Indian J. Genet., **65**(2): 99-102 (2005)

Genetic divergence and interrelationship analysis in grain amaranth (*Amaranthus hypochondriacus*) germplasm

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(Received: August 2004; Revised: March 2005; Accepted: May 2005)

Abstract

One hundred accessions (50 from India and 50 from exotic sources) of grain amaranth (*Amaranthus hypochondriacus*) germplasm were grown in complete randomised block design. Data were recorded on six quantitative and two qualitative characters. Wide range of means was observed for all the characters. All the genotypes irrespective of their place of collection were grouped into 10 different clusters. Clusters I, VII, VIII, IX and X had high genetic distance with all other clusters. Correlation and path analysis revealed the significance of inflorescence; length, no. of leaves and plant height for selecting better yielding genotypes.

Key words: Grain amaranth, protein, oil, genetic divergence, correlation, path analysis

Introduction

The genus *Amaranthus* includes a complex array of wild, weedy, and domesticated species and consists of approximately 60 species [1]. In India, the principal species giown for grain purpose are *Amaranthus hypochondriacus*, *A. cruentus*, *A. caudatus* and species grown for vegetable purpose are *A. dubius, A. lividus, A. spinosus*, and occasionally *A. viridis* and *A. hybridus*. *Amaranthus hypochondriacus* has characteristics of both *A. cruentus* and the wild sp. *A. Powelli* and may be hybrid of the two. Amaranth apart from its use as grain and green, has several other nutritional and industrial uses, such as conventional foods, potential forage, silage, green manure, and animal feed. Some desirable attributes are its high level of protein (12-19%), high level of lysine (generally low in the cereals), complementary amino acid profile, unique micro crystalline starch granules (1-3 micron diameter), and oil component (squalene), which is equivalent to whale or shark liver oil. Being a C4 plant it has very high production potential. Amaranth is one of a few dicots in which the first product of photosynthesis is a four-carbon compound. Despite all that, amaranth is still categorised as an under-utilised crop. The main attributes responsible for its limited domestication are seed-shattering before harvest, its tiny seeds, lack of public awareness on its food value, lower demand and production than conventional cereals, lack of production research and development programmes, difficulty in maintaining pure stands due to cross pollination by black seeded wild species and lack of specialised breeding projects. The current concentration on one source of germplasm has the potential to provide a setback to the development of the crop. Additional resources are needed to initiate and expand applied breeding progress to meet the needs of anticipated increase in grain amaranth production. The future of amaranth depends on the careful screening of the genotypes from the germplasm collection and we have to explore for the unusual sources of germplasm to find outstanding attributes. Brenner *et al.* [2] in their review article on genetic resources and breeding of *Amaranthus* have mentioned that only modest amount of research and plant breeding has been done in this plant. They also emphasised that in diverse collections of germplasm available in many gene banks, rapid progress could be made with little investment in screening and breeding projects.

In view of the above, present study on the evaluation of germplasn of *Amaranthus hypochondriacus*, which is the main grain species in India was taken up. Conventional breeding programme also necessitates the selection of genetically divergent parents from the available germplasm and many times breeders greatly depend upon the nature and magnitude of genetic variance of the characters under consideration and interrelationship among them before taking up hybridisation programme.

Materials and methods

The material for the present study comprised 100 accessions of amaranth germplasm collected from different parts of India (50 accessions) and donated by countries like USA (29), Nepal (4), France (4), Germany (4), Poland (3), China (2), Taiwan (2) and Russia (2). The genotypes were grown in complete randomized block design replicated thrice from June to October (3 crop seasons) 1997, 1998 and 1999. In order to maintain the genetic purity and to avoid cross- pollination, selected plants were baggged till pollination was over. A random sample of 5 of these plants from each accession was used to compute means on 8 characters *viz.*, protein content (determined by the conventional Kjeldal method), oil content (determined by non-destructive method using New Port NML), plant height, inflorescence length, number of leaves, leaf length, leaf width, and seed yield/plant.

