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## Research Report

## Open Access

## Determination of Watering Regime for Optimal Production of Hortitom 1 and Hortitom 3 Genotypes of *Solanum lycopersicum* L. (Tomatoes) under Screenhouse Conditions

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**Abstract** Water availability is a major limiting factor for tomato production, particularly under changing climate conditions. This study investigated the effects of eight watering regimes twice daily (T1), once daily (T2), every 2 days (T3), every 3 days (T4), every 4 days (T5), every 5 days (T6), every 6 days (T7), and continuous waterlogging (T8) on growth, yield, and fruit nutritional quality of two Nigerian tomato genotypes (Hortitom 1 and Hortitom 3) under screenhouse conditions. The experiment was laid out in a 2 × 8 factorial arrangement in a completely randomized design with five replicates. Both genotypes exhibited 100% survival under all non-waterlogged treatments, while continuous waterlogging (T8) resulted in 100% mortality. Hortitom 1 and Hortitom 3 attained their maximum plant height at T5 (watering every 4 days), recording 58.70 cm and 62.50 cm respectively. Fruit yield (fresh weight) was highest in Hortitom 1 under T1 (5.25 g per fruit) and in Hortitom 3 under T7 (7.75 g per fruit). Nutritional composition was significantly influenced by genotype and watering regime. Crude protein content peaked at 2.06% in Hortitom 1 under T5 and 1.85% in Hortitom 3 under T4. These results demonstrate clear genotypic differences in response to water availability. Hortitom 1 performed best under moderate water stress (T5) for vegetative growth and nutritional quality, while Hortitom 3 showed superior fruit yield under more severe water restriction (T7). Both genotypes are highly susceptible to waterlogging but tolerant to drought. The findings provide genotype-specific irrigation recommendations that can enhance water-use efficiency while maintaining or improving fruit nutritional quality in tomato production under screenhouse conditions.

**Keywords** Drought; Waterlogging; Tomato genotypes; Growth; Nutritional quality; *Solanum lycopersicum*

## 1 Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and consumed vegetables worldwide. It is valued for its rich nutritional profile, including vitamins A, C, and E, as well as lycopene antioxidants that reduce risks of cardiovascular diseases and cancers (Bai and Lindhout, 2022; Natali et al., 2025). The crop also plays key economic and industrial roles. Native to western South America, particularly Peru and Ecuador, tomatoes were domesticated in Mexico. Spanish explorers introduced them to Europe in the 16th century. Initially grown as ornamentals due to their resemblance to nightshade plants, they later became a global culinary staple (Donoso et al., 2022).

Tomatoes exhibit extensive morphological and genetic diversity. This diversity has produced genotypes adapted to various climates, diseases, and consumer preferences. Wild relatives contribute key traits, such as drought and salinity resistance from *Solanum pimpinellifolium*. Other species, including *S. peruvianum*, *S. chilense*, *S. habrochaites*, and *S. pennellii*, provide tolerances to extreme conditions, pathogens, pests, and cold (Razifard et al., 2020; Blanca et al., 2022).

Successful tomato cultivation depends on optimal environmental and agronomic factors. Well-drained loamy soils with pH 5.5-6.8 and high organic matter support root health, nutrient uptake, and disease prevention (Jones, 2021). Clay soils hinder drainage, while sandy soils require irrigation and amendments. Ideal temperatures range from 20°C to 25°C for growth, flowering, and fruiting. High temperatures (>30°C) cause flower abortion, and low



temperatures ( $<10^{\circ}\text{C}$ ) impair pollination. Farmers mitigate these using greenhouses or shade nets (Sharma et al., 2020). Adequate light is essential for photosynthesis and fruit ripening, with supplementation needed in low-light regions.

Water stress during critical growth stages reduces yields and triggers disorders like blossom-end rot. Effective countermeasures include drip irrigation (40%-60% efficiency gains), deficit irrigation, and mulching to control evaporation, regulate soil temperature, and suppress weeds (Feres and Soriano, 2020; Makhadmeh et al., 2022; Ayana and Olika, 2024). Water is central to tomato physiology, driving cell expansion, nutrient uptake, and fruit development. Drought-induced deficits limit biomass, fruit set, and nutrient profiles, while excesses cause other issues (Bastías et al., 2020; Nguyen et al., 2021; Burato et al., 2024). Genotypic variations, such as deeper roots or osmotic adjustments, enhance tolerance (Alam et al., 2021). However, limited data exist on how irrigation regimes affect growth, yield, and fruit nutritional quality in newly developed Nigerian tomato genotypes, Hortitom 1 and Hortitom 3, under screen house conditions.

In the face of water scarcity and climate variability, tomatoes require precise irrigation to sustain yields and quality (Ray and Majumder, 2024). Evaluating watering regimes for Hortitom 1 and Hortitom 3 through different irrigation methods can improve water use efficiency, root nutrient uptake, and loss reduction while maintaining nutritional content (Gheysari et al., 2021). Such insights can guide farmers toward optimal practices, enhance nutritional output for consumers, and inform breeders about genotype-environment interactions for resilient varieties (Santos et al., 2021). Therefore, this study aims to assess the impact of varying watering levels on growth, yield parameters, and fruit nutritional composition of Hortitom 1 and Hortitom 3 under screen house conditions.

## **2 Materials and Methods**

### **2.1 Location of the experiment**

This experiment was carried out at the screen house of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria (latitude  $7.2^{\circ}\text{N}$ , longitude  $5.44^{\circ}\text{E}$ ).

### **2.2 Sources of materials for the experiment**

Two tomato (*Solanum lycopersicum* L.) genotypes, Hortitom 1 and Hortitom 3, were obtained from the National Horticultural Research Institute (NIHORT), Ibadan, Oyo State, Nigeria. The soil was analyzed for physical and chemical properties using the standard methods of AOAC (1985). It was shade-dried and passed through a 2-mm sieve before analysis.

### **2.3 Soil collection and preparation**

Topsoil (0-15 cm depth) was collected from an arable farmland within the premises of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The soil was sieved to remove debris and thoroughly mixed to obtain a homogeneous medium. Approximately 14 kg of prepared soil was filled into each perforated polythene pot. Tomato seedlings raised in the nursery for 3 weeks were transplanted into perforated polythene pots filled with 14 kg of topsoil; only pots for waterlogged conditions were not perforated.

### **2.4 Experimental setup**

The potted plants were watered regularly for two weeks after transplanting for proper seedling establishment. Thereafter, they were differentially exposed to eight watering regimes: watering twice daily (T1), once daily (T2), every 2 days (T3), every 3 days (T4), every 4 days (T5), every 5 days (T6), every 6 days (T7) and completely waterlogged (T8). Pots were laid out on the screen house floor in a completely randomized design (CRD) with each treatment replicated five times. It was a  $2 \times 8$  factorial experiment with genotype as Factor A at 2 levels, and watering regime as Factor B at 8 levels. Except waterlogging condition that was permanently flooded, each potted plant received approximately 380 ml of water at every watering time. This was the volume required to keep the soil at field capacity based on 36% field capacity of the soil. Standard agronomic practices including weeding and pest control were carried out during the experiment.

## 2.5 Data collection

Plant height was measured from the soil surface to the apical bud using a meter rule. Stem girth was measured at 2 cm point above the base of the plant. The number of fully expanded leaves was counted manually on each plant. The leaf area was measured using the leaf area meter (LI-COR 300 model). The number of branches produced per plant was counted manually. At harvest, plants were carefully uprooted, washed and separated into leaves, roots and stems. Root growth was determined by measuring the root length using a meter rule, and the number of roots was counted manually. Fresh weight was measured immediately after harvest, while dry weight was obtained after oven-drying at 80°C to constant weight, using Melter PC 180. Dry weight of plant parts (roots, stems, and leaves) was also measured. Yield in terms of fresh and dry mass of the fruit was also assessed using an electronic weighing balance.

## 2.6 Laboratory analysis of tomato fruits

Dried tomato fruits were ground into fine powder for analysis. Fiber content was determined by boiling the sample in 1.25% H<sub>2</sub>SO<sub>4</sub> and 1.25% NaOH, followed by washing and drying. Other parameters of proximate composition were analyzed using the standard methods of AOAC (1985) in which the mixture was boiled until a clear solution was obtained, and allowed to cool at room temperature. The resulting solution was quantitatively transferred into a calibrated flask and completed to 25 ml with distilled water. Moisture, crude protein, crude fat, carbohydrate and ash contents were calculated using relevant formulas. N was analyzed using the macro Kjeldahl method, while P was determined using ammonium-vanadomolybdate reagent and a calibration curve. Potassium contents were assayed through flame emission photometry. calcium contents by Ethylenediaminetetraacetic acid (EDTA) titration.

## 2.7 Statistical analysis

All data collected were subjected to two-way Analysis of Variance (ANOVA) using SPSS (Version 27.0). Where significant differences were observed among treatment means, Tukey's Honest Significant Difference (HSD) test was used at 95% confidence level to perform post-hoc comparisons.

## 3 Results

### 3.1 Soil used for planting

The soil used for planting was a sandy soil with 5.60 pH, 6.19% clay, 4.29% silt, 89.7% sand, 2.89% C, 0.14% N, 9.02 mg/kg P, 6.24 mg/kg Ca, 1.84 mg/kg Mg, 0.34 mg/kg Na, 0.23 mg/100 K, 0.20 mg/kg H, and 8.86 mg/kg CEC. It had 1.12 mg/cm<sup>3</sup> bulk density, 36.13% field capacity, and 19.08% permanent wilting point.

### 3.2 Effect of watering regime on percentage survival and growth of two genotypes of *Solanum lycopersicum*

Table 1 below shows the effects of different watering regime on the survival of two *Solanum lycopersicum* genotypes. Irrigation treatments T1 to T7, applied from twice daily up to once every six days, resulted in 100% survival. In contrast, T8, which involved constant waterlogging, led to total plant death. For plant height, Hortitom1 plants measured between 41.25±3.53 cm under T7 and 58.70±6.32 cm under T5, achieving notably taller growth in T5. Hortitom 3 produced taller plants overall than Hortitom 1, with heights from 53.50±0.65 cm in T2 to 62.50±0.65 cm in T5. Stem girth in Hortitom1 varied from 2.25±0.10 cm in T6 to 2.63±0.24 cm in T1, showing no significant differences between regimes. Hortitom 3 also maintained consistent stem girth across treatments, between 2.70±0.20 cm in T5 and 2.90±0.13 cm in T2. The number of leaves in Hortitom 1 rose significantly from 11.75±0.41 leaves under T1 to 31.13±0.47 leaves under T7. Hortitom 3 displayed the opposite trend, peaking at 33.50±0.65 leaves in T1 and dropping to lower values of 19.00±0.41 in T5. Leaf area in Hortitom 1 spanned 24.15±0.31 cm<sup>2</sup> in T7 to 26.48±0.40 cm<sup>2</sup> in T1 and T3, differing significantly from other treatments. Hortitom 3 had leaf areas from 26.35±0.64 cm<sup>2</sup> in T5 to 27.31±0.08 cm<sup>2</sup> in T3. Number of roots was greater in T5 (7.50±1.04) than T7 (4.00±0.41), while root length remained similar across all treatments.

