Journal of Plant Physiology 216 (2017) 202–211

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Original article

High photosynthetic capability observed in the wheat germplasm with rye chromosomes

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ARTICLE INFO

Keywords:
Yield potential
Photosynthetic capability
Rubisco
Bread wheat
Triticale
Synthetic hexaploid wheat

ABSTRACT

Improving photosynthetic capability is one of the most important factors for increasing wheat yield potential. The photosynthetic capability of wheat germplasm with different alien chromosomes was investigated and compared with bread wheat cultivars (BC) in this study, including wheat addition lines (CA), hexaploid *triticale* (HT), octoploid *triticale* (OT), and synthetic hexaploid wheat lines (SHW). Results indicated that HT, OT, and SHW produced significantly higher biomass plant^{-1}(BMPP), with HT displaying the highest grain yield plant^{-1} (GYPP). Distinct superiority of net photosynthetic rate (Pn) and carboxylation activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was observed in HT and OT. Meanwhile, OT showed the highest expression of the Rubisco large subunit gene (*rbcL*) in the flag leaves at heading and grain-filling stages, though the coding region of *rbcL* was highly conserved in all investigated materials. Further analysis indicated that OT and Chinese Spring-rye disomic addition lines displayed higher expression of Rubisco small subunit gene (*rbcS*). Correlation analysis revealed significant and positive correlations between Pn and the expressions of *rbcL* (at both heading and grain-filling stages), the expression of *rbcS* (at heading stage), and the carboxylation activity of Rubisco (at grain-filling stage). Anatomical structure analysis of the chloroplasts showed SHW with longer chloroplasts and more chloroplast grana and grana lamella. In the present study, HT, OT, and Chinese Spring-rye disomic addition lines with rye chromosomes displayed greater photosynthetic capability than BC and SHW, and could be applied in breeding programs to improve the photosynthetic efficiency of wheat.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereals, providing approximately 20% of calories and protein in the global human diet (Braun et al., 2010). Strategies to advance yield and cropping intensity are significant for increasing the future crop production globally (Alexandratos and Bruinsma, 2012; Gregory and George, 2011). Photosynthesis is the fundamental physiological process of crop biomass accumulation during crop growth. Previous studies have classified significant associations between leaf photosynthetic rate and grain yield in wheat cultivars (Gaju et al., 2016). Improvement of photosynthetic efficiency is essential to further exploit the potential of yield increase in elite wheat in the post-green revolution era (Ort et al., 2015).

Wild germplasm can be used in physiological breeding due to its wide genetic variation of disease resistance and net photosynthetic rate. Though showing leaf morphology similar to *T. aestivum*, some of the *T. dicoccoides* accessions exhibited higher leaf CO_2 assimilation (Johnson et al., 1988). A multitude of synthetic hexaploid wheat has been hybridized with common wheat, and beneficial genes have been transferred and used in wheat breeding programs (Ren et al., 2008; Yang et al., 2010; Reynolds et al., 2015). Meanwhile, the presence of the 1RS translocation in spring cultivar “Pavon” has been shown to increase root biomass and improve tolerance to field environmental stresses when compared to Pavon (Ehdaie et al., 2003).

As a C_3 cereal, the pivotal enzyme of wheat is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), catalyzing the initial step of the Calvin-Benson cycle through carboxylation of CO_2 with ribulose-1,5-bisphosphate (RuBP). This step is acknowledged as the restrictive node in elevating photosynthetic efficiency in field crops (Spreitzer and Salvucci, 2002). Although Rubisco is the most abundant protein in plant leaves (Ellis, 1979), its poor efficiency of photosynthetic productivity can be explained by the complex enzyme reaction involved in the photosynthetic mechanism (Andersson, 2008; Tcherkez, 2013). Thus, optimizing the positive ability of Rubisco is crucial for elevating crop productivity and resource utilization (Parry et al., 2007). The functional structural sites of Rubisco are localized on its large subunits (*rbcL*) (Andersson, 2008), and its catalytic activity is influenced by the

