M. Freeling V. Walbot Editors

The Maize Handbook



Michael Freeling Virginia Walbot Editors

The Maize Handbook

With 120 Illustrations, 1 in color



Springer-Verlag New York Berlin Heidelberg London Paris Tokyo Hong Kong Barcelona Budapest Michael Freeling Department of Plant Biology University of California Berkeley, CA 94720 USA

Virginia Walbot Department of Biological Sciences Stanford University Stanford, CA 94305 USA

Library of Congress Catalog-in-Publication Data The Maize handbook / Michael Freeling, Virginia Walbot, editors.

Includes bibliographical references and index. ISBN 0-387-97826-7 (New York).—ISBN 3-540-97826-7 (Berlin)

1. Corn—Handbooks, manuals, etc. 2. Corn—Biotechnology—

Handbooks, manuals, etc. I. Freeling, Michael. II. Walbot, Virginia. SB191.M2M3275 1993

633.1'5—dc20

92-32462

Printed on acid-free paper.

© 1994 Springer-Verlag New York, Inc.

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Springer-Verlag New York, Inc., 175 Fifth Avenue, New York, NY 10010, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use of general descriptive names, trade names, trademarks, etc., in this publication, even if the former are not especially identified, is not to be taken as a sign that such names, as understood by the Trade Marks and Merchandise Marks Act, may accordingly be used freely by anyone.

Acquiring Editor: Robert Garber

Production managed by Henry Krell; manufacturing supervised by Vincent Scelta. Typeset using the editors' Microsoft Word files by Impressions, Inc., Ann Arbor, MI. Color separations and printing of the insert by New England Book Components. Hingham, MA.

Printed and bound by Edwards Brothers, Inc., Ann Arbor, MI. Printed in the United States of America.

9 8 7 6 5 4 3 2 1

ISBN 0-387-97826-7 Springer-Verlag New York Berlin Heidelberg ISBN 3-540-97826-7 Springer-Verlag Berlin Heidelberg New York

The Maize Handbook

The Maize Handbook represents the collective efforts of the maize research community to enumerate the key steps of standard procedures and to disseminate these protocols for the common good. Although the material in this volume is drawn from experience with maize, many of the procedures, protocols, and descriptions are applicable to other higher plants, particularly to other grasses.

The power and resolution of experiments with maize depend on the wide range of specialized genetic techniques and marked stocks; these materials are available today as the culmination of nearly 100 years of genetic research. A major goal of this volume is to introduce this genetical legacy and to highlight current stock construction programs that will soon benefit our work, e.g. high-density RFLP maps, deletion stocks, etc. Both stock construction and maintenance are relatively straightforward in maize as a result of the ease of crossing and the longevity of stored seeds. Crossing is facilitated by the separate staminate (tassel) and pistillate (ear) flowers, a feature almost unique to maize. On the other hand, many of the genetic methodologies utilized with maize, including the precision of record keeping, can be adapted to other plants.

Facile communication and a spirit of co-operation have characterized the maize genetics community since its earliest days. Starting in the 1930s, institutions such as annual Maize Genetics Cooperation Newsletter, the Maize Genetics Stock Center, and the annual maize genetics meeting provide continuity to the field. Each of these institutions allows geneticists to exchange information, to critique each others work, and to benefit from the enthusiasm, experience and expertise of others in their field. The Maize Handbook is clearly an extension of this desire on the part of maize geneticists, and embraced by nongenetical maize researchers as well, to share and to aid the progress of others; in modern times, the view that other scientists are cooperators rather than competitors is unusual, but it is a key part of the maize community of scholars.

The Maize Handbook covers five areas: cell biology, developmental morphology, genetic manipulations, tissue culture techniques, and molecular biology techniques. In consultation with expert section editors—Professors Jim Birchler, Ron Phillips, R. Scott Poethig, Anne Sylvester, and Sue Wessler—we developed a chapter outline and suggested authors. Without exception, each potential author contacted agreed to provide the materials requested.