Table 1 Effect of watering regime on percentage survival and growth of two genotypes of *Solanum lycopersicum*

| Parameter                    | Tomato genotype | Watering regime         |                          |                         |                          |                          |                          |                          |      |
|------------------------------|-----------------|-------------------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|------|
|                              |                 | T1                      | T2                       | T3                      | T4                       | T5                       | T6                       | T7                       | T8   |
| Plant survival (%)           | H1              | 100.00                  | 100.00                   | 100.00                  | 100.00                   | 100.00                   | 100.00                   | 100.00                   | 0.00 |
|                              | H3              | 100.00                  | 100.00                   | 100.00                  | 100.00                   | 100.00                   | 100.00                   | 100.00                   | 0.00 |
| Plant height (cm)            | H1              | 47.25±1.99 <sup>a</sup> | 53.13±9.44 <sup>a</sup>  | 46.35±2.75 <sup>a</sup> | 55.25±1.03 <sup>a</sup>  | 58.70±6.32 <sup>a</sup>  | 58.07±5.94 <sup>a</sup>  | 41.25±3.53 <sup>a</sup>  | -    |
|                              | H3              | 54.50±0.65 <sup>a</sup> | 53.50±0.65 <sup>a</sup>  | 57.50±0.65 <sup>b</sup> | 61.50±0.65 <sup>cd</sup> | 62.50±0.65 <sup>d</sup>  | 58.50±0.65 <sup>b</sup>  | 59.50±0.65 <sup>bc</sup> | -    |
| Stem girth (cm)              | H1              | 2.63±0.24 <sup>a</sup>  | 2.39±0.21 <sup>a</sup>   | 2.35±0.22 <sup>a</sup>  | 2.43±0.21 <sup>a</sup>   | 2.38±0.11 <sup>a</sup>   | 2.25±0.10 <sup>a</sup>   | 2.33±0.12 <sup>a</sup>   | -    |
|                              | H3              | 2.73±0.13 <sup>a</sup>  | 2.90±0.13 <sup>a</sup>   | 2.73±0.23 <sup>a</sup>  | 2.88±0.13 <sup>a</sup>   | 2.70±0.20 <sup>a</sup>   | 2.85±0.12 <sup>a</sup>   | 2.78±0.22 <sup>a</sup>   | -    |
| Number of leaves             | H1              | 11.75±0.41 <sup>a</sup> | 14.80±0.56 <sup>b</sup>  | 18.55±0.61 <sup>c</sup> | 21.88±0.47 <sup>d</sup>  | 24.88±0.47 <sup>e</sup>  | 28.00±0.53 <sup>f</sup>  | 31.13±0.47 <sup>g</sup>  | -    |
|                              | H3              | 33.50±0.65 <sup>c</sup> | 29.50±0.65 <sup>d</sup>  | 25.50±0.65 <sup>c</sup> | 21.50±0.65 <sup>ab</sup> | 19.00±0.41 <sup>a</sup>  | 22.50±0.65 <sup>b</sup>  | 26.50±0.65 <sup>c</sup>  | -    |
| Leaf area (cm <sup>2</sup> ) | H1              | 26.48±0.40 <sup>c</sup> | 26.10±0.38 <sup>bc</sup> | 26.48±0.22 <sup>c</sup> | 26.35±0.16 <sup>c</sup>  | 25.97±0.31 <sup>bc</sup> | 24.97±0.13 <sup>ab</sup> | 24.15±0.31 <sup>a</sup>  | -    |
|                              | H3              | 27.00±0.26 <sup>a</sup> | 27.11±0.19 <sup>a</sup>  | 27.31±0.08 <sup>a</sup> | 26.97±0.22 <sup>a</sup>  | 26.35±0.64 <sup>a</sup>  | 26.62±0.20 <sup>a</sup>  | 26.62±0.19 <sup>a</sup>  | -    |
| Number of roots              | H1              | 5.55±0.61 <sup>ab</sup> | 6.25±0.25 <sup>ab</sup>  | 4.75±1.03 <sup>ab</sup> | 6.50±0.87 <sup>ab</sup>  | 7.50±1.04 <sup>b</sup>   | 4.75±0.75 <sup>ab</sup>  | 4.00±0.41 <sup>a</sup>   | -    |
|                              | H3              | 7.25±0.85 <sup>a</sup>  | 7.25±0.25 <sup>a</sup>   | 5.25±0.25 <sup>a</sup>  | 5.00±0.71 <sup>a</sup>   | 5.50±0.65 <sup>a</sup>   | 6.50±1.32 <sup>a</sup>   | 6.75±0.48 <sup>a</sup>   | -    |
| Root length (cm)             | H1              | 5.60±0.61 <sup>a</sup>  | 4.58±0.64 <sup>a</sup>   | 5.40±0.51 <sup>a</sup>  | 5.45±0.57 <sup>a</sup>   | 4.60±0.47 <sup>a</sup>   | 3.58±0.41 <sup>a</sup>   | 3.80±0.43 <sup>a</sup>   | -    |
|                              | H3              | 6.10±0.91 <sup>ab</sup> | 5.83±0.53 <sup>ab</sup>  | 6.88±0.51 <sup>b</sup>  | 5.13±0.60 <sup>ab</sup>  | 4.20±0.63 <sup>ab</sup>  | 3.85±0.16 <sup>a</sup>   | 3.40±0.62 <sup>a</sup>   | -    |

Note: Each value is a mean ± S.E. of 5 replicates. For each value, means with the same letter(s) in superscript on the same row are not significantly different at  $P \geq 0.05$  (Tukey HSD test). T1: watering twice daily; T2: watering once daily; T3: watering every two days; T4: watering every three days; T5: watering every four days; T6: watering every five days; T7: watering every six days; T8: continuous waterlogging; H1: Hortitom 1 genotype; H3: Hortitom 3 genotype

### 3.3 Effect of water stress on biomass

The impact of watering regimes on biomass components shown in (Table 2) in two *Solanum lycopersicum* genotypes, Hortitom 1 and Hortitom 3. In Hortitom 1, fresh and dry leaf weights remained consistent across treatments at about 9.00 g and 5.51 g, respectively, while root numbers were notably higher under T5 ( $7.50 \pm 1.04$ ) than T7 ( $4.00 \pm 0.41$ ), though root length, fresh root weight, and dry root weight showed no differences. Stem biomass decreased steadily with water restriction, with fresh stem weight falling from  $15.43 \pm 0.22$  g in T1 to  $10.38 \pm 0.27$  g in T7, and dry stem weight from  $9.13 \pm 0.43$  g to  $4.53 \pm 0.28$  g. For Hortitom 3, fresh leaf weight increased significantly under T7 ( $16.75 \pm 2.10$  g) compared to T1 ( $8.50 \pm 2.06$  g), but dry leaf weight stayed similar. Root numbers did not vary, root length peaked at T3 ( $6.88 \pm 0.51$  cm), and fresh root weight rose from  $3.75 \pm 0.48$  g in T2 to  $7.75 \pm 0.48$  g in T7, with dry root weight highest in T7 ( $4.92 \pm 0.42$  g). Stem fresh and dry weights declined gradually from T1 ( $17.05 \pm 0.13$  g and  $10.03 \pm 0.41$  g) to T7 ( $13.88 \pm 0.37$  g and  $5.70 \pm 0.20$  g).

### 3.4 Phenological and yield parameter

Table 3 outlines water regime impacts on days to first flowering and fruit yield parameters in *Solanum lycopersicum* genotypes Hortitom 1 and Hortitom 3. Watering regimes impacted days to first flowering in both genotypes: Hortitom 1 flowered soonest under T1 ( $39.75 \pm 0.32$  days), with delays increasing to  $65.00 \pm 2.42$  days in T7. Hortitom 3 flowered later than Hortitom 1 in every case, starting at  $47.50 \pm 0.65$  days in T1 and extending to  $71.50 \pm 0.65$  days in T7. Hortitom 1 produced a steady  $5.63 \pm 0.13$  to  $6.50 \pm 0.20$  fruits per plant across treatments with no differences, alongside slightly reduced fresh fruit weights from  $3.75 \pm 0.63$  g to  $5.25 \pm 0.32$  g under drier conditions; notably, fruit length and breadth grew larger, from  $16.25 \pm 0.32$  cm and  $18.18 \pm 0.38$  cm in T1 to peaks of  $19.50 \pm 0.20$  cm and  $21.88 \pm 0.13$  cm in T6/T7. Hortitom 3 showed greater variability, with fruits numbering  $3.00 \pm 0.41$  to  $6.00 \pm 0.41$  (highest in T4), fresh weights climbing in T5-T7 to  $7.75 \pm 0.48$  g in T7, and dry weights maximizing at  $4.92 \pm 0.42$  g in T7 yet fruit length shrank from  $42.35 \pm 0.65$  cm in T1 to  $18.35 \pm 0.65$  cm in T7, while breadth fell from  $75.28 \pm 0.65$  cm to  $51.28 \pm 0.65$  cm.

### 3.5 Proximate and minerals composition

The results in Table 4 reveal the proximate and mineral compositions of *Solanum lycopersicum* genotypes; Hortitom 1 and Hortitom 3 across water regimes T1-T7. Hortitom 1 generally displayed higher and more variable proximate values than Hortitom 3. Moisture content in Hortitom 1 spanned  $14.28 \pm 0.05\%$  (T3) to  $19.00 \pm 0.13\%$  (T4), exceeding Hortitom 3's narrower  $15.80 \pm 0.22\%$  (T6) to  $18.71 \pm 0.04\%$  (T3). Fat remained stable and comparable, with Hortitom 1 at  $0.87 \pm 0.01\%$  (T2) to  $1.14 \pm 0.02\%$  (T5) versus Hortitom 3 from  $0.83 \pm 0.00\%$  (T1) to  $1.12 \pm 0.00\%$  (T2). Ash was broader in Hortitom 1 ( $3.66 \pm 0.01\%$  at T6 to  $5.08 \pm 0.04\%$  at T5) than Hortitom 3 ( $3.56 \pm 0.01\%$  at T6 to  $4.99 \pm 0.02\%$  at T7). Crude fiber showed Hortitom 1 ranging lower to higher ( $5.01 \pm 0.00\%$  at T2 to  $8.31 \pm 0.03\%$  at T6) compared to Hortitom 3 ( $6.03 \pm 0.01\%$  at T1 to  $8.27 \pm 0.05\%$  at T3). Crude protein was consistently superior in Hortitom 1 ( $1.10 \pm 0.01\%$  at T2 to  $2.06 \pm 0.05\%$  at T5) over Hortitom 3 ( $1.05 \pm 0.01\%$  at T1 to  $1.85 \pm 0.09\%$  at T4). Carbohydrates peaked much higher in Hortitom 1 ( $67.37 \pm 0.23\%$  at T7 to  $74.22 \pm 0.16\%$  at T2) than in Hortitom 3 ( $66.41 \pm 0.08\%$  at T7 to  $70.65 \pm 0.03\%$  at T2).

For minerals, patterns were more mixed but often favoured Hortitom 1 in range and peaks. Calcium in Hortitom 1 went from  $15.85 \pm 0.05$  mg/kg (T2) to  $22.55 \pm 0.15$  mg/kg (T6), closely matching Hortitom 3, ranging from  $14.80 \pm 0.10$  mg/kg (T3) to  $22.75 \pm 0.25$  mg/kg (T4), though the latter edged higher at its max. Potassium was notably higher in Hortitom 1 ( $25.60 \pm 0.20$  mg/kg at T6 to  $35.95 \pm 0.45$  mg/kg at T1) versus Hortitom 3 ( $24.45 \pm 0.15$  mg/kg at T4 to  $32.30 \pm 0.20$  mg/kg at T2). Magnesium spanned wider in Hortitom 1 ( $19.40 \pm 0.10$  mg/kg at T6 to  $26.15 \pm 0.15$  mg/kg at T5) than Hortitom 3 ( $18.60 \pm 0.10$  mg/kg at T5 to  $23.37 \pm 0.04$  mg/kg at T6). Iron reached a higher peak in Hortitom 1 ( $1.27 \pm 0.00$  mg/kg at T3 to  $2.01 \pm 0.00$  mg/kg at T4) over Hortitom 3 ( $1.33 \pm 0.00$  mg/kg at T4 to  $1.73 \pm 0.01$  mg/kg at T3). Phosphorus was similar, with Hortitom 1 at  $8.92 \pm 0.19$  mg/kg (T4) to  $14.04 \pm 0.07$  mg/kg (T2) and Hortitom 3 at  $9.11 \pm 0.12$  mg/kg (T6) to  $14.17 \pm 0.05$  mg/kg (T4). Nitrogen was marginally higher in Hortitom 1 ( $0.18 \pm 0.00$  mg/kg at T1/T2 to  $0.33 \pm 0.01$  mg/kg at T5) than Hortitom 3 ( $0.17 \pm 0.00$  mg/kg at T1 to  $0.30 \pm 0.02$  mg/kg at T4). These trends indicate Hortitom 1's superior nutritional profile under water stress variability.



