River. He also taught genetics and plant breeding at Woronezh at the same time. He remained in Saratov for only a few years and was then invited to Leningrad (now St. Petersburg) to head the old Botanical Institute,[**]

[*] Little is known of the expeditions to North America but in Vavilov's travel memoirs, there is an outline of a projected discussion about it. One of the entries reads, 'As Guest of the President of the University of Arizona'. On further scrutiny of the records, it was discovered that Dr H.C. Shantz has recounted an episode relating to Vavilov in his special memoirs The episode reflects the vision that Vavilov had for the people of Russia. According to Dr Shantz, on their way to Novajo and Hopi areas on l1 October 1930, they learnt that there was a telegram for Vavilov. The gist of the message was, 'There will be a State Dinner in Washington on Friday, October l7th, at which the Secretary of State and many leading American officials will be present; it is imperative that you be there. About 10 days later, a similar dinner will be given in London at which the Prime Minister and other prominent statesmen will be in attendance; it is essential that you attend.' Vavilov had to proceed to California, then to Mexico and Central America. He feared that if he accepted the social invitation there would be an interruption of his search for new plants.

Shantz writes further in his memoirs, 'Vavilov pointed out, "It is more important for the future of the people of the USSR that I visit the centres of origin of cultivated plants in Central America than attend any State Dinner."

[**] The Institute later became the Bureau of Applied Botany. The bureau was reorganised in 1924, into All-Union Institute of Applied Botany and New Crops and Vavilov became its Director. He carried out the gigantic task of organising a network of Agricultural Institutes in the USSR. At his disposal were about 400 research stations scattered all over the Soviet Union, staffed with 20 000 professional and sub-professional workers. In no other country and at no other time has an agronomist had such facilities at his disposal. In 1930, the Institute was renamed the All-Union Institute of Plant Industry (VIR). The Institute was again reorganised and now bears the name of N.I. Vavilov Institute of Plant Industry. The Institute was awarded the Order of Lenin in 1967 and the Order of Friendship of the peoples for its scientific achievements in 1975.

founded in 1894.

During this period, he published his work *Field Crops of South-East Territories*, followed by a large monograph, *Plant Immunity to Infectious Diseases* and more importantly, delineated the Law of the Homologous Series in Genetic Variability.* This law created quite a sensation and Vavilov had a real 'break' in his career, winning him a name and position internationally within the scientific world. As a result, Vavilov was often invited to participate in scientific congresses and meetings within the Soviet Union, as well as abroad. He was a forceful speaker and presented his ideas eloquently.

### CENTRES OF ORIGIN OF CULTIVATED PLANTS

Under Vavilov's leadership, a world collection of over 30 000 specimens of cultivated plants and their wild relatives was created and stored in the All-Union Institute of Plant Breeding in Leningrad. The voluminous mass of material assembled at the Institute enabled Vavilov to formulate his theory of centres of origin of cultivated plants which was published in 1926, in a book titled *The Centres of Origin of Cultivated Plants* and is now recognised as a classic. Its publication was a major event in the international scientific circle. It won him worldwide recognition and acclaim.** In formulating his ideas, Vavilov was probably much influenced by his stay at the then John Innes Horticultural Institution (UK) and 'Age and Area' hypothesis of Willis (1922). Vavilov made a profound contribution towards our understanding of agricultural origins and geographical distribution of genetic diversity in crop plants. For this work, he became one of the first recipients of the Lenin Prize, in 1926.

## Chronology of Vavilov's positions, achievements and honours

1905 Received the Polytechnical Museum Prize for his first independent research on 'Snails: the pests of winter crops'.
1909 Published his first scientific report on 'Darwinism and experimental morphology'.
1917 Elected Professor and Head of the Department of Genetics, Selection and Applied Agriculture at Seratov. Simultaneously he held the professorship at the Agricultural Institute in Woronezh, Central Russia; published a monograph entitled *Plant Immunity to Infectious Diseases*.
1919 Published his book *Field Crops of South-East Territories*.
1920 Enumerated 'The Law of Homologous Series'.
1920 At the age of 33, he was appointed Director of the All-Union Institute of Plant Industry in Leningrad–a position held by Robert Edward Regal until his death.
1925 Awarded the N.M. Przhevalsky Gold Medal for his expedition to Afghanistan (1923–24) by the Russian Geographic Society.
1926 Decorated with V.I. Lenin Prize for his work on the origin of cultivated plants.

* This law is generally believed to be as important as 'Mendeleyev's Periodic Law of Atomic Weights'. The importance of Vavilov's law is that it has a predictive value in that 'if one crop plant is found with a particular trait, then the same trait is likely to be found in unrelated species from the same geographical area'.

** Some of his generalisations have been questioned in recent times but the principal thesis of Vavilov were known and appreciated the world over, with the possible exception of his native land. A prophet is seldom respected in his own country–this was particularly true in the case of Vavilov as in the Soviet Union, his genius inspired jealousy and his integrity invited liquidation.

## MEMBERSHIP TO LEARNED SOCIETIES

In 1923, Vavilov was elected as a corresponding member of the Soviet Academy of Sciences and six years later, he was made a full member, the youngest ever to hold that rank. From 1926–35, Vavilov was elected member of the Central Executive Committee of the USSR and was also appointed a member for two years (1927–29) of a supreme organ of the Soviet Government. On 29 June 1929, he became the first President of the Lenin All-Union Academy of Agricultural Sciences (1929–35).[*] He was elected President of the All-Union Geographic Society of the Soviet Union in 1931 and subsequently held the position of Vice President of the Society (1935–40). In 1932, Vavilov attended the Sixth International Congress on Genetics in the US and was given a reception of honour as one of the world's most eminent plant scientists. He was elected President of the Seventh International Congress of Genetics to be held in 1939 at Edinburgh, Scotland–a rare honour for any geneticist. However, he could not attend the Congress because of political compulsions at home.

He was also chosen a member of the British Society of Horticulturists, the British Association of Biologists, the Academy of the Sciences at Halle (Germany), the Czechoslovakian Academy of Agricultural Sciences and numerous other national and international scientific organisations.

During a relatively short life (1887–1943) of Vavilov, he accomplished a lot. In addition to his expeditions, he travelled widely all over the world, formulated very important postulates in crop evolution, wrote over ten books (some of them published posthumously)[**] and published about 350 scientific papers. Two of his books, for example, *Geographical Regularities in the Distribution of Genes of Cultivated Plants* and *The Teaching of the Origin of Cultivated Plants,* after Darwin, are still classics.

## LYSENKO'S PERIOD

During a Congress of Genetics, Plant and Animal Breeding held in Leningrad in 1929, under the Presidentship of Vavilov, T.D. Lysenko presented a paper on the physiology of cereals. He was hailed as the discoverer of vernalisation (in fact, the phenomenon had been discovered in the US years before Lysenko gave it a name). He claimed, or it was claimed by others later, that vernalisation heralded a new era in the Soviet agriculture. Vavilov welcomed Lysenko's debut but did not comprehend on time how dangerous he could be to him and Soviet genetics. Vavilov urged scientists to test Lysenko's idea, which had been applauded by Stalin and certainly, Lysenko proved himself a master craftsman in the art of modern publicity.

The years between 1930 and 1940 were politically quite difficult for scientists working at the Institute since it was firmly based on Darwin's theories and Mendelian genetics. Vavilov was publicly accused of (a). having slowed down the development of Soviet agriculture, (b). sending worthless expeditions to gather material for his collections instead of concentrating on local varieties and (c). not being an ardent supporter of the theories of Michurian and Lysenko. Lysenko's theory[***], appeared

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[*] A.A. Niconov was the last President of the Academy before it was dissolved early in 1992.

[**] Vavilov systematically collected material for his principal work, entitled *Five continents: a scientific survey of his travels and explorations.* It was to be published in two comprehensive volumes. Some sections of these manuscripts were published in contemporary journals. After Vavilov's death, fortunately, his typist A.S. Mishina was able to retrieve or salvage portions of the major manuscript, which was published in 1962, as a booklet.

[***] Lysenko believed in the inheritance of acquired characteristics. He believed and claimed to have demonstrated that spring wheat varieties could be coaxed into becoming winter wheats and *vice versa* simply by exposing the seeds to soaking in water of different temperatures. Lysenko and his followers

to Stalin to be consistent with his views of historical materialism since it promised quick gains for Soviet agriculture. With the backing of Stalin, Lysenko's influence grew, but in spite of official support, opposition to Lysenko by Soviet scientists existed and survived.

Vavilov's frequent travels abroad aroused suspicion, and therefore, the Soviet Government suppressed scientific plant expeditions to other countries. As a consequence, he never left the Soviet Union after the spring of 1933. In 1935, Vavilov was removed from the post of President of the Lenin All-Union Academy of Agricultural Sciences, and two years later Lysenko became the President through political manipulation.

Charged for his espionage activities and efforts to harm agriculture (sabotaging Soviet agriculture*), Vavilov was arrested on 6 August 1940. He was subjected to interrogations lasting 1700 hours over a period of eleven months. He was imprisoned in Moscow and later shifted to a concentration camp in Seratov where he had begun his teaching career at the University. On 26 January 1943, he died in a prison hospital, at the age of 56, of starvation as uncovered later in the 1960s by the autopsy records. He frequently, even prophetically, said, 'Life is so short and there is so much to do', this came true, as he became a victim of Lysenkoism and ultimately a martyr to the cause of genetics .

Vavilov's death was a tragedy for the world of science and made even darker by Lysenko's ominous elevation to power by Stalin. However, Stalin's bizarre mix-up of Marxism with science unexpectedly resulted in the creation of a strong united force to free geneticists from political influence and restore Vavilov's name. By 1955, the political tide had changed, largely due to the efforts of Vavilov's Academician brother (who was then President of the Academy of Sciences), who eventually was able to help correct the wrongs. Vavilov was absolved of all 'crimes' and his name was cleared.

Lysenko's influence waned after Stalin's death in 1953 (although he retained his post as Director of Institute of Genetics until 1965). In 1958, the USSR Academy of Sciences appointed a committee to consider the physical and chemical aspects of heredity. The school of socialist biology was denounced, and Lysenkoism was declared dead for its failure to substantiate many of its claims. The reinstatement of genetics was not complete until Nikita Khrushchev's political departure in 1964. Lysenko was stripped of his position at a meeting of the USSR Academy of Sciences on 26 January 1965. After that, 80 000 biology teachers had to be reinstated and all biology books rewritten. The great leaders of Soviet dissent, Sakharov, Dubinin, Solzhenitsyn and the Medvedev brothers forced Brezhnev to allow the partial rehabilitation of Vavilov.

## Commemoration of Vavilov's Birth Centenary

To commemorate the 100th anniversary of Vavilov's birth in 1987, the USSR Academy of Sciences and the Lenin All-Union Academy of Agricultural Sciences organised a grand conference. Over 2000

were agitating to remove the study of genetics from the Universities. A wholesale dismissal of scientists and closure or complete reorganisation of research laboratories quickly followed.

* Despite the fact that Vavilov had fallen out of favour, many scientists at the Institute treasured his germplasm collection. During the seize of Leningrad, during the Second World War, all scientists but for 31 were evacuated, of which 14 reportedly died of starvation in December 1942, rather than touching the bags of grains in the gene bank. They were all students of Vavilov, who had taught them the value of genetic resources. What a true and dedicated love for their mentor! How many of us are ready to die such great death? Among the deceased were the scientist incharge of the collections and Vavilov's secretary, Mrs Brissenden from Los Angeles, who had left the US to work with Vavilov.

Soviet geneticists and breeders, as well as delegates from 40 countries, attended the conference to pay homage to a man who is aptly considered as the father of plant genetic resources conservation. On this occasion, a book in Russian titled *N.I. Vavilov–Origin and Geography of Cultivated Plants* (its English translation was brought out in 1992) was published, a medal was coined, a badge was made and a postage stamp was released in his honour. In honour of his centennial birthday anniversary, UNESCO passed a resolution proclaiming the year 1987 as the N.I. Vavilov Year. To a scholar of genetic conservation, a visit to the Vavilov Institute in St. Petersburg is a pilgrimage. A bust of Vavilov stands majestically at the top of a staircase, where at the base staff members place the flowers daily. The history of the place where Vavilov developed his theory of 'centres of origin of agricultural crops' and 'the law of homologous series' is still very much alive. The gene pool assembled at VIR has no parallels anywhere in the world. Vavilov's great contribution is remembered today in simple words of plaque at VIR, "Nikolai Ivanovich Vavilov, a prominent biologist worked here".

The exploration and conservation of plant genetic estate have assumed greater importance than when Vavilov initiated this movement over 85 years ago. Vavilov's name will be remembered forever and ever. His 'centres of origin' harbour tremendous diversity that can be used to incorporate genes into our existing crop varieties to improve yield, nutritional value, etc.

## The Importance of Vavilovian Centres of Diversity

The value of crop diversity to the breeders is immense, but no two plant breeders have completely identical objectives. Yet, despite differences of emphasis here and there, depending on the crop and the climatic, cultural and economic differences of the country where it is grown, there are underlying similarities in all breeding programmes. The basic objectives of most crop plant breeders are (a). high yields, (b). high quality, (c). high nutritional levels, (d). stability of yield (in spite of minor climatic variability), (e). pest and disease resistance and (f). stress tolerance.

To achieve these goals, genetic diversity must be made available for incorporating useable genes into existing crops. Unfortunately, much of the original genetic diversity found in the Vavilovian centre is fast disappearing and our genetic pool is gradually narrowing down as a result of man's unrestrained activities, such as overgrazing, indiscriminate lumbering, agricultural operations and burning. The man has been a major factor in altering habitats and their associations. Even the 'green revolution' which had ushered in an era of *Vanishing Yield Barriers* has resulted in significant losses in domesticated and wild biological resources, such as traditional varieties, primitive varieties or landraces owing to the practice of monoculturing. The unprecedented rate of deforestation is eliminating wild species, parental strains and many other potentially economically valuable plants that could be used in the future. It is also exterminating a number of plant species that contain hydrocarbons rather than carbohydrates in their tissues and hence could serve as a productive source of bioenergy. Considering the speed at which the world's plant gene pool is being eroded, we can predict that nearly all the remaining tropical forests that harbour tremendous biodiversity will be destroyed within a few decades. This poses a serious question that can we afford to squander our plant genetic resources. The answer is decidedly *'no'. The greatest need at present is to salvage whatever is left of our valuable genetic resources. It is recognised that the erosion of genetic resources severely threatens world food security and there is an urgent need to conserve and utilise plant genetic resources as a safeguard against an unpredictable future.* Protection of habitat is the single most effective means of conserving biodiversity – a basic requirement for sustainable development – passing on to future generations a world of undiminished options. If we fail in our efforts to respond to this call, we could justifiably be condemned by posterity for squandering

our heritage, which also belongs to future generations. Moreover, the exploration activities must therefore be pursued vigorously to collect the multitude of forms now in existence and to preserve them in germplasm banks, of course after a thorough evaluation. Plant exploration studies should be given a place of prominence in our teaching programmes at college and university levels, and then by harnessing the talents of students so trained we can educate people in the remote areas about the importance of conserving biodiversity (the ecosystems, the plant and animal species and all other microorganisms that support our life). Restoring nature where people live–reestablishing a personal link with the living world where it matters, is necessary if we are to save our planet from collapsing.