We have tried to make *The Maize Handbook* comprehensive in its coverage. There are several reference books that complement this volume. Chief among these is the annual edition of the Maize Genetics Cooperation Newsletter (MNL) in which new genetic map information and reports on gene isolation, gene action and genetic methodologies are reported in a "from the notebook" format. Maize research is a dynamic field, and it is impossible to be well informed without access to the Newsletter. For subscription

vi PREFACE

information, contact Dr. E. H. Coe, Editor, Maize Genetics Cooperation Newsletter, USDA-ARS, Curtis Hall, University of Missouri, Columbia MO 65211.

A group of inexpensive books (total cost about \$400) would also be part of a robust personal or laboratory library, but should certainly be in an institutional library for easy reference.

- The new edition of Mutants of Maize, by M. G. Neuffer, E. H. Coe, and S. Wessler due from Cold Spring Harbor Press in 1994. Color illustrations of visible mutants (naked eye polymorphisms) and cytogenetic stocks; molecular maps of cloned genes are also included.
- Corn and Corn Improvement, 1988, third edition, G. F. Sprague and J. W. Dudley, eds. American Society of Agronomy, Madison WI. This volume contains comprehensive genetic and cytogenetic information as well as more practical chapters on corn diseases, pests and agronomic practice.
- Discussions in Cytogenetics, second edition, 1992, by C. R. Burnham, Alpha Editions, Edina MN, 55435. Classical text with a dateline of about 1959. Maize research is highlighted, and presented in detail, with complete citations.
- 4. Modern Corn Production by S. R. Aldrich, W. O. Scott and E. R. Leng, 1975, second edition. A & L Publications, Champaign, Illinois. Useful reference on field practices with color illustrations.
- Maize Diseases by D. C. McGee, 1988. APS Press, St. Paul, Minnesota. Paperback book with color illustrations on disease and nutritional deficiencies.
- The Structure and Reproduction of Corn, 1949, by T. A. Kiesselbach. Nebraska Agric. Exp. Station Research Bulletin. Observations of the plant and its developmental stages are illustrated with light micrographs and camera lucida drawings. A short but classic paperback.
- 7. Anatomy of Seed Plants, second edition, 1977, by K. Esau, John Wiley & Sons, New York. Maize is among the featured species.
- 8. Compilation of North American Maize Breeding Germplasm, 1993. Authors: J. T. Gerdes, C. F. Behr, J. G. Coors, and W. F. Tracy. Editors: W. F. Tracy, J. G. Coors, and J. L. Geadelmann. Published by Crop Science Society of America, Madison, Wisconsin. This paperback lists the history of lines, and is useful in determining the likelihood of shared parentage in genetic stocks.
- The Discovery and Characterization of Transposable Elements, 1987, by B. Mc-Clintock. The collected papers of Barbara McClintock. Garland Publishing, Inc., New York.

Acknowledgments. The editors give heartfelt thanks to Melissa Quilter of the NSF Center of Plant Developmental Biology at U.C. Berkeley for her tireless efforts to assemble the initial draft of the manuscript and to complete the final text. We also thank Jill Otto of the Falconer Biology Library at Stanford University for her assistance with the verification of references. The Maize Handbook would not have been possible without the coordination and careful husbandry of the individual contributions by the section editors. Pioneer Hi-Bred International, Inc., generously financed an up-to-date genetic map prepared by Ed Coe and distributed to the maize community. The credit for the idea for The Maize Handbook rests entirely with Dr. Robert C. Garber, Life Sciences Editor of Springer-Verlag. No one in their right mind would spontaneously undertake such a large task, but we have found the 18 months of planning and editing highly rewarding and thank Rob for his persistence, his appeal to our love of maize, and his persuasive scientific arguments for initiating this project.

Contributors

Charles L. Armstrong Monsanto Agricultural Company St. Louis, MO 63198 USA

Donald Auger University of North Dakota Biology Department Grand Forks, ND 58202 USA

N.A. Avalkina
N.I. Vavilov Research Institute
Plant Industry
Department of Genetics
Sankt-Peterburg, 190000 Russia

William D. Beavis Pioneer Hi-Bred International, Inc. Johnston, IA 50131 USA

Jack B. Beckett University of Missouri Department of Agronomy Columbia, MO 65211 USA

Philip W. Becraft University of California Department of Plant Biology Berkeley, CA 94720 USA

Patricia Bedinger University of North Carolina Department of Biology Chapel Hill, NC 27599-3280 USA

