LEGACY

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IN THIS ISSUE:

Amaranth Institute Meeting

An Amaranth Institute meeting is planned for November 1996 in Sidney, Nebraska. The primary organizers of the meeting are Jane Sooby, of the High Plains Agricultural Lab, and Phil Sanders, President of the Amaranth Institute, from Sanders Farms Inc., Dalton, Nebraska. Jane Sooby can be called for additional details at (308)254-3918.

From the President of the Amaranth Institute

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Greetings from Nebraska and welcome to the 1996 edition of Legacy. As we have entered the 96 growing season I have been able to spend a lot of time thinking about where the Amaranth Institute has been and where it is going. 1996 will be Sanders Farms Inc. 16th year of working with Amaranth. I look back 16 years to a small group of individuals that put their heads together and formed the Institute. Slowly but surely growing, into a large group of dedicated people who have stopped at nothing to make the Institute what it is today.

I think back to 1980, and our first experience in growing Amaranth. We planted 10 acres of dryland Amaranth at 8 ounces per acre, in 30 inch rows. The results that fall were plants 13 feet tall, seeds that shattered everywhere, and the problem of trying to figure out, how to harvest this crop? There were so many questions, and no answers. The thing was that we never gave up and we are so glad we didn't.

We have met so many wonderful people in those past years, and have learned so much. So many things have changed from the way we raise our crop, to the way we market our crop. All of the problems we have encountered have been solved by experimentation, and by working with different individuals, many of whom are members of the Institute.

There is now doubt in my mind that Amaranth could someday be a crop every farmer may have the chance to grow, and make part of his or her rotation.

We must all remember to continue to work together as much as possible, and never give up. There are so many good things ahead for the members of the Institute that only time, dedication, and hard work will reveal. I could go on and on, but instead I encourage you all to pay your dues, and for anyone interested to become a member, or part of the Amaranth team.

I would like everyone to know that as your President, I would like to make myself available by phone or personally anytime to all of you. If you have any questions, ideas, or would like to just talk Amaranth, please call me. Anything that any of us can do to improve the Institute, or to help promote Amaranth is so important to all of us.

I would also like to invite everyone to Western Nebraska this fall to take part in the 1996 Annual Meeting of the Amaranth Institute. The University of Nebraska, and the directors of the Institute are presently planning what I think may be the best Amaranth Institute meeting and Field Day ever!

Please feel free to contact any of the directors with any suggestions or ideas.

As I close I would like to thank each and everyone of you for all your past and future efforts. And always remember, AMARANTH IS COMING!!!!

Proposed United States Standards for Grain Amaranths

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With incipient international commerce in grain amaranths, these proposed standards could form a foundation for buyers, brokers, producers, and sellers of the grain. The grain classes have been simplified to overcome the high variability for seed coat color in domesticated, wild, and weedy amaranths. Non-white seed coat colors are believed to be a cosmetic impurity in foodstuff. Nevertheless, processors who grind amaranth flour and who popped and extrude the grain often desire white amaranths. In some cases, black or brown amaranth seed classes may be sought for "soy-sauce" versions of amaranths and for contrast or product identity in applications such as maize/amaranth "corn chips." The pink seed class may be desired in sprouting and malting applications.

Grain Classes

1. White amaranths shall include those with white, cream, and off-white seed coats.
2. Brown amaranths shall include those with all chestnut, reddish-brown, dark burgundy, and all dark, medium, and light brown-shaded seed coats.
3. Pink amaranths shall includes all those with clearly-visible red or pink embryo seeds.
4. Gold amaranths shall include all those with yellow and gold seed coats.
5. Black amaranths shall include all those with black seed coats.

Each class may include not more than 5% of the other classes.

Damaged Seed Categories

1. Tarnished plant bug (*Lygus* spp.) damage
2. Combine damage
3. "Weevily or insect-infested" seed

Grain Classes

	Test Weight (lb./bu)	Moisture Content (%)	Damaged Seed (%)	Black, Brown Pink, or Gold Seed[1] (%)	Foreign Material (%)
No. 1	58	13	5	1	2
No. 2	56	14	7	3	3
No. 3[2]	54	15	8	5	5

U.S. Sample Grade: It shall be grain amaranths which do not meet the requirements for any of the grades from U.S. No. 1 to U.S. No. 3 inclusive, or which contain stones, or are otherwise of distinctly low quality.

[1] Except in classes of other non-white amaranths, where all other classes are considered excluded seed coat colors.
[2] Grain amaranths which are more than 2% sprouted shall be graded not higher than U.S. No. 3.

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McKnight Foundation Award

In April 1995, $901,500 was awarded to a group of biological, social, and economic scientists representing four institutions in Mexico and four institutions in the United States. The project is led by Dr. Robert Bye at the Universidad Nacional Autonoma de Mexico and Dr. Calvin O. Qualset at the University of California, Davis (U.S.A.). They will study ways of applying modern knowledge about genetic diversity and innovative farmer-based approaches to the genetic improvement of the milpa system of agriculture in Mexico. A complex system of food production at the village level, the milpa system centers around maize, beans, squash, and a group of leafy vegetables including amaranth.

World Wide Web (Internet) Amaranth Information

There has been an explosion of amaranth information on-line for computer users. An Alta Vista search for Amaranth in July 1996 returned 900 hits, a search for Amaranthus returned 500 hits. Many of these sites are not about our kind of amaranths. The examined sites are a jumble of fantasy fiction, recipes, plant lists, and any other topic where the word "amaranth" can be The following four sites are particularly interesting.

