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Inheritance of Isocitratase Activity and Its Relationship to Seedling Vigor in a Cross of Upland Cotton[1]

R. L. Scholl[2]

ABSTRACT

Seedlings of reciprocal F_1, reciprocal backcross, and advanced generations of a biparental cross of Upland cotton (*Gossypium hirsutum* L.) were examined for isocitratase activity and axial fresh weight after 40 to 112 hours of germination.

Results from reciprocal hybrids after 40 and 112 hours of germination indicated that level of isocitratase activity and axial growth rate were maternally inherited, but there was no evidence of cytoplasmic inheritance. Wide segregation for isocitratase activity was observed in the F_2 population after 40 and 112 hours of germination, and isocitratase activity appeared to be quantitatively inherited. In the F_2 population, relative isocitratase activity of seedlings germinated 40 hours was highly correlated with the percent of seedling fresh weight in the axis ($r=+0.843^{**}$). The association between the same two characters was small but negative for seedlings germinated 112 hours ($r=-0.431^{**}$). The strength of the association between isocitratase activity and axial growth early in germination suggests that isocitratase activity may be a good indicator of early seedling vigor.

Additional index words: **Maternal effects, Selection procedure, Quantitative inheritance.**

IN recent years the simplification of plant improvement procedures through the application of biochemical techniques has become feasible. The phenomenon of heterosis has received much attention in this regard. Hageman, Leng, and Dudley (1967) have reviewed biochemical studies relating to heterosis. Several investigators (Carpenter and Beevers, 1959; Beevers, 1961; Hock and Beevers, 1966; Breidenbach, Kahn, and Beevers 1968) have demonstrated the critical role of the glyoxylate cycle in the germination of fatty seeds. The enzymes of the glyoxylate cycle, including isocitratase and malate synthetase, are localized in the glyoxysome and are instrumental in converting the lipid reserve of seeds to carbohydrates during germination (Breidenbach et al., 1968). Roos and Sarkissian (1968) studied isocitratase activity in scutellar tissue of corn (*Zea mays* L.) inbreds and F_1 hybrids, but their results were inconclusive in relating isocitratase activity to maize seedling heteorsis.

Because of the short growing season in many areas of cotton (*Gossypium hirsutum* L.) production, cotton seeds must germinate and plants begin growth when the average temperature is still relatively cool. For example, the mean temperature for April at Charlotte, North Carolina is 15C (Henry, 1906). A number of studies have addressed different aspects of this problem. Leaching of contents from radicles of young seedlings in response to cold temperatures has been demonstrated (Christiansen, Carns, and Slyter, 1970). The mitochondria of young plants in the vegetative stage of growth are sensitive to cool temperatures (Stewart and Guinn, 1971). The enzyme, isocitratase, might be closely related to the expression of seedling cold tolerance of cotton (Mohapatra et al., 1970; Smith, 1972). The isocitratase activity of cotton cotyledons was depressed when seedlings were exposed to a temperature of 5C for 6 hours (Mohapatra et al., 1970). Isocitrate dehydrogenase was not affected in the same tissue. Both isolated glyoxysomes and intact cotyledons, when chilled, accumulated succinate which was shown to noncompetitively inhibit isocitratase activity (Smith, 1972). In addition, isocitratase activity is not detectable is cotton seeds (Carpenter and Beever, 1959) but is induced upon germination. These three examples illustrate that some physiological processes have been examined with the aim of revealing causes of cold responses of cotton. However, little has been done to determine if biochemical systems can be manipulated genetically to achieve superior cold tolerance. The current study represents an attempt to elucidate the mode of inheritance of isocitratase activity in a cross involving two Upland cotton cultivars and to ascertain the association between enzyme activity and seedling vigor in the progeny.