Table 2 Effect of watering regime on the vegetative biomass of two genotypes of *Solanum lycopersicum*

| Biomass parameter (g) | Tomato genotype | Watering regime          |                         |                          |                          |                          |                          |                          |    |
|-----------------------|-----------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|----|
|                       |                 | T1                       | T2                      | T3                       | T4                       | T5                       | T6                       | T7                       | T8 |
| Leaf fresh weight     | H1              | 9.00±1.47 <sup>a</sup>   | 9.00±1.78 <sup>a</sup>  | 8.25±1.38 <sup>a</sup>   | 5.00±1.22 <sup>a</sup>   | 8.00±0.71 <sup>a</sup>   | 7.00±0.91 <sup>a</sup>   | 5.00±0.71 <sup>a</sup>   | -  |
|                       | H3              | 8.50±2.06 <sup>a</sup>   | 9.75±1.49 <sup>ab</sup> | 13.75±1.93 <sup>ab</sup> | 12.50±0.96 <sup>ab</sup> | 12.50±1.32 <sup>ab</sup> | 13.50±0.65 <sup>ab</sup> | 16.75±2.10 <sup>b</sup>  | -  |
| Stem fresh weight     | H1              | 15.43±0.22 <sup>c</sup>  | 15.28±0.62 <sup>c</sup> | 13.85±0.67 <sup>bc</sup> | 13.03±0.91 <sup>bc</sup> | 12.23±0.34 <sup>ab</sup> | 11.63±0.36 <sup>ab</sup> | 10.38±0.27 <sup>a</sup>  | -  |
|                       | H3              | 17.05±0.13 <sup>d</sup>  | 17.43±0.38 <sup>d</sup> | 16.95±0.13 <sup>d</sup>  | 16.35±0.21 <sup>cd</sup> | 15.75±0.17 <sup>bc</sup> | 14.78±0.11 <sup>ab</sup> | 13.88±0.37 <sup>a</sup>  | -  |
| Root fresh weight     | H1              | 5.00±0.41 <sup>a</sup>   | 4.75±0.48 <sup>a</sup>  | 3.75±0.48 <sup>a</sup>   | 3.75±0.48 <sup>a</sup>   | 4.25±0.63 <sup>a</sup>   | 4.00±0.58 <sup>a</sup>   | 4.00±0.41 <sup>a</sup>   | -  |
|                       | H3              | 4.75±0.48 <sup>ab</sup>  | 3.75±0.48 <sup>a</sup>  | 5.50±0.65 <sup>abc</sup> | 4.75±0.75 <sup>ab</sup>  | 7.25±0.48 <sup>bc</sup>  | 6.67±0.88 <sup>bc</sup>  | 7.75±0.48 <sup>c</sup>   | -  |
| Leaf dry weight       | H1              | 5.51±1.74 <sup>a</sup>   | 5.55±1.42 <sup>a</sup>  | 3.75±1.85 <sup>a</sup>   | 2.16±0.85 <sup>a</sup>   | 6.60±2.92 <sup>a</sup>   | 2.04±0.79 <sup>a</sup>   | 1.14±0.29 <sup>a</sup>   | -  |
|                       | H3              | 5.86±1.69 <sup>a</sup>   | 5.98±2.23 <sup>a</sup>  | 7.29±2.05 <sup>a</sup>   | 8.34±0.60 <sup>a</sup>   | 8.43±0.78 <sup>a</sup>   | 9.29±0.96 <sup>a</sup>   | 10.48±1.09 <sup>a</sup>  | -  |
| Stem dry weight       | H1              | 9.13±0.43 <sup>b</sup>   | 8.88±0.34 <sup>b</sup>  | 7.45±0.59 <sup>b</sup>   | 7.45±0.31 <sup>b</sup>   | 5.58±0.21 <sup>a</sup>   | 5.03±0.43 <sup>a</sup>   | 4.53±0.28 <sup>a</sup>   | -  |
|                       | H3              | 10.03±0.41 <sup>d</sup>  | 9.15±0.46 <sup>cd</sup> | 8.25±0.37 <sup>bc</sup>  | 7.20±0.35 <sup>ab</sup>  | 7.03±0.41 <sup>ab</sup>  | 6.48±0.41 <sup>a</sup>   | 5.70±0.20 <sup>a</sup>   | -  |
| Root dry weight       | H1              | 0.81±0.19 <sup>a</sup>   | 1.75±0.41 <sup>a</sup>  | 0.97±0.29 <sup>a</sup>   | 0.73±0.28 <sup>a</sup>   | 1.05±0.06 <sup>a</sup>   | 0.83±0.27 <sup>a</sup>   | 0.74±0.17 <sup>a</sup>   | -  |
|                       | H3              | 2.64±0.27 <sup>ab</sup>  | 1.18±0.39 <sup>a</sup>  | 2.80±0.77 <sup>ab</sup>  | 2.56±0.60 <sup>a</sup>   | 1.82±0.38 <sup>a</sup>   | 3.10±0.36 <sup>ab</sup>  | 4.92±0.42 <sup>b</sup>   | -  |
| Total biomass         | H1              | 15.24±2.21 <sup>ab</sup> | 18.58±2.0 <sup>b</sup>  | 12.80±1.52 <sup>a</sup>  | 13.63±1.16 <sup>a</sup>  | 13.71±1.69 <sup>a</sup>  | 12.90±0.71 <sup>a</sup>  | 13.03±0.91 <sup>a</sup>  | -  |
|                       | H3              | 13.43±0.72 <sup>a</sup>  | 11.92±0.77 <sup>a</sup> | 17.65±0.88 <sup>ab</sup> | 15.57±0.97 <sup>ab</sup> | 18.38±0.93 <sup>b</sup>  | 15.71±0.47 <sup>a</sup>  | 16.35±0.21 <sup>ab</sup> | -  |

Note: Each value is a mean ± S.E. of 5 replicates. For each value, means with the same letter(s) in superscript on the same row are not significantly different at  $P \geq 0.05$  (Tukey HSD test). T1: watering twice daily; T2: watering once daily; T3: watering every two days; T4: watering every three days; T5: watering every four days; T6: watering every five days; T7: watering every six days; T8: continuous waterlogging; H1: Hortitum 1 genotype; H3: Hortitum 3 genotype

Table 3 Effect of watering regime on the phenological and yield parameters of two genotypes of *Solanum lycopersicum*

| Growth parameters                 | Tomato genotype | Watering regime         |                          |                          |                          |                          |                          |                          |    |
|-----------------------------------|-----------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|----|
|                                   |                 | T1                      | T2                       | T3                       | T4                       | T5                       | T6                       | T7                       | T8 |
| Number of days to first flowering | H1              | 39.75±0.32 <sup>a</sup> | 40.00±0.20 <sup>a</sup>  | 43.75±1.80 <sup>ab</sup> | 45.75±1.65 <sup>ab</sup> | 52.00±2.35 <sup>bc</sup> | 60.00±2.80 <sup>cd</sup> | 65.00±2.42 <sup>d</sup>  | -  |
|                                   | H3              | 47.50±0.65 <sup>a</sup> | 51.50±0.65 <sup>b</sup>  | 55.50±0.65 <sup>c</sup>  | 59.50±0.65 <sup>d</sup>  | 63.50±0.65 <sup>e</sup>  | 67.50±0.65 <sup>f</sup>  | 71.50±0.65 <sup>g</sup>  | -  |
| Number of fruit                   | H1              | 6.50±0.20 <sup>a</sup>  | 6.50±0.20 <sup>a</sup>   | 5.75±0.14 <sup>a</sup>   | 6.00±0.20 <sup>a</sup>   | 6.00±0.20 <sup>a</sup>   | 5.63±0.13 <sup>a</sup>   | 5.63±0.24 <sup>a</sup>   | -  |
|                                   | H3              | 5.50±0.65 <sup>ab</sup> | 4.50±0.65 <sup>ab</sup>  | 3.50±0.65 <sup>ab</sup>  | 6.00±0.41 <sup>b</sup>   | 3.00±0.41 <sup>a</sup>   | 5.50±0.65 <sup>ab</sup>  | 4.50±0.65 <sup>ab</sup>  | -  |
| Fruit fresh weight (g)            | H1              | 5.25±0.32 <sup>a</sup>  | 5.00±0.20 <sup>a</sup>   | 4.00±0.46 <sup>a</sup>   | 4.00±0.41 <sup>a</sup>   | 4.25±0.63 <sup>a</sup>   | 3.75±0.63 <sup>a</sup>   | 4.00±0.41 <sup>a</sup>   | -  |
|                                   | H3              | 4.75±0.48 <sup>ab</sup> | 3.75±0.48 <sup>a</sup>   | 5.50±0.65 <sup>abc</sup> | 4.75±0.75 <sup>ab</sup>  | 7.25±0.48 <sup>bc</sup>  | 6.50±0.65 <sup>bc</sup>  | 7.75±0.48 <sup>c</sup>   | -  |
| Fruit dry weight (g)              | H1              | 0.81±0.19 <sup>a</sup>  | 1.75±0.41 <sup>a</sup>   | 0.97±0.29 <sup>a</sup>   | 0.66±0.30 <sup>a</sup>   | 1.05±0.06 <sup>a</sup>   | 0.85±0.19 <sup>a</sup>   | 0.74±0.17 <sup>a</sup>   | -  |
|                                   | H3              | 2.64±0.28 <sup>ab</sup> | 1.68±0.65 <sup>a</sup>   | 2.80±0.77 <sup>ab</sup>  | 2.55±0.60 <sup>a</sup>   | 1.81±0.39 <sup>a</sup>   | 2.93±0.28 <sup>ab</sup>  | 4.92±0.42 <sup>b</sup>   | -  |
| Fruit length (cm)                 | H1              | 16.25±0.32 <sup>a</sup> | 18.00±0.20 <sup>bc</sup> | 17.50±0.20 <sup>b</sup>  | 18.50±0.20 <sup>cd</sup> | 19.25±0.14 <sup>de</sup> | 19.50±0.20 <sup>e</sup>  | 19.25±0.14 <sup>de</sup> | -  |
|                                   | H3              | 42.35±0.65 <sup>g</sup> | 38.35±0.65 <sup>f</sup>  | 34.35±0.65 <sup>e</sup>  | 30.35±0.65 <sup>d</sup>  | 26.35±0.65 <sup>c</sup>  | 22.35±0.65 <sup>b</sup>  | 18.35±0.65 <sup>a</sup>  | -  |
| Fruit breadth (cm)                | H1              | 18.18±0.38 <sup>a</sup> | 20.00±0.20 <sup>b</sup>  | 19.50±0.20 <sup>b</sup>  | 20.50±0.20 <sup>b</sup>  | 21.38±0.24 <sup>bc</sup> | 21.50±0.20 <sup>cd</sup> | 21.88±0.13 <sup>d</sup>  | -  |
|                                   | H3              | 75.28±0.65 <sup>g</sup> | 71.28±0.65 <sup>f</sup>  | 67.28±0.65 <sup>e</sup>  | 63.28±0.65 <sup>d</sup>  | 59.28±0.65 <sup>c</sup>  | 55.28±0.65 <sup>b</sup>  | 51.28±0.65 <sup>a</sup>  | -  |