## A few of the important plants in each Vavilovian centres are listed as follows (Refer to chapter 1)

- *Chinese centre*: This is the earliest and largest independent centre for the origin of cultivated plants. It includes the mountainous regions of central and western China and the adjacent lowlands. A total of 136 endemic plants are listed, among which are a few important crops, such as millet, buckwheat, soya bean, many legumes, bamboo, crucifers, onion, lettuce, eggplant, cucurbits, pear, cherry, quince, citrus, persimmon, sugarcane, cinnamon and tea.
- *Indian centre*: The two sub-centres under the Indian centre are as follows:
  - *The main centre*: It includes Assam and Burma (now called Myanmar). 117 plants are considered to be endemic, including rice, sugarcane, many legumes, mango, orange and tangerine, jute, coconut palm, oriental cotton, black pepper, cinnamon tree, eggplant, yam, etc.
  - *The Indo-Malayan centre*: It includes Indo-China and the Malay Archipelago. 55 plants are listed, including banana, coconut, sugarcane, clove, nutmeg, black pepper, Manila hemp, mangosteen, etc.
- *Central Asiatic centre*: This region includes North-West India (Punjab, Northwest Frontier Provinces and Kashmir), Afghanistan, Tajikistan, Uzbekistan (erstwhile USSR) and western Tian Shan (China). 43 plants are listed, prominent among which are common wheat, pea, beans, lentil, hemp, cotton, carrot, garlic, spinach, pistachio, apricot, almond, apple and pear.
- *Near eastern centre*: The region includes the interior of Asia Minor, all of Transcaucasia, Iran and the highlands of Turkmenistan (USSR). 83 species are included in this region. At least nine species of wheat as well as rye are indigenous to this centre. Many of the subtropical and temperate fruits (cherry, pomegranate, walnut, quince, almond and fig) and several forage crops, such as alfalfa, Persian clover and vetch are also native to this region.
- *Mediterranean centre*: This region includes the borders of the Mediterranean Sea. 84 plants are known to have originated here, including olives and many cultivated vegetables (garden beet, cabbage, turnips, rhubarb, asparagus); forage plants (Egyptian clover, white clover, crimson clover); oil-yielding plants (rape, black mustard); wheats (durum and emmer) and ethereal oil and spice plants (caraway, anise, thyme, peppermint, sage, hops).
- *Abyssinian centre*: Comprises Abyssinia (now Ethiopia), Eritrea and parts of Somalia. 38 species are native to this region. Wheat and barley are especially rich in diversity and others include sesame, castor bean, coffee and okra.
- *South-Mexican and Central American centre*: This centre includes the southern parts of Mexico, Guatemala, Honduras and Costa Rica. Plants native to this region are extremely

varied and include maize, bean, squash, chayote, sweet potato, red pepper, upland cotton, sisal, papaya, guava, cacao and tobacco.

- *South American centre*: The three following sub-centres are included under this centre:
  - *The Peruvian-Ecuadorean-Bolivian centre*: Mainly consist of high mountainous areas representing the centre of pre-Inca civilisation. Plants native to the Puna and Sierra uplands are also included. This centre is known to be the original home of many potato species, tomato, lima bean, pumpkins, red pepper, coca, Egyptian cotton, quinine tree and tobacco.
  - *The Chiloe centre*: An island near the coast of southern Chile is thought to be the region of origin of the common potato.
  - *The Brazilian-Paraguayan centre*: It is believed to be the region of origin of groundnut, cassava, pineapple, rubber tree and cashew nut.

## Vavilovian Centres of Diversity: A Critical Appraisal

Although the general idea of the Vavilovian concept of centres of diversity remains unchanged, some of the generalisations have been questioned recently in the light of new evidence. Kuckuck (1962), however, concluded that Vavilov would certainly have introduced changes to his theories in view of our increased knowledge of the origins and evolution of cultivated plants. In fact, Vavilov did work on his concept of gene centres, modifying it until his death in 1943, at the age of 56. He began with five centres in 1926 but raised them to eight with three sub-centres (Vavilov, 1935, 1951). Later, authors such as Darlington and Janaki Animal (1945) increased the number of centres to 12. In 1956, Darlington brought it to 15 and subsequently in 1973 to 16, while Zhukovsky (1968, 1975), one of Vavilov's colleagues, proposed a series of 12 'macrocentres' or 'megagene' centres, covering almost the whole world. The thought-provoking alternative schemes of critics of the Vavilovian concept of centres of diversity, such as that of Harlan and Hawkes are included in the text to give a clear idea of the gene centre theory (refer to chapter 1). However, before doing so, it is important that we critically look at the situation that exists today. A crop-by-crop analysis reveals that the study of the origins and evolution of cultivated plants is much more complex and intricate than it was formerly conceived by Vavilov.

1. Vavilov's contention that a region with the greatest genetic diversity is also the centre of origin is no longer tenable (Schiemann, 1939; Gökgöl, 1941; Harlan, 1951, 1969, 1970; Kuckuck, 1962; Smith, 1969; Brücher, 1969; Zohary, 1970). Maize and tomato would serve as two classical examples of crops in which variability may present misleading information on the place of domestication. The centre of diversity of maize is in Peru, yet archaeological evidence and distribution of wild relatives indicate conclusively that maize was domesticated in Mexico (Mangelsdorf, 1974). Similarly, in tomato the centre of diversity (Mexico) and the distribution of related wild species (western South America) do not coincide. Here again archaeological, historical and linguistic data tend to suggest Mexico as the centre of domestication (Jenkins, 1948).
2. Vavilov postulated that a primary centre is marked by a high frequency of dominant alleles towards the centre and recessive genes towards the periphery is not tenable. Gökgöl (1941) pointed out that it was impossible to pinpoint such a centre for wheat. Brieger (1961) did

not find one for maize, nor Zeven (1967, 1972) for oil palm (*Elaeis guineensis* Jacq.) and neither Hanelt (1972) for *Vicia faba* L..

3. Vavilov's claim that the centres of development and diversity are limited exclusively to the mountainous regions is no longer certain. Brieger (cf. Kuckuck, 1963) emphasised that maize exhibits the same, or even greater, diversity in the plains as in the highlands.
4. Some crops, such as bottle gourd, *Lagenaria siceraria* (Molina) Standl., may even have no centre of diversity, but were probably domesticated repeatedly throughout the range of their wild ancestors (Harlan, 1976). Sorghum is another example of a crop for which neither a centre of diversity nor a centre of origin is evident from the distribution of variation alone.
5. Many crops either did not originate in the Vavilovian centres at all or originated in more than one centre. Balsam apple (*Momordica balsamina* L.), Bitter gourd (*Momordica charantia* L.), Aramina fibre (*Urena lobata* L.) and Olona (*Touchardia latifolia* Gaud.) are a few of the examples that could not be listed in one of the gene centres. In oats, there is no single centre of diversity since they must have spread with other cereals as weeds of cultivation to many different regions and with several different crops.
6. Some crops seem to have evolved all over the geographical range of the species or to have been domesticated over a vast area. Examples are sorghum, common bean, banana and *Brassica campestris* L.
7. Several crops can be traced to very limited and specific origins, and did not spread appreciably. Examples are *Ensete ventricosa* (Welw.) Cheesm. in Ethiopia; *Digitaria iburua* Stapf. in West Africa; *Setaria geniculata* (Lam.) Beauv. in ancient Mexico and *Panicum sonorum* Beal in modern Mexico. In these cases, the centre of origin and the centre of such diversity as exists would coincide.
8. In some crops, centre of domestication cannot be determined precisely for lack of suitable evidence.

In the attempt to meet the criticism that many crops originated outside Vavilov's centres of origin, Zhukovsky (1968) put forward his idea about 'megagene centres' where species were domesticated. These enlarged centres cover much of the world's land surface, leaving out only Canada, Brazil, southern Argentina, northern Siberia and small nations, such as Norway and Britain (Figure W4.4). He divided the world into regions and implied that there were no centres. The concept hardly seemed to agree with the concept of 'centres' at all. In addition, he gave 'microgene centres' of wild species genetically related to cultivated plants. The megagene centres, as shown in Figure W4.4, are as follows:

1. China
2. Indochina-Indonesia
3. Australia-New Zealand
4. Indian subcontinent
5. Central Asia
6. West Asia
7. Mediterranean coastal and adjacent regions
8. Africa
9. Europe-Siberia
10. Central America
11. Bolivia-Peru-Chile
12. North America

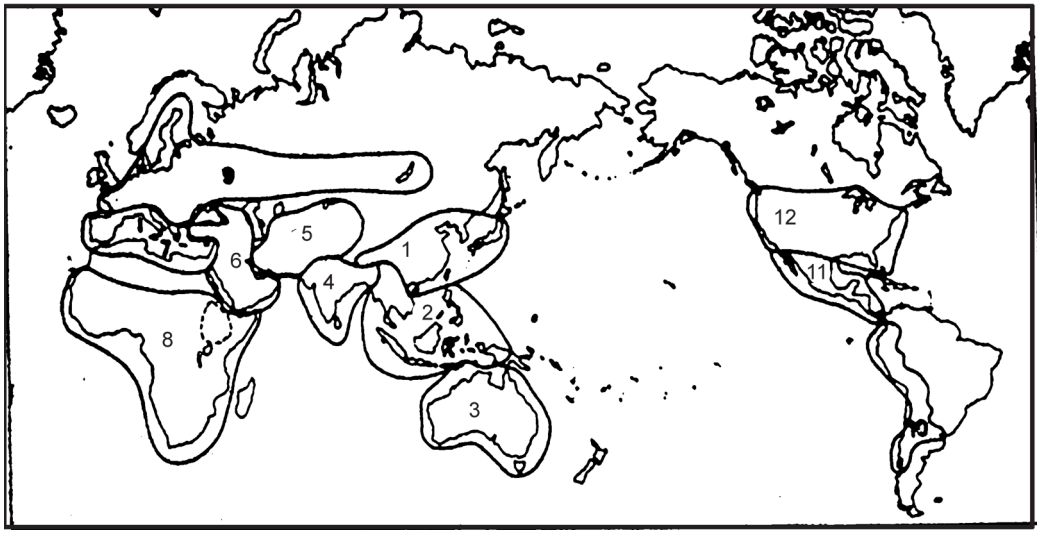


**Figure W4.4.** Megagene centres of cultivated plants, according to Zhukovsky (1968).

Web Resource 5

# Plant Introduction Activities: An Indian Perspective

Plant introduction has been defined as the orderly transfer of a cultivated species or variety to a new habitat, following the usual procedures of quarantine, evaluation, multiplication and distribution. If well organised, plant introduction is one of the most powerful tools for agricultural improvement in developing countries. Biologically, it is the adaptation of plant material to the new environment. In fact, some of the exotics have acclimatised in certain regions so well that they proved superior in yield to their local counterparts.

According to Allard (1960), plant introduction refers to acquisition of superior varieties by importing them from other areas, and it may be classified into two following categories:

- *Primary introduction*: It refers to the direct use of the introduced variety or plants, without any alteration in the original genotype, for commercial cultivation into a new environment. For example, the Australian wheat variety *Ridley* is now grown on several thousands of hectares in the hills of Uttarakhand and Himachal Pradesh. The variety was found to be resistant to brown and black rusts, moderately resistant to loose smut, and with good yield and grain quality. The Australian oat *Kent* has become popular in Punjab, Haryana, Delhi and the *Terai* regions of Uttarakhand. Its foliage provides green fodder while the grains can be processed as white oat by the breakfast industry. The US peas *Bonneville* and *Early Badger* have spread over the northern plains of India; *Sioux* variety of tomato – a high yielding American variety is suitable for growing in hilly regions of India. Among the recent primary introductions in India are the Mexican dwarf or semi-dwarf wheat varieties (*T. aestivum*), Sonora 64 and Lerma Rojo, and semi-dwarf rice varieties Taichung Native 1 from Taiwan and IR8 and IR28 from IRRI, Philippines.
- *Secondary introduction*: The introduced variety may be hybridised with indigenous varieties to transfer one or few characters from this variety to its local counterparts. Examples of secondary introduction are 'Kalyansona' and 'Sonalika' wheat varieties selected from material introduced from CIMMYT, Mexico. A few semi-dwarf rice varieties have also been developed through hybridisation with introduced varieties. Some other exotic accessions of maize, sorghum, bulrush or pearl millet (*Pennisetum* spp.), American cottons, linseed

and many other crops have been and are being used to improve upon their indigenous counterparts by employing selection and hybridisation. Likewise, 'Pusa Ruby' and 'Pusa Early Dwarf'–the reputed tomato hybrid varieties were bred by hybridising the local variety 'Meeruti' with the US varieties 'Sioux' and 'Red clouds', respectively.

Both types of introduction activity have run parallel since the beginning of agri-horticulture. However, the emphasis has shifted from primary to secondary progressively.

## Plant Introduction Agencies in India

In early days, plant introduction in India was random and made for the most part by missionaries, traders, travellers, invaders, explorers or naturalists. Some of the introductions were fortuitous and other conscious. In India, for example, the Muslim invaders brought cherries and grapes from Afghanistan and Iraq by AD 1300, and the Portuguese introduced a host of New World crops, such as maize, groundnut, chillies, potato, sweet potatoes, guava, custard apple, papaya, pineapple, cashew and tobacco by the seventeenth century. In addition, the British East India Company introduced tea, litchi and loquat from China; cabbage, cauliflowers and other winter vegetables from the Mediterranean region and annatto (*Bixa orellana* L.–source of dye) and mahogany timber from the West Indies in the last quarter of the eighteenth century. Thus, the voyages of exploration, first by the Portuguese and Spanish and later by other European nations, brought new crop materials from the New to the Old World and *vice versa*, creating the most important revolution in the history of agriculture.

Plant introduction activities started in the pre-independent India in 1946. A section of 'Plant Introduction' was established in the Botany Division of Indian Agricultural Research Institute (IARI), New Delhi. The task was assigned to Late Dr Harbhajan Singh, who was then an Assistant Botanist of this new section. Later in 1956, the section was converted into a full-fledged Plant Introduction and Exploration Organization, and subsequently in 1961, it became independent as Division of Plant Introduction, headed by Dr Harbhajan Singh. The division was reorganised into an independent national organisation and was initially named as National Bureau of Plant Introduction (NBPI). NBPI was later rechristened as National Bureau of Plant Genetic Resources (NBPGR) in 1976 by the Indian Council of Agricultural Research (ICAR), with its headquarters at the Pusa Campus and Experimental Farm at Issapur village, near Delhi. The institute has 12 regional stations/base centres located in diverse agroclimatic zones of the country. NBPGR is the nodal organisation for developing, operating and coordinating the Indian Plant Genetic Resource System. The Bureau comprises of the following five divisions:

- Germplasm Exchange
- Plant Quarantine
- Plant Exploration and Collection
- Germplasm Evaluation
- Germplasm Conservation

The Bureau has also established the National Facility for Plant Tissue Culture Repository (NFPTCR) and Cryopreservation Unit with funds provided by the Department of Biotechnology. It also houses three All India Coordinated Research Projects, namely, Medicinal and Aromatic Plants, Underutilised Plants and Arid Legumes.

## National Gene Bank

The Indian National Gene Bank has been established by NBPGR to conserve national heritage of germplasm collections in the form of seeds, vegetative propagules, tissue/cell cultures, embryos and gametes. Based on the experience gained from working with a built-in cold storage vault obtained from UK in 1983, four modules (two units of 100 $m^3$ and two of 176 $m^3$ capacity) have been installed for long-term storage of seeds of orthodox species kept in laminated aluminium foils at –20 °C after drying them to 5% moisture content. Electric supply is backed up by a standby diesel generator. Vegetatively propagated clonal materials and recalcitrant species are being maintained under field conditions. The Bureau has a strong programme of *in vitro* conservation and cryopreservation of a large number of species.

