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Soil water stress attenuate the growth and development but enhance the saponin synthesis of *Panax notogesing* during flowering stage

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ABSTRACT

Water stress can affect the growth and secondary metabolites of plants. Thus, this study identified the effects of soil water conditions on the growth and development of *Panax notoginseng*, especially saponin synthesis. *Panax notoginseng* was grown in different soil water conditions (0.40, 0.55, 0.70, 0.85, 1.00 field capacity) for 30 days, and related indicators were determined every 10 days. *Panax notoginseng* could not survive under the content of 0.40 and 1.00 field capacity. Thus, their cultures were set at 0.55, 0.70, and 0.85 field capacity for experimental purposes. The results showed that agronomic traits, leaf water contents, chlorophyll contents, and photosynthetic efficiency in 0.70 field capacity were significantly higher than in 0.55 and 0.85 field capacity. In addition to flowers, contents of soluble total sugars and proteins increased significantly in all the parts under 0.55 and 0.85 field capacity. Compared with 0.70 field capacity treatment, total saponin content of aboveground organs increased significantly under 0.55 and 0.85 field capacity. And 18 key genes increased significantly but 2 key genes changed insignificantly in saponin synthesis pathway. These results suggested that 0.70 field capacity was the most suitable soil water condition for flowering stage *Panax notoginseng* and produced the best agronomic traits, while water stress (0.55 and 0.85 field capacity) produce more saponin contents by improving the expression of key genes in saponin synthesis pathway.

1. Introduction

Panax notoginseng (Bruk) F.H.Chen, commonly known as "notoginseng", is a perennial herb that belongs to the *Araliaceae* family of the genus *Panax* (Briskin, 2000). It is used to promote blood circulation, counteract blood stasis, relieve swelling and pain, and treat cardiovascular and cerebrovascular diseases (Ng, 2006; Yoshikawa et al., 2003). *Panax notoginseng* is the largest ingredient in Yunnan Baiyao, compound Danshen dripping pills, Xuesaitong, and other traditional Chinese medicines. Yunnan Province, the herb's primary producing area, provides more than 98% of the total radix notoginseng on the Chinese market (Liu et al., 2015).

Yunnan is located on a large plateau with a monsoon climate. Affected by global climate changes, this area has suffered five successive years of drought, with the situation worsening year by year. *Panax notoginseng* grows under cool conditions, and its growth has been seriously affected by drought in Yunnan. In 2015, the drought area included more than 80% of the total planting area of *P. notoginseng*, and irrigation accounted for 11.7% of the total planting cost. Irrigation-related spending tends to increase as drought conditions intensify.

Currently, *P. notoginseng* cultivation suffers from a lack of scientific guidance, and irrigation factors such as time, methods, and water use largely depend on experiences. This lack of data increases costs and creates a significant waste of water resources. Even worse, it leads to waterlogging and affects the normal growth of *P. notoginseng*. Reasonable, scientifically-based irrigation represents a promising strategy to save cost, improve efficiency, and enhance yields. Bazargani et al. (2011) discovered that appropriate deficit irrigation during the ripening period of pomegranate not only improves the fruit's content of anthocyanin and secondary metabolites but also saves 6% to 11% of irrigation water. Similarly, Masoero et al. (2013) claimed that reasonable deficit irrigation during corn's different growing periods can reduce irrigation water use and guarantee the yield and nutritional value of high oil corn.

Appropriate water supply represents a critical aspect of crop growth, because water content has an important effect on plant

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Table 1
Primer sequence of key genes in saponin synthesis pathway.