The genetic divergence among genotypes was computed by means of Mahalanobis' D^2 technique [3, 4]. The difference between the varieties for the set of characters taken was tested according to the procedure of Wilks [5]. The genotypes were grouped into clusters following Tocher's method as described by Rao [6]. The relative contribution of characters towards divergence was estimated using canonical analysis. Significance of differences among genotypes was tested using Wilks' criterion. Genetic variability parameters, correlation, and path analysis were analysed as proposed by Johnson *et al.* [7] and Dewey and Lu [8].

Results and discussion

Means and genetic variance: The analysis of variance showed a wide range of variation and significant differences for all the characters (Table 1). The coefficients of variation at phenotypic (PCV) and genotypic (GCV) level were high for seed yield, no. of leaves, leaf length and inflorescence length and medium to low for other characters. The differences in the magnitude of PCV and GCV were more for quantitative characters indicating more influence of environment in their governance, whereas it was less for qualitative characters *viz.*, protein and oil content indicating consistency in the expression of these characters irrespective of growing conditions. Guillen *et al.* [9] suggested phenotypic plasticity as the main source of variation and it had the greatest value for all the characters being at least 30-fold greater than the genetic variance estimates. Heritability was high for protein content, oil content and seed yield, moderate for inflorescence length, leaf length, and low for plant height, leaf width and no. of leaves. Moderate to low estimates of broad sense heritability indicates that improvement through selection would be limited. Genetic advance expressed as percentage of mean was high for seed yield, leaf length, inflorescence length, no. of leaves and moderate to low for other characters. Relationship of heritability and genetic advance also give an idea about the type of gene action. It was found that all the characters were under the influence of both additive and non additive gene action which suggests that simple selection methods alone will not be effective hence hybridisation followed by selection would be a better choice for amaranth improvement. Other workers have also reported the role of additive and non-additive gene action and emphasise recombinant breeding for grain amaranth improvement.

Genetic divergence: Using "V" statistics, the analysis of dispersion for the test of significance of difference in the mean values based on Wilks' criterion revealed significant differences among the genotypes for the aggregate 8 characters (X^2 = 1331.84 at 792 d.f.). On the basis of relative magnitude of D^2 values, the 100 genotypes were grouped into 10 clusters each having 35, 27, 12, 8, 6, 4, 3, 2, 2 and 1 genotypes, respectively (Table 2). The composition of clusters revealed that genotypes of a cluster originate from a wide range of eco-geographical areas, thereby suggested that genetic differences and similarities among the genotypes were irrespective of the areas. This allows us to select parents for hybridisation on the basis of genetic diversity and not merely on the basis of eco-geographical isolation.

Cluster grouping: The cluster distance was ranging from 0.00-11.24 within clusters and 15.94-104.44 between clusters. This indicated that the clusters were homogenous within themselves and heterogeneous between themselves. The highest inter-cluster genetic distance was found between cluster V & VIII (104.44) closely followed by VII and X (101.27), VI and VII (100.89), IV & VIII (97.53) and I & VIII (89.06). The distance was lowest between clusters V and VI (15.94) followed by III and X (19.31), IV & VI (20.73), I & VII

Table 1. Parameters of genetic variability

Characters	Range		Mean	Heritability	Genetic advance	CV (P)	CV(G)
	Mini.	Maxi.					
Protein content (%)	11.15	17.31	13.71	80.55	30.86	18.65	16.73
Oil content (%)	9.65	12.71	11.33	75.93	23.60	15.16	13.20
Plant height (cm)	58.20	289.10	157.02	50.55	31.63	30.38	21.60
Inflorescence length (cm)	10.60	110.45	50.68	69.00	57.69	35.75	29.68
No. of leaves	14.00	136.25	56.99	51.29	41.95	39.71	28.44
Leaf length (cm)	5.90	90.50	19.17	62.48	53.24	41.42	32.74
Leaf width (cm)	3.70	19.20	11.13	48.49	26.83	26.95	18.78
Seed yield/ plant (g)	10.50	99.05	44.30	75.65	70.37	45.17	39.29

