The Role of Tissue Culture and Novel Genetic Technologies in Crop Improvement

Proceedings of the Third Conference of the International Plant Biotechnology Network (IPBNet)

Nairobi, Kenya
January 8-12, 1989

Sponsored by the
Tissue Culture for Crops Project
Colorado State University
Fort Collins, Colorado 80523

Supported by the
United States Agency
for International Development

Colorado State University

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OF THE

THIRD CONFERENCE OF THE INTERNATIONAL

PLANT BIOTECHNOLOGY NETWORK (IPBNet)

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Published by the Tissue Culture for Crops Project (TCCP)

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The Tissue Culture for Crops Project is a program of the United States Agency for International Development, implemented by Colorado State University under Cooperative Agreement No. DAN-4137-A-00-4053-00.

ACKNOWLEDGMENTS

The Tissue Culture for Crops Project extends its sincere thanks to the following organizations who donated funds to support IPBNet Conference speakers and participants and without which the Conference would not have been possible:

AID/African Bureau
AID/Barbados
AID/Indonesia
AID/Kenya
AID/Morocco
AID/Thailand
AID/Uganda
AID/Washington D.C.
Chiang Mai University, Thailand
Chulalongkorn University, Thailand
Ciba-Geigy Limited
CIMMYT (International Corn and Wheat Improvement Center)
CIP (International Potato Center)
The Egyptian Government
FAO (Food and Agriculture Organization)
IAEA (International Atomic Energy Agency)
IAV Hassan II, Morocco
ICRISAT (International Crops Research Institute for the Semi-Arid Tropics)
IDRC (International Development Research Center)
IFS (International Foundation for Science)
IITA (International Institute of Tropical Agriculture)
INTSORMIL (International Sorghum/Millet CRSP)
KARI (Kenya Agricultural Research Institute)
MIAC (Mid-America International Agricultural Consortium)
Monsanto Company
McCormick and Company, Inc.
Pioneer Hi-Bred International, Inc.
[illegible]
The Rockefeller Foundation
Twyford International, Inc.
UNEP (United Nations Environmental Program)
UNESCO (United Nations Educational, Scientific, and Cultural Organization)

PROBLEMS AND PROSPECTS FOR GRAIN LEGUME BREEDING

Y. L. Nene, J. P. Moss, and C. L. L. Gowda

ABSTRACT

Grain legumes are an important source of nutrition, and contribute a substantial part of dietary protein in many parts of the world. Groundnut (*Arachis hypogaea* L.) is also an important source of edible oil.

There are large differences in yield per unit area, even ten-fold increases, between different locations, and between experimental plots and farmer's fields. Reduced yields are due to a range of factors, primarily climatic, edaphic and biotic. Among the major yield reducers are pests and diseases, drought, and adverse soil conditions.

In addition to breeding for yield *per se*, breeders have incorporated resistances to pests and diseases and other yield constraints. However, the primary gene pool that is easily accessible to the breeder lacks many of the characters needed.

A range of techniques need to be applied to grain legumes to overcome these constraints. Embryo rescue may be sufficient where the gene can be found in close relatives, but in many cases more sophisticated techniques are needed.

The priorities in breeding efforts for different crops will be discussed.

INTRODUCTION

Grain legumes are an important source of nutrition, and contribute a substantial part of dietary protein in many parts of the world. They are grown on a wide range of soil types and under varying conditions from cool temperate zones to humid tropics. Some are widely distributed; others, such as mungbean (*Vigna radiata* L. Wilczek) are more localized but still occupy an important position. Groundnut and soybean (*Glycine max* L. Merr) are also important sources of edible oil.

Grain legumes are important crops of developing countries (Table 1); in many cases they are essential to the nutrition of the population and the economy of the country.

Table 1. Areas (million ha) Under cultivation of selected important grain legumes, 1986.