Enzyme	Genes	Senes primer sequence	Anti-senes primer sequence
Acetyl-CoA acetyltransferase	*ACAT*	5′ TTCCATGCCACCAGCCACA 3′	5′ GGTCAAGCACCTGCAAGACAA 3′
3-hydroxy-3-methylglutaryl coenzyme-A synthase	*HMCAS*	5′ GTTGCGAAGTCCCAGTACGATGT 3′	5′ GGATGTTGACCTTCACGAAGACG 3′
3-hydroxy-3-methylglutaryl coenzyme-A reductase	*HMCAR*	5′ TTCTGGAAATTATTGCTCGGATAAG 3′	5′ AGGCTGCCACATTTGTCTTCAAC 3′
Mevalonate kinase	*MVK*	5′ TTGAATCTCCTGCTTCGGATGA 3′	5′ CCTCCACCACCAGCTCCTGT 3′
Phosphomevalonate kinase	*PMK*	5′ ATGCTACTATGAAAATGGAAGGAGTC 3′	5′ TATGAACTGCCGAAATGCCAGA 3′
Isopentenyl diphosphate isomerase	*IDI*	5′ TGTACCGAGAATCCGAGCTTATAGA 3′	5′ ATGCTCTCCCCACTTCCCATC 3′
Geranylgeranyl pyrophosphate synthase	*GGPS*	5′ ATCTATTGGGACAGAAGGGCTAGTA 3′	5′ AAATGGCTCCTAAAACTACAGATGC 3′
Geranylgeranyl diphosphate synthase	*GGR*	5′ ATGTTCTGAAGGAATTTCGAGTGTG 3′	5′ TCAAGAATATCATCCACCACCTGAA 3′
Squalene epoxidase	*SE*	5′ GACCCTTATGCGATCTCCATGACT 3′	5′ AATTCCTCCGAGGCTCAGATAATC 3′
Dammarenediol- synthase	*DS*	5′ TGACAACAGTGAAGCAGTTCGTAAG 3′	5′ CAAACATAAGACCTAGCATAGCCCA 3′
Cycloartenol synthase	*CAS*	5′ ACAAGCATCAGATGGCTCATGGTA 3′	5′ CCAACCACCAGAAGCAAGTTGT 3′
Panaxnotoginseng actin	*PnACT2*	5′ TCCAAGGGTGAATATGATGAATCG 3′	5′ AACCTCTCCAAAGAGAATTTCTGAGT 3′
1-deoxy-D-xylulose-5-phosphate synthase	*DXPS*	5′ GACCCTGGAGGACCTAACAT 3′	5′ TGGTCAGGTCTATGAGGCAAT 3′
1-deoxy-D-xylulose-5-phosphate reductoisomerase	*DXPR*	5′ GTCCTGCTCTTGTATGATTATGG 3′	5′ TCAATGTCTTTCACTTGCTCC 3′
2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	*ispD*	5′ GATGACGATTCTTCAACTGGG 3′	5′ TCTTTGGCCTTTTCCTCCTG 3′
4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	*ispE*	5′ TATTATTGCTGCGGGTTGTG 3′	5′ CGTCTTCAAGTGTCTTTGGTGTA 3′
2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	*ispF*	5′ GAGTGAGCCTCGCAACCTTT 3′	5′ ACTCTGTTATGCCGCTACTTCTAT 3′
4-hydroxy-3-methylbut-2-enyl-diphosphate synthase	*ispG*	5′ AGGCTTTTGAGTCTCCATCCA 3′	5′ ATCGTGGAGACCTTGGTTCG 3′
4-hydroxy-3-methylbut-2-enyl diphosphate reductase	*HDR*	5′ ACTTTCTTTTGGGCGACCTT 3′	5′ ACCGTTTGGTAGCCAGTTTT 3′

morphogenesis and metabolism. The osmotic stress caused by drought induces the production of reactive oxygen species, which damage cell structures. Meanwhile, leaf stomata close, reducing the gas exchange rate, inhibiting photosynthesis, and slowing down the accumulation of dry matter (Wang et al., 2008). On the other hand, excessive soil water conditions causes damage to plants, leading to root hypoxia and reductions in metabolism (Ricoult et al., 2006).

Studies have investigated the effects of soil water conditions on the growth, development, and physiology of several economic crops, but such studies rarely consider medicinal plants like *P. notoginseng*. Appropriate water content represents a key factor to ensure the quality and yield of *P. notoginseng*. Therefore, it is important to establish irrigation rules for *P. notoginseng* cultivation. In this study, the effects of different soil water conditions on the growth, development, physiological and gene expression characteristics of 2-year-old *P. notoginseng* under flowering stage was demonstrated.

