Micropropagation of Orchids

Volume I

This greatly expanded and updated edition of this classic reference work provides the user with comprehensive details of all procedures for propagating orchids through the culture of tissues *in vitro* (micropropagation).

Presented in two volumes, the book opens with a detailed and illustrated historical section which covers the discoveries, developments, and people which made micropropagation possible. The next section discusses in detail the principles and components of culture media which are used in micropropagation. The main component of the two volumes, however, is the third section. This comprises the detailed procedures for the culture of over 100 orchid genera. Classical, established, and the very latest techniques of clonal propagation of organs, tissues, and cells are presented. Every one of the listed methods includes tables of complete recipes for a wide range of culture media.

This new edition will continue to be *the* key reference for all those interested in and involved in orchid micropropagation, growing, and production.

Dr Joseph Arditti majored in floriculture as an undergraduate and received his doctorate from the University of Southern California in 1965. After serving as a lecturer for one year at the University of Southern California, he accepted a faculty position at the University of California, Irvine in 1966 where he taught General Botany, Horticulture, and Plant Physiology while engaging in research on various aspects of orchid biology in the US, Indonesia, Singapore, and Malaysia. Dr Arditti retired in 2001 and is now Professor Emeritus. He continues to write and travel worldwide to lecture at scientific meetings and to orchid groups. He is acknowledged to be one of the world's leading experts on orchid biology and propagation.

Micropropagation of Orchids

Second edition

Volume I

Joseph Arditti
Professor Emeritus
Developmental and Cell Biology
University of California, Irvine

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For
my 22-year-old son Jonathan
. . . yet again

My brother Mordi . . . again
and his Virginia Quintana

And the memory of my parents
Salomon (1902–1993) and Rebecca (1905–1997) Arditti

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Preface

Micropropagation of Orchids was "born" in 1974 when I initiated my *Orchid Biology, Reviews and Perspectives* (*OB*) series. Since a book containing only scientific literature reviews on orchid topics did not seem to have much of a financial or sales future I decided to include in the first volume a practical appendix entitled *Clonal Propagation of Orchids by Means of Tissue Culture – A Manual*. My hope was that the manual would attract buyers. I wrote the appendix while on sabbatical leave at one of my most favorite places on earth (the Bogor Botanical Gardens in Indonesia) using a portable manual typewriter which was a gift from my late father. Tissue culture propagation was relatively new then and the existing methods and literature citations were covered in 90 pages. The appendix accomplished its mission by attracting buyers and the series got its start in 1977.

By 1990 the first volume of *Orchid Biology, Reviews and Perspectives* was out of print and second hand copies were in considerable demand because of the appendix. The tail was wagging the dog. It was time to update and expand the manual and write a book on the subject which had acquired a name by then: micropropagation. I invited my colleague Dr. Robert Ernst to join me and we expanded the manual into *Micropropagation of Orchids* (*MO*1). It was published by John Wiley and Sons in 1993. *MO*1 included all of the procedures in the manual and almost all of the methods published between 1974 and 1990 in its 682 pages (nearly 7.6 times as many pages as the manual).

Like *OB*I, *MO*1 went out of print about 15 years after it was published. Demand for second hand copies rose quickly. Many people wrote me asking where to find a copy. By the year 2000 it was clear that the time had come to write a second edition of *MO*. I retired on July 1, 2001 and started to write a few months after that. My plan was to include in *MO*2 all the procedures in *MO*1 because they are still useful, and as many of the methods that were published after 1990 as possible (and hopefully all). What I found was that more new methods were published between 1990 and the year 2000 than from 1949 to 1990 (the period covered by the manual and *MO*1). Suggestions that orchid micropropagation was a mature field with a decreased number of publications seem to have been grossly exaggerated (to paraphrase Mark Twain) and completely unrealistic. This meant that writing would take a long time and it did. I finished the first draft in early 2004, edited it after that and stopped adding procedures on May 1, 2004, my 72nd birthday. There is no question

that many new methods will be published in the years after that, but *MO*3 will have to be written by someone else.