## Indian National Plant Genetic Resources Programme (IN-PGRP)

NBPGR is gradually developing and strengthening the IN-PGRP by linking up the National Base Collection (maintained under long-term storage at NBPGR) with 30 National Active Germplasm Sites responsible for different crops where germplasm collections are evaluated and multiplied under field conditions backed by medium-term storage facilities. Germplasm Advisory Committees for different crops have been set up to advise the Bureau regarding improving the capability, efficiency and effectiveness of its services.

## International Collaboration

International Board for Plant Genetic Resources (IBPGR), earlier re-named as International Plant Genetic Resources Institute (IPGRI),[*] has its office for South and Southeast Asia located in NBPGR campus. NBPGR implements work plans developed under memorandum of understanding (MoU) between ICAR and IBPGR. Besides funding several projects operated by NBPGR, IBPGR also sponsors meetings of national PGR programme leaders of South Asian Region as well as International Workshops on Genetic Resources of different crops native to this region. FAO and IBPGR also sponsor Regional Training Courses on Conservation and Utilizations of Genetic Resources of Local Crops of Agricultural Importance in South Asia and adjoining region to be conducted by NBPGR.

Besides working closely with IBPGR, the NBPGR also collaborates actively with several International Agricultural Research Centres (IARCs) like ICRISAT, IRRI, ICARDA and CIMMYT. It exchanges plant germplasm with more than 80 countries and implements work plans developed under bilateral, regional and international agreements.

### MANDATE

The NBPGR has the following national mandate:

- To plan, conduct and coordinate plant explorations for collection of diversity in germplasm of cultivated plants, their wild relatives and naturally occurring species of economic importance.
- To undertake introduction and exchange of plant germplasm for research purpose.

[*] In 2006, IPGRI and INIBAP (International Network for the Improvement of Banana and Plantain) became a single organization and subsequently changed their operating name to "Biodiversity International" with its Headquarters in Maccarese, Rome, Italy.

- To examine seed and plant propagules under exchange for the presence of associated pests and pathogens and also to salvage healthy material from the infected/infested/contaminated samples.
- To undertake and promote characterisation, evaluation and documentation of plant germplasm collections and their distribution to user scientists.
- To undertake and promote conservation of plant genetic resources on a long-term basis employing *in vivo, in vitro* and cryopreservation techniques and also to assist *in situ* conservation efforts.
- To develop and operate the National Database for storage and retrieval of information on plant genetic resources.
- To conduct basic researches for providing a sound scientific back up to its services.
- To develop and operate the National Herbarium of Crop Plants and their Wild Relatives.
- To organize suitable training programmes at the national, regional and international levels.
- To develop and implement work plans based on MoU and bilateral agreements.

**REGIONAL STATIONS AND BASE CENTRES**

*1. Akola, Maharashtra
*2. Bhowali, Uttarakhand
*3. Cuttack, Odisha
4. Hyderabad, Andhra Pradesh
*5. Jodhpur, Rajasthan
6. Ranchi, Jharkhand
*7. Shimla, Himachal Pradesh
*8. Shillong, Meghalaya
*9. Thrissur, Kerala

In addition to the NBPGR, there are some other agencies for plant introduction. Forest Research Institute, Dehradun (Uttarakhand) has an independent plant introduction organisation which looks after introduction, maintenance and testing of germplasm of forest tree species. The Botanical Survey of India was established in 1890 with the responsibility of introduction, testing and maintenance of plant materials of botanical and medicinal interest.

But currently, introduction and improvement of medicinal plants is being looked after by the NBPGR. The Central Research Institutes for various crops, e.g. tea, coffee, sugarcane, potato, tobacco, rice, cotton, rubber, etc., introduce, test and maintain plant materials of their interest. But their activities are co-ordinated by the NBPGR, which has the ultimate responsibility for introduction activities. Plant material may also be introduced by individual scientists, universities and other research organisations but all the requests for introductions into India must be routed through NBPGR, New Delhi.

## Procedure of Plant Introduction

Plant introduction is one of the oldest and very effective methods for crop improvement and it consists of the following steps: procurement of germplasm, quarantine, cataloguing, evaluation, multiplication and distribution.

* Regional Stations and the other two are Base Centres.

***Procurement of Germplasm***

For acquiring propagating materials of plants, scientists submit their requests to NBPGR. If the Bureau is unable to meet their requirements from its own stock or from other available sources in the country, then it attempts to obtain from their counterparts or other agencies in various countries where it has a liaison for the exchange of germplasm. Generally the materials are obtained through correspondence as gifts, in exchange of germplasm. Sometimes the germplasm has to be purchased. NBPGR has links with IBPGR, Rome, more than 80 countries and 8 International Agricultural Crops Research Centres that provide free exchange of plant genetic resources from their own stocks or else helps in arranging the supply of germplasm from other sources. At times it may become necessary to organize exploration to collect the required germplasm.

All these propagating materials (seeds, tubers, runners, suckers, stolons, bulbs, root cuttings or other genetic stocks) with proper packing are sent to NBPGR under phytosanitary conditions, and are collected personally by their representative from the foreign post office or the airline office or at other port of entry.

***Quarantine***

All the introduced germplasm is thoroughly inspected for contamination with insect pests; plant pathogens (bacterial, fungal and viral diseases), weeds, soil-borne diseases and packing material of plant origin, etc. Materials that are suspected to be contaminated are fumigated or undergo other treatments to destroy contaminants. If necessary, the materials are grown in isolation for close observation to prevent spreading of diseases. The entire process is known as quarantine and every country has framed regulations which are known as quarantine rules.

Seed samples of introduced germplasm exchanged every year are examined for quarantine clearance/ phytosanitary certification, both by the donor and the receiving country. Consignments without authentic phytosanitary certification from the source country to the effect that the germplasm is free from pernicious weeds, diseases and pests are confiscated and destroyed.

***Cataloguing***

Every introduction received is given an entry number, and information regarding the name of the species and variety, place of origin, adaptation and characteristics. The plant materials are grouped into three categories:

1. Exotic collections are given the prefix 'EC'
2. Indigenous collections are marked 'IC'
3. Indigenous wild collections are designed IW'

It is therefore possible to identify from the prefix whether the plant is local or introduced strain, and if local or indigenous strain, whether wild or cultivated.

***Evaluation***

To assess the potentials of introduction, these are grown in different substation of the Bureau to study their resistance under environments favouring heavy attack by the diseases and pests. In case of those

crops for which Central Research Institutes are established, e.g. rice, sugarcane, potato, tobacco, coffee, etc., the introductions are evaluated and maintained by respective institutes.

Generally, the introduced varieties perform poorly because they are often not adapted to the new environment. Sometimes, their performance in the new environment improves with the number of generations grown there. The process that leads to adaptation of a variety to a new environment over a period of time is known as acclimatisation and is brought about by a faster multiplication of those genotypes that are well adapted to the new habitat. These are then distributed to farmers for commercial cultivation.

## Purpose of plant introduction

The chief objective of pant introduction is to improve the plant wealth of the country and the main aims may be grouped as follow:

1. *To obtain an entirely new crop plant/species,* e.g. many of our important crops
2. *To serve as new varieties.* Sometimes, introductions are released as superior varieties. The Mexican semidwarf wheat varieties, Sonora 64 and Lerma Rojo; Semidwarf rice varieties TNI and IR8 are recent examples of introduction.
3. *To be used in crop improvement.* Often the introduced germplasm is used in hybridisation with local varieties to develop superior varieties. Pusa Ruby tomato was derived from a cross between Meeruti and Sioux - an introduction from USA.
4. *To save the crop from diseases and pests.*Hevea rubber was introduced into Southeast Asia from South America to protect it from South American Leaf Blight (SALB disease), caused by *Microcyclus ulei* (P. Henn.) Arx.
5. *For scientific studies:* Collection of plants has been used in studies on biosystematics, evolution and origin of plant species. Japanese wheat carrying Norin 10 dwarfing genes were crossed onto Local American variety Brevor to break linkage barrier. This led to the development of Gaines-a winter US wheat which hold the world record of 13.5 t/ha.

## Merits of plant introduction

- It is the easiest method of crop improvement.
- It helps in obtaining quickly and at low cost the best or elite variety available as it happened in semidwarf Mexican wheats and Taiwan rice varieties..
- It provided an entirely new crop. Many of our major crops like potato, tobacco, maize, cashew nut, groundnut, etc., were introduced from the New World.
- To evolve superior varieties in comparison to their counterparts by crossing the introduced variety with the local elite varieties. Sharbati Sonora with amber grain was derived from Sonora 64 through mutation. The grains have high protein content as compared to the parent variety.
- Crops may be introduced into disease-free areas to protect from damage, e.g. coffee (pathogen *Hemileia vastatrix* Berk. & Br. causing coffee rust, as it happened in Sri Lanka). Rubber tree was introduced into Southeast Asia to prevent it from South American Leaf Blight caused by *Microcyclus ulei.*

- It helps in introducing new germplasm to incorporate resistance into the existing varieties, thus to prevent genetic erosion or to protect genetic variability.

## DEMERITS OF PLANT INTRODUCTION

The uncontrolled plant introduction may lead to the introduction of many new weeds, harmful insect pests and pathogens along with the introduced materials. Some of which are listed here:

1. **Weeds:** *Argemone mexicana* L., *Lantana camara* L., *Eichhornia crassipes* (Mart.) Solms, *Parthenium* spp., *Phalaris* spp. have become noxious weeds.
2. **Diseases:** Late blight of potato was introduced into Ireland from Europe; Flag smut of wheat was introduced from Australia; coffee rust came from Sri Lanka (earlier known as Ceylon) in 1876; Bunchy top of banana was introduced from Sri Lanka in 1940.
3. **Insect pests:** The potato tuber moth came from Italy in 1900 while woolly aphid of apple and fluted scale of *Citrus* were introduced to India along with plant introduction.
4. Some introduced species of *Eucalyptus* from Australia have wrought havoc with the ecological balance - water table has gone down as a result of rapid depletion of subsoil water reserve.

# Appendix I – Radiocarbon Dating

Radiocarbon dating, a method of estimating the age of prehistoric objects containing carbon, was discovered and developed at the University of Chicago by an American Physicist Willard F. Libby and his colleagues during the period from 1946 to 1954. Later in 1960, Dr. Libby was awarded the Nobel prize for this outstanding accomplishment.

The principle of radiocarbon dating is elegantly simple. The radioactive isotope of carbon ($^{14}C$) is naturally produced in the upper atmosphere by the bombardment of nitrogen-14 with free neutrons from primary cosmic radiation. A neutron on entering the nitrogen nucleus knocks out a proton, thereby converting nitrogen atom to $^{14}C$ as indicated below.

$$^{14}N + n \rightarrow {}^{14}C + H^{+}$$

These $^{14}C$ atoms formed in nature are quickly oxidised to radioactive carbon dioxide which is mixed with ordinary carbon dioxide in a fixed proportion. Green plants, through their photosynthetic apparatus, are able to incorporate some $^{14}C$ in building carbohydrates. Through food chains, $^{14}C$ enters all other plant and animal life. All living organisms, thus, contain radioactive $^{14}C$ and normal carbon in a fixed proportion: about one trillionth ($10^{-12}$) of a gramme of $^{14}C$ occurs with each gramme of ordinary nonradioactive carbon in living organisms. As long as the animal or plant is alive, there is continual exchange with atmospheric radioactive carbon, i.e, the amount of radioactive carbon assimilated by it from food just equals the amount undergoing decay in the tissues. Once the organism dies, it no longer incorporates $^{14}C$ into its tissues and the amount of $^{14}C$ gradually diminishes with ageing according to known radioactive disintegration laws and eventually reforms the nitrogen-14 from which it was made. The decay of $^{14}C$ takes according to the equation.

$$^{14}C \rightarrow + \overline{\beta} + {}^{14}N$$

The older the organic remains, the lower is the proportion of $^{14}C$ in them. Thus physicists, by comparing the amount of residual $^{14}C$ in them. Thus physicists, by comparing the amount of residual $^{14}C$ in the dead tissues and the proportion of $^{14}C$ in living plant tissues, can determine the approximate age of any plant fragment up to about 40,000 years old. The half life (the time in which half the number of $^{14}C$ atoms present initially undergo radioactive decay) of $^{14}C$ is believed to be 5730 years. The number of $^{14}C$ atoms thus expected to be left after time lapse of 5730, 2 × 5730, and 3 × 5730 years would be $(1/2)$, $(1/2)^2$, $(1/2)^3$, respectively of the number of atoms originally present. Assuming 2000 $^{14}C$ atoms were originally present, after 5730 years, there would be 1000 atoms left; after 11460 years (2 × 5730), 500 atoms; after 17190 years (3 × 5730), 250 atoms etc. Since the disintegration rate of radioactive substances is extraordinarily immutable and is independent of the

chemical state, temperature, pressure and other physical conditions on the surface of the earth, the rate of disappearance of $^{14}C$ from a sample bears absolute relationship to time.

This method has been applied to a wide variety of materials such as wood and other plant remains uncovered during archaeological excavations; parchment, cloth, charcoal, peat, marine and fresh water shells, other carbonate deposits, ancient manuscripts, mummy cases and other artifacts of organic origin. About one ounce of such materials is sufficient for radiocarbon dating; the whole process taking three to four days. More than 80 laboratories all over the world are investigating the age of these materials. The radiocarbon technique as it stands today is summarized below:

After thorough physical cleaning (removal of younger intrusive rootlets of crude oil etc.), the sample is treated with strong acids to remove calcium carbonate that might have been deposited secondarily by precipitation from ground water or other chemical alterations that might have occurred. Then the material is burnt to carbon dioxide in a sealed combustion chamber. The purified radioactive carbon dioxide (freed from traces of radon, oxides of nitrogen and sulphur and some other products of incomplete combustion) is either measured directly in a sensitive Geiger counter, or is converted to elementary carbon black by passing carbon dioxide over hot magnesium; the carbon is then measured in a Geiger counter of somewhat different design. In some laboratories, the carbon dioxide is converted into methane or acetylene and the radioactivity measured as for carbon dioxide.

Since then the precision of radioactive measurements have improved and bristlecone pine (*Pinus aristata* Engelm.) tree rings as old as 8200 years have become available for analysis. Findings reveal that the atmospheric radiocarbon level prior to 1000 BC deviates measurably from the contemporary level. In the year 6200 BC it was roughly* eight per cent above the present day level. This departure form the present level mans that a sample with a true age of 8200 years will be dated by radiocarbon dating as 7500 old. The ultimate cause of $^{14}C$ variations with time has generally been ascribed to temporal fluctuations in the cosmic radiation that bombards the upper atmosphere and produces terrestrial $^{14}C$. Whatever may be the cause, it is obvious that $^{14}C$ dates lack the accuracy that a traditional anthropologist would like to have to trace events in human history.**

Unlike $^{14}$carbon dating, optically stimulated technique (OSL) is a new dating method widely used to determine the age with precision and accuracy of ancient materials such as the geological sediments and fired pottery shards etc., ranging form decades upto 400,000 years or more. The credit for the discovery of OSL in radiation domisetry should go to Albrecht, H.O. and Mandeville, C.F. (1956). Among other methods are infrared stimulated luminescence (RSL) and thermoluminescence (TL). In light of newer techniques, the timelines of the so called 'cradle of civilisation' such as Indus Valley, Mesopotamian (in the valley of the Tigris and Euphrates) or Tehuacan Valley (in the present day Mexico) etc. shall have to be re-written. Recent archaeological findings have suggested that the Indus Valley civilisation was at least 8,000 years old, not around 5,500 old as earlier believed.

