Effects of Cultivation Methods on Agronomic Traits, Yield, and Quality of Isatis indigotica (Postprint)

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Abstract

Cultivation methods exert substantial influence on the yield and quality of traditional Chinese medicinal materials. To determine the optimal cultivation practice for Isatis indigotica in the Hexi Corridor oasis irrigation zone, a field experiment was conducted using a two-factor randomized block design, with cultivation patterns (flat planting and ridge planting) and mulching methods (no mulching, white film mulching, and black film mulching) as factors, establishing six treatments. Isatis indigotica plant samples were collected at monthly intervals from sowing to harvest, and root agronomic trait indices, Banlangen yield, and the content of the marker compound (R,S)-goitrin were measured across the six cultivation methods. The results demonstrated that different cultivation methods significantly affected the accumulation dynamics of root length, root diameter, and root dry weight per plant, as well as Banlangen yield and quality. Ridge planting with black film mulching produced the longest duration of rapid root growth, the most developed root system, and superior values for root length, root diameter, and root dry weight per plant. Banlangen yield peaked under ridge planting with black film mulching (4 514.4 kg • hm 2) and was lowest under ridge planting with white film mulching (3 116 kg • hm 2); conversely, (R,S)-goitrin content was highest under ridge planting with white film mulching (2.61 g kg 1) and lowest under ridge planting without mulching (0.137%). Across all cultivation methods, the growth of main root length, root diameter, and root dry weight per plant in Isatis indigotica exhibited an "S" -shaped temporal trend, consistent with the Logistic growth model. The appropriate cultivation method for Banlangen production from Isatis indigotica in the Hexi Corridor oasis irrigation zone is ridge planting with black film mulching.

Full Text

Effect of Cropping Pattern on Agronomic Characteristics, Yield and Quality of Radix Isatidis

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Abstract: Cropping pattern significantly affects the yield and quality of Chinese medicinal materials. To determine the optimal cultivation method for *Isatis indigotica* in the irrigated oasis region of the Hexi Corridor, we conducted a field experiment using a two-factor randomized block design with cultivation model (flat planting and ridge planting) and mulching method (no mulch, white film mulch, and black film mulch) as factors, establishing six treatments. Plant samples were collected monthly from sowing to harvest to measure root agronomic traits, radix isatidis yield, and (R,S)-epigoitrin content under the six cultivation methods.

The results showed that different cultivation methods significantly affected root length, root diameter, cumulative dynamics of root dry weight per plant, radix isatidis yield, and quality. Ridge planting with black film mulch produced the most vigorous root system with the longest rapid growth duration, achieving the highest values for root length, root diameter, and root dry weight per plant. Ridge planting with black film mulch yielded the highest radix isatidis production (4,514.4 $kg \cdot hm^{-2}$), while ridge planting without mulch produced the lowest yield (3,116 $kg \cdot hm^{-2}$). The (R,S)-epigoitrin content was highest under ridge planting with white film mulch (2.61 $g \cdot kg^{-2}$) and lowest under ridge planting without mulch (0.137%). Under all cultivation methods, main root length, root diameter, and root dry weight per plant exhibited S-shaped growth trends over time, fitting the Logistic growth model. Ridge planting with black film mulch is recommended as the suitable cultivation method for radix isatidis production in the Hexi Corridor oasis irrigation area.

Keywords: *Isatis indigotica* Fort.; Cropping pattern; Logistic model; Agronomic characteristics; Radix isatidis yield; Epigoitrin content

Introduction

Isatis indigotica Fort. is a biennial cruciferous herb whose dried leaves and roots are used medicinally; the root is known as Banlangen (radix isatidis), the leaf as Daqingye, and the processed product as Qingdai. Both Daqingye and

Banlangen possess heat-clearing and detoxifying properties and are widely used to treat colds, influenza, and viral infections. The plant is cultivated mainly in Gansu, Heilongjiang, Hebei, and Anhui provinces, with Gansu and Heilongjiang being the major production regions.

In 2012, Minle County in Gansu Province was designated the "Hometown of Radix Isatidis in China" by the Chinese Specialty Products Hometown Promotion Committee. Previous research on cultivation methods in this region has focused on black film mulching techniques, ridge planting with film mulching intercropped with *Vaccaria segetalis*, and flat planting with black film dibbling. However, most studies have concentrated on apparent yield changes, with limited theoretical investigation of agronomic trait indices and root growth dynamics.

