

**EFFECT OF ALIEN CYTOPLASMS
ON PHOTOSYNTHESIS
AND RELATED TRAITS IN COTTON
(*GOSSYPIUM BARBADENSE* L.)**

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Abstract

The photosynthetic apparatus is dually inherited with components being contributed by both the nuclear and cytoplasmic genomes, which must function together for photosynthesis to be efficient. The objective of the present study is to determine if the replacement of the cultivated tetraploid cotton cytoplasm (AD2) with alien ones affects photosynthesis and other gas exchange parameters. Ten different alloplasmic lines with cytoplasms A2, B1, C1, D2-2, D3-d, D8, E1, AD1, AD2 and AD5 in 57-4 nuclear background (AD2) were grown in field plots with three replications. Leaf photosynthetic rate (PN), stomatal conductance (COND), intercellular CO₂ concentration (C_i), transpiration rate (E) and water use efficiency (WUE) were determined at four different sampling dates from July 25 to August 19. Alloplasmic lines with wild diploid cytoplasms had 7.2 to 24.3% higher PN than 57-4, with a mean of 11.3%, while the cytoplasms from A2, AD1 and AD5 had less effect on PN (5% higher than 57-4). The exotic cytoplasms, especially wild ones, had markedly positive effects on COND of water, but generally had no significant effect on C_i, E and WUE. The positive effects of exotic cytoplasms on PN and COND did not persist over the last sampling date. There were significant correlations among PN, COND and E.

Introduction

Photosynthesis, one of the principal components of crop yields, is controlled by many genes located on both chromosomes and cytoplasmic genome(s). Theoretically, a nuclear-cytoplasmic combination could be synthesized that would increase the efficiency of photosynthesis or other energy functions. We know that nuclear genes influence photosynthesis. For example, major gene mutants, such as leaf type (Pettigrew et al., 1993), frego bract (Zhang et al., 1993), virescent (Benedict et al., 1972) and nectariless (Zhang et al., 1990) have been shown to influence cotton leaf photosynthetic rates (PN). Genetic variation of PN among cotton species, cultivars and F1 hybrids has also been detected (El-Sharkawy et al., 1965; Leidi et al., 1993; Pettigrew and Meredith, 1994; Well et al., 1988; Zhang et al., 1995). Pettigrew and Meredith (1994) showed that genetic variation of PN existed in upland cottons with higher performance in some modern cultivars, and found a

significant positive association between PN during the boll filling period and lint yield. However, Leidi et al. (1993) found that significant differences in PN among upland cottons do not persist over time. This difference of PN among genotypes is due to the genetic variation of the nuclear genome since upland cottons share a common cytoplasm. El-Sharkawy et al. (1965) found significant differences in PN among cotton species. Differences in cytoplasms among cotton species was also detected by chloroplast DNA polymorphisms (Wendel and Albert, 1992), which may account in part for the PN differences among species. However, Zhang et al. (1991) did not detect any marked effect of exotic cotton cytoplasms on PN in a Chinese upland cotton background based on in-vitro single leaf measurements. Cytoplasmic effects on in vivo PN and other gas exchange parameters in the *Gossypium barbadense* (AD2) background have received little attention. In this study, our objectives were to (i) determine if exotic cytoplasms including A2, B1, C1, D2-2, D3-d, D8, E1, AD1 and AD5 in the AD2 background had any effect on gas exchange parameters, and (ii) investigate the relationships among those gas exchange parameters.

Materials and Methods

In 1996, ten alloplasmic lines with 57-4 (AD2) nuclear background, developed by Stewart (1990), were grown in field plots of the University of Arkansas, Agricultural Experiment Station, Fayetteville, AR. The total number of backcrosses to the recurrent parent, 57-4, was a minimum of four generations. Seeds were planted in peat pots in the greenhouse on May 1, and transplanted one month later in a Captina Silt Loam soil (Typic Fragiadult) 1 ft apart in 50 ft rows. The experimental design was a complete randomized block with three replications. Conventional weed and insect control measures were utilized as needed.

