



Accessing the agronomic and photosynthesis-related traits of high-yielding winter wheat mutants induced by ultra-high pressure



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ABSTRACT

To generate new wheat breeding lines, ultra-high pressure (UHP) (using castor oil as the medium) was used to induce mutant lines from the wheat variety ‘Yanzhan 4110’. Three high-yielding mutant lines (Gaoya 2, Gaoya 3 and Gaoya 9) were selected from 1580 mutants induced by UHP and were evaluated for agronomic and photosynthesis-related traits in this study. Our results showed that these mutant lines retained higher grain yield than Yanzhan 4110 and exhibited higher biomass and harvest index, significantly higher plant height, spikelet numbers per spike and higher kernel numbers per spike but similar or lower thousand-kernel weight ($\alpha = 0.05$). The reading of relative leaf chlorophyll content (SPAD) and photosynthetic traits of the high-yielding mutant lines varied to different degrees. The mutant lines had higher SPAD and higher stomatal conductance (Gs) than Yanzhan 4110 at two grain-filling stages. The net photosynthetic rate (Pn), apparent mesophyll conductance, Gs and transpiration rate (Tr) of Gaoya 2 were significantly higher than those of Yanzhan 4110 at the mid grain-filling stage (GF-2) ($\alpha = 0.05$). The decreases in SPAD, Pn, Gs, transpiration efficiency (TE) and Tr in the high-yielding mutant lines were smaller than those in Yanzhan 4110 during the early grain-filling stage (GF-1) and GF-2. Fluorescence parameters, including F0, Fm, Fv, Fv/F0, and Fv/Fm, were higher in the three high-yielding mutant lines, while qN was significantly lower at GF-1 and GF-2 compared with those in Yanzhan 4110 ($\alpha = 0.05$). Higher qP and Φ PSII values were also observed in the three high-yielding mutant lines. These results indicate that wheat mutations induced by UHP exhibited high yield, improved photosynthetic efficiency, decreased thermal dissipation and altered photoinhibition.

1. Introduction

Wheat is the one of the most important agricultural crops, providing the primary food supply in the Weibei rain-fed highland area in Shaanxi, China. Wheat yield improvement has been a priority for studies throughout the world. Great achievements have been made in wheat production through genetic improvement, developing the locally adapted varieties and management practices such as weed control and fertilizer utilization (Reynolds et al., 2009, 2012; Fischer and Edmeades, 2010). In the Weibei rain-fed highland area in Shaanxi, some factors still limit the survival and productivity of wheat. These factors include low rainfall, changes in soil structure due to excessive fertilizer use and limited genetic diversity caused by cultivar

domestication and artificial selection, resulting in decreased drought resistance (Shen et al., 2013; Budak et al., 2013). Therefore, the development of high-yielding wheat lines that are well adapted to current and future climatic conditions is one viable means of increasing wheat production and reducing agriculture costs. Low grain yield for wheat has been attributed in part to low photosynthetic and physiological capacity (Xue et al., 2002) and/or to early leaf senescence during the grain-filling stage (Borrell et al., 2000). The primary components of grain yield are the spike number per hectare, kernel number per spike, and thousand-kernel weight. Adjustments to the grain yield and yield components are often used for improving the wheat yield (Slafer et al., 2014). Improvement of some physiological traits has been important for increased yield in the past, although traits were not selected

Abbreviations: UHP, ultra high pressure; SPAD, reading of relative leaf chlorophyll content; Pn, net photosynthetic rate; g_m , apparent mesophyll conductance; Gs, stomatal conductance; TE, transpiration efficiency; Tr, transpiration rate; Ls, stomatal limitation; F0, minimum fluorescence yield of a dark-adapted leaf; Fm, maximum fluorescence yield of a dark-adapted leaf; Fs, steady-state fluorescence; Fm', maximum fluorescence yield of a light-adapted leaf; Fv, variable fluorescence; Fv/F0, potential activities; Fv/Fm, maximum photochemical efficiency; qN, non-photochemical quenching coefficients; qP, photochemical quenching coefficient; Φ PSII, the quantum efficiency of photosystem II; GF-1 stage, the early grain-filling stage; GF-2 stage, the mid grain-filling stage

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consistently (Landivar et al., 2010).