Chapter 1 in *MO*1 had presented a reasonably accurate history of orchid micropropagation, but I was made to remove parts of the story which placed the discovery and its discoverers in proper perspective because they questioned established dogma and the claims of the presumed discoverer. Professor (now emeritus) Abraham D. Krikorian and I used the excised parts as the basis for an extensive and precise history of orchid micropropagation, which was published in the *Botanical Journal* of the Linnean Society of London. This article served as the foundation for the history chapter in the present edition of *MO* which pulls no punches, tells the story as it happened, and places all historical figures in proper perspective. In retrospect I regret buckling down under intense pressure and allowing the history chapter in *MO*1 to be emasculated. I apologize for my lapse in good sense and momentary weakness. History must be reported as it really happened even if the actual facts may offend some people (even friends) because (to quote E. Mach, 1838–1916) "It is hardly possible to state any truth strongly without apparent injustice to some other." I thank Professor Krikorian for allowing me to quote liberally from our joint publication.

Chapters 2 and 4 were rewritten and revised not due to any shortcomings but because I decided to emphasize a few points more strongly, add information, and reword or restate several subjects. Both chapters are longer as a result of these changes.

With one exception, reviews of *MO*1 were very positive. The sole exception was critical of the absence of: (1) information about the use of colchicine to increase chromosome numbers in orchids, and (2) advice on how to combat internal contamination. This current edition also does not have information on the use of colchicine because this topic is not part of micropropagation. It belongs in a book on cytology, cytogenetics, or bioengineering. In view of the large amount of information that had to be included in *MO*2 my criteria for inclusion were very strict, narrow, and based entirely on the last half of the term micro*propagation*. Bioengineering, cytology, cytogenetics, physiology, molecular biology, and seed germination were excluded. As is obvious, even with this strict policy, the book grew to be very large.

Only published information was included in the manual and *MO*1 and is part of *MO*2. No published information on internal contamination in orchid cultures and how to handle it was available when the manual and *MO*1 were written. And, I could find none when writing this edition. Thus, when faced with the choice of presenting or not presenting non-existent (i.e., not available in the literature) information I chose the latter. However, I did include information on a variety of antibiotics and anticontaminants because they can be used to combat any contamination.

Most published papers on orchid micropropagation and tissue culture techniques contain information on media, culture conditions, and procedures which is sufficient for a presentation in *MO*, but some do not. When information was missing I made a few logical assumptions. For example, if a paper did not describe culture conditions in detail I assumed that standard culture room temperature and illumination will be suitable. In the few cases of truly atrocious papers, I made more (perhaps too many) assumptions and also called attention to their low quality.

Many years ago a thoughtful reviewer of one of my early papers pointed out that I failed to evaluate the quality and content of several articles that were mentioned in a review of the literature. He/she indicated that a certain amount of expertise is

implied in the writing of a paper or a review (and by implication a book) and that readers have a right to expect evaluations, criticisms, praise, opinion, and advice from an expert. *MO*1 was largely devoid of such comments, but I added several to this edition when they were called for. Some of these comments are negative. They may cause unhappiness in some quarters and/or generate criticism, but I think that the reviewer of long ago was right in suggesting that readers have a right to expect guidance and the opinions of an author who presumes to write a review or a book.

