PN-AAN-169
ISN: 29843

U.S. National Academy of Sciences

Workshop on

"Priorities in Biotechnology Research for International Development"

Washington, D.C., July 26-30, 1982

Perspectives in Biotechnology Research from the Point of View of Third World Agriculture

by

Dr. M. S. Swaminathan
Director General
International Rice Research Institute
Los Baños, Laguna, Philippines
and Independent Chairman, FAO Council

I. Introduction:

Biotechnology in a broad sense encompasses many facets of the management and manipulation of the biological system. In 1981, the European Federation of Biotechnology defined this branch of science as "the integrated use of biochemistry, microbiology and chemical engineering in order to achieve the technological application of the capacities of microbes and cultured cells." A recent offshoot of biotechnology research is genetic engineering involving gene splicing and recombinant DNA cloning. There has been considerable public and political interest in this field of technology because of reports of breakthroughs of great potential significance to life on earth appearing from time to time in fields such as the development of new nitrogen fixing plants, single-cell edible protein, new crops resistant to pests, bacteria for use in waste recycling and pollution control, petrochemical substitutes, use of gene therapy to correct monogenic

diseases such as sickle cell anemia, use of genetic screening for the isotation of genes responsible for birth defects, microbial production of human insulin, interferon and growth hormones, as well as the general increase of the knowledge base of immunological processes including cancer (Stewman et al, 1981).

It is widely believed that it is in agriculture and health that biotechnology will probably have its most important impact. Bollinger (1980) has called tissue culture "the botanical equivalent of the laser, in that there are more potential applications than originally conceived," since the vast panorama of possibilities for the use of tissue culture techniques in agriculture, animal husbandry, fisheries and forestry is just unfolding.

Fears have also been expressed about the potential dangers inherent in some fields of biotechnology research such as genetic engineering. In addition, doubts have been voiced from time to time about the wisdom of undue priority in the allocation of resources to new technologies, often at the cost of intensifying work using already successful and well-tested techniques (Sprague et al, 1980). In addition to considerations of safety and strategy, the other aspect of concern is the growing secrecy attached to biotechnology research because of commercial considerations.

Developing countries are naturally attracted to the potential applications of biotechnology research in solving problems of hunger, energy supply, and quality of life improvement. The precise priorities of developing countries would naturally vary widely. The National Institute of Biotechnology and Applied Microbiology set up in the

Philippines, for example, has accorded priority to research on (a) bio-fuels; (b) nitrogen fixation; (c) food fermentation; (d) plant hydrocarbons; (e) antibiotics, vaccines and microbial insecticides; and (f) biomass production. The National Biotechnology Board set up by the Government of India of which I have had the privilege of being the first Chairman has chosen genetic engineering, photosynthesis, tissue culture, enzyme engineering, alcohol fermentation and immuno-technology as areas of immediate interest. Nearly every developing country has plans or programmes for harnessing the tools of biotechnology for national development. It is, therefore, important that a realistic understanding of the problems and potentials associated with biotechnology research becomes widespread. I consider this conference as a very important milestone in this respect. Since food is the first among the hierarchical needs of man, I would like to discuss priorities in biotechnology research as related to agriculture.

II. Agricultural Scenario and Balance Sheet:

Current world food stocks are satisfactory because of the good harvests of the last few years in North America as well as most countries of Asia (Figure 1). The situation on global food security, however, is far from satisfactory since fluctuations in production caused by weather aberrations, pest epidemics and unfavorable public policies could be considerable. Both FAO and the World Food Council have been emphasizing the need for greater interest in the development of enduring national, regional and global food security systems. The present satisfactory level of global food reserves should hence be regarded only as a breathing

spell during which greater efforts for elevating and stabilising food production should be launched.

If the reasons why the world as a whole has been able to keep food production slightly above the rate of population growth are analysed, we find that new technologies leading to the development of high yielding and management responsive varieties of wheat, rice and other major food crops have been important factors. A major agricultural asset of many developing countries is the existence of a vast untapped production reservoir arising from the prevailing gap between potential and actual yields even at current levels of technology. An important agricultural liability is the shrinking land resource for crop production. Farms in many countries in Southeast Asia are getting smaller and smaller in size. In most of the states of India, the average size of a farm holding is less than one hectare. Therefore, enhanced food production will have to come largely from productivity improvement and a greater intensity of cropping. This is where the high yield technology is of particular relevance. For example, in the Philippines an additional area of about 3.2 million hectares will be needed to achieve the 1980 rice production level, had there been no yield improvement since 1960 (Table 1). In India, over 34 million hectares of additional land will be needed to produce the quantities of rice and wheat the country harvested in 1979 but for the productivity advance made since the mid-60s (Table 2).

In spite of this impressive progress, the vulnerability of world food production systems particularly to weather conditions is very great because of the following factors:

a) Much of the surplus food grains available for supply to other countries on commercial or concessional terms come from a few countries such as the USA, Canada, Australia and New Zealand (Figure 2).

b) About 16 crops and 7 farm animals provide the bulk of human food needs (Figures 3 and 4). Consequently, if the production of one of the major crops like wheat or rice is adversely affected over large geographic areas, there could be acute food scarcity.

c) The escalating cost of energy and the consequent rise in the cost of food production lead to a slow diffusion of new technology which is based to a great extent on purchased inputs. When the cost of production is high, the risk involved is also greater. The cost, risk and return structure of farming determines the extent of investment of both money and effort a farm family will make. Even in Africa where the per capita food production declined during the 70s, there are examples of a sharp increase in production when farmers were given a remunerative price. This is particularly important in many developing countries where the arable area per agricultural worker is declining (Figure 5).

The small and fragmented holdings create problems in efficient management. Also, serious problems of soil erosion and land degradation arising from indiscriminate deforestation are encountered in many developing countries. According to FAO, every year 0.6% of the existing forest cover disappears in Asia. This is equivalent to 1.8 million hectares every year or 3.5 hectares per minute. On a global basis, 21 hectares of tropical forest are disappearing every minute. A recent study by a

Commitee set up by the Government of India on the assessment of fuel-wood supply and requirement has shown that in contrast to the present requirement of 133 million tonnes of fuel wood per year, current efforts are sufficient to provide only 39 million tonnes. The Committee therefore came to the conclusion that at least 1.5 million hectares of land should be planted annually under quick growing fuel wood species (Swaminathan, 1982). Scientific land use thus becomes exceedingly important if the food, fuel, fodder, fiber and other needs of the population are to be adequately met.

The FAO study on "Agriculture: Toward 2000" has provided a wealth of valuable statistics. For example, the study has shown that for 90 developing countries as a group, the net cereals deficit of 36 million tonnes in 1978-79 will be doubled by 1990 and again doubled by 2000. In other words, 18 years from now the gap between demand and supply in these countries will be 144 million tonnes. Sixty percent of total cereals were consumed as feed in developed countries and the demand for animal products tends to increase with increased income. FAO has further projected that by year 2000, 7% of the population of developing countries would still remain undernourished. Can we integrate appropriate segments of emerging technologies with traditional ones to find speedy and effective solutions to the problems of undernutrition and malnutrition?

III. Agricultural production strategies:

The major pathways of productivity improvement will have to be increased yields and a greater intensity of cropping. Multiple cropping

is possible in the tropics and subtropics because of the abundance of sunshine throughout the year. The major constraint will be water availability. However, most developing countries are making major investments in bringing more land under irrigation (Table 3). For both higher productivity and intensity of cropping, there will be need for more nutrient supply. Many of the soils in Southeast Asia and Latin America suffer from mineral stress (Figure 6). Hence, plant nutrition, soil and plant health care as well as irrigation require concurrent attention. At the same time, steps will have to be taken to expand the area under cultivation through the reclamation of soils affected by factors retarding healthy crop growth. For example in South and Southeast Asia, about 86.5 million hectares can be made more productive provided problems of salinity, alkalinity and other adverse soil conditions can be rectified (Table 4). Fortunately it is possible to breed varieties tolerant to some of the adverse soil factors (Table 5).

Developing countries face the dilemma of having to improve both production and consumption simultaneously. This will be possible under the economic conditions prevailing in many of them only if the cost of production can be kept as low as possible so that the output price can be kept at a level which is reasonable to a majority of consumers. The alternative is to subsidize both input and/or output. Most developing countries cannot bear the financial burden involved in extensive farmer and consumer subsidies. Since energy in the form of fertilizer, irrigation and tillage and postharvest technology operations is an important component in the cost of production, fossil fuel requirements will have to be reduced. Thus, the challenge to those in charge of technology develop-

ment is to bring about a continuous improvement in the productivity of major farming systems per unit of land, water, time and energy without detriment to the long term production potential of soil. Can biotechnology help in this task? I would like to take rice as an example and illustrate the scope for integrating recent advances in biotechnology with the techniques already in use.

IV. Biotechnology Applications in Rice

In its study on "Agriculture: Toward 2000," FAO has projected the need for an additional production of 300 million tons of paddy between 1974-76 and the end of the century. The rate of growth in world demand for rice during the 1980s is expected to be of the order of 2.9% a year. In contrast to the other cereals, the demand for rice would remain overwhelmingly for direct use as human food. If past trends in demand and production continue, the gross import requirement of rice of the developing countries would rise from 8.3 million tons of paddy in 1974-76 to 33 million tons in 2000. Through an expansion in the area under irrigation and through productivity improvement, it should be possible for developing countries to meet 200 million tons (paddy) of the additional demand through greater home production (Table 6). For this purpose, there is need for an optimum blend of packages of technology, services and public policies.

It takes several years of breeding and selection work for useful genes from a suitable donor strain to get incorporated into a commercially popular variety. The breeding of the rice variety, IR36, which now occupies an area of over 10 million hectares in Asia took about 7 years.

Seed production on a scale necessary to cover large areas takes another 2 to 3 years. Thus, it takes about 10 years from the time a cross is made to the time it makes a widespread impact, even when 2 to 3 crops can be grown in a year. Through techniques like "Rapid Generation Advance," even 4 crops of rice can be taken in some strains in a year.

