

Variability in 'Plainsman' Grain Amaranth

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Grain amaranth is a pseudo-cereal that played an important role as human food in the ancient civilizations of America. Current interest in amaranth resides in the fact that it exhibits a high nutritional value, a C₄ photosynthetic pathway, a great amount of genetic diversity, and phenotypic plasticity (Downton 1972; Hauptli 1977; Jain et al. 1979; Kauffman 1981; National Research Center 1984). In 1992, a cooperative amaranth breeding program between the Rodale Research Center and the University of Nebraska resulted in the release of the cultivar 'Plainsman', an interspecific hybrid between *Amaranthus hypochondriacus* L., a gold seeded selection from Mexico, and *Amaranthus hybridus* L., a black seeded selection from Pakistan. Breeding objectives for 'Plainsman' development were early maturity, light seed color, and short plant height. Single plant selection in earlier generations (F₂ to F₅) and mass selection in advanced generations (F₆ to F₇) was used (Baltensperger et al. 1992). 'Plainsman' maturity is very early in Western Nebraska, reaching maturity 110 days after planting. Evaluation tests in Nebraska, Colorado, Missouri, Minnesota, and South Dakota indicated that 'Plainsman' was one of the most promising amaranth cultivars for the United States. In 1994, approximately 1200 ha of 'Plainsman' were grown (Baltensperger 1992; Myers 1994) but field observations indicated a great deal of variation. The pollination mechanism of amaranth is complex in nature, varying from low to high outcrossing rates and furthermore being strongly affected by the environment (Jain et al. 1982; Hauptli and Jain 1985). The complexity of its pollination mechanism along with the breeding strategy employed in its development provided some evidence to suspect that some residual genetic variability is still present in 'Plainsman'. However, the large phenotypic plasticity observed in the amaranths might be the cause of such variability. Phenotypic plasticity has been defined as the physiological and/or morphological alteration by an organism in response to environmental differences (Schiliching 1986) and plastic variance as the amount of variation due to the environment and due to the genotype by environment interaction ($\sigma_{Pt}^2 = \sigma_E^2 + \sigma_{G \times E}^2$). Plasticity has been defined as the ratio of plastic variance to total phenotypic variance (Scheiner and Goodnight 1984). Further improvement by selection within 'Plainsman' requires more knowledge about the cause of variability in seed production traits. The objectives of this study were to investigate the amount of morphological variation present in 'Plainsman', to estimate the genetic and environmental components of variance in agronomic traits in a random sample of 'Plainsman', and to predict the response to selection within 'Plainsman' under a selfing-selection scheme.

METHODOLOGY

'Plainsman' foundation seed from the Foundation Seed Division of the University of Nebraska was planted on 6 Jan 1995 in 140 pots. Sixty days after planting, plants were self pollinated by covering the panicles with pollinating bags. The panicles were hand-harvested 120 days after planting, allowed to dry at 32°C for three days, threshed, and cleaned by hand. Morphological traits measured included color of the main stem during the seed-filling period, inflorescence color during seed-filling period, branching index based on presence or absence of primary, secondary, or tertiary branches developed from the main stem, and inflorescence compactness just before harvesting.

In 1995, the 140 self pollinated families were planted at the High Plains Agricultural Laboratory near Sidney, Nebraska, and the Panhandle Research and Extension Center at Scottsbluff. At Sidney, a non-irrigated trial was planted on 16 June in a Duroc loam (Pachic Haplustol) soil and an irrigated trial on 17 June in a Keith silt loam (Aridic Arguistoll) soil. The irrigated trial at Scottsbluff was planted on 18 July in an Otero loam (Fluventic Haplustol) soil using a Wintersteiger air seeder. Transplanting and hand thinning was necessary to achieve uniform populations at each experiment. Distance between plants was 0.15 m for the Sidney non-irrigated plots (51,000 plants/ha), 0.3 m for the Sidney irrigated plot (25,500 plants/ha), and 0.3 m at Scottsbluff (25,500 plants/ha). For all experiments, nitrogen was applied before planting at a rate of 100 kg/

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ha and 85 kg/ha was applied at panicle emergence at Scottsbluff. The plots were hand-weeded.