James A. Birchler University of Missouri Division of Biological Sciences Columbia, MO 65211 USA Steven P. Briggs Pioneer Hi-Bred International, Inc. Johnston, IA 50131 USA

Anne B. Britt University of California Department of Botany Davis, CA 95616 USA

Anne H. Broadwater University of North Carolina Department of Biology Chapel Hill, NC 27599-3280 USA

Thomas E. Bureau University of Georgia Botany Department Athens, GA 30602 USA

Benjamin Burr Brookhaven National Lab Biology Department Upton, NY 11973 USA

Frances A. Burr Brookhaven National Lab Biology Department Upton, NY 11973 USA

Wayne R. Carlson University of Iowa Botany Department Iowa City, IA 52242 USA

G.K. Carswell
CIBA-Geigy Corporation
Agricultural Biotechnology
Research Unit
Research Triangle Park, NC 27709-2257
USA

XX CONTRIBUTORS

Vicki Chandler
University of Oregon
Department of Biology
Molecular Biology Institute
Eugene, OR 97403 USA

Ming T. Chang Garst/ICI Seeds Slater, IA 50244 USA

Jychian Chen Yale University Department of Biology New Haven, CT 06511 USA

Ping-Chin Cheng
State University of New York
Department of Electrical and Computer
Engineering
Buffalo, NY 14260 USA

Paul S. Chomet
DeKalb Plant Genetics
Mystic, CT 06355-1958 USA

Jancie K. Clark University of North Dakota Biology Department Grand Forks, ND 58202 USA

B. Gregory Cobb University of Florida Department of Vegetable Crops/IFAS Gainesville, FL 32611 USA

Ed H. Coe
United States Department of Agriculture
—ARS
University of Missouri
Department of Agronomy
Columbia, MO 65211 USA

Karen Cone University of Missouri Columbia, MO 65211 USA

R. Kelley Dawe University of California Department of Molecular and Cell Biology Berkeley, CA 94720

Stephen L. Dellaporta Yale University Department of Biology New Haven, CT 06511 USA

Ellen Dempsey Indiana University Biology Department Bloomington, IN 47401 USA

Jeffrey R. de Wet Pfizer Central Research Groton, CT 06340 USA

John Doebley University of Minnesota Department of Plant Biology St. Paul, MN 55108 USA

Hugo K. Dooner DNA Plant Technology Corporation Oakland, CA 94608-1239 USA

Gregory G. Doyle
United States Department of Agriculture
—ARS
University of Missouri
Columbia, MO 65211 USA

Edwin R. Duke University of Florida Department of Vegetable Crops/IFAS Gainesville, FL 32611 USA David J. Earp