Another recent example of the time taken for transferring a desirable gene from one genetic background to another is the work on high lysine corn. The opaque-2 gene discovered at Purdue University in the early sixties has been associated with several undesirable traits. It is only after 12 years of patient research that scientists of the International Maize and Wheat Research Center in Mexico (CIMMYT) have been able to combine the high lysine character with other desirable traits.

Thus, food production in the eighties will have to depend largely on the material already in the plant breeders' assembly line. Immediately the task of R&D establishments should be the bridging of the gap between potential and actual yields in small farmers' fields by helping to eliminate the constraints responsible for this gap. Any new research programme planned and initiated now will be relevant largely to increasing and stabilising production in the nineties. It is essential, therefore, that in agricultural research and development planning, both the short and the long term goals and needs are given appropriate and concurrent attention.

I shall take yield improvement and nutrient supply in rice as examples of research goals where the application of tissue culture and genetic engineering techniques could be of great value.

1. Raising the ceiling to yield

The potential and actual experimental rice yields under two levels of incident solar radiation, representing wet and dry season in monsoon Asia are indicated in Figure 7. The term "favorable conditions" refers to the conditions under which diseases, insects, water, fertilizers and cultural practices do not limit rice growth and yield. The data suggest that further increases of rice yield by 2.2 t/ha for wet season and 3.5 t/ha for dry season would be realistic targets. The assumptions underlying this conclusion are as follows:

1) Estimated potential yields were calculated assuming that the amount of incident solar energy during grain filling period and efficiency of energy use by a crop ultimately determine the yield.
2) Six and 9 t/ha yields were chosen for Experiment Station's yields. These two yields can be easily produced with a good variety and appropriate management. Yields higher than the above have been reported but those were obtained by exceptionally favorable conditions or by "a special treatment" such as CO_2 enrichment before or after heading.
3) 300 and 550 cal/cm^2/day represent levels of incident solar energy during ripening period of rice crop in monsoon Asia during the wet and dry seasons respectively. In Australia and California, USA much higher levels of solar energy i.e. 600 700 cal/cm^2/day are available.
4) 3.0% of energy use efficiency is given for wet season and 2.5% for dry season. Energy use efficiency is expected to decrease as the level of incident solar energy increases. The highest

recorded values are 3.7% by Murata (1967) and 3.3% by Japanese International Biological Programs (1975). But these high values were obtained for the vegetative growth stage. During ripening period, senescence is unavoidable and hence the energy use efficiency during ripening period is inevitably lower than during vegetative stage. Differences in energy use efficiency between the estimated potential yields and Experiment Station's yield are 0.7~0.8%.

2. Methods of bridging the gap between actual and potential yields

The physiological factors that may contribute to reducing the gap between actual and potential yields are indicated below. In such research, the following points should be kept in view:

I. Increasing biomass production
   - A. Fast leaf area development
   - B. Low maintenance respiration
   - C. Low photorespiration

II. Increasing crop's sink size
   - A. Large spikelet number per shoot
   - B. Large grain size
   - C. Greater partition of assimilates into spikelet formation
   - D. Increase in harvest index (up to 0.6)

III. Assuring better grain filling
   - A. Slow senescence
   - B. Maintenance of healthy root system

IV. Increasing lodging resistance
   - A. Stiff culm
   - B. Slow senescence
   - C. Maintenance of healthy root system

1) In the past, slower initial growth was correlated with higher yields because old varieties were tall and susceptible to lodging. With short-statured and lodging-resistant varieties, fast initial growth is one requirement for maximum crop photosynthesis. In theory, the land should be covered with leaves as early as possible; the leaves should be erect to increase crop photosynthesis.

2) Dark respiration is divided into two components, i.e., growth respiration and maintenance respiration. Growth respiration can not be changed unless biochemical pathways are modified. Maintenance respiration is used for protein turnover and active ion transport. It is possible to look for a variety with a lower maintenance respiration.

3) Total elimination of photorespiration in rice may increase net photosynthesis by 50%.

4) Reduction in maintenance respiration and photorespiration should increase leaf photosynthesis.

5) Sink size is generally limiting rice yield under favorable conditions.

6) To increase sink size, the number of spikelets per shoot and grain size must be simultaneously increased. This attempt is now underway in the Plant Physiology Department of IRRI.

7) In a physiological sense, greater partition of assimilates into spikelet formation before heading will result in a larger sink size. Young spikelets compete for assimilates with flag leaf and culm. This process is probably under hormonal control. In terms of morphology, short culm and small flag leaf favor greater partition of assimilates into spikelets.

8) Increased sink size should be coupled with increased harvest index. Modern indica varieties have a harvest index of about 0.5. This could be increased to 0.6.

9) Slow senescence assures active photosynthesis during grain filling period and contributes to increased lodging resistance. Leaf sheath contributes to the breaking strength of the shoot by 30-60%.

10) Maintenance of healthy root system is important, in addition to water and nutrient uptake, for (a) production of cytokinin, a plant hormone responsible for senescence, and (b) better anchorage of crop stand to prevent root lodging.

11) Stiff culm must be considered in addition to short plant stature. Increased importance of lodging resistance can be clearly illustrated in the following way. With a harvest index of 0.5, 8.2 tons of straw supports 8.2 tons of grains (estimated potential yield); total biomass becomes 16.4 tons. When harvest index is increased to 0.6, 5.5 tons of straw will have to support 8.2 tons of grains. Thus, straw must be much stiffer even though total biomass production is less. (5.5 + 8.2 - 13.7 tons). Special attention should be given to this character since lodging resistance is much less in recent varieties like IR36, IR42 and IR50 in comparison with IR8.

3. The role of biotechnology research

The possible applications of biotechnology to rice improvement are indicated below. Even though basic techniques have been developed and known in the past, the application of these techniques to crop improvement is a new avenue of research.

Tissue and Cell Culture

1. Induction and selection of useful mutants at the cellular level	o Salt tolerance o Aluminum toxicity tolerance o High lysine and high protein o Low photorespiration o Disease resistance o Low oxygen tolerance
2. Embryo culture	o Intra- and interspecific hybridization
3. Anther and pollen culture	o Reducing breeding time
4. Protoplast fusion	o Interspecific and intergeneric hybridization o Hybrid rice improvement o Azoila improvement
Genetic Engineering	o Incorporation of nitrogen fixation genes

In order for genetic engineering to be useful for improvement of higher plants such as rice, further advances in tissue and cell culture techniques are indispensable. This is because modification of single cells is the end of research in microorganisms but it is just the first step in higher plants. In higher plants, cells must be modified, multiplied, and regenerated into the whole plant. In addition, the modified traits must be manifested in the whole plant and retained for succeeding generations. In tissue and cell culture, the following two problems require intensive research:

- o Understanding mechanisms of genotypic differences in plant regeneration and methods to overcome such genotypic differences.
- o Methods to regenerate plants from long-term cultured callus and cell suspension culture. Without solving the above two problems, usefulness of tissue and cell culture, and hence genetic engineering may remain restricted to a very narrow range of materials and problems.

4. Some immediate applications

1) Among various techniques in tissue and cell culture, induction and selection of useful mutants at the cellular level is probably the most promising approach to rice improvement. At IRRI, work on salt tolerance and aluminum toxicity tolerance is now underway. Work on high lysine and high protein rice will soon be initiated.

2) Low photorespiration has been studied at several laboratories in the world. At the moment, tissue culture is used to reduce photorespiration rate of tobacco plants. In addition, photorespiration inhibitors are also being studied intensively in several laboratories.

3) Tissue culture has been used to increase lysine content in rice (Scheffer, G.W.). The results indicate that there has been more increase in protein and only slight increase in lysine content of grains.

4) Disease resistance can be increased by tissue culture when particular toxins are directly involved in the disease development. Application of tissue culture to sheath blight resistance will soon be initiated at IRRI.

5) Embryo culture has been used to achieve interspecific hybridization at IRRI. No results have been so far obtained. Because of simplicity in the technique, embryo culture may be useful in some instances where post-fertilization abnormalities impair embryo development.

6) Anther culture may not be useful in rice breeding unless further advances are made in plant regeneration and pollen culture. In

China, several rice varieties were produced by anther culture but these are japonica varieties. Plant regeneration problems hamper usefulness of anther culture in most indica varieties.

7) Incorporation of nitrogen fixation genes into rice by genetic engineering is the most ambitious subject. We now know that at least 17 genes are involved in the nitrogen fixation system. We still do not know if manipulation of such large number of genes will be possible. In view of the widespread interest in this field, problems relating to nitrogen fixation are dealt with in some detail separately.

5. Biological nitrogen fixation in rice

Wetland rice has been grown without nitrogen fertilizer for centuries. Long term fertility experiments in both temperate and tropical regions have indicated that about 50 kg N/ha were absorbed by every crop of rice in no nitrogen plots. Most of this amount is compensated by biological nitrogen fixation.

Wetland is quite suitable for biological nitrogen fixation. First, floodwater provides a suitable site for phototrophic nitrogen fixing organisms. Second, the anaerobic soil provides also suitable site for anaerobic nitrogen fixation. Nitrogen fixation is principally an anaerobic process. Third, rice root provides another site for biological nitrogen fixation. Wetland rice can stimulate biological nitrogen fixation by supporting nitrogen 'rhizobacteria' (bacteria that live in and around the root). But dryland rice has little ability to do this.

Major nitrogen fixing agents in wetland rice soils are -- 1) free-living blue-green algae, 2) blue-green algae symbiotic with a water fern

Azolla, 3) heterotrophic nitrogen fixing bacteria associated mainly with root of rice, 4) heterotrophic nitrogen-fixing bacteria growing on plant residues, and 5) rhizobia in symbiosis with aquatic legumes.

