GENETIC STUDIES IN HEAVY METAL TOLERANT PLANTS II. ZINC TOLERANCE IN *AGROSTIS TENUIS*

D. W. GARTSIDE* and T. McNEILLY†

Department of Botany, University of Liverpool, P.O. Box 147, L69 3BX

Received 17.xii.73

SUMMARY

The genetics of zinc tolerance in *Agrostis tenuis* was examined using the diallel technique. Evidence is presented that the genetic control of zinc tolerance in *A. tenuis* is dominant and directional with a high additive genetic variance. The polygenic control of zinc tolerance in *A. tenuis* has been established.

1. INTRODUCTION

In a previous paper in this series (Gartside and McNeilly, 1974), an account of the genetic control in *Anthoxanthum odoratum* was given. Using both the diallel analysis (Jinks, 1954; Hayman, 1954) and pair cross techniques, evidence was presented that the control of zinc tolerance in this species was both directionally dominant and under polygenic control.

In this present investigation, the diallel analysis has again been used in order to elucidate the genetic control of zinc tolerance in *Agrostis tenuis* Sibth. Using a 10×10 parental diallel cross involving both zinc-tolerant and non-tolerant genotypes, the components of the genetic variance are discussed.

2. MATERIAL AND METHOD

Ten genotypes of *A. tenuis* were collected from the lead/zinc mine at Trelogan in Flintshire during the winter of 1970-71. Material was collected from as diverse and non-localised areas as possible in order to avoid sampling clones of the same genotype. In addition, five non-tolerant genotypes were collected from adjacent non-contaminated pasture land. Single tiller plants were planted in John Innes No. 2 compost in 14-cm pots and grown over winter in a plastic tunnel house, without additional heat or light. In August 1971, each genotype was divided into 20 ramets, these being grown in the same conditions as described above. Plants were grown over winter in the plastic tunnel house without additional heat, and under natural day-length conditions. Flowering occurred in May 1972, and a diallel set of crosses was made at this time between eight tolerant genotypes (10, k, E, D, A, 7, 16, C) and two non-tolerant genotypes (n8 and n2). Crosses were made in 48×15 cm non-moisture-proof glassene bags with the bases of the individual inflorescences wrapped in non-absorbent cotton wool. Two inflorescences of each genotype were used in each cross. Inflorescences

* Present address: Unit of Genetics, Department of Plant Biology, University of Hull, HU6 7RX.

† Present address: Department of Botany, University of Waikato, Hamilton, New Zealand.

were bagged 24 hours prior to anthesis, and the bags removed after 7 days to allow a natural seed maturation process. Excessive humidity within the bags was avoided by removal of the flag leaves prior to bagging. All genotypes were tested for selfing by isolating three inflorescences and testing subsequently for viable seed production. Seeds were collected in August 1972, allowed to dry at room temperature, and planted in J.I. No. 2 compost in seed trays and grown in heated glasshouse conditions under a 14-hour photoperiod. After 8 weeks, ten F_1 progeny of each cross, together with reciprocal F_1 progeny, were taken at random and planted individually in J.I. No. 2 compost in 14-cm pots and placed in the plastic tunnel house. The pots were arranged in a completely randomised block. After 12 weeks, 20 tillers of four of each F_1 family (including two reciprocals) together with parental material were tested for zinc tolerance by the method described by McNeilly and Bradshaw (1968), using 7.5 p.p.m. zinc solution and deionised water. Tolerance testing was performed in a growth room at a constant temperature of 20° C., a relative humidity of 85 per cent, and under continuous illumination provided by both fluorescent tubing and tungsten red filament bulbs.

3. DIALLEL DEFINITION AND ASSUMPTIONS

A diallel crossing system may be defined as one in which n genotypes are chosen and intercrossed leading to n^2 combinations. Although parental material is usually inbred, outbreeding lines may be used.

A diallel table is an arrangement in squares of n^2 observations (see table 1) from a set of diallel crosses among n parental lines. The rows and columns of the square correspond to the values of the offspring of each parent, while the n parental values form the diagonals of the table. It is, therefore, easy to assess whether or not differences occur between reciprocal crosses. Whether or not these are significant, means of reciprocal values may be calculated such that both rows and columns contain the same values, these being termed arrays.

