Variation Patterns of Thebaine in Papaver somniferum and Effects of Water-Nitrogen Coupling on Thebaine: Postprint

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Abstract

Using HPLC method, the thebaine content in roots, stems, leaves, and capsules of annual poppy plants at different growth stages after flowering was analyzed and determined. By investigating the variation patterns of thebaine in poppy plants and the effects of water-nitrogen coupling on thebaine, this study provides a basis for the rational development and management of this special medicinal plant resource. The results showed that the thebaine content in annual poppy plants varied as follows: in capsules, 1.81%-4.54%, reaching the highest level at maturity; in leaves, 0.30%-0.68%, highest at late swelling stage and lowest at harvest; in roots, 0.03%-0.28%, highest at early swelling stage and lowest at harvest; in stems, 0.23%-0.60%, showing a decreasing trend with the lowest at harvest. The thebaine content in upper, middle, and lower stem parts ranged from 0.42%-0.97%, 0.15%-0.60%, and 0.13%-0.37%, respectively. Drip irrigation amount and nitrogen application rate had significant effects on the thebaine content in annual poppy capsules. The optimal water-nitrogen coupling condition for capsule quality was I130N14, i.e., when the drip irrigation amount was 130 $m^3 \cdot (667\ m^2)^{1}$ and the nitrogen application rate was 14 kg · $(667\ m^2)^{1}$, the thebaine content reached its maximum.

Full Text

Preamble

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Title: Changes in Thebaine Content in Poppy (Papaver somniferum) and Effects of Water-Nitrogen Coupling

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Abstract

High-performance liquid chromatography (HPLC) was employed to determine thebaine content in roots, stems, leaves, and capsules of annual poppy plants at different growth stages following flowering. This study investigated the dynamic patterns of thebaine accumulation in poppy and the effects of water-nitrogen coupling under drip irrigation to provide a scientific basis for the rational development and management of this specialized medicinal plant. Results demonstrated that thebaine content varied significantly across organs and developmental stages. In capsules, thebaine ranged from 1.81% to 4.54%, peaking at maturity. Leaf thebaine content ranged from 0.30% to 0.68%, reaching its maximum during the late expansion stage and declining to its lowest at harvest. Root thebaine exhibited the lowest values (0.03%-0.28%), peaking during early expansion and dropping to minimum levels by harvest. Stem thebaine decreased progressively from 0.23% to 0.60%, with harvest-time values being the lowest. Within stems, thebaine gradient followed the pattern: upper stem (0.42%-0.97%) > middle stem (0.15%-0.60%) > lower stem (0.13%-0.37%). Both irrigation volume and nitrogen application rate exerted significant effects on capsule thebaine content. The optimal water-nitrogen combination for maximizing thebaine concentration was identified as I130N14, corresponding to an irrigation volume of 130 $m^3 \cdot (667\ m^2)^{1}$ and nitrogen application of 14 $kg \cdot (667\ m^2)^{1}$.

Keywords: poppy, thebaine, variation pattern, water-nitrogen coupling, influence

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Introduction

Poppy (*Papaver somniferum* L.), a member of the Papaveraceae family commonly known as opium poppy, is an annual or perennial herbaceous plant (National Pharmacopoeia Committee, 2000) that serves as a specialized medicinal resource. Its latex and capsules contain over 20 alkaloids, including morphine (C H NO), thebaine (C H NO), cocaine (C H NO), codeine (C H NO), noscapine (C H NO), and papaverine (C H NO) (Li et al., 2012). While morphine represents the primary alkaloid with potent analgesic, sedative, antitussive, and antidiarrheal properties, its strong addictive potential and substantial social harm are well-documented. Heroin, pethidine, and methadone all derive from morphine. Thebaine, an opiate isoquinoline alkaloid, functions as a crucial intermediate in pharmaceutical synthesis (Huo, 1999), enabling production of buprenorphine, naloxone, nalbuphine, and etorphine. Thebaine derivatives such as buprenorphine offer superior safety profiles compared to morphine and methadone, effectively treating opioid dependence by suppressing withdrawal symptoms while preventing drug abuse (Xing et al., 2015). These compounds can completely replace heroin and methadone in clinical detoxification therapy without inducing withdrawal symptoms.