A brief description of each system, its limitations and research needs is given for the information of those who may be interested in undertaking research in this field. Problems of biological nitrogen fixation in rice culture were discussed by Watanabe and App (Nitrogen and Rice 1979), and general problems as technologies for developing countries were described in the publication on microbial processes published by the National Academy of Sciences.

6. Blue-green algae

Extensive surveys of literatures on blue-green algae in rice soils were published by IRRI (Roger and Kulasooriya 1980). Inoculation of blue-green algae is widely used in Egypt, India and Burma. Preparation of inoculum was well developed as small scale village biotechnology. The average of increase of rice yield by inoculation over to uninoculated control was calculated to be 16% from the published results (Table 7).

Little is known about the mechanism of rice yield increase due to inoculation. To fill the gap in our knowledge on the relationship between inoculation and grain yield, studies on the availability of algal nitrogen and ecology of blue-green algae have been initiated at IRRI.

Although the preparation and transport of inoculum is easier than Azolla, the establishment of inoculated BGA is rather sporadic. Little is known about the conditions leading to the success of inoculation and about the factors to govern the survival of the inoculated BGA. Indigenous

BGA and algae predator and mineral deficiencies in soil would limit the establishment of inoculated BGA.

BGA is still not a familiar tool for gene engineering, because only a limited number of strains are free from bacteria and no vector of gene information has been found. Some of the urgent research requirements are:

1) Strains should be collected and selected for environmental adaptation as well as nitrogen fixation.
2) Information is needed on how to encourage the growth of desirable organisms and discourage the growth of undesirable indigenous algae and predators.
3) Low cost sources of controlling predator, like Neem (Azadirachta indica) seed, should be sought.
4) Possibility of gene transfer suitable to BGA should be sought. Protoplast fusion would be one of the techniques.

7. Azolla-Anabaena symbiosis

Recently, the potentiality of azolla as nitrogen fixing green manure crop suitable to rice culture has been recognized by many researchers, agricultural administrators and peasants. Azolla pinnata can fix at the maximum about 3 kg N/ha daily and A. filiculoides 10 kg N/ha. Azolla has been used in China and northern Vietnam for centuries. Recently, trials to use this as green manure crop have been made in other Asian countries. In the International Network of Soil Fertility and Fertilizer Evaluation for Rice (INSFFER) organized by IRRI, the effect of azolla green manuring was examined. It was confirmed that one crop

of azolla gave rice yield increase equivalent to that obtained by 30 kg N/ha of urea (Table 8).

In southern Philippines, the Azolla technique was successfully introduced in an area amounting to several thousand hectares. Farmers say that two crops of azolla (before transplanting and after harvest of rice) could replace chemical nitrogen fertilizer. IRRI maintains Azolla collections including 6 species and makes screening of strains suitable for the use in the tropics.

Because of high protein content, azolla is also called a potential source of animal and fish nutrition and can be used by farmers with small land holdings.

8. Application of biotechnology to Azolla improvement

Azolla requires a high level of phosphorus fertility in soil, lower temperature (preferably less than 30°C), good water control and the control of insects which cause serious damage, particularly in the tropics.

Areas that meet these requirements would be limited in rainfed tropical rice culture. Azolla must be maintained throughout the year as vegetatively growing plant and must be transferred and inoculated in the fresh state. Azolla forms sporocarps (male and female organs) and these sporocarps can be used for germplasm maintenance and propagation. But the conditions to induce these artificially are not known and artificial mating is not yet successful. If we could cross Azolla species by asexual recombination, the improvement of strains would be much promoted. This is where protoplast fusion may be of value. Protoplast fusion can also be tried between Azolla and Anabaena (Figure 8).

9. N_2-fixation associated with wetland rice

Since the discovery of a sensitive assay of detecting nitrogen fixation through acetylene reduction, many scientists have reported nitrogen fixation, though to a small extent, of non-nodulating plants, particularly graminaceous plants, in association with nitrogen fixing bacteria living in and around the root. C_4 grasses in the tropics and wetland plants (including rice) showed higher activities than others. Despite the enthusiasm of earlier days, there is a dearth of published nitrogen balance data and we need more studies in this field.

Direct evidence of nitrogen fixation associated with plant by using $^{15}N_2$ was obtained for wetland rice, and Digitaria and Paspalum. In wetland rice soils nitrogen fixed by the heterotrophic bacteria associated with rice may be about 5-10 kg N/ha. Various groups of N_2-fixing bacteria were isolated from the stem and roots of wetland rice. At the highest, nitrogen-fixing bacteria occupied 90% of the heterotrophic bacteria isolated from wetland rice root.

Inoculation of bacteria isolated from roots (Azospirillum) to plants was sometimes positive in its impact on growth and nitrogen fixation. But the results were very variable. Differences of cultivars in their ability to support N_2-fixation in root zone have been reported. Screening of varieties which are higher in stimulating N_2-fixation in root zone is one way to increase N_2-fixation by this mechanism.

Despite many reports of 'potentiality' of nitrogen fixation in root zone of non-nodulating plants, convincing data pinpointing the importance of this mechanism in nitrogen supply in the soil and nitrogen nutrition

are very limited. Despite many reports of the presence of Azospirillum and inoculation of this bacteria, convincing data establishing the actual involvement and dominance of this bacteria in N_2-fixation of plants grown in soil are scarce. Little is known of the physiology of this associative N_2-fixation for it to be used effectively. Little is known of the plant characteristics to support it more efficiently.

Conditions for successful inoculation are complicated. Mechanisms of beneficial effects to plant by inoculation still remain to be clarified.

Sesbania, Aesynomyne and Neptunia are aquatic leguminous plants which can grow in aquatic environment. It is noteworthy that Sesbania rostrata and Aesynomine indica form stem nodules. Because of stem nodule, nitrogen fixation by Sesbania rostrata is less inhibited by NH_4-N than ordinary root-nodule bearing leguminous plants. Sesbania sesban is widely used in the tropics as an intercropping among rice.

Because of height of plant, Sesbania competes with rice plants at its later growth stage. Sesbania is, thus, grown at a late stage of the first crop of rice, and is allowed to grow further after harvest of rice crop and used for fertilizer to the second crop. This practice is used in Vietnam. Collection of Sesbania species and strains is needed to exploit this possibility.

10. Comprehensive and integrated use of biological nitrogen fixation in rice culture

None of the nitrogen fixing systems mentioned so far can be the sole source of nitrogen in rice cultivation. Combinations of possible sources should be used based on a clear understanding of the relative importance of each nitrogen fixing agent under different environmental,

cultural and social conditions. For example, azolla is more suitable in double cropping of rice in irrigated area than in rainfed area. In contrast, BGA technique can be easily adopted in rainfed area. Continuous flooding is favorable in the establishment of high biomass by indigenous BGA. In such cases, inoculation of every crop would not be effective because of well established indigenous BGA biomass.

Nitrogen fixation by BGA and heterotrophic bacteria would be able to co-exist, because ecological niches are different and BGA is active mainly in early stage of rice, while N_2-fixation associated with root is active during the reproductive stage.

The final goal of biotechnology of N_2-fixation is to minimize dependence on chemical nitrogen fertilizer through the use of biofertilizers.

IRRI scientists believe that the continuation and strengthening of currently operating systemsof interaction between N_2-fixing bacteria and rice root would be the most feasible way to increase N_2-fixation associated with rice. In view of the fact that living root is inhabited with 10^7-10^9 cells of bacteria per gram dry root, the introduction of Nif genes by genetic engineering techniques is worthy of attempt. Most of heterotrophs isolated from rice roots have N_2-fixing ability, through their N_2-fixing activity was ten times lower than most active N_2-fixing bacteria. If we could increase the N_2-fixing activity of these 'rhizobacteria' of rice and give them the ability of derepression (the ability to excrete NH_4 to outside cells), we would be able to expect progress.

Another encouraging feature is that most of these 'rhizobacteria' have the ability to utilize H_2 gas. Because paddy field produces hydrogen gas from organic matter, the ability of dominant N_2-fixing 'rhizobacteria'

to use H_2 would be interesting. If we prove or improve the ability of these bacteria to use H_2 as energy source, we could say that wetland rice root is quite unique in recycling energy, wasted as H_2. IRRI would be willing to cooperate in such research with scientists and institutions who are interested in increasing rice yield without an associated increase in the consumption of mineral fertilizers.

11. Protoplast fusion

The University of Nottingham and IRRI are jointly undertaking a project to utilize the scope offered by protoplast fusion in the following areas:

a) Hybrid rice production: The People's Republic of China has nearly 6 million hectares under hybrid rice. These are based on cytoplasmic male sterile lines identified in varieties adapted to temperate conditions. It is now necessary to develop speedily male sterile lines and restorers in varieties characterized by adaptation to tropical conditions as well as by good combining ability. Also the scope for heterosis in root growth and early seedling vigour will have to be studied. For this purpose, it is proposed to combine the tools of somatic and sexual genetics. It will be necessary to use anther culture for production of haploids in order to maintain the chromosome number at the diploid level (Figure 9).

b) Protoplast fusion and hybrid cell regeneration will also be used in interspecific hybridization in *Azolla*. Also attempts will be made to bring about fusion between cells of *Azolla* and *Anabaena*.

12. Many applications of tissue culture techniques

Among other applications of tissue culture techniques will be standardization of methods for breeding perennial plants like coconut, rubber and quick-yielding fuel trees. In coconut, for example, there are diseases of unknown etiology such as cadang-cadang in the Philippines, root wilt in India, etc. In the midst of severely infested coconut palms, healthy palms with a high yield potential also occur. Coconut is an obligatorily cross pollinated plant. Therefore, it becomes difficult to fix desirable genotypes. This is where vegetative propagation of disease resistant and high yielding palms through tissue culture will be of particular value.