A replications-in blocks experimental design with two replications at each location was used. At each location, the experiment included ten blocks each containing a set of 14 randomly chosen families. The sets were kept together between replications and locations with a different randomization of families within each set. Single-row plots 5 m long with 0.76 m row spacing were used. Morphological traits measured were the same as those measured in the greenhouse. In addition, days to panicle emergence, flowering, and maturity, plant height, stem diameter, panicle length, grain yield per plant (the last four averaged over 5 plants randomly chosen per plot), and 1000-seed weight were determined. Morphologic data was analyzed by using a Chi-square goodness-of fit test between observations collected in the greenhouse (G_0 generation) and those collected in the field (G_1 generation). The least square mean method was used for the analysis of the agronomic data using Statistical Analysis System (SAS Institute, Inc. 1989). The analysis of data combined over locations was performed using a random linear model where the hypothesis test for genetic differences among families within blocks was $H: \sigma_f^2 = 0$. Components of variance were estimated by equating the observed mean squares to the expected mean squares from the combined analysis of variance. The standard errors associated with these estimates were calculated as described by Anderson and Bancroft (1952). Broad sense heritability (H) was calculated as the ratio of the genetic variance to the phenotypic variance, $H = \sigma_f^2 / \sigma_p^2$ where σ_f^2 = genetic variance among families, and σ_p^2 = phenotypic variance. Plasticity (P_t) was calculated as the ratio of the plastic variance to the sum of the variance components, $P_t = \sigma_{pt}^2 / \sigma_{pt}^2 + \sigma_f^2 + \sigma_e^2$ where σ_{pt}^2 = plastic variance, σ_f^2 = genetic variance among families, and σ_e^2 = residual variance. Approximate standard errors estimated for H and P_t were computed as $SE(H) = SE(\sigma_f^2) / \sigma_p^2$ and $SE(P_t) = SE(\sigma_{pt}^2) / \sigma_{pt}^2 + \sigma_f^2 + \sigma_e^2$. The predicted genetic gain from selection Gp was calculated as $Gp = k \sigma_f^2 / \sigma_p$ where k = standardized selection differential, σ_f^2 = genetic variance among families, σ_p = estimated phenotypic standard deviation.

EXPERIMENTAL RESULTS

Frequency distributions of morphological traits of selfed plants grown in the greenhouse (G_0) and of resulting progeny grown in the field in Scottsbluff (G_1) had three or four classes for all traits in both G_0 and G_1

Table 1. Chi-square test of differences of classes within four morphological traits in two generations of 140 selfed families from the cultivar ‘Plainsman’.

Trait	Class ^z	Generation ^y		χ^2
		G_0	G_1	
Branching index	No branches	56	49	21**
	Few branches at the bottom	37	26	
	Few branches at the top	41	39	
	Branches all along the stem	6	18	
Stem color	Green	13	13	18**
	Pink base	110	119	
	Red or darker base	17	0	
Inflorescence color	Green	1	2	13**
	Dark amaranthine	125	129	
	Light amaranthine	14	1	
Inflorescence density	Lax	92	51	127**
	Near lax	15	47	
	Near dense	15	32	
	Dense	18	2	

^z Classes within traits were established following Brenner (1994).

^y G_0 based on 140 individuals grown in the greenhouse, G_1 , Based on 132 individuals grown in the field (Scottsbluff).

** significant at 1% level.

generations, with one class being larger than the others (Table 1). No branches was the most frequent class for branching index with 39% of the total across generations. Pink-base stem color was present in 85%, while dark amaranthine inflorescence color was present in 93% of the plants. Lax density was the most prevalent class for inflorescence density with 52% of the plants. A Chi-square test of goodness-of fit to a $G_0:G_1$ ratio was highly significant for all the characteristics studied (Table 1), indicating that expression of these traits was greatly affected by the environment. It is evident that ‘Plainsman’ is characterized by a large amount of morphological variation, which is higher for branching index and inflorescence density than for stem and inflorescence color.

Panicle emergence and flowering days, and days to maturity were uniform among families (data not shown). Substantial variation over locations for all the agronomic traits was found (Table 2). Weather conditions contributed to this variation during the growing season. In late June, a hailstorm occurred at the Sidney non-irrigated location decreasing the plant population at this experiment to 10,000 plants/ha.