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http://dx.doi.org/10.1016/j.jplph.2017.06.012
Received 9 March 2017; Received in revised form 25 June 2017; Accepted 28 June 2017
Available online 04 July 2017

expression of its different forms of small subunits (*rbcS*), which are encoded in the nucleus by a multigene family (Morita et al., 2014). The small subunits are more inclined to vary the catalysis and modify the dynamic structure of the complex L_8S_8 Rubisco, as supported by an analysis of mutant and natural Rubisco structures (Van Lun et al., 2011). Meanwhile, environmental drivers affecting the CO_2 availability for carboxylation or directly shifting the photosynthetic limitations between Rubisco and RuBP regeneration determine to what extent Rubisco kinetics are optimally suited to maximize the CO_2 assimilation rate (Galmes et al., 2014). A previous study illustrated that genetic changes can contribute to the evolution of photosynthesis, and parallel amino acid replacements were associated with adaptive changes in Rubisco (Kapralov et al., 2012). To understand the recombination diversity of Rubisco catalytic properties, Whitney et al. (2011) revealed that Ile in C_4 plant substitution of Met in C_3 in the large subunit acts as a catalytic switch through transplastomic tobacco lines expressing native and mutated Rubisco large subunits (L-subunits) from *Flaveria pringlei* (C_3), *Flaveria floridana* (C_3-C_4), and *Flaveria bidentis* (C_4). Similarly, Ishikawa et al. (2011) introduced the small subunit (*rbcS*) of high kcat Rubisco from the C_4 plant sorghum (*Sorghum bicolor*) into rice (*Oryza sativa*) and significantly enhanced the catalytic turnover rate of Rubisco.

The photophosphorylation, photosynthetic electron transport, and reaction exhibited fluctuations as the structure of the leaf blade and activity of chloroplasts changed, and ultimately influenced the photosynthetic rate (Nir and Poljakoff-Maybep, 1967). A study by Fellows and Boyer (1976) proposed that altering the conformation of the lamellar membranes of leaves and structure of chloroplasts at various water conditions can influence the PS II activity. Consequently, by examining leaf anatomical features related to C_4 photosynthetic activity, significant changes in photosynthetic activity were observed in cuticle thickness, stomatal size and frequency, mesophyll anatomy, and interveinal distance; variations in those characteristics could be attributed to ontogenetic differences (Miranda et al., 1981). These results inspired us to examine the variable structure of chloroplasts in different types of wheat germplasm.

The goal of this study was to identify the variations in photosynthetic characteristics and yield traits among wheat germplasm with different alien chromosomes, and to clarify the physiological, molecular, and anatomical bases causing these variations. Therefore, the expression levels of genes encoding the large subunit (*rbcL*) and small subunit (*rbcS*) of Rubisco, the carboxylation activity of Rubisco, and the ultrastructure of flag leaf were analyzed further, with the prospect that they are likely to improve photosynthetic efficiency in bread wheat breeding.

2. Materials and methods

2.1. Plant materials and field experiments

Five types of wheat germplasm were used in this study (Table 1), including bread wheat cultivars (BC), disomic alien chromosome addition lines (CA) with the Chinese Spring background, hexaploid *Triticale* lines (HT), octoploid *Triticale* lines (OT), and synthetic hexaploid wheat lines (SHW). With the exception of HT-Certa and HT-Woh45, the CA, HT, OT, and SHW lines were generously provided by Professor Tsujimoto Hisashi (Arid Land Research Center, Tottori University, Japan). All materials used in this study were selected with similar phenology from a pre-screen experiment conducted with 108 wheat germplasm in the 2013–2014 growing season.

