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Research Paper

Water availability effects on plant growth, seed yield, seed quality in *Cassia obtusifolia* L., a medicinal plant

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ABSTRACT

The seeds of *Cassia obtusifolia* L. are widely used in traditional Chinese medicine and health tea. The objective of this study was to investigate the effect of water availability on plant growth, seed yield, and seed quality in *C. obtusifolia* for improving its cultivation. Pot and field irrigation experiments were conducted in Qingdao of China in 2013 and 2014, respectively. Seven water irrigation treatments including 100%, 90%, 80%, 70%, 60%, 50% and 40% of field capacity (FC) in pot experiments while five water irrigation treatments including 100%, 85%, 70%, 55% and 40% of field capacity (FC) in field experiments were applied on the plant of *C. obtusifolia*, respectively. In both experiments, the results showed that different drought stress significantly resulted in different reduction in plant growth and seed yield attributes of *C. obtusifolia*, and more pronounced with the severity of drought. However, as for the seed quality attributes, both results showed that weak and moderate drought stress significantly increased the content of anthraquinones (plant secondary metabolites) while significantly decreased the content of protein (plant primary metabolites) in the seeds of *C. obtusifolia*, except that strong drought stress significantly decreased both of them. Notably, the weak drought stress (70% FC in pot and in field) did not decrease the seed yield significantly which resulted in a biggest harvest index among other treatments and thus resulted in the highest anthraquinones yields per plant. In addition, results showed that strong and moderate drought stress affected the proportion between hydrophilic and lipophilic anthraquinones in seed of *C. obtusifolia* significantly. Our study suggested that a certain of deficit irrigation has the potential to compromise seed yield and seed quality of *C. obtusifolia*, which can be used as a practical irrigation strategy for cultivation improving of *C. obtusifolia* as a medicinal plant.

1. Introduction

The ripe seed of *Cassia obtusifolia* L. called as "Jue-ming-zi" in Chinese, has been widely used as traditional herbal medicine or health tea in China for improving eyesight, alleviating constipation, and lowering hypertension and hyperlipidemia (China Pharmacopoeia Committee, 2010; Hao et al., 2001; Xiao, 2002). Several classes of bioactive metabolites have been identified from the seeds of *C. obtusifolia*, including anthraquinones, sterols, triterpenoids and xanthones etc. (Hao et al., 2001; Sob et al., 2010; Tang et al., 2015; Xiao, 2002; Xu et al., 2012; Zhang et al., 2009), in which anthraquinones are the most abundant group of active metabolites in the seeds of *C. obtusifolia* and the contents of aurantio-obtusin and chrysophanol are important indicators for the quality of raw herbal materials and related products of *C. obtusifolia* seeds (China Pharmacopoeia Committee, 2010). Numerous health promoting activities, such as anti-cancer, neuroprotective and anti hypercholesterolemic effects, and inhibition of oxidative modification of low-density lipoprotein, have been reported particularly in *C. obtusifolia* seed extracts (Ju et al., 2010; Kim et al., 2011; Patil et al., 2004; Sob et al., 2010; Tang et al., 2015). Consumer awareness and knowledge about the health benefits of *C. obtusifolia* have greatly stimulated marketing and consumption of *C. obtusifolia* seed products, and the demand for this plant has increased rapidly in recent years in Asia. The cultivation of *C. obtusifolia* was developed widely in 29 countys of 7 provinces of China including Qingdao of Shandong Province. Thus, there is considerable interest in the development of cultivation technology to improve both yield and quality of this medicinal plant.

The yield and quality of medicinal plants when cultivated are strongly influenced by growing conditions such as temperature, light regime, soil nutrient supply, soil water availability, etc (Guo et al., 2013; Ncube et al., 2012; Ncube and Van Staden, 2015; Ramakrishna and Ravishankar, 2011). Water availability is a most

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important abiotic factor influencing all plant physiological process including primary and secondary metabolism during whole plant life cycle including vegetative and reproductive growth. Water availability deviating from the optimum has an effect on the rate of relevant physiological processes, thereby constituting a water stress to the plant. Water stress is generally considered as negative factor being responsible for severe yield losses in agriculture. However, a large numbers of studies manifested that plants exposed to water stress accumulate higher concentrations of secondary metabolites, and thus a better quality resulted in many medicinal plants (Kleinwächter and Selmar, 2015; Kleinwächter et al., 2015; Li et al., 2011; Selmar and Kleinwächter, 2013a,b). This poses a dilemma for a herb grower: to yield or to quality? The need for an irrigation regime suitable for balancing the yield and quality in medicinal plant cultivation is therefore of vital importance.

With *C. obtusifolia* though widely cultivated in China, water availability induced variations in yield and quality have not been fully understood, and whether water availability treatments have the potential to compromise yield and quality have not been investigated deliberately. Therefore, searching for an irrigation schedule for cultivation control of this herbal medicine that would cater for a balance between yield and quality is a major justification of the present study. Meanwhile, this study try to provide a preliminary foundation for elucidating the mechanisms of variations in yield and quality of this herbal medicine under different water availability.