Amaranth Grain Production in Nebraska
http://ianrwww.unl.edu/ianr/pubs/nebfacts/nf91-35.htm

Best of Health, Amaranth Resources Inc.
http://hlthmall.com/healthmall/amaranth/welcome.html

Distributions of wild *Amaranthus* species in Texas
http://www.csdl.tamu.edu/FLORA/bonapfams/bonxxama.htm

Amaranth Germplasm
http://www.ars-grin.gov/ars/MidWest/Ames/crops/amaranth.html

Amaranth Products

Amaranth Foaming Bath and Shower Gel. Floris, 89 Jermyn Street, London, England.

Floris has a line of elegantly packaged bath products with "Amaranth" on the label. There is no amaranth content listed on the package.

Callaloo. Product of Jamaica, imported by Eve Sales Co., Bronx, N.Y. 10473. On the label "**For that true west Indian taste Blue Mountain Country Callaloo**". Callaloo is a traditional Jamaican name for amaranth leaves eaten as a vegetable. It was $1.95 for a 19 oz (539g) can in Pammel Grocery, Ames, Iowa.

Amaranth Publications

Alfalfa as a companion crop for control of *Lygus lineolaris* (Hemiptera: Miridae) in amaranth. K.M. Clark, W.C. Bailey, and R.L. Myers. 1995 Journal of the Kansas Entomological Society 68(2):143-148.

Since the amaranth was more attractive to *Lygus* bugs than the alfalfa, the authors concluded that no useful control was achieved.

Cereal and nut bars, nutritional quality and storage stability. A.M. Estévez, B. Escobar, M. Vásquez, E. Castillo, E. Araya, and I. Zacarías. 1995. Plant Foods for Human Nutrition 47:309-317.

The bars with toasted amaranth grain had better acceptability under conditions of accelerated storage than bars with puffed amaranth grain.

Effect of wilting and processing on the nitrate and nitrite contents of some Nigerian leaf vegetables. Ike E. Ezeagu, Mich A. Fafunso. 1995. Nutrition and Health 10:269-275.

Nitrite increased and nitrate decreased during wilting. Sustained high consumption of nitrite can be harmful especially to infants. So vegetable amaranth foliage is best prepared fresh rather than wilted.

Pigweed identification: a pictorial guide to the common pigweeds of the Great Plains. M.J. Horak, D.E. Peterson, D.J. Chessman, L.M. Wax. 1994. Cooperative Extension Service, publication S-80, Kansas State University, Manhattan.

An eleven page booklet.

Weed control gone awry. Greg D. Horstmeier. 1996. Farm Journal, 120(February):18-19.

A group of three similar dioecious (separate male and female plants) amaranth field weed species have become "the weed of the decade" in soybean production. They have evolved resistance to ALS inhibitor herbicides such as Pursuit and become harder to control. Combinations of tillage and switching herbicides are recommended. There are many similar articles in other agronomy publications.

Diversifying U.S. crop production. Jules Janick, Melvin G. Blase, Duane L. Johnson, Gary D. Jolliff, Robert L. Myers. 1996. Council for

Agricultural Science and Technology, issue paper number 6, Ames, Iowa.

A twelve page booklet summarizing the benefits of crop diversity and new crops. The Jefferson Institute as proposed, would be a center to sponsor new crops development with federal funding. Such a center could help develop amaranth and many other crops.

Morphophysiological variation in some Mexican species of vegetable *Amaranthus*: evolutionary tendencies under domestication. Christina Mapes, Javier Caballero, Eduardo Espita, Robert A. Bye. 1996. Genetic Resources and Crop Evolution 43:283-290.

A comparison the plant morphology of Mexican grain and vegetable types.

Threshing cylinder speed affects germination of *Amaranthus cruentus* L. Seeds. Palaniappa Krishnan, Thomas A. Evans, and Wallace G. Pill. 1994. Hortscience 29(6):652-654.

Seed damage during threshing caused abnormal seedlings. Loss of viability in storage was greater for the damaged seeds than for undamaged seeds.

Identifying suitable regions for amaranth production using a geographic information systems approach. Robert L. Myers. 1994. American Journal of Alternative Agriculture 9:122-126.

A map of suitable regions was developed. The author speculates that high suitability might correlate with successful sorghum production.

Priming improves germination and emergence of combine-harvested *Amaranthus cruentus* L. Seeds. Wallace G. Pill, Thomas A. Evans, and Palaniappa Krishnan. 1994. Hortscience 29(6):655-658.

Vitamin C in leaves and seed oil composition of the *Amaranthus* species. Dhan Prakash, B.D. Joshi, and M. Pal. 1995. International Journal of Food and Nutrition 46:47-51.

Both the vitamin C and the seed oil content varied between accessions. The vitamin C was up to 288 mg/100 g of leaf material. The seed oil content was up to 13.2 percent.

Genetic variation in iron bioavailability from *Amaranthus* species. Anusuya Rangarajan. 1995. Publ. #9619894. Dissertation Abstracts International. Volume 57. Issue 2B.

Comparison of yield and properties of amaranth starches using wet and dry-wet milling processes. J. Uriyapongson and P. Rayas-Duarte. 1994. Cereal Chemistry 71:571-577.

Pasting curves and other properties were estimated for two cultivars.

Contributions to the botany and nutritional value of some wild *Amaranthus* species (Amaranthaceae) of Nuevo Leon, Mexico. Pedro Wesche-Ebeling, Ratikanta Maiti, GracielaGarcia-Diaz, Diana I. Gonzalez, and Fernando Sosa-Alvarado. 1994. Economic Botany 49:423-430.