	World	Dev'd Countries	Dev'g Countries	Africa	N\C America	South America	Asia
Beans, Dry[1]	26.2	2.1	24.1	2.6	3.1	6.2	12.9
Chickpeas[1]	10.5	0.1	10.3	0.4	0.2	<0.1	9.7
Pigeonpea[2]	4.1	0.1	4.0	0.5	<0.1	<0.1	3.5
Groundnuts[1]	19.7	0.9	18.8	5.4	0.8	0.4	13.1
Pulses, Total[1]	68.4	12.4	56.0	10.9	3.9	6.7	35.7

[1] From FAO Production Yearbook, 1986.
[2] From International Pigeonpea Newsletter 5:10-14.

Areas of production are defined by both edapho-climatic and socio-economic factors, and within these areas, production in each crop has been limited by a number of constraints. These can be classified broadly into biotic, such as pests and diseases, and abiotic, such as inclement soil and climatic conditions, and drought. In addition, there are constraints on availability of inputs (Table 2). Although there have been concerted international and national efforts to overcome these constraints, much remains to be done.

Table 2. National and International Activities in Grain Legume Improvement

<u>Constraints</u>

- Management
  - Pests, diseases, soil conditions, drought
  - Government policies/Infrastructure
  - Lack of inputs, marketing facilities

<u>Improvement Efforts</u>

- International Efforts
  - Consultative Group on International Agricultural Research
    - ICRISAT : Groundnut, Chickpea, Pigeonpea
    - CIAT : Dry beans (*Phaseolus*)
    - IITA : Cowpea, Soybean
    - ICARDA : Faba beans, Lentils, Chickpea
- National Efforts
  - Developed countries
    - Rapid dissemination and use of new technologies
    - Improved management
  - Developing countries
    - Lack of inputs
    - Aware of new technology, but:
      - Extension services inadequate
      - Management often suboptimal

Recent advances in biotechnology have opened up new vistas for crop scientists and offer new hope to the farmer. This paper outlines the major constraints to production of some grain legumes, and suggests how these may be addressed. Groundnut, beans (mostly *Phaseolus*), chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L. Millsp.) are important in Asia, and groundnut, beans, and cowpeas (*Vigna unguiculata* L. Walp) are important in Africa (Table 1). Their importance has been recognized by the Consultative Group on International Agricultural Research (CGIAR) by their inclusion in the mandate of one or more international agricultural research institutes.

There are some excellent books on grain legumes and pests and diseases (Summerfield and Bunting 1980, Summerfield and Roberts 1985) and on tropical legume pathology (Allen 1983); it is not our intention to give a complete review of the subject, but to highlight some of the outstanding problems.

CONSTRAINTS TO PRODUCTION

Biotic Stresses

Grain legumes are attacked by a wide range of fungal, bacterial and viral diseases, and a host of insect and other pests. Some of these are distributed throughout the whole growing area of the crop, while others are restricted to certain continents (*e.g.* Groundnut Rosette Virus to Africa) or are only of local significance.

Fungal diseases. High levels of resistance to **ascochyta blight,** (*Ascochyta rabiei* Pass. Lab.), or **botrytis gray mold** (*Botrytis cinerea* Pers., ex Pers.) are not available in cultivated chickpeas. The levels of resistance available are about 5 on a 1-9 scale (1=resistant, 9=susceptible), and the recovery of such resistance in the progenies is low. A few related *Cicer* species have slightly higher levels of resistance (2 or 3 rating); however, these do not cross with chickpea, and embryo rescue may be needed to produce hybrids from these crosses.

Cowpea grown in the humid forest belt of West Africa is attacked by a wide range of diseases, including **cercospora leafspot** (*Cercospora canescens*), **powdery mildew** (*Erysiphe polygoni*), **fusarium wilt** (*Fusarium oxysporum*), **fusarium root rot** (*Fusarium solani*), **ascochyta blight** (*Ascochyta phaseolorum*), **phytophthora stem rot** (*Phytophthora vignae* and *Phytophtora cactorum*), **and verticillium wilt** (*Verticillium albo-atrum*), for which resistances are available. There is no reported resistance, however, to **anthracnose** (*Colletotrichum lindemuthianum*), **pythium stem rot** (*Pythium aphanidermatum*), **web blight** (*Rhizoctonia solani*), and **brown rust** (*Uromyces appendiculatus*), which are major causes of crop loss (Singh and Allen 1980).

