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# Coronatine enhances drought tolerance in winter wheat by maintaining high photosynthetic performance

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ABSTRACT

Coronatine (COR) is a phytotoxin produced by *Pseudomonas syringae*. Its structure is similar to those of jasmonates (JAs), which play diverse roles in multiple plant biotic and abiotic defenses. However, the biological activity of COR is 1000 times greater than the activity of JA. In addition to being involved in the JA pathway, COR affects plant photosynthetic efficiency. In this study, we examined wheat blade pretreatment with COR. Blades treated with COR remained green longer than those of control plants under drought stress conditions, resulting in less yield loss with COR treatment. To investigate the mechanism of COR in drought resistance further, we employed two-dimensional gel electrophoresis technology and matrix-assisted laser desorption/ionization mass spectrometry to sequester and identify key proteins. Six COR-inducible proteins that are located in the chloroplast and involved directly in photosynthesis were found. The wheat homologue of protein gi|326509937 is degradation of periplasmic proteins 1 (DEGP1) in *Arabidopsis*, which is a response to photosystem II reparation, and was maintained at a low level with COR treatment. Finally, we measured levels of chlorophyll and photosynthetic performance to reveal the phenotypic effect of COR. Taken together, the results demonstrate that COR enhances drought tolerance by maintaining high photosynthetic performance.

## 1. Introduction

Food security depends mainly on the yield gain of major cereal crops, but the annual rate of crop yield improvement is not keeping pace with the projected future demand for food (Grassini et al., 2013). Drought is the most important abiotic stress limiting crop yields (Araus et al., 2002; Jiangkang, 2016). Most agricultural crop production relies on rainfall during the growing season, so droughts can be potentially catastrophic and can have unpredictable impacts on crop yields. Wheat (*Triticum aestivum* L.), one of the main cereal crops worldwide, is particularly susceptible to drought stress during the jointing and grain filling stages (Noorka et al., 2009). Drought directly affects plant growth and grain mass during these stages. It has been classified as one of the major adversities for wheat (Aimin et al., 2017; Boyer, 1982; Samarah et al., 2009).

Photosynthesis is one of the key processes affected by drought stress, and reduction of photosynthesis leads to decreased plant growth and grain yield (Chaves et al., 2003; Du and Bramlage, 1992; Flexas et al., 2004; Lawlor and Tezara, 2009). Pinheiro and Chaves (2011) revised the current status of the physiological limitations to photosynthesis under drought and performed a meta-analysis covering more than 450 papers published in the last 15 years with the goal of strengthening our understanding of the complex network of interactions and regulations of photosynthesis in plants subjected to drought stress. Many measures can be used to improve photosynthetic efficiency under drought, including irrigation, fertilization, and spraying of plant growth regulators. In a previous study, we found that the plant growth regulator coronatine (COR) could maintain more stable chloroplast structure under heat stress (Yuyi et al., 2015).

COR is a chlorosis-inducing non–host-specific phytotoxin produced by several *Pseudomonas syringae* pathovars (Aaron and Mark, 2009; Bender et al., 1999). It is a structural mimic of jasmonates, but it is more active. COR and jasmonates share the same receptor, coronatine insensitive 1, in plants (Chini et al., 2007; Uppalapati et al., 2005; Jianbin et al., 2009). Moreover, COR can lead to leaf chlorosis, anthocyanin production, ethylene emission, auxin synthesis (Feys et al., 1994; Uppalapati et al., 2008), opening of stomata allowing bacterial entry, bacterial growth in the apoplast, systemic susceptibility, and

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disease symptoms (Paul and Silke, 2006; Zheng et al., 2012). However, microdoses of COR play important roles in resisting abiotic stress, such as improving salt stress tolerance in cotton (Zhixia et al., 2008), cold resistance in cucumber (Wang et al., 2009), heat resistance in wheat (Yuyi et al., 2015), and drought tolerance in rice and soybean (Ai et al., 2008; Ling et al., 2013; Peng et al., 2009). Previous studies focused mainly on explaining the mechanism by which COR enhances drought resistance in the reactive oxygen species (ROS)-dependent signal and abscisic acid (ABA) pathway during the wheat seedling stage. However, our study demonstrates the mechanism of COR-induced drought tolerance at the molecular level by functional proteomics combined with field testing. This research will provide guidance for the application of COR in agricultural production.

## 2. Materials and methods

### 2.1. Materials and growth conditions

The experiment was conducted at the Shangzhuang Experimental Station of China Agricultural University. The field soil type was silty loam, the previous crop was corn, and the maximum capacity was 24.50%. The organic matter content of the top layer of soil was 12.45 g/kg, and the nitrogen, total phosphorus, total potassium, available phosphorus, and available potassium contents were 1.56 g/kg, 0.617 g/kg, 10.34 g/kg, 19.54 mg/kg, and 97.75 mg/kg, respectively.

