Akshay Kumar Chakravarthy
Venkatesan Selvanarayanan *Editors*

Experimental Techniques in Host-Plant Resistance

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Foreword

The last five decades have witnessed rapid progress in the development of crop varieties resistant to insect pests. Advances in instrumentation technology, biotechnology and molecular biology have allowed scientists to adopt newer approaches and devise more precise techniques for identifying plants containing resistance and/or tolerance to insect pests. Improved insights into the genetics and breeding of crop plants for resistance have further facilitated developments in host plant resistance studies. Current research efforts focused on durable pest resistance form the most important programmes in food crops, horticulture and forestry, where pest-resistant plants continue to be economically and ecologically superior to broad-spectrum insecticides. Identifying and utilizing pest-resistant varieties remain very cost-effective, often requiring less time than transgenic techniques. Continued growth in the human population and the associated need for increased food availability reinforce the pressing demand for efficient high-throughput plant germplasm screening schemes to identify and exploit sources of resistance.

The book *Experimental Techniques in Host-Plant Resistance* contains 30 chapters contributed by 60 authors, who addressed the needs for the development of more precise yet rapid methods of identifying and implementing insect crop plant resistance. They have attempted to collate information on standardized procedures used to determine resistance mechanisms; molecular marker-assisted selection of resistance; durability of insect-resistant plants in varying climatic regimes; and understanding the role of resistance in tritrophic interactions. I am hopeful the book will educate all researchers about the need to adopt refined methods to investigate and identify insect-resistant plants. The use of such improved techniques and technologies will greatly benefit the large-scale cultivation of insect-resistant crops across diverse landscapes and human communities.

Department of Entomology, Kansas State University, Manhattan, KS, USA
March 2019

C. Michael Smith

Preface

The earliest land plants evolved around 450 million years ago from aquatic plants devoid of vascular systems. The diversification of flowering plants (angiosperms) during the Cretaceous period is associated with speciation in insects. Early insect herbivores were mandibulate, but the evolution of vascular plants led to the co-evolution of other forms of herbivory, such as leaf feeding, sap sucking, leaf mining, tissue boring, gall forming and nectar feeding. Plant defence against biotic stress is an adaptive evolution by plants to increase their fitness. Plants use a variety of strategies to defend against damage caused by herbivores. Plant defence mechanisms are either inbuilt or induced. Inbuilt mechanisms are always present within the plant, while induced defences are produced or mobilized to the site where a plant is injured. Induced defence mechanisms include morphological and physiological changes and production of secondary metabolites.

Host plant resistance is one of the eco-friendly methods of pest management. It protects the crop by making it less suitable or tolerant to the pest. While books on the theoretical aspects of host plant relationships/resistance are available, an exclusive book on the practical aspects is lacking. There is a wide gap between the theory and the experimental procedures required for conducting studies on host plant resistance for the postgraduate students and young researchers. A dire need for a book on practical aspects was strongly felt. Initially, a practical manual was prepared which eventually evolved into the present book. We hope this book, *Experimental Techniques in Host-Plant Resistance*, will be useful. The book provides information on major aspects of screening crop germplasms, sampling techniques, genetic and biochemical basis of HPR, behavioural studies on plant volatiles and some of the recent approaches in HPR. Besides the detailed procedures, the 'references', 'further reading' and 'illustrated examples' provide the additional material for the benefit of readers and workers alike. Illustrated examples provide at a glance, the tools and experimental setups for executing the techniques. The examples can also serve to help evolve an idea or understand the principle based on which the techniques have been developed. It further broadens one's prospective to meet the desired objective or goal. We hope this will further kindle interest in researchers to develop improved techniques in host plant resistance.

Bangalore, Karnataka, India — Akshay Kumar Chakravarthy
Chidambaram, Tamil Nadu, India — Venkatesan Selvanarayanan
March 2019

Acknowledgements

Host Plant Resistance is indeed a dynamic field, and researchers are continuously refining and updating techniques for studying different aspects of host plant resistance/relationships. It was a daunting task to collate documented information and concisely put it in a volume. The editors thank the contributors for their time and attention to write chapters on different aspects of host plant resistance. The chapters have been categorized under the following five parts: (I) Prerequisites for Host Plant Resistance Studies; (II) Instrumentation in Host Plant Resistance Studies; (III) Techniques in Host Plant Resistance Studies; (IV) Ecological and Climatographic Factors in Host Plant Resistance Studies; and (V) Genetics, Plant Breeding and Molecular Tools in Host Plant Resistance Studies. 60 contributors have written chapters for this book. The wide-ranging techniques can be applied across several species of insects with slight modifications or changes. Often, the screening germplasm results are not comparable directly, and we hope this book will go a long way in rendering the data comparable.

