

MATERNAL AND RECIPROCAL EFFECTS ON SEEDLING CHARACTERS IN *ARABIDOPSIS THALIANA* (L.) HEYNH¹

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ABSTRACT

Five early growth characters were examined in six races of *Arabidopsis thaliana* (L.) Heynh, their reciprocal F_1 hybrids (1974) and F_1 by tester hybrids, using a seventh race as a paternal tester. Three of the five characters were also examined at two nutrient levels in reciprocal F_1 hybrids (1972) of all seven races. Analyses of F_1 and F_1 by tester hybrids revealed significant maternal effects in all characters examined in F_1 hybrids (1972) and in root length and plant weight of F_1 (1974) and F_1 by tester hybrids. Significant reciprocal effects were found for plant weight in F_1 by tester hybrids and for seed weight, percentage of germination and root length in F_1 (1974) and F_1 by tester hybrids. The presence of significant maternal and/or reciprocal components in both F_1 (1974) and F_1 by tester diallels suggests that differences in maternal cytoplasm rather than maternal genotype *per se* were responsible for much of the variation resulting from these non-direct genetic effects.

THE genetics of quantitative traits has been examined in numerous plant species. Most of these studies have emphasized general and specific combining ability effects while, for the most part, ignoring maternal and reciprocal effects. In those studies which have examined maternal effects in quantitative characters in plants, such effects were generally thought to be of two types: those originating from differences in the maternal environment provided to the developing seed by the female parent, and those originating from differences in cytoplasm. Much of the work on maternal inheritance and reciprocal differences, as well as theories concerning the mechanisms by which they might operate, is presented in review articles by CASPARI (1948), JINKS (1964), MATHER and JINKS (1971) and SAGER (1972).

The purpose of this study was to determine the existence of reciprocal differences in quantitative characters of *Arabidopsis* seedlings and, should these differences exist, to determine whether they originate from differences in the maternal environment provided by the female parent to developing seeds or from differences in cytoplasm or its interactions with other effects. The use of a male tester to obtain F_1 by tester hybrids through crosses with reciprocal F_1 progenies

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resulting from a diallel system of crosses involving inbred lines can provide a means by which maternal effects resulting from differences in mothering ability can be differentiated from those resulting from differences in cytoplasm. Since the tester serves only as a male parent in crosses with reciprocal F_1 hybrids, the differences in cytoplasmic lineage present in reciprocal F_1 hybrid pairs would be maintained in corresponding F_1 by tester hybrids. This is under the assumption that the female parent is responsible for the cytoplasmic composition of its offspring. Reciprocal F_1 by tester hybrids, however, develop on maternal parents which are genetically identical. Therefore, there should be no reciprocal differences in F_1 by tester hybrids resulting from differences in maternal environment, if it is assumed that the maternal environment provided by the female parent is conditioned entirely by its nuclear genotype. Reciprocal differences or maternal effects present in F_1 by tester hybrids can therefore only be logically attributed to differences in cytoplasm or in its interaction with nuclear genotype.

MATERIALS AND METHODS

Seven races of *Arabidopsis thaliana* (L.) Heynh served as the source of the experimental material examined in this study. These races included Estland, Martuba, Rschew, Clayton, Basel, Tsu Islands, and Catania. *Arabidopsis* is predominantly a self-fertilizing plant and the laboratory inbred lines which served as parental stocks were considered homozygous.

F₁ hybrids (1972): All possible F_1 hybrids, including reciprocals, were obtained from hand pollinations of the seven races in a 7×7 diallel crossing system.

F₁ hybrids (1974): All possible F_1 hybrids, including reciprocals, were obtained from hand pollinations involving all races except Catania in a 6×6 diallel system of crosses. These hybrids served as the maternal stock from which all F_1 by tester hybrids were derived.

F₁ × T hybrids: F_1 hybrids obtained in the 6×6 diallel were crossed as females by a tester (Catania). In all cases Catania was used only as the male parent.

All parental genotypes for a given study were grown and mated during the same time period in controlled environmental chambers in an attempt to standardize the extra-plant prenatal environment of developing seeds.

Culturing techniques

All plants were cultured aseptically in test tubes containing a mineral agar substrate. Two levels of aseptic agar were used in the study. F_1 hybrids (1972) were cultured at optimum and low (1/12 mineral level of optimum) nutrient levels while F_1 hybrids (1974) and $F_1 \times T$ hybrids were grown only at the optimum nutrient level.

Seeds were not pre-treated in any way before being sown other than washing with a solution consisting of equal volumes of 95% ethanol and 1 N hydrogen peroxide to remove any surface contaminants. After being sown, seeds underwent a cold treatment for a period of four days and then were placed in numbered wooden blocks and moved to a growth chamber having a constant source of incandescent and fluorescent light which provided 1500 foot-candles. The temperature inside the test tubes was maintained at approximately 25°. During the growth of the plants, each wooden block was moved to a new random position daily.