The four *Amaranthus* species were found to be very nutritious. None had oxalates at harmful levels. The nitrate levels were high enough to be toxic, although similar to those found in spinach. Most of the nitrate would leach into cooking water during food preparation.

Physical properties of starch from two genotypes of *Amaranthus cruentus* of agricultural significance in China. 1995. Huaixiang Wu, Shaoxian Yue, and Harold Corke. Starch 47(8):295-297.

The two types differed in gelanitization temperature and other properties. Cultivars with known properties could become required for new food uses.

Effect of home processing on ascorbic acid and β-carotene content of spinach (*Spinacia oleracia*) and amaranth (*Amaranthus tricolor*) leaves. 1995. Shashi Kala Yadav, and Salil Sehgal. Plant Foods for Human Nutrition. 47:125-131.

The recommended way to conserve these nutrients is by refrigerator storage, quick blanching for only 5 minutes, pressure cooking, or drying them in an oven.

The research and development of grain amaranth in China. Yue, S.X., H.L. Sun, F.D. Tang (eds.). 1993. Chinese Agricultural Science and Technology Publishing House, Beijing, China.

The book is 467 pages long, and includes 123 short papers. The English language summary is 24 pages long.

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Variability in Amaranthus spp. Cultivar 'Plainsman'

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Among the amaranth genera, three grain species, namely *Amaranthus hypochondriacus, A. cruentus,* and *A. caudatus,* have played an important role as source of human food in the ancient civilizations of America. The current interest in amaranth has become apparent with the discovering of several attributes of this pseudo-cereal (high nutrient value, tolerance to dry and drought

conditions suitable to be produced in semiarid regions, and high amount of genetic variability present in land race populations suggesting potential success in breeding)(2,5). In 1991 the University of Nebraska and the Rodale Research Center released the cultivar 'Plainsman', an interspecific hybrid between *A. hypochondriacus* and *A. hybridus*, which has been tested in areas of production including Nebraska, Colorado, Missouri, Minnesota, and South Dakota, with very promising results(1). In fact, Plainsman is currently the most widely grown grain amaranth in the United States, where approximately 1200 ha. are devoted to its production each year. Improvement of Plainsman requires knowledge of the genetic variability involved in traits affecting seed production, but little is known in this regard. The objectives of this study are to estimate the genetic and environmental components of variance in quantitative traits in a random population of Plainsman, to predict response to selection under an S1 family-selection scheme, and to estimate the phenotypic and genetic correlations among traits affecting seed yield.

Literature Review

Substantial evidence shows amaranth is an inbred-outbred crop with a breeding system similar to crops like sorghum (*Sorghum spp.*), cotton (*Gossypium hirsutum*), pearl millet (*Pennisetum glaucum*), and broad bean (*Vicia faba*)(3). The mixed mating system in amaranth opens new perspectives for the improvement of grain amaranth providing suitable techniques from both the self-pollinated and cross-pollinated crops(7). Improvement of grain amaranth has been carried out mostly using self-pollinated breeding methods(6). Single plant selection and mass selection has been extensively applied for amaranth improvement. Also, interspecific and intraspecific hybridization has been suggested as potential method for amaranth improvement(6). The literature reports successful interspecific and intraspecific crosses in amaranths in which heterosis is expressed for biomass yield(8). The proven mixed mating system and the occurrence of nuclear-cytoplasmic male sterility in some populations suggests that recurrent selection methods applied first to cross-pollinated crops like maize and later on to mixed-mating system crops like sorghum could be used for amaranth breeding(7). Recurrent selection has traditionally been applied to cross-pollinated crops although under certain conditions it is applicable to those having a mixed-mating system. Based mainly on the fact that sorghum is a mixed-mating system crop and that male sterility is available, Dogget (1972) proposed to apply recurrent selection methods to sorghum populations to identify and select promising materials. Substantial evidence exists in regard to the occurrence of male sterility in amaranth populations which could be used for recombination (intra or interpopulation crossing) in amaranth breeding(9). Studies on breeding of sorghum random mating populations show the advantages of using S1 family selection(4).

Materials and Methods

Early in 1995 one hundred and forty S0 families from Plainsman cultivar were obtained under greenhouse conditions at the University of Nebraska, Lincoln. The corresponding 140 S1 families were evaluated in the field in the summer of 1995 using a Replications-in Block Experimental Design with two replications and repeated in three locations, Scottsbluff (irrigated land), and Sidney (irrigated and non-irrigated land). Each block consisted of a set of 14 S1 families

chosen at random. The set per block remained the same in the second replication but the families per set were randomized again. The traits studied included stem color, inflorescence color, branching index, inflorescence density, plant height, seed-head length, seed yield/plant, and 1000-seed weight. Table 1 shows some simple statistical results of observations taken on 140 S0 families grown in the greenhouse. The distribution frequency of the quantitative traits suggests segregation is still occurring the cultivar Plainsman. Agronomic traits present a normal distribution with a relatively small amount of variation among S0 families. At present, results of the field evaluation are under progress. These results will provide the basis for the genetic improvement beyond the cultivar Plainsman.

Table 1. Frequency distributions and simple statistics for morphologic and agronomic traits in 140 S0 families derived from the cultivar Plainsman.