Analysis of variance showed variation among locations for all of the traits ($p < 0.01$), suggesting that the growing conditions at each location were different (Table 3). Family differences were found for plant height and stem diameter ($p < 0.01$) and 1000-seed weight ($p < 0.05$). Family by location interactions were found for plant height ($p < 0.01$) and stem diameter ($p < 0.05$), suggesting these traits were more susceptible to environmental changes. It appears that ‘Plainsman’ possesses a genetic structure composed mainly by homozygous-heterogeneous lines. The analysis of variance components indicated that the genetic components were small compared to the other variance estimates (Table 4), which reinforces the idea about the homozygous structure

Table 2. Mean, maximum, minimum values and their corresponding standard errors (se) of five agronomic traits measured in 140 selfed families from the cultivar ‘Plainsman’.

Parameter	Plant height (cm)	Stem diameter (cm)	Panicle length (cm)	Grain yield per plant (g)	1000-seed weight (g)
Sidney (non irrigated)					
Max.	152	3.0	66	15.7	1.1360
Min.	69	1.0	25	0.7	0.5480
Mean	118	1.8	46	9.1	0.6642
SE	13.4	0.35	10.05	4.45	0.0324
Sidney (irrigated)					
Max.	174	6.8	75	--	--
Min.	104	1.6	29	--	--
Mean	155	2.3	50	--	--
SE	8.52	0.3	6.81	--	--
Scottsbluff (irrigated)					
Max.	228	3.8	93	52.3	1.0797
Min.	120	1.6	34	11.5	0.6130
Mean	170	2.6	59	26.8	0.7133
SE	10.54	0.25	6.08	7.90	0.0364
Combined over locations ^z					
Max.	228	6.8	93	52.3	1.1360
Min.	69	1.0	25	0.7	0.5480
Mean	147	2.2	51	18.3	0.6900
SE	11.02	0.32	7.94	6.57	0.0346

^zPlant height, stem diameter and panicle length averaged over the three locations. Grain yield per plant and 1000-seed weight averaged over locations Sidney (non irrigated) and Scottsbluff.

of 'Plainsman'. The family by location (genotype by environment) interaction variance component was greater than the genetic variance component, suggesting that most of the variability in 'Plainsman' was plastic. A small negative estimate for grain yield per plant was obtained, but since it is small, it can be ignored. The phenotypic variance was greater than the genetic and the family by location components of variance for all the traits. Residual error variance estimates showed larger values than the rest of the components, except for plastic variance. The estimated error variances among locations were relatively uniform for 1000-seed weight, stem diameter, and plant height but not for panicle length and grain yield per plant where a 1:3 error variance

Table 3. Combined analysis of variance of five agronomic traits measured in 140 selfed families from the cultivar 'Plainsman' tested at Sidney (irrigated and non irrigated conditions) and Scottsbluff, Nebraska, in 1995.

Source	df	Mean squares					
		Plant height (cm)	Stem diameter (cm)	Panicle length (cm)	df	Grain yield/plant (g)	1000-seed weight (g)
Locations (L)	2	161592.4**	36.34**	9201.7**	1	29171.3**	0.2321**
Blocks (B)	9	552.6**	0.58**	136.7*	9	160.2**	0.0025*
B × L	18	707.3**	0.26**	242.9**	9	65.5 NS	0.0006 NS
Replications/B/L	30	110.9 NS	0.17*	59.6 NS	20	63.7 NS	0.0023 NS
Families/B	130	227.5**	0.16**	72.4 NS	129	43.7 NS	0.0016*
F/B × L	251	169.5**	0.13*	71.4 NS	115	37.5 NS	0.0014 NS
Error	326	121.5	0.10	63	170	43.1	0.0012
C.V. (%)		7	15	15		36	5

*, ** significant at 5% and 1% levels.

Table 4. Estimates of components of variance and their associated standard errors (SE) of five agronomic traits in 140 selfed families from the cultivar 'Plainsman'.

Variance component ^z	Plant height (cm)	Stem diameter (cm)	Panicle length (cm)	Grain yield/plant (g)	1000-seed weight (g)
Family (σ_f^2)	11.04 ± 6.05	0.006 ± 0.004	0.19 ± 2.08	1.99 ± 2.17	0.0000 ± 0.0001
Family by location ($\sigma_{f \times l}^2$)	26.21 ± 9.74	0.015 ± 0.008	4.57 ± 4.39	-3.47 ± 3.88	0.0001 ± 0.0000
Phenotypic (σ_p^2)	43.31 ± 5.33	0.031 ± 0.004	13.78 ± 1.69	14.03 ± 1.61	0.0005 ± 0.0001
Plastic (σ_{pt}^2)	709.62 ± 485.49	0.168 ± 0.109	42.63 ± 27.98	157.06 ± 127.60	0.0013 ± 0.0010
Residual (σ_e^2)	121.54 ± 9.49	0.108 ± 0.008	63.05 ± 4.92	43.15 ± 4.65	0.0012 ± 0.0000