The Logistic growth curve is widely applied in crop cultivation and ecological modeling to describe plant growth cycles, yield prediction, dry matter accumulation, and population dynamics. Due to the invisible nature of root growth environments and measurement limitations, root system modeling has developed slowly. Previous studies have demonstrated that Logistic curves effectively fit the relationship between root growth and planting time in *Salvia miltiorrhiza* and *Adenophora tetraphylla*, and that flax root volume and dry weight growth follow Logistic equations.

Building on previous research methods and integrating practical cultivation techniques, this study established a two-factor randomized block field experiment with cultivation model and mulching method as factors, comprising six treatments. We investigated the effects of different cultivation methods on root agronomic traits, radix isatidis yield, and (R,S)-epigoitrin content, clarified the cumulative dynamics of root traits and differences in yield and active component content, and developed Logistic models for main root length, root diameter, and root dry weight per plant. This theoretical analysis of biomass changes under different cultivation methods provides a basis for selecting optimal cultivation practices for *I. indigotica* in the Hexi Corridor.

1.1 Materials and Experimental Site

Seeds were collected in July 2014 from the Chengtai Pharmaceutical Plantation Base in Liuba Town, Minle County, Gansu Province. The field experiment was conducted from May to November 2015 at the Liuba Town Agricultural High-tech Park in Minle County. The experimental site is located at 100°43 28 E, 38°38 36 N, at an altitude of 1,824 m. The climate is arid, with the first frost occurring on October 25, a frost-free period of 140 days, 1,300 sunshine hours during the crop growth period (April–August), and total annual solar radiation of 587.58 $kJ \cdot cm^{-2}$. The average daily maximum temperature from May to November 2015 was 22.26°C, with precipitation of 125.4 mm. Detailed precipitation and temperature changes during the experiment are shown in [Figure 1: see original paper].

The experimental soil was irrigation-silted soil with a sandy loam texture and moderate fertility; the previous crop was potato (*Solanum tuberosum*). The topsoil (0-20 cm) contained 17.8 $g \cdot kg^{-1}$ organic matter, 98.6 $mg \cdot kg^{-1}$ available nitrogen, 157.5 $mg \cdot kg^{-1}$ available potassium, and 10.7 $mg \cdot kg^{-1}$ available phosphorus.

1.2 Experimental Design

The experiment employed a two-factor randomized block design with cultivation model (flat planting and ridge planting) and mulching method (no mulch, white film mulch, and black film mulch) as factors. The six treatment combinations were: flat planting without mulch (PN), flat planting with white film mulch (PW), flat planting with black film mulch (PB), ridge planting without mulch (LN), ridge planting with white film mulch (LW), and ridge planting with black film mulch (LB). Detailed field operations are described in .

Each treatment had three replicates, totaling 18 plots. Plot size was 3 m × 5 m (15 m^2). Inter-plot spacing was 40 cm, with 1 m protective rows on all sides. Black and white polyethylene (PE) films were 80 cm wide and 0.008 mm thick. Plant spacing was 10 cm × 15 cm.

1.3 Field Management

Twenty days before sowing, 450 $m^3 \cdot hm^{-2}$ of irrigation water was applied. Ten days before sowing, the land was deeply plowed and leveled. Basal fertilizer was applied at rates of 180 $kg \cdot hm^{-2}$ nitrogen, 75 $kg \cdot hm^{-2}$ phosphorus pentoxide, and 85 $kg \cdot hm^{-2}$ potassium oxide, incorporated into the soil in a single application. Sowing occurred on May 10 at a rate of 100 $kg \cdot hm^{-2}$. Film-mulched plots were dibbled on the film, while non-mulched plots were directly dibbled. Seedlings emerged on June 5, and final thinning was completed on June 15. Two irrigations of 600 $m^3 \cdot hm^{-2}$ each were applied on August 20 and September 20. Two inter-tillage weedings were performed during the cultivation period. Chemical control of cabbage caterpillars was conducted in August; no diseases were observed. Harvest occurred on November 5.