Note: Each value is a mean ± S.E. of 5 replicates. For each value, means with the same letter(s) in superscript on the same row are not significantly different at  $P \geq 0.05$  (Tukey HSD test). T1: watering twice daily; T2: watering once daily; T3: watering every two days; T4: watering every three days; T5: watering every four days; T6: watering every five days; T7: watering every six days; T8: continuous waterlogging; H1: Hortitom 1 genotype; H3: Hortitom 3 genotype

Table 4 Effect of watering regime on the fruit proximate and mineral compositions of two genotypes of *Solanum lycopersicum*

| Proximate/Mineral composition | Tomato genotype | Watering regime          |                         |                          |                         |                          |                         |                          |    |
|-------------------------------|-----------------|--------------------------|-------------------------|--------------------------|-------------------------|--------------------------|-------------------------|--------------------------|----|
|                               |                 | T1                       | T2                      | T3                       | T4                      | T5                       | T6                      | T7                       | T8 |
| Moisture (%)                  | H1              | 17.61±0.06 <sup>d</sup>  | 15.13±0.13 <sup>b</sup> | 14.28±0.05 <sup>a</sup>  | 19.00±0.13 <sup>e</sup> | 15.00±0.02 <sup>b</sup>  | 16.41±0.08 <sup>c</sup> | 17.44±0.06 <sup>d</sup>  | -  |
|                               | H3              | 18.65±0.00 <sup>cd</sup> | 15.86±0.05 <sup>a</sup> | 18.71±0.04 <sup>d</sup>  | 16.80±0.22 <sup>b</sup> | 18.01±0.01 <sup>c</sup>  | 15.80±0.22 <sup>a</sup> | 18.21±0.03 <sup>cd</sup> | -  |
| Fat (%)                       | H1              | 1.06±0.06 <sup>b</sup>   | 0.87±0.01 <sup>a</sup>  | 1.12±0.00 <sup>b</sup>   | 1.02±0.03 <sup>a</sup>  | 1.14±0.02 <sup>b</sup>   | 1.13±0.02 <sup>b</sup>  | 1.07±0.06 <sup>b</sup>   | -  |
|                               | H3              | 0.83±0.00 <sup>a</sup>   | 1.12±0.00 <sup>c</sup>  | 0.99±0.02 <sup>abc</sup> | 0.93±0.08 <sup>ab</sup> | 0.97±0.01 <sup>abc</sup> | 0.84±0.01 <sup>ab</sup> | 1.01±0.01 <sup>bc</sup>  | -  |
| Ash (%)                       | H1              | 5.02±0.00 <sup>d</sup>   | 3.68±0.01 <sup>a</sup>  | 4.19±0.06 <sup>b</sup>   | 3.70±0.01 <sup>a</sup>  | 5.08±0.04 <sup>d</sup>   | 3.66±0.01 <sup>a</sup>  | 4.50±0.01 <sup>c</sup>   | -  |
|                               | H3              | 4.05±0.00 <sup>b</sup>   | 3.89±0.03 <sup>b</sup>  | 3.60±0.10 <sup>a</sup>   | 4.64±0.00 <sup>c</sup>  | 4.94±0.02 <sup>d</sup>   | 3.56±0.01 <sup>a</sup>  | 4.99±0.02 <sup>d</sup>   | -  |
| Crude fibre (%)               | H1              | 5.39±0.02 <sup>a</sup>   | 5.01±0.00 <sup>a</sup>  | 7.47±0.12 <sup>c</sup>   | 5.67±0.12 <sup>b</sup>  | 7.93±0.08 <sup>cd</sup>  | 8.31±0.03 <sup>d</sup>  | 8.01±0.21 <sup>cd</sup>  | -  |
|                               | H3              | 6.03±0.01 <sup>a</sup>   | 7.24±0.00 <sup>c</sup>  | 8.27±0.05 <sup>c</sup>   | 6.59±0.01 <sup>b</sup>  | 7.94±0.08 <sup>d</sup>   | 7.90±0.07 <sup>d</sup>  | 7.95±0.02 <sup>d</sup>   | -  |
| Crude protein (%)             | H1              | 1.12±0.01 <sup>a</sup>   | 1.10±0.01 <sup>a</sup>  | 1.68±0.01 <sup>c</sup>   | 1.99±0.02 <sup>d</sup>  | 2.06±0.05 <sup>e</sup>   | 1.34±0.02 <sup>b</sup>  | 1.74±0.01 <sup>c</sup>   | -  |
|                               | H3              | 1.05±0.01 <sup>a</sup>   | 1.26±0.00 <sup>b</sup>  | 1.83±0.00 <sup>d</sup>   | 1.85±0.09 <sup>d</sup>  | 1.54±0.01 <sup>c</sup>   | 1.53±0.01 <sup>c</sup>  | 1.45±0.02 <sup>bc</sup>  | -  |
| Carbohydrate (%)              | H1              | 69.81±0.10 <sup>c</sup>  | 74.22±0.16 <sup>e</sup> | 71.27±0.02 <sup>d</sup>  | 68.65±0.28 <sup>b</sup> | 68.80±0.11 <sup>b</sup>  | 69.16±0.12 <sup>c</sup> | 67.37±0.23 <sup>a</sup>  | -  |
|                               | H3              | 69.43±0.01 <sup>b</sup>  | 70.65±0.03 <sup>c</sup> | 66.63±0.20 <sup>a</sup>  | 69.21±0.19 <sup>b</sup> | 66.61±0.05 <sup>a</sup>  | 70.39±0.13 <sup>c</sup> | 66.41±0.08 <sup>a</sup>  | -  |
| Calcium (mg/kg)               | H1              | 20.40±0.10 <sup>d</sup>  | 15.85±0.05 <sup>a</sup> | 17.40±0.00 <sup>b</sup>  | 21.80±0.30 <sup>e</sup> | 18.50±0.10 <sup>c</sup>  | 22.55±0.15 <sup>e</sup> | 20.40±0.30 <sup>d</sup>  | -  |
|                               | H3              | 18.50±0.10 <sup>c</sup>  | 17.05±0.15 <sup>b</sup> | 14.80±0.10 <sup>a</sup>  | 22.75±0.25 <sup>d</sup> | 17.80±0.30 <sup>bc</sup> | 16.65±0.15 <sup>b</sup> | 17.50±0.30 <sup>bc</sup> | -  |
| Potassium (mg/kg)             | H1              | 35.95±0.45 <sup>e</sup>  | 30.65±0.05 <sup>c</sup> | 27.45±0.35 <sup>b</sup>  | 27.70±0.20 <sup>b</sup> | 31.05±0.35 <sup>c</sup>  | 25.60±0.20 <sup>a</sup> | 32.80±0.30 <sup>d</sup>  | -  |
|                               | H3              | 28.60±0.10 <sup>bc</sup> | 32.30±0.20 <sup>e</sup> | 29.30±0.20 <sup>c</sup>  | 24.45±0.15 <sup>a</sup> | 27.65±0.15 <sup>b</sup>  | 30.60±0.10 <sup>d</sup> | 28.35±0.25 <sup>bc</sup> | -  |
| Magnesium (mg/kg)             | H1              | 25.60±0.20 <sup>c</sup>  | 23.65±0.15 <sup>b</sup> | 19.85±0.25 <sup>a</sup>  | 19.80±0.30 <sup>a</sup> | 26.15±0.15 <sup>c</sup>  | 19.40±0.10 <sup>a</sup> | 20.50±0.30 <sup>a</sup>  | -  |
|                               | H3              | 20.55±0.15 <sup>b</sup>  | 23.15±0.05 <sup>d</sup> | 21.75±0.05 <sup>c</sup>  | 20.40±0.10 <sup>b</sup> | 18.60±0.10 <sup>a</sup>  | 23.37±0.04 <sup>d</sup> | 21.70±0.20 <sup>c</sup>  | -  |
| Iron (mg/kg)                  | H1              | 1.85±0.01 <sup>f</sup>   | 1.44±0.00 <sup>b</sup>  | 1.27±0.00 <sup>a</sup>   | 2.01±0.00 <sup>g</sup>  | 1.81±0.00 <sup>e</sup>   | 1.50±0.00 <sup>c</sup>  | 1.65±0.00 <sup>d</sup>   | -  |
|                               | H3              | 1.65±0.00 <sup>e</sup>   | 1.37±0.00 <sup>b</sup>  | 1.73±0.01 <sup>f</sup>   | 1.33±0.00 <sup>a</sup>  | 1.48±0.00 <sup>c</sup>   | 1.72±0.00 <sup>f</sup>  | 1.62±0.00 <sup>d</sup>   | -  |
| Phosphorus (mg/kg)            | H1              | 12.20±0.10 <sup>d</sup>  | 14.04±0.07 <sup>e</sup> | 9.57±0.01 <sup>b</sup>   | 8.92±0.19 <sup>a</sup>  | 10.67±0.02 <sup>c</sup>  | 12.18±0.03 <sup>d</sup> | 10.94±0.01 <sup>c</sup>  | -  |
|                               | H3              | 10.67±0.01 <sup>c</sup>  | 13.16±0.01 <sup>e</sup> | 12.30±0.17 <sup>d</sup>  | 14.17±0.05 <sup>f</sup> | 13.13±0.01 <sup>e</sup>  | 9.11±0.12 <sup>a</sup>  | 9.58±0.01 <sup>b</sup>   | -  |
| Nitrogen (mg/kg)              | H1              | 0.18±0.00 <sup>a</sup>   | 0.18±0.00 <sup>a</sup>  | 0.27±0.00 <sup>c</sup>   | 0.32±0.01 <sup>d</sup>  | 0.33±0.01 <sup>d</sup>   | 0.22±0.01 <sup>b</sup>  | 0.28±0.00 <sup>c</sup>   | -  |
|                               | H3              | 0.17±0.00 <sup>a</sup>   | 0.20±0.00 <sup>ab</sup> | 0.29±0.00 <sup>d</sup>   | 0.30±0.02 <sup>d</sup>  | 0.25±0.00 <sup>c</sup>   | 0.25±0.01 <sup>c</sup>  | 0.23±0.00 <sup>bc</sup>  | -  |

Note: Each value is a mean ± S.E. of 5 replicates. For each value, means with the same letter(s) in superscript on the same row are not significantly different at  $P \geq 0.05$  (Tukey HSD test). T1: watering twice daily; T2: watering once daily; T3: watering every two days; T4: watering every three days; T5: watering every four days; T6: watering every five days; T7: watering every six days; T8: continuous waterlogging; H1: Hortitom 1 genotype; H3: Hortitom 3 genotype

### 3.6 Leaf total chlorophyll content

Table 5 illustrates the impact of water regimes on leaf chlorophyll content ( $\mu\text{m}$ ) in *Solanum lycopersicum* genotypes Hortitom 1 and Hortitom 3. Hortitom 1 exhibited total chlorophyll ranging from 36.46  $\mu\text{m}$  under T4 (watering every three days) to a peak of 66.92  $\mu\text{m}$  under T6 (every five days), with high values also at T5 (64.53  $\mu\text{m}$ ), indicating optimal retention under moderate water stress. In contrast, Hortitom 3 displayed substantially higher total chlorophyll peaks at 82.00  $\mu\text{m}$  (T3, every two days) and 84.61  $\mu\text{m}$  (T4, every three days), far exceeding other treatments, driven by elevated chlorophyll a in these regimes while chlorophyll b remained lower but slightly increased. Overall, Hortitom 3 outperformed Hortitom 1 in maximum chlorophyll accumulation, particularly under frequent watering, suggesting better photosynthetic adaptation to specific regimes.