Leaf photosynthetic rate, stomatal conductance (COND), intercellular CO₂ concentration (C_i), transpiration (E), and water use efficiency (WUE) were measured on the fourth leaf (disease-free, fully sunlit) from the top on one plant in each plot using a Li-6200 portable photosynthesis system (Li-COR, Lincoln, NE, USA). The measurements were taken at four different sampling dates (7/25, 8/02, 8/06 and 8/19) within one hour between 900 and 1130 local time with individual leaves oriented perpendicular to the sun. The leaf temperatures at each sampling date were 30-32, 30-32, 33-35 and 29-31°C, respectively. The photosynthetic photon flux densities reaching the adaxial leaf surfaces ranged from 1500 to 2200 μmol m⁻² s⁻¹ on all measurements.

All data were analyzed by analysis of variance and correlation analysis based on alloplasmic means at each measurement date was used to assess the relationships among different gas exchange parameters (SAS Institute Inc., Cary, NC).

Results and Analysis

Fluctuation of gas exchange parameters: The results are shown in Table 1. PN was highest on the first measure date (7/25) and decreased thereafter through the third sampling date, showing a recovery on the fourth date following a period of high temperature and water stress conditions. Cotton plants showed a high water transpiration rate (E) during early August, resulting in the lowest water use efficiency (WUE). However, stomatal conductance reached a maximum at the second sampling date (8/02) and then declined.

PN: The alloplasmic lines with wild cytoplasm exhibited higher PN than the recurrent parent, 57-4 with AD2 cytoplasm, on the first three sampling dates, except for the D3-d alloplasmic line with a lower value on 8/06. Only the E1 and D3-d alloplasmic lines had a higher PN on the fourth sampling date (Table 2). E1 cytoplasmic line, one of the highest PN lines, was 24.3% higher than 57-4 on average across the four sampling dates. Averaged across the four sampling dates, all other wild cytoplasmic lines showed 7.2 to 11.8% higher PN than 57-4, with a mean of 11.3%. Because of large variation in replications, the differences were not significant in all cases. However, among the alloplasmic lines with cultivated or tetraploid cotton cytoplasm, A2 and AD1 cytoplasmic lines had PN performance very similar to 57-4 during four different measuring dates except for the AD1 cytoplasmic line at 7/25. The PN of 57-4 and the AD5 cytoplasmic line was expected to be similar because of their phyletic relationship, however, PN in the AD5 alloplasmic line was 12% greater than the AD2 line.

COND: In nearly all cases, the exotic cytoplasmic lines showed higher COND than 57-4 (Table 3). Wild cytoplasmic lines increased COND by 12.2-85.5% with an average 53% increase over the recurrent parent. The A2 cytoplasmic line resembled 57-4 with only a 6.2% increase. AD1 and AD5 cytoplasmic lines had much higher COND than 57-4 with 35.5 and 32.1% average increase, respectively.

E: Exotic cytoplasmic lines had higher water transpiration rate (E) on the first two sampling dates, and lower E on the last two dates (Table 4). Averaged across the four dates, A2 and AD1 cytoplasmic lines were similar to 57-4, indicating similarity among these three cytoplasm, a result also shown in PN. AD5 had 15.3% higher E numerically than 57-4, but the difference was not statistically significant. E for all wild cytoplasmic lines averaged from 6.3 to 13.7% higher than 57-4, but there were no statistically significant differences at 5% level.

Ci: Intercellular CO₂ concentration (Ci) varied little in these experiments. Except that the E1 cytoplasmic line had consistently lower Ci perhaps because of higher PN, all

cytoplasmic lines had very similar Ci (Table 5), indicating no significant effect of cytoplasm on Ci.

WUE: Except that E1 and C1 cytoplasmic lines had consistently higher water use efficiency (WUE) than 57-4, no consistent differences were noted among alloplasmic lines, which averaged 1.1-6.5% below 57-4 (Table 6). Although exotic cytoplasm had positive effects on PN, they did not increase water use efficiency because of the increase in water transpiration at the same time.

Correlation among parameters: There were significant positive associations among PN, COND and E (Table 7), indicating higher PN is correlated with higher COND and E. So, increasing PN and decreasing or maintaining E is a difficult task. However, obtaining high PN can be achieved by different mechanisms (Pettigrew and Meredith, 1994). The association of PN and COND was also observed by Leidi et al. (1993). A positive correlation between COND and Ci was also detected. There was no significant correlation between Ci and E. Higher stomatal conductance of water generally generates higher CO₂ conductance. Since WUE is the ratio of PN to E, failure to observe the expected association between WUE and PN, or WUE and E was surprising. In fact, significant negative correlations were detected between COND, Ci, or PN, and WUE in some cases. This means that higher PN was at the expense of higher water transpiration in some alloplasmic lines.