Three characters were measured in the F_1 hybrids cultured in 1972. These included germination time (the number of days from the time that the seeds were moved to the chamber to emergence of the radicle), root length (cm) of the primary root 13 days after seeding, and green plant weight (mg) measured 19 days after seeding. These traits were also measured in the plants grown in 1974, in addition to percentage of germination and seed weight (mg) per 50 seed lot. Data for percentage of germination were transformed to angles prior to statistical analyses.

Statistical analysis

Separate diallel analyses were performed on F_1 (1972) combined over nutrient levels, F_1 (1974) and $F_1 \times T$ data excluding selfs for each character. The model used in each case is similar to that presented by COCKERHAM (1963). The model used for F_1 hybrid data is:

$$Y_{ijk} = u + g_i + g_j + s_{ij} + m_i + r_{ij} + a_k + e_{ijk}$$

where

$$i, j = 1, 2 \dots p, \text{ (line indices)}$$

$$k = 1, 2 \dots k, \text{ (replication indices)}$$

and where

$$s_{ij} = s_{ji}$$

and Y_{ijk} is the plot mean resulting from the cross of the i^{th} maternal parent and the j^{th} paternal parent in the k^{th} replicate, g_i is the nuclear genetic effect of the i^{th} parent, g_j is the nuclear genetic effect of the j^{th} parent, s_{ij} is the nuclear genetic interaction effect of the i^{th} and j^{th} parents, m_i is the effect of the i^{th} maternal cytoplasm, $r_{ij} = (gm)_{ii} + (gm)_{ij}$ is the sum of interaction effects of the i^{th} cytoplasm and the nuclear genetic contributions of the i^{th} and j^{th} parents, a_k is the effect on all crosses in the k^{th} replicate and e_{ijk} is the experimental error associated with the Y_{ijk} observation. This model was extended to include nutrient level effects and their interactions for F_1 hybrids cultured in 1972.

The model for $F_1 \times T$ data is:

$Y_{(ij)tk} = u + g_i/2 + g_j/2 + g_t + s_{it}/2 + s_{jt}/2 + m_i + r_{(ij)t} + a_k + e_{(ij)tk}$ where the effects are as previously defined except g_t is constant and $r_{(ij)t} = (gm)_{ii}/2 + (gm)_{ij}/2 + (gm)_{it}$ is the sum of the interaction effects of the i^{th} maternal cytoplasm with the i^{th} , j^{th} and t^{th} nuclear contributions.

The equations used for obtaining the sums of squares are those presented by YATES (1947). The expected mean squares for all partitions of variation were obtained by calculating the expected values of the sums of squares in terms of the original model and dividing by degrees of freedom. In deriving the expected mean squares, it was assumed that the g 's, s 's, m 's, r 's and e 's were uncorrelated random variables with their squares having expectations of σ_G^2 , σ_S^2 , σ_M^2 , σ_R^2 and σ^2 , respectively. The mean square expectations in terms of variance components for the F_1 and $F_1 \times T$ models are given in Tables 1 and 2, respectively. Table 3 presents the mean square

TABLE 1
Expected mean squares for the F_1 model of the diallel design

Source	M.S.	$E(MS)$
Replications	M_6	
General	M_5	$\sigma^2 + k\sigma_R^2 + 2k\sigma_S^2 + k(p-2)\sigma_M^2/2 + 2k(p-2)\sigma_G^2$
Specific	M_4	$\sigma^2 + k\sigma_R^2 + 2k\sigma_S^2$
Maternal	M_3	$\sigma^2 + k\sigma_R^2 + kp\sigma_M^2/2$
Reciprocal	M_2	$\sigma^2 + k\sigma_R^2$
Error	M_1	σ^2

TABLE 2
Expected mean squares for the $F_1 \times T$ model of the diallel design

Source	M.S.	$E(MS)$
Replications	M_6^*	
General	M_5^*	$\sigma^2 + k\sigma_R^2 + k\sigma_S^2 + k(p-2)\sigma_M^2/2 + k(p-2)\sigma_G^2/2$
Specific	M_4^*	$\sigma^2 + k\sigma_R^2 + k\sigma_S^2$
Maternal	M_3^*	$\sigma^2 + k\sigma_R^2 + kp\sigma_M^2/2$
Reciprocal	M_2^*	$\sigma^2 + k\sigma_R^2$
Error	M_1^*	σ^2