The author of a second edition has the advantage of hindsight since comments by readers, discussions with friends, opinions by users of the book, and statements by reviewers point to strengths and weaknesses. What I learned from comments about *MO*1 is that users of that book appreciated having the following:

- redundancy ["move the tissue to the first medium (Table XYZ-1) and then to the second solution (Table XYZ-2) before returning it to the first substrate (Table XYZ-1) and then taking it to the third (Table XYZ-3)" was liked by users who when asked and given a choice preferred this type of writing to "move the tissue to the first medium and then to the second solution before returning it to the first substrate and then taking it to the third"],
- repetitions ("tell me how to prepare a sterilant every time I need to use it, don't refer me to 100 pages back"),
- details ["dissolve it in 70% ethanol (73 ml 95% ethanol diluted to 100 ml with distilled water)" was preferable to "dissolve it in 70% ethanol"],
- clarity (short declarative sentences rather than long and involved ones),
- unambiguous instructions ("don't give me a choice between two sterilants, tell me which one to use"),
- simple language ("don't use a long chemical name if the compound has a trade-name or list both"), and
- self-standing procedures ("list all media and solutions with every procedure, don't tell me to use the medium in Table JOA-1 on p. xxx first, then the solution in Table MA-3 on p. yyy, the substrate in Table VQ-9 on p. zzz after that and finish with Table SUN-8 on p. aaa. This will make me leaf through the book endlessly in search of media and I will not like it").

I wrote *MO*2 in the same manner. What several users did not like was the fact that in many cases tables that pertained to one procedure were mixed with pages that contained text about another method. I reorganized this edition so that tables and text which relate to a procedure are together. Tables and sometimes text do not always fill a page. To not have empty spaces throughout the book and avoid mixing procedures in such cases I added illustrations, chemical formulae, and miscellaneous information or historical vignettes in boxes. These items are not numbered because some may have to be removed due to space limitations which could arise during typesetting. I hope that readers and users of the book will find these items to be interesting and even illuminating. They can be ignored by those who will find them uninteresting and distracting.

Ideally every procedure in this edition and in the previous versions would have been tested before being included in the book. However, given the number of procedures this is clearly impossible due to limitations of time, laboratory facilities, funds, and availability of orchids for experimentation. In fact, it would be illegal to import

some species due to CITES. Therefore procedures are presented without having been tested in the hope that they do work. However, it is reasonable to assume that procedures which are affected by the previous history of the donor plant may not work with plants that were grown under different conditions.

I could not have written this book without help from many individuals and sources. My thanks go to:

- Professor P. N. "Dhanny" Avdhani, National University of Singapore, my friend since 1969 for good ideas and stimulating discussions,
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- Suan Wong (Mrs. C. S. Hew) for an extensive retrospective literature search which produced a very detailed and inclusive printout of relevant publications. I could not obtain such a printout from the library of my own institution, the University of California, Irvine,
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- Kathryn Kjaer, University of California, Irvine library for tending to my current awareness profile and literature searches efficiently, willingly, and well (she is not in charge of retrospective searches),
- Hideka Kobayashi for finding and sending me many papers from journals which are not available at the University of California, Irvine library (the selection of plant science journals in the UCI library is meager and reflects the negative attitude toward plant sciences in the School of Biological Sciences),
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- Dr. John Yong, Nanyang Technological University in Singapore for allowing me to use illustrations, and
- all those who allowed me to use illustrations from their web sites and/or publications.

My special thanks go to my son Jonathan Omar for just being around and to my brother for maintaining my computers when they needed it and making it easier for me to write.

I also thank Ward Cooper, my editor; Jane Andrew, easily the best copy editor I have ever worked with and her assistant Pat Croucher for ferreting out errors, finding omissions, correcting mistakes, and generally making the book much better; as well as Rosie Hayden, Caroline Milton, Rachel Moore, Delia Sandford, Nancy Whilton, and the rest of the staff at Blackwell Publishing for their high level of professionalism.

Most of all I thank my Jonathan for being my son and making my life better by just being around.

Joseph Arditti
Irvine, California

Preface to the First Edition

Photocopy machines, pocket calculators, and micropropagation of orchids through tissue culture appeared on the scene almost simultaneously, and the world has not been the same since. Now it is hard to imagine how it was ever possible to get along without these advances. Those who are in their thirties and forties have never known a world without them. The large, slow, and primitive copiers of the 1960s gave rise to the small, fast, and versatile photocopiers of today. Pocket calculators became smaller (some even moved to the wrist in combination with watches) and more sophisticated. It is possible to suggest that one evolutionary branch led from them to the personal and "notebook" computers of today.