1.4 Measurement Methods and Logistic Modeling

Destructive sampling was conducted at 45, 75, 105, 135, and 165 days after sowing and at harvest. Ten plants were randomly selected from each plot each time (six sampling events total). Complete root systems were excavated, cleaned of soil, and transported to the laboratory. After washing with tap water and air-drying, measurements included root length (cm), root diameter (cm), and root dry weight (g) per plant, with averages calculated as observed values.

On November 5, roots were harvested by plot, cleaned of soil and impurities, and air-dried in a ventilated area to produce radix isatidis. Plot root yield was measured and converted to yield per hectare ($kg \cdot hm^{-2}$). Dried radix isatidis samples were ground, passed through a 60-mesh sieve, and stored in reagent bottles. After all measurements were completed, (R,S)-epigoitrin content was determined according to the method specified in the 2015 edition of the *Pharmacopoeia of the People' s Republic of China*.

The Logistic growth model was used to quantitatively describe the dynamic accumulation of root agronomic traits:

$$y = \frac{K}{1 + e^{A+Bx}}$$

where y is the average value of each trait, x is days after sowing ($x \in [45, 180]$), K is the growth limit value, and A and B are equation parameters.

The Logistic curve equation can be transformed into a linear equation:

$$\ln\left(\frac{K-y}{y}\right) = A + Bx$$

where y_i are the average values of each trait at equal intervals of measurement days.

The model was used to estimate key growth parameters including start time of rapid accumulation period, end time of rapid accumulation period, end time of accumulation period, time of maximum accumulation rate, maximum accumulation rate, and durations of rapid and slow growth periods .

1.5 Data Processing and Statistical Analysis

Data were organized and analyzed using Microsoft Excel 2003 and SPSS 17.0. Origin 8.0 software was used for graphing.

Results

2.1.1 Dynamics of Main Root Length

Under all cultivation methods, measured main root length of *I. indigotica* showed an S-shaped growth trend [Figure 2: see original paper]A. Root length increased slowly during the seedling stage, then accelerated with a growth peak occurring 75-105 days after emergence. The highest daily increment (0.22 $\text{cm} \cdot \text{d}^{-1}$) was observed in treatment LB. Subsequently, root length increase gradually slowed, reaching maximum values at harvest in all treatments.

Logistic model fitting based on measured values yielded the parameters shown in . Using days after sowing as the independent variable, theoretical root length values were estimated and plotted [Figure 2: see original paper]B. All treatments exhibited typical Logistic growth trends. The model fit the data well ($R^2 > 0.9$, $P < 0.01$). Theoretical maximum root length followed the order: LB > PW > PB > LW > PN > LN. The rapid growth period started 34-39 days after sowing, lasted 50-63 days, with maximum growth rates of 0.28-0.34 cm · d^{-1}. The time to reach maximum growth rate was 63-86 days, slow growth duration was 61-78 days, and root length growth ended at 152-176 days.

Treatment LB had the longest root growth duration and largest theoretical root length, indicating that ridge planting with black film mulch extended the root elongation period and favored high yield. PN and LN showed similar root growth durations and the smallest theoretical root lengths, demonstrating that mulching contributed more to root length growth than non-mulching. Under flat planting, white film mulch was more beneficial than black film, while under ridge planting, black film outperformed white film. Overall, ridge planting with black film mulch was most conducive to main root length increase.

2.1.2 Dynamics of Main Root Diameter

Measured root diameter under all cultivation methods showed an S-shaped growth trend [Figure 3: see original paper]A. Root diameter was in the initiation phase at 45-75 days, entered rapid growth at 75-105 days (increasing from 0.52 cm to 1.07 cm), then gradually transitioned to slow growth after 105 days, reaching maximum values around 165 days. By 105 days after emergence, root diameter stabilized at 0.98-1.17 cm across treatments, with minimal subsequent increase. At harvest, treatment LB achieved the maximum root diameter (1.17 cm), significantly different from other treatments ($P < 0.05$), while other treatments showed no significant differences ($P > 0.05$).

