

LEGACY

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IN THIS ISSUE:

From the President of the Amaranth Institute

Larry A. Walters p.1-3

Amaranth Research in the Nebraska Panhandle

David D. Baltensperger p.7

Survival Strategies of the Peasants Amaranth Project

Davidson K. Mwangi p.7-8

Amaranth Progress in India

B.D. Joshi p.8-9

Amaranth Sprouts at Disney Farms

Kenneth Disney p.9-10

Expression of C4 photosynthesis genes in grain amaranth.

James O. Berry p.10-13

Response of some Grain Amaranths to Shortday and Longday Ecological Conditions. p.13-14

T. Badra

Amaranthus Germplasm at the North Central Regional Plant Introduction Station

David M. Brenner p.14

Rapid Cycling of Grain Amaranths

James W. Lehmann p.15-19

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The 1995 Annual Meeting

The 1995 Amaranth Institute annual meeting will be held October 22-25 in conjunction with the Third National Symposium on New Crops, in Indianapolis, Indiana. Dr. Robert Myers will present a paper "Amaranth: Health Food Fad or True

New Crop Opportunity?". Meeting information is available from Dr. Jules Janick, Indiana Center for New Crops and Plant Products, Purdue University, 1165 Horticulture Bldg., West Lafayette, IN 47907-1165, Phone:317-494-1329, Fax:317-494-0391. The New Crops Symposium will be preceded by The Association for the Advancement of Industrial Crops meeting October 21-22 in Indianapolis. Registration materials are available from: Dr. David A. Dierig, AAIC, U.S. Water Conservation Laboratory, 4331 E. Broadway, Phoenix AZ 85040.

From the President of the Amaranth Institute

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It is a privilege to have this opportunity to address the members and friends of the Amaranth Institute once again, now some years beyond the original Amaranth Growers Association and American Amaranth Institute. I accept the challenge and responsibility to shore up and revitalize the Institute's service to its members by utilizing the resources and talents of its membership.

However, there is great news!

The AMARANTH INSTITUTE lives on! A major thanks goes to volunteer members such as: Philip Sanders, Jim Lehmann, Richard Wilson, David Baltensperger, Robert Myers, Bill Breene, Patricia Rayas-Duarte, Terry Cunningham, Bill Bunger, Bill Bennett, Tom Frantzen, Duane Berglund, Roger Matkin, Arris Sigle, Leon Weber, Al Schneiter, Eugene Sanders, Terry Walters, and Ricardo Bressani; who, with their contributions of energy and active assistance, are moving the Amaranth Institute forward through new ideas and continued promotion of Amaranth (production - markets - research - applications - sales). I hope the revitalization that is occurring within the Amaranth industry will continue to provide growth and benefits to our many professionals, (i.e., farmers, researchers, educators, marketers, brokers, processors, customers, and interested parties).

The Amaranth Board, as part of its responsibility to the future of Amaranth as a viable crop, endeavors to assist you in experiencing success in the Amaranth industry through the Amaranth Institute by taking advantage of the talent and experience offered by its membership. The Board also plans to promote Amaranth awareness and usage by being a resource.

I would also like to introduce you to the current officers and directors of the Amaranth Institute:

President

Larry Walters

Vice-President

Philip Sanders

Secretary/Treasurer

Jim Lehmann

Directors

Robert Myers

Bill Breene

Patricia Rayas-Duarte

Richard Wilson

David Baltensperger

Legacy

David Brenner

To all of these people I wish to say THANK YOU! Without these long standing Amaranth pioneer friends, and their supporting efforts, the Amaranth industry and its future, may well have been lost, since the Amaranth Institute was close to its weakest survival point late last year. However, with a board meeting held on March 18, 1995, exciting new energy and ideas are again working to push the Amaranth Institute towards the next decade. Participants who braved the winter weather to attend the March Amaranth Institute meeting were Larry Walters, Philip Sanders, Richard Wilson, and Jim Lehmann, plus input from Robert Myers, David Baltensperger and Bill Breene.

The following is a general outline of the meeting which included discussion of and ways to implement the following topics:

Secretary Report:

- Alternative Crop meeting (Oct. 21-24, 1995 Dr. Robert Myers to be a speaker) Indianapolis, Indiana
- Amaranth Institute (Fall / Winter) meeting late November or early December
- Amaranth Grower membership status
- dues
- membership drive
- newsletter update
- Amaranth specifications
- seed standards
- Amaranth crop pricing
- production guide update
- market ideas to promote Amaranth
- export standards
- research needed
- University research update
- growers / production update
- joint meeting with alternative crop groups
- organic standards and organic Amaranth certification program
- Amaranth Institute Trademark
- Industrial / Broker / Trader memberships
- new ideas
- Legacy search for reports
- other topics

-- social time

Complete minutes of the Board meeting will be in the upcoming newsletter. In any case, the meeting had a full agenda, as always, with too much to do and too little time to do it. But, the board worked very well together, and we managed to get through the agenda, resolve some important issues, set some priorities and assign a huge workload to our board and a few volunteer members.

Since the meeting, we have made slow, but good progress with standing committee assignments. A list of committee chairpersons and committee members will be available from the board president.

There is much more that I want to share with you, but I recognize this is a busy planting time and Amaranth growers are already busy in the fields. We hope the Amaranth growers have a good planting crop response despite the weird weather. We of the board appreciate your letters, faxes, and telephone calls supporting Amaranth efforts, and I want you to know that your entire Amaranth Institute Board appreciates what you are doing for Amaranth agriculture and trade. Thank you.

AMARANTH NEWS

Robert Myers moves to Washington DC

Rob Myers, Amaranth Institute president from 1992 to 1994 left the University of Missouri Agronomy Department. He is now The National Director for USDA Sustainable Agriculture Programs. His address is: Room 3351, South Building, Ag. Box 0910, Washington DC, 20250-0910.