Wild relatives of common bean (*Phaseolus vulgaris*) are good sources of resistance to fungal diseases. Resistance to **anthracnose** (*Colletotrichum lindemuthianum* (Sacc. and Magn., Bri. and Cav.), **rust** (*Uromyces phaseoli* (Pers. Wint.) and angular **leafspot** (*Phaeoisariopsis griseola* Sacc., Ferraris), has been identified in **scarlet runner** (*P. coccineus*), (CIAT 1987), but as numerous races of these pathogens exist, further work is needed to introduce stable resistance into *P. vulgaris*. *Phaseolus coccineus* is also resistant to **ascochyta blight** (*Ascochyta phaseolorum* Sacc.) and **beanfly** (*Ophiomyia phaseoli* Tryon.). Tepary bean (*P. acutifolius*) is resistant to **common bacterial blight** (*Xanthomonas phaseoli*), (CIAT 1988) and **leaf hoppers** (*Empoasca kraemeri*).

Early leaf spot, caused by Cercospora arachidicola and **late leaf spot** caused by Phaeoisariopsis personata, result in extensive defoliation in groundnuts and subsequent yield losses. Although there is some resistance in A. hypogaea, the best sources of resistance are the wild species. Arachis cardenasii is resistant to **late leaf spot**, and many lines have been produced incorporating this resistance. Resistance to **early leaf spot** has been identified in species of section Erectoides (ICRISAT 1987) which have not been crossed with A. hypogaea.

Resistance to **groundnut rust**, caused by Puccinia arachidis, has been identified in the cultivated germplasm. There are indications that a different gene is involved in the resistance that has also been reported in the wild species (Singh et al. 1984), and use of this could lead to improved stability of resistance. Germplasm with resistance from wild species have been produced at ICRISAT (Moss et al. 1988).

Bacterial diseases. Bacterial diseases have not been given high priority in many breeding programs, as bacterial infections do not frequently cause epidemics; however, yield losses can be as high as 40% (Allen 1983) and bacterial diseases are often difficult to control. Some resistance is available—such as to **bacterial leaf spot** and **stem canker** (Xanthomonas cajani)—in accessions of pigeonpea from Africa. Disease incidence often depends on presence of damage to the plant, either by insect or mechanical means, after which the bacteria invade the plant and multiply rapidly. Only a few genera of bacteria cause crop losses, and some species infect a wide range of legumes. Selection of resistance may be possible in cell cultures. A gene, with action similar to that of the viral coat protein gene, could be isolated and used, though there does not seem to be an urgent need at present.

Viral diseases. Viruses have a well-established reputation for devastating legume crops, as there is a wide range of virus diseases whose effects range from minimal yield loss to total destruction of the crop. The true picture is often confounded by difficulties of identification.

Peanut Stripe Virus has received much attention in the last few years. Previously of restricted distribution, it has spread to many countries, and is both seed-borne and aphid (Aphis craccivora Koch) transmitted. Extensive field screening of the germplasm has so far failed to find resistance in A. hypogaea, but resistance has been reported in A. diogoi, A. helodes and other wild species (Culver et al. 1987).

Virus diseases of cowpea are very important. Reported yield losses can be as high as 80% for cowpea severe mosaic. Sunn-hemp mosaic and cowpea banding mosaic can each result in losses of 40% or above (Singh and Allen, 1980). There are no reports of resistance to these viruses.

Pests. Moderate levels of resistance have been found in pigeonpea against **pod borer** (Helicoverpa armigera Hubner) and **pod fly** (Melanagromyza obstusa Malloch). These resistances are being incorporated into breeding lines. Moderate levels of resistance are available in chickpea for **pod borer** (Heliocoverpa); however, Heliocoverpa remains a major problem for both chickpea and pigeonpea, and a higher degree of resistance is needed to stabilize production.

The **pod borer** (Maruca testulalis), is the major world wide pest of cowpea. (Singh and Allen 1980, Steele et al. 1985). Some wild cowpeas and related species are resistant to **pod borer** Maruca (Singh, pers comm.).