13. Genetic engineering

There has been an impressive growth in published material on different aspects of genetic engineering research as applied to crop improvement. A conference convened in 1980 by The Rockefeller Foundation on "Genetic Engineering for Crop Improvement" has resulted in an excellent synoptic account of possibilities and problems (Rachie and Lyman, 1981). The meetings organized by UNIDO for developing a project for the establishment of an International Centre for Genetic Engineering and Biotechnology have also provided valuable information. I have reviewed elsewhere (Swaminathan, 1981) the potential value of an integration of emerging and current techniques of plant breeding.

It is clear from a survey of published literature that we need more knowledge of the structure and organization of genetic information in plants together with an understanding of processes related to gene

regulation and expression. A somatic cell population directly isolated from the plant represents a valuable source of genetic variation of interesting practical consequences. If we gain a better understanding of the biochemical processes that control cell differentiation and the development of organs with specialized functions in whole plants, cellular totipotency could be put to many uses. Dr. Peter Day has suggested that a start can be made in analyzing the comparatively small genomes of chloroplasts and mitochondria by sequencing their DNAs and cataloguing the proteins and enzymes they code for.

An area of research which needs urgent attention is the development of quick yielding fuel trees. All aspects of research related to "energy agriculture" deserve the highest priority, if further damage to soil health as a result of deforestation and soil erosion is to be arrested in the tropics and subtropics (Figure 10). For example, can we produce annual varieties of bamboo by crossing bamboo with sugarcane and other appropriate crops? Leguminous shrubs and trees provide considerable opportunities for improvement. Now that an International Research Institute for Winged Bean (*Psophocarpus tetragonolobus*) has been established in Sri Lanka, it should be possible to initiate a research programme designed to develop erect and self-standing strains of winged bean.

In the choice of problems, it is obvious that we should select those which defy solution through already available techniques. Solving a problem rather than worshipping a tool should be the goal. Such research tools or combination of tools which can help us to reach the desired objective surely and speedily should be chosen. This does not mean that speculative and imaginative research by gifted and capable

scientists which may or may not yield the results we seek should not be supported. In the interest of the generations yet to be born, imaginative research should always receive support without a prior judgement on their ultimate value.

14. Needs of developing countries

Developing countries are naturally concerned about the need for avoiding technological obsolescence and for profiting from the most recent advances in technology. While science helps to advance the frontiers of knowledge and hence is of value irrespective of the country or source of origin of the new knowledge, technology to be effective in advancing the frontiers of production has to be compatible with specific ecological, socio-economic and socio-cultural factors. At the same time, technologies which can help to purchase time and which will facilitate quantum jumps in production and prosperity, will be of particular interest to developing countries. Biotechnology has raised considerable hopes in this respect.

In order to assist Third World Countries to gain both a realistic appreciation of the usefulness of the new techniques and practical benefits from them, it will be desirable to extend assistance in the following areas.

A. Choice of research priorities and implementation of programmes

To avoid later disappointments and dismay, careful thought should be given to the choice of research fields where the new tools will help to accelerate progress and to solve problems which have so far defied solution. This is particularly important if the research goals have

applied objectives. The following institutional structures may be helpful in this respect.

a) Setting up International and Regional Brain Banks: So far banks like the International Bank for Reconstruction and Development (World Bank), Asian Development Bank, etc. have been established mainly for rendering financial support to worthwhile development projects. We now need to give thought to the organization of "Brain Banks" from which countries can receive objective and up-to-date advice on technology choice and transfer. This has become particularly urgent in the context of growing commercialization of skills and know-how and of enlarging secrecy around the knowledge base of discovery. To be successful, the Brain Banks will obviously need the support and guidance of leading scientists and technologists, who are not only authorities in their respective fields of specialization but are also humanists. Members of such "Brain Banks" should be men and women who are wedded to the concept outlined by Albert Einstein in the following words:

> "Concern for man himself and his fate must always form the chief interest of all technical endeavours in order that the creation of our minds shall be a blessing and not a curse."

Can a beginning be made at this conference in organizing an "International Brain Bank for Sustainable Development"?

b) Institutional Twinning: Appropriate institutions in developing and developed countries can enter into twinning arrangements to maximize the benefits from their complementary strengths.

This will be very helpful to purchase time and sharpen priorities. The BIFAD programme of USAID is an important step in this field.

c) Investigator-initiated Tie-up: In addition to inter-institutional collaboration, there should be scope for individual scientists in developed and developing countries to collaborate on projects of mutual interest. Simplified administrative procedures and appropriate financial support mechanisms will be needed to foster such collaboration.

d) Periodic Seminars and Workshops: Organization of Seminars and Workshops in developing countries in the frontier areas of science and technology under the joint auspices of the Science Academies of developed and developing countries will be helpful to review progress, clarify issues and determine priorities. Periodically, seminars should also be held for Policy Makers and Political Leaders in order to familiarize them with the state of the art in biotechnology and help them to make investment decisions based on a scientific understanding of the likely returns.

B. Research resources

The major research resources needed for organizing effective national programmes are: a) Trained manpower,

b) Chemicals and equipment, and

c) Information and literature.

Assistance in these areas will be of value for strengthening the national research programmes. In manpower development, priority should

be accorded to filling critical gaps in internal competence. Training and help will also be needed in the design of laboratories with adequate safety measures. Where possible, help should be given to assist in the manufacture of enzymes, radio-chemicals, etc. within the country. The information network should help in spreading knowledge not only on the scientific aspects but also on the legal (patent regulations, plant breeders' rights, etc.) and commercial aspects of biotechnology applications.

For imparting training and for information dissemination, institutions like INTSOY (International Soybean Program) could be promoted.

C. International centres and programmes

Several ideas including the setting up of international and regional centres are now being considered by UN and other organizations. Immediately, a modified version of the TOKTEN scheme (Transfer of Know-how through Expatriate National will be useful. A review of six years of experience gained under the UNDP-sponsored TOKTEN scheme made at a workshop held in Islamabad, Pakistan in January, 1982, has resulted in several useful recommendations. The TOKTEN scheme itself in my view needs to be broad-based in some selected fields like biotechnology so as to include not only expatriate nationals but also other appropriate experts.

In December 1982, the opportunities now available for the appropriate integration of emerging and traditional technologies in fields like bio-technology, micro-electronics, computer sciences and satellite imagery and communication will be discussed at a workshop to be held at IRRI under the sponsorship of the U.N. Advisory Committee on Science and Technology for Development. It is through the planned and purposeful integration of the old and the new that we can sow the seeds for sustainable developmer

REFERENCES

Bollinger, W.H. 1980. Sustaining renewable resources: Techniques from Applied Botany. In Techniques from Applied Botany, Academic Press, New York.

Chakrabarty, A.M. (ed.) 1978. Genetic Engineering. CRC Press, 198 p.

Conger, B.V. (ed.) 1981. Cloning agricultural plants via invitro techniques. CRC Press, 273 p.

Flores, H.E., R.K. Sawhney and A.W. Galston. 1981. Protoplasts as vehicles for plant propagation and improvement. In Advances in Cell Culture, Vol. 1, Academic Press, 241-279.

IRRI (Int. Rice Res. Inst.). 1980. Innovative approaches to rice breeding. Selected papers from the 1979 International Rice Research Conference. 182 p.

IRRI (Int. Rice Res. Inst.). 1980. Rice tissue culture planning conference. 28-30 April 1980.

Panopoulos, N.J. (ed.). 1981. Genetic engineering in the plant sciences. Praeger Publishers, 271 p.

Rachie, K.O. and J.M. Lyman. 1981. Genetic engineering for crop improvement. Proceedings of a conference organized by the Rockefeller Foundation in May, 1980. 254 p.

Rains, D.W., R.C. Valentine and A. Hollaender (eds.). 1980. Genetic engineering of osmoregulation. Symposium on genetic engineering of osmoregulation, Plenum Press, New York & London, 381 p.

Research with Recombinant DNA. 1977. An Academy Forum. U.S. National Academy of Sciences, 295 p.

Sprague, G.F., D.E. Alexander and J.W. Dudley. 1980. Plant breeding and genetic engineering: a perspective. Bioscience:30, 17-21.

Stewman, S. and D. Loncoln. 1981. Recombinant DNA breakthroughs in agriculture, industry and medicine. Futures:13, 128-140.

Swaminathan, M.S. 1981. Plant breeding in preparation for the 21st century. Meghmad Saha Medal lecture, Indian National Science Academy, New Delhi, 17 p.

Swaminathan, M.S. 1982. Report of the fuelwood study committee of the Planning Commission, Government of India, 46 p.

Table 1. Rice production, area, and yield in the Philippines, 1960, 1970, 1980[a].

Year	Production rough rice ($x\ 10^3$ t)	Area ($x\ 10^3$ ha)	Yield (t/ha)
1960	3705	3198	1.16
1970	5343	3112	1.72
1980	7723	3459	2.23

[a]About 3.2 million ha more would have been needed to achieve the 1980 production level at 1960 yield level.

Source: Philippine Ministry of Agriculture, Bureau of Agricultural Economics

Table 2. Impact of new technology on yield and land requirement in India.

	Year	Area ($x\ 10^3$ ha)	Yield (t/ha)	Production ($x\ 10^3$ t)	Area saved ($x\ 10^3$ ha)
Rice	1966-70	36,360	0.98	35,770	-
"	1979	40,480	1.33	53,770	14,331
Wheat	1961-66	13,191	0.83	10,950	-
"	1979	22,560	1.57	35,510	20,222

Source: Ministry of Agriculture, Government of India.

Table 3. Increase in areas under irrigation (millions of ha) during the period 1961-65 (annual average to 1977).

	1961-65	1977	Increase (%)
Africa	5.8	7.8	13.4
Asia	100.0	128.8	28.8
South America	4.9	6.5	32.6
All developing countries	110.0	144.9	31.7
All developed countries	39.0	52.9	35.6
World	149.0	198.0	32.9

Data: FAO

Table 4. Problem rice lands of South and Southeast Asia.