TABLE 3

Expected mean squares for the $F_1 - (F_1 \times T)$ model of the diallel design

Source	M.S.	$E(MS)$
General	M_5^{**}	$\sigma^2 + (3/8)k\sigma_R^2 + (3/2)k\sigma_S^2 + k(p-2)\sigma_G^2/4$
Specific	M_4^{**}	$\sigma^2 + (3/8)k\sigma_R^2 + (3/2)k\sigma_S^2$
Maternal	M_3^{**}	$\sigma^2 + (3/8)k\sigma_R^2$
Reciprocal	M_2^{**}	$\sigma^2 + (3/8)k\sigma_R^2$
Error	M_1^{**}	σ^2

expectations for the $F_1 - (F_1 \times T)$ model which was used in the analysis of differences between F_1 (1974) and $F_1 \times T$ hybrids. Assuming equal variances of the different types of maternal \times nuclear interaction effects, i.e., $\sigma^2(gm)_{ii} = \sigma^2(gm)_{ij} = \sigma^2(gm)_{it}$, then σ^2_R (Table 2) = $(3/4)\sigma^2_R$ (Table 1). This assumption was utilized for the expectations in Table 3.

RESULTS AND DISCUSSION

F₁ hybrids (1972)

Mean squares and their significance levels, as determined by appropriate F -tests were obtained from analyses of these data combined over nutrient levels and are presented in Table 4. Nutrient level effects accounted for a large portion of the variation observed in root length and plant weight. The significance of interactions between nutrient level and general combining ability effects for these traits suggests that genotypic differences also exist in the abilities of F_1 hybrids to react to changes in the levels of nutrients used.

Maternal effects accounted for the only significant variation among parental sources, excluding interaction effects, in all traits. Estimated maternal components were the greatest in magnitude and were the only components which consistently approached significance when compared with their standard errors.

TABLE 4

Diallel analyses of F_1 hybrids combined over nutrient levels

Source	d.f.	Mean squares		d.f.	Mean squares Germination time
		Root length	Weight		
Nutrients	1	22.2688**	3062.6148**	1	.0100
Reps/Nut.	14	.3285**	88.4708**	10	.9130**
General	6	1.5944	269.6347	6	.4883
Specific	14	.0908	17.7552	14	.0743
Maternal	6	1.1172**	172.5159**	6	1.8066**
Reciprocal	15	.1456	8.5955	15	.1333
$G \times N$	6	.5123**	43.1010**	6	.2133
$S \times N$	14	.0831	8.6999	14	.1243
$M \times N$	6	.1419	9.3994	6	.2217
$R \times N$	15	.0890	6.8311	15	.1620
Error	557	.0874	7.2448	408	.1328
Total	654			501	

** Significant at the 1% level.

The design used did not permit the determination of the exact origin of the reciprocal differences observed in the traits examined. Possible biological explanations for these differences, however, fall into two main categories: those concerned with the environment provided to the developing seed by the maternal parent, and those concerned with cytoplasm. In this experiment these effects were nested in the maternal partition of variance and could not be separated; consequently, the F_1 (1974) and $F_1 \times T$ hybrids were examined.

F₁ (1974) and F₁ × T hybrids

Separate diallel analyses of variance were run on F_1 and $F_1 \times T$ hybrid data for each character. The mean squares obtained in these analyses and their levels of significance as ascertained by appropriate F-tests are presented in Table 5. General combining ability effects, reflecting additive genetic effects, were found to be significant for all characters except root length in F_1 hybrids, but were not found to be significant in $F_1 \times T$ hybrids. Dominance or epistatic effects of nuclear genes did not appear to play a significant role in any of the traits examined in either F_1 or $F_1 \times T$ hybrids, as was indicated by the lack of significance of specific combining ability effects. Maternal and/or reciprocal effects were found to be significant for all characters examined in both F_1 and $F_1 \times T$ hybrids.

The variance components associated with each source of variation and their standard errors estimated from the diallel analyses of F_1 and $F_1 \times T$ data are given in Table 6. While additive genetic effects, as indicated by the relative size of estimated general combining ability components of variance, do account for a large portion of the variation observed in root length in F_1 and $F_1 \times T$ hybrids and in plant weight in F_1 hybrids, the significance of maternal and/or reciprocal partitions of variation in the diallel analyses indicates that the variation observed

