Theor. Appl. Genet. 11-15 (1979)

THEORETICAL AND APPLIED GENETICS

© by Springer-Verlag 1979

# Variations Among Races of *Arabidopsis thaliana* (L.) Heynh for Survival in Limited Carbon Dioxide

R.K. Sharma, B. Griffing and R.L. Scholl
Department of Genetics, The Ohio State University, Columbus, Ohio (USA)

**Summary.** Races of a $C_3$ plant species, *Arabidopsis thaliana*, were screened for time of survival when enclosed in an air-tight chamber with a $C_4$ plant species (*Zea mays* L.). This has been suggested as a method of detecting and selecting for increased photosynthetic efficiency among $C_3$ genotypes. The $C_4$ plant should, in such conditions, reduce the atmospheric $CO_2$ concentration below the compensation point of the $C_3$ plant, resulting in its eventual death. Significant differences were found among the *A. thaliana* races for survival time; some races survived only one week in competition with corn while others survived more than two weeks. Two races, chosen for contrasting survival in closed chambers, were hybridized and compared to their $F_2$ progeny for survival time. Substantial genetic segregation occurred among the $F_2$, and a number of transgressive segregates having survival times superior of both parents were identified. Also significant heterosis was observed in the $F_2$ population.

**Key words**: Photosynthetic efficiency – Transgressive segregation – $CO_2$ compensation point

## Introduction

Recent research has shown that two distinct types of photosynthetic carbon fixation pathways exist among plants. These are designated as '$C_3$' and '$C_4$' (Hatch, Osmond and Slayter 1971; Zelitch 1971; Black 1973). Plants of $C_4$ species in addition to having a four-carbon instead of a three-carbon organic acid as the product of $CO_2$ fixation, exhibit different bundle sheath anatomy, have a lower $CO_2$ compensation point and under many conditions have higher net photosynthesis than $C_3$ plants (Meinder 1962; Moss 1962; El-Sharkaway et al. 1967; Downton and Tregunna 1968; Moss and Musgrave 1971). Plants of $C_3$ species photorespire, presumably contributing to their decreased photosynthetic efficiency. It has been suggested that improvement in productivity of $C_3$ crops might be achieved by altering their photosynthetic and/or photorespiratory rates toward those of $C_4$ species (Marx 1973; Zelitch 1974). This would be accomplished by either selection within the $C_3$ species or by converting a $C_3$ species to a $C_4$ through hybridization and selection.

A few genetic studies of photosynthetic efficiency have already been conducted. In several of these, attempts were made to take advantage of the differences in $CO_2$ compensation points of $C_3$ and $C_4$ plants by the enclosure of different genotypes of $C_3$ species in air-tight chambers with $C_4$ plants (Menz et al. 1969; Moss 1970). $C_3$ genotypes that survived under these conditions should have had a lower $CO_2$ compensation point, designated '$\Gamma$', (Heath and Orchard 1957) and, by implication, increased photosynthetic efficiency. This method was used to screen the USDA world collection of soybeans (Cannel et al. 1969), approximately 10,000 oat seedlings and more than 50,000 wheat seedlings (Moss and Musgrave 1971). No low $\Gamma$ plants were found in these studies. However, in another study involving two varieties of tall fescue (a $C_3$ species), small genetic variability was detected for survival time in closed chambers (Nelson et al. 1975). This variability was not associated with increased net photosynthesis but rather with increased dark respiration.

The present study was designed to explore further the extent of genetic variability for survival in closed chambers in a $C_3$ species. The $C_3$ laboratory plant, *A. thaliana*, was chosen for this study because it can be grown in large numbers on a defined medium under exactly controlled conditions (i.e. in test tubes within plant growth chambers). The specific objectives of the study were: 1) to define a simple quantitative measure of competitiveness for $CO_2$; 2) to define optimum experimental conditions for distinguishing genetic variability for this measure of photosynthetic efficiency; 3) to determine if *A. thaliana* races differ in ability to survive in limiting atmospheric

0040-5752/79/0054/0011/$ 1.00

$CO_2$; and 4) to determine the inheritance of survival in limiting $CO_2$ in a cross between two races contrasting for the trait. By the quantitative screening of a diverse collection of races it may be possible to estimate the genotypic variation in $CO_2$ competitiveness in an entire species. Subsequent physiological examination of such variants should provide a more conclusive test of whether or not this kind of screening can detect variation for photosynthetic efficiency.