Mass rapid clonal propagation of orchids led to the development of similar procedures for other plants and eventually to the isolation and culture of cells and protoplasts. The combination of molecular biology and tissue, cell, and protoplast culture is the basis of plant biotechnology that holds the promise of improved crops, safer chemicals, and perhaps a better environment. Tissue culture was and is used for the mass rapid clonal propagation of outstanding hobby crosses and commercial cut-flower cultivars of orchids. In the former case it resulted in reduced prices of desirable plants to levels within the reach of most growers, whereas in the latter instance it is responsible for the tremendous growth of the orchid cut-flower industry in Thailand, Singapore, Malaysia, Indonesia, and other countries.

Research on orchid tissue culture as a means of micropropagation is being carried out in many laboratories all over the world. Papers based on this research are published in numerous journals and in several languages (but fortunately for English-speaking people mostly in English). Even with computerized literature searches it is not always possible to trace all existing papers because some publications are not recorded in the relevant databases. Some papers (in both popular and obscure journals) do not contain enough details to be useful for the average grower or even the experienced scientist. Moreover, most orchid growers and propagators are familiar with only one or at most two languages (usually their own and English). These limitations may deny some growers access to certain methods. The tissue culture propagation appendix in *Orchid Biology, Reviews and Perspectives*, Vol. 1, written by one of us (J. A.), to provide access to most available methods in the mid-1970s, is now outdated. This book is intended to update the appendix by including all the information it contains as well as procedures that have developed since

it was written. However, despite all our efforts we may not have included all existing methods.

Procedures must be presented in a clear easy-to-follow format to be useful. Comments by users and reviewers suggest that the format used in *Orchid Biology*, Vols. 1 (tissue culture) and 2 (seed germination), is appropriate, and we have adopted it for this book. Procedures for which complete details are not available are described briefly.

Despite the bewildering number of formulations in this book, only a relatively small core of basic media are used for orchid tissue culture. These media are usually modified to meet the needs of individual orchids or the preferences of researchers. In writing this book we were faced with the need to choose between two formats: (1) listing only basic media and indicating modifications in each procedure, or (2) providing complete recipes in every case. We selected the latter despite the enormous amount of additional work it entailed because it is more convenient and (in computer jargon) more "user-friendly."

It is easier to follow a table that includes all components of a medium than to try to make sense of instructions in the following form: "for buds use Doe's medium, but replace 2 mg of hormone *X* with 1.5 mg of growth regulator *Y* and 0.5 mg of hormone *Z*. Also add 6 mg of hormone *W* in place of substance *V*. Replace vitamin A with an equal amount of vitamin B, and leave out vitamin C. Finally, add concoction RX7 instead of extract 300ZX. For stem explants use 1 mg hormone *A*, 0.5 mg growth regulator *B*, and 0.25 mg substance *T*. Do not alter the vitamin mixture, but use less agar and replace extract 300ZX with filtrate D1600, except for older stems when juice TR6 must be employed at 100 ml l^{-1}."

To provide a wide selection we have included in this book most, perhaps all, available methods for clonal propagation of orchids through tissue culture. Testing all of these procedures clearly would have been an impossible task, and for this reason we do not have firsthand experience with many of them. The outlines we present are based on the literature and as a consequence are limited by the amount of detail and degree of accuracy of each original communication.

Orchid nomenclature is in a constant flux and subject to disagreements among taxonomists. Rather than determine the "correctness" of names, we have chosen to use the ones employed by original authors.

Another point to keep in mind is that procedures are sometimes suitable only for certain cultivars, hybrids, species, and genera grown under specific conditions. This fact is not always evident. For these and other reasons we cannot guarantee success for any of the procedures and cannot assume responsibility for failures. Those who wish to propagate expensive and/or rare plants would be well advised to experiment first with less valuable ones.