The winter wheat variety was Nongda211. Basic seedlings were established at 220 plants per $m^2$, and six irrigation treatments were set for the field test (Table 1). Each plot was 2 × 4 m (area, 8 $m^2$). A randomized block design and three repetitions were used. A unified irrigation of 50 mm was used before winter (December 10), irrigation of 50 mm water per plot was used at the recovering stage (March 10), and subsequent irrigation amounts and timing followed the treatment plans shown in Table 1. An irrigation water meter was used to measure the quantity of water. COR (concentration of 0.1 μM) was sprayed one week before irrigation.

### 2.2. Photosynthetic performance

Wheat with the same vigor was selected and marked during the jointing stage. A chlorophyll meter (SPAD-502; Konica Minolta, Inc., Japan) was used for determination of SPAD values in the jointing stage (April 20) and the grouting period (May 25). New fully expanded leaves of 15 plants in each plot were selected to measure the SPAD values, and the leaf SPAD values were determined by averaging three points (tip, middle, and base) on each leaf.

Net photosynthetic rate (Pn) and gas exchange parameters were determined using a portable photosynthesis meter (Li-6400; LI-COR, Lincoln, NE, USA) in the jointing stage (April 20) and filling stage (May 25). The measurement conditions were set as follows: 400 μmol $mol^{-1}$ $CO_2$ concentration, 1200 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density, and 20 °C (April 20) and 28 °C (May 25), respectively. Fifteen wheat flag leaves were selected in each replication (plot) between 9:00 and 11:00 A.M. on a sunny and windless day.

**Table 1**
Irrigation amounts (mm) and dates (month/day).

| Treatment | Jointing Water (mm) | Filling Water (mm) |
|---|---|---|
| Control | 50 | 50 |
| Control + COR | 50 | 50 |
| B | 0 | 20 |
| B + COR | 0 | 20 |
| G | 20 | 0 |
| G + COR | 20 | 0 |

Note: Jointing water was applied on April 5th; filling water was applied on May 1st.

### 2.3. Protein extraction

One gram of wheat leaves was collected and ground in liquid nitrogen. Then, protein extraction was performed according to Rinalduccia et al. (2011) and Yuyi et al. (2015). The powder was suspended (1 g/ml) in chilled lysis buffer in acetone containing 0.007% dithiothreitol (DTT) and 1% plant protease inhibitor cocktail (Bio-Rad, Hercules, CA, USA). The mixture was incubated at −20 °C for at least 1 h and centrifuged at 12,000 rpm for 15 min. The supernatant was then collected.

### 2.4. Two-dimensional electrophoresis (2-DE)

Isoelectric focusing (IEF) was performed using the Bio-Rad Protean IEF Cell System according to Yuyi et al. (2015). Immobilized pH gradient (IPG) strips (24 cm, pH 4–7; Bio-Rad) were passively rehydrated overnight with 750 μg protein containing 1% carrier ampholyte. The total product time × voltage applied was 63,500 V h. The strips were subsequently reduced (1% DTT, 15 min) and alkylated (2.5% indoleacetic acid [IAA], 15 min) during the equilibration step (30 min in 50 mM Tris–HCl [pH 8.8], 6 M urea, 30% glycerol v/v, 1% sodium dodecyl sulfate polyacrylamide [SDS], bromophenol blue). The equilibrated strips were placed on SDS-polyacrylamide gels. Protein spots were stained using Coomassie Brilliant Blue (CBB).

### 2.5. 2-DE image analysis

Two-dimensional gel images were digitized using a flatbed scanner (Image Scanner-II; GE Healthcare, Buckinghamshire, UK) with a resolution of 300 dpi according to Yuyi et al. (2015). Image analysis was carried out with an Image Master 7.0 (GE Healthcare). Spot quantity values were normalized in each gel by dividing the raw quantity of each spot by the total quantity of all spots included in the standard gel. The average spot quantity value and its variance coefficient in each group were then determined. The least significant difference (LSD) test was used to determine significant differences among group means.

### 2.6. Protein identification by tandem mass spectrometry

Protein spots were cut out from Coomassie blue–stained gels and subjected to trypsin digestion according to Shevchenko et al. (1996) and Yuyi et al. (2015). Peptide mixtures were separated using an Autoflex2 (Nova Biomedical, Brook, Germany) system. A sample volume of 1 μL was loaded.

### 2.7. Functional annotation and statistical analysis

Data for gene function analysis were downloaded from the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov/) and The Arabidopsis Information Resource website (http://www.arabidopsis.org). The data were analyzed statistically according to a randomized block design using SAS statistical software. The LSD was calculated for data found to be significant at $P \leq 0.05$.

## 3. Results

### 3.1. Effects of COR on wheat morphology and yield

With the goal of determining whether COR alleviates drought stress in wheat, three irrigation treatments and six treatments in total were established for the field test (Table 1). For each treatment, unified irrigation of 50 mm before winter (December 10) and 50 mm at the recovering stage (March 10) was applied per plot. In addition to this unified irrigation, the irrigation amounts and timing for the treatments shown in Table 1 were followed. Irrigation water meters were used to measure water quantity. COR was sprayed one week before irrigation.