Problem	Area (millions of ha)
Salinity	53.2
Alkalinity	7.0
Acid sulfate soil condition	5.3
Peat soil condition	21.0
Total	86.5

Source: Ponnamperuma, IRRI.

Table 5. Performance of rice varieties/lines tolerant of problem soils.

Soil problem	Variety or line	Yield (t/ha)
Salinity	IR50, IR5657-44	3.6
Alkalinity	IR36, IR4595-4	3.6
Strong acidity	IR42, IR4683-54	4.2
Peat	IR42, IR8192-31	3.1
Aluminum toxicity	IR43, IR6115-1	3.8
Phosphorus deficiency	IR52, IR8192-200	4.4
Zinc deficiency	IR36, IR8192-31	2.9
Iron deficiency	IR36, IR52	2.8

Source: IRRI Annual Report (1981).

Table 6. Area, yield and production of rice (paddy) in developing countries.

Land and Water Class	Harvested Area (Million ha) 74-76	2000	Yield (t/ha) 74-76	2000	Production (million tons) 74-76	2000
Rainfed	62.6	57.8	1.5	2.1	95.4	123.8
Irrigated	36.5	64.8	2.7	4.1	97.6	264.9
Total	99.1	122.6	1.9	3.2	193.0	388.7

Source: FAO: Agriculture Toward 2000.

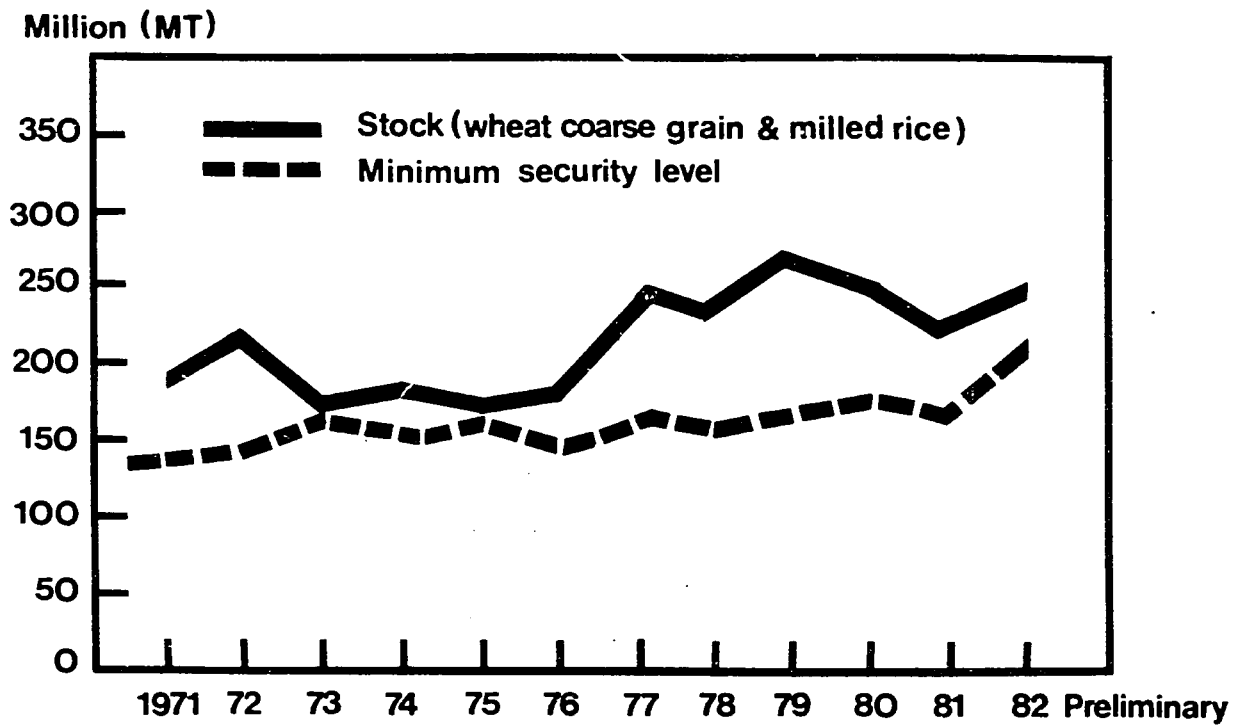
Table 7. Average effects of algalization in field experiments.

	Control	Algalization	Grain yield increase over control (kg/ha)
	Grain yield (kg/ha)	Over control (%)	
Mean	3016	16.1	485
Number of data	30	87	87

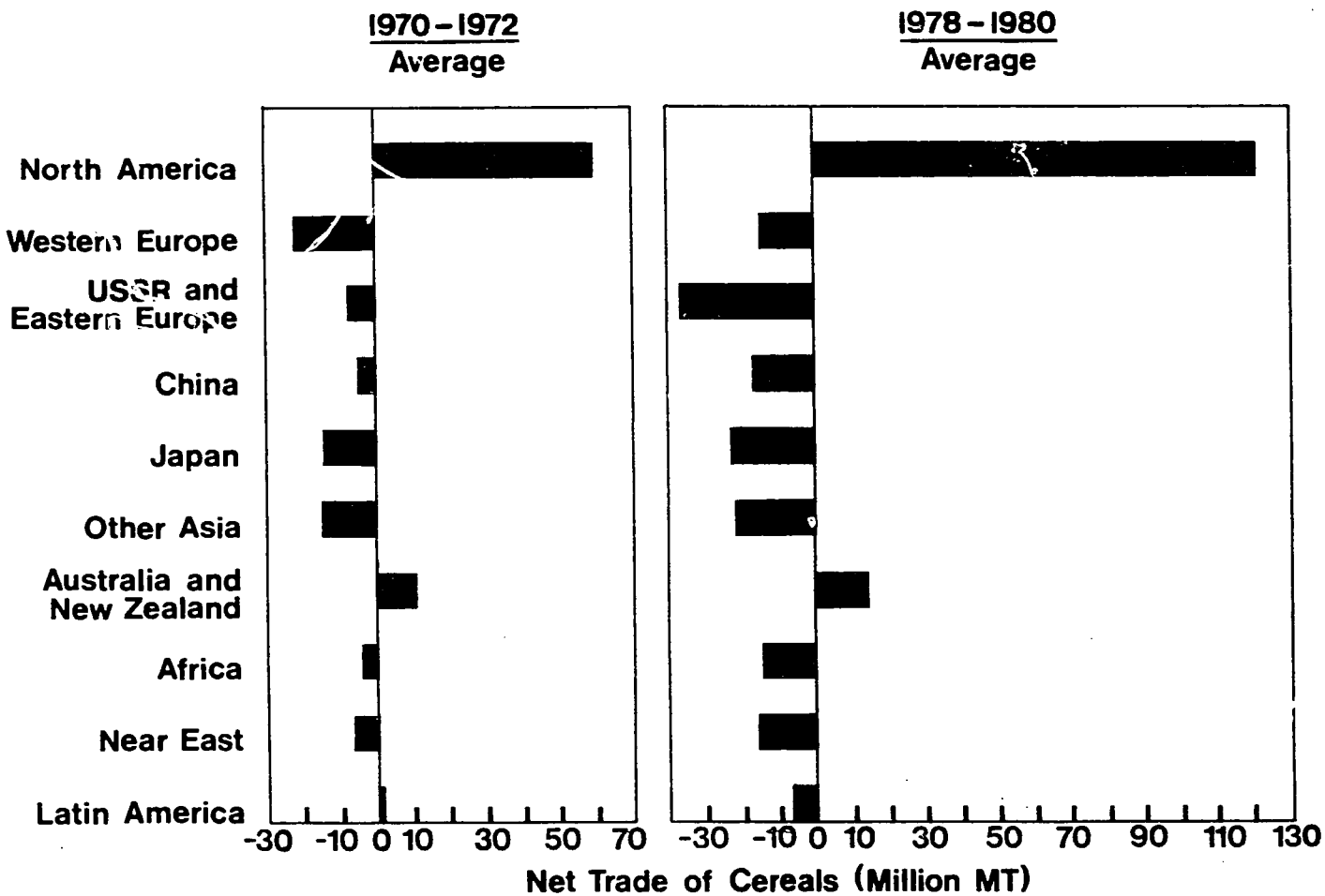
Modified by Roger and Kulasooriya (1980).

Table 8. The effect of azolla incorporation as compared with chemical nitrogen fertilizer (INSFFER 1979, 1980).