Orchid tissue culture research is an active field, and new procedures were published while we were writing the book. This means that we had to add new methods to earlier sections while writing later ones (e.g., add a procedure to *Cymbidium* while writing about *Vanda*). If all figures and tables were to be numbered consecutively this would have meant constant renumbering. To avoid this onerous task we used prefixes to number the tables and illustrations in each section. These prefixes are the abbreviations of generic (natural and hybrid) names adopted by the Sander's List of Orchid Hybrids. In cases where abbreviations do not exist in the List we devised provisional ones that follow its format.

Full appreciation of present procedures requires a knowledge of their history, which is the reason for the first chapter. We thank Dr. Abraham (Abe) D. Krikorian, Department of Biochemistry, State University of New York, Stony Brook, for providing some of the information in this chapter through his excellent reprints and several informative discussions. However, the opinions in the chapter are our own.

Conversations with those who have used the appendices in *Orchid Biology, Reviews and Perspectives*, Vols. 1 and 2, indicated that a more general discussion of methods and procedures would be of benefit, especially to those who may not be completely familiar with the methodology. Chapter 2 was written to meet this need. Not all orchid laboratories have access to reference books that contain conversion factors, lists of abbreviations, definitions of units, information about reagents, and similar data. Chapter 2, which contains some information of such nature, is intended to make this book as much as possible a self-standing reference.

Detailed indices are indispensable tools in a book like this. Preparing such indices manually is an extremely unpleasant task. The indexing capabilities of word-processing programs cannot be used to prepare book indices since they must be based on page proofs, which are not stored in files. A computer program written especially for this purpose by Kevin J. Hackett in 1983–1986 for *Orchid Biology, Reviews and Perspectives*, Vol. 4, and modified in 1989 by Handajany Suryadharma and Ling Shao (computer science students from Indonesia and Hong Kong, respectively, at the University of California, Irvine) made indexing this book much easier.

We thank those who provided us with illustrations; they are acknowledged in the captions. We are grateful to the following for reading and commenting on parts of the manuscript and/or engaging us in helpful discussions: P. N. Avadhani, C. S. Hew, and A. N. Rao (Botany Department, National University of Singapore), Djunaidi Gandawijaja (Bogor Botanical Gardens, Indonesia), Abdul Karim B. Abdul Ghani (Botany Department, University Kebangsaan Malaysia, Bangi, Selangor, Malaysia), Franz Hoffmann (University of California, Irvine), Syoichi Ichihashi (Department of Biology, Aichi University of Education, Aichi, Japan), Helen Nair (Botany Department, University of Malaya, Kuala Lumpur), Leslie Paul Nyman (California State Polytechnic University, Pomona, California), Tim Wing Yam (formerly of the University of Hong Kong, then a postdoctoral fellow in our laboratory and now at the Singapore Botanic Gardens), and Gu Zhuping (Biology Department, Lanzhou University, Lanzhou, China).

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A book of this type requires special editorial and production skills, and the staff at Wiley were more than equal to the task. We thank our editor Philip C. Manor and the staff, including Jennifer Dowling, Ruth Ellowitz, Melanie Field, Joanne Kelman, Maggie Kennedy, and the copyeditor Susan Middleton for being so competent, helpful, and efficient.

Finally, two personal comments: First, both of us came to the United States as young adults without resources (J.A. did not even have a high school education or diploma and still does not). We feel that adopting this great and free country as our own was the wisest decision we ever made. The United States accepted us, gave us the same opportunities it affords its native sons, and thereby allowed us to become all we could be. Even more importantly, it gave our children (J.A.'s seven year-old Jonathan and R.E.'s Nina and Olivia and their children) the opportunity to be born as American citizens. No one could ask for more. We are deeply grateful and very appreciative. Second, we met in 1966 and have worked together ever since. Publication of this book will mark 25 years of fruitful collaboration. We are happy for that.