North American Amaranth Growers Association

Phil Sanders, Vice-President of the Amaranth Institute, organized the first meeting of this group which took place on June 24, 1995. The twenty grower-members plan to market amaranth grain as a group. They are looking for more members.

Call Phil Sanders at 308-377-2231 for more information.

Amaranth Marketing Group

This group was recently formed. The North American Amaranth Growers Association has an agreement with the Amaranth Marketing Group to market its 1995 crop. AMG will be marketing conventionally grown as well as certified organic amaranth grain this coming year. The shipment point will be western Nebraska. For further information please contact Steve Boese at Amaranth Marketing Group, Box 2458, Dearborn, Michigan 48123 Phone: 313-535-2506 Fax: 313-535-4466.

The Australian New Crops Newsletter

It is published two times a year. The January 1995 issue was 24 pages long. It has good international coverage. Write to: Ian Wood, Principal Editor, I.M. Wood and Associates, 258 Bielby Road, Kenmore Hills Qed 4069. Phone/Facsimile (07)378-5911.

Legacy is held in Libraries

Legacy is listed in databases from libraries of: The New York Botanical Garden, The University of Nebraska, and the ATTRA appropriate technology organization in Arkansas.

NEW AMARANTH PRODUCTS

Best of Health Amaranth Bread

This bread is available in a local bakery in Ames, Iowa. The address on the package is: AMARANTH RESOURCES, INC., 139 East William Street, Albert Lea, Minnesota 56007. It is made with popped amaranth grain and has a beautiful garnish of popped amaranth.

Govinda's Bliss Bar

A new amaranth product has appeared in the Ames, Iowa natural food store. Several different kinds of 2.4 oz (68g) bars combine amaranth seeds with other ingredients such as sunflower seeds, walnuts, sesame seeds, raisins,

dates, and honey. The amaranth seeds appear to be half-popped, perhaps parched. They are delicious and worth trying at \$1.69 each (retail). The address on the label is: Govinda's, 2651 Ariane Drive, San Diego, CA 92117.

ULTIMA High Protein Corn & Amaranth All Natural Tortilla Chips

This new amaranth product is beautifully packaged in 11 oz (312g) plastic bags. The package text emphasizes the nutritional advantages of amaranth's excellent amino acid composition. The address on the package is: ULTIMA FOODS, Waukegan, IL 60087.

AMARANTH ELECTRONIC INFORMATION

A Gateway to the NewCROP Information System

This online information resource will provide a window to new and specialty crops, literature, newsletters, a directory of new crop researchers, announcements and upcoming events, and coverage of the Indiana Center for New Crops & Plant Products at Purdue University. Questions about the NewCROP system can be sent to amots@uga.cc.uga.edu by E-mail. The NewCROP World Wide Web (WWW) can be reached by accessing your local WWW link and connecting to:
<http://newcrop.hort.purdue.edu>
(The editors of the NewCROP Information system have offered to put issues of Legacy on their database. The Amaranth Institute Board of Directors will decide if this should be done.)

New Crops Electronic Bulletin Board

This bulletin board is run from Purdue University. The procedure to subscribe is simple; if you have access to E-mail. Send the following message to:
LISTSERVE@VM.CC.PURDUE.EDU
Nothing should be placed in the subject box, but the following should be the body of the text:
SUBSCRIBE NEWCROPS <Your name,

Institution>
Confirmation of the subscription will be sent. To post information or questions to the group, the message should be sent by E-mail to: NEWCROPS@VM.CC.PURDUE.EDU

Germplasm Resources Inventory (GRIN)

This computer database has the records for the United States Department of Agriculture, National Plant Germplasm System collections. Included in the collections are the 3,000 amaranth accessions maintained at the North Central Regional Plant Introduction Station in Ames, Iowa. The information is of interest to specialists working on plant breeding, taxonomy, or other research projects. It is available on the internet. E-mail inquiries should be sent to: grin@ars-grin.gov.

Access through WWW is:
<http://www.ars-grin.gov>
Access through Gopher is:
gopher gopher.ars-grin.gov

Missouri Botanical Garden Databases

These databases include information on chromosome counts for *Amaranthus* species and for other kinds of plants. Access through WWW is:
<http://straylight.tamu.edu/MoBot/welcome.html>
Then select the Missouri Botanical Garden gopher.

NEW AMARANTH LITERATURE

NCRIPS *Amaranthus* genetic resource management program.

D. Brenner. 13 pages. A November 1994 draft is available, from the author, for review and comment (North Central Regional Plant Introduction Station, Agronomy Department, Iowa State University, Ames, Iowa 50011, USA).

This planning document reviews the goals and problems of an amaranth germplasm collection. Comments are sought.

Perspectives on the cultivation of amaranths in Argentina. G. Covas. 1994. Estación Experimental Agropecuaria Anguil, Instituto Nacional de Tecnología Agropecuaria, CC 11 (6326) Anguil - La Pampa, República Argentina.

This new booklet, in Spanish, is on Amaranth cultivation in Argentina. It is 10 pages long.

Nitrogen fertilizer and cultivar effects on yield and nitrogen-use efficiency of grain amaranth. A. Elbehri, D.H. Putnam and M. Schmitt. 1993. *Agronomy Journal* 85:120-128

Increasing the nitrogen application increased yields, but also increased lodging. The authors suggest selecting new cultivars that have a greater harvest index, and nitrogen harvest index.

Biological Control of the shoot disease of *Amaranthus hybridus* caused by *Choanephora cucurbitarum* with *Bacillus subtilis*. F.E.O. Ikediugwa, A.E. Emoghene and P.O. Ajiodo. 1994. *Journal of Horticultural Science* 69(2):351-356

Foliage treated with the *Bacillus* was protected from the fungal disease.