Physical and chemical mutagens are two basic means of mutation breeding, which can induce mutations and enrich genetic diversity. Physical mutagenesis has been conducted using energy sources such as X-, β -, and γ -rays and neutrons (Jia and Li, 2008). EMS (an alkylating agent, ethyl methanesulfonate) is a widely used chemical mutagen that produces a high frequency of nucleotide substitutions (Yadav et al., 2016). Ultra-high pressure (UHP) is a new technology used in the food processing industry (Toro-Funes et al., 2014). With added pressure (100–1000 MPa) as the power factor and a liquid (usually soil, water or a combination of water and soil) as the medium of transmission, materials (such as food) are pressurized in a UHP vessel at room temperature or low temperature (below 100 °C) conditions. The use of UHP technology has achieved food sterilization and material modification, generated new organizational structures, and resulted in changes in food quality and some physical and chemical reaction rates (Chen, 2005; Zhang et al., 2008).

UHP technology was applied for the first time in 1986 in Japan (Kinugasa et al., 1993), and it has been studied and used as an alternative to traditional treatments for food and mutations for decades (Huang et al., 2007; Liu et al., 2013b). UHP technology is the subject of a growing body of research on the production of food, sterilization and the development of processed foods (Huang et al., 2007; Gonzalez and Barrett, 2010; Bajovic et al., 2012; Chen et al., 2012; Pina-Pérez et al., 2012; Balasubramaniam et al., 2015). Pressure is one of the basic thermodynamic variables that impacts protein conformation, and UHP has been widely used in physics and biology for thermodynamic and kinetic studies on bioprocesses and biosystems. High hydrostatic pressure treatment (using water as the medium) has received considerable attention in the field of rice breeding, and some successes have been reported for selecting high-yielding and high-quality rice mutant lines (Bai et al., 2003a,b; Li et al., 2003a,b). In addition, UHP has many advantages, including heritable variation induction, mutation stability in offspring, simple equipment, lower costs, non-polluting technology and minimal hazards to humans and the environment, compared with other mutation induction technology (Wang et al., 2010; Liu et al., 2013a).

The effect of UHP treatment (using castor oil as the medium) on wheat mutation has been previously reported. The castor oil is inexpensive and environmentally friendly, has a good flow properties and the thermal insulation (Ogunniyi, 2006). The average seedling height and fresh weight of the wheat were both greater when water-packed wheat seeds were treated with 95 MPa of pressure for various times (4 h, 8 h, and 12 h) compared with seeds not treated with pressure (Liang et al., 2005). Wang et al. (2010) noticed that the wheat protein of wheat varieties was ruptured and systematized and that the starch granule was broken when vacuum-packed wheat seeds were subjected to 200 MPa of pressure, with a pressure-rising time of 6 min and a pressure-holding time of 6 h. Wang et al. (2013) reported that the total number of gliadin spectra was decreased but that some dough-related qualities were improved in wheat mutant strains induced by 120-MPa UHP treatment. A study by Xiong et al. (2014) focused on drought resistance variation of wheat mutant lines generated by UHP and found that some wheat mutants had significantly different drought resistance coefficients ($DCR = \text{Mean}_{\text{water-stressed}}/\text{Mean}_{\text{well-watered}}$) compared with the control, which was not treated with 120-MPa UH Bai et al. (2003a,b) and Li et al. (2003b) noted that high hydrostatic pressure treatment (using water as the medium) could be an effective means of selecting for rice cultivars with high yield and high quality. However, little research has been conducted to investigate the photosynthesis of wheat mutant strains induced by UHP. Photosynthetic characteristics and agronomic traits were reported to be altered in wheat mutant lines generated by 120-MPa UHP (Liu et al., 2013b).