The editors are grateful to Mr. Subhash S., Dr. Doddabasappa B., Mr. Kumar K. P., Mr. Nitin K. S. and other postgraduate students and faculty from different universities in India. The editors are also thankful to the vice chancellors of the universities in India for encouragement. Select photos and line diagrams have also been taken from few books and other sources mentioned under “references”; we are thankful for the contribution. This has been done primarily for the benefit of a large number of undergraduate and postgraduate students and also for the research scholars who constitute a major share of the readership. The contribution from the selected books/publishers/authors/editors for this compilation is gratefully acknowledged by the editors. Colleagues from entomology across several universities and institutes in India and abroad have made valuable contributions to the book. We indeed are lucky to have the Foreword from a fine expert on host plant resistance, Dr. C. Michael Smith, Kansas State University, USA. We are highly indebted and thankful to Dr. C. Michael Smith. We also take this opportunity to thank Dr. S. Lingappa and Dr. P. Narayanaswamy who inspired us and stimulated ideas on insect–plant relationships. The editors are grateful to them all. Finally, the editors are thankful to Springer India Private Ltd., New Delhi, for their deep interest and enthusiasm in publishing this book.

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Dr. A. K. Chakravarthy Head and Principal Scientist, Division of Entomology and Nematology, he is an author of many books and over 300 scientific papers and 50 chapters in Entomology and Natural history. His interest includes insects, birds, bats, rodents and mammals. With nearly four decades of experience in teaching, research and extension, Dr. Chakravarthy has been the investigator for over 35 research projects and has guided more than 25 students for post-graduation. A Ph. D. from Punjab Agriculture University and fellow from IARI, New Delhi, he is a member of several national and international scientific academia, an adviser, a panellist, a referee, reviewer and editor and is associated with publication of several national and international journals worldwide. A field-orientated, widely travelled biologist, he is actively working on novel approaches in integrated pest management, host plant Interaction, vertebrate pest management, biodiversity and environmental conservation issues. Recently, he has published books titled, *New Horizons in Insect Science: Towards Sustainable Pest Management*, *Economic and Ecologi cal Significance of Arthropods in Diversified Ecosys tems: Sustaining Regulatory Mechanisms* and *Arthropod Diversity and Conservation in the Tropics and Sub-tropics*, The Black spotted, Yellow Borer, *Conogethes punctiferalis* Guenee and Allied Species by Springers.

Dr. V. Selvanarayanan Professor of Entomology, Faculty of Agriculture, Annamalai University, he has 25 years of teaching and research experience. He was a gold medallist in post-graduation, and his doctoral research resulted in identification of new species of tomatoes resistant to insects. He was awarded with the Best Research Paper Award for 2006 and Distinguished Teacher Award for 2010 at Annamalai University. He has authored more than 80 research articles and 4 books. He is a fellow of Plant Protection Association of India and life member in many professional societies. He has spearheaded many externally funded research projects. He is passionate about travelling and has visited the UK, USA, UAE, China and other countries. He enjoys interacting with peers and delivers invited lectures in scientific meetings. As additional responsibilities, he served as Deputy Controller of Examinations and as Head, Department of Entomology at Annamalai University. He is on the editorial board of select scientific journals.

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Part I
Prerequisites for Host Plant Resistance Studies

Introduction

V. Selvanarayanan

Abstract Since the seventeenth century, efforts have paved way for developing insect-resistant varieties. Resistant varieties are compatible with biological and cultural practices. Resistant varieties are especially of value in low-income group crops. Resistant varieties may enhance the efficacy of insecticides and environment quality. On the whole, resistant varieties form an ideal means of pest suppression.