Chinese poppy cultivars are classified into two categories based on their primary alkaloid composition. The first category, exemplified by white poppy (*Papaver somniferum* L.), contains morphine as the dominant alkaloid, with research focusing on seed viability (Li et al., 2012; Chang et al., 2010), morphine distribution across plant parts (Luo et al., 2012), and relationships between morphine content and cultivation practices (Wang et al., 2010). The second category, represented by perennial red poppy (*Papaver orientale* L.), contains thebaine as the principal alkaloid, with studies investigating analytical methods for thebaine quantification (Huo, 1999; Song and Liu, 2005) and pollen germination characteristics (Wei et al., 2009). However, research on thebaine content dynamics in annual poppy materials and corresponding cultivation techniques remains limited. This study examines the dynamic changes in thebaine content across different aboveground organs of annual poppy after flowering and evaluates the impacts of various water and nitrogen management strategies, thereby providing a scientific foundation for rational development and management of this specialized medicinal plant resource.

Materials and Methods

1.1 Experimental Site Description

The experimental site was located at the interface between plain and desert 戈壁 regions, characterized by a continental temperate arid climate. The area featured a groundwater table at 100 m depth, annual precipitation of 220 mm, annual evaporation of approximately 3000 mm, annual sunshine duration of 2915.1 hours, and a frost-free period of 167 days. The soil type was calcareous irrigation desert soil with 1.20% organic matter, 0.15% total nitrogen, 82 mg · kg ^{1} alkali-hydrolyzable nitrogen, 49 mg · kg ^{1} available phosphorus, and 371 mg · kg ^{1} available potassium. The topsoil layer (0–20 cm) had a bulk density of 1.23–1.33 g · cm ^{3} and a maximum field water capacity of 30.2%.

1.2 Experimental Design

A split-plot design was employed with two factors: drip irrigation volume (I) as the main plot and nitrogen application rate (N) as the subplot. Three irrigation levels were established: I90 (90 m^3 · (667 m^2) ^{1}, equivalent to 135 mm), I110 (110 m^3 · (667 m^2) ^{1}, 165 mm), and I130 (130 m^3 · (667 m^2) ^{1}, 195 mm).

Four nitrogen rates were implemented: N8, N11, N14, and N17 (corresponding to 8, 11, 14, and 17 kg pure nitrogen · (667 m²) ¹, respectively), resulting in 12 treatment combinations with three replications. Each plot covered 32 m² with 1 m spacing between plots, arranged randomly. To minimize lateral water seepage and nitrogen movement between adjacent plots, 1 m buffer zones were maintained. Irrigation water was supplied via tap water and measured using water meters installed at each plot inlet.

The experiment utilized under-mulch drip irrigation with 140 cm wide plastic film covering 120 cm of the bed surface. Row spacing followed a wide-narrow configuration of 30/15 cm, with plant spacing of 12.7 cm, achieving a planting density of 18,900 holes · (667 m²) ¹. The drip irrigation system consisted of 40 mm diameter branch pipes and 16.0 mm diameter drip tapes with emitter flow rates of 2.8 L · h ¹ and 30 cm emitter spacing. Drip tapes (two per film) were installed between wide rows, resulting in 45 cm spacing between drip lines in the field [Figure 1: see original paper].

The detailed irrigation schedule is presented in Table 1 . Basal fertilizers, including 5 kg · (667 m²) ¹ nitrogen, 10 kg P O · (667 m²) ¹ phosphorus, and 5 kg K O · (667 m²) ¹ potassium, were applied prior to planting. The remaining nitrogen was applied in three split applications through the drip system during the bolting, flowering, and early capsule expansion stages at a 4:2:4 ratio.

1.3 Instruments and Reagents

Instruments: Waters 991 HPLC system (USA) equipped with a 486 UV detector, solid-phase extraction columns (C8, 250 mg) from Tianjin Woji Technology Co., Ltd., and MILLENNIUM data processing software.

Reagents: Thebaine reference standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products. Acetonitrile and methanol were HPLC-grade; potassium dihydrogen phosphate and sodium heptanesulfonate were analytical-grade; distilled water was prepared in-house.

1.4 Chromatographic Conditions

Column: Hypersil C18 (5 m, 4.6 nm × 250 nm) packed with octylsilane-bonded silica gel. Mobile phase: potassium dihydrogen phosphate (0.025 mol · L ¹): sodium heptanesulfonate (0.0025 mol · L ¹): acetonitrile = 1:1:1.5 (v/v/v). Flow rate: 1.0 mL · min ¹. Detection wavelength: 254 nm. Injection volume: 10 L.