Joseph Arditti and Robert Ernst
Irvine, California
September 1990

CHAPTER ONE

History

A reasonable case can be made that new orchid propagation methods were always in the forefront of the biotechnology (or at least propagation methods) of their time. The first method for orchid seed germination (Moore, 1849; for reviews see Arditti, 1984; Yam et al., 2002*a*) was a radical departure from the manner in which other seeds were germinated 155 years ago. David Moore's (1807–1879) approach was innovative and a major horticultural and biological advance.

Half a century after Moore's discovery, Noël Bernard (1874–1911) made another quantum jump when he formulated a method for symbiotic germination of orchid seeds in vitro (Bernard, 1899, 1909; F. Bernard, 1990; for reviews see Boullard, 1985; Arditti, 1990; Rasmussen, 1995; Yam et al., 2002*a*). His is probably the first method for in vitro propagation of any plant. It utilizes what were at the time modern and advanced microbiological procedures. Bernard also predicted that a day would come when orchid growers would have laboratories as part of their establishments. This is the case at present not only for orchids, but also for other plants.

Lewis Knudson's (1884–1958) method for the asymbiotic germination of orchid seeds (Knudson, 1921, 1922; for reviews see Arditti, 1984, 1990; Yam et al., 2002*a*) was the first procedure for in vitro propagation of any plant in pure (i.e., axenic) culture. His method was a significant conceptual and technological innovation which foreshadowed modern biotechnology.

David Moore may have based his work (Moore, 1849) on reports that orchid seeds can germinate if scattered at the base of a mature plant. However Bernard's discovery and method were not based on any previous procedures and/or research by others. They were solely a result of his brilliance (Bernard, 1899, 1909; Boullard, 1985; Arditti, 1990; F. Bernard, 1990; Yam et al., 2002*a*). Knudson developed the asymbiotic method as a result of a sharp mind, incisive reasoning and on the basis of his own pioneering research with other plants (Knudson 1921, 1922; for a review see Arditti, 1990). The micropropagation of orchids by means of tissue culture has a more complex history, which is not free of controversy and includes unusual episodes (Arditti, 1977*b*, 1985, 2001; Arditti and Arditti, 1985; Torrey, 1985*b*; Arditti and Krikorian, 1996; Easton, 2001).

Terminology

As is very often the case, popular usage brought about some confusion regarding several terms associated with micropropagation. There is also some misuse. Given these facts, it is appropriate to describe and define a number of relevant terms at the outset (others are in the glossary, which please see). A number of the definitions presented here are taken from three scholarly and thoughtful reviews (Krikorian and Berquam, 1969; Krikorian, 1975, 1982).

Cell culture is the culture of isolated cells in vitro.

Clone as a term was introduced in 1903 and is based on the Greek word *clon*, which means twig, spray, or slip of the type used for vegetative propagation (Weber, 1903; Krikorian, 1982). Originally referred to plants produced through vegetative propagation methods like cuttings, layering, budding, and grafting, clone signified that "plants grown from . . . vegetative parts are not individuals in the ordinary sense, but are simply transplanted parts of the same individual, and in heredity and all biological and physiological senses such plants are the same individual" (Weber, 1903, cited by Krikorian, 1982). Because this definition can be applied to plants produced in vitro from a variety of explants, the term clone is now also used to describe individuals propagated in this manner. However, it is necessary to keep in mind that the tissue culture process, especially if the tissues are proliferated extensively, can be mutagenic and therefore some clones produced in vitro may not be completely (1) genetically uniform, or (2) similar to other clones or ramets of the same hybrid or cross.

Explant is a portion of a plant (tissue, organ, a few cells, or part of a callus mass) taken for culture in vitro. In practice the term is sometimes assumed to imply a relatively small amount of tissue, but this usage is neither universal nor a requirement.

Medium is a liquid or solidified solution used for the culture of explants, callus, organs, cells, protoplasts, etc. *Medium* is the singular form of the word whereas the plural is *media*. Using "media" as singular is incorrect. Therefore, it is proper to speak of "one medium" and "many media." "One media," "many medias," and "several mediums" are incorrect and simply bad English.