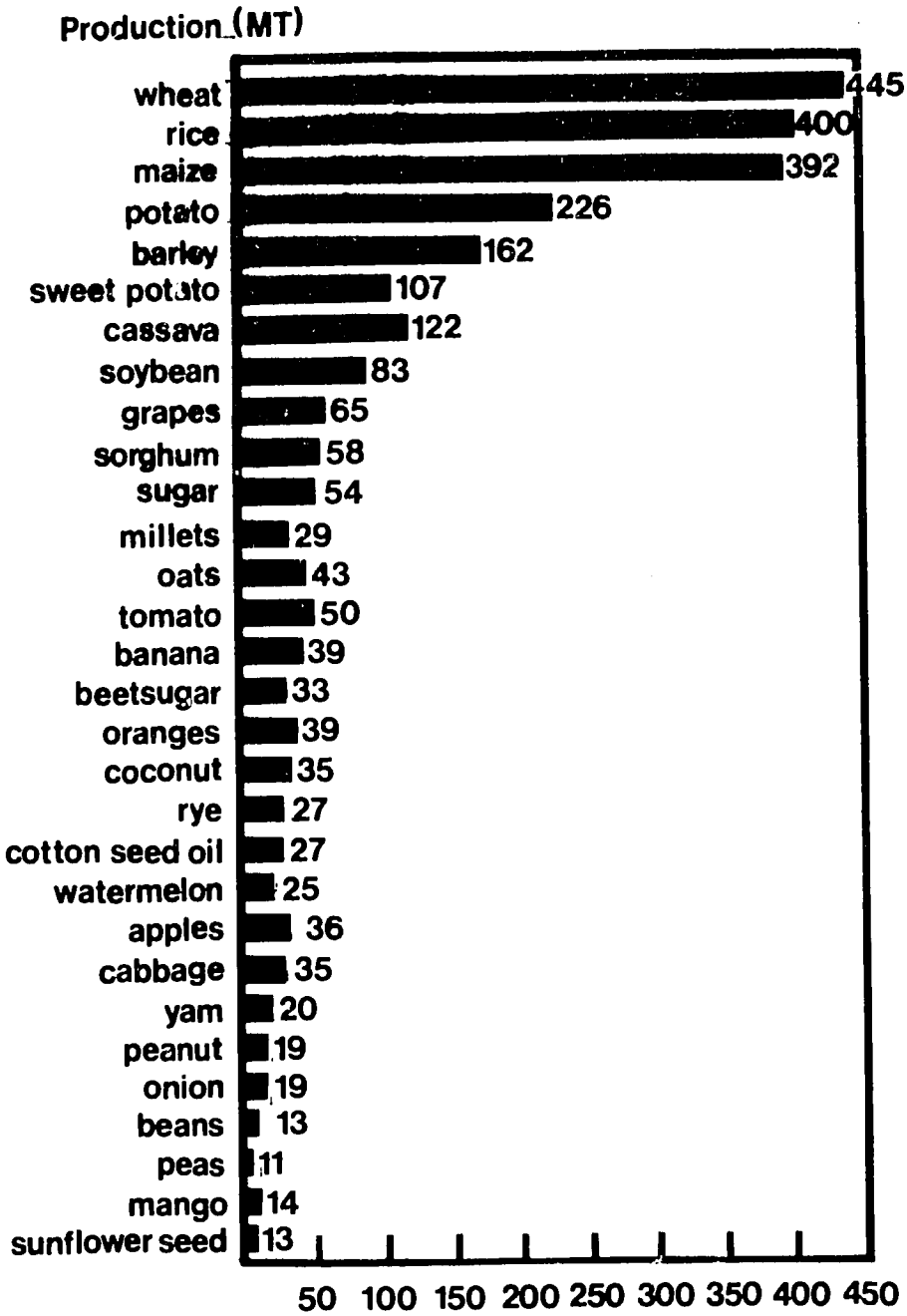
Treatment	Grain yield ton/ha (index)	
	1979	1980
1. No nitrogen	2.6 (100)	3.2 (100)
2. 30 kg N/ha urea split	3.2 (122)	3.8 (123)
3. 60 kg N/ha urea split	3.7 (141)	4.2 (139)
4. Azolla grown before transplanting	3.2 (122)	4.0 (123)
5. Azolla after transplanting and incorporated	3.1 (118)	3.9 (123)
6. The same as #5, but not incorporated	3.1 (119)	4.0 (123)
7. 30 kg N/ha + azolla before transplanting	3.7 (143)	4.4 (140)
8. 30 kg N/ha + azolla after transplanting	3.5 (134)	4.4 (135)
9. Azolla before and after transplanting	3.6 (139)	4.2 (137)
Number of sites	13	19



World carry-over stocks and minimum security levels of cereals (including CHINA and USSR)
Fig.1



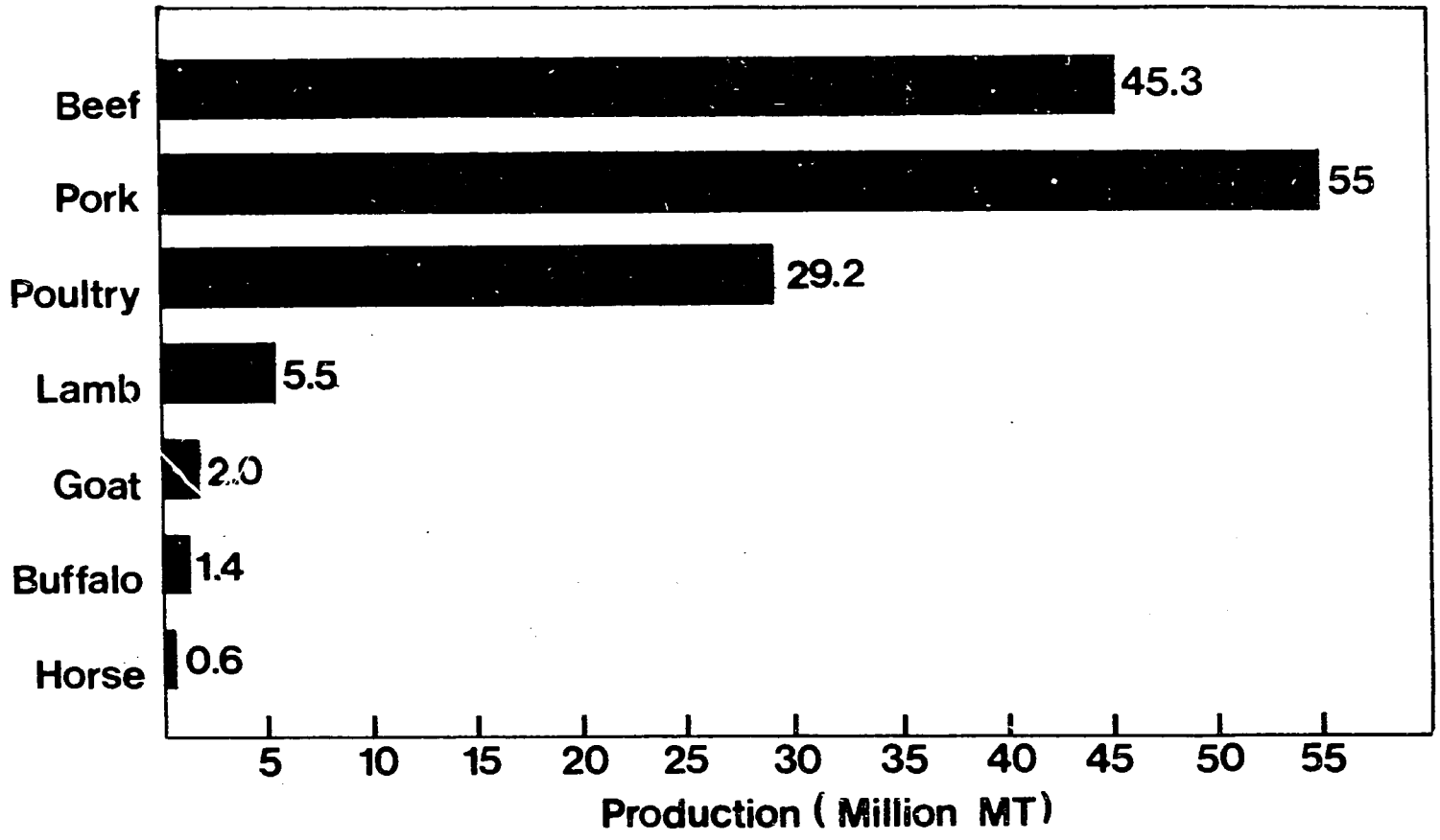
World's increasing dependence of the grain exports of a few countries; USA and Canada supply most of the grain.
Fig.2



Annual production of the world's major food crops (1980)

Source: FAO Production Year Book, 1980

Fig. 3



Annual production of the world's major animal products

Source : FAO Production Year Book, 1980

Fig. 4

ARABLE AREA PER AGRICULTURAL WORKER (ha). (FAO data)