^zPlant height, stem diameter, and seed-head length estimates based on a combined analysis over three locations: Sidney (irrigated and non-irrigated) and Scottsbluff. Grain yield per plant and 1000-seed weight estimates based on a combined analysis over two locations: Sidney (non-irrigated) and Scottsbluff.

Table 5. Broad sense heritability (H) and plasticity (Pt) estimates and their associated standard errors.

Parameter	Plant height (cm)	Stem diam. (cm)	Panicle length (cm)	Grain yield/plant (g)	1000-seed wt. (g)
Broad sense heritability (H)	0.25±0.14	0.19±0.13	0.01±0.15	0.14±0.15	0.00±0.20
Plasticity (P _t)	0.84±0.58	0.60±0.39	0.40±0.26	0.77±0.63	0.52±0.40

Table 6. Predicted gain (G_p) from selection per year using three different standardized selection differentials (k) in five agronomic traits from the cultivar 'Plainsman'. Numbers in parenthesis correspond to genetic gain expressed as a percentage of the mean.

Standardized selection differential (k)	Expected genetic gain				
	Plant height (cm)	Stem diameter (cm)	Panicle length (cm)	Grain yield/plant (g)	1000-seed weight (g)
2.64 (1%)	4.43 (3)	0.09 (4)	0.13 (0)	1.41 (8)	0.00 (0)
2.06 (5%)	3.46 (2)	0.07 (3)	0.10 (0)	1.10 (6)	0.00 (0)
1.75 (10%)	2.94 (2)	0.06 (3)	0.09 (0)	0.93 (5)	0.00 (0)

proportion among locations was observed (data not shown). Plastic variance components as defined by Scheiner and Goodnight (1984) had the greatest value for all five agronomic characters, and plastic variance was at least 30 fold greater than the genetic variance. It is evident that separation of genetic variation from phenotypic variation is not easy in amaranth cultivars (Kauffman 1981).

Estimates of broad sense heritability showed the largest heritability for plant height, followed by stem diameter, grain yield per plant, and panicle length. A zero heritability was observed for 1000-seed weight (Table 5). These estimates do not agree with those reported in the literature. Espitia (1994), in a population of amaranth races, found very high heritability of 0.92 for plant height and a moderately high heritability of 0.43 for grain yield. Joshi (1986), studying Indian landraces of amaranth, found high heritability of 0.77 for 1000-seed weight, 0.63 for inflorescence length, and 0.61 for plant height. Since the heritability estimates were small for the five agronomic characteristics studied, it is concluded that improvement for these traits through selection would be limited within this cultivar. Estimates of plasticity were high for all of the agronomic traits studied, indicating that they were greatly affected by the environment.

Estimates of the predicted gain from selection per year using three standardized selection differentials corresponding to a selection of the upper 10%, 5%, and 1% families based on estimates over locations show a relatively high genetic gain for plant height, a low genetic gain for yield per plant and stem diameter, and no genetic gain for 1000-seed weight (Table 6).

CONCLUSION

The breeding biology of amaranth is complex in nature being strongly affected by the environment (Jain et al. 1982; Hauptli and Jain 1985). Based on theoretical considerations (Allard 1960; Simmonds 1979; Fehr 1987) the breeding method employed in the development of 'Plainsman' seems appropriate, although self-pollination was assumed to be the prevalent reproductive system in the developing populations (Weber and Kauffman 1990; Kauffman 1981; Schulz-Schaeffer et al. 1991). Under a single plant selection scheme, it is generally accepted that at the F₅ generation near homozygosity is reached, so preliminary yield trials may begin at F₆ generation (Allard 1960; Simmonds 1979). However, some residual genetic variability is retained, and it might be present indefinitely. Evidence of this has been found in cotton (*Gossypium hirsutum*) and sorghum (*Sorghum* spp.). Homologous pairing, new mutations, and recombination of linkage blocks promoted by homozygosity have been suggested as possible causes (Simmonds 1979). Amaranths in general are considered to have an intrinsic ability to attenuate the effects of strong environmental variations (Kauffman 1981).