Logistic model fitting yielded the parameters in . Theoretical root diameter growth dynamics plotted using days after sowing as the independent variable showed typical Logistic trends [Figure 3: see original paper]B. The model fit well ($R^2 > 0.9$, $P < 0.01$). Theoretical maximum root diameter followed the order: LB > PN > PW > PB > LW = LN. Rapid growth started 31-47 days after sowing, lasted 42-60 days, with maximum growth rates of 0.017-0.020 cm · d^{-1}. Time to maximum growth rate was 59-72 days, slow growth duration was 53-75 days, and growth ended at 141-176 days.

Treatment LB had the longest root diameter growth duration and largest theoretical value, indicating that ridge planting with black film mulch extended the growth period and favored high yield formation. LW and LN had the smallest theoretical values, showing that ridge planting with white film or without mulch was unfavorable for root diameter growth. Compared with ridge planting, flat planting showed superior root diameter growth, though final diameter increase was slightly less than ridge planting with black film. Overall, ridge planting with black film mulch significantly promoted root system development, enhanced root diameter increase, and extended the growth period.

2.1.3 Dynamics of Root Dry Weight per Plant

Measured root dry weight per plant under all cultivation methods consistently showed S-shaped growth trends [Figure 4: see original paper]A. During the growth period, root dry weight increased from 0.27 g to 7.51 g. Before 105 days after emergence, root dry weight increased rapidly from 0.27 g to 6.46 g, with average daily increments of 0.06-0.09 $g \cdot d^{-1}$. After 135 days, the increase slowed to 0.02-0.03 $g \cdot d^{-1}$ across treatments. At harvest, all treatments reached maximum values, with treatment LB showing the highest root dry weight, significantly different from other treatments ($P < 0.05$).

Logistic model fitting produced the equations and parameter characteristics shown in . Theoretical root dry weight growth dynamics plotted using days after sowing as the independent variable exhibited typical Logistic trends [Figure 4: see original paper]B. The model fit well ($R^2 > 0.9$, $P < 0.01$). Theoretical maximum root dry weight per plant followed the order: LB > PB > LW > PW > LN > PN. Rapid growth started 60-69 days after sowing, lasted 37-43 days, with maximum growth rates of 0.062-0.090 $g \cdot d^{-1}$. Time to maximum growth rate was 76-86 days, slow growth duration was 46-53 days, and growth ended at 143-164 days.

Treatment LB had the largest theoretical root dry weight value, indicating the highest individual plant yield, while flat planting without mulch had the smallest theoretical value and lowest yield. The overall performance followed the pattern: black film > white film > no mulch, demonstrating that black film contributed most to root dry weight accumulation, while no mulch resulted in significantly lower root dry weight and was unfavorable for yield improvement.

2.2 Effects of Cultivation Methods on Radix Isatidis Yield

Cultivation methods significantly affected radix isatidis yield [Figure 5: see original paper]. ANOVA showed that cultivation model and mulching had F-values of 6.503 and 100.48, respectively ($P < 0.05$), reaching significant levels. The interaction between cultivation model and mulching was highly significant ($F = 80.552$, sig. = 0.000), indicating both factors substantially influenced yield. Yield ranking was LB > PB > PN > PW > LN > LW. Ridge planting with black film mulch achieved the highest yield (4,514 $kg \cdot hm^{-2}$), while ridge planting with white film mulch had the lowest (3,116 $kg \cdot hm^{-2}$). No significant differences were observed between flat planting without mulch, flat planting with black film, and ridge planting with white film ($P > 0.05$).

2.3 Effects of Cultivation Methods on (R,S)-Epigoitrin Content

Cultivation methods significantly affected (R,S)-epigoitrin content in radix isatidis [Figure 6: see original paper]. ANOVA revealed significant differences among treatments, with both cultivation model and mulching method influencing epigoitrin content. The F-values for cultivation model and mulching were 11.764 and 256.481, respectively ($P < 0.05$), with a highly significant interaction ($F = 338.765$, sig. = 0.000). Ridge planting with white film mulch had the highest epigoitrin content, followed by flat planting with black film and ridge planting with black film, while ridge planting without mulch had the lowest. No significant difference was found between flat planting with white film and ridge planting with black film (P > 0.05).