Some other methods such as accelerator mass spectrometry (AMS) and conventional liquid scintillation counting (LSC) are also being used to date materials of antiquity.

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* Radiocarbon samples form industrialised areas indicate $^{14}C$ radioactivity appreciably lower than samples from non-industrial areas.

** Although radiocarbon dating is a good method for dating biological remains and sediments, yet for an accurate determination of age, conventional radiocarbon dates must be calibrated by tree ring chronologies (for further details see, *An Introduction to Tree-Ring Dating* by Stokes, M.A *et al.*, 1968; University of Chicago Press). Also, it is not reliable beyond 50,000 years. Uranium/thorium (U/T) series dating has extended this limit to 300,000 years, but the samples have to be of marine origin. Raisbeck and Yiou of Orsay have now claimed that it seems feasible to date bones and calcium carbonate formations by measuring calcium carbonate formations by measuring calcium ($^{41}Ca$), up to an age of few million years. It sounds quite promising but it has to be verified experimentally.

# Appendix II – Glossary of Some Medical Terms

**Abortifacient**: an agent that promotes abortion.
**Addiction**: inability to resist indulgence in some habit, especially strong dependence on a drug.
**Aflatoxin**: specific mycotoxin secreted by *Aspergillus*; a liver toxin that can induce tumours.
**Allergy**: an exaggerated reaction or sensitivity of the body cells to specific substances such as antigens and allergens.
**Analgesic**: a pain reliever which does not induce loss of consciousness; anodyne.
**Anemia** (or anaemia): a condition in which the equilibrium between blood production and blood loss is disturbed, causing impoverishment either in the number of erythrocytes, the quantity of haemoglobin or the volume of packed red blood cells.
**Anesthesia**: induction of the loss of tactile sensibility (numbness) , especially of pain.
**Anodyne**: = analgesic.
**Anthelmintic**: a drug which is used for combating intestinal worms.
**Anticoagulant**: an agent which prevents blood from clotting.
**Anticonvulsant**: agent that prevents or stops convulsion (muscle relaxant).
**Antipyretic**: a drug that relieves or reduces fever.
**Antisomniac**: substance used to treat insomnia or inability to sleep.
**Antispasmodic**: a drug which relieves muscular spasm and has a sedative effect on the nerves.
**Antitussive:** agent that prevents or relieves cough.
**Aperient:** substance employed to evacuate the bowels; includes laxative (mild purgative like mineral oil) and cathartics (powerful purgative such as castor oil).
**Aphrodisiac**: a drug which arouses sexual desire.
**Arteriosclerosis** (eq. = atherosclerosis): a condition characterized by thickening and hardening of the walls of the arteries as a result of accumulation of lipid substances and progressive diminution in the size of the lumen.
**Arthritis**: inflammation of joints.
**Asthma**: a chronic inflammation of the bronchial tubes.
**Astringent**: an agent which shrinks and hardens tissues, thereby lessening secretion.
**Beri-beri**: charactrised by extreme fatigue and polyneuritis; other symptoms are oedema (retention of interstitial fluid in the connective tissues) and cardiovascular disturbances. This illness is caused by an acute deficiency of vitamin $B_1$ (thiamine.)

**Bronchitis**: an inflammation of the air passages.

**Cancer**: any malignant growth formed by uncontrolled proliferation of certain cells (no longer controlled by the genetic plan governing the orderly division of normal cells) with varying chromosome numbers. Cancerous cells enter the bloodstream, to be carried to more distant parts of the body where they form similar growths (metastases). Malignant cells also spread throughout the body through lymphatic vessels.

**Carcinogen**: any physical or chemical agent that tends to produce a cancer.

**Caries**: bacterial infection of the enamel and dentin of the tooth leading to decalcification and cavitation (decay).

**Carminative** (= flatulence): any substance that promotes expulsion of gas (or reduced formation of a gas) in the stomach.

**Catarrh**: the discharge form inflamed mucous membranes, such as those lining the nasal cavity or stomach.

**Cathartic** (= purgative, evacuant): a fairly powerful purgative (agent used to evacuate the bowels).

**Cerebral haemorrhage**: a stroke resulting from the bleeding of a vessel into the brain.

**Cerebral thrombosis**: stroke resulting from a blood clot blocking a cerebral vessel.

**Colic**: pain due to spasmodic contraction of the abdomen.

**Counterirritant**: an agent which produces irritation in one part of the body to relieve pain or inflammation elsewhere.

**Curare**: general name applied to the dried extract obtained from several different South American arrow poisons (such as *Chondrodendron tomentosum* and several species of *Strychnos*) used to secure muscle relaxation.

**Delirium**: an extreme mental disturbance marked by excitement, restlessness and rapid succession of confused and unconnected ideas.

**Demulcent**: a drug having a soothing action on the skin and mucous membranes.

**Depressant**: an agent that reduces the functional activity; a sedative.

**Dermatitis**: inflammation of the skin.

**Diabetes**: metabolic disorder affecting insulin production, giving rise to sugar in the urine and also an increase in the level of glucose in the blood.

**Diaphoretic**: a drug which tends to induce copious perspiration.

**Diarrhoea**: abnormal frequency and fluidity of faecal discharges.

**Diphtheria**: an infectious disease of the throat and the air passage.

**Diuretic**: a drug that increase the secretion and discharge of urine.

**Dropsy**: a disease marked by an excessive collection of a watery fluid in body tissues or cavities.

**Dysentery**: an infectious disease, causing acute diarrhoea and discharge of mucus and blood.

**Dyspepsia**: the impaired digestion of food.

**Emetic**: an agent that induces vomiting.

**Emollient**: a drug that allays irritation of the skin and alleviates swelling and pain.

**Epilepsy**: a chronic nervous disorder marked by attacks of unconsciousness or convulsions. Electro-encephalography (i.e. recording of the electrical activity of the brain by means of electrodes fixed on the scalp) enables the diagnosis of epilepsy.

**Ergotism**: a disease caused by intake of bread infected with the parasitic fungus ergot: symptoms include spasms, cramps, burning sensation in the extremities, hallucinations and induction of abortion.

**Expectorant**: a drug that helps the removal of catarrhal matter and phlegm from the bronchial tubes.
**Febrifuge** (= antipyretic): an agent which reduces fever.
**Flatulence**: a disorder in which there is excessive accumulation of gas in the alimentary canal.
**Gangrene**: tissue death resulting from inadequate blood supply, direct injury or infection.
**Goitre**: a chronic enlargement of the thyroid gland.
**Gout**: painful disease affecting the joints, resulting from faulty purine metabolism.
**Habituation**: intense desire, or hunger for a particular drug, also called psychological dependence or craving.
**Hallucination**: caused by agents that alter the state of consciousness, that is, produce change in perception (of time, space and of self), changes in mood and changes in thought.
**Haemolysis**: the breakdown of red blood cells; the cell contents diffuse into blood plasma.
**Haemostatic** (= styptic): a substance which stops or staunches bleeding.
**Hypertension**: abnormally high constructive tension in blood vessels; usually revealed as high blood pressure.
**Hypnotic** (= soporific): sleep-inducing drug.
**Hypotension**: a state of abnormally low blood pressure.
**Infusion**: an extract obtained by steeping the drug in water.
**Jaundice**: a condition characterised by a raised level of bilirubin (breakdown product of haemoglobin), producing a yellowness of mucous membranes, including the eyes.
**Laudanum** (or ladanum): opium dissolved in alcohol; tincture of opium.
**Laxative**: a mild purgative.
**Liniment**: a liquid pharmaceutical preparation for external use, usually oily and applied by rubbing.
**Migraine**: periodic severe attacks of headache, commonly affecting only one side of the head.
**Mutagen**: an agent that elicits a mutation.
**Narcotic**: an agent capable of causing a depression in the central nervous system.
**Nausea**: a feeling that vomiting is about to begin.
**Necrosis**: death of tissues, usually in localised areas.
**Neuralgia**: nerve pain.
**Neuritis**: nerve inflammation.
**Oxytocic**: hastening childbirth.
**Panacea**: universal remedy or cureall.
**Paralysis**: a disease wherein there is loss of power of voluntary movement in any part of the body.
**Peristalsis**: rhythmic contraction and relaxation of smooth muscles lining the intestine promoting the movement of food.
**Piles**: an inflamed condition of the veins in the region of rectum.
**Psychotomimetic**: an agent which produces conditions that mimic mental disorders.
**Purgative**: an agent that stimulate peristaltic action and bowel evacuation.
**Pyorrhoea**: a disease marked by purulent discharge form the gums.
**Rheumatism**: a term used for pains in the muscles,. Joints and certain tissues.
**Rickets**: a deficiency disease caused by insufficiency or absence of vitamin D and by insufficient exposure to sunlight. It occurs during the period of growth in infants and is accompanied by disturbance of bone formation and by skeletal deformation.
**Scabies**: an itching skin disease caused by mite.

**Scurvy**: a deficiency disease caused by lack of vitamin C in the diet, and is characterised by multiple haemorrhage, especially of the gums, gastrointestinal disturbances and loss of teeth.

**Sedative**: a drug that reduces excitement, irritation, and pain (or has a soothing or sleep-producing action).

**Spasm**: an intense, uncontrolled muscular contracton.

**Soporific**: a drug that induces sleep.

**Stomatitis**: inflammation of the mouth.

**Stomachic**: a drug that stimulates the appetite, thereby promoting the functional activity of the stomach.

**Styptic**: an agent which checks bleeding.

**Tranquiliser**: a drug used to calm or soothe a person without directly inducing sleep.

**Ulcer**: an open sore (peptic ulcer, ulceration of the stomach, oesophagus or duodenum by the action of the acid gastric juices).

**Vermifuge**: a drug that expels intestinal worms.

**Vesicant**: an irritating substance producing blisters on the skin.

# Appendix III – International Agricultural Research Centres and Institutes

| *Institute* | *Areas of research* | *Founded* | *Location* |
|---|---|---|---|
| International Rice Research Institure (IRRI) | Rice | 1960 | Philippines |
| International Maize and Wheat Improvement Center (CIMMYT) | Wheat, maize, barley, triticale | 1966 | Mexico |
| International Institute of Tropical Agriculture (IITA) | Maize, rice, cowpeas, soyabeans lima beans, root and tuber crops | 1968 | Nigeria |
| International Center of Tropical Agriculture (CIAT) | Field beans, cassava, rice, corn, forages | 1969 | Colombia |
| West African Rice Development Association (WARDA) | Rice | 1971 | Liberia |
| International Potato Center (CIP) | Potatoes | 1972 | Peru |
| International Crops Research Institute for the Semi-arid Tropics (ICRISAT) | Sorghum, millets, chickpeas, pigeon peas, groundnuts | 1972 | India |
| *International Board for Plant Genetic Resources (IBPGR) | Genetic resources for crops | 1973 | Italy |
| International Livestock Center for Africa (ILCA) | Livestock systems | 1974 | Ethiopia |
| International Laboratory for Research on Animal Diseases (ILRAD) | Trypanosomiasis and east coast fever | 1974 | Kenya |
| International Food Policy Research Institute (IFPRI) | Studies on food policy | 1975 | USA |
| International Center for Agricultural Research in Dry Areas (ICARDA) | Wheat, barley, lentils, broad beans, oilseeds; cotton | 1976 | Station in Syria & Lebanon |
| International service for National Agricultural Research (ISNAR) | Promotion of national research programmes | 1980 | The Hague. Netherlands |

*Now known as International Plant Genetic Resources Institute—IPGRI.

# Appendix IV – Names of Countries (Old and New)

| *Old name* | *New name* |
|---|---|
| *African continent* | |
| Abyssinia | Ethiopia |
| Belgian Congo (also known as Democratic Republic of the Congo or Congo, Leopoldville) | Zaire |
| British Somaliland + Italian Somaliland | Somalia |
| Casamance | Senegal |
| French Equatorial Africa | Cameroon, Central African Empire, Chad, Congo (Brazzaville), Gabon |
| French Guinea | Guinea |
| French West Africa | Dahomey, Guinea, Ivory Coast (Now named Cote Divoire), Mail, Mauritania, Niger, Togo, Upper Volta |
| Gold Coast | Ghana |
| Ivory Coast | Cote Divoire |
| Madagascar | Malagasy, Republic of |
| Northern Rhodesia | Zambia |
| Nyasaland | Malawi |
| Oubangui-Chari | Central African Republic (Now renamed Central African Empire) |
| People's Republic of the Congo (also known as Congo-Brazzaville) | Congo |
| Republic of Cameroon (French Cameroons) + British* South Cameroon | Cameroon |

*Contd.*

* Northern part of the British Cameroons joined the Federation of Nigeria in a 1961 Plebiscite.

*Contd.*

| *Old name* | *New name* |
|---|---|
| Southern Rhodesia | Zimbabwe |
| Spanish Guinea | Equatorial Guinea |
| Tanganyika | Tanzania |
| United Arab Republic | The Arab Republic of Egypt |
| Zanzibar and Pemba | Now part of Tanzania |
| *Asian continent* | |
| Asia Minor | Asian part of Turkey ( = Anatolia) |
| Burma | Myanmar |
| Ceylon | Sri Lanka |
| Cambodia | Khmer Republic (now Democratic Kampuchea) |
| Formosa or Nationalist China | Taiwan |
| Indochina | Laos, Vietnam |
| Netherland or Dutch East Indies | Indonesia: it includes Borneo (part), Celebes, Java, Sumatra, Timor (part), Irian (Old Dutch New Guinea) , Amboina |
| North Borneo | Sabah |
| North Korea | Democratic People's Republic of Korea |
| North Yemen | Yemen Arab Republic |
| Pakistan (East) | Bangladesh |
| Persia | Iran |
| Siam | Thailand |
| South Korea | Republic of Korea |
| South Yemen | People's Democratic Republic of Yemen |
| Southern part of the Malay Peninsula, excluding Singapore ( = Western Malaysia) ,+ much of North Borneo (renamed Sabah)+ Sarawak (= Eastern Malaysia) | Malaysia |
| The Republic of Vietnam ( = South Vietnam) + Democratic Republic of Vietnam (= North Vietnam) | Vietnam |
| *American continent* | |
| British Guiana | Guyana |
| British Honduras | Belize |
| Netherland or Dutch Guiana | Surinam |

# Appendix V – Names of Families (Old and New)

**Names of Families**

| *Old name* | *Alternative name* |
|---|---|
| Cruciferae | Brassicaceae |
| Compositae | Asteraceae |
| Guttiferae | Clusiaceae |
| Gramineae | Poaceae |
| Labiatae | Lamiaceae |
| Leguminosae* | Fabaceae |
| Palmae | Arecaceae |
| Umbelliferae | Apiaceae |

* If the three subfamilies of Leguminosae are raised to family level then the alternative name cited above is used for the Papilionaceae and others are named as Caesalpiniaceae and Mimosaceae.