Morphologic traits					Agronomic traits			
	G	**GbP**	**Pr**		**x̄**	**Max**	**Min**	**Std. Dev.**
Stem Color	13	110	17	Plant height (cm)	125.45	171.00	85.00	±17.42
	G	**P**	**R**		**x̄**	**Max**	**Min**	**Std. Dev.**
Inflorescence color	1	14	125	Seed-head length (cm)	54.39	82.00	25.00	±11.05
	1	**3**	**6**	**9**	**x̄**	**Max**	**Min**	**Std. Dev.**
Inflorescence density	92	15	15	18	Seed-yield/plant (gr) 22.78	37.82	12.45	±4.32
	1	**3**	**6**	**9**	**x̄**	**Max**	**Min**	**Std. Dev.**
Branching index	56	37	41	6	1000 Seed weight(gr) 0.55	0.63	0.46	±0.03

Literature Cited

1. Baltensperger D.D., Weber L.E., Nelson L.A. 1992. Registration of 'Plainsman' Grain Amaranth. Crop Sci. 32:1510-1511.

2. Hauptli H. 1977. Agronomic Potential and Breeding Strategy for Grain Amaranths. In Proc. 1st Amaranth Sem., Rodale Press, Emmaus, PA, July 29.71.

3. Hauptli H. and Jain S. 1985. Gentic Variation in Outcrossing Rate and Correlated Floral Traits in a Population of Grain Amaranth (*A. cruentus*). Genetica 66: 21-27.

4. Jan-Orn J., C.O. Gardner, and W.M. Ross. 1976. Quantitative Genetic Studies of the NP3R Random-mating Grain Sorghum Population. Crop Sci. Vol. 16, 489-496.

5. Kauffman Ch. 1981. Grain Amaranth Varietal Improvement: Breeding Program. Rodale Research Center. Rodale Press, Inc.

6. Kauffman Ch. and L. Weber. 1990. Grain Amaranth. In: Advances in New Crops. Janick J. and Simons J.E. (ed.), Tember Press, Portland, OR, 127.

7. Kulakow P.A. and Hauptli H. 1994. Genetic Characterization of Grain Amaranth. In: Amaranth. Biology, Chemistry, and Technology. Paredes-Lopez O. (ed.) CRS Press, Inc. 9-22.

8. Lehman J.W., Clark R.L., and Frey K.J., 1991. Biomass Heterosis and Combining Ability in Interspecific and Intraspecific Matings of Grain Amaranths. Crop Sci. 31: 1111-1116.

9. Peters I. and Jain S. 1987. Genetics of Grain Amaranths, III. Gene-cytoplasmic male sterility. J. Hered. 78:251-256.

New Amaranth Production Manual

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A new amaranth production manual has received approval for funding as a USDA Extension publication. This would take the place of the manual, *Amaranth Grain Production Guide,* last published in 1990 by the Rodale Research Center and the Amaranth Institute.

Authors David Baltensperger and Jane Sooby of the University of Nebraska-Lincoln, would like to collect as much current information on amaranth production as possible. If you have production information, slides, or photos of amaranth or field operations, or suggestions of what you think is important for a production manual, please call Sooby at 308-254-3918. Information will be gathered throughout the growing season. The publication date is estimated to be sometime in the winter of 1997.

Proposed Outline

I. Planting
   a. timing
   b. depth
   c. equipment
   d. seeding rate
   e. fertilization requirements

II. Growing
   a. water requirements
   b. weed control
   c. insects in amaranth
   d. amaranth disease

III. Harvesting
   a. timing
   b. yields - factors that influence and how much to expect
   c. equipment
   d. seed production: standards

IV. Processing
   a. quality
   b. cleaning
   c. storing
   d. transporting

V. General Topics
   a. dryland production
   b. organic production
   c. amaranth's place in a rotation
   d. amaranth varieties

Another Amaranth Pest: Blister Beetles

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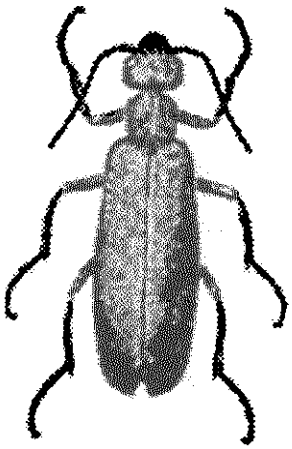
Blister beetles belong to the beetle family, Meloidae. Blister beetles get their name from the chemical, cantharidin, in their elytra. Elytra are the leathery, outer wings you see that cover the

hind wings. They commonly meet in a straight line down the back of the insect. If this beetle walks on your skin, you might develop blisters.

These beetles used to be called "old-fashioned potato bugs" because they were a common pest of potatoes before the Colorado potato beetle became so prominent. Blister beetles are fairly good sized - being from ½ to 1 inch long. They are about four times as long as they are wide (see the accompanying diagram).



Typical blister beetle

Several species of blister beetle attack potatoes, tomatoes, eggplants, sweet potatoes, beans, peas, soybeans, cowpeas, melons, pumpkins, onions, spinach, beets, carrots, peppers, Swiss chard, radishes, cabbages, corn, oats, barley, alfalfa, clover, cotton, clematis, aster, chrysanthemum, zinnia, amaranth, and several other crops and weeds. More than 310 species of these beetles are found in all parts of the U.S. and Canada. There are 3,000 species worldwide.

The larvae of these beetles are actually 'good guys'. They hatch from eggs laid in the soil and then they feed on grasshopper eggs. They are an important biological control agent for grasshoppers. The adult, however, is not a good guy. The adult feeds on the foliage and flower parts of the plants attacked. The adults normally appear about midsummer. Frequently, whole populations emerge about the same time and can do considerable plant damage before they are even noticed.