## Materials and Methods

### *Genetic Stocks and Growth of Plants*

Thirty-three races of *A. thaliana*, which were originally obtained from the standard Laibach collection (Röbbelen 1965), were used. These races are designated by code names which are abbreviations of the site names at which they were first collected. All races originated from single plants and have been maintained by self-fertilization. Therefore each race may be regarded as a homozygous clone. Also, homozygous accessions of the closely related species, *A. pumila* (Steph.) Busch and *A. korshinskyi* (Botsch.) Busch were used in one experiment.

*Arabidopsis* seeds were planted on 6.7 ml of an agar medium containing inorganic salts (Langridge 1957) in 18 X 150 mm test tubes. Plants were grown at 25°C (after an initial 48 hour cold treatment at ∿4°C to induce germination) under continuous illumination of 27 $w/M^2$ of 400-700 nm radiation from cool white fluorescent lamps. Relative humidity was maintained at 70-80 percent.

### *Experimental Treatment of Plants*

The apparatus for evaluating photosynthetic efficiency was similar to that devised by Menz et al. (1969). The $C_3$ (*Arabidopsis*) genotypes were placed in a small (51 X 25 X 20 cm) air-tight chamber (Fig. 1) with plants of a $C_4$ species (*Zea mays* seedlings). The $C_4$ species should reduce the $CO_2$ concentration in the chamber below the compensation point of the $C_3$ species. This would eventually result in the death of the $C_3$ plants. Age at death was recorded for each plant and analyzed as a quantitative trait. The corn plants were grown in vermiculite moistened with water containing major and minor mineral salts.

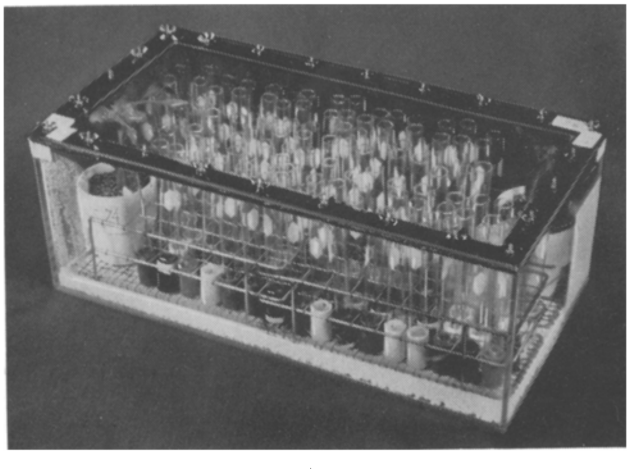

**Fig. 1.** Photograph of plexiglass chamber containing corn and *Arabidopsis* plants (in 18 X 150 mm test tubes)

### *Design of Experiment*

Effect of Number of Corn Plants on *Arabidopsis* Survival: The objectives of this experiment were to observe the effect of the presence of corn plants in a closed chamber on the survival of different *Arabidopsis* species and to determine the optimum number of corn plants to be used in each chamber for further studies. Four different numbers of corn seedlings (0, 4, 6, 8) were placed in separate chambers with *Arabidopsis* plants. Equal numbers (52) of *Arabidopsis* plants ten days of age from two species (*A. pumila* and *A. korshinskyi*) were placed in each chamber to determine if the species responded differently to the same treatments. Numbers of dead plants belonging to each species in each chamber were recorded daily. The experiment was terminated after a two-week period. The proportion of dead plants associated with each treatment at the time of termination was calculated and expressed as a percentage. The percentage data were analyzed by a two-way analysis of variance using a fixed-effect model. An angular transformation of the data was used to ensure normality (Li 1969).

Screening of *A. thaliana* races: Several experiments were conducted with the primary objective to screen races of *A. thaliana* for survival in closed chamber. Each experiment was conducted within a single chamber, and six corn plants were placed in each chamber. A secondary objective of these experiments was to determine the optimum age to differentiate the *A. thaliana* races with regard to survival. Thus, in different experiments, *Arabidopsis* plants of different ages were used. All races were not necessarily represented by equal numbers of plants. Data were collected daily on survival. The number of days that each plant survived in a closed chamber was analyzed.