Allergy recipes: super foods. M.H. Jones. 1993. revised edition. Mast Enterprises Inc., 2615 N. Fourth St. #616, Coeur d'Alene, ID 83814-3781

This booklet features amaranth as one of the six best alternatives to wheat.

Vitamin E isomers in grain amaranths (*Amaranthus* sp.). J.W. Lehmann, D.H. Putnam and A.A. Qureshi. 1994. *Lipids* 29:177-181

Amaranth grain has significant levels of tocotrienols which could be beneficial as antioxidants and in reducing cholesterol. The levels vary between the kinds of amaranths.

Diseases of *Amaranthus* spp. caused by *Pythium aphanidermatum* and *Macrophomina phaseolina*. J.D.

Mihal and E.R. Champaco. 1993. *Canadian Journal of Botany* 71:1219-1223

Pythium is a problem in warm wet years in Missouri. Some cultivars are more susceptible than others.

Factors influencing the biocontrol of tumble pigweed (*Amaranthus albus*) with *Aposphaeria amaranthi*. A.S. Mintz, D.K. Heiny and G.A. Weidemann. 1992. *Plant Disease* 76(3):267-269

Growth chamber and field studies were conducted to evaluate this fungus as a herbicide on an amaranth species. The amaranth plants were killed.

Alkali wet-milling characteristics of pearled and unpearled amaranth seed. D.J. Myers and S.R. Fox. 1994. *Cereal Chemistry* 71(1):96-99

This method is capable of recovering 52% of the available starch at a purity of greater than 98%. The starch is potentially useful because of its unique microcrystalline granules.

Incidence of weed seed in cow (*Bos* sp.) manure and its importance as a weed source for cropland. J.M. Pleasant and K.J. Schlather. 1994. *Weed Technology* 8:304-310

Cows distribute wild weedy amaranth seeds, and many other weeds in manure.

Iranian breads supplemented with amaranth flour. M. Samiyi and H.L. Ashraf. 1993. *International Journal of Food Science and Technology* 28:625-628.

Replacement of up to 30% of wheat in three Iranian breads similar to those in other semitropical countries produced little adverse effect on sensory attributes and improved calculated nutrient composition.

Species identification by RAPD analysis of grain amaranth genetic resources. D.K. Transue, D.J.

Fairbanks, L.R. Robinson, and W.R. Andersen. 1994. Crop Science 34:1385-1389.

The research was at Brigham Young University, in Provo, Utah. The RAPD analysis is a new DNA-based bio-technology method of classifying plants. This study validated the J.D. Sauer taxonomic system for classifying the three cultivated grain amaranth species. The future of this line of research should include examination of more of the approximately 60 *Amaranthus* species.

Grain amaranth (*Amaranthus* species)

J.T. Williams and D. Brenner, pages 129-186 in Cereals and Pseudocereals 1995 J.T. Williams (ed) Chapman and Hall, London £45.00. The publisher can be phoned at 212-564-1060 in the US and at 0264-342923 in the UK.

This book has chapters on seven under-utilized crops. The amaranth chapter is a recent review of breeding and utilization. It includes a revised key to the cultivated grain species, and other information about taxonomy and germplasm.

Isolation and characterization of starch from amaranth flour. J. Zhao and R.L. Whistler. Cereal Chemistry 71(4):392-393.

The authors developed a simple procedure for isolation of amaranth starch with well-formed, non-cemented granules. The resulting product is low in protein and fat and is white.

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Amaranth Research in the Nebraska Panhandle

David D. Baltensperger
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4502 Avenue I
Scottsbluff, NE 69361

Amaranth research in the Nebraska panhandle area will be supervised again this year by Dr. David D. Baltensperger at the University of Nebraska's Panhandle Research and Extension Center in Scottsbluff. Mr. Fernando Roger Guillen Portal, an M.S. student in the Department of Agronomy, will be conducting research on improving uniformity of seed size and color, seed maturation, and plant height by selecting within Plainsman cultivar variation. Mr. Guillen-Portal has studied amaranth in his home country of Bolivia, and is looking forward to developing improved cultivars.

We will be conducting amaranth variety trials this season, and will help coordinate the regional trials. We have received the germplasm collection from Dr. Robert Myers for several cultivars and amaranth species including *Amaranthus cruentus*, *A. hybridus*, and *A. hypochondriacus*. Please contact us to obtain seed for the regional trials or for other germplasm, specify the desired quantity of each type. This material is all duplicated at the USDA Plant Introduction Station, Ames, Iowa.

Survival Strategies of the Peasants Amaranth Project

Davidson K. Mwangi
Seed & Food Research
Development and Production Center
P.O. Box 376
Nanyuki, Kenya

This program started with 50 small-scale farmers and by the end of last year I had achieved 1,050 small scale farmers in the Nyeri and Nyandarua Districts. In this program I do the following:

(1) Transfer my research findings directly to the small-scale farmers.

(2) Each farmer allocates 1/4 of an acre.

(3) Educate the farmers in a group which includes Ministry of Agriculture technical staff posted in the area, on how to cultivate amaranth as a food crop.

(4) Supply free amaranth seeds enough to plant 1/4 acre.

(5) Organize workshops for the farmers and the ministry of Agriculture technical staff and teach the groups how to prepare the amaranth as food at home. The workshops are held in the farmer's home using the farmer's equipment. In these workshops I supply all materials which include, amaranth seeds for popping, and all flours including wheat, maize, sorghum, millets, and rice. In potato growing areas we have made amaranth/potato mash foods, etc. Out of this program the women are now making infant amaranth food. We have also replaced sorghum, and millets with amaranth 100%, maize, wheat, rice, and even oats by 50%.

(6) Visit every small-scale farmer at least once a month to advise further.