# Appendix VI – International Agricultural Research Institutes, National Bureau and National Research Centres/Organisations

*Acronyms*
(with the name of location given in parenthesis)

| | |
|---|---|
| ARS | Agricultural Research Service |
| ASRB | Agricultural Scientists Recruitment Board |
| BARC | Bhaba Atomic Research Center (Mumbai, (Bombay) , Maharashtra |
| CAZRI | Central Arid Zone Research Institute (Jodhpur, Rajasthan) |
| CCRI | Central Coffee Research Institute (Chickmagalur, Karnataka) |
| CCRI | Central Coir Research Institute (Alleppy, Kerala) |
| CDRI | Central Drug Research Institute– CSIR (Lucknow, UP) |
| CFTRI | Central Food and Technological Research Institute – CSIR (Mysore, Karnataka) |
| CIMAP | Central Institute for Medicinal and Aromatic Plants – CSIR (Lucknow, UP) |
| CICR | Central Institute for Cotton Research (Nagpur, Maharashtra) |
| CIRCT | Central Institute for Research on Cotton Technology (Mumbai, Maharashtra) |
| CIHNP | Central Institute of Horticulture for Northern Plains (Lucknow, UP) |
| CISH | Central Institute for Subtropical Horticulture (Lucknow, UP) |
| CITH | Central Institute for Temperate Horticulture (Srinagar, J & K) |
| CMRS | Central Mango Research Station (Malihabad, UP) |
| CPCRI | Central Plantation Crops Research Institute (Kasaragod, kerala) |
| CPRI | Central Potato Research Institute (Shimla, Himachal Pradesh) |
| CRIDA | Central Research Institute for Dryland Agricultural (Hyderabad, AP) |
| CRRI | Central Rice Research Institute (Cuttack, Odisha) |
| CRSARD | Centre for Research on Sustainable Agricultural and Rural Development (Chennai, (Madras), Tamil Nadu) |
| CSIR | Council of Scientific and Industrial Research (New Delhi) |
| CSSRI | Central Soil Salinity Research Institute (Karnal, Haryana) |
| CTCRI | Central Tuber Crops Research Institute (Trivandrum, Kerala) |
| CTRI | Central Tobacco Research Institute (Rajahmundry, AP) |
| CTRL | Cotton Technological Research Laboratory (Mumbai, Maharashtra) |

| | |
|---|---|
| DARE | Department of Agricultural Research and Education, Government of India |
| DBT | Department of Biotechnology, Ministry of Science & Technology, Government of India (New Delhi) |
| DNE | Department of Non-Conventional Energy, Government of India |
| FRI | Forest Research Institute and College (Dehradun, Uttaranchal) |
| IARI | Indian Agricultural Research Institute (Pusa, New Delhi) |
| ICAR | Indian Council of Agricultural Research (New Delhi) |
| IGFRI | Indian Grassland and Fodder Research Institute (Jhansi, UP) |
| IIHR | Indian Institute of Horticultural Research (Bangalore, Karnataka) |
| IIPK | Indian Institute of Pulses Research (Kanpur, UP) |
| IISR | Indian Institute of Spice Research — (Calicut, Kerala) |
| IISR | Indian Institute of Sugarcane Research (Lucknow, UP) |
| ILRI | Indian Lac Research Institute (Ranchi, Jharkhand) |
| IPR | Intellectual Property Right |
| IBPGR | International Board of Plant Genetic Resources (now known as International Plant Genetic Resources Institute—IPGRI, Rome, Italy) |
| ICGEB | International Center for Genetic Engineering and Biotechnology (New Delhi, India) |
| ICRISAT | International Crops Research Institute for the Semi-arid Tropics (Hyderabad, India) |
| IDRC | International Development Research Centre (Canada) |
| IFPRI | International Food Policy Research Institute (USA) |
| IITA | International Institute of Tropical Agricultural (Ibadan, Nigeria) |
| ILTAB | International Laboratory for Tropical Agricultural Biotechnology (La Jolla, California, USA) |
| ILDIS | International Legume Database and Information Service |
| IPGRI | International Plant Genetic Resource Institute (earlier known as IBPGR) (Rome, Italy) |
| ISNAR | International Service for National Agricultural Research (The Hague –Netherlands) |
| IUCN | International Union for the Conservation of Nature and Natural Resources (Now known as World Conservation Union ) |
| JARI | Jute Agricultural Research Institute (Barrackpore, West Bengal) |
| JTRL | Jute Technological Research Loboratories, Kolkata, West Bengal, (Now known as National Institute of Research on Jute & Allied Fibre Technology — NIRJAFT) |
| KVK | Krishi Vigyan Kendra |
| NAAR | National Academy for Agricultural Research (New Delhi) |
| NAARM | National Academy of Agricultural Research Management (Hyderabad, Andhra Pradesh ) |
| NABARD | National Bank for Agricultural and Rural Development |
| NAFED | National Agricultural Cooperative and Marketing Federation of India |
| NBPGR | National Bureau of Plant Genetic Resources (Pusa Campus, New Delhi) |
| NBRI | National Botanical Research Institute (formerly National Botanical Garden) — (Lucknow, UP) |
| NHRDF | National Horticultural Research and Development Foundation (Nasik, Maharashtra) |

| | |
|---|---|
| NIRJAFT | National Institute of Research on Jute & Allied Fiber Technology |
| NRIAH | National Research Institute for Arid Horticulture (Bikaner, Rajasthan) |
| NRCMAP | National Research Centre for Medicinal and Aromatic Plants (Anand, Gujarat) |
| NRCPB | National Research Centre for Plant Biotechnology (IARI, New Delhi) |
| NRCOP | National Research Centre for Oilpalm (Eluru, Andhra Pradesh) |
| NRCOG | National Research Centre for Onion and Garlic (Nasik, Maharashtra) |
| NRI | National Research Institute (UK) |
| ODA | Overseas Development Administration (UK) |
| *ODNRI | Overseas Development Natural Resources Institute (UK) |
| PBR | Plant Breeders Right |
| RBG | Royal Botanic Gardens (Kew, UK) |
| RRII | Rubber Research Institute of India & Rubber Board (Kottayam, Kerala) |
| RRL | Regional Research Laboratory—CSIR (Jammu-Tawi) |
| RRIM | Rubber Research Institute of Malaysia (Kuala Lumpur, Malaysia) |
| SBI | Sugarcane Breeding Institute (Coimbatore, Tamil Nadu) |
| SAARC | South Asian Association of Regional Countries |
| SEPASAL | Survey of Economic Plants for the Arid and Semi-arid Lands |
| SIDA | Swedish International Development Agency |
| TPI | Tropical Products Institute (Now known as Tropical Development and Research Institute) (UK) |
| TRIPS | Trade Related Intellectual Property Rights |
| TBGRI | Tropical Botanical Garden and Research Institute (Palode, Kerala) |
| TERI | The Energy and Resources Institute (formerly known as Tata Energy and Resources Institute) Lodhi Road, New Delhi. |
| TES | Tocklai Experimental Station (Jorhat, Assam) |
| TIFR | Tata Institute of Fundamental Research (Bombay, Maharashtra) |
| UNDP | United Nations Development Programme |
| UNEP | United Nations Environment Programme |
| UNESCO | United Nations Educational Scientific and Cultural Organization |
| UNIDO | United Nations Industrial Development Organization |
| UPOV | Union for the Protection of New Plant Varieties |
| USDA | United States Department of Agriculture |
| USAID | United States Agency for International Development |
| WARDA | West African Rice Development Association |
| WCU | World Conservation Union (earlier Known as IUCN) |
| WHO | World Health Organization |
| WIPO | World Intellectual Property Organization |
| WTO | World Trade Organization |
| WWF | Worldwide Fund for nature |

* ODNRI was formed by the amalgamation of the Tropical Development and Research Institute and the Land Resources Development Centre. The Institute is the scientific arm of Britain's Overseas Development Administration (ODA)

# Appendix VII – Some Other Common Useful Trees

| *Botanical name* | *Family* | *Common English name* | *Indian (Hindi name)* | *Time of flowering* |
|---|---|---|---|---|
| *Acacia auriculiformis* A. Cunn. ex. Benth. | Fabaceae | Australian phyllode acacia or wattle | Akashmuni | October-Novermber |
| *A. catechu* (L.f.) Willd. | Fabaceae | Black cutch, catechu | Khair | August-September |
| *A nilotica* (L.) Willd. ex. Del. ssp. *indica* (Benth.) Brenan | Fabaceae | Acacia | Babul, Kikar | July-December |
| *Adansonia digitata* L. | Malvaceae | Baobab or Monkey-Bread tree | Gorakh imli | July |
| *Adina cordifolia* Hook. | Rubiaceae | Yellow teak, Saffron teak | Haldu | March-May |
| *Albizia lebbeck* Benth. | Fabaceae | Koko, Lebbeck Tree | Kala Siris | March-May |
| *A. procera* (Roxb.) Benth. | Fabaceae | White Siris | Safed Siris | July-August |
| *Alstonia scholaris* R. Br. | Apocynaceae | Devil Tree, Dita bark tree | Chatian, Satwin | December-March |
| *Anogeissus latifolia* Wall. | Combretaceae | Yon | Dhaura | September-January |
| *Anthocephalus cadamba* Miq. | Rubiaceae | Kadam | Kadamba | June-July |
| *Artocarpus altilis* (Park.) Fosberg | Moraceae | Breadfruit | Vilayati phanas | |
| *A. integra* (Thunb.) Merrill | Moraceae | Jackfruit | Kathal | November-January |
| *A.lakoocha* Roxb. | Moraceae | Monkey Jack, Lakoocha | Barhal, Dahua | March |

*Contd.*

*Contd.*

| *Botanical name* | *Family* | *Common English name* | *Indian (Hindi name)* | *Time of flowering* |
|---|---|---|---|---|
| *Azadirachta indica* A. Juss. | Meliaceae | Neem | Neem, Margo | April |
| *Bauhinia purpurea* L. | Fabaceae | Camel Hoof Tree, Mountain Ebony | Purple Kachnar, khairwal | March-April |
| *B. variegata* L. | Fabaceae | The kachnar or Variegated Bauhinia | Kachnar (baisakhi) | February-March |
| *Butea monosperma* Roxb. | Fabaceae | The Flame of the Forest or Dhak | Dhak, palas | January-April |
| *Callistemon lanceolatus* Sweet | Myrtaceae | The Scarlet Bottle Brush Tree | Lal botal Brush | March and October |
| *Carissa carandas* L. | Apocynaceae | Karanda | Karaunda | January-April |
| *Caryota urens* L. | Arecaceae | Toddy palm, Fishtail palm | Mari, Ram goah | Throughout the year |
| *Casuarina equisetifolia* L. | Casuarinaceae | Beefwood tree, She-oak, Indian Chritmas tree | Jangli Saru | September-October |
| *Cassia fistula* L. | Fabaceae | Indian Laburnum | Amaltas | April-June |
| *Cochlospermum religiosum* (L.) Alson | Cochlosper-maceae | Yellow Silk Cotton | Kumbi, Pili kapas | February-April |
| *Cordia dichotoma* Forsk. (Syn. *C. obliqua* Willd.) | Boraginaceae | Lasora | Lasora | March-April |
| *Delonix regia* Rafin (Syn. *Poinciana regia* Bojer) | Fabaceae | Gulmohur | Gul Mohur | April-May |
| *Dillenia indica* L. | Dillieniaccae | Elephant Apple | Chalta | June |
| *Elaeocarpus augustifolius* Bl. (Syn. *E. ganitrus* Roxb. ex. G. Don) | Elaeocarpaceae | Utrasum Bead Tree | Rudraksh | May-June |
| *Emblica officinalis* Gaertn. (Syn. *Phyllanthus emblica* L.) | Euphorbiaceae | Emblic myrobalan | Amla, Anwala, Aonla | February-May |
| *Enterolobium saman* Prain (Syn. *Pithecellobium saman* Benth.; *Samanea saman* Merrill. ) | Fabaceae | Rain Tree | Belati Siris or Jangal Zalebi | March-October |
| *Erythrina indica* Lam. | Fabaceae | Indian Coral Tree | Mandara, Pangri, Pangara | February-May |
| *Ficus benghalensis* L. | Moraceae | Banyan | Bargad | June-Sept. |

*Contd.*

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| *Botanical name* | *Family* | *Common English name* | *Indian (Hindi name)* | *Time of flowering* |
|---|---|---|---|---|
| *F. elastica* Roxb. | Moraceae | Indian Rubber Tree | Bor, Atta Bor | — |
| *F. krishnae* C.DC. | Moraceae | Krishna's Fig, Krishna's Buttercup | Makahan Katori | — |
| *F. religiosa* L. | Moraceae | Bo-Tree, Peepul tree or Sacred fig | Pipal | April-June |
| *Gmelina arborea* Roxb. | Verbenaceae | Gumhar, White Oak, Coomb teak | Gumhar, Gamari | February-May |
| *Grevillea robusta* Cunn. ex R.Br. | Proteaceae | Silver or Silky Oak | — | March-May |
| *Grewia tiliaefolia* Vahl. | Tiliaceae | Dhaman | Daman, Dhaman | April-July |
| *Holarrhena antidysenterica* (L.) Wall. ex DC. | Apocynaceae | Easter Tree | Kurchi | February-August |
| *Jacaranda mimosiefolia* D. Don | Bignoniaceae | Jacaranda | Nil gul mohur | March-May |
| *Kigelia pinnata* DC. | Bignoniaceae | Sausage Tree | Jhar Phanoos | April-May |
| *Lagerstroemia lanceolata* Wall. | Lythraceae | Bondara, Nana | Benteak | |
| *L. speciosa* Pers. | Lythraceae | The Queen Flower or Pride of India | Jarul | June-July |
| *Leucaena leucocephala* (Lamk.) de Wit | Fabaceae | Horse tamarind, White popinae | Subabul | June-August |
| *Llanea coromandelica* (Houtt.) Merrill | Anacardiaceae | Jhingan | Jhingan | December-April |
| *Madhuca indica* Gmel. | Sapotaceae | Mohwa | Mahua, Mahwa | March-April |
| *Magnolia grandiflora* L. | Magnoliaceae | Bull bay, Laurel Magnolia | Him champa | August-September |
| *Mallotus philippensis* (Lamk.) Muell.-Arg. | Euphorbiaceae | Kamala tree, Monkey face tree, Kumkum Tree | Kamela | November-January |
| *Manilkara hexandra* (Roxb.) Dubard (Syn. *Mimusops hexandra* Roxb.) | Sapotaceae | — | Khirni | November-February |
| *Melia azedarach* L. | Meliaceae | Persian Lilac, The Bead Tree | Dake, Bakain | February-March |
| *Michelia champaca* L. | Magnoliaceae | Champac | Champ, Champa | April-September |

*Contd.*

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| *Botanical name* | *Family* | *Common English name* | *Indian (Hindi name)* | *Time of flowering* |
|---|---|---|---|---|
| *Mimusops elengi* L. | Sapotaceae | Spanish Cherry | Maulsari | January-March |
| **Moringa oleifera* Lam. | Moringaceae | Drumstick Tree, Horse-Radish Tree, Bakul Tree | Sainjana | January-April |
| *Morus alba* L. | Moraceae | White mulberry | Shatoot, Tut | February-March |
| *Nyctanthes arbor-tristis* L. | Oleaceae | Coral Jasmine Tree of Sorrow | Harsinghar | August-December |
| *Parkinsonia aculeata* L. | Fabaceae | Jerusalem Thron | Vilayti kikar | November to February |
| *Phoebe goalparensis* Hutchins. | Lauraceae | Bensum, Assam teak | Bonsum, | — |
| *Pinus roxburghii* Sar. | Pinaceae | Long-leaved pine or Three-leaved pine | Chir | — |
| *P. wallichiana* A.B. Jackson | Pinaceae | Indian blue pine or Five-leaved pine | Kail | — |
| *Platanus orientalis* L. | Platanaceae | Oriental Plane or European Plane tree | Chinar | April-may |
| *Plumeria alba* L. | Apocynaceae | White frangipani | — | — |
| *P. rubra* L. | Apocynaceae | Temple or Pagoda tree | Son champa | February-October |
| *Polyalthia longifolia* Thev. | Annonaceae | Mast Tree or Ashok | Asoka, devdar | February-April |
| *Pongamia pinnata* (L.) Pierre (Syn. *P. glabra* Vent.) | Fabaceae | Pongam, | Karanja, Kanji | April-June |
| *Pterocarpus dalbergioides* Roxb. | Fabaceae | Andaman Padauk Andaman reewood | Paduk | — |
| *P. marsupium* Roxb. | Fabaceae | Kino tree, Malabar Kino | Bijasar, Bijasal | May-June |
| *Roystonea regia* C.F. Cook | Arecaceae | Royal palm. Bottle Palm | — | — |
| *Salvadora oleoides* Decne. | Salvadoraceae | — | Barapilu, pilu Jhal | March-May |
| *S. persica* L. | Salvadoraceae | Mustard tree | Chotta Pilu | November-May |

*Contd.*

* The leaves and fruits of this 'miracle tree' are an astonishingly rich source of calcium (440mg/100 g) , iron, vitamins B, A and C (when raw) and of proteins.