Plant Gene Expression Center

Albany, CA 94710 USA

Jesús Escudero

Friedrich Miescher-Institut

CH-4002, Basel, Switzerland

Lewis Feldman

University of California

Department of Plant Biology

Berkeley, CA 94720 USA

Robert J. Ferl

University of Florida

Department of Vegetable Crops

Gainesville, FL 32611 USA

Witold Filipowicz

Friedrich Miescher-Institut

CH-4002 Basel, Switzerland

John Fowler

University of California

Department of Plant Biology

Berkeley, CA 94720 USA

Michael Freeling

University of California

Department of Plant Biology

Berkeley, CA 94720 USA

Michael Fromm

Monsanto Company

St. Louis, MO 63198 USA

Susan Gabay-Laughnan

University of Illinois

Urbana, IL 61801 USA

Walton C. Galinat

University of Massachusetts, Amherst

Eastern Agricultural Center

Waltham, MA 02154-8096 USA

Daniel R. Gallie

University of California

Department of Biochemistry

Riverside, CA 92521-0129 USA

Burle G. Gengenbach

University of Minnesota

Department of Agronomy and Plant Genetics

St. Paul, MN 55108 USA

A.D. Genovesi

DeKalb Plant Genetics

DeKalb, IL 60115 USA

Steve Goff

CIBA/Geigy Biotechnology

Research Triangle Park, NC 27709-2257 USA

Inna Golubovskaya

N.I. Vavilov Research Institute Plant Industry

Department of Genetics

St. Petersburg, 190000 Russia

Ben Greene

Plant Gene Expression Center USDA—ARS

Albany, CA 94710 USA

Richard I. Greyson

University of Western Ontario

Department of Plant Sciences

London, Ontario N6A 5B7 Canada

Z.K. Gzebennikova

N.I. Vavilov Research Institute Plant Industry

Department of Genetics

St. Petersburg, 190000 Russia

Sarah Hake

Plant Gene Expression Center USDA—ARS

Albany, CA 94710 USA

xxii Contributors

L. Curtis Hannah University of Florida Department of Vegetable Crops/IFAS Gainesville, FL 32611 USA

Christian Harms
CIBA-Geigy Corporation
Research Triangle Park, NC 27709-2257
USA

Tim Helentjaris University of Arizona Plant Science Department Tucson, AZ 85721 USA

Jane Hershberger Stanford University Department of Biological Science Stanford, CA 94305-2040 USA

Manfred Heun University of Arizona Plant Science Department Tucson, AZ 85721 USA

Ken A. Hibberd Plant Science Research, Inc. Minnetonka, MN 55343 USA

Thomas K. Hodges Purdue University Department of Botany and Plant Pathology West Lafayette, IN 47907 USA

Barbara Hohn Friedrich Miescher-Institut CH-4002, Basel, Switzerland

Erin E. Irish University of Iowa Departments of Botany and Biology Iowa City, IA 52242 USA Nurul Islam-Faridi
CIMMYT
International Maize and Wheat
Improvement Center
06600 Mexico, D.F. Mexico

David C. Jewell
CIMMYT
International Maize and Wheat
Improvement Center
06600 Mexico, D.F. Mexico

Robert J. Jones University of Minnesota Department of Agronomy and Plant Genetics St. Paul, MN 55108 USA

Heidi F. Kaeppler University of Minnesota Department of Agronomy and Plant Genetics Plant Molecular Genetics Institute St. Paul, MN 55108

Jerry L. Kermicle University of Wisconsin Laboratory of Genetics Madison, WI 53706 USA

I.S. Kim Purdue University Department of Botany and Plant Pathology West Lafayette, IN 47907 USA

Bryan Kindiger USDA—ARS Southern Plains Research Station Woodward, OK 73801 USA

Julie Anderson Kirihara Plant Science Research, Inc. Minnetonka, MN 55343 USA

Tamás Kiss Friedrich Miescher-Institut CH-4002 Basel, Switzerland Ted Klein E.I. DuPont Medical Products Division Glasgow, DE 19702 USA

Karen E. Koch University of Florida Department of Vegetable Crops/IFAS Gainesville, FL 32611 USA

Richard V. Kowles St. Mary's College of Minnesota Biology Department Winona, MN 55987 USA

C. Kramer CIBA-Geigy Corporation Agricultural Biotechnology Research Unit Research Triangle Park, NC 27709-2257 USA

Barbara Lane University of California Department of Plant Biology Berkeley, CA 94720 USA

Jane A. Langdale
University of Oxford
Department of Plant Sciences
Oxford OX1 3RB United Kingdom

Brian A. Larkins University of Arizona Department of Plant Sciences Tucson, AZ 85721 USA

John R. Laughnan University of Illinois Urbana, IL 61801 USA

Michael Lee Iowa State University Department of Agronomy Ames, IA 50011 USA Avraham A. Levy
The Weizmann Institute of Sciences
Plant Genetics Department
Rehovot, 76100 Israel

S.M. Livingston University of Minnesota Plant Molecular Genetics Institute Department of Agronomy and Plant Genetics St. Paul, MN 55108 USA

Kenneth R. Luehrsen Stanford University Department of Biological Science Stanford, CA 94305-2040 USA

Leszek A. Lyznik Purdue University Department of Botany and Plant Pathology West Lafayette, IN 47904 USA

Marjorie P. Maguire University of Texas Zoology Department Austin, TX 78712 USA

Eileen C. Matz Brookhaven National Lab Biology Department Upton, NY 11973 USA

Albrecht Melchinger Universität Hohenheim Institut für Pflanzenzüchtung W-7000 Stuttgart 70 Federal Republic of Germany

Maria A. Moreno Yale University Department of Biology New Haven, CT 06511 USA

xxiv Contributors

R. Michael Mulligan
University of California
Department of Developmental and
Cell Biology
Irvine, CA 92717 USA

Oliver Nelson University of Wisconsin Department of Genetics Madison, WI 53706 USA

