Comparative Physiological and Metabolomic Analysis of Arabidopsis Accessions and Their F? Hybrids

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Abstract

Enhancement of growth development has contributed greatly to worldwide agricultural yields demanded by a growing human population. The superior hybrid performance of F_1 hybrids over their parents is defined as heterosis or hybrid vigor, which has been studied widely by many plant breeding specialists and geneticists. Recently, changes in transcriptomes, epigenetic factors (DNA methylation and small RNA), or metabolomes between F_1 hybrids and parents have been reported. However, knowledge of the molecular mechanism underlying heterosis remains fragmented. As an increase in biomass is characterized by multiple factors, it is conceivable that a variety of factors due to individual differences and growth environments are responsible.

Photoperiods and carbohydrate sources are two important environmental factors controlling plant growth and development. The two widely used accessions, Col and C24, may affect regulation of physiological traits in response to daylight length and exogenous sucrose. Moreover, F_1 hybrids of Col and C24 showed a heterotic phenotype in early developmental stages. Most studies have focused their analysis of natural variation on limited growth conditions or accession in various growth conditions. Further research will be necessary to acquire a better understanding of the functional system. We conducted this study to evaluate the extent of metabolic changes among two *Arabidopsis* accessions, Col and C24, and their F1 hybrid under SD (12 h light and 12 h dark) and LD (16 h light and 8 h dark) photoperiods with/without exogenous sucrose.

We found that the length of the photoperiod showed a significant positive effect on Col, C24, and F_1 hybrids, whereas exogenous sucrose strongly affected plant growth in Col and F_1 hybrids. Comparative metabolome profiling among Col, C24, and their F_1 hybrids by GC-TOF-MS revealed that almost all metabolites were regulated in a genotype-specific manner rather than

by growth conditions. We found alterations in TCA intermediates correlated with enhanced biomass under LD conditions. The presence of exogenous sucrose leads to higher accumulation of seven identified metabolites including four sugars, a polyamine and two amino acids in C24, whereas no such accumulation was observed in Col and F_1 hybrids. Moreover, these metabolites indicated significant interactions between genotypes and external sucrose by three-way analysis of variance ($P < 0.01$). These results provided new insights into how plant metabolomic functions respond to environmental conditions that affect plant growth and development.

Next, we took advantage of *Arabidopsis* intraspecific hybrids to identify heterosis-related metabolites. In this portion of the study, the establishment of a new cultivation system was attempted to minimize environmental factors as much as possible. To equalize the growth stage of the plants, F_1 hybrids and their parents were checked for the timing of germination and sowing time points were switched.

A total of 200 *Arabidopsis* accessions were used in this study. In the selection process, late-flowering accessions were eliminated. The accessions with significant differences in seed size to Col (as the control accession) were continuously omitted because of the effect on seed germination time and seedling growth rates. Twenty-eight accessions were selected to generate F_1 hybrids with controlled growth conditions and a matched germination time. Effects of the parent-of-origin were determined on seed area and germination time from 56 hybrids. On the basis of seedling biomass, we established divergent groups according to heterosis level. The combinations with high heterosis of F_1 hybrids were associated with a statistically significant increase of 6.1% to 44% ($P < 0.05$) over BPV, whereas the low vigor and no-heterosis hybrids ranged from −19.8% to 9.8% over BPV.

We then conducted metabolomic analysis of growth and development during the early developmental stage of hybrids indicating high heterosis, including Col/C24 and Col/Rld-1, and low heterosis, including Col/Ws-0 and Col/L*er*-1. Our finding suggests that one of the key factors controlling growth development is change in TCA cycle intermediates coupled with metabolic support of the higher fumarate/malate ratio, which is associated with enhanced biomass on either photosynthetic response or high heterosis. The main function of the TCA process is not only to produce energy, but also reported to photosynthesis and nitrogen assimilation. In summary, we hypothesize hybrids with high heterosis can speed up TCA flux to produce meet energy demands for enhanced biomass production. Further study is needed to reveal the epigenomic, genomic, and/or other factors that play a master role in controlling differences in the TCA cycle. Such progress would contribute to meeting growing food demands and sustainable development for the near future.