Keywords Resistant Varieties · Role in pest management · History · Resistant variety · Ecology

1 Introduction

Plants acquired insect-resistant characters due to their co-evolution with insects. Since crop domestication was initiated by mankind, exploitation of desirable plant types was common. Intensive agriculture enabled more research impetus on exploiting crop varieties possessing insect tolerance or resistance. An insect-resistant crop cultivar yields more than a susceptible cultivar. Use of such insect-resistant/insect-tolerant cultivars evinces enhanced productivity without harming the benign environment. The genetic diversity of many crop plants offers ample scope for exploitation of resistant traits. The relationship between an insect and crop cultivar is influenced by the kind or mechanism of resistance, namely, antixenosis (non-preference), antibiosis and tolerance (Painter 1958). Resistance in a crop cultivar is exerted due to the presence of biophysical or biochemical factors resulting in any one of the above mechanisms of resistance. Cultivated varieties, wild lines, land races and other distantly related genera of crop plants are gathered and screened for resistance at the field and/or glasshouse conditions, and promising resistant sources are being exploited since early period, for large-scale cultivation or for future breeding programmes.

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2 Early Success

Since the eighteenth century, extensive attempts have paved way for developing insect-resistant crop varieties. In 1788, early maturing varieties of wheat were developed to escape from Hessian fly, *Mayetiola destructor* (Say), damage (Chapman 1826). This was followed by the identification of wheat cultivar "Underhill" in New York as resistant to Hessian fly (Havens 1792). Apple varieties "Winter Majetin" and "Northern Spy" were recommended as resistant to woolly aphid, *Eriosoma lanigerum* (Hausmann) (Lindley 1831). Later, the entire wine industry of France was devastated by incidence of phylloxera, *Daktulosphaira vitifoliae* (Fitch). To overcome this infestation, grafts of rootstocks of American grapes, *Vitis labrusca*, with the scions of French grapes, *Vitis vinifera*, were popularized which evinced revival of the vine industry (Smith 2005). With the advent of intensive agriculture, development and use of insect-tolerant/insect-resistant crop varieties gained prominence.

The International Rice Research Institute (IRRI), Manila, Philippines, developed improved varieties that possessed resistance to key insect pests and diseases. Rice variety IR36 was found resistant to brown planthopper, green leafhopper, stem borers, gall midge, blast, bacterial blight and tungro. This cultivar was planted in about 11 million ha in the world which enhanced the economic returns of rice growers and processors exponentially (Khush and Brar 1991).

3 Role in Pest Management

By reducing pest numbers, resistant varieties helped to shift the pest/predator ratios in favour of biological control (Heinrichs 1994). Varietal resistance is usually compatible with biological control but may also have an adverse effect on natural enemies. In field studies at IRRI, Philippines, the brown planthopper, *Nilaparvata lugens* (Stal), and spider *Lycosa pseudoannulata* (Bosenberg and Strand) ratio increased with the level of susceptibility from ASD7 and IR36, both highly resistant rice cultivars, to IR42 and Triveni, moderately resistant cultivars to IR8 and TN1, susceptible cultivars. The resistant plant may also enhance the predatory activity. Predation rate of the mirid bug, *Cyrtorhinus lividipennis* Reutger, when feeding on the first instar brown planthopper nymphs increased on the resistant cultivar, IR36, compared to the susceptible IR8.

Use of resistant varieties is most useful in crops of low value where yields fluctuate due to weather and other factors. Resistant varieties are of special significance for countries like India where holdings are small and farmers are not equipped to take up other methods of pest suppression. Physical and mechanical control methods are the oldest and offer several primitive ways of suppressing the pests.

For example, *Lablab niger* Medick creeper type is generally tolerant to pod borers compared to field-type *L. niger*. Collection and destruction of larvae would be easier on resistant/tolerant cultivars than susceptible cultivars (Chakravarthy 1978). Chakrabarthy et al. (1970) reported grape varieties, namely, Bangalore Blue and Bhokri, to be resistant to the defoliator, *Adoretus devanceli* (Blanch), than Bangalore Purple, Khalili and Anab-e-shahi grapes, and hence it was convenient to pick and destroy defoliator on resistant than susceptible grape varieties.

Host plant resistance may enhance the efficacy of insecticides. Evaluation of insecticides in rice indicated that they cause higher mortality of plant hoppers and leafhoppers feeding on resistant than on susceptible rice varieties (Heinrichs 1994). The mortality of brown planthopper reared on either moderately resistant ASD7 or a highly resistant cultivar "Sinna Sivappu" was higher than when feeding on a susceptible TN1 cultivar. The LD_{50} of white-backed planthopper was 9.4 on the susceptible variety TN1 treated with ethylan but only 2.8 on moderately resistant N22.