The use of the diallel analysis procedure as devised by Hayman (1954) and Jinks (1954), is strictly valid only where the following assumptions are valid:

1. No genotype $\times$ environment interaction,
2. Homozygous parents,
3. Disomic segregation,
4. No maternal effects,
5. No non-allelic interaction,
6. No multiple alleles,
7. Uncorrelated gene distribution.

Failure to meet any of these conditions will cause a characteristic disturbance of the expected relationship between array covariance Wr and array variance Vr (Jinks, 1954; Dickinson and Jinks, 1956; Jinks, 1956).

1. The design of the experiment precludes any estimate of genotype $\times$ environment interaction, but since the degree of tolerance remained unaltered over a 2-year period, including estimates taken on material directly from the mine, and the same material after 2 years in cultivation, it is unlikely that $G \times E$ interaction would prove to be a significant source of bias.

TABLE 1

The index of tolerance for a 10×10 diallel with two replicates in two columns and their mean value in the third column in each array

	n8			n2			10			k			E			D			A			7			16			C		
n8	0	0	0	0	0	0	16	8	12	8	12	10	10	10	10	12	24	18	20	20	20	30	40	35	30	36	33	36	40	38
n2	0	0	0	0	0	0	10	10	10	6	8	7	3	9	6	25	27	26	25	25	25	26	30	28	40	44	42	35	41	38
10	6	10	8	0	8	4	10	10	10	6	6	6	12	20	16	30	38	34	30	34	32	34	38	36	46	46	46	44	44	44
k	10	10	10	5	9	7	3	9	6	15	9	12	18	22	20	29	29	29	26	34	30	38	38	38	30	34	32	35	39	37
E	8	12	10	6	6	6	11	13	12	18	26	22	6	18	12	26	30	28	26	30	28	34	36	35	40	40	40	38	38	38
D	18	26	22	18	22	20	30	30	30	20	30	25	24	28	26	20	30	25	36	28	32	46	50	48	38	46	42	50	42	46
A	20	28	24	24	26	25	40	48	44	34	42	38	26	30	28	31	29	30	28	28	28	40	44	42	48	48	48	40	48	44
7	38	40	39	24	32	28	33	39	36	36	40	38	45	45	45	40	40	40	41	43	42	30	36	33	43	49	46	46	46	46
16	40	50	45	32	36	34	30	30	30	40	48	44	42	38	40	38	46	42	45	43	44	45	55	50	36	40	38	40	46	43
C	36	40	38	30	30	30	45	35	40	41	49	45	40	40	40	40	48	44	44	44	44	44	48	46	51	35	43	46	50	48

2. *Agrostis tenuis* is almost exclusively an exogamous species, although a small proportion of selfing may occur. In such a species, it is likely that a high degree of heterozygosity will occur. From the Wr/Vr regression graph, it can be seen that the array points lie close to the limiting parabola, a characteristic disturbance associated with heterozygosity of the parental lines. In spite of this bias, which will reduce b and the intercept of the Wr/Vr regression with the Wr axis, the diallel may be analysed in the normal manner. The regression of Wr on Vr will have a slope not significantly different from unity if the remaining assumptions are valid (Jinks, 1954).

3. Cytological studies (Jones, K., pers. comm.) have suggested that *A. tenuis* is a tetraploid ($2n = 28$). Normal disomic segregation, however, has been shown to occur and no chromosomal abnormalities have been found.

4. The non-significant c mean square in the analysis of variance table (table 2), indicates no maternal effects.

TABLE 2
Analysis of variance. 10 × 10 diallel

Item	d.f.	SS	M.S.	V.R.	P%
<i>a</i>	9	15892.0	1765.8	110.5	0.1
<i>b</i>	45	3246.0	72.0	4.5	0.1
<i>b1</i>	1	848.7	848.7	53.3	0.1
<i>b2</i>	9	99.1	11.0	0.7	n.s.
<i>b3</i>	35	2298.2	65.4	4.1	0.1
<i>c</i>	9	137.0	15.2	0.9	n.s.
<i>d</i>	36	543.0	15.1	0.9	n.s.
TOTAL	99	19818.0	—	—	—
Error	100	1598.0	15.9	—	—

The assumptions of no epistasis, no multiple alleles, and uncorrelated gene distributions, are difficult to evaluate separately. If, however, all assumptions are valid, the regression of Wr/Vr over all arrays is expected to be a straight line. Since the regression of Wr/Vr in this investigation did not differ significantly from unity ($b = 0.90 \pm 0.04$), it can be suggested that the above items would not represent a significant source of bias.