MATERIALS AND METHODS

Parents used to generate the genetic materials examined were TH 149-8-5 and CQ-1, referred to hereafter as P_1 and P_2, respectively. Both of these parental lines were derived from bulk self-pollination of individual plant selections from the commercial varieties 'TH 149' (P_1) and 'Carolina Queen' (P_2). The two parents differ significantly in isocitratase activity at approximately 2 and 5 days after initiation of germination, and the isocitratase activity of TH 149 might be less sensitive to chilling depression than that of Carolina Queen (Smith, Fites, and Noggle, 1971). Reciprocal crosses were made between P_1 and P_2 in the winter of 1970-71 and again in the field in 1971. Each parent was represented by 1 row of 50 plants in the field and a group of 12 plants in the greenhouse. Also, F_1 plants were backcrossed reciprocally and F_2 plants were individually self-pollinated in the field. Flowers were selfed and crossed in proportionate numbers each time pollinations were made and were distributed uniformly along the row. Thus, cross- and self-pollinations involving the same maternal parent were made on the same plants at the same time.

Maternal genotypic effects and maternal environmental effects associated with the parent row were confounded. Effects from interaction of the maternal genotype with all levels of the environment were also confounded with genotypic effects. However, environmental effects were minimized by growing the parents in adjacent rows in the field and on the same bench in the greenhouse.

Seeds for all entries in an experiment were harvested at the same time and stored together; the seeds of a given genotype were bulked. Seed sources for the reciprocal F_1 and reciprocal backcross progenies were separated according to maternal parent with the following exceptions: F_2 and F_3 seed used were not identified with respect to cytoplasm and are referred to in the text as "F_2 bulk" and "F_3 bulk," respectively. Individual F_2 plants of unidentified cytoplasm were selfed to generate F_3 families. In addition to the reciprocal hybrids and segregating progenies, two groups of F_2 seedlings were examined, one with P_1 and the other with P_2 cytoplasm. The seed stocks for these were generated by bulk-harvesting self-pollinated seed from

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[2] Research Assistant (now Assistant Professor, Department of Genetics, Ohio State University, Columbus, OH 43210).

$P_1 \times P_2$ and $P_2 \times P_1$, respectively. Seeds for this study were developed on a group of 15 greenhouse plants of each parent grown in the summer of 1971.

Seeds to be germinated were ginned and acid-delinted, and their coats were pierced. Seeds were germinated on wet, rolled paper towels in the dark at 25 C and were harvested at intervals from 40 to 112 hours. Twenty seedlings of each entry were used for all experiments except in the F_2 segregation study where 14 seedlings were harvested after 40 hours and 11 seedlings were harvested after 112 hours of germination. Experiments were replicated over time. The F_2 segregation study was replicated four times, and the experiments involving reciprocal crosses were replicated five or six times. Direct comparison of activity among entries was made only when the entries were run simultaneously to avoid unknown environmental influences on relative rate of plant development and the rate of change of enzyme activity. F_3 families from F_2 plants were examined at both harvest intervals. Eighty-two families were common to both harvest times. The entries were randomly grouped into five blocks. Seedlings were dissected into cotyledons and axes (radicles and hypocotyls), and fresh weight was determined for both. The cotyledons were washed with distilled water, then homogenized with a mortar and pestle in 1.7 ml of cold potassium phosphate (0.05*M*, pH 7.5) per cotyledon pair. The homogenate was filtered through a double thickness of cheesecloth and centrifuged at 15,000 $\times$ *g* for 20 minutes. The supernatant served as the source of crude enzyme. Isocitratase was assayed using the method of Dixon and Kornberg (1959) except that 15 μmoles of dl-isocitrate (trisodium salt) were substituted for 5 μmoles of potassium L_s isocitrate.

RESULTS AND DISCUSSION

Reciprocal Crosses—Maternal Inheritance

The general trend of observed isocitratase activity during germination (Table 1) was similar to that previously reported (Carpenter and Beevers, 1959; Smith, 1972). After 40 hours of germination significant differences between reciprocal F_1 hybrids were noted for enzyme activity and growth as measured by axial fresh weight. F_1 seedlings from P_2 maternal plants had higher activity than those from P_1. This indicates one of two modes of inheritance: 1) cytoplasmic inheritance (control by genetic factors outside the nucleus) or 2) maternal inheritance (control by the nuclear genotype of the parent on which the seed was produced).