Of interest is the substance cantharidin, which is found in the beetles' body. Cantharidin is a powerful irritant and highly toxic by ingestion or absorption from skin and mucous membranes. It can cause severe gastroenteritis, nephritis, collapse and even death may occur. This substance from a European species is the "Spanish fly" of commerce. It was once thought to be an aphrodisiac and now is used to treat certain urogenital diseases. It was reported that blister beetles trapped in alfalfa hay were fed to valuable race horses in Florida. Even though the beetles were dead the horses died from cantharidin poisoning.

Much of the information for this report came from the following references. If you are interested in more facts about blister beetles or cantharidin, these references will provide you with additional details.

Arnett, R. H., Jr. 1985. Order 24: Coleoptera. *In* American Insects: A Handbook of the Insects of America North of Mexico. Van Nostrand Reinhold Co., New York. p. 356.

Metcalf, R. L. and R. A. Metcalf. 1993. Chapter 14: Insects injurious to vegetable gardens and truck crops. *In* Destructive and Useful Insects: Their Habits and Control (5th edition). McGraw-Hill, Inc., New York. p 14.45 - 14.46.

Windholz, M., S. Budavari, R. F. Blumetti, and E. S. Otterbein (eds). 1983. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. (10th edition). Merck and Co., Inc., Rahway, NJ. p. 241-242.

An Amaranth Tour of China

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In August of 1995, I was privileged to take a 10-day tour of some amaranth research projects in Hong Kong and China. The trip was supported by a grant from the government of Hong Kong to Dr. Mei Sun and Dr. Harold Corke at the University of Hong Kong.

Dr. Mei Sun and her students are using molecular markers to investigate relationships among the species of *Amaranthus*. Her husband, Dr. Harold Corke, studies biological variation in starch properties of amaranth grain. Corke and collaborators have recently published measurements of amaranth starch properties (Wu et al. 1995). I have cooperated with their research by sending amaranth seed samples from the United States National Plant Germplasm System collection, at the North Central Regional Plant Introduction Station, where I am the Amaranth Curator.

My tour started in Hong Kong. I visited research labs and met graduate students working with my hosts. We also had time to taste fresh durian fruit, and to climb "The Peak", a mountain at the center of Hong Kong Island.

In China, I traveled with Harold Corke and his graduate student, Wu Huaixiang. They, with many local cooperators had a series of replicated plantings of selected amaranth seed starch types. We visited some of the plantings. Wu coordinated all the arrangements for airline flights and lodging, so the trip was very easy for us.

In Beijing, we met with Professors Shaoxian Yue and Hongliang Sun, of the Chinese Academy of Agricultural Sciences. Both have been active with grain amaranth since the early 1980s when they brought seed samples from the Rodale Research Center in the United States to China. Those samples are the seed source for some 80,000 hectares (197,600 acres) of amaranths grown per year in China. Local grain and vegetable amaranth types have been less important in the recent amaranth expansion in China.

We visited Associate Professor Li Yunsheng, at the Chifeng Institute of Agricultural Sciences, in Chifeng, Inner Mongolia. He is experimenting with soy sauce production from black amaranth seeds. He has also developed colorful orange and two-colored forms of *Amaranthus cruentus*. After the touring his research plots, we visited the beautiful open grasslands where grazing animals are herded on open range. I was surprised to see these open prairies, which were green from late August rains. In much of China, August is a rainy month, and is followed by dry weather in the autumn.

The last research plots we toured were those of Professor Tang Defu at the Northeast University of Agriculture in Harbin. The plots were very well cultivated. We also visited the nearby town of Hailun where a natural-farming methods conference was in progress.

The main amaranth growing areas in China are near Kunming in Yunnan and Chongqing in Sichuan, but lack of time did not permit our visit to those areas. With 80,000 hectares in production the Chinese have more land in amaranth production than any other country. Their main cultivar is a selection from the Rodale Research cultivar RRC 1011, also known as R104. This is a tall *Amaranthus cruentus* developed by the RRC in 1977. In the United States, many growers prefer shorter cultivars to reduce lodging. Tall plants produce more forage which is greatly valued in China. Apparently the main Chinese use of amaranth is for high-protein pig fodder.

Harold Corke recommends selecting alternate forms from existing cultivars that have seeds with either translucent, or opaque endosperm. This would prepare for potential diverse marketing opportunities of the two endosperm types. These two forms of white seeds are mixed in most seed lots, and can be distinguished by careful observation. Harold Corke and I agreed that selecting parent seeds from mixed seed lots of the two types would be a start toward developing true breeding varieties.

Chinese development of amaranth is summerized in a 1993 book edited by Yue et al.. The following information is extracted from Yue's book. Chinese researchers are developing amaranth for: amaranthine pigment, grazing, silage, poultry feed, fish food, pollen source for honey bees, soy sauce, noodles, and other human foods. The emphasis has been on amaranth use within China, rather than for export. The agronomic research includes studies of salt tolerance, time of planting, and intercropping. Amaranth for silage is sometimes grown between rows of corn.

References:

Wu, Huaixiang, Shaoxian Yue, Hongliang Sun, and Harold Corke. 1995. Physical properties of starch from two genotypes of *Amaranthus cruentus* of agricultural significance in China. Starch 47:295-297.

Yue, S.X., H.L. Sun, F.D. Tang (eds.). 1993. The research and development of grain amaranth in China. Chinese Agricultural Science and Technology Publishing House, Beijing, China.

Copy number of C4 photosynthetic genes in Amaranthus hypochondriacus.

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Amaranth uses the C4 pathway of photosynthesis, which allows it to be very efficient in the assimilation of atmospheric CO2 into biologically useful molecules (Hatch, 1987; Wang et al., 1992; Furbank and Taylor, 1995). This pathway requires numerous specialized leaf adaptations that occur at several levels, including morphological development (development of kranz leaf anatomy), biochemical metabolism(an additional CO2 assimilation pathway), and gene expression(enhancement as well as cell type-specific localization of expression for genes encoding C4 enzymes).