Table 2. Grouping of genotypes into different clusters

Clusters	Genotypes	Source
I	IC 16636, IC 35598, IC 35602, IC 35611, IC 35612, IC 38269, IC 38477, IC 38527, IC 42318, IC 47437, IC 107098, IC 107536, IC 42258, NIC 22535, NIC 22539, NIC 22541, EC 18874, EC 169648, EC 198135, EC 272969, EC 289386, EC 289406, EC 333747, EC 341797, EC 321556, EC 321560, EC 321561, EC 321567, EC 322032, EC 358458, EC 359410, EC 359411, EC 359413, EC 359419, EC 359421	Shimla, Kinnaur, Kullu, Maharasthra, Pauri, Almora, Pithoragarh, Nepal, USA, China, USSR, France, Poland
II	IC 35414, IC 35531, IC 35576, IC 35595, IC 35600, IC 35619, IC 35634, IC 35635, IC 38184, IC 42315, IC 42316, IC 94086, IC 107194, NIC 22543, NIC 22561, EC 153062, EC 198126, EC 321557, EC 321559, EC 328889, EC 354979, EC 355797, EC 359437, EC 359441, EC 359442, EC 359443, EC 377614	Kullu, Shimla, Kinnaur, Maharashtra, Almora, Garhwal, USA, Germany, China, Nepal, France
III	IC 17926, IC 35415, IC 35586, EC 120051, EC 157315, EC 169611, EC 169792, EC 198125, EC 328878, EC 321563, EC 359430, EC 333745	Taiwan, Poland, Germany, USA, Maharasthra, Kinnaur, Almora
IV	IC 35665, IC 42308, NIC 22536, EC 321560, EC 359417, IC 42289, IC 35599, NIC 22544	Maharasthra, Kullu, USA
V	IC 42302, IC 42325, NIC 22562, EC 289389, EC 289412, EC 170317	USSR, Germany, Kinnaur, Alrmora
VI	IC 35523, IC 42317, EC 329696, EC 333744	Germany, USSR, Lahaul & Spiti, Maharasthra
VII	IC 32533, EC 169624, IC 42319	Maharasthra, USA, Pithoragarh
VIII	IC 35408, NIC 22554	Maharasthra, Kinnaur
IX	NIC 22556, EC 351944	Kinnaur, Taiwan
X	EC 321558	USA

(21.66) and I & VII (22.76). Cluster VIII showed maximum genetic distance and cluster VI showed maximum closeness to all other clusters. The genotypes were also grouped (arbitrary) on the basis of seed colour and inflorescence length although these may not have much direct relevance for breeding but it was observed that of the 100 germplasm lines 51 lines had white seeds, 27 had creamish sesds, and 12 had golden seeds. The inflorescence of the genotypes ranged from green (41), yellowish-green (19), light yellow (23) and red shades (17). This grouping was done just to find out whether arbitrary classification has any correlation with cluster grouping done on the basis of genetic distance. But no relationship was found in both the classifications. It also highlighted the importance of genetic diversity analysis for better breeding gain.

Cluster means: The diversity in the present materials was also supported by the appreciable amount of variation among cluster means for different characters. Cluster V showed highest mean for protein content (16.41%), cluster II for oil content (12.51%), cluster VIII for plant height (207.85 cm) and no. of leaves (119.15), cluster IX for inflorescence length (62.28 cm) and seed yield/plant (86.53 g) and cluster III for leaf length (25.72 cm) and leaf width (12.87 cm). The canonical analysis showed that of the total variation, the first three canonical roots accounted for 82.41%. The first root accounted for 38.39% of the variation, followed by 22.47% for the second and 11.55% for the third. The contribution of different characters towards divergence was high by no. of leaves, inflorescence length, leaf width, seed yield and protein content as was supported by other workers [10].

Interrelationship analysis: Seed yield showed significant positive correlation with plant height, inflorescence length, no. of leaves and leaf width, significant negative association with oil content and no association with protein content and leaf length (Table 3). Protein content showed significant negative association with plant height, leaf length and leaf width. Inflorescence length showed positive association with oil content, plant height, no. of leaves, and leaf width. Correlations among different characters can be used to select a desired plant type. However, the values we