To differentiate between the above-mentioned types of inheritance, the reciprocal F_1 plants were self-pollinated to yield two sets of F_2 seed with different cytoplasms. When seedlings germinated from these seed sources were compared, isocitratase activity after 40 hours of germination for F_2 plants with P_1 cytoplasm (1.65 μmoles glyoxylate min^{-1} gfw^{-1}) was not significantly different from that for F_2 plants with P_2 cytoplasm (1.72 μmoles glyoxylate min^{-1} gfw^{-1}; gfw = grams fresh wt). Therefore, for isocitratase activity after 40 hours germination, maternal nuclear inheritance was favored over cytoplasmic inheritance to explain the results obtained.

Table 1. Isocitratase activity of cotyledons and growth of axes after three different intervals of germination for reciprocal F_1, parent, and advanced generation seedlings from field-grown parents.

	Isocitratase			Axial fresh weight		
	40 hours	76 hours	112 hours	40 hours	76 hours	112 hours
	μmoles glyoxylate min^{-1} gfw^{-1}			mg/seedling		
P_1	1.94 c*	7.02 a	4.90 a	43 b	293 ab	664 a
$P_1 \times P_2$	1.95 c	6.08 ab	4.70 ab	44 b	277 b	680 a
$P_2 \times P_1$	3.29 a	5.82 b	3.53 c	68 a	324 a	606 b
P_2	2.70 b	5.48 b	3.58 c	64 a	264 b	540 c
F_2 bulk	2.69 b	5.74 b	4.15 bc	63 a	285 b	644 ab
F_3 bulk	2.73 b	5.66 b	3.95 c	67 a	272 b	664 a

* Means followed by the same letter were not significantly different at the 5% level according to Duncan's New Multiple Range Test.

Table 2. Isocitratase activity of cotyledons and growth of axes after two different intervals of germination for reciprocal backcross, parent, and F_2 seedlings from field-grown parents.

	Isocitratase		Axial fresh weight	
	40 hours	112 hours	40 hours	112 hours
	μmoles glyoxylate min^{-1} gfw^{-1}		mg/seedling	
P_1	1.32 d*	3.92 a	27 c	672 ab
$P_1 \times F_1$	1.39 cd	3.82 ab	28 bc	693 a
$P_2 \times F_1$	2.15 a	3.12 bcd	37 bc	561 e
P_2	2.16 a	2.68 d	46 a	511 f
F_2 bulk	1.76 b	2.62 d	37 ab	645 bc
$F_1 \times P_1$	1.58 bcd	3.62 abc	32 bc	618 cd
$F_1 \times P_2$	1.63 bc	3.06 cd	35 bc	600 d

* Means followed by the same letter were not significantly different at the 5% level as evaluated with Duncan's New Multiple Range Test.

Isocitratase activity was high and probably near its peak at 76 hours (Table 1). No reciprocal differences were observed. In fact, P_1 showed the only significant deviation from uniform activity for all entries. On the other hand, the pattern of activity after 112 hours of germination conformed to a maternal mode of inheritance (Table 1). The reciprocal F_1 seedlings differed for isocitratase level but both had activities very similar to those of their respective maternal parents. The reciprocal F_2 study gave no indication of cytoplasmic inheritance for 112-hour activity. Means for F_2 plants with P_1 and P_2 cytoplasms, respectively, were 2.62 and 2.49 μmoles glyoxylate min^{-1} gfw^{-1}.

There was excellent agreement between isocitratase activity and axial growth (Table 1) at 40 hours of germination. Axial fresh weight was also correlated with isocitratase activity after 112 hours of germination, but not as highly as after 40 hours. However, these differences could have been a result of the larger seeds harvested from P_1 than from P_2 whether produced by self- or cross-pollination. Studies discussed below indicate that axial weight at this stage is associated directly with the original seed size.