The farmers are very happy as they can now store amaranth longer than other grains and it is less expensive than other grains.

As the program expands here I have a very serious problem. I am now faced with a cash problem. I need to educate the farmers, and I need to travel to more areas. I need more staff to assist me in my seed breeding program, seed distribution to the farmers, field

supervision, and workshop presentations. Should you have in mind any person, group, or organization, who would be willing to assist me in this program please recommend that I receive financial assistance.

Amaranth Progress in India

B.D. Joshi
National Bureau of Plant Genetic
Resources
Regional Station
Phagli, Shimla-171004
INDIA

The Himalayas are endowed with rich genetic diversity of amaranths and are considered to be its secondary center of origin. Amaranth cultivation is widely spread in the mountain regions above 1500 m and extends up to 3000 m elevation. The crop is also sporadically grown in the Northern plains, Central and Southern India. In the Himalayas two grain species, i.e. *Amaranthus hypochondraicus* and *A. caudatus* are commonly grown, whereas *A. cruentus* is of rare occurrence.

To date, the National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Shimla, has mounted a total of 45 crop specific/region specific multi-crop explorations and consequently a germplasm collection of 3000 accessions, including exotic introductions representing about 25 species, has been built-up. The germplasm collected so far has been evaluated for 40 descriptors and descriptor states. A catalog of 990 accessions for 31 descriptors has been published, and another updated catalog of about 2000 accessions with 40 descriptors is ready for publication. After multi-locational trials all over the country, a variety *Annapurna*, with yield potential of 25 q/ha has been released for general cultivation in the Himalayas, as well as in the plains. This has also been accepted as a standard check in the National

Trials. A core set of 100 collections has been identified to represent the whole variability available in the germplasm holdings. Part of the evaluated material has been sent to the National Gene Bank, New Delhi and the rest of the germplasm is being maintained in medium term storage (Rh 35% and temperature 4-5 C°) at NBPGR, Regional Station, Shimla.

The collections identified for various economic characters, have been supplied to the breeders for further improvement in this crop. Seeds of the variety *Annapurna* and other promising accessions have also been supplied to extension agencies, Department of Agriculture, agricultural universities, research institutions and farmers of the country.

However, there is still more to be done to bridge the gaps in what has been done and what is to be done in this crop. There is a need to have finer grid collection for locating the genes for most important characters viz. early maturity, dwarf types, bold (large) seededness and non-shattering types etc. from the North-West, North-East and Southern hill regions of India. To represent the variability from all over the world there is a need to collect germplasm from all of the countries and also to develop an International Cooperative Network for testing the germplasm under diverse agro-climatic conditions. There is an urgent need to identify a chain of breeding and testing centers in this country to develop a well elaborated breeding program for developing improved varieties to meet the local needs. The crop is highly nutritious and there is a need to undertake in-depth studies to assess its quality parameters. The crop also needs to be popularized in the industrial sector, such as the food industry, cosmetic industry and pharmaceuticals for various uses. Pushing up of the crop into private sector will increase the area,

production, and quality of the crop.

In India, apart from NBPGR, other organizations involved in amaranth improvement work are HP Agr. Univ., Palampur, VPKAS, Almora, G.B. Pant Agri. Univ. Hill campus, Ranichauri, Tamil Nadu Agri. Univ. Coimbatore, IIHR, Bangalore (vegetable types), Orrisa Agri. Univ. Bhuvneshwar, NBRI, Lucknow (ornamental types) and Punjab Agri. Univ. Ludhiana (fodder types).

Amaranth Sprouts at Disney Farms

Kenneth Disney
Disney's Better Way, Inc.
Lodgepole, Nebraska
69149-2544

The following briefly describes my experience sprouting the edible amaranth grain variety *Plainsman*.

The *Plainsman* amaranth that we used in our sprouting and milling facility was produced on our Organic Crop Improvement Association (OCIA) certified farm, "Disney Farms" from seed acquired through the Nebraska Crop Improvement Associations, Foundation Seed Division. It was developed primarily for grain production and its uniform height, approximately 42 inches at maturity in our area under dryland production.

Our interest in sprouting amaranth arose from our desire to find new ways to utilize this crop, which is grown on approximately 1,500 acres in our area by five OCIA certified producers. Although its milling and baking qualities are well documented, little research has been done in the area of sprouting, an area of promising growth (not only in well established East and West Coast markets, but the Front Range of Colorado as well, where we intend to market the majority of our production).

We began sprouting amaranth in September of 1994 and continued to do experimental sprouting until December of 1994. Our experience

with amaranth sprouting has brought us to these conclusions:

1. Most amaranth varieties, with the exception of *Plainsman*, do not have a high germination rate (90% or above) which is a critical factor in sprouting yield, (the increase in weight from a pound of dry seed to the finished sprout). Typically a yield ratio of 1:8 to 1:10 in alfalfa and clover can only be achieved through high germination seed.

2. *Plainsman* amaranth can be grown quickly to harvest maturity, usually 72 hours in a drum system, which was used to sprout our amaranth. Maturity was determined to be the point at which the emerging leaves opened, displaying the brilliant red or maroon color. This was usually at a height of 3/4 inch. If grown longer than 72 hours the plant would increase substantially in size and the leaves would turn an off-green and red color. This was not preferred, as the bright red at the earlier stage gave salads a distinctive contrast between the greens and the red amaranth.

3. At all stages of growth the amaranth exhibited a very distinctive aftertaste, which 95% of consumers disliked. This distinctive aftertaste grew more pronounced with each day of growth and was the reason we chose to harvest at the earliest stage possible. The aftertaste has been attributed to the plants instinctive defensive mechanism and allelochemic process which causes the plant to produce a very offensive taste, (to deter predatory activity by insects and animals, including humans, apparently).