Tim Nelson Yale University Department of Biology New Haven, CT 06511-8112 USA

M.G. Neuffer University of Missouri Plant Sciences Unit Columbia, MO 65211 USA

Kathleen J. Newton University of Missouri Division of Biological Sciences Columbia, MO 65211 USA

Dayakar R. Pareddy Dow Elanco Agricultural Biotechnology Division Champaign, IL 61820 USA

M.V. Parthasarathy Cornell University Division of Biological Sciences Section of Plant Biology Ithaca, NY 14853 USA

Earl B. Patterson University of Illinois Department of Agronomy Urbana, IL 61801 USA Anna-Lisa Paul University of Florida Department of Vegetable Crops Gainesville, FL 32611 USA

J.F. Petolino Dow/Elanco Champaign, IL 61824-4011 USA

Ronald L. Phillips
University of Minnesota
Department of Agronomy and Plant Genetics
Plant Molecular Genetics Institute
St. Paul, MN 55108 USA

R. Scott Poethig
The University of Pennsylvania
Department of Biology
Plant Science Institute
Philadelphia, PA 19104-6018 USA

A. J. Pryor CSIRO Division of Plant Industry Canberra ACT 2601 Australia

Natasha V. Raikhel Michigan State University Plant Research Laboratory East Lansing, MI 48824-1312 USA

S.W. Ritchie Purdue University Department of Botany and Plant Pathology West Lafayette, IN 47907 USA

Steven Rodermel Iowa State University Department of Botany Ames, IA 50011 USA

Scott D. Russell University of Oklahoma Department of Botany and Microbiology Norman, OK 73019-0245 USA Steven E. Ruzin University of California Center of Plant Developmental Biology Department of Plant Biology Berkeley, CA 94720 USA

Liang Schweitzer
University of Minnesota
Institute for Advanced Study in Biological
Process Technology
Department of Chemical Engineering
and Materials Science
St. Paul. MN 55108 USA

J.C. Sellmer Purdue University Department of Botany and Plant Pathology West Lafayette, IN 47907 USA

Jack C. Shannon The Pennsylvania State University Department of Horticulture University Park, PA 16802 USA

William F. Sheridan University of North Dakota Biology Department Grand Forks, ND 58202 USA

R.D. Shillito CIBA-Geigy Corporation Agricultural Biotechnology Research Unit Research Triangle Park, NC 27709-2257 USA

Neelima Sinha Plant Gene Expression Center USDA—ARS Albany, CA 94710 USA

Laurie G. Smith Plant Gene Expression Center USDS—ARS Albany, CA 94710 USA David A. Somers
University of Minnesota
Department of Agronomy and Plant Genetics
Plant Molecular Genetics Institute
St. Paul. MN 55108 USA

Friedrich Srienc
University of Minnesota
Institute for Advanced Study in Biological
Process Technology
Department of Chemical Engineering
and Materials Science
St. Paul, MN 55108 USA

Chris J. Staiger
John Innes Institute
Department of Cell Biology
Norwich NR4 7UH
United Kingdom

Anne W. Sylvester University of Idaho Department of Biological Sciences Moscow, ID 83843 USA

Gary A. Thompson University of Arizona Department of Plant Sciences Tucson, AZ 85721 USA

Marguerite J. Varagona Michigan State University Plant Research Laboratory East Lansing, MI 48824-1312 USA

Julie M. Vogel University of California Department of Plant Biology Berkeley, CA 94720 USA

Virginia Walbot Stanford University Department of Biological Sciences Stanford, CA 94305-2040 USA

xxvi Contributors

D.B. Walden
University of Western Ontario
Department of Plant Sciences
London, Ontario N6A 5B7 Canada

Elsbeth Walker
Yale University
Department of Biology
New Haven, CT 06511 USA

Christine A. Warren Stanford University Department of Biological Sciences Stanford, CA 94305-2040 USA

David F. Weber Illinois State University Genetics Group Department of Biological Sciences Normal, IL 61761 USA

Clifford F. Weil University of Idaho Department of Biological Sciences Moscow, ID 83843 USA Susan R. Wessler University of Georgia Department of Botany Athens, GA 30602 USA