Results from the reciprocal backcross experiments (Table 2) tend to confirm the hypothesis of maternal control. In only one case (F_2 bulk vs $F_1 \times P_1$ at 112 hours) the enzyme activity of seedlings produced from cross-pollination was different from that of seedlings produced by self-pollination of the same parent. Axial weight of P_2 differed from that of $P_2 \times F_1$ at both 40 and 112 hours of germination.

Reciprocal Crosses—Genetic Interaction

The observed differences among the progeny of this cross suggest the presence of genetic interaction. The genotype $P_2 \times P_1$ showed isocitratase activity for 40 hour germination outside the range of parental activity (Table 1). A deviation from the midparent value, as great as that of the maternal parent (P_2), can be explained by maternal inheritance, but the additional deviation encountered can only be caused by an interaction between the seed and the maternal genotypes. It is unlikely that this result represents an error deviation since an independent study (Scholl, unpublished), utilizing seed of the same genotypes harvested from greenhouse plants, produced similar results. This study also verified the differences between reciprocal F_1's for both 40 and 112-hour germination.

If isocitratase activity is maternally inherited, the entry designated "F_2 bulk" in Table 1 represents the F_1 genotype. Dominance effects may have been present because the mean of this entry for 40 hours of germination was near that of the higher parent. However, the F_2 bulk was intermediate when compared to the two reciprocal F_1 hybrids. Also the F_3 bulk was equal to the F_2 bulk and did not regress toward the midparent value as would be the case with dominance. Therefore, the evidence does not wholly support a conclusion that significant dominance effects are present. For seedlings germinated 112 hours, activity of the F_2 bulk was intermediate to the parents indicating a probable lack of dominance.

F_2 progeny Segregation

Figure 1 shows the distribution of 40-hour isocitratase activity for progeny from five blocks (groups) of F_2 plants. Corrections for block effects were made by subtracting the appropriate block effect from the mean of each entry. In each block, the parental extremes expected from the reciprocal F_1 seedlings were exceeded, whereas the F_2 progeny mean was near the F_2 bulk control. No simple inheritance pattern was discernible from examination of the distribu-

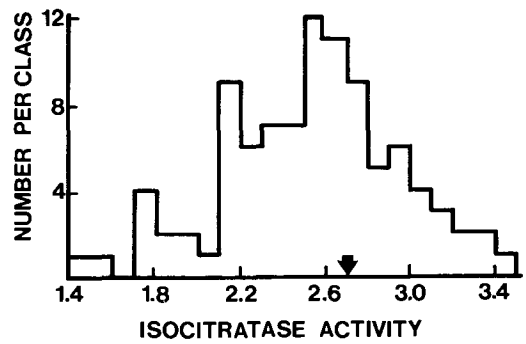

Fig. 1. F_2 progeny distribution for 40-hour isocitratase activity (μmoles glyoxylate min^{-1} gfw^{-1}) adjusted for block effect. Arrow indicates mean for F_1 control (F_2 bulk); LSD .05 = 0.49.

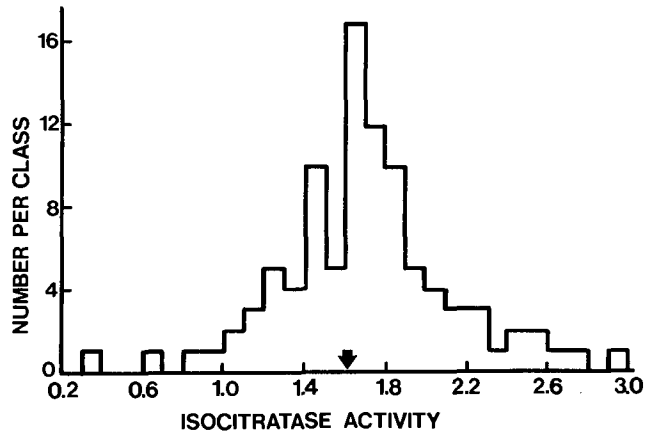

Fig. 2. F_2 progeny distribution for 112-hour isocitratase activity (μmoles glyoxylate min^{-1} gfw^{-1}) adjusted for block effect. Arrow indicates mean for F_1 control (F_2 bulk); LSD .05 = 0.67.