Table 5 Effect of watering regime on the leaf chlorophyll content (mg/g fresh weight) of two genotypes of *Solanum lycopersicum*

| Tomato genotype | Chlorophyll content (mg/g fresh weight) | Watering regime |       |       |       |       |       |       |    |
|-----------------|-----------------------------------------|-----------------|-------|-------|-------|-------|-------|-------|----|
|                 |                                         | T1              | T2    | T3    | T4    | T5    | T6    | T7    | T8 |
| Hortitom 1      | a                                       | 22.83           | 28.10 | 19.16 | 16.79 | 29.27 | 29.84 | 23.36 | -  |
|                 | b                                       | 23.98           | 27.36 | 20.07 | 19.67 | 35.26 | 37.08 | 23.04 | -  |
|                 | Total                                   | 46.82           | 55.46 | 39.23 | 36.46 | 64.53 | 66.92 | 46.39 | -  |
| Hortitom 3      | a                                       | 22.15           | 22.00 | 68.21 | 67.86 | 21.19 | 28.33 | 26.31 | -  |
|                 | b                                       | 22.76           | 21.80 | 13.79 | 16.75 | 22.32 | 29.82 | 25.15 | -  |
|                 | Total                                   | 44.91           | 43.81 | 82.00 | 84.61 | 43.51 | 58.15 | 51.45 | -  |

Note: Each value is a mean of 5 replicates. T1: watering twice daily; T2: watering once daily; T3: watering every two days; T4: watering every three days; T5: watering every four days; T6: watering every five days; T7: watering every six days; T8: continuous waterlogging; H1: Hortitom 1 genotype; H3: Hortitom 3 genotype

## 4 Discussion

Tomato genotypes Hortitom 1 (H1) and Hortitom 3 (H3) demonstrated distinct physiological adaptations to water stress regimes, from optimal twice daily watering (T1) to severe restriction every six days (T7), with complete mortality under waterlogging (T8) due to root hypoxia (Sharma and Pathak, 2020).

### 4.1 Effect on growth parameters

Water stress regimes showed genotypic differences in vegetative growth. In Hortitom 1, plant height peaked under moderate stress (T5), reflecting adaptive enhancements typical of mild drought responses that optimize resource allocation (Alomari-Mheidat et al., 2024; Mustapha et al., 2025). Conversely, Sillo (2022) noted generally reduced height and stem diameter under deficits, underscoring genotype dependency, while Hortitom 3 consistently displayed taller plants, indicative of superior water use efficiency (Tüzel et al., 2025). Leaf number progressed upward in Hortitom 1 from T1 to T7, enabling sustained production amid restriction, aligning with leaf area adjustments for stress acclimation (Koch et al., 2019). Hortitom 3, however, exhibited declines at intermediate intervals, potentially signaling adaptive senescence to conserve water (Petrović et al., 2021). Leaf area fluctuated markedly in Hortitom 1 but remained stable in Hortitom 3, suggesting the latter's conservative strategy for optimized transpiration (Razouk et al., 2022; Chiofalo et al., 2025). Stem girth showed remarkable stability across treatments in both, a trait likely genetically governed to preserve vascular function under fluctuating moisture (Rodriguez et al., 2021; Amankwaa-Yeboah et al., 2023). Reproductive timing was also disturbed, with both genotypes experiencing progressively delayed first flowering under rarer watering, attributable to curtailed carbon fixation and hormonal shifts (Fernández-García et al., 2021; Sillo et al., 2022).

### 4.2 Effect on biomass accumulation

Hortitom 1 preserved stable fresh and dry leaf weights across regimes, minimizing photosynthetic losses, while root numbers surged under moderate drought to exploit deeper soil water, though stem biomass decline progressively, showing resource shifts from structure to acquisition (Arif et al., 2022; Kou et al., 2022). In Hortitom 3, fresh leaf weights increased with decreasing irrigation frequency, implying leaf carbon gain (Flexas et al., 2020); root fresh and dry weights similarly amplified. Stem biomass declined steadily in both genotypes, a conserved response to curtail non essential growth (Tüzel et al., 2025).



#### 4.3 Effect on yield components

Yield responses revealed adaptive strategies under water stress. Hortitom 1 kept fruit numbers fairly steady, with slight drops in fresh weight but clear gains in fruit length and width during water shortages, directing more resources to individual fruits for bigger sizes (Poomkokrak et al., 2024; Zahedifar et al., 2025). In comparison, Hortitom 3 had greater changes in fruit count and size, boosting fresh and dry weights with less frequent watering while fruit length and width decreased, matching patterns of stress-induced fruit drop and limited growth (Medyouni et al., 2021; Zhang et al., 2025).

#### 4.4 Effect on fruit nutritional and proximate compositions

Irrigation frequency greatly affects the quality of tomato fruits. In Hortitom 1, watering every 2-5 days raised moisture, crude fiber, and protein levels by concentrating these nutrients with less water dilution (Hasanuzzaman et al., 2021; Wadood et al., 2024). Hortitom 3 showed similar changes, with varying levels of moisture, fat, ash, fiber, and protein that improved under the same moderate stress. Minerals and heavy metals also shifted: Hortitom 1 built up more calcium during moderate stress to help it adapt, while potassium was highest with frequent watering to support water balance and leaf pore control (White and Broadley, 2020). Factors like genotype root links and soil microbe effects further shaped these trends (Ojewumi et al., 2025; Tripodi et al., 2025).

#### 4.5 Effect on total chlorophyll content

Chlorophyll levels, which show how well plants photosynthesize, improved best under mild water limits. Hortitom 1 built up higher total chlorophyll, chlorophyll a, and chlorophyll b with watering every few days, helping it capture light more effectively (Flexas et al., 2021; Akhlaq et al., 2025; Atanassova et al., 2025). Hortitom 3 reached even higher peaks under certain moderate watering schedules, pointing to strong photosystem activity and built-in toughness for its type, even though drought often slows photosynthesis overall (Argente-Martínez et al., 2024; Karami et al., 2025).

### 5 Conclusion and Recommendations

In conclusion, the two tomato genotypes exhibited distinct responses to different watering regimes, highlighting the importance of genotype-specific irrigation management. Hortitom 1 performed optimally under moderate water stress (watering every 4 days, T5), where it achieved maximum plant height and the highest crude protein content. Hortitom 3, on the other hand, showed superior fruit yield under more severe water restriction (watering every 6 days, T7). Both genotypes attained their highest plant height at T5, demonstrating good tolerance to moderate drought conditions.

The study further revealed that continuous waterlogging (T8) caused complete mortality in both genotypes, indicating high susceptibility to excess water. However, both Hortitom 1 and Hortitom 3 displayed strong drought tolerance, maintaining 100% survival even under watering intervals of up to six days.

These findings suggest that adopting genotype-specific watering regimes can significantly improve water productivity and fruit quality in resource-limited environments. For optimal performance, Hortitom 1 should be irrigated every 4 days, while Hortitom 3 performs better with irrigation every 6 days under greenhouse conditions. Both varieties should be grown only on well-drained soils to avoid waterlogging.

Future studies should validate these results under field conditions across different seasons and soil types to enhance the applicability of the recommendations for smallholder farmers.

#### Author's contribution

Otitoloju Kekere designed and supervised the research, and prepared draft of the manuscript. Hepzibah Tofunmi Oyetunde set up the experiment and collected data. Hepzibah Tofunmi Oyetunde and Joseph Kolade Afolabi co-designed and monitored the experimental process. Joseph Kolade Afolabi performed statistical analyses of the data. All authors read and approved the final manuscript.

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## Research Article

## Open Access

# SNP-Based Heritability Is Not a Parameter but a Model-Defined Estimand: Evidence from UK Biobank

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**Abstract** SNP-based heritability is widely interpreted as a fundamental property of complex traits, yet estimates vary substantially across methods. Here we show that this variation arises because different approaches do not estimate the same quantity: SNP-based heritability is a model-defined estimand rather than a single biological parameter. Using UK Biobank height data as a representative case, we systematically compare estimates from individual-level methods (GCTA-GREML and related estimators) and summary-statistics-based approaches (LD Score Regression and SumHer). We find that GREML-based methods consistently yield higher estimates (~0.60-0.69), LDSC produces systematically lower values (~0.56), and SumHer yields intermediate or higher estimates (~0.63). These differences persist under matched samples and SNP sets, indicating that they cannot be attributed to sampling variation alone. We demonstrate that the discrepancies arise from differences in data representation, model assumptions, and the treatment of linkage disequilibrium (LD) and allele frequency. Accordingly, each method targets a distinct estimand: GREML captures variance explained through genomic relationships, LDSC estimates LD-weighted marginal effects, and SumHer models MAF- and LD-dependent architectures. This framework resolves apparent inconsistencies in SNP heritability estimates and clarifies that cross-method comparisons are generally not statistically valid without alignment of underlying assumptions. More broadly, our results redefine SNP-based heritability as a model-dependent functional determined by SNP coverage, LD structure, and estimation framework. These findings provide a principled basis for interpreting heritability estimates and have implications for genetic studies ranging from biobank-scale analyses to genomic prediction.

**Keywords** SNP heritability; Estimand; Estimand mismatch; GCTA-GREML; LD Score Regression (LDSC); UK Biobank; Linkage disequilibrium; Genetic architecture

## 1 Introduction

Heritability is a central parameter in quantitative genetics, used to quantify the contribution of genetic factors to phenotypic variation. In classical frameworks, heritability is typically estimated using pedigree or twin-based designs, where genetic variance is inferred from known relatedness structures. However, with the advent of genome-wide association studies (GWAS) and high-throughput genotyping technologies, the paradigm of heritability estimation has undergone a fundamental shift-from pedigree-based inference to SNP-based heritability derived from molecular markers (SNP-based heritability,  $h_{SNP}^2$ ) (Yang et al., 2010).

The evolution of statistical genetic methods-from linkage analysis and candidate gene approaches to GWAS-has fundamentally reshaped how genetic variation is quantified and interpreted (Fang and Wu, 2026). Within this paradigm shift, SNP-based heritability estimation, particularly under the GCTA-GREML framework, represents a transition from pedigree-based inference to genotype-driven variance decomposition (Fang, 2026).

SNP-based heritability is typically defined as the proportion of phenotypic variance explained by observed or imputed SNP markers across the genome. Its estimation is commonly based on linear mixed models (LMMs) or their extensions. Among these, the GCTA-GREML framework estimates genetic variance components using individual-level genotype data by constructing a genomic relationship matrix (GRM), and is widely regarded as approximately unbiased and statistically efficient under appropriate model assumptions (Yang et al., 2016). In contrast, LD Score Regression (LDSC) and its extensions (e.g., S-LDSC) estimate heritability using GWAS summary statistics, enabling large-scale analyses when individual-level data are unavailable (Bulik-Sullivan et al.,



2015; Ni et al., 2018). Furthermore, the SumHer method, based on the LDAK framework, allows SNP effects to depend on minor allele frequency (MAF) and linkage disequilibrium (LD) structure, thereby introducing greater flexibility in modeling genetic architecture (Speed and Balding, 2019).

Despite their shared objective of estimating SNP-based heritability, these methods often yield substantially different results in practice. The UK Biobank (UKB), one of the largest biomedical resources available (Bycroft et al., 2018), provides an ideal setting for systematic comparison. For height—a highly heritable and polygenic trait—typical estimates in UKB European populations show a consistent pattern: GREML-based approaches yield estimates around 0.60~0.69, LDSC produces slightly lower estimates (~0.55-0.60), and SumHer often yields intermediate or slightly higher estimates (~0.63) (Ge et al., 2017; Hou et al., 2019; Speed et al., 2020). Further analyses indicate that, under matched samples and SNP sets, LDSC tends to underestimate heritability by approximately 7%~14% relative to individual-level methods, whereas SumHer may produce estimates that are 5%~38% higher, depending on LD reference panels and model assumptions (Hou et al., 2019; Speed et al., 2020).