2. Materials and methods

2.1. Plant and experimental site

2-year-old flowering stage *P. notoginseng* seedlings with similar growth vigor were used in this study. The experiment was conducted in the shading greenhouse of faculty of life science and technology, Kunming University of Science and Technology (24° 51′ 0″ N, 102° 52′ 2″ E, altitude 1835 m). During the experiment, the daily average temperature was 25 °C, the daily maximum difference in temperature is 10 °C.

2.2. Experiment treatment

PVC pots (18.5 cm height, 22 cm width, 54 cm length) were used as experimental units. Each pot was filled with 8 kg soil. Soil nutrient status was as follows: 35.71 g/kg organic matter, 2.29 g/kg total nitrogen, 2.34 g/kg total phosphorus, 7.83 g/kg total potassium, 101.23 mg/kg available nitrogen, 39.53 mg/kg available phosphorus, and 207.94 mg/kg available potassium. The soil sample was taken from the plowed soil of the idle field with 0.26 field capacity (FC). Soil water content was confirmed by gravimetric methods (Xia et al., 2016). Five different soil absolute water content were adopted in present study: 0.40, 0.55, 0.70, 0.85 and 1.00 FC. The method for determination of field capacity (Klute, 1986) was as follows: soil sample was collected by ring sampler. Then put soil sample and ring sampler into tank, and water was added into the tank until water covered the top of ring sampler. Soaked the ring sampler and soil sample for 48 h until their

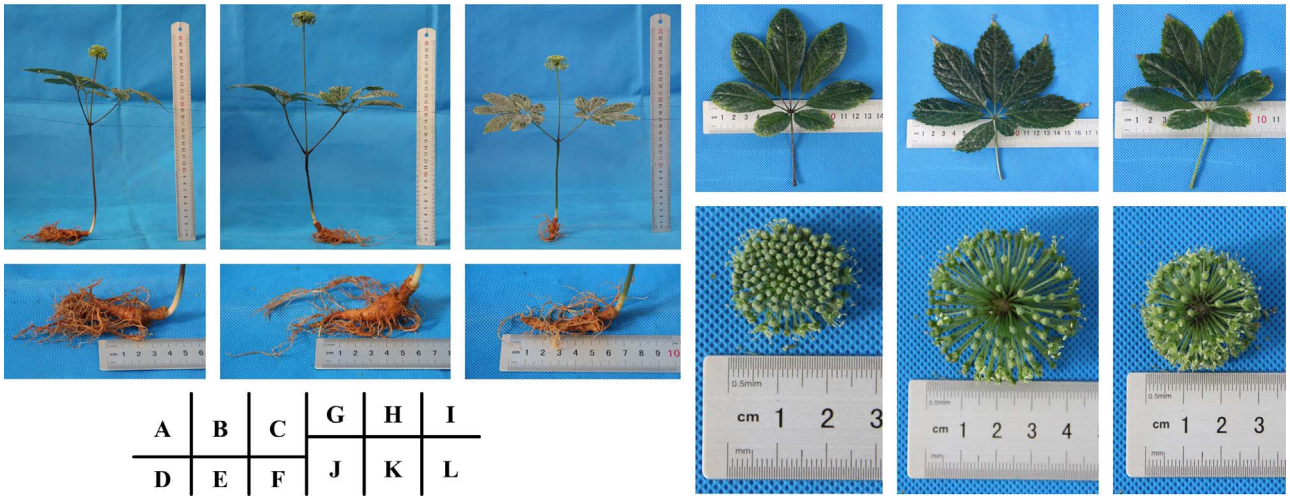

Fig 1. Effects of different field capacity on the agronomic traits of 2-year-old flowering stage *P. notoginseng*.
Whole plant (A), root (D), leaf (G) and flower (J) under 0.55 FC treatment for 30 d; Whole plant (B), root (E), leaf (H) and flower (K) under 0.7 FC treatment for 30 d; Whole plant (C), root (F), leaf (I) and flower (L) under 0.85 FC treatment for 30 d.