4. The very small size of the sprouted plant, with a near hair-like stem, was a constant problem for adequate shelf life, (the amount of time a sprout can be displayed in a grocer's produce case) which for alfalfa is 7 to 12 days. The shelf life for our amaranth was less than a week, usually five days, which is not adequate for commercial production and barely adequate for fresh farmers markets. Adequate drying of the sprouts before packaging in quart sized ziplock veggie bags was hampered by the fact that the excess water present on the sprout after harvest could not be properly removed with a centrifuge. Excessive moisture in a packaged sprout product greatly reduces shelf life.

As a result of the above mentioned problems, commercial production of amaranth sprouts has been discontinued for the time being, until a variety with much larger seed size and a milder aftertaste can be found.

We feel that there is still promise for amaranth sprouts meeting our requirements, as there is a nice market for this unique and nutritious sprout, however small it may be.

Expression of C4 Photosynthesis Genes in Grain Amaranth

James O. Berry
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Buffalo, NY 14260

Amaranth uses the highly specialized C4 photosynthetic pathway, which allows this plant to be very efficient in the assimilation or fixation of atmospheric CO₂ into biologically useful molecules. C4 plant species

possess a specialized leaf organization which is characterized by the presence of two separate photosynthetic cell types, the mesophyll and bundle sheath cells. Photosynthetic enzymes present in one or the other of these two cell types function together and work as a "CO₂ pump" to concentrate CO₂ in leaf bundle sheath cells where carbon fixation occurs. The result is that C4 plants are much more efficient than the more common and less specialized C3 plants, particularly at high temperatures and in marginal desert or arid environments.

C4 photosynthesis requires interactions between enzymes which are specifically compartmentalized to one or the other cell type and selective cell type-specific expression of genes encoding the C4 enzymes. The focus of our research is to investigate developmental signals and mechanisms that regulate the cell type-specific expression of nuclear- and plastid-encoded genes which produce enzymes of the C4 photosynthetic pathway in amaranth, a dicotyledonous C4 plant.

We have developed several molecular biology tools for analyzing gene expression in amaranth. These include genomic (phage and cosmid) libraries, a cDNA expression library from light-grown leaves and cotyledons, and antibodies against RuBPCase, PEPCase, and the mitochondrial NAD-dependent malic enzyme. We have developed several new techniques for amaranth molecular biology research, including procedures for isolating high molecular weight nuclear DNA, for isolating mRNA, for separating bundle sheath and mesophyll protoplasts, and for transient gene expression analysis using biolistic ("gene gun") transformation of intact leaves and cotyledons.

By using amaranth as a model C4 dicot, we are continuing to achieve new insights into mechanisms and processes responsible for the development of C4 photosynthetic

capacity in higher plants. In addition, basic research with amaranth will contribute to the development of this plant as an agriculturally important crop for food production and for industry.

C4 gene expression is coupled to photosynthetic metabolism. Ribulose 1, 5-bisphosphate carboxylase (RuBPCase) is present in all plants and is the enzyme responsible for incorporating, or fixing, CO₂ from the atmosphere into biological molecules. In the mature leaves of a C₄ plant such as amaranth, RuBPCase is found only in the chloroplasts of leaf bundle sheath cells, and is not present in the chloroplasts of mesophyll cells. However, in very young leaves and cotyledons RuBPCase genes are expressed in both bundle sheath and mesophyll cells in a pattern characteristic of plants that utilize the less specialized C₃ photosynthetic pathway (Wang et al, 1992; 1993a; 1993b; Ramsperger and Berry, 1995). We have shown that photosynthetic gene expression changes during leaf development, from the initial default "C₃-like" developmental pattern (RuBPCase is present in both bundle sheath and mesophyll cells, gene expression is not cell-type specific) to the more specialized "C₄-type" pattern of expression (RuBPCase is specifically localized only to bundle sheath cells, gene expression has become cell-type specific). The shift from C₃-like to C₄-type RuBPCase expression occurs in the basipetal (apex to base) direction, so that bundle sheath cell-specific expression of RuBPCase was observed initially at the apex of young leaves and progressed rapidly down toward the leaf base.

Our data demonstrate very clearly that this "C₃-to-C₄" transition in photosynthetic gene expression is coordinated with the carbon sink-to-source transition, the process in which a leaf is converted from a net importer (sink) to a net exporter (source) of

photoassimilates (sugars produced by photosynthetic carbon fixation) (Wang et al, 1993a). As specific regions of the leaves went through the developmental and metabolic transition from carbon sink to carbon source, the cellular localization of RuBPCase gene expression in these regions shifted as well. Our findings link together molecular and physiological developmental processes that were not previously known to be related and suggest that changes in photoassimilate accumulation or transport may influence or signal changes in the cell-type specific expression of RuBPCase genes. We believe that the changes in photosynthetic gene expression occurring near the sink-source transition zone may reflect wider changes in gene expression and in cell-to-cell communication that are occurring as the leaf tissue reaches developmental and metabolic maturity. To determine how photosynthesis and carbon transport affect bundle sheath-specific gene expression, we are now analyzing photosynthetic transport and function in C₃ and C₄ regions of the young leaves.

The majority of this work has been done using *Amaranthus hypochondriacus*. Recently we have found that *A. tricolor* can also be very useful for this research, since the three color leaves that emerge during later phases of development in this species have both photosynthetic (green) and non-photosynthetic (red and yellow) regions. These different regions show different patterns of cell-specific RuBPCase localization, suggesting that photosynthetic function might be correlated with the establishment of C₄ gene expression (Ramsperger and Berry, unpublished results).