It has been hypothesized that plasticity and heterozygosity act in an opposite way. According to one hypothesis, phenotypic plasticity should increase as heterozygosity decreases due to the increase in developmental instability caused by deleterious homozygous recessive genes. Another hypothesis considers plasticity and heterozygosity as two antagonistic conditions in the sense that they represent alternative methods to deal with environmental heterogeneity. Thus, a population in which a consistent plastic response is observed has no need for genetic variation, and vice versa (Schlichting 1986). Yet under another hypothesis, plasticity and heterozygosity might well be expressed together so that a population could respond to an extremely variable environment by becoming both more plastic and more genetically variable (Scheiner and Goodnight 1984). The study showed the large extent to which environmental conditions affect the expression of morphological and agronomic traits in a population exhibiting a small degree of genetic variability, and that genetic improvement through a selfing–selection scheme would be limited.

REFERENCES

- Allard, R.W. 1960. Principles of plant breeding. Wiley, New York. p. 115–125.
- Anderson, R.L. and T.A. Bancroft. 1952. Statistical theory in research. McGraw-Hill, New York. p. 313–335.
- Baltensperger, D.D., L.E. Weber, and L.A. Nelson. 1992. Registration of ‘Plainsman’ grain amaranth. *Crop Sci.* 32: 1510–1511.
- Brenner, D. 1994. Characterizing system for *Amaranthus* germplasm. NCRPIS. USDA/ARS. Iowa State Univ, Ames. p. 2–7.
- Dowton, W.J.S. 1972. *Amaranthus edulis*: A high lysine grain amaranth. *World Crops J.* 20. p. 25.
- Espitia-Rangel, E. 1994. Breeding of grain amaranth. p. 23–38. In: O. Paredes-Lopez (ed.), *Amaranth: Biology, chemistry, and technology*. CRC Press, Boca Raton, FL.
- Fehr, W. 1987. Principles of cultivar development: Theory and technique. Vol. I. McGraw-Hill, New York. p. 332–337.
- Hauptli, H. 1977. Agronomic potential and breeding strategy for grain amaranths. p. 71–78. In: Proc. 1st. Amaranth Sem. Rodale Press, Emmaus, PA.
- Hauptli, H. and S. Jain. 1985. Genetic variation in outcrossing rate and correlated floral traits in a population of grain amaranth (*Amaranthus cruentus* L.). *Genetica* 66:21–27.
- Jain, S.K., K.R. Vaidya, and B.D. Joshi. 1979. Collection and evaluation of Indian grain amaranths. p. 123–128. In: Proc. 2nd. Amaranth Conf. Rodale Press, Emmaus, PA.
- Jain, S.K., H. Hauptli, and K.R. Vaidya. 1982. Outcrossing rate in grain amaranths. *J. Hered.* 73: 71–72.
- Joshi, B.D. 1986. Genetic variability in grain amaranth. *Indian J. Agr. Sci.* 56:574–576.
- Kauffman, C. 1981. Grain amaranth varietal improvement: Breeding program. OGFRC. Rodale Press, Emmaus PA.
- Myers, R.L. 1994. Regional amaranth variety test. *Legacy* 7(1). The Amaranth Institute, Briceyn, MN. p. 5–8.
- National Research Council. NRC. 1984. *Amaranth: Modern prospects for an ancient crop*. National Academy Press, Washington, DC.
- SAS Institute Inc., SAS/STAT. 1989. User’s guide version 6, 4th. ed. Vol. 2. SAS Institute Inc., Cary, NC.
- Scheiner, S.M. and C.J. Goodnight. 1984. The comparison of phenotypic plasticity and genetic variation in populations of the grass *Danthonia spicata*. *Evolution* 38:845–855.
- Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. *Annu. Rev. Ecol. Syst.* 17:667–693.
- Schulz-Schaefer, J., D.E. Baldrige, H.F. Bowman, G.F. Salknecht, and R.A. Larson. 1991. Registration of ‘Amont’ grain amaranth. *Crop. Sci.* 31:482–483.
- Simmonds, N.W. 1979. Principles of crop improvement. Longman, London.
- Weber, L.E. and C. Kauffman. 1990. Plant breeding and seed production. p. 115. In: *Amaranth: Perspectives on production, processing, and marketing*. Minnesota Ext. Service, St. Paul.