Comparison of parental races and hybrid progeny: Two races, chosen from the previous experiment for differential survival, and their $F_2$ hybrid progeny were compared for survival in closed chambers. Twenty plants of each parent and 80 of their $F_2$ progeny were enclosed in each of two chambers 16 days after planting. Other conditions, including the number of corn plants used, were the same as for the racial screening study. Time of survival was recorded for each plant and analyzed. Single degree-of-freedom contrasts, i.e., linear and quadratic, were constructed to evaluate parental differences and heterotic effects (Netter and Wasserman 1975).

## Results and Discussion

### *Effect of Corn Plants on Survival of Arabidopsis in Closed Chambers*

The effects of differing numbers of corn plants on survival of plants of the two *Arabidopsis* species are given in Table 1. Since all plants of both species survived two weeks of enclosure in the chambers unless corn plants were added, it is logical to conclude that death of *Arabidopsis* plants was due to competition for $CO_2$. Although no significant differences were detected among the 4, 6, and 8 corn plant treatments, the mean mortality values

**Table 1.** Mortality percentages for two species of *Arabidopsis* when competing with different numbers of corn plants

| Treatment number of corn plants | Species *A. pumila* | *A. korshinskyi* | Mean[a] for two species |
|---|---|---|---|
| 0 | 0.0 | 0.0 | 0.0 a |
| 4 | 34.6 | 42.3 | 38.4 b |
| 6 | 69.2 | 82.6 | 75.9 b |
| 8 | 34.6 | 94.2 | 64.4 b |
| Overall species mean | 34.6 | 54.7 | 44.7 |

[a] Means followed by the same letter do not differ significantly (P = 0.05), as determined by Duncan's New Multiple Range Test

suggest a slight trend for increased mortality with increase in numbers of corn plants. Such a minimal effect could occur if all the treatments quickly exerted stress on the *Arabidopsis* plants. Six corn plants per chamber were chosen for use in subsequent experiments since this was the smallest number of plants certain to produce near-maximum mortality. Mortality percentages did not differ significantly between the species (P = 0.05).

## *Screening of A. thaliana Races for Survival*

For each of the five racial screening experiments significant differences were found (see F-values, Table 2). Since most experiments involved different races it can be inferred that there exists a wealth of genetic variation for survival under limited $CO_2$ in *A. thaliana.* This is somewhat surprising in view of past findings with similar experiments (Cannel et al. 1969: Moss and Musgrave 1971). Relative survival times of races were extremely reproducible. For example, races DI, PI and CT were all grown in both experiments in which the chambers were closed when the plants were 16 days old. While overall survival time was quite different in these two experiments the differences between survival times of these races were almost identical. Time of enclosure appeared to have little effect on the relative ranking of races. For example, races such as CT and DI were ranked similarly when inclosed at two ages (16 and 19 days). Also, PI exhibited intermediate survival when enclosed at either 10 or 16 days. Thus, not only does substantial racial variation exist for survival in limiting $CO_2$ but the closed-chamber technique was very efficient in identifying such variability.

Comparisons of relative variabilitv among the six experiments were used to determine an optimum age at which plants should be enclosed in the airtight chambers. The F-values for the racial mean squares (Table 2) suggested that the variability for survival was greater at 16 and 19 days than for 11 or 13 days. Sixteen-day-old plants were preferred since enclosures at this age allowed more time for exceptional survival ability to be expressed before the onset of natural senescence.