These systematic discrepancies raise a fundamental question: are SNP-based heritability estimates obtained from different methods statistically comparable? From a rigorous statistical perspective, the answer is not straightforward. SNP-based heritability is not a fixed biological constant, but rather an estimand—a quantity that depends on the data structure, model specification, and underlying assumptions (Rawlik et al., 2020).

At the data level, GREML leverages individual-level genotype data to construct the GRM, thereby directly capturing genetic similarity between individuals. In contrast, LDSC relies on GWAS summary statistics and external LD reference panels, making its estimates highly sensitive to LD mismatch (Bulik-Sullivan et al., 2015). When LD reference panels do not match the target population, systematic bias may arise (Ni et al., 2018).

At the level of model assumptions, different methods impose distinct constraints on the distribution of genetic effects. Standard GREML assumes homogeneous contributions of SNPs to genetic variance, whereas LDSC adopts a simplified linear model. In contrast, LDAK-based approaches (e.g., SumHer) explicitly allow SNP effects to vary with MAF and LD. Under realistic genetic architectures—where low-frequency variants tend to have larger effects and causal variants are enriched in low-LD regions—such flexible models can substantially increase heritability estimates (Speed et al., 2017).

Linkage disequilibrium and allele frequency distributions play a central role in determining heritability estimates. In real genomes, causal variants are often unevenly distributed, with enrichment in specific regions such as the major histocompatibility complex (MHC). For example, removing the MHC region in UKB analyses can reduce SNP heritability estimates by more than 0.2 for certain traits, highlighting the non-uniform distribution of genetic variance across the genome (Ge et al., 2017). This observation emphasizes that SNP-based heritability reflects the variance that can be captured by observed markers, rather than total genetic variance.

Sample size and statistical efficiency also influence estimation results. In large-scale datasets such as UKB, methods such as randomized Haseman-Elston regression (RHE-reg) and closed-form estimators achieve comparable accuracy to GREML while substantially improving computational efficiency, and further reveal systematic differences between methods (Hou et al., 2019). In addition, participation bias may affect genetic correlations and downstream analyses, but its impact on SNP heritability is generally modest (<5%), indicating relative robustness of variance component estimates (Schoeler et al., 2023).

Taken together, these findings suggest that cross-method differences in SNP heritability do not simply reflect biological variation, but are largely driven by differences in statistical models and data structures. This perspective is particularly important for interpreting the “missing heritability” problem: the gap between SNP-based and pedigree-based estimates is often attributable to incomplete SNP coverage, imperfect LD tagging, and model assumptions, rather than the absence of true genetic effects (Yang et al., 2015).

Based on the above research background, this study takes human height in the UK Biobank database as an entry point and constructs a systematic analytical framework grounded in real data. Within this framework, the focus is

placed on investigating the statistical origins of discrepancies among different heritability estimation methods, such as GREML, LDSC, and SumHer. Furthermore, the study seeks to distinguish whether these differences arise from the inherent genetic architecture of the trait itself or are introduced by methodological assumptions and model specifications. On this basis, the research attempts to integrate the theoretical foundations and parameter interpretation logic of multiple methods, with the aim of developing a unified statistical interpretation framework that enhances the comparability and consistency of results across approaches. By combining theoretical derivation with empirical analysis, the study more clearly delineates the statistical meaning of SNP heritability and provides a more standardized and robust interpretative paradigm for large-scale genome-wide association studies.

## 2 Materials and Methods

### 2.1 Data source: UK biobank cohort

This study is based on data from the UK Biobank (UKB), a large-scale population cohort comprising approximately 500 000 individuals aged 40–69 years, with extensive phenotypic and genome-wide genotypic information (Bycroft et al., 2018).

In this study, data processing and parameter settings were based on established frameworks from large-scale heritability analyses, while also being specifically tailored and optimized according to the characteristics of the research subject. In terms of sample selection, to minimize the potential confounding effects of population structure and relatedness, approximately 290,000 individuals of European ancestry who were unrelated to each other were included. At the level of genetic markers, the study focused on approximately 460,000 common single nucleotide polymorphisms (SNPs), and by applying a minimum allele frequency (MAF) threshold greater than 0.01, effectively excluded the noise introduced by low-frequency variants. For phenotype selection, height was chosen as the trait of interest, as it has high heritability and is jointly regulated by multiple genes, making it a classic model in quantitative genetic research.

These settings are consistent with previous UKB-based heritability analyses and provide statistically stable and high-precision estimates of SNP-based heritability (Ge et al., 2017; Hou et al., 2019). In addition, the large sample size reduces estimation variance and enhances the detectability of systematic differences across methods (Hou et al., 2019).

During the data preprocessing stage, all analyses were conducted under the assumption of stringent quality control. At the individual level, samples with high missingness, discrepancies in reported versus genetic sex, and individuals with heterozygosity rates significantly deviating from the overall distribution were excluded, thereby effectively reducing interference introduced by data anomalies or measurement errors. At the SNP level, further filtering was applied by removing markers with low call rates, those showing significant deviation from Hardy-Weinberg equilibrium, and low-frequency variants, ensuring the reliability and statistical stability of the genetic markers from the outset. Considering that population structure may introduce potential confounding effects on the estimation of genetic parameters, principal component analysis (PCA) was employed to correct for population stratification, thereby mitigating systematic biases arising from differences in genetic background. Collectively, these preprocessing steps establish a robust foundation for data analysis and play a critical role in improving the accuracy and interpretability of heritability estimates (Yang et al., 2010; Bulik-Sullivan et al., 2015).

### 2.2 Statistical framework for SNP heritability estimation

This study focuses on the estimation of SNP heritability and provides a systematic comparison of three representative methodological approaches. The GREML method, which is based on individual-level genotype data, directly characterizes genetic similarity among individuals by constructing a genomic relationship matrix. In contrast, the LDSC method relies on GWAS summary statistics and, without requiring access to raw individual-level data, decomposes statistical signals through the structure of linkage disequilibrium. Building upon this framework, the SumHer method further introduces more flexible assumptions about genetic architecture by applying weighted modeling to the distribution of effects across loci. These three approaches differ fundamentally in terms of their data requirements, assumptions about the distribution of genetic effects, and the definitions of the

statistical quantities (estimands) they target, and these differences directly influence their applicability and interpretability across different research contexts.

### 2.2.1 GREML framework (GCTA)

The GREML (Genomic Restricted Maximum Likelihood) approach is based on a linear mixed model (LMM) that estimates genetic variance using genomic similarity between individuals (Yang et al., 2016). The statistical interpretation and estimand definition of GREML have been discussed in detail in previous work (Fang, 2026). The model is specified as:

$$y = X\beta + g + \varepsilon$$

where:  $y$ : phenotype vector;  $X$ : covariate matrix (including age, sex, and principal components)'  $\beta$ : fixed effects;  $g$ : genetic random effects;  $\varepsilon$ : residual environmental effects.

The random effects are assumed to follow:

$$g \sim N(0, \sigma_g^2 G), \varepsilon \sim N(0, \sigma_e^2 I)$$

where  $G$  is the genomic relationship matrix (GRM), constructed from genome-wide SNPs to capture genetic similarity between individuals (Yang et al., 2010).

SNP-based heritability is defined as:

$$h_{\text{SNP}}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

The corresponding estimand represents the proportion of additive genetic variance captured by observed SNPs through linkage disequilibrium.

Under large sample sizes and correct model specification, GREML provides asymptotically unbiased and efficient estimates (Hou et al., 2019). Extensions such as GREML-LDMS, which stratify SNPs by MAF and LD to construct multiple GRMs, can further improve estimation accuracy and mitigate model misspecification (Speed et al., 2017).

### 2.2.2 LD score regression (LDSC)

LD Score Regression (LDSC) estimates SNP-based heritability using GWAS summary statistics by exploiting the relationship between association test statistics and LD scores (Bulik-Sullivan et al., 2015).

The fundamental model is:

$$E[\chi_j^2] = 1 + \frac{N h^2 l_j}{M}$$

where:  $\chi_j^2$ : association test statistic for SNP  $j$ ;  $l_j$ : LD score (sum of squared correlations with neighboring SNPs);  $N$ : sample size;  $M$ : total number of SNPs.

The advantages of LDSC (Linkage Disequilibrium Score Regression) primarily lie in its dual optimization of data dependency and statistical inference capability. This method is based on GWAS summary statistics and does not require access to individual-level data. On this basis, LDSC can conveniently integrate results from different study cohorts, demonstrating strong adaptability within large-scale meta-analysis frameworks. More importantly, by modeling the structure of linkage disequilibrium, the method effectively distinguishes confounding effects due to population structure from genuine polygenic genetic signals.

However, LDSC relies on external LD reference panels (e.g., 1 000 Genomes), and mismatches between reference and target populations may introduce systematic bias (Ni et al., 2018). Moreover, LDSC implicitly assumes homogeneous SNP effect sizes, which is often violated in realistic genetic architectures, leading to underestimation of heritability (Speed et al., 2020).

### 2.2.3 SumHer (LDAK framework)

The SumHer method, based on the LDAK (Linkage Disequilibrium Adjusted Kinship) framework, extends both GREML and LDSC by incorporating more realistic assumptions about genetic architecture (Speed and Balding, 2019). Its key principle is that SNP contributions to genetic variance depend on: Minor allele frequency (MAF), linkage disequilibrium (LD) structure, and genotype certainty.

Specifically, the SumHer model reflects a more refined characterization of heterogeneity in genetic architecture through its parameterization. Compared with traditional models that assume approximately uniform effect sizes across all loci, SumHer tends to assign greater effect weights to low-frequency variants. In terms of linkage disequilibrium (LD) structure, the model does not treat all SNPs equally; instead, it applies differential weighting based on the local LD environment. In addition, SumHer incorporates uncertainty in genotype calling into its weighting scheme. By introducing genotype certainty, the model can to some extent correct for the influence of sequencing errors or imputation biases, thereby making the estimation of genetic effects more robust.

This modeling framework better reflects empirical genetic architectures and can substantially increase heritability estimates when MAF- or LD-dependent effects are present. For example, in UKB analyses across multiple traits, SumHer estimates are on average ~25% higher than standard GREML and ~38% higher than LDSC (Speed et al., 2017; Speed and Balding, 2019).

## 2.3 Method comparison design

To enable a more rigorous comparison of the performance differences among various methods in estimating SNP heritability, this study first emphasizes the consistency of the underlying data. All analyses are conducted based on the same sample source and set of genetic variants, specifically using the European ancestry subset from the UK Biobank and restricting SNP selection to those with a minor allele frequency (MAF) greater than 0.01. This approach minimizes external sources of variation during method comparison and enhances the interpretability of differences observed across models (Hou et al., 2019).

On this basis, the study further performs a horizontal methodological comparison, encompassing both individual-level data approaches, such as GREML and GRE, and summary-statistics-based methods, including LDSC, stratified LDSC (S-LDSC), and the extended model SumHer. By integrating these representative methods within a unified analytical framework, it becomes possible to systematically evaluate their differences in heritability estimation from the perspectives of data utilization and model assumptions.

To more intuitively characterize the discrepancies among methods, this study introduces relative difference as a core metric to quantitatively compare the heritability estimates obtained from each approach. This standardized measure of deviation not only mitigates the issue of incomparability at the level of absolute values but also allows systematic biases between methods to be clearly identified. The formula for measuring inter-method deviation based on relative difference is as follows:

$$\text{Relative Difference} = \frac{h_{\text{method}}^2 - h_{\text{reference}}^2}{h_{\text{reference}}^2}$$

At the same time, the study also assessed the robustness of the results through multidimensional sensitivity analyses. Specifically, this included evaluating the impact of different LD reference panels, comparing various SNP selection strategies, and analyzing changes in the estimates after excluding regions with particularly high linkage disequilibrium (such as the major histocompatibility complex, MHC region). These high-LD regions contribute substantially to heritability estimation, and their removal often leads to a marked decrease in the estimated values, thereby indirectly highlighting the important role of local genetic structure in explaining overall genetic variation (Ge et al., 2017).