Mericlone was proposed by Mr. (at the time Lieutenant) Gene Crocker (Fig. 1-1) who originated it by condensing the words "meristem" and "clone." It was popularized by the late Gordon W. Dillon, long-time editor of the *American Orchid Society Bulletin* and executive secretary of the American Orchid Society (Dillon, 1964). This term is a very clever merchandizing tool, but, as has already been pointed out (Krikorian, 1982; Arditti and Krikorian, 1996), "mericlone" is unfortunate for several reasons:

1. It is a linguistic abomination as for example: "to mericlone," "mericloning," "to make a mericlone," "this plant has been mericloned," "to mericlone a mericlone" (meaning that a plant produced in vitro will be/is/was propagated a second time in the same manner), or "meristemmer" (Rutkowski, 1967). Fortunately no one seems to have bestowed upon him/herself the title of "mericloner" in the title of an article.
2. It is inaccurate since in most cases the explant is a shoot tip, not a meristem.

FIG. 1-1–1-6. **Early plant physiology and tissue culture researchers. 1. Lieutenant and later orchid grower Gene Crocker (source: Joseph Arditti (JA)). 2. Professor Gottlieb Haberlandt (White, 1943). 3. Professor Hans Fitting (photograph by Brigitta H. Flick, signature from a letter to JA). 4. Professor Kenneth V. Thimann (photograph from University of California Santa Cruz website, signature from autographed book owned by JA). 5. Professor Frits W. Went (photograph and signature from Went, 1990). 6. Professor Johannes van Overbeek (photograph from Skoog, 1951, signature from a letter to JA). Flowers below Fig. 1-6 (one facing left and the other right) are of *Schoenorchis funcifolia*, a Javanese orchid, whose carbon dioxide fixation was studied by Professor Went at the Bogor Botanical Gardens between 1927 and 1933 (orchid drawings from Smith, J. J. 1914. *Die Orchideen von Java*. E. J. Brill, Leiden, the Netherlands).**

3 The term is not really necessary because in principle there is no difference between cloning in vitro or through standard vegetative propagation methods.

4 It is misleading since it implies that all plants produced by this method are identical, which is not the case. Separate rules govern the naming of orchid crosses, clones, and mutants (Batchelor, 1982). These rules and the terminology they employ belong to the realms of plant and orchid taxonomy and nomenclature and are beyond the scope of this book.

Meristem is a well-defined term that describes the apex of a shoot tip. In common usage, especially among orchid growers, this term is erroneously used to describe the shoot-tip apex, which includes the apical meristem and some leaf primordia.

Micropropagation was first proposed in 1968 and defined as an aseptic procedure for the asexual production of plantlets from organs, tissues, and cells bypassing the sexual process or other means of asexual propagation (Krikorian, 1982; Hartman and Kester, 1983). This term should never be used to describe in vitro seed germination as is being done in the literature (see Perner, 1999, for one example).

Organ culture pertains to the culture of isolated juvenile or mature organs (leaves, roots, buds, shoot tips, flowers).

Ortet, from the Latin *ortus* (origin), was coined in 1929 to designate the "original plant of seedling origin from which members of a clone or ramets have originated" (Stout, 1929; Krikorian, 1982).

Protocorm, a term coined by Melchior Treub in 1890 (not by Noël Bernard as stated by Dr. Phillip Cribb in the first volume of *Genera Orchidacearum*, which was published in 1999), was applied to orchids by Bernard between 1899 and 1910. It refers to the small spherical, tuber-like bodies, formed by germinating orchid seeds (spherules is an incorrect name for these structures and must not be used). The term must not be used to describe similar bodies formed from explants or tissues in vitro (see below).