**Table 2.** Mean days-to-death for *A, thaliana* when competing with corn plants under limited $CO_2$ as affected by race and age of plants when enclosed

| Age of enclosed plants (days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | | 11 | | 13 | | 16 | | 16 | | 19 | |
| Race | Longevity[1] | Race | Longevity[1] | Race | Longevity[1] | Race | Longevity[1] | Race | Longevity[1] | Race | Longevity[1] |
| Mu 2079 | 7.6 a | SI | 8.1 a | TA | 8.5 a | DI | 13.3 a | DI | 5.0 a | WS | 8.0 a |
| WK | 8.1 ab | MT | 8.2 ab | Pet 7 | 8.5 a | PI | 15.7 b | Pet 7 | 6.0 ab | SCH | 9.0 b |
| HM | 8.4 bc | RLD | 8.3 ab | LA | 8.6 ab | CT | 17.1 c | PI | 7.0 bc | DI | 10.7 c |
| VI | 8.4 bc | EST | 8.3 ab | GUCK | 8.8 ab | | | TA | 7.5 bc | LI | 10.8 c |
| PI | 8.5 bc | CHI | 8.4 ab | FR | 9.2 ab | | | WS | 7.8 bcd | Mu1013 | 12.1 d |
| FR | 8.5 bc | BLA | 8.5 ab | SA | 9.2 abc | | | WK | 8.2 cde | TU | 12.2 d |
| PF | 8.6 bc | CT | 8.8 bc | KS | 9.3 abc | | | GIE | 8.7 cdef | WI | 13.0 d |
| DA | 8.8 bc | LS | 9.0 c | GR | 9.6 bc | | | WIL | 9.0 def | CT | 15.0 e |
| KOL | 9.0 c | | | Marb | 10.2 cd | | | Marb | 9.2 def | | |
| WIL | 9.1 c | | | GIE | 10.6 d | | | MT | 9.4 ef | | |
| | | | | | | | | WI | 9.4 ef | | |
| | | | | | | | | CT | 9.5 ef | | |
| | | | | | | | | DA | 10.2 f | | |
| | | | | | | | | LI | 10.5 f | | |
| F[2] = 3.56[5] | | F[2] = 2.66[3] | | F[2] = 5.27[5] | | F[2] = 13.93[5] | | F = 10.47[5] | | F = 39.61[5] | |

1 Means followed by the same letter do not differ significantly at P = 0.05 (Duncan New Multiple Range Test)
2 F = ratio of racial mean square to error mean square from the analysis of variance
3 Significant, 0.01 < P < 0.05
4 Significant, 0.005 < P < 0.01
5 Significant, P < 0.005

### Comparison of Parental Races and $F_2$ Progeny

The variable 'days-to-death' was examined in the inheritance study of the two *Arabidopsis* races and their $F_2$. As expected, a large difference between parents for survival in competition with corn existed (Fig. 2). This difference was statistically significant (see linear mean square, Table 3). Also the quadratic sum of squares (Table 3) was significant indicating some heterosis for survival time. The existence of this heterotic effect raises the interesting possibility that the photosynthetic phenomena might affect heterosis of growth in this species. $F_1$ progeny were not examined in this study due to the difficulty of producing a sufficient quantity of hybrid seed.

Comparison of the distributions of the parental and $F_2$ populations (Fig. 2) suggests that there was much greater variability for survival time in the $F_2$ population than in the parental populations. In fact, there was substantial transgressive segregation in the $F_2$ population; over one-fourth of the $F_2$ progeny (45 out of 160) survived longer than plants from either parent. This suggests that recombination for longer survival occurred readily. Therefore recurrent selection for increased survival time would probably be successful in segregating populations such as the one generated in this study.

**Table 3.** Analysis of variance for days-to-death for two *Arabidopsis* races and their $F_2$ progeny when competing with corn plants for limited $CO_2$

| Source | Degrees of freedom | Mean square | F |
|---|---|---|---|
| Genotype (G) | 2 | 206.560 | 28.373[a] |
| Linear | 1 | 270.112 | 37.103[a] |
| Quadratic | 1 | 143.008 | 19.643[a] |
| Chamber (C) | 1 | 2.669 | 0.366 |
| G X C | 2 | 3.772 | 0.518 |
| Error | 234 | 7.280 | |

[a] Significant, $P < 0.005$.