Cell-specific RuBPCase gene expression in isolated chloroplasts. We have used separated bundle sheath and mesophyll chloroplasts to

investigate mechanisms controlling the cell type-specific expression of the chloroplast-encoded gene for the large subunit (LSU) of RuBPCase, the *rbcL* gene (Boinski et al, 1993). A major advantage to using amaranth for these studies is that intact, separated bundle sheath and mesophyll protoplasts can be rapidly prepared from fully expanded leaves, and chloroplasts can then be isolated from the separated protoplasts. It is very difficult to obtain bundle sheath protoplasts from C4 monocots because the thick bundle sheath strands are very resistant to cell wall degradation.

While the *rbcL* mRNA accumulated only in bundle sheath chloroplasts, transcriptional run-on analysis demonstrated that the *rbcL* gene was transcribed to a similar degree in both types of chloroplasts. Therefore, the specific accumulation of the *rbcL* transcript in bundle sheath and not in mesophyll chloroplasts of mature amaranth leaves is due, at least in part, to regulation at the post-transcriptional level. Possibly this control is mediated by differential processing or stabilization of the *rbcL* transcripts. We are currently using extracts prepared from the separated bundle sheath and mesophyll chloroplasts to investigate in greater detail the post-transcriptional control of cell-type specific RuBPCase gene expression. Stability of *rbcL* mRNA is being determined in soluble extracts prepared from the two chloroplast types. In addition, because RNA binding proteins have been correlated with differential mRNA accumulation in other systems, we are using these extracts to determine if cell type-specific RNA binding proteins can be identified that interact with the *rbcL* transcript.

The C4 photosynthetic NAD-dependent malic enzyme of amaranth mitochondria. Amaranth is classified as an NAD-ME type C4

plant and utilizes a unique malic enzyme, located in the bundle sheath mitochondria, to decarboxylate C4 acids for refixation by RuBPCase (Long et al, 1994). We have found that this enzyme is regulated by light at the level of mRNA accumulation, is bundle sheath cell-specific at all stages of leaf development, and can be turned off at post-transcriptional levels by darkness. We have isolated the promoter of this gene, and have used "gene gun" transformation to investigate the expression of a promoter-GUS gene construct in intact leaves. It appears that this construct is expressed only in bundle sheath cells and that cell specificity is conferred by the promoter region. We are in the process of constructing transgenic tobacco plants to investigate the cell-specific and light-mediated regulation of this C4 amaranth gene construct in a C3 plant and to investigate the mitochondrial transport of this unique photosynthetic enzyme.

Photosynthetic gene expression is regulated by light. One of the most exciting aspects of gene regulation in amaranth is that very rapid and dramatic changes in the synthesis of the RuBPCase occurs at the level of protein synthesis (translation). These translational changes in gene expression can be induced and studied simply by changing illumination conditions (Berry et al, 1990). RuBPCase synthesis is rapidly induced at the level of translational initiation when dark-grown seedlings are transferred to light (light-shift), and is rapidly blocked at the level of translational elongation when light-grown seedlings are transferred to darkness (dark-shift). We are investigating the regulation of the RuBPCase at these two translational steps. Potential sites of translational pausing are being identified in purified polyribosomes, and changes in phosphorylation for several

ribosome-associated proteins have been shown to occur in response to changes in illumination. In addition, new translational gene constructs are being produced for "gene gun" transformation to determine which regions of RuBPCase mRNAs are responsible for light-mediated translational regulation.

The process of protein translation is an integral part of the overall pathway of gene expression in all organisms. Rapid changes in the translation of specific mRNAs may be a mechanism for adapting to sudden environmental changes that could affect the survival of the organism. While little is currently known about translational control in plants, amaranth seedlings demonstrate one of the clearest examples of this form of regulation. Amaranth therefore provides an ideal system in which to study rapid, light-mediated changes in the expression of genes that produce photosynthetic proteins.

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Response of Some Grain Amaranths to Shortday and Longday Ecological Conditions

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Three-hundred-eleven stocks of grain amaranth were evaluated for their performance and adaptability under Nigeria's shortday tropical conditions and Ontario, Canada's longday conditions. The plantings in Nigeria were September 1984, May 1985, and March 1986 at latitude 07°27'N, longitude 03°51'E, 150 M above sea level. The Canadian planting was in Ontario planted July 1, 1991 at latitude 43°41'N, 79°38'W, 11 M above sea level.

Plants were shorter in Nigeria than in Canada, and the terminal inflorescences were shorter in Nigeria than in Canada. In Nigeria populations of *A. cruentus*, and *A. cruentus* X *caudatus* branched all along the stem; while populations of *A. caudatus* and *A. hypochondriacus* excessively branched, all near the stem base. In Canada, no

populations produced branches. The 6th leaves were much narrower and shorter in Nigeria than in Canada.

Ontario's longday regime, with a high light integral, favors the studied genotypes, resulting in greater seed yields than those produced in Nigeria's short daylength.

Amaranthus Germplasm at the North Central Regional Plant Introduction Station

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The National Plant Germplasm System has a collection of more than 3,000 *Amaranthus* accessions, 1,376 of which are available in June 1995 for distribution to researchers, at no cost.

This collection was assembled to conserve amaranth types that would otherwise be abandoned when subsistence amaranth growers switch to new crops, and to introduce amaranth as a new crop for the United States. Each accession was collected separately (usually from a farmer's field) and is maintained separately. When accessions are grown for seed production the plants are grown in separate plastic tents to prevent pollen contamination between accessions.

The collection has more than 100 accessions from each of the following countries: Ecuador, India, Mexico, Nepal, Nigeria, and Peru. The Rodale Research Center sent us all of their landrace and wild *Amaranthus* germplasm in 1990. The best represented species are *A. caudatus*, *A. cruentus*, *A. hypochondriacus*, and *A. tricolor*. Thirty *Amaranthus* species are in the collection. My goal as the curator,

is to acquire more of the 60 *Amaranthus* species.