Discussion

In plant phylogenetic history, the nuclear genome co-evolves with cytoplasmic genomes (chloroplast DNA, cpDNA and mitochondria DNA, mtDNA) to develop a harmonious mechanism of interaction within species. When other specific cytoplasm are introduced into cultivated cotton nuclear genomes, the cytoplasm-nuclear interaction usually results in abnormal phenomena such as male sterility, external ovules, reduced or increased anther numbers and decreased yield. Because cpDNA and mtDNA are related to photosynthesis and respiration, respectively, the genes responsible for those physiological processes in different cotton species may have different DNA sequences. Photosynthetic genes such as the chloroplast gene encoding the large subunit of ribulose-1,5-bisphosphate carboxylase vary among species (Clegg, 1993). Wendel (1989) showed that tetraploid cotton species (AD) had very similar cpDNA genome. AD chloroplast genomes are very close to that of A-genome species, and differ from those of F, B, E, D and C genomes, ascendingly (Wendel and Albert, 1992). When the exotic cytoplasm replaced that of the cultivated tetraploid cotton (AD2), the interaction between the exotic cytoplasm and the cultivated tetraploid nuclear genome resulted in altered physiology. In these experiments, positive effects of the exotic cytoplasm on PN and COND were detected. However, A2, AD1 and AD5 cytoplasm had less effects on PN and COND than the

wild diploid cytoplasm. The cytoplasmic genomes can partially explain the higher PN in wild cotton species.

Residual genetic variation may exist in some of the alloplasmic lines due to insufficient backcross. This may partially account for the high variability among replicates for PN and other gas exchange parameters. Leidi et al. (1993) also observed high intra-genotypic variation for PN, E and COND, attributed partially to changing environmental conditions during measuring. An additional backcross has been made, which will be used to conduct similar experiments in the future.

Conclusions

1. Because of high temperature and water stress, lowest PN was observed during early August.
2. Wild cytoplasm generally had a positive effect on PN, while cytoplasm from cultivated or tetraploid cottons had less effect.
3. Exotic cytoplasm, especially wild ones, had a markedly positive effect on stomatal conductance of water, which was significantly correlated with PN and E.
4. Exotic cytoplasm had no significant effect on transpiration, water use efficiency or intercellular CO₂ concentration.
5. The cytoplasmic effects on PN and COND may be strongly influenced by crop age or physiological status.

Reference

Benedict, C. R., K. J. McCree, and R. J. Kohel. 1972. High photosynthetic rate of chlorophyll mutant of cotton. *Plant Physiol.* 49: 968-971.

Clegg, M. T. 1993. Chloroplast gene sequences and the study of plant evolution. *Proc. Natl. Acad. Sci. USA.* 90: 363-367.

El-Sharkawy, M., J. D. Hesketh, and H. Muramoto. 1965. Leaf photosynthetic rates and other growth characteristics among 26 species of *Gossypium*. *Crop Sci.* 5: 173-175.

Leidi, E. E., J. M. Lopez, M. Lopez, and J. C. Gutierrez. 1993. Searching for tolerance to water stress in cotton genotypes: photosynthesis, stomatal conductance and transpiration. *Photosynthetica* 28: 383-390.

Pettigrew, W. T., and W. R. Meredith, Jr. 1994. Leaf gas exchange parameters vary among cotton genotypes. *Crop Sci.* 34: 700-705.

Pettigrew, W. T., J. J. Heitholt, and K. C. Vaughn. 1993. Gas exchange differences and comparative anatomy among cotton leaf-types. *Crop Sci.* 33: 1295-1299.

Stewart, J. McD. 1990. New cytoplasm for cotton. *Proc. Beltwide Cott. Prod. Res. Conf.* p69-70.

Well, R., W. R. Meredith, Jr., and J. R. Williford. 1988. Heterosis in upland cotton: II. Relationships of leaf area to plant photosynthesis. *Crop Sci.* 28: 520-525.

Wendel, J. F. 1989. New world tetraploid cottons contain Old world cytoplasm. *Proc. Natl. Acad. Sci. USA.* 86: 4132-4136.

Wendel, J. F., and V. A. Albert. 1992. Phylogenetics of the cotton genus (*Gossypium*). Character-state weighted parsimony analysis of chloroplast-DNA restriction site data and its systematic and biogeographic implications. *Systematic Bot.* 17: 115-143.