The field experiments were conducted during two winter cropping seasons (October to June 2014–2015 and 2015–2016) in the Institute of Water Saving Agriculture in Arid Regions of China, Northwest A & F University, Yangling, Shaanxi, China (34°7′N, 108°4′E). Materials were planted with two replications in a randomized complete block design; each line was planted in three rows 1.8 m in length, with 25 cm between rows and 6.67 cm between plants. Fungicides and insecticides were applied as required to prevent insect and disease damage. The distributions of monthly rainfall and temperature in the growing seasons are presented in Fig. S1 and Fig. S2.

2.2. Agronomic traits investigation

At the maturity stage, 10 plants were harvested randomly from each material for each replication. Yield-related agronomic traits, including spike length (SL), number of spikelet spike^{-1}(SN), and number of grains spike^{-1}(GNPS), were recorded. The plants were dried in the sun and biomass plant^{-1} (BMPP), grain yield plant^{-1} (GYPP), and thousand kernel weight (TGW) were determined.

2.3. Assessment of net photosynthesis rate

At the heading (Z55) and grain-filling (Z73) stages, net photosynthesis rate (Pn) was measured in healthy flag leaves using the Li-6400 portable photosynthesis system (LI-COR Biosciences, USA). Measurement conditions were set as follows: 400 μmol mol^{-1} CO_2 concentration, 1200 μmol m^{-2}s^{-1} photosynthetic photon flux density (PPFD), and 20 °C. Five plants were selected for each line in each replication between 9:00 and 11:00 a.m. on a sunny and windless day.

2.4. Expression analysis of rbcL and rbcS

The sequences encoding Rubisco large subunits (*rbcL*) of different *Triticeae* were obtained from GenBank (http://www.ncbi.nlm.nih.gov/nucleotide/), including *Aegilops tauschii* (LN626630), *Elymus cilliaries* (HQ652780), *Elymus trachycaulus* (JN965509), *Leymus mollis* (KC483088), *Secale cereale* (rye, LN626639.1), *Agropyron elongatum* (AY836174), *Triticum aestivum* (AY328025), and *Turgidum* (KJ614401). Primers (Table S1, *rbcL*-F and *rbcL*-R) were designed based on the consistent section of the coding region to detect the general expression of *rbcL* in flag leaves of all genotypes. The conserved coding region of the Rubisco small subunit (*rbcS*) in *Triticum aestivum* (AB042068), *Turgidum* (AJ635206), *Aegilops tauschii* (AB020938), and *Secale cereale* (rye, AB020942) was used to design primers (Table S1, *rbcS*-F and *rbcS*-R) for the specific and total expression of *rbcS* in BC, Chinese Spring-rye disomic addition lines (TACBOW0018, TACBOW0029), HT, OT, and SHW. The reference gene *TaActin* was used for background standardization using the primers of *Actin*-F and *Actin-R* (Table S1).

Fully expanded flag leaf samples were taken at heading (Z55) and grain-filling (Z73) stages, respectively. A three-step RNA extraction was carried out using a modified hot phenol method; the cDNA samples were synthesized and stored at −20 °C for subsequent analysis (Zheng et al., 2015). The cDNA sample from each genotype was replicated three times as per specifications of the SYBER Premix ExTaq Kit (Takara, Dalian) and qRT-PCR were conducted using ABI7300 real time PCR system (Applied Bio Systems, USA). The reaction mixture consisted of 10 μL 2X SYMBER MIX, 0.4 μL of each of the forward and reverse primers (0.8 μM), and 1.5 μL template cDNA (100 ng); ddH_2O was added to the final volume of 20 μL. The qRT-PCR reaction was programmed as initial denaturation at 95 °C for 20 s, followed by 40 cycles at 95 °C for 5 s, and 60 °C for 30 s. Relative expression of the target gene was calculated as:

$$NE = \frac{2^{-CTX}}{2^{-CTR}}$$

NE indicates the relative expression of the target gene, 2 is the primer efficiency, CTX indicates CT values of the target gene, and CTR indicates the geometric mean of values of the reference gene (Ramakers et al., 2003; Rieu and Powers, 2009).