Despite research on protein, ribonuclease and processed foods, the effects of UHP (using castor oil as the medium) reported in previous studies on wheat focused mainly on the effects on wheat seed

germination and seedling growth. There is relatively little research on wheat mutants induced by UHP, particularly on the changes in agronomic traits, leaf gas exchange and chlorophyll fluorescence among high-yielding wheat mutant lines.

This study selected and isolated three high-yielding wheat mutant lines using UHP (using castor oil as the medium) based on previous results in our program. The objectives of this study were (1) to compare three high-yielding wheat mutant lines and the control, Yanzhan 4110, in yield components, SPAD, and leaf gas exchange and fluorescence parameters and (2) to increase our knowledge of the use of UHP in wheat mutant induction and breeding.

2. Materials and methods

2.1. Plant materials

The winter wheat variety Yanzhan 4110 was introduced to our program from National Crop Variety Regional Test Station, Yangling in 2006. This variety exhibits high and stable yield, early maturity, high tiller number and low spike number. Yanzhan 4110 was used in this experiment for the induction of mutations by UHP using castor oil as the medium. A total of 4000 seeds of Yanzhan 4110 were treated under 120 MPa for eight hours in September of 2007 at the College of Food Science and Engineering, Northwest A & F University, Yangling, China. The seeds treated by UHP and the control Yanzhan 4110 seeds were planted in the field; the plots were 1.0 m long and 2.5 m wide, with 0.1 m between seeds, 10 rows and 0.25 m between rows. Approximately 1580 plants with similar early maturity and higher kernel numbers per spike than Yanzhan 4110 were regenerated from the M1 generation. A total of 27 lines exhibited higher kernel numbers per spike and higher grain yield in the field in 2008, 2009 and 2010. Of these 27 lines induced by UHP, three highest-yielding wheat mutant lines, Gaoya 2, Gaoya 3 and Gaoya 9, were selected.

2.2. Ultra-high pressure treatment

Wheat seeds were first soaked in water for six hours and then packed with water in polyethylene bags (20 × 28 mm). Water-packed seeds were packed again to prevent contact between the pressurization fluid (castor oil) and the suspension. The UHP treatment was performed using a 15L-800 High Pressure Food Processor (jointly developed by the College of Food Science and Engineering of Northwest A & F University and Bao Tou KeFH High Pressure Technology Co., Ltd., Inner Mongolia, China) as described by Liang et al. (2005) and Wang et al. (2010). The seeds were treated with 120-MPa pressure for eight hours. The temperature of the UHP vessel was thermostatically controlled at 20 °C throughout the treatment.

2.3. Field experiments

The experiment was conducted in the research unit of the North Campus, Northwest A & F University, Yangling, Shaanxi, China (34°20'N, 108°24'E, elevation 526 m), during the 2011–2012 and 2012–2013 growing seasons (October to June). The field experimental plots were arranged in a randomized complete block design with three replicates. The three high-yielding mutant lines and Yanzhan 4110 were planted at a rate of 300 seeds per m². Each plot was 2.5 m × 1.5 m, with 10 rows and 0.25 m between rows. The soil is loess soil, had the organic matter of 11.3 g kg⁻¹, total N content of 0.80 g kg⁻¹, the available N content of 7.5 mg kg⁻¹, the available P content of 14.7 mg kg⁻¹, the available K content of 109.5 mg kg⁻¹ and pH of 8.0 for the 0–20 cm soil layers at the seeding of wheat field. The N, P and K fertilizers were applied with incorporating urea (N, 46%), diammonium phosphate (P₂O₅, 45%; N, 18%) and potassium sulphate (K₂O, 48%) into the 0–20 cm layers before wheat seeding. Herbicides, fungicides, and insecticides were applied as required. The rainfall from

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