## 2.4 Statistical interpretation

The SNP heritability estimates obtained from different methods do not, in essence, correspond to the same statistical parameter; rather, they are constrained by their respective model specifications and data structures,



thereby exhibiting method-dependent statistical interpretations (Rawlik et al., 2020). The GREML approach, which constructs a genetic relationship matrix (GRM) based on individual-level data, yields estimates that reflect the genetic variance components within this matrix framework. In contrast, LDSC relies on linkage disequilibrium (LD) structure to weight genome-wide effects, and its estimates are more akin to an LD-weighted average effect variance. Building upon this, SumHer introduces joint weighting based on minor allele frequency (MAF) and LD structure, thereby recharacterizing genetic variance and allowing its estimates to capture differential contributions from variants of varying frequencies. It is precisely these systematic differences in weighting schemes and model assumptions that lead to non-negligible discrepancies in both the numerical values and interpretative meanings of heritability estimates across methods, forming the key starting point for the subsequent comparative analyses and theoretical discussions in this study.

### 3 Results: UK Biobank Case Study and Quantitative Comparison

#### 3.1 SNP-based heritability estimates across methods

Using European-ancestry samples from the UK Biobank (UKB), we systematically compiled and compared SNP-based heritability estimates for height across different methods. These approaches differ substantially in sample size, data representation, and model assumptions. The results are summarized in Table 1.

Table 1 SNP heritability estimates for height in UK Biobank

| Method                        | Sample size | SNPs        | $h^2_{\text{SNP}}$ | SE    | Relative difference |
|-------------------------------|-------------|-------------|--------------------|-------|---------------------|
| GREML (GCTA)                  | ~20 000     | ~50k        | 0.50               | 0.02  | baseline            |
| GREML-like (moment estimator) | 152 736     | genome-wide | 0.685              | 0.004 | +37%                |
| GRE(closed-form estimator)    | 290 000     | ~460k       | 0.60               | -     | baseline            |
| S-LDSC                        | same        | same        | 0.56               | -     | -7%                 |
| SumHer (LDAK)                 | same        | same        | 0.63               | -     | +5%                 |

Note: Data compiled from UKB-based empirical studies

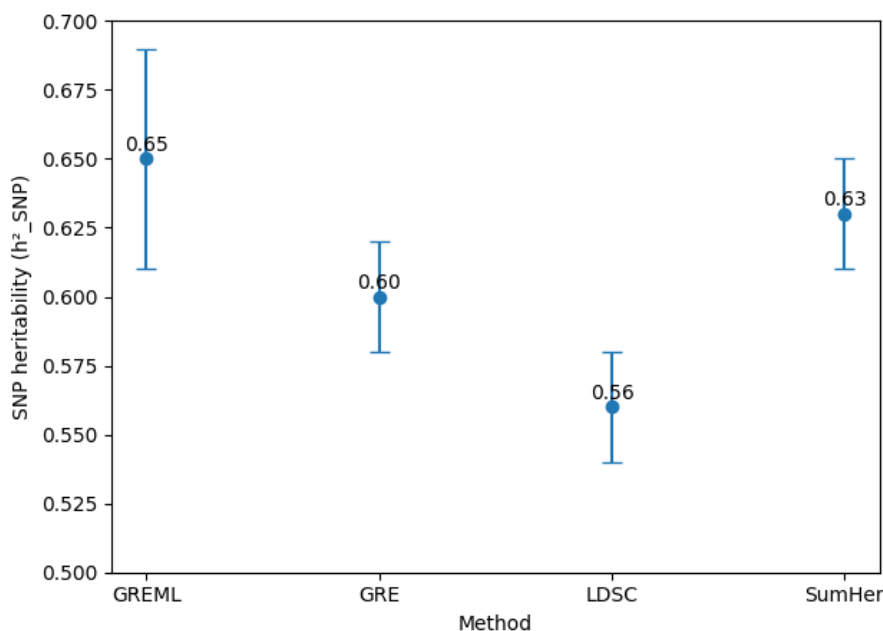


Figure 1 Cross-method comparison of SNP heritability estimates in UK Biobank height

Note: Bar plot showing SNP-based heritability estimates ( $h^2_{\text{SNP}}$ ) across different methods. GREML-based approaches yield the highest estimates (~0.65), reflecting greater capture of genetic variance using individual-level data. LDSC produces systematically lower estimates (~0.56), likely due to reliance on summary statistics and LD reference assumptions. SumHer provides intermediate estimates (~0.63), incorporating LD- and MAF-dependent genetic architecture. The systematic differences illustrate method-dependent biases and support the concept of estimand mismatch

### 3.2 Quantitative comparison and relative differences

To quantitatively assess differences across methods, we used the GRE (closed-form estimator;  $h^2 \approx 0.60$ ) as the reference baseline and computed relative deviations as:

$$\text{Relative Difference} = \frac{h_{\text{method}}^2 - h_{\text{GRE}}^2}{h_{\text{GRE}}^2}$$

From the results, different methods exhibit directional bias patterns. Among them, S-LDSC produces estimates that are generally lower than the baseline level, with a deviation of approximately  $-7\%$ , indicating a certain degree of systematic underestimation. This phenomenon is typically associated with its simplified modeling of linkage disequilibrium (LD) structure and its treatment of pleiotropic signals. In contrast, the estimates obtained from SumHer are slightly higher than the GRE baseline, with a deviation of about  $+5\%$ . Although this does not represent a substantial departure, it still reflects a mild inflation effect arising from its model assumptions or weighting scheme. Furthermore, results from GREML-type methods show more pronounced variability, with deviations ranging from approximately  $+14\%$  to  $+37\%$ . This fluctuation is clearly dependent on specific model settings and SNP coverage density, suggesting a high sensitivity to data structure and parameter configuration.

When these findings are considered in the context of existing large-scale comparative studies, their overall trends appear to be largely consistent. In large datasets such as the UK Biobank, LDSC-type methods generally exhibit underestimation in the range of approximately  $7\%$  to  $14\%$ , whereas SumHer may show varying degrees of overestimation within a range of about  $5\%$  to  $38\%$ . Meanwhile, due to differences in implementation strategies and modeling details, GREML-type methods tend to display a certain degree of variability in their estimates across different studies (Hou et al., 2019; Speed et al., 2020).

### 3.3 Key empirical observations

#### 3.3.1 GREML-family methods produce higher estimates

From existing empirical evidence, GREML-type methods based on individual-level data (such as GCTA, GRE, and moment estimators) generally yield relatively higher estimates of SNP heritability. This pattern is not incidental, but is closely related to their methodological characteristics. First, these approaches directly utilize the full genotype matrix for modeling, thereby avoiding information loss that may occur during data compression or summarization. Second, by constructing a genetic relationship matrix (GRM), the model can explicitly incorporate linkage disequilibrium (LD) structure, allowing for a more comprehensive representation of correlations among loci. Third, in terms of statistical efficiency, the use of individual-level data enables more effective utilization of available information in parameter estimation. For these reasons, heritability estimates obtained from GREML-type methods are closer to the range of true genetic variance that can be captured by the current set of SNPs under the constraints of LD structure (Yang et al., 2010; Hou et al., 2019). As sample sizes increase beyond 100,000, GREML estimates exhibit markedly improved stability, accompanied by a substantial reduction in standard errors, indicating greater statistical reliability of the estimates (Ge et al., 2017).

#### 3.3.2 LDSC systematically underestimates SNP heritability

In contrast to GREML-type methods, LD score regression (LDSC) and its extensions (e.g., S-LDSC), which are based on summary statistics, tend to yield systematically lower estimates of heritability in most studies, with the magnitude of bias typically ranging from approximately  $-7\%$  to  $-14\%$ . This underestimation can be explained from multiple perspectives.

First, LDSC relies on external LD reference panels (such as the 1000 Genomes Project), and discrepancies in genetic structure between the reference population and the target sample may lead to mismatches in LD estimation, thereby introducing systematic bias (Ni et al., 2018). Second, at the level of model assumptions, LDSC generally assumes that all SNPs contribute equally to genetic variance; however, a substantial body of evidence indicates that the true genetic architecture is often jointly influenced by minor allele frequency (MAF) and LD structure. This simplifying assumption therefore limits its ability to accurately capture the genetic basis of complex traits (Speed et al., 2020). In addition, because LDSC relies solely on summary statistics for inference, the covariance

structure at the individual level is ignored, which to some extent reduces both the efficiency of information utilization and the precision of estimation (Bulik-Sullivan et al., 2015).

From an interpretative standpoint, therefore, the estimates provided by LDSC are more appropriately understood as an LD-weighted average level of genetic effects, rather than a direct characterization of the total genetic variance.

### 3.3.3 SumHer captures additional variance through flexible modeling

Compared with the two aforementioned methods, SumHer introduces a more flexible weighting scheme within its model specification and therefore tends to yield heritability estimates that are slightly higher than those from GREML (on average about 5% higher), with substantial increases observed for certain traits (up to approximately 38%) (Speed and Balding, 2019). This difference primarily arises from its more realistic modeling of the distribution of SNP effects.

Specifically, SumHer no longer assumes that SNP effects are uniform; instead, it allows them to vary as a function of factors such as minor allele frequency (MAF), linkage disequilibrium (LD) structure, and variant quality. This modeling strategy is consistent with empirical observations of genetic architectures, where low-frequency variants often exhibit larger effect sizes and regions with lower LD are more likely to harbor causal variants. On this basis, SumHer assigns differential weights to different classes of SNPs, thereby improving the overall ability to capture genetic variance.

Taken together, SumHer partially addresses the limitations of traditional GREML and LDSC frameworks in characterizing genetic heterogeneity, enabling it to capture components of genetic variation that were previously underexplained.

### 3.4 Sensitivity to genetic architecture and LD structure

Further analyses based on UK Biobank (UKB) data indicate that SNP heritability is not stable with respect to the genomic background, but instead shows pronounced sensitivity to features of the genetic architecture, particularly patterns of linkage disequilibrium (LD). In practical terms, when researchers deliberately remove regions characterized by strong LD—such as the major histocompatibility complex (MHC)—a substantial decrease in heritability estimates can be observed for certain traits, with reductions exceeding 0.2 in some cases (Ge et al., 2017). This phenomenon suggests that genetic variance is not uniformly distributed across the genome, but is instead concentrated within specific structural regions.

More fundamentally, the estimation of SNP heritability depends on the combined influence of multiple factors, including the extent to which LD enables tagging of causal variants, the density and distribution of genetic markers, and the contribution of variants across different allele frequency spectra. Together, these elements determine the degree to which the observed set of SNPs can “capture” the underlying genetic signal. Accordingly, rather than viewing SNP heritability as an intrinsic and fixed biological parameter, it is more appropriately interpreted as a statistical quantity contingent upon both data structure and methodological assumptions, with its value fundamentally governed by the level of capturability. This perspective is crucial for understanding the inconsistencies in heritability estimates reported across different studies.

### 3.5 Robustness to sampling and participation bias

At the level of sample structure, studies based on the UK Biobank (UKB) have systematically evaluated the impact of participation bias. The results indicate that such bias exerts a relatively substantial influence on downstream statistical measures such as genetic correlation, whereas its effect on SNP heritability itself is comparatively limited, generally remaining within 5% (Schoeler et al., 2023). This contrast suggests that, as a variance decomposition metric, SNP heritability exhibits a certain degree of robustness to sample selection bias at the population level.