TABLE 5

Diallel analyses of F₁ and F₁ × T hybrids

Source	Mean squares		d.f.	Mean squares		Mean squares		d.f.	Mean squares
	d.f.	Root length		d.f.	Plant weight	d.f.	Germ. time		Percent germ.
<i>F₁ hybrids</i>									
Reps	19	1.2701**	19	165.9598**	19	.1721**	372.0379**		
General	5	1.7067	5	64.6637**	5	.2414*	1033.1920**	5	.2000**
Specific	9	.2289	9	5.7777	9	.0408	145.5122	9	.0240
Maternal	5	.4407*	5	10.2303*	5	.0698	301.7200	5	.0700
Reciprocal	10	.0927*	10	2.1909	10	.3655**	1826.6770**	10	.0780**
Error	546	.0449	544	1.3244	551	.0574	120.1974	60	.0020
<i>F₁ × T hybrids</i>									
Reps	19	1.1716**	19	185.6064**	19	.1379**	214.0895**		
General	5	.9042	5	27.2786	5	.1552	189.3000	5	.1300
Specific	9	.1151	9	3.7727	9	.1787	255.2888	9	.0180
Maternal	5	.5514**	5	16.9489*	5	.4438	415.4820	5	.1200
Reciprocal	10	.0934**	10	4.1169**	10	.1750**	535.8130**	10	.0490**
Error	550	.0196	549	.9524	551	.0362	88.3211	60	.0030

** Significant at the 1% level.

* Significant at the 5% level.

TABLE 6

Diallel components of variance and standard errors (in parentheses)

Variance components	Root length	Plant weight	Germination time	Percentage of germination	Seed weight	
σ_G^2	F ₁	.0078(.0057)	.3345(.2178)	.0025(.0016)	11.9020(4.7086)	.0230(.0790)
	F ₁ × T	.0121(.0131)	.3738(.3976)	-.0051(.0050)	.3558(20.1982)	.0280(.0140)
σ_S^2	F ₁	.0034(.0054)	.0897(.0656)	-.0081(.0120)	-42.0291(18.9848)	-.0270(.0320)
	F ₁ × T	.0010(.0019)	-.0172(.1164)	.0002(.0570)	-14.0262(12.2338)	-.0320(.0120)
σ_M^2	F ₁	.0058(.0038)	.1340(.0992)	-.0049(.0026)	-25.4160(12.7162)	-.0026(.0160)
	F ₁ × T	.0076(.0049)	.2139(.1535)	.0045(.0041)	-2.0055(5.1954)	.0230(.0210)
σ_R^2	F ₁	.0024(.0019)	.0433(.0449)	.0154(.0075)	85.3240(37.2886)	.0760(.0320)
	F ₁ × T	.0037(.0019)	.1582(.1414)	.0069(.0035)	23.8746(10.9454)	.2545(.0077)
σ^2	F ₁	.0449	1.3233	.0547	120.1974	.0002
	F ₁ × T	.0196	.9524	.0362	88.3211	.0003

in the material examined herein cannot be explained solely in terms of direct genetic effects.

The same problem as to the origin of observed reciprocal differences seen with regard to the F₁ hybrids (1972) once again presents itself with regard to the F₁ hybrids (1974). The degree of transience seen in reciprocal differences from one generation to the next, however, can be used to differentiate between effects resulting from differences in mothering ability and/or effects from physiological interactions between particular seed and pollen parents, and effects of cytoplasmic origin. Since a reciprocal difference resulting from differences in mothering ability would be conditioned by the genotype of the maternal parents as well as the particular environment to which the female was exposed, reciprocal differences of the same size and magnitude would not be expected to occur in succeeding generations. On the other hand, effects which were entirely cytoplasmic should be seen to the same degree among progenies with a common cytoplasmic lineage, regardless of the nuclear genetic composition of either parent. The consistency of this type of behavior is, of course, contingent on the degree to which the cytoplasmic genes or other factors function autonomously of nuclear genes; the lack of autonomy would be measured by σ_r^2 .

Since the tester served as the male parent in all crosses producing F₁ by tester hybrids, the cytoplasmic composition of these progenies in terms of cytoplasmic DNA should be identical with that of the F₁ hybrid serving as the maternal parent. In this way, the differences in cytoplasmic origin in individual reciprocal F₁ hybrid pairs were maintained in the F₁ × T hybrids. However, the nuclear genetic composition of reciprocal hybrids that served as maternal parents of F₁ by tester progenies was identical. Reciprocal differences among F₁ hybrids should have disappeared in F₁ × T hybrids to the extent that they were a result of differences in mothering ability under direct nuclear genetic control. This was

not seen to occur with regard to any of the characters examined in the study. In all cases, significant maternal or reciprocal effects were found in both F_1 and $F_1 \times T$ hybrids (See Table 5). Further, maternal effects present in $F_1 \times T$ hybrids were not found to differ significantly from those present in F_1 hybrids (1974) with regard to any character except percentage of germination when differences between F_1 and $F_1 \times T$ hybrids were analyzed (i.e., analysis of $(Y_{ij} - Y_{ji}) - (Y_{(ij)t} - Y_{(ji)t})$). This suggests that the reciprocal differences noted herein cannot be strictly attributed to differences in mothering ability *per se* but must be tied in some way to cytoplasmic influences or their interactions.

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