1.5.1 Water Volume Calculation

Irrigation volume calculations require specification of soil area and depth; when depth is unspecified, a 1 m soil profile is assumed. The calculation formula is:

Water volume [m³ · (667 m²) ¹] = Water layer thickness (H, mm) × 0.667

1.5.2 Sample Collection and Thebaine Determination

At harvest, capsules were collected from each treatment, seeds were removed, and mixed capsule shell samples were prepared. For the N14I110 treatment, sampling was conducted at six key growth stages beginning at flowering: flowering, petal fall, early expansion, late expansion, maturity, and harvest. Ten plants were randomly selected per stage. Samples were separated into roots, stems, leaves, and capsules; stems were divided into upper, middle, and lower thirds; capsules were crushed and deseeded, separating main and branch capsules. All samples were air-dried initially, then oven-dried at 60–70 °C to constant weight in paper bags. Dried samples were weighed, ground, passed through a 40-mesh sieve, placed in labeled envelopes, and stored in a dry, ventilated location for analysis.

For analysis, 0.2 g of powdered sample was placed in a 200 mL volumetric flask, extracted with 50 mL of 5% acetic acid via ultrasonication for 30 min, cooled to room temperature, diluted to volume with 5% acetic acid, and filtered through a 0.45 m membrane. A 10 L aliquot was injected for HPLC analysis. External standard calibration was performed using peak area quantification.

1.5.3 Data Analysis

All data were processed using Excel and SPSS 17.0 statistical software.

Results

2.1 Thebaine Variation Patterns

Following flowering, poppy enters the reproductive growth phase, during which thebaine predominantly accumulates in capsules, followed by stems and leaves, with roots containing the lowest concentrations. At maturity, thebaine distribution followed the descending order: capsule > leaf > stem > root [FIGURE:2, FIGURE:3].

2.1.1 Thebaine Content Changes in Capsules As shown in Figure 2 [Figure 2: see original paper], thebaine content in poppy capsules ranged from 1.81% to 4.54% across developmental stages. From petal fall to maturity, main capsule thebaine content was lower than that of branch capsules; however, during flowering and from maturity to harvest, main capsules exhibited higher thebaine levels. Both main and branch capsules reached peak thebaine concentrations at maturity (4.54% and 4.43%, respectively), with subsequent declines post-maturity, though main capsules maintained higher levels than branch capsules.

Main capsule thebaine content ranged from 2.33% to 4.54%, displaying a "decrease-increase-decrease" pattern. The minimum occurred at petal fall (2.33%), while maxima were observed at flowering (4.24%) and maturity (4.54%). Content decreased from flowering to petal fall, then increased progressively to the peak of 4.54% at maturity, followed by a post-maturity decline.

Branch capsule thebaine exhibited a "increase-decrease" trend, rising continuously from flowering (1.81%) to maturity (4.43%), then declining post-maturity in parallel with main capsules.

2.1.2 Thebaine Content Changes in Roots and Leaves As illustrated in Figure 3 [Figure 3: see original paper], thebaine content followed the pattern stem > leaf > root from flowering to early expansion, shifting to leaf > stem > root from early expansion to harvest. Root thebaine was the lowest among all organs (0.03%-0.28%), peaking at early expansion (0.28%) and declining progressively to the minimum at harvest (0.03%). Leaf thebaine increased during early developmental stages (flowering to late expansion), reaching maximum concentration at late expansion (0.68%), then decreased to the lowest value at harvest (0.30%).

2.1.3 Thebaine Content Variation in Different Stem Sections Stem thebaine content ranged from 0.23% to 0.60%, decreasing progressively from flowering to harvest [Figure 3: see original paper]. The vertical distribution consistently followed upper stem > middle stem > lower stem, with respective ranges of 0.42%-0.97%, 0.15%-0.60%, and 0.13%-0.37%, all declining throughout the growth period [Figure 4: see original paper].