The C4 pathway enzymes function together as a "CO2 pump" to concentrate CO2 in bundle sheath cells in the vicinity of ribulose 1,5-bisphosphate carboxylase (RuBPCase), thereby increasing photosynthetic efficiency and reducing metabolically wasteful photorespiration. Our laboratory is investigating the developmental and environmental signals that regulate the expression of genes encoding four principle enzymes of the amaranth C4 pathway (reviewed in Berry, 1995). These enzymes are listed below.

Ribulose 1,5-bisphosphate carboxylase (RuBPCase) is bundle sheath cell-specific in mature leaves of light-grown amaranth plants. It is located in the chloroplasts and is the principal enzyme in photosynthetic carbon fixation. RuBPCase is composed of 8 chloroplast-encoded large subunits (LSU, 55 kD) and 8 nuclear-encoded small subunits (SSU, 14 kD) (Ellis, 1981; Miziorko and Lorimer, 1983.).

NAD-dependent malic enzyme (NAD-ME) is bundle sheath cell-specific and decarboxylates malate to pyruvate in bundle sheath mitochondria, thereby releasing CO2 for re-fixation by RuBPCase. This nuclear-encoded enzyme is composed of two 65 kDa a subunits (NAD-MEL) and two 60 kDa b subunits (NAD-MES) and is located in the mitochondria (Long et al., 1993).

Phosophenolpyruvate carboxylase (PEPCase) is mesophyll cell-specific. This is the initial CO2 fixation enzyme in C4 plants, combining CO2 with phosphoenolpyruvate (PEP) to form oxaloacetate. This nuclear-encoded enzyme is composed of four identical 100 kD subunits and is located in the cytoplasm (O'Leary, 1982; Rydzik and Berry, 1996).

Pyruvate orthophosphate dikinase (PPdK) is mesophyll cell-specific and produces PEP, the initial CO2 acceptor molecule in C4 plants. This nuclear-encoded enzyme is composed of four 100 kD subunits and is located in the chloroplasts (Matsuka et al., 1988).

We have cloned and characterized genes encoding each of these C4 enzymes, and have determined the gene copy number of the nuclear-encoded genes. While we have discussed gene copy number for some of these C4 genes in previous manuscripts (Klessig and Berry, 1983; Wang et al., 1992), we show here some of the original data, summarize the results, and describe the methods used for these determinations.

Gene copy number can provide important information about the possible origins of C4 genes encoding enzymes such as PEPCase, PPdK, or NAD-ME. These enzymes have no photosynthetic function in C3 plants, but in amaranth they have acquired enhanced levels of expression and cell-specific localization patterns essential for their specialized photosynthetic roles in the C4 pathway (Wang et al., 1992; 1993a; 1993b; Boinski et al., 1993; Long et al., 1994; Ramsperger et al., 1996). In addition, this information can be very important if these genes are to be used as molecular markers for genetic analysis in amaranth.