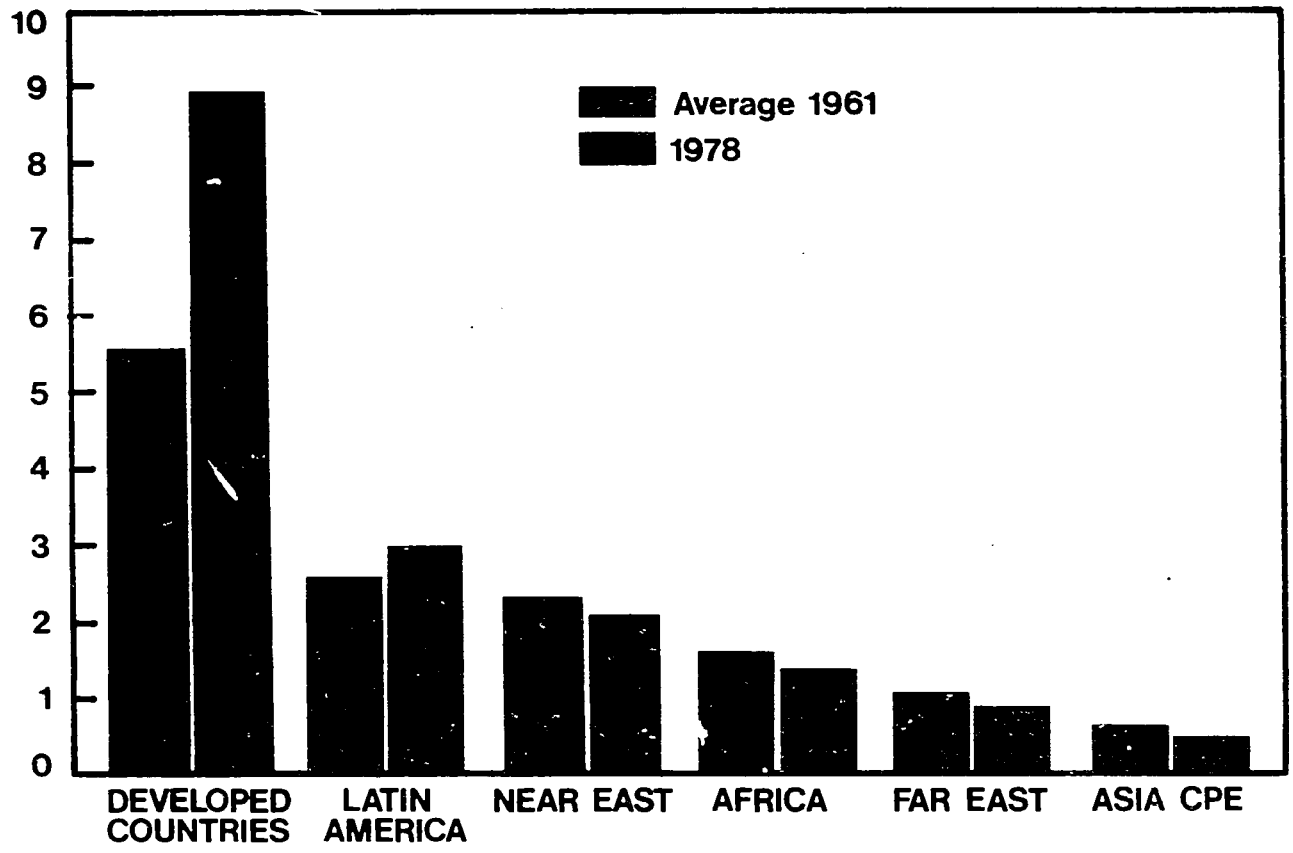


Fig.5

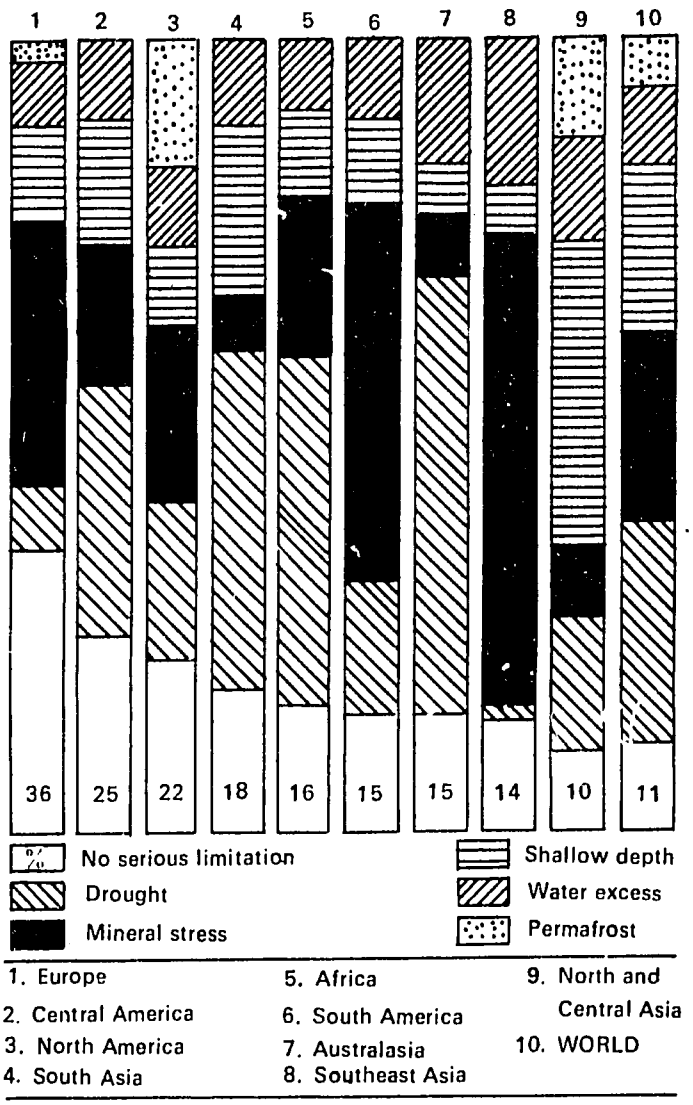


Fig. 6. Regional distribution of soils with or without limitations for agriculture. Source: Allen (1980).