In addition to long-term conservation, the collection is used for research. We provide germplasm for plant breeding, systematics, ornamental horticulture, entomology, and pathology, and other projects. We are well suited to supply projects that screen diverse samples for unusual qualities needed for crop improvement.

The following recent additions to the collection are of special interest, and are now available for distribution:

PI 568125 to PI 568131 *Amaranthus hypochondriacus*: These seven accessions segregate for male sterility. The male sterility is useful to plant breeders because it makes crosses between plants easier to control than is the case with normal male fertile plants. They are derived from the cultivar *Plainsman* which they resemble. The selections from *Plainsman* were made by David Brenner in 1992. They grow 1.5 m tall; and have white or black seeds.

PI 566897 'Kerala Red' *Amaranthus cruentus* from Kerala, India: It has attractive, intensely red foliage, and can be used as an ornamental. In India it is used as a vegetable spinach. The foliage flavor is mild and pleasant. It grows 2 m tall, the seeds are black. Because of the intense coloring, it would be a good choice for amaranth pigment research.

PI 584523 *Amaranthus hypochondriacus* donated by Dr. Peter Kulakow of Salina, Kansas: It has a determinate inflorescence which may be related to synchronized seed-grain maturity. This accession could become useful as a parent of new grain cultivars. The 1.3 m tall plants are resistant to lodging; their seeds are black.

Rapid Cycling of Grain Amaranths

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Rapid turnover of generations is vital to many aspects of plant breeding, including establishment of segregating populations, inbreeding, backcrossing, recurrent selection, and population development. Although many key quantitative crop traits must ultimately be tested or selected in a field environment, generation advancement and intermating for traits not requiring a field environment can substantially improve genetic gain (Fehr, 1987). Typically, such work is done in off-season or winter greenhouses. Rapid cycling of generations is also an asset in classical and plant molecular biology, where mutants of Arabidopsis thaliana (L.) Heynh. have been widely studied.

Because some amaranth species can complete a life cycle in 5-6 weeks under short day (SD) photoperiods, they have been proposed as tools for genetic studies like Arabidopsis and Drosophila (the fruit fly) (Walton, 1968a). The relatively large number of chromosomes in Amaranthus (n=16 or 17) may be an advantage over the number in Arabidopsis (n=5) when studying quantitative traits (Walton, 1968a) and in mimicking the breeding behavior of partially allogamous crops like cotton (Walton, 1968b). Rapid amaranth cycling at the rate of six or more amaranth generations per year has been suggested (Walton, 1968a).

The objectives of this study were to i) test selected amaranth accessions for rapid cycling traits under SD conditions, and ii) to test the same accessions under stressed vs. non-stressed growing media.

LITERATURE REVIEW

Photoperiod responsiveness of grain amaranths, (A. hybridus L. (Townsend, 1977; Brennan, 1981), Amaranthus hypochondriacus L., A. cruentus L., and A. caudatus L. and their relatives varies greatly. Murray (1938) detected early flowering in amaranth hybrids, such that:

"under exceptional conditions...a few plants with 3-4 miniature flowers [were] definitely mature in 6 days time from germination (described as emergence of cotyledons from the soil)."

Similarly, Amaranthus retroflexus L., a weedy relative of the grain amaranths, will flower 3 days after seedling emergence when treated on a single 8 h cycle (Koller et al. 1977b).

Allard and Garner (1940) described an indeterminate flowering response for the amaranth family, Amaranthaceae, although they characterized Amaranthus hybridus as an obligatory or facultative short day plant. Fuller (1949) found A. caudatus to be a SD plant while Zabka (1961) qualified the results of Fuller to include a "sensitive period" for inflorescence development. Because the species behaved differently under long day (LD) (18 hours light) conditions, Zabka (1961) suggested that it is not truly an obligate SD plant. Kohli (1978) also postulated that A. caudatus f. albiflorus, A. caudatus f. caudatus, and A. tricolor L. var. tritis were qualitative SD plants which displayed divergent photoperiod requirements for reproductive initiation. In subsequent study of all three species under 8 hr SD regimes, Kohli et al. (1980) demonstrated synthesis of 3-5 new proteins, which they considered requisite to floral initiation.

Kulakow and Jain (1985) concluded that a single dominant

earliness gene was present in backcrosses of an A. retroflexus X A. cruentus mating, which was most clearly seen in controlled 8 hour photoperiods and not always evident in the field. On the other hand, Pandey (1985) investigated flowering in A. hypochondriacus matings and found that recessive alleles contributed to early maturity.

MATERIALS AND METHODS

Eleven amaranth accessions were chosen from the North Central Regional Plant Introduction Station's germplasm to represent diverse types (Kauffman & Reider, 1986) (Table 1).

EXPERIMENT I. Ten amaranth accessions (Table 1) were planted in a randomized complete block design with two replications. Accessions were sown in 50% soil/50% peat mix (Sunshine No. 3, Fisons Western Corp., Vancouver, Canada¹) in the greenhouse, with 50 pots per accession. After seven days, seedlings were thinned to two per Jiffy pot (Jiffy Products [N.B.] Ltd., Shippegan, Canada). A growth chamber was maintained at a constant 65° F. and a photoperiod regime of 8 hours light/16 hours dark. Irradiation was provided by 400 W., high-pressure sodium horticulture lights (Energy Technics, York, PA) which typically accelerate floral initiation in most herbaceous plants (Cathey and Campbell, 1979).

Traits measured were: i) number of leaves 24 days after planting, ii) node number at 70 days after planting, iii) height at 70 days after planting, and iv) number of seeds at 70 days after planting. Statistical analyses were conducted with SAS (1985) and MSTAT-C (1990). Seed data was transformed by the equation: $(X + 0.5)^{1/2}$.