Location for figure 1

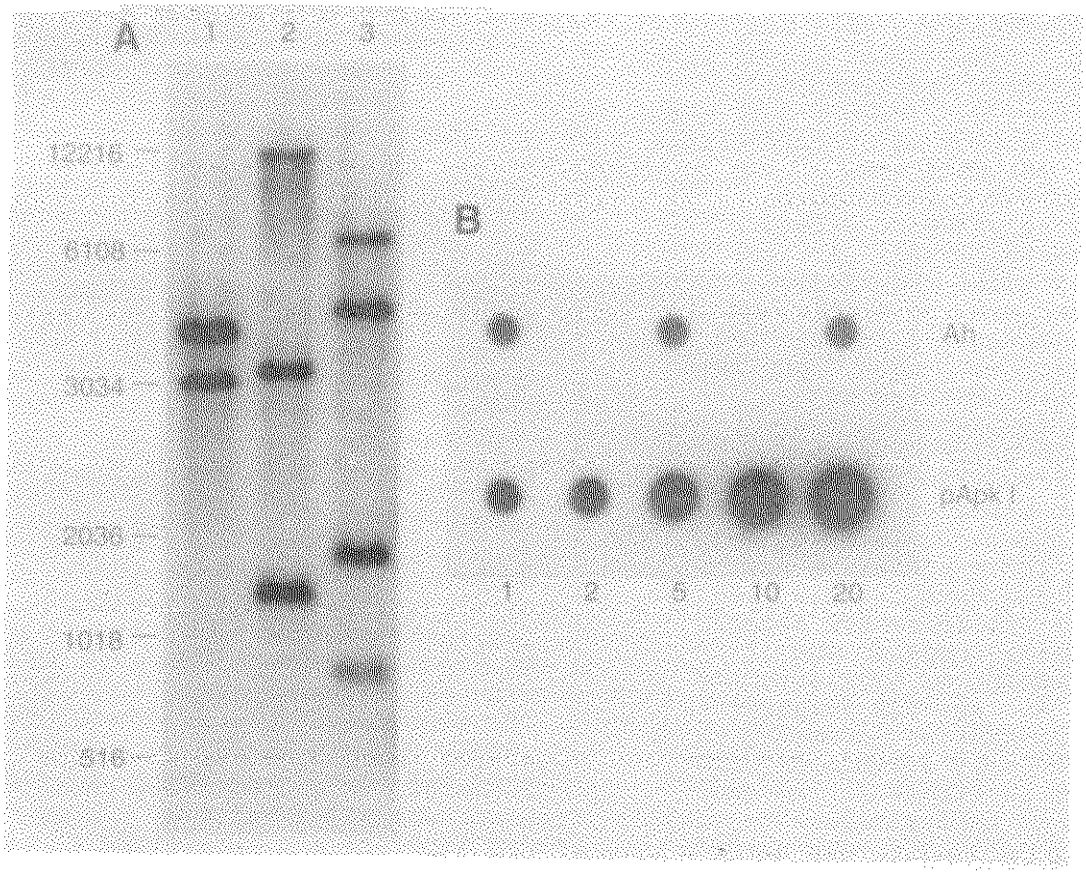

Figure 1. Determination of PPdK gene copy number. (A) A gel-purified pApk1 cDNA fragment was 32P-labeled by random primer labeling and hybridized to 10 mg of total amaranth nuclear DNA per lane, digested with EcoRI (lane 1), BamHI (lane 2), or HindIII (lane 3). (B) The PPdK probe was hybridized to a dot blot containing 10 mg of amaranth nuclear DNA(Ah, three dots in top row), and to 1, 2, 5, 10, and 20 copy amounts of PPdK cDNA insert (pApkI, bottom row). Hybridized blots were exposed to X-ray film for 1 - 2 days.

Methods

The cDNA clones used for these analyses were as follows. For RuBPCase SSU (rbcS), pAss1 (Berry et al., 1985); for PEPCase and PPdK, pApe1 and pApk1, respectively, (Wang et al., 1992); for NAD-ME a subunit, pMel1 (Long et al., 1994). Total nuclear DNA was isolated from amaranth leaves and cotyledons and southern blots or dots blots were hybridized using single copy hybridization conditions as previously described (Klessig and Berry, 1983). Gel-purified fragments of each cDNA insert were used as hybridization probes. For Southern blots, 10 mg of total amaranth nuclear DNA, digested with the appropriate restriction enzymes, were loaded into each lane of an agarose gel. Single copy or multiple copy amounts of cDNA restriction fragments for each gene were also loaded (mixed together with 5 mg of calf thymus carrier DNA). The DNAs were electrophoresed and transfered to nitrocellulose paper as described (Klessig and Berry, 1983). For dot blots, 10 mg of total restriction digested nuclear DNA, or single and multiple copy amounts of restriction digested DNA from each cDNA clone (with carrier DNA), was absorbed to nitrocellulose membrane using a dot-blot apparatus (BRL hybri-dot).

Gene copy amounts for each cDNA were calculated based on the previously determined *A. hypochondriacus* genome size of 1.65 x 109 bp (Klessig and Berry, 1983). Two methods were used for quantitation of hybridization signals. First, we visually compared and estimated the amount of hybridization to 10 mg of total nuclear DNA relative to amounts of hybridization occurring to 1, 2, 5, 10, and 20 copy amounts of each probe, as shown in Figure 1. Second, relative rates of hybridization to total nuclear DNA and to single copy amounts of each probe on a Southern blot were quantitated using a phosphorimager (Molecular Dynamics) equipped with ImageQuant version 4.2 software, as shown in Figure 2.

Results and Discussion

Examples of each method of gene copy determination are shown in Figures 1 and 2. We have found that some, but not all, of the amaranth photosynthetic enzymes are encoded by multigene families.

We have previously reported that RuBPCase SSU gene sequences in amaranth, as in most plant species, are present in 5-10 copies per genome (Klessig and Berry, 1983). The more accurate phosphorimager analysis described here indicates that there are approximately 8 copies of rbcS per genome (data not shown).

Location for figure 2

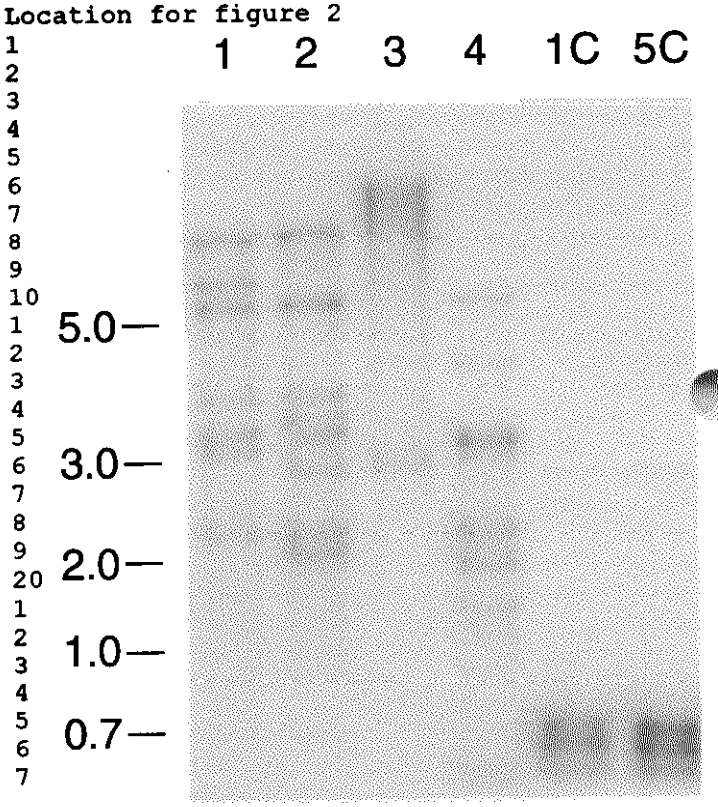

Figure 2. Determination of NAD-ME gene copy number. A gel-purified pMel1 cDNA fragment was 32P-labeled by random primer labeling and hybridized to 10 mg of total amaranth nuclear DNA per lane, digested with EcoRI (lane 1), EcoRI and BamHI (lane 2), BamHI (lane 3), and HindIII (lane 4), and to single copy (1C) and five copy (5C) amounts of pMel1 digested with XhoI and EcoRI. Hybridization signals were recorded on a phosphorimager, quantitated using ImageQuant version 4.2 software, and

printed using a dye-sublimation printer.