Certain insects in addition to infesting crop plants also transmit pathogens causing diseases and thus serve as vectors. Host plant resistance is an effective means to manage both the disease and the vector. For instance, certain banana varieties showed resistance to bunchy top because of its less susceptibility to the banana aphid, *Pentalonia nigronervosa* Coq., the bunchy top vector (Verghese 2001; Hooks et al. 2009). Certain species of citrus like *Citrus nobilis*, *C. aurantifolia* and *C. rashmi* had significantly lower populations of the vector aphid (*Toxoptera* sp.), from among 22 species and 3 hybrids (IIHR 1984). Birch et al. (1992) showed the use of restriction fragment length polymorphism (RFLP) analysis, in distinguishing biotype of the virus vector aphid, *Amphorophora idaei* Borner, thus aiding in raspberry screening.

The effect of resistant host plants on pest population is specific, cumulative and persistent. It offers no environmental hazards and is eco-friendly. It is compatible with other pest management methods. However, the occurrence or selection of pest biotypes limits the effectiveness of resistant variety. Resistant factors may be incompatible with desired economic characters. In many crops, a variety resistant to one pest may be susceptible to another pest. Pod borer-resistant cultivars of *Lablab niger* are susceptible to the aphid, *Aphis craccivora* Koch (Chakravarthy 1992). Similarly, a variety resistant to insect pests may be susceptible to diseases as in the case of sesame wherein capsule borer-resistant varieties are susceptible to phyllody disease (VijaiAnandh and Selvanarayanan 2005).

Considering the above concepts, development of resistant varieties of crops should be a recurrent, continuous and concerted attempt. Substantial success has been achieved in resistance breeding of crops to manage plant pests and diseases. Buddenhangen (1991, 1996) argued that the popularity of insecticides, and even insect-based integrated pest management, has reduced efforts in resistance breeding. Attempts on resistance breeding are generally focused on exploiting qualitative vertical resistance. This approach is convenient because high levels of resistance can be achieved and because the method is compatible with breeding schemes used for enhancing crop performance through control of major genes. However, its gene-for-gene nature can sometimes lead to its breakdown of resistance due to selection pressure on the pest species. Classic examples of such resistance breakdown in insect pests include the Hessian fly, *Mayetiola destructor* Say, and green bug, *Schizaphis graminum* (Rondani), on wheat and the brown planthopper, *Nilaparvata lugens* Stal.; green leafhopper, *Nephotettix virescens* (Distant); and rice gall midge, *Orseolia oryzae* (Wood-Mason), on rice. While plant resistance breeding is often identified as a component of integrated pest management (IPM), its actual integration with other pest control methods in IPM systems has been limited. In most cases, breeding programmes have sought single technology solutions to pest problems (i.e. complete resistance), much like insecticides. This is often hard to achieve for some pests, e.g. stem borers in cereals, and while partial resistance can be obtained as in field bean, *L. niger*, to pod borer (Chakravarthy 1978, 1992). Partially resistant varieties, however, may have a value when viewed in an IPM context, where the contribution of natural enemies and other factors complements their effects (Thomas and Wage 1993).

Integrated pest management (IPM) approach can also contribute to the durability of plant resistance to pests. The action of mortality factors, such as natural enemies, can reduce pest populations and thereby delay the selection pressure to overcome the resistance factors. The role of natural enemies in maintaining durability in this manner has been demonstrated (Gould 1996).

In view of the above-said advantages, exploring, exploiting and employing host plant resistance will envisage eco-friendly pest management, for which realistic cooperation among entomologists, pathologists and plant breeders is needed. For developing insect-resistant cultivars, proper practical expertise and standard protocols are necessarily to be followed. Only such attempts will enable development of crop varieties with durable resistance (Figs. 1, 2, 3, 4, 5, 6, 7 and 8).