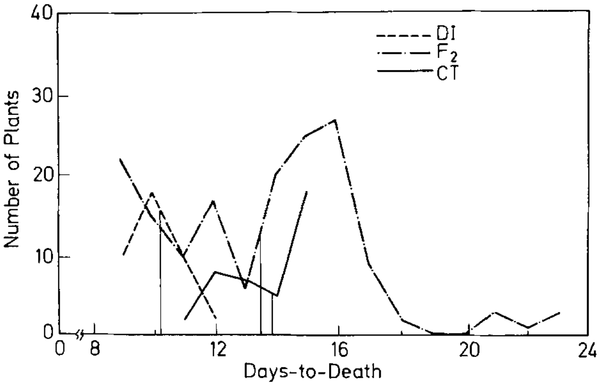

**Fig. 2.** Distributions and means for days-to-death for two *A. thaliana* races and their $F_2$ when competing with corn plants for limited $CO_2$. (Means are represented by vertical lines descending from the associated distributions)

### Physiological Implications

In the past, several attempts have been made to screen genotypes of various field crops under reduced $CO_2$ (Cannel et al. 1969; Moss 1970; Moss and Musgrave 1971). However, these studies did not yield successful results because they were designed to detect $C_4$ plants within a $C_3$ species. Because of the complexity of differences between the two photosynthetic types, it is highly unlikely that a mutant $C_4$ plant would be found in a $C_3$ species. Therefore, a logical alternative is to search for quantitative rather than qualitative differences in photosynthetic capacity within a $C_3$ species as measured by differences in survival length under reduced $CO_2$. Although a somewhat similar procedure was followed by Nelson et al. (1975), the range of survival times was apparently greater with *Arabidopsis* (from 9 to 23 days in the $F_2$ population).

It has been assumed in most studies of this type that survival of a particular $C_3$ genotype in competition with a $C_4$ species indicates superior photosynthetic efficiency (Menz et al. 1969). It is almost certain that survival time is closely related to $CO_2$ metabolism in *Arabidopsis*, since the plants did not die unless corn plants were present. Also, opportunity for action of extraneous environmental influences was minimized by conducting the experiments in small, highly controlled chambers. Assuming survival is related to limiting $CO_2$, the basic question remains as to precisely how the observed phenomenon correlates with various physiological processes associated with $CO_2$ metabolism. Since $CO_2$ exchange rates and parameters of associated biochemical processes were not measured in this study, the answer to the above question can only be conjectured.

Races of other species have been shown to differ for characteristics such as net photosynthetic rate (Avratovščuková 1968; Curtis et al. 1969; Heichel and Musgrave 1969a & b; Asay et al. 1974), and rate of photorespiration (Wilson 1972; Zelitch and Day 1973). For tall fescue, intrapopulation variations of survival in limited $CO_2$ have been shown to be associated with the rate of dark respiration (Nelson et al. 1975). In the present *Arabidopsis* study the variability was so large and widespread it might ultimately be attributable to both photosynthetic and respiratory sources. Therefore it is clear that appropriate physiological and biochemical studies should be made on those *Arabidopsis* races which now have been characterized in terms of their survival ability.

The final question that should be raised is the relationship of survival time under competition for limited $CO_2$

and crop productivity. The conceptual argument is that the survival variable is a direct measure of photosynthetic efficiency, and that an increase in crop productivity automatically would result from increased photosynthetic efficiency. This argument has not been tested directly although it was noted that the $F_2$ hybrid (DI × CT) did exhibit heterosis for dry weight accumulation under limited $CO_2$ (Sharma 1976).

## Acknowledgment

The authors thank Dr. S.S.Y. Young for helpful suggestions provided throughout this work.

## Literature

Asay, K.H.; Nelson, C.J.; Horst, G.L.: Genetic variability for net photosynthesis in tall fescue (*Festuca arundinacea* Schreb). Crop Sci. *14*, 571-574 (1974)

Avratovšćuková, N.: Differences in photosynthetic rate of leaf discs in five tobacco varieties. Photosynthetica *3*, 149-160 (1968)

Black, C.C.: Photosynthetic $CO_2$ fixation in relation to net $CO_2$ uptake. Ann. Rev. Plant Physiol. *24*, 253-286 (1973)

Cannel, R.A.; Brun, W.A.; Moss, D.N.: A search for high net photosynthetic rate among soybean genotypes. Crop Sci. *9*, 840-841 (1969)