Protocorm-like body (PLB) is the proper term for structures that resemble protocorms and are formed by tissue explants and/or callus in vitro. This term was coined by Georges Morel in his first English language article on shoot-tip culture (Morel, 1960). It is the only first that can be attributed to him in connection with orchid micropropagation.

Protoplast culture should be applied only to cultures of isolated protoplasts (cells whose walls have been removed).

Ramet, based on the Latin *ramus* (branch), is an independent member of a clone (Stout, 1929; Krikorian, 1982).

Seedling is a young plant obtained from seed. This term may not be used to describe young plants obtained through tissue culture. The proper term for these is "plantlets."

Shoot tip is a meristem with several subjacent leaf primordia. This is the orchid explant generally cultured under the name "meristem."

Tissue culture is often used inappropriately to describe the culture of organs, tissues, cells, and protoplasts in vitro. This term should be applied only to the culture of tissues or tissue explants (meristems, callus sections, parenchyma pieces, tuber portions, and the like), not protoplasts, cells, or organs.

Origins of Orchid Micropropagation

Orchid micropropagation did not originate suddenly and de novo in the mind of one person despite a self-serving effort to create such an impression (Morel, 1960). The roots of orchid micropropagation are intertwined with the history of tissue

culture but they also have other origins (this chapter was revised extensively with new information, some of it taken verbatim or nearly so, from Arditti and Krikorian, 1996; I thank Professor Emeritus Abraham D. Krikorian for allowing me to use both text and photographs). Its origins lie in several lines of research and came from the work of many scientists, some of them well known and others not as appreciated as they should be (Arditti and Krikorian, 1996). The different lines of research will be discussed separately and brought to where they converged and gave rise to orchid micropropagation as it is known and practiced at present. A short outline of the history of plant hormones will also be presented because these substances are of critical importance to the culture in vitro of plant cells, tissue, and organs as well as to the differentiation of cultured plantlets (see Krikorian, 1995, for a more extensive history).

Plant Hormones and Propagation Additives of Plant Origin

Inclusion of plant hormones in culture media used for tissue culture, to control development and differentiation in vitro, and micropropagation is taken for granted at present. Yet, a century ago the existence of plant hormones was only being suggested.

Auxins

Gottlieb Haberlandt (1854–1945; Fig. 1-2), Professor of Plant Physiology in Berlin, was the first to propose the existence of plant hormones by stating that pollen tubes affect ovary growth through the release of substances he called *Wuchsenzyme* ("growth enzymes") and suggesting that if vegetative cells were cultured together with pollen tubes "perhaps the latter would induce the former to divide" (Haberlandt, 1902, English translation by Krikorian and Berquam, 1969; Arditti and Krikorian, 1996; Laimer and Rücker, 2003).

Pollen tubes do indeed release a substance which brings about post-pollination phenomena and ovule development in orchids. This was first shown by Hans Fitting (1877–1970; Fig. 1-3), before he became Professor of Botany in Bonn in his work with *Phalaenopsis* pollinia and pollination at the Bogor (then Buitenzorg) Botanical Gardens (Kebun Raya) in Indonesia (at the time the Netherlands Indies) in 1909 (Fitting, 1909*a*, 1990*b*, 1910, 1911, 1921 and a number of letters to JA in 1968 and 1969; for reviews see Arditti, 1971*a*, 1979, 1984, 1992; Avadhani et al., 1994). Fitting, who was "The first investigator to work with hormones and active extracts in plants" (Went and Thimann, 1937), went on to become one of the most prominent plant physiologists of his time and chancellor of the University of Bonn immediately after World War II.

He named the substance *Pollenhormon* and thus became the first plant scientist to use the word hormone in connection with plants and to suggest that they produce hormones.

From the time he named it (Fitting, 1909*a*, 1990*b*) and until his death (in letters to JA) Fitting maintained that *Pollenhormon* was a specific substance or hormone different from auxin. Present evidence suggests that Fitting's extracts in Bogor probably contained several substances including auxin (see Avadhani et al., 1994, for a