2. Materials and methods

2.1. Experimental site

Pot experiments were conducted during croping season of *C. obtusifolia* under natural day/night conditions from April to October 2013 in the greenhouse at School of Pharmacy, Qingdao University (latitude 36.08° N and longitude 120.34° E, Qingdao City, Shandong Province of China). Field experiments were conducted during croping season of *C. obtusifolia* under natural day/night conditions from April to October 2014 in the Medicinal Plant Garden of Lao Mountain of Qingdao City (latitude 36.14° N and longitude 120.63° E, Shandong Province of China). Both the experimental area are in north-temperate marine monsoon climate zone with annual average rainfall of 660.7 mm, annual average pan evaporation of 1612.0 mm, annual average temperature of 12.4 °C, annual average sunshine hours of 2531.5 h and annual average relative humidity of 70%. The monthly average temperature and rainfall are given in Table 1 throughout the growing periods of *C. obtusifolia* in Qingdao. Experimental field soil was a light loam texture with a field capacity (FC) of 0.27 $cm^3\ cm^{-3}$, a wilting point (WP) of 0.09 $cm^3\ cm^{-3}$, and had organic matter content of 12.70 $g\ kg^{-1}$, available N content of 253.06 $mg\ kg^{-1}$, available P content of 63.00 $mg\ kg^{-1}$ and available K content of 109.89 $mg\ kg^{-1}$ in topsoil, respectively. The pH of the soil was 7.4 and 7.5 in 2013 and 2014. The electrical conductivity of the soil was 0.6 $dS\ m^{-1}$ in 2013 and 0.5 $dS\ m^{-1}$ in 2014. Same soil was passed through a 0.5 cm mesh sieve when used for pot experiments. No additional fertiliser was applied during the growth of *C. obtusifolia* in both pot and field experiments. Experimental seeds of *C. obtusifolia* were collected from cultivated plants of *C. obtusifolia* in the Medicinal Plant Garden of Lao Mountain of Qingdao City at every last harvest season for next cultivation season.

In pot experiments, healthy mature seeds of uniform size were selected, surface sterilised with 95% ethanol for 20 min, washed in distilled water thoroughly 5–6 times. Ten sterilised seeds were hand sown in each pot (each containing the above mentioned soil of 103 kg in 65 cm deep and 40 cm mean diameter) on April 1, 2013 and irrigated with tap water to FC to facilitate germination. Each pot was placed on an electronic weighing device in a rain excluded and well ventilated environmental conditions. On the 20th day after sowing (DAS), the seedlings were thinned to three uniform strong seedlings per pot. Plants were irrigated with tap water to FC until the 50th DAS (May 20, 2013).

In field experiments, healthy mature seeds of *C. obtusifolia* were directly sown in furrows in each plot on April 1, 2014 and watered to FC to facilitate germination. Each plot was 4 m wide by 5 m long with 8 plant rows. Seedlings were thinned to plant density of 20 plants/m^2 by hand at the third-leaf stage. Plants were watered to FC until the 50th DAS (May 20, 2014). A drip irrigation system was used in the experimental plots. A 16 mm diameter polyethylene pipe with in-line drippers at 0.5 m intervals was placed on one side of each plant row. Drip laterals were placed around 10 cm away from the plant. The mean discharge of the emitters was 2.5 $l\ h^{-1}$. Each plot had a valve to control irrigation. A pressure gauge and a water meter were installed upstream to manually adjust the operating pressure and measure water applications. Irrigation water was filtered and pumped from a reservoir (Irrigation water quality: Na^+ = 0.19 $meq\ L^{-1}$, Ca^{2+} = 2.98 $meq\ L^{-1}$, Mg^{2+} = 1.41 $meq\ L^{-1}$, K^+ = 0.09 $meq\ L^{-1}$, Cl^- = 0.34 $meq\ L^{-1}$, HCO_3^- = 4.26 $meq\ L^{-1}$, pH = 7.5). In the field treatments, rainfall was excluded from the plots by metallic structures covered with transparent polyethylene sheet allowing manual covering depending on environmental conditions, trying to keep the polyethylene film extended only during rainfall events. These rain-shelters had no walls, to ensure good ventilation even when covered and the minimum possible alteration of the plants microclimate.

2.2. Experimental design and water deficit treatment

Pot treatments were imposed from the 51th to the 204th DAS (May 21, 2013 to October 21, 2013). Seven water treatments were predefined in this experiments including 100% of FC as control and 6 levels of deficit irrigation at 90%, 80%, 70%, 60%, 50% and 40% of FC, respectively. At the beginning of pot treatments from the 51th to the 70th DAS (May 21, 2013 to June 9, 2013), water loss of all the pots were determined by weighing the pots daily using the electronic weighing devices. Until soil water depletion reached their respective target levels, different group of pots were weighed on every three days and watered to their respective weight on every three or six or nine days to maintain the soil moisture content at 100%, 90%, 80%, 70%, 60%, 50% and 40% of FC, respectively (Table 2). There were seven replications per treatment arranged in a completely randomized block design, totally 49 pots. In order to eliminate the interference of plant weights, one pot from each replication kept with same weight soil but without plants was weighed and watered in its respective way for monitoring soil water loss in the absence of the plants. Plants of all treatments were observed and recorded for plant growth and seed yield attributes from the beginning of water availability treatments, and till they were sampled at harvest (October 21 in 2013) for determination of plant growth, seed yield and quality attributes.

Field treatments were imposed from the 51th to the 191th DAS (May 21, 2014 to October 8, 2014). Five water treatments were predefined in this experiments including 100% of FC as control and 4 levels of deficit irrigation at 85%, 70%, 55% and 40% of FC, respectively. At the beginning of field treatments from the 51th to the 71th DAS (May 21, 2014 to June 10, 2014), the soil water contents (SWC) of all the plots were determined daily by a portable soil moisture monitoring system (Diviner 2000, Sentek Pty Ltd., Australia). After the SWC reached their respective predefined target levels, different plots were determined continuely on every three days, and then