4. RESULTS

The results were analysed by the method of Mather and Jinks (1971) and the analysis of variance table is shown in table 2. Array covariance/array variance results are expressed graphically in Fig. 1.

Additive genetic variance was shown by the significant *a* item, while the significant *b* item indicates a significant non-additive component. Directional dominance for zinc tolerance was shown by the significant *b1* item, while the non-significant *b2* item indicates a symmetrical distribution of genes within the parental lines. Hayman (1954) points out that when the average frequencies of those alleles displaying dominance are equal in the parents, the parental contributions balance out and there are no detectable differences in dominance between arrays. The non-significant *c* and *d* items indicate no reciprocal differences.

It has been mentioned earlier that the Wr/Vr regression was not significantly different from unity, and since the correlation of $Wr + Vr$ and the

parental index of tolerance was significant ($r = -0.95$), directional dominance for zinc tolerance was indicated. The position of the array points along the Wr/Vr regression gives a measure of the relative numbers of dominant alleles within the parental lines. Parents having a greater frequency of dominant alleles have a low covariance/variance value and appear nearest to the origin, whilst parents having a low frequency of dominant alleles have a high covariance/variance value and appear furthest away from the origin.

FIG. 1.—Array covariance/array variance results for zinc tolerance in *A. tenuis*. (Array points with the prefix n refer to non-tolerant parental material, while array points without a prefix refer to parents collected from zinc-contaminated waste.)

In the homozygous diallel the degree of dominance may be judged from the intercept of the Wr/Vr regression of the Wr axis. In the heterozygous diallel it is impractical to determine the degree of dominance since the bias introduced by the heterozygous parental lines will tend to displace the Wr/Vr regression towards the limiting parabola, thus underestimating the degree of dominance.

The ecological significance of a high additive genetic variance has been discussed by Gartside and McNeilly (1974) with respect to *Anth. odoratum*. It is suggested that despite the long half-life of *A. tenuis*, gene flow from surrounding non-tolerant populations adjacent to the mine site would be

sufficiently high as to introduce new genetic variability into the mine population (Jain and Bradshaw, 1966; Antonovics, 1968). In this way, both heterozygosity and a high additive genetic variance would be maintained.

5. REFERENCES

- ANTONOVICS, J. 1968*b*. Evolution in closely adjacent plant populations. VI. Manifold effects of gene flow. *Heredity*, 23, 507-524.
- DICKINSON, A. G., AND JINKS, J. L. 1956. A generalised analysis of diallel crosses. *Genetics*, 41, 65-77.
- GARTSIDE, D. W., AND McNEILLY, T. 1974. Genetic studies in heavy metal tolerant plants. I. Zinc tolerance in *Anthoxanthum odoratum* L. *Heredity*, 32, 287-297.
- HAYMAN, B. I. 1954. The analysis of variance of diallel crosses. *Biometrics*, 10, 235-244.
- JAIN, S. K., AND BRADSHAW, A. D. 1966. Evolutionary divergence among adjacent plant populations. I. The evidence and its theoretical analysis. *Heredity*, 21, 407-441.
- JINKS, J. L. 1954. The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties. *Genetics*, 39, 767-788.
- JINKS, J. L. 1956. The F_2 and backcross generations from a set of diallel crosses. *Heredity*, 10, 1-30.
- MATHER, K., AND JINKS, J. L. 1971. *Biometrical Genetics*, 2nd Ed. Chapman & Hall, London.
- McNEILLY, T. AND BRADSHAW, A. D. 1968. Evolutionary processes in populations of copper tolerant *Agrostis tenuis* Sibth. *Evolution*, 22, 108-118.