Zhang, Jinfa, Jizhong Sun, and Jinlan Liu. 1990. Photosynthesis related characters of nectariless cotton. *J. Huazhong Agric. Univ.* 9: 59-63.

Zhang, Jinfa, Jizhong Sun, and Jinlan Liu. 1991. Effect of exotic cytoplasm on photosynthetic traits in upland cotton. *China Cottons* 18(1): 17-18.

Zhang, Jinfa, Jizhong Sun, and Jinlan Liu. 1993. Effect of frego bract on photosynthetic traits in upland cotton. *J. Huazhong Agric. Univ.* 12: 328-332.

Zhang, Jinfa, Zhongchi Deng, Bing Wang, Kaijing Zuo, Jinlan Liu and Jizhong Sun. 1995. A preliminary investigation of photosynthetic characteristics in the interspecific hybrid between *Gossypium hirsutum* and *G. barbadense*. *J. Huazhong Agric. Univ.* 14: 115-119.

Table1. Variation of gas exchange parameters across different dates.

| Parameter | 7/25 | 8/2 | 8/6 | 8/19 |
|---|--------|--------|--------|--------|
| PN ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | 21.25 | 18.04 | 15.75 | 19.61 |
| COND ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | 0.48 | 1.56 | 0.54 | 0.33 |
| E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | 5.56 | 14.40 | 9.31 | 3.65 |
| Ci ($\mu\text{l CO}_2 \text{ l}^{-1}$) | 169.00 | 238.83 | 213.38 | 158.15 |
| WUE ($\text{mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$) | 3.85 | 1.27 | 1.74 | 5.57 |

Table 2. Photosynthetic rate ($\mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in different alloplasmic lines of cotton

| Cytoplasm | 7/25 | 8/2 | 8/6 | 8/19 | Mean |
|-----------|-------|-------|-------|-------|-------|
| A2 | 17.81 | 15.87 | 13.35 | 19.69 | 16.68 |
| B1 | - | 20.17 | 17.14 | 20.17 | 19.16 |
| C1 | 21.98 | 17.89 | 17.52 | 16.87 | 18.57 |
| D2-2 | 20.44 | 18.63 | 16.11 | 18.77 | 18.49 |
| D3-d | 21.14 | 18.46 | 12.87 | 21.02 | 18.37 |
| D8 | 23.30 | 17.73 | 15.65 | 17.70 | 18.60 |
| E1 | 22.33 | 22.72 | 18.68 | 21.47 | 21.30 |
| AD1 | 21.35 | 16.37 | 14.85 | 20.52 | 18.27 |
| AD2 | 17.79 | 15.80 | 14.51 | 20.44 | 17.14 |
| AD5 | 23.27 | 16.73 | 16.81 | 19.43 | 19.06 |

Table 3. Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) in different alloplasmic lines of cotton

| Cytoplasm | 7/25 | 8/2 | 8/6 | 8/19 | Mean |
|-----------|-------|-------|-------|-------|-------|
| A2 | 1.005 | 3.025 | 1.033 | 0.766 | 1.457 |
| B1 | - | 6.037 | 1.361 | 0.841 | 2.746 |
| C1 | 1.389 | 4.044 | 1.549 | 0.642 | 1.906 |
| D2-2 | 1.088 | 5.271 | 1.812 | 1.025 | 2.299 |
| D3-d | 1.181 | 2.934 | 0.983 | 0.979 | 1.519 |
| D8 | 1.587 | 5.263 | 1.316 | 0.747 | 2.228 |
| E1 | 1.230 | 4.146 | 1.612 | 0.903 | 1.973 |
| AD1 | 1.282 | 3.774 | 1.403 | 0.926 | 1.846 |
| AD2 | 0.983 | 2.360 | 1.201 | 0.873 | 1.354 |
| AD5 | 1.323 | 3.361 | 1.650 | 0.834 | 1.792 |