2.2 Effects of Water-Nitrogen Treatments on Capsule Thebaine

The primary economic product of poppy, besides seeds, is the capsule shell, whose thebaine content determines product quality. Figure 5 [Figure 5: see original paper] reveals that at nitrogen rates of 8-14 $kg \cdot (667\ m^2)^{1}$, capsule thebaine content increased with irrigation volume. Under N8, increasing irrigation from 90 to 110 and 130 $m^3 \cdot (667\ m^2)^{1}$ elevated thebaine content from 4.29% to 4.54% and 4.62%, respectively. Under N14, maximum thebaine (5.02%) occurred at 110 $m^3 \cdot (667\ m^2)^{1}$. At the highest nitrogen rate (N17), thebaine peaked at 5.02% under 110 $m^3 \cdot (667\ m^2)^{1}$ irrigation, but declined to 4.27% under 130 $m^3 \cdot (667\ m^2)^{1}$.

Under identical irrigation regimes, capsule thebaine content at I110 (110 $m^3 \cdot (667\ m^2)^{1}$) with N8, N11, N14, and N17 was 4.54%, 4.55%, 4.93%, and 5.02%, respectively. At I130 (130 $m^3 \cdot (667\ m^2)^{1}$), corresponding values were 4.62%, 4.69%, 5.02%, and 4.27%.

Variance analysis indicated significant treatment differences. Main plot effects showed I110 and I130 were significantly different from I90 (which had lower thebaine), while I110 and I130 did not differ significantly. Subplot effects revealed N8, N11, and N17 were significantly different from N14, but not from each other. Water-nitrogen interactions were significant: treatments I90N8, I90N14, I90N17, I110N8, I110N11, and I130N8 showed no significant differences among them, nor did I110N14, I110N17, I130N11, and I130N14. However, I110N17 and I130N14 differed significantly from all other treatments, achieving the highest thebaine content of 5.02%.

Comprehensive evaluation identified the optimal water-nitrogen combination as I130N14, corresponding to an irrigation volume of 130 $m^3 \cdot (667\ m^2)^{1}$ and nitrogen application of 14 $kg \cdot (667\ m^2)^{1}$.

Discussion and Conclusion

Previous research on perennial red poppy reported thebaine contents of 1.49%, 0.55%, 0.28%, and 1.80% in roots, stems, leaves, and capsules, respectively, with capsules containing the highest concentration followed by roots, while leaves had the lowest (Huo, 1999). Morphine distribution in poppy follows the pattern capsule > leaf > stem, with content ranges of 2.567%-3.455% in capsules, 1.742%-2.653% in leaves, and 0.128%-0.467% in stems (Luo et al., 2012). Our study on annual poppy demonstrated that thebaine concentrated primarily in capsules across all developmental stages, with the highest content in capsules, moderate levels in stems and leaves, and the lowest in roots. While thebaine enrichment in capsules is consistent with previous findings, the lowest content occurred in roots rather than leaves as reported for red poppy, likely attributable to varietal differences and the perennial nature of red poppy, which relies on roots for survival and varietal maintenance.

From flowering to maturity, capsule thebaine content increased continuously, reaching maximum at maturity. Root and leaf thebaine declined to minimum values at harvest (0.03% and 0.30%, respectively, from ranges of 0.28%-0.03% and 0.68%-0.30%). Stem thebaine decreased progressively to 0.23% at harvest, with vertical distribution consistently showing upper stem (0.97%-0.42%) > middle stem (0.60%-0.15%) > lower stem (0.37%-0.13%).

As capsules represent the primary economic product (besides seeds), their alkaloid content determines product quality. Mature perennial red poppy capsules contain 2.534%-2.732% thebaine (Song and Liu, 2005) or 1.80% (Huo, 1999). In contrast, our annual poppy material achieved substantially higher thebaine concentrations at maturity, with main and branch capsules reaching 4.54% and 4.43%, respectively, indicating greater economic value.

Wang et al. (2010) investigated water regulation effects on morphine content in poppy under mulched drip irrigation, finding that increased irrigation frequency from squaring to early full-bloom stages enhanced capsule morphine content, while continuous irrigation during late full-bloom caused sharp declines. Our study identified the optimal water-nitrogen combination for annual poppy capsule thebaine as I130N14 (130 $m^3 \cdot (667\ m^2)^{1}$ irrigation, 14 $kg \cdot (667\ m^2)^{1}$ nitrogen). Thebaine content increased with irrigation volume at nitrogen rates of 8-14 $kg \cdot (667\ m^2)^{1}$, but at 17 $kg \cdot (667\ m^2)^{1}$ nitrogen, maximum thebaine occurred at 110 $m^3 \cdot (667\ m^2)^{1}$ irrigation.

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Note: Figure translations are in progress. See original paper for figures.