David P. West Jacques Seed Company Research Department Prescott, WI 54021 USA

Michelle H. Williams University of Florida Department of Environmental Horticulture Institute of Food and Agriculture Services Gainesville, FL 32611-0670 USA

Georgia L. Yerk
University of Minnesota
Department of Agronomy and Plant Genetics
Plant Molecular Genetics Institute
St. Paul, MN 55108 USA

Contents

| Preface | | V |
|---|---------|-----|
| Contributors | ; | xix |
| I. Development and Morphology | ••• | 1 |
| Fertilization and Embryogeny in Maize WILLIAM F. SHERIDAN AND JANICE K. CLARK | • • • • | 3 |
| 2. The Maize Shoot | • • • • | 11 |
| 3. The Maize Leaf | | 17 |
| 4. The Maize Root | | 29 |
| 5. Morphology and Development of the Tassel and Ear Ping-Chin Cheng and Dayaker R. Pareddy | | 37 |
| 6. Gametogenesis in Maize | | 48 |
| 7. The Patterns of Plant Structures in Maize | | 61 |
| 8. Genetics and the Morphological Evolution of Maize JOHN DOEBLEY | •••• | 66 |
| 9. Overview of Key Steps in Aleurone Development VIRGINIA WALBOT | **** | 78 |
| II. Cell Biology | **** | 81 |
| 10. Light Microscopy I: Dissection and Microtechnique Anne W. Sylvester and Steven E. Ruzin | | 83 |
| 11. Light Microscopy II: Observation, Photomicrography, and Image Analysis STEVEN E. RUZIN AND ANNE W. SYLVESTER | | 95 |
| 12. Scanning Electron Microscopy | | 108 |

X CONTENTS

| | 13. | Freezing Methods, and Immunolocalization | 1Ω |
|----|-----|--|-----|
| | | M. V. Parthasarathy | 10 |
| | 14 | Techniques for Histology of Maize Megaspores and | |
| | 14. | Embryo Sacs | 35 |
| | | S. D. Russell and D. P. West | ,,, |
| | 15 | Indirect Immunofluorescence: Localization of the | |
| | 10. | Cytoskeleton | 40 |
| | | C. J. Staiger | 10 |
| | 16 | Immunocytochemistry for Light and Electron Microscopy 14 | 49 |
| | 10. | M. J. Varagona and N. V. Raikhel | |
| | 17. | Immunolocalization of Nuclear Proteins | 58 |
| | | Laurie G. Smith | |
| | 18. | In situ Hybridization 10 | 65 |
| | | JANE A. LANGDALE | |
| | 19. | Photography of Maize | 80 |
| | | PHILIP W. BECRAFT | |
| | | | |
| | _ | - C- P-1 - 1 | |
| Ш. | Ger | netics Protocols | 87 |
| | | | |
| | 20. | Genetic Experiments and Mapping 1 | 89 |
| | | E. H. COE | |
| | 21. | Growing Maize for Genetic Studies 1 | 97 |
| | | M. G. Neuffer | |
| | 22. | A Nine-Step Way to Characterize a Morphological | |
| | | Mutant | 09 |
| | 0.0 | MICHAEL FREELING AND JOHN FOWLER | 40 |
| | 23. | Mutagenesis | 12 |
| | 24 | M. G. Neuffer Gene Tagging with Ac/Ds Elements in Maize | 10 |
| | 24. | STEPHEN L. DELLAPORTA AND MARIA A. MORENO | 19 |
| | 25 | Using Cytogenetics to Enhance Transposon Tagging | |
| | 20. | with Ac Throughout the Maize Genome | 34 |
| | | DONALD AUGER AND WILLIAM F. SHERIDAN | JŦ |
| | 26. | Transposon Tagging with Spm | 40 |
| | 20. | KAREN CONE | 10 |
| | 27. | Transposon Tagging with Mutator 2 | 43 |
| | | PAUL S. CHOMET | |
| | 28. | Mapping Genes with Recombinant Inbreds | 49 |
| | | BENJAMIN BURR, FRANCES A. BURR AND EILEEN C. MATZ | |
| | 29. | The Placement of Genes Using waxy-Marked Reciprocal | |
| | | Translocations 2 | 55 |
| | | JOHN R. LAUCHNAN AND SUSAN CARAV-LAUCHNAN | |