*Contd.*

| *Botanical name* | *Family* | *Common English name* | *Indian (Hindi name)* | *Time of flowering* |
|---|---|---|---|---|
| **Saraca asoca* (Roxb.) De Willd | Fabaceae | Ashoka tree | Ashok | February-March |
| *Schleichera oleosa* (Lour.) Oken | Sapindaceae | Lac Tree | Kusum | April-May |
| *Semecarpus anacardium* L.f. | Anacardiaceae | Marking-Nut Tree | Dhobi-nut or Bhilawa | May-August |
| *Sesbania sesban* (L.) Merr. | Fabaceae | Sesban | Balmota, Jayanti | February-March |
| *Spathodea campanulata* Baeav. | Bignoniaceae | African Tulip Tree, Scarlet-bell Tree, Faountain or Squirt-Tree | — | March-April |
| *Syzygium cuminii* (L.) Skeel (Syn. *Eugenia jambolana* Lamk.) | Myrtaceae | Jambolana, Java Plum | Jamun | March-June |
| *Tabernaemontana divaricata* (L.) R. Br. | Apocynaceae | Crape-Jasmine | Chandni, Chamela | May-October |
| *Tamarindus indica* L. | Fabaceae | Tamarind | Imli | May-June |
| *Terminalia arjuna* (Roxb.) Wight & Arn. | Combretaceae | Arjun Terminalia | Arjun | April-July |
| *T. bellirica* (Gaertn.) Roxb. | Combretaceae | Belleric myrobalan | Bahera | February-May |
| *T. myriocarpa* Heurck & Muell. –Arg. | Combretaceae | Hollock | Hollock | February-May |
| *Thevetia peruviana* (Pers.) K. Schum. (Syn. *T. nerifolia* Juss. ex Steud.) | Apocynaceae | Yellow Oleander | Pili Kaner | Throught the year |

* Sita, wife of Rama, when abducted to Sri Lanka was kept in a garden among groves of Ashoka tree which is why it is also known as 'Sita Ashok', and should therefore be not confused with Ashok or Mast Tree (*Polyalthia longifolia*)

# Appendix VIII – Shrubs, Climbers and Some Exotics

## Shrubs

| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Abutilon striatum* Dicks. ex Lindl. | Malvaceae | Spotted flowering maple | Guatemala |
| *Acalypha godseffiana* Mast. | Euphorbiaceae | Threadleaf, copperleaf | New Guinea |
| *A. hispida* Burm. f. | Euphorbiaceae | Red-Hot Cat's tail, chenille plant | East Indies |
| *A.wilkesiana* Muell.-Arg. | Euphorbiaceae | Copperleaf, Jacob's Coat, Beefsteak Plant | South Sea Island, Fiji |
| *Acokanthera spectabilis* Hook f. | Apocynaceae | Winter green, Bushman's poison bush | Africa |
| *Allamanda neriifolia* Hook. | Apocynaceae | Golden Trumpet Bush, Oleander Allamanda | Brazil |
| *A.schottii* Pohl. | Apocynaceae | Bush Allamanda | South America |
| *Aphelandra squarrosa* Nees | Acanthaceae | Zebra plant, Saffron spike | Tropical America |
| *A.sinclairiana* Nees | Acanthaceae | Coral aphelandra | Tropical America |
| *Asclepias curassavica* L. | Aselepiadaceae | False ipecacuanha,Blood flower | Tropical America |
| *A.physocarpa* (Mey.) Sch. (Syn. *A fruticosus* Schltr.) | Asclepiadaceae | Balloon Plant | Tropical America |
| *Barleria cristata* L. | Acanthaceae | Philippine violet | India |
| *B.prionitis* L. | Acanthaceae | — | Tropical Africa and Asia |
| *Bauhinia acuminata* L. | Fabaceae | Dwarf white Bauhinia, Orchid Tree, Red Bauhinia | Southeast Asia |

*Contd.*

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| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *B.galpinii* N.E. Br. | Fabaceae | Pride of Cape | Tropical Africa |
| *Beloperone guttata* L. | Acanthaceae | Shrimp plant | Tropical America (Mexico) |
| *Brunfelsia calycina* (Hook.) Benth. (syn. *Franciscea hopeana* Benth.) | Solanaceae | Yesterday-Today-and-Tommorrow, Brazil raintree, Morning-Noon-and-Night, Kiss Me Quick | South America and the West Indies |
| *Brya ebenus* A.DC. | Fabaceae | Jamaica ebony, Green ebony | Central America and West Indies |
| *Buddleia (Buddleja) davidii* A. Franchet | Loganiaceae | Butterfly Bush | Central and Western China |
| *B.madagascariensis* Lamk. | Loganiaceae | Nicodemia Yellow buddleia | Malagasy Rupublic |
| *Caesalpinnia pulcherima* Swartz. (syn. *Poinciana pulcherrima* L.) | Fabaceae | Pride of Barbados or Peacock flower, Dwarf Poinciana | West Indies, South America |
| *Calliandra haematocephala* Hassk. | Fabaceae | Blood-Red Tassel flower, powder puff tree | Northern South America |
| *C.houstonii* Benth. | Fabaceae | — | — |
| *Cassia alata* L. | Fabaceae | Candlestick senna, candle bush, winged cassia, Ringworm cassia | Tropical America |
| *C. biflora* L. | Fabaceae | — | Tropical America |
| *Catesbaea spinosa* L. | Rubiaceae | Lily Thorn, prickly apple | Florida and the West Indies |
| *Cestrum diurnum* L. | Solanaceae | King of the Day (Day Jasmine) | West Indies, South America |
| *C.nocturnum* L. | Solanaceae | Queen of the night | West Indies |
| *C.elegans* Schlecht. | Solanaceae | — | Mexico |
| *Clerodendrum fragrans* (Vent.) Willd. | Verbenaceae | Burma cone-Head, Fragrant clerodendron | Japan |
| *C.inerme* (L.) Gaertn. | Verbencaceae | Forest Jasmine | India |
| *C.paniculatum* L. | Verbencaceae | Kashmir bouquet, pagoda flower, Quezonia | Southeast Asia, Japan, New Guinea |
| *Codiaeum variegatum* L. | Euphorbiaceae | Croton | Malaysia |
| *Crossandra undulaefolia* Salisb. | Acanthaceae | Fire cracker flower | India, Sri Lanka |
| *Daedalacanthus nervosus* T . Anders. | Acanthaceae | Maze plant flower | India |

*Contd.*

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| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Erythrina blakei* Hort. ex. Parker | Fabaceae | Blake's Coral tree, Indian Coral tree | — |
| *E. crista-galli* L. | Fabaceae | Coral tree, Cockspur Coral tree | Brazil |
| *E. resupinata* Roxb. | Fabaceae | — | — |
| *Euphorbia cotinifolia* L. | Euphorbiaceae | Hierbamala white Christmas | West Indies, Mexico to Venezuela |
| *E. leucocephala* Lotsy ex Klotz. | Euphorbiaceae | White poinsettia, White lace euphorbia, White Chritstmas | West Indies, Mexico and Guatemala |
| *E. pulcherrima* Willd. (syn. *Poinsettia pulcherrima* R. Grah. | Euphorbiaceae | Poinsettia Christmas flower | Mexico and Central America |
| *Excoecaria bicolor* Hassk (syn. *E. cochinchinensis* Lour.) | Euphorbiaceae | Chinese croton | Southeast Asia |
| *Fortunella japonica* (Thunb.) Swingle (syn. *Citrus japonica* Thunb.) | Rutaceae | Kumquat (*Narangi*) | East Asia, Malaysia |
| *Gardenia jasminoides* Ellis (syn. *G. florida* L.) | Rubiaceae | Cape jasmine, Gardenia | China |
| *G. lucida* Roxb. (syn. *G. resinifera* Roth.) | Rubiaceae | Gambi Resin | India and Myanmar |
| *Gmelina asiatica* L. | Verbenaceae | Badhara bush, Asiatic beech-berry | Eastern Asia, northern Australia |
| *Graptophylum pictum* (L.) Griff. | Acanthaceae | Caricature plant | Southeast Asia, Polynesia |
| *Gustavia insignis* Linden ex Hook. | Lecythidaceae | Gustavia, Scarlet bush | South Africa |
| *Hamelia patens* Jacq. | Rubiaceae | Rat poison tree, Fire bush | Tropical and Subtropical America |
| *Hamiltonia suaveolens* Roxb. | Rubiaceae | Bain champa | India, China |
| *Hibiscus mutabilis* L. | Malavceae | Confederate rose, Changeable rose, Cotton rose | China |
| *H. rosa-sinesis* L. | Malavaceae | China rose, Chinese Hibiscus | China |
| *H. schizopetalus* (Mast.) Hook. f. | Malavaceae | Japanese Lantern, Coral Hibiscus, Fringed Hibiscus | Tropical East Africa |
| *H. syriacus* L. | Malavaceae | Rose of Sharon | Syria |

*Contd.*

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| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Holmskioldia sanguinea* Retz. | Rubiaceae | Chinese hat plant, Parasol or Cup and Saucer plant | India |
| *Hydrangea macrphylla* A.P. DeCandolle (syn. *H. hortensis* Sm.) | Saxifragaceae | French or Chinese Hydrangea, Common Hydrangea | China |
| *Ixora chinensis* Lamk. | Rubiaceae | Chinese Ixora | China |
| *I. coccinea* L. | Rubiaceae | Flame of the woods | East Indies |
| *I. parviflora* Vahl. (syn. *I. arborea* Roxb.) | Rubiaceae | Torchwood Ixora, Small-flowered Ixora | India |
| *Jacobinia carnea* (Lindl.) Nicholson | Acanthaceae | Flamingo plant, Brazilian plume flower | Brazil |
| *Jacquinia ruscifolia* Jacq. | Theophrastaceae | Thorny Jaquinia | Tropical America |
| *Jasminum humile* L. | Oleaceae | Yellow Jasmine, Italian Jasmine | Tropical Asia |
| *J. pubescens* Willd. (syn. *J. multiflorum* (Burm. f.) Andr.) | Oleaceae | Downy Jasmine, Star Jasmine | China, India |
| *J. sambac* (L.) Ait. | Olaceae | Arabian Jasmine, Tuscan Jusmine | India |
| *Jatropha gossypifolia* L. | Euphorbiaceae | Bellyache Bush | Brazil |
| *J. multifida* L. | Euphorbiaceae | Coral plant | South America |
| *J. integerrima* Jacq. (syn. *J. panduraefolia* Andr.) | Euphorbiaceae | Peregrina Jatropha, Fiddle-Leaved Jatropha | West Indies |
| *J. podagrica* Hook. | Euphorbiaceae | Guatemala Rhubarb, Gouty Foot, Physic nut | Central America |
| *Justicia gendarussa* Burm. f. | Acanthaceae | — | India, Myanmar |
| *J. ovata* (Walt.) Lindau | Acanthaceae | Loose water willow, Pride of India | Southern USA |
| *Lagerstoremia indica* L. | Lythraceae | Crape myrtle | China |
| *Lantana camara* L. (syn. *L. aculeata* L.) | Verbenaceae | Shrub verbena, Yellow sage, Cloth of Gold | Jamaica, Tropical America |
| *L. sellowiana* Link & Otto. | Verbenaceae | Weeping or Trailing lantana | — |
| *Lawsonia inermis* L. (syn. *L. alba* Lamk.) | Lythraceae | Tree mignonette | North Africa, South-West Asia |
| *Magnolia fuscata* Andr. | Magnoliaceae | Banana Magnolia, Port Wine Magnolia | China |
| *Malpighia coccigera* L. | Malpighiaceae | Singapore Holly | West Indies |
| *M. glabra* L. | Malpighiaceae | Barbados cherry, Jamaica cherry | Tropical America, West India |

*Contd.*

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| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Malvaviscus arboreus* Cav. | Malvaceae | Wax Mallow, Sleeping Mallow, Giant Fire Dart, Turk's Cap, Fire Cracker Hibiscus | South America |
| *Manihot esculenta 'variegata'* Crantz | Euphorbiaceae | — | Tropical America |
| *Melastoma malabathricum* L. | Melastomataceae | Indian Rhododendron, Malabar Gooseberry | Tropical Asia, Australia |
| *Memecylon ellipticum* Thwaites | Melastomataceae | — | Tropical Africa, Asia, Australia |
| *Muehlenbeckia platyclada* (F. Muell.) Meissn. (syn. *Coccoloba platyclada* F. Muell. ex Hook.) | Polygonaceae | Ribbon Bush, Centipede Plant, Tapeworm Plant | New Guinea, Australia, New Zealand |
| *Murraya paniculata* (L.) Jack. | Rutaceae | Chinese Box, Orange Jasmine, Mock Orange | India |
| *Mussaenda erythrophylla* Schum. & Thonn. | Rubiaceae | Red Flag Bush | Africa |
| *M. philippica* A Rich. | Rubiaceae | Flagbush | Philippines |
| *M. frondosa* L. | Rubiaceae | Paper chase tree, Oholoy's Tree | Tropical Asia |
| *M. luteola* Delilie | Rubiaceae | The Yellowish Dhobu's Tree | Tropical Africa |
| *Nandina domestica* Thunb. | Berberidaceae | Sacred Chinese Bamboo | China |
| *Nerium oleander* L. (syn. *N. odorum* Soland., *N. indicum* Mill.) | Apocynaceae | Oleander, Sweet scented oleander | Mediterranean, Asia and Japan |
| *Ochna jabotapita* L. (syn. *O. squarrosa* L.) | Ochnaceae | Bird's Eye Bush | Asia, Africa |
| *Oncoba spinosa* Forsk. | Flacourtiaceae | Bride of the desert | Tropical Africa |
| *Pachystachys lutea* Nees | Acanthaceae | Lollipop plant, Candle plant, Golden shrimp plant | West Indies, South America |
| *Pentas lanceolata* (Forsk.) Schum. | Rubiaceae | Egyptian star cluster | Tropical Africa |
| *Plumbago capensis* Thunb. | Plumbaginaceae | Cape leadwort | South Africa |
| *P. zeylanica* L. | Plumbaginaceae | Ceylon Leadwort, White Leadwort | East Indies |
| *Polyscias balfouriana* Baily (syn. *Aralia balfouriana* Hort. ex Andr. | Araliaceae | Aralia | Asia and Pacific |
| *Punica granatum* L. | Punicaceae | Pomegranate | East Mediaterranean and Palestine |