Leaf hoppers (Empoasca spp.), **bruchids** (Zabrotes subfasciatus and Acanthoscelides obtectus), and **whitefly** (Bemisia tabaci) are pests of beans worldwide. **Whitefly** is more important as a vector of bean golden mosaic virus than in its role of yield reducer. **Bean pod weevil** (Apion godmani) is important in Latin America, and **bean fly** is the worst pest of beans in Africa. Resistance to **beanfly** has been identified in P. coccineus (CIAT 1987), and to **leaf hoppers** in P. acutifolius (CIAT 1988). Resistance to **bruchids** has been found in wild forms of P. vulgaris (CIAT 1988).

Abiotic Stresses

Many of the resource-poor farmers of the world who rely on grain legumes as their major source of protein grow them on soils which are low in nitrogen and phosphate, in areas which are subject to drought. Crops grown on acid soils are adversely affected by aluminum or manganese toxicity. This is particularly true for beans. Cowpea is grown under the same conditions as the major crops with which it is co-cultivated, which tend to be in the low input, rainfed systems of subsistence farming. Many of the cowpeas grown therefore suffer from low fertility levels and low moisture availability.

Soil salinity is increasingly important where chickpea and pigeonpea are grown. Cultivated germplasm does not have appreciable levels of resistance, but a moderate level of tolerance to salinity has been found in Atylosia albicans and A. platycarpa, and needs to be transferred to pigeonpea. Possibilities exist for single cell selection in suspension cultures.

Drought is especially limiting in groundnut production. The plants are susceptible to drought at flowering and at pod filling. The effect of end of season drought has been reduced by breeding short-duration varieties, but there is still a need to breed shorter duration varieties to fit crop rotations. A similar approach is applicable to chickpea which is grown on residual moisture.

Temperature extremes are another threat to which tolerance is needed. Cold tolerance would be valuable in chickpea when grown as a winter crop, and Cicer microphyllum has been identified as a source of resistance.

RECOMMENDATIONS

Wild species of many legumes are good sources of resistance. The first priority, therefore, is to screen the wild germplasm to identify desirable traits. Techniques of hormone treatment to prevent pod abortion and maintain ovule growth, and techniques of ovule and embryo rescue are widely applicable; however techniques, timings, concentrations, and media used are specific to each crop and need further attention.

Use of wide crosses in legumes is problematic as resulting hybrids have many undesirable features. Many generations of backcrossing are needed to regain the adaptability of the cultivated parent. The use of vectors containing resistance genes from wild species to transform adapted cultivars overcomes these problems, and opens up the possibility of transferring these genes into completely unrelated genera.

Some solutions may have wide applicability in legumes, such as the use of the Bt and similar genes for insect resistance, and the use of gene coding for viral coat proteins to provide virus resistance. These genes have wide applicability, and should be transferred to legumes when the techniques for plant regeneration have been developed. Major thrusts are called for in these fields, both to transfer available genes to grain legumes, and to search for other genes.

Although biotechnology holds promise for the legume breeder, many of the grain legumes are not easy to regenerate from callus or single cells, and this aspect should receive attention. Much basic work is needed before the full potential of the germplasm, and the new techniques to utilize the available variation, can be realized.

Selection in cell suspension cultures shows promise for improving a number of traits. This technique could be applied for salinity tolerance and for disease resistance where no suitable source can be found in the existing germplasm.

There are a number of techniques which assist the legume breeder in crop improvement. The application of anther culture to generate haploids is one such technique. Isolation of the gene for resistance and subsequent development of probes for gene detection and vectors for asexual transfer would be beneficial in breeding efforts. Asexual transformation has the advantage of introducing a desirable gene without disturbing the adaptation of the recipient cultivar.

CONCLUSIONS

Biotechnology adds a new phase to the ongoing process of crop improvement in grain legumes. Some goals have been achieved by conventional means, and there are a number of adapted lines with resistance to certain pests and diseases which are, or shortly will be, contributing to increased yields. Much remains to be done, however—especially in the field of pest and disease resistance—to improve and stabilize yields of these important crops.

ACKNOWLEDGMENTS

The authors acknowledge the help of Dr. S.R. Singh, Director, Grain Legume Improvement Program, IITA, and of the Legume Breeders, ICRISAT, in preparation of this paper.

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