Curtis, P.E.; Ogren, W.L.; Hageman, R.H.: Varietal effects in soybean photosynthesis and photorespiration. Crop Sci. *9*, 323-327 (1969)

Downton, W.J.S.; Tregunna, E.G.: Carbon dioxide compensation: its relation to photosynthetic carboxylation reactions, systematics of Graminae and leaf anatomy. Can. J. Bot. *46*, 207-215 (1968)

El-Sharkaway, M.; Loomis, R.S.; Williams, W.A.: Apparent reassimilation of respiratory $CO_2$ in different plant species. Physiol. Plantar. *20*, 171-186 (1967)

Hatch, M.D.; Osmond, C.D.; Slayter, R.O.: Photosynthesis and Photorespiration. New York: Wiley Interscience 1971

Heath, O.V.S.; Orchard, B.: Temperature effects on minimum intercellular space $CO_2$ concentration. Nature *180*, 180-181 (1957)

Heichel, G.H.; Musgrave, R.B.: Varietal differences in net photosynthesis of *Zea mays* L. Crop Sci. *9*, 483-486 (1969a)

Heichel, G.H.; Musgrave, R.B.: Relation of $CO_2$ compensation concentration to apparent photosynthesis in maize. Plant Physiol. *44*, 1724-1728 (1969b)

Langridge, J.: The aseptic culture of *Arabidopsis thaliana* (L.). Heynh. Aust. J. Biol. Sci. *10*, 243-252 (1957)

Li, J.C.R.: Statistical Inference I.: Ann Arbor, Michigan: Edwards Bros. Inc. 1969

Marx, J.L.: Photorespiration. Key to increasing plant productivity. Science *179*, 365-367 (1973)

Meinder, H.: The minimum intercellular space $CO_2$ concentration ($\Gamma$) of maize leaves and its influence on stomata movement. J. Exp. Bot. *13*, 284-293 (1962)

Menz, K.M.; Moss, D.N.; Cannel, R.A.; Brun, W.A.: Screening for photosynthetic efficiency. Crop Sci. *9*, 692-694 (1969)

Moss, D.N.: The limiting $CO_2$ concentration for photosynthesis. Nature *193*, 587 (1962)

Moss, D.N.: Laboratory measurement and breeding for photosynthetic efficiency. Prediction and Measurements of Photosynthetic Productivity. Proceedings of the IBP/mm Technical Meeting., pp. 323-330. Wageningen, The Netherlands: Center of Agricultural Publishing and Documentation. 1970

Moss, D.N.; Musgrave, R.B.: Photosynthesis and crop production. Advances in Agronomy *23*, 317-336 (1971)

Nelson, C.J.; Asay, K.H.; Patton, K.D.: Photosynthetic responses to tall fescue to selection for longevity below $CO_2$ compensation point. Crop Sci. *15*, 629-633 (1975)

Netter, J.; Wasserman, W.: Applied Linear Statistical Models. Homewood, Illinois: Richard D. Irwin Inc. 1975

Röbbelen, G.: The Laibach collection of natural races. Arabid. Inf. Serv. *2*, 36-47 (1965)

Sharma, R.K.: Genetic analysis of photosynthetic efficiency in Arabidopsis thaliana (L.). Ph.D. thesis. Columbus, Ohio: Ohio State University. 1976

Wilson, D.: Variation in photorespiration in Lolium. J. Exp. Bot. *23*, 517-525 (1972)

Zelitch, I.: Photosynthesis, Photorespiration and Plant Productivity. New York: Academic Press 1971

Zelitch, I.: Improving the energy conversion in agriculture. Am. Soc. Agron. Specl. Publ. *22*, 37-50 (1974)

Zelitch, I.; Day, P.R.: The effects on net photosynthesis of pedigree selection for low and high rates of photorespiration in tobacco. Plant Physiol. *52*, 33-37 (1973)

Received September 15, 1978
Communicated by O.E. Nelson

R.K. Sharma
Laboratory of Biochemical Genetics
National Institute of Environmental Health
Sciences – NIH
P. O. Box 12233
Research Triangle Park, N.C. 27709
USA

B. Griffing
R.L. Scholl
Department of Genetics
The Ohio State University
1735 Neil Avenue
Columbus, Ohio 43210
USA