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| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Quassia amara* L. | Simaroubaceae | Quassia, Surinam Bitterwood | America and Africa |
| *Ravenia spectabilis* Griseb. (syn. *Limonia spectabilis* Lindl.) | Rutaceae | — | Cuba and Brazil |
| *Rondeletia odorata* Jacq. | Rubiaceae | Sweet-smelling Rondelitia | Cuba |
| *Ruellia rosea* (Nees) Hemsl. | Acanthaceae | Wild Petunia | South America, Brazil |
| *Russelia equisetiformis* Schlecht. and Cham. (syn. *R. juncea* Zucc.) | Scrophulariaceae | Coral bush, Coral plant, Coral blow, Fountain bush, Fire cracker plant | Mexico and Central America |
| *R. sarmentosa* Jacq. | Scrophulariaceae | Coral blow | — |
| *Sambucus canadensis* L. | Sambucaceae | American elder | Canada, Eastern North America |
| *Sanchezia nobilis* Hook. f. | Acanthaceae | Brilliant-flowered Sanchezia | Ecuador |
| *Solanum grandiflorum* Ruiz. and Pav. (syn. *S. macaranthum* Dunal, *S. wrightii* Benth.) | Solanaceae | Potato Tree | Tropical South America (Brazil, Chile) |
| *Stachytarpheta indica* (L.) Vahl. | Verbenaceae | Brazilian Tea-Tree | West Indies |
| *Streptosolen jamesonii* Benth. | Solanaceae | Marmalade Bush, Orange Browallia | Colombia, Ecuador |
| *Strobilanthes dyerianus* Mast. | Acanthaceae | Persian shield | Myanmar (Burma) |
| *Strophanthus wallichii* A. DC. | Apocynaceae | — | South Africa |
| *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult. | Apocynaceae | Crepe Jasmine, Adam's Apple | India |
| *Tecoma stans* (L.) H.B.K. | Bignoniaceae | Yellow bell, Yellow trumpet flower | Central and South America |
| *Tecomaria capensis* (Thunb.) Spach. | Bignoniaceae | Cape Honey Suckle | Central and South America |
| *Vitex agnus-castus* L. | Verbenaceae | Chaste Tree, Hemp Tree | Southern Europe, Western Asia |
| *Woodfordia fruticosa* (L.) Kurz. (syn. *W. floribunda* Salisb.) | Lythraceae | Fire-flame bush, Dharu-Dhao | India |
| *Wormia burbidgii* Hook f. (syn. *Dillenia burbidgii* Mart.) | Dilleniaceae | — | Northern Borneo |

## Some Beautiful Climbers

| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Adenocalymma alliaceum* Miers. | Bignoniaceae | Garlic vine | South America |
| *A.comosum* DC. | Bignoniaceae | Yellow trumpet vine | South America |
| *Allamanda cathartica* L. | Apocynaceae | Golden trumpet | Brazil |
| *A. violacea* Garden. | Apocynaceae | Purple allamanda | South America (Brazil) |
| *Antigonon leptopus* Hook. & Arn. | Polygonaceae | Coral Vine, Mexican Creeper, Pink Vine, Queen's Jewel, Chain of Love | Mexico |
| *Aristolochia grandiflora* Sw. | Aristolochiaceae | Pelican flower, Swan flower, Duck flower | Guatemala |
| *A.ornithocephala* Hook. | Aristolochiaceae | The Bird's head flower, the Bird's head Birthwort | Brazil |
| *Argyreia nervosa* Boj. (syn. *A. speciosa* Sweet.) | Convolvulaceae | Woolly morning glory, Elephant Creeper, Silver morning glory | India, China and Indonesia |
| *Artabotrys odoratissimus* R. Br. | Annonaceae | Climbing Ylang-ylang | Tropical Africa, Indo-Malaysia |
| *Banisteria laevifolia* Juss. | Malpighiaceae | — | Brazil, Paraguay |
| *Bauhinia vahli* W. &A. | Fabaceae | Climbing Bauhnia, Camel's Foot Climber | — |
| *Beaumontia grandiflora* (Roxb.) Wall. | Apocynaceae | Nepal Trumpet Creeper | India |
| *Bougainvillea spectabilis* Willd. | Nyctaginaceae | Bougainvillea | South America |
| *Campsis grandiflora* (Thunb.) K. Schum. (syn. *Tecoma grandiflora* Delaun.) | Bignoniaceae | Chinese Trumpet creeper | China, Japan |
| *C. radicans* Seem (syn. *Tecoma radicans* Juss.) | Bignoniaceae | Trumpet Climber, Trumpet vine | North America |
| *Cardiospermum helicacabum* L. | Sapindaceae | Love-in-a puff, Balloon vine, Blaster creeper | Asia, Africa |
| *Cissus discolor* Blume (syn. *Vitis discolor* (Blume) Dalziel) | Vitaceae | Rex Begonia vine, Painted cissus | Tropical SE Asia |
| *Clematis gouriana* Roxb. ex DC. | Ranunculaceae | Gourian clematis | China |
| *C. paniculata* Thunb. | Ranunculaceae | Virgin's Bower, Sweet Clematis | North America, North Asia |
| *Clerodendurm splendens* G. Don. | Verbenaceae | Glory Bower | West and Central Africa |

*Contd.*

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| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *C. thomsonae* Balf. | Vernenaceae | Bleeding Heart vine, Glory Bower | W. Africa |
| *Clitoria ternatea* L. | Fabaceae | Mussel-shell creeper | Asia and Singapore |
| *Cobaea scandens* A.J. Cav. | Polemoniaceae | Cup and Saucer vine, Purple Bell Climber, Cathedral Bells | Tropical America (Mexico) |
| *Combretum coccineum* (Sonn.) Lam. | Combretaceae | Flame vine | Malagasy Republic |
| *C. comosum* G. Don. | Combretaceae | — | Tropical and South Africa |
| *Congea tomentosa* Roxb. | Verbenaceae | Shower orchid, Lavender wreath | Thailand and Myanmar, NE India |
| *Cryptostegia grandiflora* (Roxb.) R. Br. | Asclepiadaceae | Indian Rubber vine | Malagasy Rupublic |
| *C. madagascariensis* Boj. | Asclepiadaceae | — | — |
| *Doxantha unguis-cati* (L.) Rehd. (syn. *Bignonia unguis-cati* L.) | Bignoniaceae | Cat's Claw, Yellow Trumpet Vine, Hug me Tight | |
| *Echites caryophyllata* Roxb. | Apocynaceae | — | Florida, Mexico, NE Colombia, West Indies |
| *Ficus pumila* L. (syn. *F. repens* Rottl.) | Moraceae | Creeping Fig, Ivy-Like Fig. | Japan and China |
| *Gloriosa rothschildiana* O' Brien | Liliaceae | Glory lily, Red Climbing Lily | South Africa |
| *G. superba* L. | Liliaceae | Glory Lily | Tropical Africa |
| *Gmelina philippensis* Cham. (syn. *G. hystrix* Schult. ex Kurz.) | Verbenaceae | Parrot's Beak, Ching-Chai | Philippines |
| *Hiptage madablota* Gaertn. [syn. *H. benghalensis* (L.) Kurz.] | Malpighiaceae | Cluster Hiptage | India and Malaya |
| *Holmskioldia sanguinea* Retz. [syn. *H. rubra* (Juss.) Pers.] | Verbenaceae | Cup and Saucer plant, Chinese Hat Plant | Subtropical Himalayas |
| *Hoya carnosa* R. Br. | Asclepiadaceae | Porcelain flower, wax plant, wax flower | South Asia, Australia, Polynesia |
| *Ipomoea bonanox* (L.) House (syn. *I. alba* L.) | Convolvulaceae | Moon flower | Tropical America |
| *I.palmata* Forsk. | Convolvulaceae | Native Morning glory, Railway Creeper | South America |

*Contd.*

*Contd.*

| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *I. purpurea* (L.) Roth | Convolvulaceae | Common Morning glory | Tropical America |
| *Jacquemontia violacea* (Vahl.) Choisy. [syn. *J. pentantha* (Jacq.) G. Don.] | Convolvulaceae | Skyblue Cluster vine, pentantha | Tropical America |
| *Jasminum angustifolium* Vahl. | Oleaceae | Wild Jasmine | South India |
| *J. auriculatum* Vahl. | Oleaceae | Joohi | India |
| *J. grandiflorum* L. | Oleaceae | Spanish Jasmine, Common Jasmine | NW Himalayas |
| *J. officianale* L. | Oleaceae | White jasmine, common Jasmine | Persia, North India |
| *Lonicera japonica* Thunb. | Caprifoliaceae | Japanese honeysuckle | China, Japan, Taiwan |
| *L. sempervirens* L. | Caprifoliaceae | Trumpet honeysuckle | Southern USA |
| *Pandorea jasminoides* (Lindl.) K. Schum. (syn. *Tecoma jasminoides* Lindl.) | Bignoniaceae | The Bower Plant of Australia | Australia |
| *Passiflora caeruela* L. | Passifloraceae | Blue Passion-flower | Brazil |
| *P. coccinea* Aubl. | Passifloraceae | Red passion flower, Red Granadilla | South America |
| *P. edulis* Sims. | Passifloraceae | Passion fruit, Purple Granadilla | Brazil |
| *P. holosericea* L. | Passifloraceae | Silky-leafed passion flower | South America |
| *P. quadrangularis* L. | Passifloraceae | Giant granadilla, Passion flower, Apple ball | Tropical America |
| *P. violacea* Vell. | Passifloraceae | Purple passion flower | Brazil |
| *Petrea volubilis* L. | Verbenaceae | Purple wreath, Sandpaper vine, Queen Wreath | Tropical America |
| *Phaedranthus buccinatoria* (DC.) A. Gentry (syn. *Bignonia cherere* Lindl.) | Bignoniaceae | Blood trumpet vine | Mexico |
| *Philodendron scandens* Micans | Araceae | Heart leaf or velvet philodendron | Topical America |
| *Podranea ricasoliana* (Tanf.) Sprague [syn. *Pandorea ricasoliana* (Tanf.) Baill.] | Araceae | Pink trumpet vine | South Africa |
| *Porana paniculata* Roxb. | Convolvulaceae | Bridal creeper | India |
| *Pseudocalymma alliaceum* (Lam.) Sandwith [syn. *Adenocalymma alliaceum* (Lam.) Miers] | Bignoniaceae | Garlic vine | Tropical America (Guatamela) |

*Contd.*

*Contd.*

| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Pyrostegia venusta* (Ker.-Gwal) Miers (syn. *Bignonia venusta* Ker.) | Bignoniaceae | Flame, Golden shower vine, Orange trumpet vine | Brazil |
| *Quamoclit coccinea* Moench. | Convolvulaceae | Star Ipomoea | North Mexico, Arizona (US) |
| *Q. pinnata* (L.) Bojer (syn. *Ipomoea pinnata* Hochst. ex Choisy.) | Convolvulaceae | Cypress vine, Cardinal climber, Indian pink creeping tuberose | Tropical America |
| *Quisqualis indica* L. | Combretaceae | Rangoon creeper | SE Asia |
| *Scindapsus aureus* Engler (syn. *Rhaphidophora aurea* (Lind. & Andre) Birdsey; *Epipremnum aureum* (Lind. & Andre) Bunting) | Araceae | Golden Pathos or Money Plant, Devil's Ivy | Solomon Islands |
| *Senecio confusus* Britten | Asteraceae | Mexican flame vine, Mexican daisy | North America, Mexico |
| *Stephanotis floribunda* Brongn. | Asclepiadaceae | Madagascar Jasmine, Clustered Wax flower | Malagasy Republic |
| *Stigmaphyllon ciliatum* (Lamk.) A. Juss. | Malpighiaceae | Brazilian gold vine, Orchid vine | Tropical America |
| *S. periplocifolium* (Desf.) A. Juss. | Malpighiaceae | Sweet pea, Monarch Amazon vine | Tropical America and Cuba |
| *Strophanthus grandiflorus* N.E. Br. | Apocynaceae | Sand forest poison rope | Tanzania, Mozambique |
| *S. gratus* (Benth.) Baill. | Apocynaceae | Cream fruit, Indian rubber vine | Tropical West Africa |
| *Syngonium podophyllum* Schott. | Araceae | Arrowhead vine, Goosefoot plant, African evergreen | Mexico |
| *Thunbergia alata* Boj. | Acanthaceae | Black-eye Susan | South Africa |
| *T. fragrans* Roxb. | Acanthaceae | White thunbergia, Sweet clock vine | India and Myanmar, China |
| *T. grandiflora* Roxb. | Acanthaceae | Sky vine, Blue trumpet vine | India |
| *T. mysorensis* T. Aderson | Acanthaceae | Brick and Butte vine | Southern India |
| *Trachelospermum jasminoides* Lamk. (syn. *Rhynchospermum jasminoides* Lindl.) | Apocynaceae | Star Jasmine | China |
| *Tristellateia australis* A. Rich. | Malpighiaceae | Golden Rod, Galphimia vine, Maiden's Jealousy | Malaysia, Australia |
| *Vallaris heynei* Spr. (syn. *V. dichotoma* Wall. ex Don. | Apocynaceae | — | Southeast Asia and Malaysia |

*Contd.*

*Contd.*

| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Vernonia elaeagnifolia* DC. | Asteraceae | Vernonia creeper, Curtain creeper | Myanmar |
| *Wisteria sinensis* (Simms) DC. | Fabaceae | Chinese wisteria | China |

** Some of the annual climbers are: *Pharbitis purpurea, P. tricolor, Quamoclit coccinea and Q . lobata (Mina lobata)*, and *Calonyction aculeatum* of the family Convolvulaceae; *Clitora ternatea* (Fabaceae); *Cobaea scandens* (Polemoniaceae); *Maurandia barclayana* (Scrophulariaceae); *Thunbergia alata* (Acanthaceae); *Tropaeolum peregrinum* and *T. majus* (Tropaeolaceae); and *Lathyrus odoratus* (Fabaceae)

## Exotics

| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *Alstroemeria aurantiaca* D. Don. | Amaryllidaceae | Chilean Lily, Peruvian Lily | South America |
| *A.versicolour* Ruiz and Pavon | Amaryllidaceae | — | South America |
| **Banksia australis* R. Br. | Proteaceae | Banksia | Australia |
| *Eustoma grandiflorum* (Raf.) Shinn (syn. *Lisianthus russellianum* (Don.) Griseb) | Gentianaceae | Praire gentian | USA |
| *Leucospermum cordifolium* (Salib. ex Knight) Rourke (syn. *L. nutans* R. Br.) | Proteaceae | Nodding Pincushion | South Africa |
| *Liatris spicata* (L.) Willd. | Asteraceae | Button snakeroot, Blazing star | East and South USA |
| *Protea cynaroides* (L.) L. | Proteaceae | Giant Protea, King Protea | Cape Province (S. Africa) |
| *P. grandiceps* Tratt. | Proteaceae | Princess Protea, Red Sugar bush, Peach Protea | South Africa |
| *Heliconia psittacorum* L. | Musaceae | Parrot's Plantain, Wild Bird of paradise | Tropical America |
| *H. rostrata* Ruiz and Pavon | Musaceae | Hanging lobster claw, Beaked Heliconia | Tropical America |
| *Strelitzia reginae* Banks | Musaceae | Bird of Paradise Flower, | South Africa |
| ***S. nicolai* Regel & Koern. | Musaceae | Giant Bird of Paradise, Natal Wild Banana, Blue Strelitzia | South Africa |
| *Anthurium andreanum* Linden | Araceae | Tail flower | Tropical Central and South America |

*Contd.*

*Contd.*

| *Botanical name* | *Family* | *Common name* | *Native place* |
|---|---|---|---|
| *A.crystallinum* Lind. & Andre | Araceae | Crystal Anthurium | Tropical America (Colombia, Peru) |
| *A.scherzerianum* Schott. | Araceae | Flaming flower or flame flower, Pinter's palette | Costa Rica |

* It is named after Sir Joseph Banks, the renowned British botanist who travelled with Captain Cook in 1770.