Discussion

Plant organ development during growth follows a “slow-fast-slow”S-shaped curve. Previous studies have shown that the seedling emergence process of *Codonopsis pilosula* follows an S-shaped trend fitting the Logistic equation, and that growth dynamics of *I. indigotica* organs conform to this pattern. Our results confirm that main root length, root diameter, and root dry weight of *I. indigotica* all exhibit S-shaped growth trends that are well-fitted by Logistic curves, consistent with previous research.

During early growth, good field ventilation and light transmission promoted transport of photosynthates to roots, facilitating rapid root trait development. As new leaves continuously emerged, plant space decreased, suppressing photosynthetic capacity and growth, leading to slowed growth rates of root length, diameter, and dry weight in later stages, reaching lifetime maxima about 10 days before harvest. Model fitting revealed that root length, diameter, and dry weight all followed Logistic curves with four distinct phases: gradual increase, rapid increase, slow increase, and termination. Ridge planting with black film mulch was particularly beneficial for increasing root diameter and dry weight. Ridge planting created a three-dimensional field structure, enhanced population photosynthetic capacity, extended the aboveground growth period, prevented premature senescence, and provided favorable conditions for yield improvement. Film mulching increased soil temperature, improved soil structure, and created advantageous conditions for dry matter accumulation, establishing the foundation for high yield.

Compared with flat planting, both ridge formation and mulching significantly affected crop growth, yield, and quality. Previous research has demonstrated that film mulching increases yield through enhanced growth and water consumption, improves soil moisture content, modifies soil structure, promotes early and uniform emergence, and improves yield and quality. Mulching has been shown to improve crop growth in saline-alkaline soils and significantly increase grain yield. However, some studies have reported yield reductions with mulching, such as a 15.8% decrease in potato tuber yield under film mulching. Our study demonstrates that black film mulching benefits *I. indigotica* yield, consistent with most previous findings. The superior yield effect of black film results from improved soil environment and temperature, plus effective weed suppression that reduces nutrient competition, providing more favorable growth conditions.

Ridge planting creates beneficial micro-ecological environments that promote crop growth. Previous studies have shown that ridge planting increases yield by enhancing soil respiration and extending leaf functional duration, promoting photosynthate accumulation and translocation to grains. Our results indicate that ridge planting favors root dry matter accumulation in *I. indigotica*, establishing the foundation for high yield. Overall, ridge planting outperformed flat planting, with ridge planting plus black film mulch producing the highest yield by improving population structure, light use efficiency, and growth conditions.

Secondary metabolite accumulation in medicinal plants is a complex physiological process influenced by plant characteristics, environmental conditions, and developmental stages. The core evaluation index for medicinal material quality is the chemical composition that objectively represents clinical efficacy. Our study demonstrates that cultivation methods significantly affect (R,S)-epigoitrin content, with ridge planting plus white film mulch producing the highest content and ridge planting without mulch the lowest. The combination of ridge planting and white film mulch was most conducive to epigoitrin accumulation. Ridge planting elevated soil temperature for root growth, while white film facilitated sunlight transmission and improved light use efficiency, creating favorable conditions for epigoitrin accumulation. However, epigoitrin synthesis is complex, and the specific effects of different cultivation methods on its biosynthetic pathway require further investigation.

Medicinal plants are cultivated to provide raw materials, where component content ultimately determines medicinal value, making yield-only pursuit inappropriate. Comprehensive evaluation should consider both yield and quality based on active component yield per unit area. Integrating yield and quality considerations, ridge planting with black film mulch proved optimal for both radix isatidis yield and quality in the Hexi region, while ridge planting without mulch was poorest.

Conclusion

Root trait dynamics of *I. indigotica* conform to the Logistic growth model. Across treatments, mulching produced larger theoretical maximum values than non-mulching, with earlier and longer rapid accumulation periods. Ridge planting with black film mulch showed significantly higher theoretical values for root length, diameter, and dry weight than other treatments, while flat planting without mulch and ridge planting without mulch showed the smallest values, demonstrating the clear yield benefits of black film mulching and ridge planting. Ridge planting with black film mulch produced the highest yield per unit area, while ridge planting with white film mulch produced the highest epigoitrin content. Considering both radix isatidis yield and epigoitrin content, ridge planting with black film mulch is the optimal cultivation method.

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