Fig. 1 Hessian fly – *Mayetiola destructor*. (Source: www.bugwood.com)

Fig. 2 Grape phylloxera – *Daktulosphaira vitifoliae*. (Source: BugGuide.Net)

Fig. 3 Woolly aphid – *Eriosoma lanigerum*. (Source: barmac.com.au)

Fig. 4 Camellia or black citrus aphid – *Toxoptera aurantii*. (Source: www.aphotofauna.com)

Fig. 5 Banana aphid – *Pentalonia nigronervosa*. (Source: http://www.musarama.org)

Fig. 6 IR 20 rice variety resistant to key pests. (Source: http://www.agritech.tnau.ac.in/)

Fig. 7 IR 36 rice variety resistant to key pests. (Source: http://www.agritech.tnau.ac.in)

Fig. 8 Brown planthopper –*Nilaparvata lugens*. (Source: http://www.pestnet.org)

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Germplasm Exploration and Collection

A. K. Chakravarthy and V. Selvanarayanan

Abstract A prerequisite for detecting sources of resistance to insect pests is to have diversified crop germplasm. Information on major sources for obtaining germplasm plays a crucial role in developing resistant crop varieties. This chapter indicates 220 sources of germplasm centres for more than 25 crop plants. Monoculture and intensive cultivation practices endanger the local germplasms, and care should be taken to conserve them. Wild species of the cultivated crops need also to be conserved. Local expertise, manpower and resources need to be best utilized for identifying resistant sources.

Keywords Germplasm collection · Utilization · Germplasm sources · Conservation

1 Introduction

Genetic resources of crop plants are diverse and offer ample scope for exploration and exploitation. Russian geneticist N.I. Vavilov emphasized that the success of crop improvement programmes depends on the strength of the germplasm gathered. Vavilov and his coworkers identified that China, India, Central Asia, Asia Minor, the Mediterranean, Ethiopia, Central America and western-central South America are the great centres of floral genetic diversity in the world (Maps 1 and 2).

Germplasm collections may consists of (1) improved modern cultivars under cultivation, (2) unimproved or purified cultivars no longer in cultivation, (3) breeding stocks developed by breeders but not released for cultivation, (4) land races, (5) weed races and (6) wild species (Panda and Khush 1991).

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2 Major Sources of Crop Germplasm

Extensive efforts of centres of excellence in the world have culminated in the maintenance of huge germplasm of crop plants. Early efforts of Russian geneticist Vavilov and his colleagues beginning in 1916 enabled the establishment of the world's first scientific gene bank at St. Petersburg, Russia, comprising more than 250,000 accessions. Since then, many crop-specific and general gene banks have been established. A major impetus to germplasm exploration, collection and preservation was given since the inception of the International Board for Plant Genetic Resources (IBPGR) in 1974 by the Consultative Group for International Agricultural Research (CGIAR). Later in 1991, it was rechristened as the International Plant Genetic Resources Institute (IPGRI). In 1994, it was given autonomous status as one of the centres of CGIAR. Subsequently, IPGRI took over the governance of International Network for Improvement of Banana and Plantain (INIBAP). During 2006, both organizations merged and christened as Bioversity International with headquarters in Rome, Italy. Since inception of IBPGR, genetic resources of all crops are maintained.

Besides Bioversity International, other international agricultural research centres such as CIAT (beans, cowpeas), Cali, Columbia; CIP (cassava, potato), Lima, Peru; CIMMYT (maize, wheat), Mexico City, Mexico; ICARDA (legumes), Aleppo, Syria; ICRISAT (millet, sorghum), Patancheru, India; IITA (cassava, cowpeas), Ibadan, Nigeria; and IRRI (rice), Los Baños, Philippines, maintain major germplasm holdings. In addition to such international institutes, national-level germplasm preservation centres are established, like the National Bureau of Plant Genetic Resources (NBPGR) in India or Plant Gene Resources of Canada (PGRC). Many sources of germplasm also exist in foreign national seed collections maintained by voluntary organizations and private seed companies.

The following is the list of major sources of germplasm of important crops (Batugal et al. 2005; Khan 2007; Smith 2005).

2.1 *Banana/Plantain, Musa* spp.