Table 3. Summary of linear regressions involving several characteristics of the segregating F_2 progeny.

Dependent variable†	Independent variable‡	Correlation coefficient
ENZ112	ENZ40	-.588**
AFW40	ENZ40	.705**
%AFW40	ENZ40	.843**
AFW112	ENZ112	.265*
AFW112	ENZ112-ENZ40	.206
%AFW112	ENZ112	-.431**
%AFW112-%AFW40	ENZ112	.604**
%AFW112-%AFW40	ENZ112-ENZ40	.287**

*, ** Significant at the 5 and 1% level, respectively.
† AFW40 = axis fresh weight/seedling (mg), 40-hour germination.
AFW112 = axis fresh weight/seedling (mg), 112-hour germination.
%AFW40 = percent of fresh weight as axis, 40-hour germination.
%AFW112 = percent of fresh weight as axis, 112-hour germination.
‡ ENZ40 = isocitratase activity (ΔO. D./min per gfw of cotyledon), 40 hours.
ENZ112 = isocitratase activity (ΔO. D./min per gfw of cotyledon), 112 hours.

tions in single or combined blocks. The proximity of the F_2 progeny mean to that of the F_1 suggests a lack of dominance in the inheritance of isocritratase activity in this particular population, as does the apparent symmetry of the distribution.

The F_2 progeny distribution for isocitratase activity of seedlings germinated 112 hours is given in Fig. 2. Distribution appears to be symmetrical around the F_1 mean which, as noted previously, is near the midparent value. This distributional pattern is typical of a polygenic trait inherited in an additive fashion.

Isocitratase activity 40 hours after germination was negatively correlated with activity 112 hours after germination (Table 3). This result is consistent with the hypothesis that genotypes which develop activity earlier in germination deplete their lipid reserve earlier. If the residual lipid content of the cotyledons is the determining factor for isocitratase level late in germination under etiolated conditions this type of correlation is to be expected. Indeed, isocitratase activity coincides closely with the state of lipid utilization in cotyledons of germinating fatty seeds (Carpenter and Beevers, 1959).

Association of Isocitratase Activity with Seedling Vigor

Accumulation of fresh weight at 40 hours was closely related to isocitratase activity in the F_2 population (ENZ40) whether the weight was expressed on a per plant basis (AFW40) or as a percentage of the total seedling weight (%AFW40) (Table 3). (The actual distribution of F_2 growth parameters are not shown.) The correlation was strong enough to suggest that isocitratase activity or very closely allied glyoxysomal characteristics might have limited early seedling growth. Even if the enzyme was not directly involved with the control of seedling growth it still must have been very closely associated with those factors that influence relative growth.

Whereas the simple regression of 40-hour axial weight on 40-hour isocitratase was highly significant, the only term that measurably improved the goodness of fit was cotyledonary fresh weight (CFW40) (Table 4). This parameter was probably a reasonable approximation to a correction for seed size. Although a number of models were tested, only those for which all coefficients were statistically significant were included in the summary. Exceptions were made for simple regression models whose independent variables were included in a significant multiple model. The appar-

Table 4. Summary of pertinent multiple regression models for the F_2 progeny population.*

Dependent variable†	Independent variables†	Coefficient of determination
AFW40	CFW40 (ns)	.028
	CFW40 ENZ40	.652
%AFW40	CFW40	.138
	CFW40 ENZ40	.718
AFW112	AFW40	.276
	CFW112	.069
	CFW112 AFW40	.314
	CFW112 AFW40 ENZ112	.353
%AFW112	AFW40 (ns)	.027
	CFW112	.134
	ENZ40	.221
	CFW112 AFW40	.179
	CFW112 ENZ112	.236
	ENZ40 ENZ112	.258
	CFW112 ENZ40	.302
%AFW112	ENZ112 (ENZ112)²	.226
	CFW112 ENZ112 (ENZ112)²	.280