ESTIMATED POTENTIAL AND EXPERIMENT STATION'S YIELDS DURING WET AND DRY SEASONS

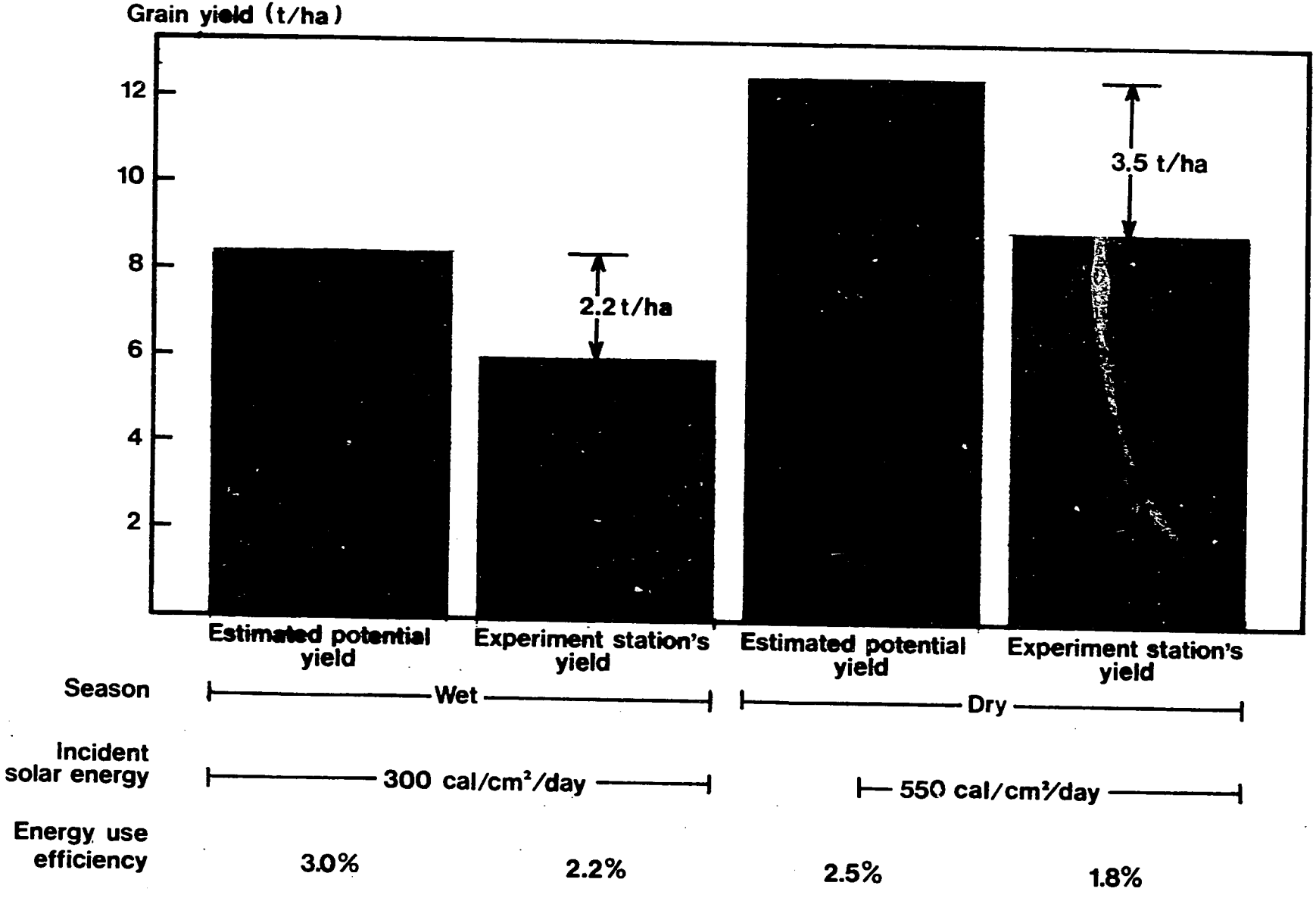


Fig.7

POTENTIAL APPLICATION OF PROTOPLAST FUSION IN AZOLLA

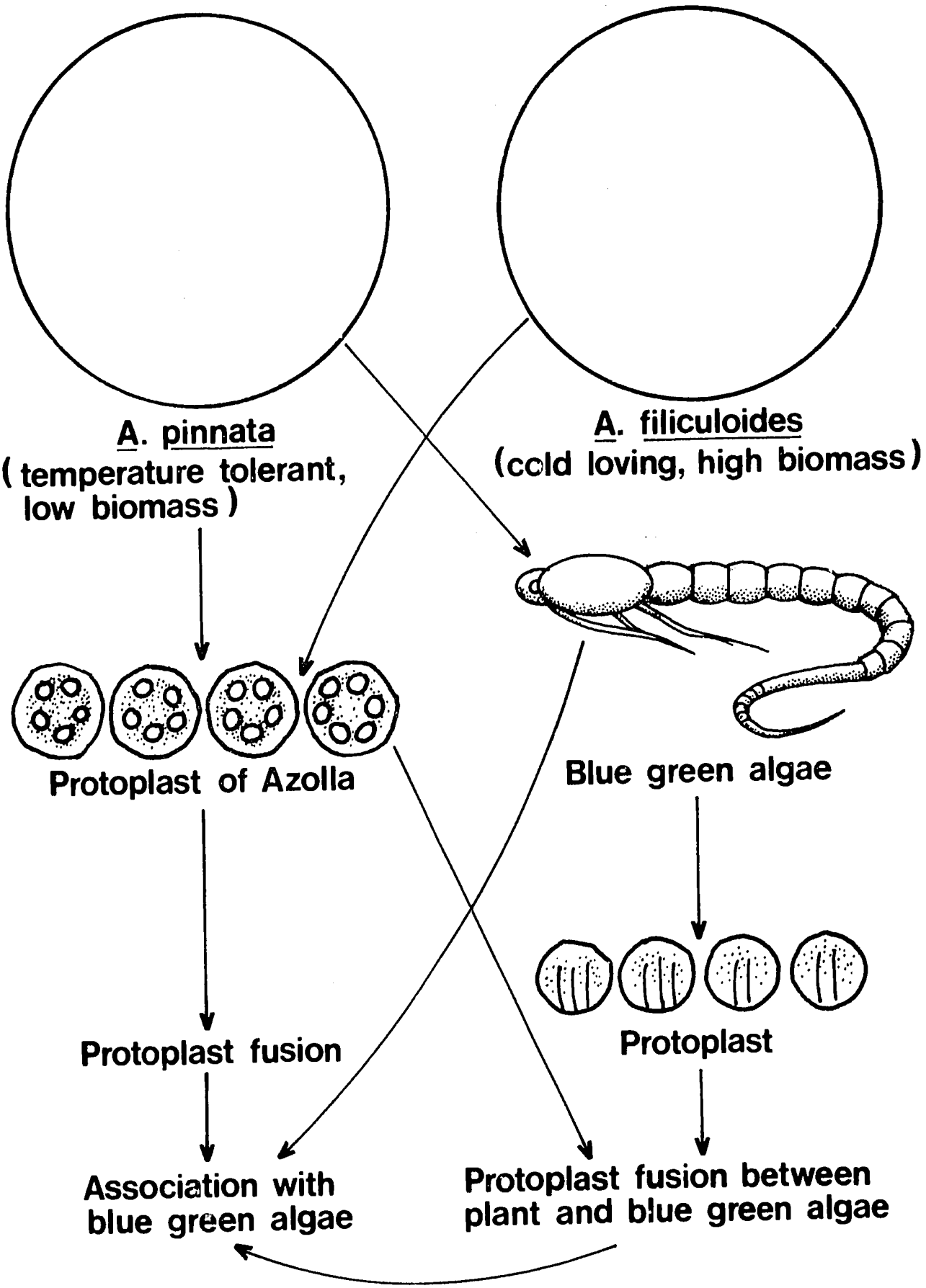
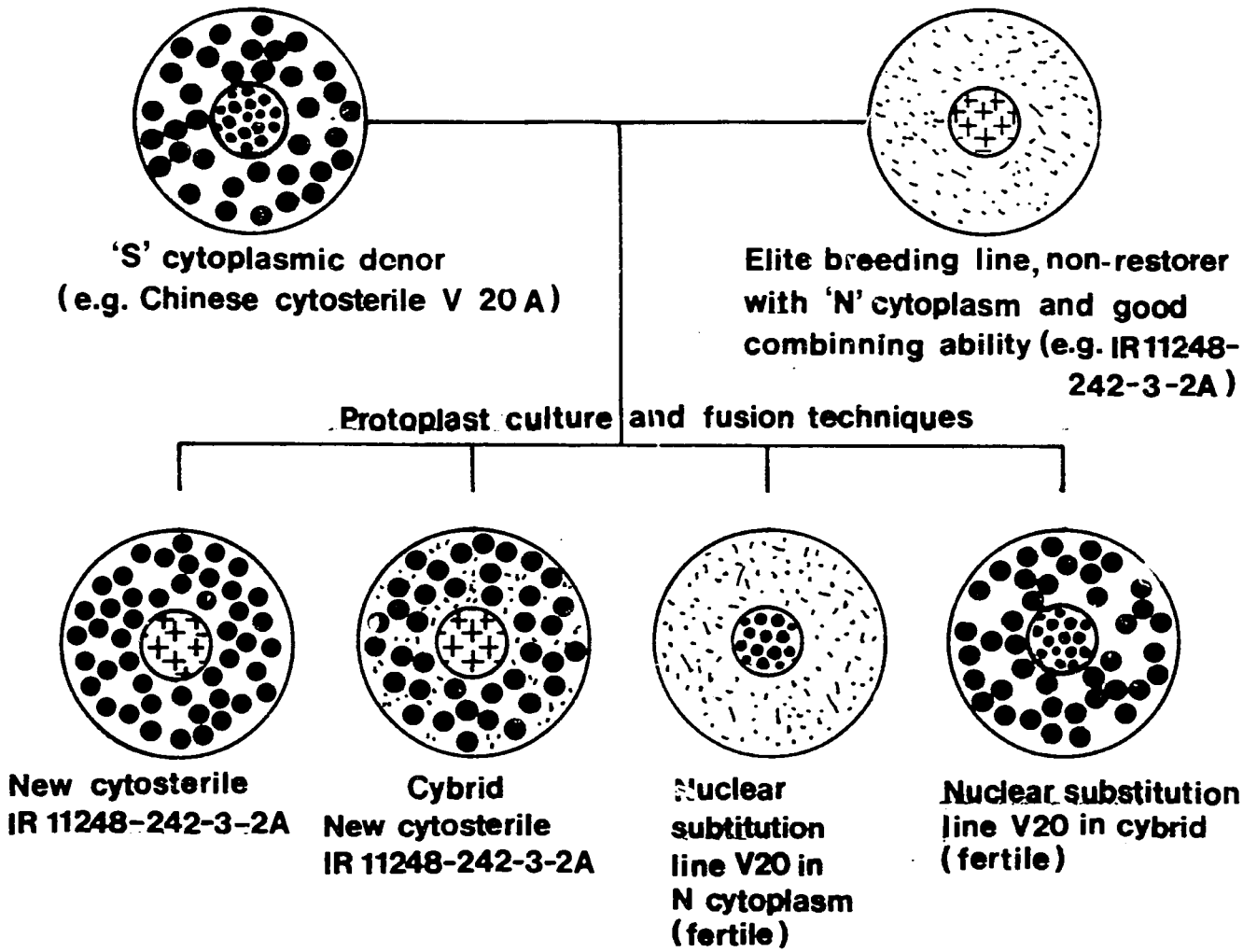


Fig. 8



Use of protoplast culture and fusion techniques to develop new cytoplasmic male sterile lines in rice.

Fig. 9

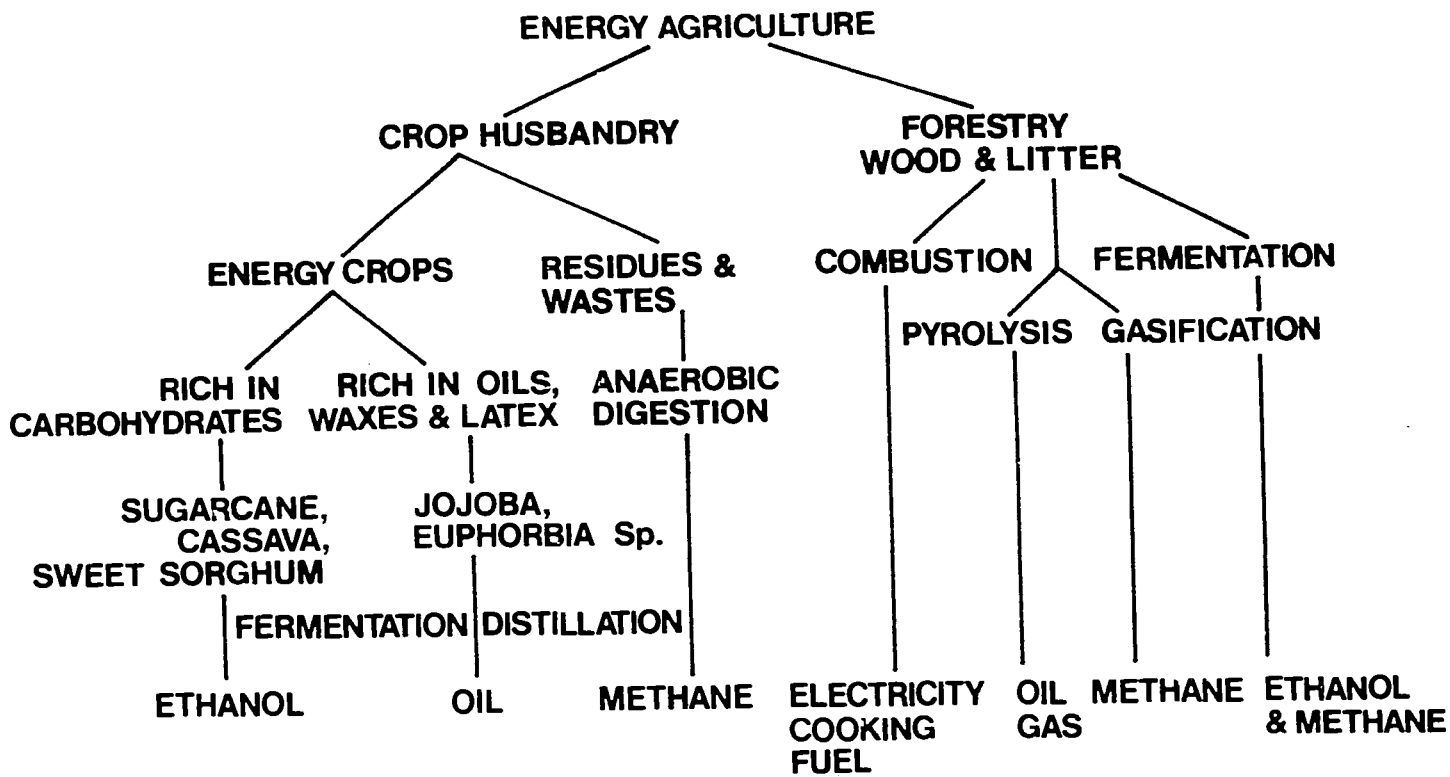

ENERGY AGRICULTURE
CROP HUSBANDRY
FORESTRY
WOOD & LITTER
ENERGY CROPS
RESIDUES &
WASTES
COMBUSTION
FERMENTATION
PYROLYSIS
GASIFICATION
RICH IN
CARBOHYDRATES
RICH IN OILS,
WAXES & LATEX
ANAEROBIC
DIGESTION
SUGARCANE,
CASSAVA,
SWEET SORGHUM
JOJOBA,
EUPHORBIA Sp.
FERMENTATION
DISTILLATION
ETHANOL
OIL
METHANE
ELECTRICITY
COOKING
FUEL
OIL
GAS
METHANE
ETHANOL
& METHANE

Fig. 10