Bioversity International Musa Germplasm Transit Centre, Belgium
Indonesian Tropical Fruit Research Institute, Solok, West Sumatra, Indonesia
Institut des Sciences Agronomiques du Rwanda (ISAR), Rubana, Rwanda
National Research Centre for Banana (ICAR), Tiruchirappalli, India

2.2 *Barley,* Hordeum vulgare*; Oat,* Avena sativa*; and Rye,* Secale cereale

International Center for Agricultural Research in the Dry Areas (ICARDA), Syria

International Maize and Wheat Improvement Center (CIMMYT), Mexico
Waite Agricultural Research Institute Barley Collection, Adelaide, Australia
Ohara Institute for Agricultural Biology, Okayama University, Kurashiki, Japan
Agricultural Research Institute, Kromeriz, Czech Republic
Swedish Seed Association, Svalov, Sweden
USDA-ARS National Small Grains Collection, Aberdeen, Idaho, USA
Biotechnology and Biological Sciences Research Council (**BBSRC**) Cereal Collections, Germplasm Resources Unit, John Innes Centre, Norwich, UK

2.3 Bean, Phaseolus *Species, and Cowpea,* Vigna *Species*

Centro Internacional de Agricultura Tropical (CIAT), Colombia
Instituto Nacional de Investigaciones Agricolas (INIA), Mexico
International Institute of Tropical Agriculture (IITA), Nigeria
University of Cambridge, UK
USDA-ARS Western Regional Plant Introduction Station, Pullman, Washington, USA
N.I. Vavilov Institute of Plant Industry, St. Petersburg, Russia
Indian Institute of Vegetable Research, Varanasi, India

2.4 Capsicum

Asian Vegetable Research and Development Center (AVRDC), World Vegetable Centre, Tainan, Taiwan
Germplasm Bank of the Agricultural Experimental Station, La Consulta INTA, Argentina
International Center for Tropical Agriculture (CIAT), Cali, Colombia
Indian Institute of Horticultural Research, Bengaluru, India
Indian Institute of Vegetable Research, Varanasi, India

2.5 Castor

AGES Linz - Austrian Agency for Health and Food Safety/Seed Collection, Austria
Agricultural Research Station Teleorman, Teleorman County, Romania
Biodiversity Conservation and Research Institute, Ethiopia
Centro Nacional de Pesquisa de Algodao (CNPA), Brazil
Comunidad de Madrid. Universidad Politécnica de Madrid. Escuela Técnica Superior de Ingenieros Agrónomos. Banco de Germoplasma, Spain
Indian Institute of Oilseeds Research, Hyderabad, India

EMBRAPA/CENARGEN, Brasilia, Brazil
Empresa Baiana de Desenvolvimento Agricola SA, Brazil
Faculty of Agriculture, University of Zagreb, Croatia
Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research, Germany
Genetic Resources Institute, Azerbaijan
Gobierno de Aragón. Centro de Investigación y Tecnología Agroalimentaria. Recursos Forestales, Spain
Institute for Agrobotany, Hungary
Institute for Plant Genetic Resources 'K. Malkov', Bulgaria
Institute of Biodiversity Conservation (IBC), Ethiopia
Institute of Botany, Azerbaijan
Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, China
Institute of Crop Science (CAAS), China
Institute of Field and Vegetable Crops, Maksima Gorkog, Serbia
Institute of Oil Crops Research, China
Institute of Oil Crops, Ukraine
Instituto Agronomico de Campinas, Brazil
Maize Research Institute, Zemun Polje, Serbia
Medicinal and Aromatic Plants Research Station Fundulea, Romania
Millennium Seed Bank Project, Seed Conservation Department, Royal Botanic Gardens, Kew, UK
N.I. Vavilov All-Russian Scientific Research, Institute of Plant Industry, Russia
National Bureau of Plant Genetic Resources (NBPGR), India
National Genebank of Kenya, Crop Plant Genetic Resources Centre, KARI, Kenya
Plant Breeding and Acclimatization Institute, Poland
Research Station of Medicinal Crops, Ukraine
USDA-ARS-PGRCU, Griffins, Georgia, USA

2.6 Cassava, **Manihot esculenta**

Centro Internacional de Agricultura Tropical (CIAT), Colombia
Central Tuber Crops Research Institute, Thiruvananthapuram, India
International Institute of Tropical Agriculture (IITA), Nigeria
National Root Crops Research Institute, Umudike, Nigeria
National Crops Resources Research Institute, Kampala, Uganda

2.7 Chickpea, **Cicer arietinum***, and Lentil,* **Lens culinaris**

Ethiopian Gene Bank, Addis Ababa, Ethiopia