*All regression coefficients in all models included are significant at the 5% level unless indicated as being not significant (ns).
†Variable definitions are the same as for Table 3 with the following additions:
CFW40 = fresh weight of cotyledon/seedling (mg), 40 hours.
CFW112 = fresh weight of cotyledon/seedling (mg), 112 hours.

ent unimportance of a quadratic term for enzyme activity indicates that even the higher enzyme levels encountered early in germination were strongly limiting on seedling growth. Thus, genotypes with enzyme activity greater than that encountered in the present experiment should be expected to have a concomitantly greater capacity for growth.

Fresh weight of axis after 112 hours of germination was less closely related to enzyme activity. Although the linear relationships between isocitratase activity (ENZ112) and both axial fresh weight (AFW112) and percent of fresh weight in the axis (%AFW112) were significant (Table 3), isocitratase activity accounted for a relatively small percentage of the variability for either.

Because both enzyme activity and the rate of fresh weight accumulation were in a dynamic state during the period studied, an attempt was made to correlate net change in fresh weight with observed enzyme activities for 40 and 112 hours of germination. The change in percent of fresh weight in the axis between 40 and 112 hours (%AFW112-%AFW40) was much more closely associated with 112-hour enzyme activity (ENZ112) than was total axial weight after the 112-hour period (AFW112) (Table 3). Multiple regression models did not appreciably improve this agreement (Table 4).

The factors most closely related to 112-hour axial weight were coytledonary size and axial weight at 40 hours. After correction for these factors, enzyme activity may not have been limiting at the 112-hour develomental stage. The observed correlations between percent of fresh weight as axis and isocitratase activity may have been spuriously high since cotyledonary weights were necessary to calculate both parameters.

Implications on Physiological Control Mechanisms and Future Breeding Methodology

The most striking result from this study is that the maternal nuclear genotype controlled relative isocitratase activity during the first 5 days of germination. The actual mechanism of maternal control was not investigated although some factor which controlled enzyme activity directly could have been transmitted through the seed. Enzyme activity is generally assumed to reflect concentration or efficiency of the given enzyme. However, many other factors influence activity, particularly when crude preparations are examined. Thus, the results reported here did not necessarily demonstrate any actual differences in enzyme quantity or quality or that maternal effects are the rule since only one cross was examined.

However, it is possible to arrive at some conclusions regarding the source of the observed differences in activity and, in particular, the mode in which maternal control was exerted. Maternal control is inconsistent with the possibility of isozyme variation for isocitratase being the cause of observed differences between the parents. The form (s) of the enzyme present should be a function of the seed genotype rather than the maternal genotype, regardless of the developmental stage at which the enzymatic protein is synthesized. However, the relative amount or rate of supply of the ultimate substrate of the glyoxylate cycle is especially attractive as a possible explanation of the maternal control of isocitratase activity. Seed characteristics, including total lipid content (Brim, Schutz, and Collins, 1968; Singh and Hadley, 1968) and the level of specific fatty acids are known to be controlled maternally.

Isocitratase activity was sufficiently repeatable at both 40 and 112-hour harvests so that genotypes could be ranked confidently without excessive replication either within or across environments. Therefore, heritability for the enzyme, although not determined in the present study, was probably high. Selection among individual seedlings from the same maternal plant (or genotype) would have been ineffective.

Although early axial growth was very closely related to isocitratase activity in the populations studied, it was influenced less by levels of this enzyme after 112 hours. Not determined in this study were the carryover effects of the apparent initial advantage afforded those seedlings that initiate isocitratase activity early. Although it is true that the relative parental means of both enzyme activity and axial growth reversed themselves after the initial surge by P_1, this was not so in the segregating population as evidenced by the small but significant association between percent of fresh weight as axis at 112 hours (%AFW112) and enzyme activity at 40 hours. If isocitratase activity is closely enough associated with continued plant vigor, then sufficient genetic variability exists at least in the segragating population studied here to select for the indirect improvement of overall performance.

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