EXPERIMENT II. Ten amaranth accessions (Table 1) were planted in a split-plot design with three replications. Two growth media (sand and 50% soil/50% peat mix as in Experiment I) were randomly allocated to main plots and ten

accessions or genotypes were randomly allocated to sub-plots. Accessions were sown and thinned as in Experiment I. A growth chamber was maintained at a constant 90° F. and a photoperiod regime of 8 hours light/16 hours dark. Irradiation was provided by 400 W., high-pressure sodium horticulture lights. Traits measured were: i) days to flowering and ii) number of leaves after 24 days. Statistical analyses were conducted with SAS (1985) and MSTAT-C (1990).

RESULTS & DISCUSSION

EXPERIMENT I. Means are given for all traits in Table 2. Of the four traits measured, only the mean squares for number of seeds set at 91 days after planting (DAP) was significant ($P = 0.01$). Three accessions, Ames 5634, Ames 2027 and K 343, had 28, 13, and 8 mature seeds, respectively. Six of the ten accessions tested set no mature seed under these photoperiod and temperature conditions.

The original experimental design for Experiment I included three replications and another photoperiod regime. At the relatively low temperature (65° F.) for enzymatic and photosynthetic activity, many seedlings succumbed to damping off, Pythium spp., a common opportunistic fungi in cold, wet soils; thus, many materials were lost.

IMPLICATIONS. If a rapid cycling system is to be effective, then rapid flowering and seed maturity are imperative. Under the photoperiod and temperature regimes employed here, four generations per year could be obtained with a growth chamber. The larger number of seeds set by Ames 5634, the same species as the major domestic grain lines, suggests that it may be a source of earliness genes. It would be highly desirable to study the inheritance of this earliness to determine both the number of genes controlling earliness and whether or not there is photoperiod insensitivity.

Due to the high loss of experimental materials to Pythium spp. (a common, opportunistic fungi in cold, wet soils), fungicides or soil drenches might be desirable. Alternatively, sixty-five degrees F. may be too low a temperature to sustain growth in most amaranth accessions, i.e., photosynthesis must offset maintenance respiration.

EXPERIMENT II. Mean squares for both days to flowering and number of leaves after 24 days were highly significant ($P= 0.01$) and significant ($P= 0.05$). A Spearman's rank correlation for both traits measured was non-significant at 0.28

($P = 0.43$). Mean squares for both traits were also non-significant when the growth media types were tested on the main plot error.

IMPLICATIONS. The three accessions, Ames 5634, Ames 2248, and K 343, flowered at least 20 days ahead of the other accessions (Table 3). These three accessions are potential sources of earliness genes in rapid cycling programs. Additionally, they represent both grain species, A. cruentus and A. hypochondriacus, which are usually sterile when hybridized.

Table 1. Names, species, types, and origins for Amaranthus accessions used in rapid cycling Experiments I and II.

<u>Accessions</u>	<u>Species</u>	Comments
R 158	CRU	High growth rate stock
K 343	HYP	Cultivar
RRC 1011	CRU	Standard grain type
Ames 2027	HYP	
Ames 2248	CRU	Gold seed coat
RRC 3250	CRU	Segregating population from Peruvian breeding program
Ames 5175	CRU ¹	Promising genotype observed in 1986 germplasm fields at Ames, IA
Ames 5176	CRU	Promising genotype observed in 1986 germplasm fields at Ames, IA
PI 433228	CRU	Guatemalan grain unimproved
PI 451711	CRU	Mexican grain unimproved
Ames 5634	HYP ²	Potential source of earliness genes

* CRU = Amaranthus cruentus L., HYP = A. hypochondriacus L.

¹ Ames 5175 was used in Experiment II only.

² Ames 5634 was used in Experiment I only.

Table 2. Means for ten amaranth accessions grown under a 8 hour light/16 hour darkness photoperiod at 65° constant temperature (Experiment I).

Accessions	No. of Seeds	No. of Seeds ¹ (transformed data)	Height (cm)	No. of Nodes	No. of Leaves
R 158	0	0.7	12.9	3.8	4.1
PI 433228	0	0.7	8.0	2.5	9.2
K 343	7.5	3.2	17.2	3.3	3.3
PI 451711	0	0.7	12.1	2.7	3.2
RRC 1011	0	0.7	13.4	3.1	4.6
Ames 2027	13.4	3.7	16.6	3.6	3.1
Ames 2248	4.2	2.1	13.9	2.4	3.1
RRC 3250	0	0.7	12.5	3.8	5.8
Ames 5176	0	0.7	15.0	4.6	5.0
Ames 5634	28.1	5.3	13.8	4.9	4.7
CV, %	--	31.9	19.3	36.9	23.4
LSD (0.05)	--	1.3	NS ²	NS	NS

¹ Data was transformed by the equation $(X + 0.5)^{1/2}$

² NS is not significant.

Table 3. Means for ten amaranth accessions grown under a 8 hour light/16 hour darkness photoperiod at 90° constant temperature (Experiment II).

Accessions	Days to flowering	No. of leaves after 24 days
RRC 1011	66.3	2.53
Ames 2027	36.2	2.10
R 158	67.5	2.23
Ames 5175	63.3	2.62
Ames 5176	64.8	2.57
PI 433228	62.7	2.53
Ames 2248	35.5	2.15
RRC 3250	58.3	2.18
K 343	38.7	1.97
PI 451711	67.3	1.81
CV, %	6.0	18.0
LSD (0.05)	3.9	0.47

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PUBLICATIONS FOR SALE

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Back issues of Legacy are \$3 each for members and \$5 each for non-members. Bumper stickers are \$2 each.

*1990 Amaranth Grain Production Guide, 36 pp. is OUT OF PRINT A revision is planned for 1995.

Legacy is edited by David Brenner, North Central Regional Plant Introduction Station, Iowa State University, Ames, Iowa 50011. Manuscripts and information for publication in Legacy are very welcome.

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