PEPCase genes are also very abundant in amaranth, with approximately in 10-20 copies per genome (data not shown; Wang et al., 1992). We have previously shown that RNA transcripts with homology to the amaranth PEPCase gene were present in the bundle sheath cells of morphologically differentiated leaves, even though immunolocalization with PEPCase antisera clearly demonstrated that PEPCase enzyme accumulated specifically in mesophyll cells (Wang et al., 1992). Since there are many copies of the PEPCase gene in amaranth, it is possible that individual genes are expressed differently in bundle sheath and in mesophyll cells. PEPCase transcripts in bundle sheath cells may encode a non-C4 form of PEPCase which is not recognized by our antisera, or they may represent a population of nonfunctional mRNAs with homology to the PEPCase probe.

PpdK (Figure 1) and NAD-MEL a subunit (Figure 2) gene sequences in amaranth are present at low copy number, with only one or two copies per genome. It is therefore unlikely that the original "C3-forms" of these genes were duplicated during the development of C4 capacity in amaranth. Instead, the single genes encoding each of these enzymes appear to have acquired new regulatory processes to allow for their enhanced expression and cell-specific localization in amaranth leaves and cotyledons.

Although NAD-ME a subunit gene appears to be present as a single copy, it shows a very complex restriction pattern (Figure 2). It is likely the gene encoding this C4 enzyme is very large, and we suspect that sequence analysis of an NAD-ME genomic clone will reveal the presence of multiple introns.

Molecular and structural analysis of C4 genes in amaranth will allow us to better understand the origin and complex regulation patterns of this specialized and highly efficient photosynthetic pathway. In addition, we hope that by investigating the number and organization of these and other genes in amaranth, we might aid in the development of a system of genetic analysis for this ancient and potentially agronomically important crop plant.

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References

Berry, J.O., B.J. Nikolau, J.P. Carr, and D.F. Klessig. 1985. Transcriptional and post-transcriptional regulation of ribulose 1,5-bisphosphate carboxylase gene expression in light- and dark-grown amaranth cotyledons. Mol. Cell. Biol. 5:2238-2246.

Berry, J.O. 1995. Expression of C4 photosynthesis genes in grain amaranth. Legacy, The Newsletter of the Amaranth Institute, Volume VIII No. 1:10-13.

Boinski, J.J., J.-L. Wang, P. Xu, T. Hotchkiss, and J.O. Berry. 1993. Post-Transcriptional Control of Cell Type Specific Gene Expression in Bundle Sheath and Mesophyll Chloroplasts of *Amaranthus hypochondriacus*. Plant Mol. Biol. 22:397-410.

Ellis, R.J. 1981. Chloroplast proteins: synthesis, transport and assembly. Annu. Rev. Plant Physiol. 32: 111-137.

Furbank, R.T. and W.C. Taylor. 1995. Regulation of photosynthesis in C3 and C4 plants: a molecular approach. The Plant Cell 7: 797-807.

Klessig, D.F. and J.O. Berry. 1983. Improved filter hybridization method for detection of single copy sequences in large eukaryotic genomes. Plant Mol. Biol. Rep. 1(4): 12-18.

Long, J.J., J.-L.Wang, and J.O. Berry. 1994. Cloning and Analysis of the C4 Photosynthetic NAD-dependent Malic

Enzyme of Amaranth Mitochondria. J. Biol. Chem. 269: 2827 - 2833.

Matsuka, M., Y. Ozeki, N. Yamamoto, H. Hirano, Y. Kano-Murakami, and Y. Tanaka. 1988. Primary structure of maize pyruvate, orthophosphate dikinase as deduced from cDNA sequence. J. Biol. Chem. 263: 11080-11083.

Miziorko, H. M., and G.H. Lorimer. 1983. Ribulose-1,5-bisphosphate carboxylase-oxygenase. Annu. Rev. Biochem. 52: 507-535.

O'Leary, M.H. 1982. Phosphoenolpyruvate carboxylase: an enzymologist's view. Ann. Rev. Plant Physiol. 33: 297-315.

Ramsperger, V.C., R.G. Summers, and J. O. Berry. 1996. Photosynthetic Gene Expression in Meristems and Initial Leaf Development in the C4 Dicot Amaranthus. Plant Physiol. (in press).

Rydzik, E. and J.O. Berry. 1996. The C4 Photosynthetic Phosphoenolpyruvate Carboxylase (PEPCase) from Grain Amaranth (accession number L49175) (PGR95-135). Plant Physiol. 110:713.
Wang, J.-L., Klessig, D.F., and J.O. Berry. 1992. Regulation of C4 gene expression in developing amaranth leaves. The Plant Cell 4:173-184.

Wang, J.L., J.J. Long, T. Hotchkiss, and J.O. Berry. 1993. Regulation of C4 gene expression in light- and dark-grown amaranth cotyledons. Plant Physiol. 102: 1085-1093.

Wang, J.-L., R. Turgeon, J.P. Carr, and J. O. Berry. 1993. Carbon Sink-to-Source Transition is Coordinated with Establishment of Cell-Specific Gene Expression in a C4 Plant. The Plant Cell 5:289-296.

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