Table 4. Water transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) in different alloplasmic lines of cotton

| Cytoplasm | 7/25 | 8/2 | 8/6 | 8/19 | Mean |
|-----------|-------|--------|--------|-------|-------|
| A2 | 4.718 | 14.233 | 8.433 | 3.400 | 7.696 |
| B1 | - | 16.633 | 9.150 | 3.400 | 9.728 |
| C1 | 5.913 | 14.300 | 10.767 | 2.967 | 8.487 |
| D2-2 | 4.988 | 14.767 | 9.733 | 4.433 | 8.480 |
| D3-d | 5.828 | 13.933 | 8.067 | 3.900 | 7.932 |
| D8 | 6.515 | 14.967 | 8.867 | 3.467 | 8.454 |
| E1 | 5.376 | 14.400 | 10.067 | 3.467 | 8.328 |
| AD1 | 5.566 | 13.767 | 8.667 | 3.933 | 7.983 |
| AD2 | 5.083 | 12.533 | 9.333 | 3.633 | 7.646 |
| AD5 | 6.078 | 14.433 | 9.967 | 3.933 | 8.603 |

Table 5. Internal CO₂ concentration ($\mu\text{l CO}_2 \text{l}^{-1}$) in different alloplasmic lines of cotton

| Cytoplasm | 7/25 | 8/2 | 8/6 | 8/19 | Mean |
|-----------|--------|--------|--------|--------|--------|
| A2 | 169.57 | 236.27 | 204.53 | 147.00 | 189.34 |
| B1 | - | 240.87 | 212.95 | 155.93 | 203.25 |
| C1 | 168.57 | 235.80 | 213.87 | 145.00 | 190.81 |
| D2-2 | 161.87 | 228.23 | 220.03 | 175.27 | 196.40 |
| D3-d | 173.00 | 240.40 | 211.87 | 166.03 | 197.83 |
| D8 | 181.40 | 244.33 | 210.73 | 159.57 | 199.01 |
| E1 | 158.10 | 255.73 | 211.50 | 153.03 | 194.59 |
| AD | 171.50 | 233.17 | 214.17 | 162.97 | 195.45 |
| AD2 | 166.30 | 229.73 | 216.30 | 162.23 | 193.64 |
| AD5 | 170.70 | 243.50 | 217.80 | 154.50 | 196.63 |

Table 6. Water use efficiency ($\text{mmol CO}_2 \text{mol H}_2\text{O}^{-1}$) in different alloplasmic lines of cotton

| Cytoplasm | 7/25 | 8/2 | 8/6 | 8/19 | Mean |
|-----------|-------|-------|-------|-------|-------|
| A2 | 3.770 | 1.114 | 1.657 | 5.824 | 3.091 |
| B1 | - | 1.206 | 1.856 | 6.186 | 3.083 |
| C1 | 3.871 | 1.298 | 1.646 | 6.188 | 3.251 |
| D2-2 | 4.252 | 1.238 | 2.082 | 4.300 | 2.968 |
| D3-d | 3.661 | 1.327 | 1.644 | 5.419 | 3.013 |
| D8 | 3.584 | 1.209 | 1.757 | 5.191 | 2.935 |
| E1 | 4.193 | 1.633 | 1.856 | 6.295 | 3.494 |
| AD1 | 3.870 | 1.212 | 1.709 | 5.199 | 2.998 |
| AD2 | 3.626 | 1.258 | 1.557 | 6.064 | 3.125 |
| AD5 | 3.830 | 1.171 | 1.673 | 4.992 | 2.917 |

Table 7. Correlation analysis of gas exchange parameters

| Parameter | Date | Ci | E | PN | WUE |
|-----------|------|--------|---------|---------|---------|
| COND | 7/25 | 0.2034 | 0.9128 | 0.9086 | 0.2284 |
| COND | 8/2 | 0.2774 | 0.4844 | 0.9358 | -0.5920 |
| COND | 8/6 | 0.7999 | 0.6392 | 0.8268 | 0.1398 |
| COND | 8/19 | 0.7788 | 0.8021 | 0.9295 | -0.5530 |
| Ci | 7/25 | | 0.2527 | -0.1136 | -0.7502 |
| Ci | 8/2 | | -0.0086 | 0.1609 | -0.1210 |
| Ci | 8/6 | | 0.2588 | 0.6056 | -0.0837 |
| Ci | 8/19 | | 0.3076 | 0.7573 | -0.7699 |
| E | 7/25 | | | 0.8654 | 0.0005 |
| E | 8/2 | | | 0.6382 | 0.2857 |
| E | 8/6 | | | 0.7625 | 0.3764 |
| E | 8/19 | | | 0.7045 | -0.0819 |
| PN | 7/25 | | | | 0.4859 |
| PN | 8/2 | | | | -0.5435 |
| PN | 8/6 | | | | -0.0441 |
| PN | 8/19 | | | | -0.7425 |