Table 3. Correlation coefficients at phenotypic (P) and genotypic (G) levels

Characters	2	3	4	5	6	7	8
1 P	0.12	–0.37*	–0.06	–0.08	–0.24*	–0.28*	0.09
1 G	–0.18	–0.50*	–0.07	–0.15	–0.31*	–0.45*	–0.12
2 P		–0.21*	0.27*	–0.25*	–0.04	–0.11	–0.44*
2 G		–0.65*	0.46*	–0.34*	0.02	–0.22*	–0.50*
3 P			0.32*	0.37*	0.32*	0.27*	0.23*
3 G			0.57*	0.50*	0.80*	0.34*	0.41*
4 P				0.47*	0.15	0.41*	0.25*
4 G				0.84*	0.04	0.47*	0.46*
5 P					–0.30*	–0.33*	0.42*
5 G					–0.49*	–0.80*	0.64*
6 P						0.05	0.13
6 G						0.24*	0.06
7 P							0.25*
7 G							0.54*

Significant at p = 0.05

get from the correlation analysis sometimes do not reflect the results true to type and selection based on these values give poor or no response to selection. Because a character showing positive correlation may not have direct effect on seed yield but may contribute to yield via other characters. In the present material, direct positive effects of inflorescence length, no. of leaves and leaf length and negative direct effects of oil content, plant height and leaf width were observed on seed yield (Table 4). These direct effects reflect the impact of these characters on seed yield as some of the characters having negative direct effect have shown significantly positive correlation with seed yield. It also indicated that many times positive correlation between two characters could be a result of high indirect effects via other characters. For instance, positive correlation of seed yield with plant height was mainly contributed via oil content, no. of leaves and leaf length and of leaf width via leaf length and no. of leaves. On the other hand when we look at various correlations the protein content showed no correlation with seed yield but showed negative direct effect at genotypic level. In this case direct effect might have nullified by the positive effects via plant height, seed yield and inflorescence length.

Table 4. Path analysis at phenotypic (P) and genotypic (G) level taking yield as dependent variable (direct effects in bold)

Characters		1	2	3	4	5	6	7
1	P	**0.07**	–0.21	0.29	0.17	–0.03	–0.22	0.22
1	G	**–0.22**	0.33	0.40	0.26	–0.11	–0.36	0.29
2	P	0.11	**–0.16**	0.23	–0.14	–0.16	–0.04	0.11
2	G	0.19	**–0.51**	0.42	–0.18	–0.26	0.14	0.28
3	P	–0.09	0.21	**–0.42**	–0.25	0.49	0.22	–0.13
3	G	0.11	0.34	**–0.80**	–0.35	0.58	0.35	–0.22
4	P	–0.12	–0.32	–0.11	**0.34**	0.63	–0.11	0.14
4	G	0.17	–0.38	–0.25	**0.68**	0.74	–0.21	–0.27
5	P	0.08	0.12	–0.30	–0.14	**0.49**	0.21	–0.44
5	G	0.30	0.22	–0.61	–0.23	**0.76**	0.32	–0.69
6	P	0.16	–0.11	–0.59	0.16	0.18	**0.08**	0.22
6	G	0.23	–0.18	–0.74	0.21	0.20	**0.12**	0.31
7	P	–0.01	–0.15	–0.15	0.04	0.33	0.29	**–0.11**
7	G	0.05	–0.25	–0.27	0.07	0.61	0.44	**–0.36**

Residual effects (P) = 0.16 Residual effects (G) = 0.21

The study concluded that genetic diversity is more important for selecting parents for hybridization than eco-geographical isolation. Genotypes from the clusters I, VII, VIII, IX. and X may yield better recombinants if chosen for breeding programs. Beside seed yield, Inflorescence length, no. of leaves and plant height are found important characters for selecting better yielding genotypes. Genotypes rich in protein content (EC 289389, EC 289412, EC 170317, EC 322032, NIC 22541, 1C 42302, 1C 16636 and 1C 17926) and oil content (1C 42316, 1C 35415, NIC 22561, NIC 22541, EC 321558, EC 328889 and EC 359442) can be selected for quality breeding programs as both the characters have shown high heritability. In general the amaranth grain contain 6-10% oil content, which is mostly found with in germ, but in the material under study it was higher and ranging from 9.65-12.71. Looking at the meager number of varieties released so far not only in India but also across the globe, careful recombinant selection of genotypes from the diverse gene pool will be of great help to amaranth breeding projects and production.

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