** The species name *nicolai* was given in honour of Czar Nicholas of Russia not after Nicolai Ivanovitch Vavilov — the famous Russian geneticist and phytogeographer.

Some of the garden ornamental annuals are given for the benefit of readers:
*Ageratum houstonianum and A. conyzoides* (Floss Flower) (Asteraceae), *Alcea rosea* (syn. *Althaea rosea*) (Hollyhock) (Malavaceae), *Alyssum maritimum* (Sweet Alyssum) (Brassicaceae), *Amaranthus splendens* (Love-lies-bleeding) (Amaranthaceae), *Antirrhinum majus* (Snapdragon) (Scrophulariaceae), *Aster amellus* (Perennial Aster/Michaelmas Daisy) (Asteraceae), *Callistephus chinensis* (China Aster) (Asteraceae), *Calendula officinalis* (Pot Marigold) (Asteraceae), *Campanula latifolia* (Bell Flower) (Campanulaceae), *Celosia cristata* (Cock's Comb) (Amaranthaceae), *Chrysanthemum coronarium* (Crown Daisy) (Asteraceae), *Cineraria hybrida* var. *grandiflora* (Cineraria) (Asteraceae), *Coreopsis grandiflora* (Tick Seed) (Asteraceae), *Cosmos bipinnatus* (Mexican Aster) (Asteraceae), *Dahlia pinnata* (Asteraceae), *Delphinium ajacis* (Larkspur) (Ranunculaceae), *Dianthus chinensis* (Indian Pinks, Carnations) (Caryophyllaceae), *Gaillardia drummondii* (Blanket Flower) (Asteraceae) *Gomphrena globosa* (Bachelor's Button) (Amaranthaceae), *Helianthus annuus* (Sunflower) (Asteraceae), *Helichrysum bracteatum* (Everlasting Flower) (Asteraceae), *Helipterum roseum* (*Acroclinium roseum*) (Acroclinium) (Asteraceae), *Iberis amara* (Candytuft) (Brassicaceae), *Impatiens balsamina* (Balsam) (Balsaminaceae), *Ipomoea purpurea* (Morning Glory) (Convolvulaceae), *Kochia scoparia* var. *trichophylla* (Summer Cyprus), *Lathyrus odoratus* (Sweet Pea) (Fabaceae), *Linum usitatissimum* (Flax) (Linaceae), *Nicotiana (alata) grandiflora* (Flowering Tobacco) (Solanaceae), *Papaver rhoeas* (Shirley/Corn Poppy) (Papavaeraceae), *Petunia hybrida* (Petunia) (Solanaceae), *Phlox drummondii* (Polemoniaceae), *Portulaca grandiflora* (Rose Moss/Table Rose) (Portulacaceae), *Pimpinella monoica* (Lady's Lace) (Apiaceae), *Rudbeckia fulgida* var. *speciosa* (Cone Flower) (Asteraceae), *Salvia splendens* (Scarlet Sage) (Lamiaceae), *Schizanthus hybridus* var. *grandiflora* (Butterfly Flower) (Solanaceae), *Tagetes erecta* (African Marigold) (Asteraceae), *Torenia flava* (Scrophulariaceae), *Tropaeolum majus* (Nasturtium) (Tropaeolaceae), *Verbena grandiflora* (Verbenaceae), *Viola tricolor* (Garden Pansy) (Violaceae), *Zinnia elegans* (Zinnia) (Asteraceae).

# Appendix IX – Microchemical Tests for Major Plant Reserve Food Materials

## Carbohydrates

Among the most commonly occurring carbohydrates are the monosaccharides (reducing sugars, notably glucose and fructose) the disaccharides (non-reducing sugars, especially sucrose or table sugar) and the polysaccharides (mainly starch and cellulosic cells walls). Sucrose is the principal metabolite but other sugars notably fructose and glucose are also present. Sugars in plants are often stored as starch.

### GENERAL TEST FOR CARBOHYDRATES

To 2 ml of the test solution add 2-3 drops of Molisch's reagent (5% α-naphthol in ethanol). Mix it well and then pour 2 ml of concentrated sulphuric acid gently down the side of test tube to form a lower acid layer. A purple ring formed at the junction of the two liquids indicates the presence of carbohydrates. Repeat the above test using water instead of carbohydrate solution – a negative test results.

> Furfural or (furfural derivatives) so formed during acid hydrolysis react with α-naphthol to form a purple product.

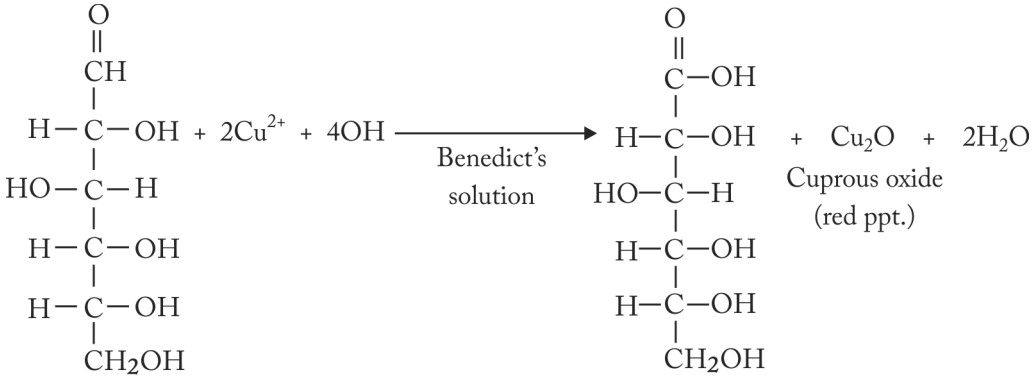
(*A) Distinction between reducing and non-reducing sugars*

(*i*) To 1 ml of test solution add 2 ml of freshly prepared (1:1) solution of Fehling A and B. Boil the mixture. The formation of brownish-red or brick-red precipitate indicates the presence of reducing sugars. The cupric hydroxide present in Fehling's solution is reduced to red cuprous oxide on heating with reducing sugars (having free aldehydic or ketonic groups).

> Monosaccharides (such as glucose) which contains an aldehyde group are known as aldolases whilst those which contain keto group, such as fructose, are ketoses. Those sugars with a free, or potentially free, aldehyde or keto groups are thus known as reducing sugars.

(*ii*) To about 2 ml Benedict's qualitative reagent add 5 drops of test solution. The mixture is then boiled. A brick-red, yellow or green precipitates indicates the presence of reducing sugar.

> Benedict's and Fehling's solution contain the cupric ion, $Cu^{2+}$, which gives the reagent a blue colour. When glucose is added to Benedict's solution, the aldehyde group is oxidized to a carboxylic acid group, while the cupric ion $Cu^{2+}$, is reduced to the cuprous ion, Cu+. The products are gluconic acid an insoluble solid, a precipitate of cuprous oxide, $Cu_2O$.




Glucose Gluconic acid

The intensity of the colour is a measure of the concentration of glucose present.

**The disaccharide (cane-sugar) will give a negative test in both the reactions**

**(*B) Hydrolysis test for non-reducing sugars***

To about 5 ml of sucrose solution (or cane-sugar juice) add 5 drops of concentrated hydrochloric acid and then heat it for 5 minutes. The solution is made slightly alkaline with 10% NaOH. With this hydrolysed solution, perform the Fehling's or Benedict's test as described earlier. Appearance of yellow or red precipitates of cuprous oxide indicates the formation of reducing sugars during hydrolysis.

> Cane-sugar when boiled with HCI yields reducing sugars.

**(*C) Test for starch***

A test solution containing starch gives a blue colour with a very weak solution of iodine (0.3 g. iodine, 1.5 g Kl, 100 ml of water.) The blue colour disappears on heating but returns on cooling.

Iodine-stained starch grains preparations appear blue when viewed under the microscope. Different types of starch grains can be recognized on the basis of their structural features such as accentric or concentric organization; simple or compound nature (for details see the text).

> Starch stains red when composed entirely (100%) of amylopectin as in the case of waxy corn; only the amylose fraction gives an intense blue colour with iodine.

## Cellulose

The best known structural polysaccharide is cellulose which is made up of a linear chain of glucopyranose units linked by β–1, 4–glycosidic bonds. Inulin occurs as a reserve polysaccharide in certain members of Asteraceae and Campanulaceae.

(*i*) Mount cotton fibres in water on s slide and place the cover slip on. Irrigate the mount with a few drops of concentrated sulphuric acid and then with iodine solution. Draw the liquid away from the cover glass by placing a filter paper at the opposite end. Nose the cotton threads are coloured blue.

This test can be performed with a lump of cotton fibres kept in a watch glass. Pour a little of iodine solution. Then fibres are just given a touch of sulphuric acid with a glass rod otherwise with excess sulphuric acid the colour will turn black.

> Cellulose is hydrolysed by concentrated sulphuric acid into a colloidal hydrocellulose which forms a blue absorption compound with iodine.

(*ii*) The cotton fibres or jute fibres turn blue when treated with chlor-zinc-iodine (Schultze's solution)

(*iii*) To the cotton fibres kept in a watch glass add a few drops of iodine. These are coloured yellow.

## Proteins

(*i*) ***Xanthoproteic test***

To the solution in a test tube add a few drops of strong nitric acid. A cloudy white precipitate is formed which on warming turns yellow. Cool it and then add ammonium hydroxide sufficient enough to make it basic. The colour change to orange.

(*ii*) ***Biuret test***

To a few ml of protein solution add about 1 ml of dilute solution of caustic soda (40%). Then put 'few drops' of copper sulphate solution. A bluish violet colour is produced.

(*iii*) ***Millon's test***

Add a few drops of Millon's reagent to a test tube containing few ml of protein solution. A white precipitate is formed which on boiling turns into a reddish-brown flocculation. The colour deepens further when $NaNO_2$ solution is added.

Millon's reagent is prepared by adding one part of mercury to two parts of strong nitric acid in the cold. After the mercury has completely dissolved (a little gentle warming may be necessary) the solution is diluted with two volumes of water.

## Oils and Fats

(*i*) Shake a little groundnut or mustard oil with an alcoholic solution of Sudan III or Sudan IV (prepared by dissolving 0.5 g in 100 ml of 70% alcohol). The oil will be stained red.

*or*

Boil the aqueous suspension containing oil (obtained by crushing oilseeds such as groundnut or castor with the help of a pestle and mortar) in a test tube. A translucent layer appears on

the surface. Pour a little of Sudan III and shake it lightly. A red colour in the upper layer indicates the presence of fat.

*or*

Satin the cut sections of plant material with Sudan III. The oil droplets are stained red. The cuticle, if present, will take up red stain (e.g., *Nerium* leaf or *Capsicum* fruits). Repeat the experiment with a transverse section of young maize root and try to locate the casparian band in the endodermis.

| Cutinised and suberized walls (as in the potato peel or skin) turn red with Sudan III. |
|---|

(*ii*) Add few drops of 1% osmic acid to oil or fat in a test tube. A black colour is produced.

## Lignin

(*i*) To jute fibres or coir fibres or a newspaper strip (even a match-stick handle) add few drops of acidified phloroglucinol (a saturated solution of phloroglucinol in 18% hydrochloric acid.) Lignin in the cell walls stains cherry red.

(*ii*) Treat jute fibres with a solution of aniline hydrochloride or aniline sulphate. The fibres turn bright yellow in colour.

(*iii*) To the lignin containing plant material kept in a watch-glass add 1% potassium permanganate solution. Keep it for five minutes (the Maule test). After thorough washing in water place it in dilute hydrochloric acid (2%) for a minute. Wash it again and keep in a solution of 2% ammonium hydroxide or sodium bicarbonate. Lignin is stained deep red to brown.

*or*

Sections with lignified tissue can be treated in a similar manner and then mounted in 2% ammonium hydroxide.

## Pectin

To a little pulp (obtained by pressing some fleshy fruits like apple or melon) add few drops of methylene blue. Pectic substances in the cell wall are stained violet.

## Tannins

Mount thin sections of tea leaves or rose stem with 10% aqueous solution of ferric chloride. The cells containing tannins turn black.

## Callose

Stains T.S. and L.S. of *Cucurbita* stem for 15-20 minutes with an aqueous solution of aniline blue and then mount in glycerine. Callose, present in the perforations of sieve plates, is stained blue.

# Appendix X – Aquatic Plants used as a Source of Human Food

| *Botanical name* | *Family* | *English name* | *Indian name* | *Centre of origin* | *Main uses* |
|---|---|---|---|---|---|
| *Colocasia esculenta* (L.) Schott | Araceae | Taro | Arvi, Kachalu | South-east Asia | Starchy tuberous roots are edible; used as potato substitute |
| *Cyrtosperma chamissonis* (Schott) Merr. (syn. *C. edule* Schott) | Araceae | Swamp Taro | — | East Asia, Malaysia, Pacific | Corms are eaten cooked as a vegetable or made into flour |
| *Eleocharis dulcis** (Burm. f.) Trin ex Henschel (syn. *E. tuberosa* Schult.) | Cyperaceae | Chinese water chestnut | — | East Asia | Tubers are a vegetable delicacy throughout the Orient, common ingredient in chopsuey and meat and fish dishes |
| *Euryale ferox* Salisb. | Euryalaceae | Gorgan nut, Fox nut | Makhana | South China, Tropical Asia | Seeds are roasted and eaten |
| *Ipomoea aquatica* Forsk. (syn. *I reptans* Poir.) | Convolvulaceae | Water spinach | — | India, South-east Asia, Taiwan and Southern China | Young leaves and stems used as vegetable; also used in pickles |

*Contd.*

*Contd.*

| *Botanical name* | *Family* | *English name* | *Indian name* | *Centre of origin* | *Main uses* |
|---|---|---|---|---|---|
| *Nasturtium officinale* R. Br. | Brassicaceae | Watercress | Brahmi sag | Europe and northern Asia | Used as fresh-salad or as a cooked green vegetable |
| *Nelumbo nucifera* Gaertn. | Nelumbonaceae | Sacred lotus or Indian lotus | Kamal, kanwal (fruiting torus known as 'Kamalgatta', 'Kaul chapni') | China, Japan, India | Farinaceous rhizomes (*Kamal-kakdi,*** *bhen*) are cooked as vegetable or pickled: seeds are eaten raw or roasted |
| *Sagittaria sagittifolia* L. | Alistmataceae | Arrow-head | Chotakut | Temperate regions of Europe and Asia | Tubers are eaten as vegetable |
| *Trapa bispinosa* Roxb. [syn. T. *natans* L. var. *bispinosa* (Roxb.) Makino] | Trapaceae | Water chest-nut, Caltrops, singhara nut | Singhara or Paniphal | Eurasia and Africa | Fruits are eaten raw, dried kernels are ground into a meal (substitute for cereal flour), |
| T. *bicornis**** Osbeck. | Trapaceae | Water caltrop, Buffalo nut | Singhara | Eurasia and Africa | Consumed on the Hindu fasting days, the *Navratras* |
| *Zizania aquatica* L. | Poaceae | American wild rice | — | North America, Asia | Staple food for tribal people, good feed for duck, and other waterfowl |

* It is sedge, whose round, crisp-fleshed corms are commonly used in Western-style, Chinese food and it should not to be confused with the unrelated *Trapa* species.

** Lotus stem (*Kamal-Kakdi* or *Bhen*) contains about 3007.0 mg of potassium/100 gm.

*** The fruits resemble the head of a bull.