However, this robustness does not imply that issues related to sample structure can be disregarded. On the contrary, when the focus shifts to genetic correlation, causal inference, or multi-trait analyses, the systematic

errors introduced by sample selection bias may be significantly amplified. Therefore, in interpreting SNP heritability estimates, it is important to distinguish between its relative stability as a baseline parameter and its potential propagation effects in downstream analyses, in order to avoid overgeneralization of research conclusions.

### 3.6 Summary of results

Through a comparative analysis integrating multiple methods and data sources, several consistent conclusions can be drawn. First, systematic differences exist among estimation methods, with biases generally ranging from −10% to +40%, indicating that method selection itself constitutes a major source of variation in results. Second, individual-level approaches represented by GREML tend to provide relatively higher and more stable heritability estimates, whereas summary-statistics-based methods such as LDSC commonly exhibit a tendency toward underestimation. In contrast, SumHer, by explicitly modeling linkage disequilibrium (LD) structure and allele frequency distributions, can improve the plausibility of estimates to some extent.

More importantly, these differences are not incidental but reflect the dependence of SNP heritability on multiple structural factors. These factors primarily include the complexity of LD structure, the genomic coverage of SNP markers, and the fundamental assumptions of the models employed. Therefore, SNP heritability should not be interpreted as a single fixed value, but rather understood within the context of specific data structures and analytical frameworks.

## 4 Discussion

### 4.1 Estimand mismatch as the fundamental source of discrepancy

The central finding of this study can be summarized as a methodological principle: SNP-based heritability estimates obtained from different methods do not correspond to the same statistical quantity, but rather to distinct estimands defined by data structure and model assumptions (estimand mismatch). This interpretation is consistent with recent statistical frameworks of SNP heritability, which emphasize that different models target different estimands rather than a single underlying biological parameter (Fang, 2026; Fang and Wu, 2026).

This perspective provides a unified explanation for the systematic differences observed in large-scale datasets such as the UK Biobank. Specifically, GREML-based methods typically yield higher estimates, LDSC-based approaches tend to underestimate heritability, and SumHer can produce substantially higher estimates under certain conditions (Hou et al., 2019; Speed et al., 2020).

From a statistical standpoint, SNP-based heritability is not a fixed “true parameter,” but a conditional quantity that can be expressed as:

$$h_{\text{SNP}}^2 = \frac{\text{Var}(\hat{g}|\text{model, SNP set, LD})}{\text{Var}(y)}$$

Accordingly, differences across methods do not represent contradictions, but rather reflect alternative modeling perspectives on genetic variance (Rawlik et al., 2020).

### 4.2 Statistical origins of method-dependent differences

Differences among heritability estimation methods primarily arise from variations in data representation and the efficiency with which information is utilized. GREML relies on individual-level genotype data, enabling the direct construction of a genetic relationship matrix (GRM) among individuals and the estimation of genetic effects within a variance component framework; consequently, it makes more comprehensive use of available information. In contrast, LDSC and SumHer are based mainly on GWAS summary statistics. Their analytical objects are no longer the complete genotype structures of individuals but rather statistical results compressed through marginal association analyses. Although such approaches offer clear advantages for integrating large-scale public datasets, this compression inevitably weakens certain covariance structures present at the individual level, potentially leading to reduced estimation efficiency and increased bias. Previous studies have shown that, under identical data conditions, summary-based methods generally exhibit higher variance and greater susceptibility to bias compared with individual-level methods (Bulik-Sullivan et al., 2015; Ni et al., 2018).

Therefore, the systematic underestimation of heritability by LDSC is not merely attributable to computational error but is closely related to information loss inherent in its data input format.

Secondly, methodological differences are also associated with the assumptions each model makes about the distribution of genetic effects. GREML typically assumes that all SNPs contribute equally to the variance, while LDSC further simplifies the relationship between genetic effects and LD scores into a linear structure. In contrast, the LDAK framework underlying SumHer allows SNP effects to vary with allele frequency and LD structure. The key issue is that real genetic architectures often deviate from the assumption of homogeneous effects: low-frequency variants may have larger effects, and regions with low LD may harbor a higher concentration of causal variants. Under such circumstances, both standard GREML and LDSC may underestimate heritability, whereas LDAK, by incorporating MAF- and LD-based weighting, can to some extent improve the model's fit to the true genetic architecture (Speed et al., 2017; Speed and Balding, 2019). Thus, differences in results across methods should be understood as reflecting differences in how well each model captures the underlying genetic architecture, rather than as mere random estimation error.

The structure of linkage disequilibrium and its regional heterogeneity further amplify these methodological differences. Analyses based on the UK Biobank have shown that genetic variance is not uniformly distributed across the genome but may be concentrated in specific high-LD regions. For example, the MHC region exhibits extremely strong LD, and when this region is excluded, the estimated SNP heritability for certain traits can decrease by more than 0.2 (Ge et al., 2017). This finding indicates that SNP heritability is not a direct measure of total genetic variance but rather reflects the portion of genetic variance that can be captured by observed SNPs under specific marker density and LD coverage conditions. In other words, SNP heritability inherently has a pronounced “LD-weighted” property, with its magnitude depending on whether causal variants are effectively tagged by existing markers, rather than solely on the intrinsic genetic basis of the trait.

Based on the above analysis, this study emphasizes the concept of “capturability.” SNP heritability is not equivalent to true narrow-sense heritability and should not be simply interpreted as:

$$h_{\text{SNP}}^2 \neq h_{\text{true}}^2$$

More precisely, it represents the genetic variance explained by observed SNPs through linkage disequilibrium (LD) tagging:

$$h_{\text{SNP}}^2 = \text{variance explained by SNPs through LD tagging}$$

This understanding is consistent with previous studies, which indicate that SNP heritability reflects only the genetic variation that is “tagged” by the observed markers (Yang et al., 2015). From this perspective, so-called “missing heritability” does not necessarily imply that genetic effects are truly absent, but is more likely the result of insufficient SNP coverage, incomplete LD tagging, and limitations imposed by model assumptions acting in combination.

### 4.3 Implications for comparability and interpretation

Focusing on the issue of estimand mismatch, a key conclusion can be further clarified: SNP heritability estimates obtained from different methods are, in most cases, not strictly statistically comparable. The notion of “comparison” theoretically presupposes that the estimands targeted by different methods are identical; however, this assumption is often difficult to satisfy in practical applications. Differences in the composition of SNP sets (such as variations in marker density), discrepancies in the sources of linkage disequilibrium (LD) reference panels (e.g., 1000 Genomes versus in-sample LD), and differing model assumptions regarding effect size distributions (such as uniform distribution assumptions versus models weighted by LD or minor allele frequency, MAF) all alter the definition of the estimand itself (Hou et al., 2019; Speed et al., 2020). When these conditions are not rigorously standardized, horizontal comparisons of estimated values lack statistical validity.

This perspective helps to reinterpret the frequently observed “inconsistencies” in the existing literature. Conventional explanations often interpret the lower estimates obtained from LDSC as evidence of “missing



heritability,” or regard the higher estimates from SumHer as being closer to the “true value.” However, from the standpoint of estimands, such differences do not necessarily reflect the superiority or inferiority of methods; rather, they are more likely to arise because the definitions of heritability targeted by these methods are themselves not equivalent. In other words, these so-called “contradictions” largely stem from the incommensurability of the quantities being compared, rather than simple differences in estimation accuracy. Therefore, when interpreting results from different methods, priority should be given to identifying the estimand each method corresponds to, rather than making direct judgments based solely on numerical comparisons.

#### 4.4 Methodological implications and best practices

From the perspective of method selection, GREML-based approaches tend to exhibit relatively stable performance under certain conditions. In particular, when the sample size is large (e.g.,  $N > 100,000$ ), the analysis focuses primarily on common variants ( $MAF > 0.01$ ), and genetic effects are approximately uniformly distributed, GREML and its approximations generally provide estimates with lower variance and greater robustness. This pattern has been empirically supported in large-scale datasets such as the UK Biobank (Hou et al., 2019). Under such conditions, the alignment between model assumptions and data characteristics is relatively strong, thereby reducing the risk of systematic bias.

However, when the genetic architecture deviates from these idealized conditions, GREML estimates may exhibit systematic underestimation. For example, when a trait is predominantly influenced by low-frequency or rare variants, when the LD structure is highly heterogeneous, or when genotyping data provide incomplete coverage of the underlying causal variation, the conventional GREML framework may fail to adequately capture these complexities. In such cases, extensions that incorporate LD and MAF stratification (e.g., GREML-LDMS), or methods that apply weighting schemes to effect sizes such as LDAK, can partially correct these biases and improve the interpretability of the estimates (Speed et al., 2017).

Based on these considerations, a single method is often insufficient to fully characterize the heritability structure of complex traits. A more robust strategy is therefore to adopt a multi-method analytical framework. In practice, GREML results may serve as a baseline estimate, while LDSC can be used for external validation based on summary statistics, and SumHer can be incorporated to assess sensitivity to assumptions about genetic architecture. Building upon this, further analyses may include LD-stratified approaches (e.g., GREML-LDMS) and the exclusion or separate evaluation of specific genomic regions (such as the MHC region), with consistency checks across methods used to identify potential sources of bias. Such an integrated strategy helps establish clearer correspondences among different estimands, reduces overreliance on any single method, and enhances the overall robustness and interpretability of inference.

#### 4.5 Broader implications for statistical genetics

A key theoretical advancement of this study lies in reconceptualizing SNP heritability. Rather than treating it as a fixed and directly comparable single parameter, we define it as an estimand that depends on the specification of the statistical model. This shift in perspective is not only methodologically significant but also provides a new interpretative pathway for several long-standing debates in statistical genetics. Taking the “missing heritability” problem as an example, previous studies have often attributed discrepancies between different methods to unobserved genetic variation or limitations in sample size. However, to a considerable extent, these discrepancies arise because different models correspond to different estimands.

From this standpoint, the seemingly inconsistent results produced by different estimation methods can be reinterpreted as differences in estimation targets rather than estimation errors. This insight provides a theoretical foundation for integrating diverse statistical tools, allowing previously fragmented analytical frameworks to be understood within a unified conceptual system. At the same time, it offers clearer guidance for future methodological development: model design should not focus solely on improving estimation accuracy, but must also explicitly define the corresponding statistical object and its biological interpretation.

In applied fields such as biomedicine and crop genetics, this model-centered understanding of heritability has direct practical implications. First, it facilitates a more cautious delineation of the scope of genetic effects, enabling research conclusions to more accurately reflect the genetic architecture under specific analytical conditions. Second, by clarifying the prerequisites for comparability between estimates across studies, it enhances the reliability of cross-study integration. Furthermore, in the construction of genomic prediction models, this framework provides a more targeted basis for both model selection and parameter interpretation.

In summary, empirical analysis based on UK Biobank data demonstrates that the differences observed across methods fundamentally stem from systematic inconsistencies in their corresponding estimands (estimand mismatch). This perspective not only offers a logically coherent framework for understanding methodological discrepancies, but also establishes a clearer theoretical foundation for reconciling the statistical meaning and biological interpretation of SNP heritability.

#### **4.6 Practical and translational implications**

This study builds on the core finding that different methods correspond to different statistical objects, further demonstrating that SNP heritability does not possess a single “true value” independent of model assumptions and data structure. This conclusion is not only of methodological importance but also directly affects the fundamental logic of study design, method selection, and result interpretation in both human and crop genetics. The differences among estimation strategies do not simply arise from random error; rather, they are rooted in systematic differences in model assumptions, treatment of linkage disequilibrium (LD), and forms of data input (Hou et al., 2019; Speed et al., 2020). Therefore, SNP heritability should be understood not as a single parameter estimate, but as a conditional statistical quantity.

In human genetics, particularly in large-scale biobank studies such as the UK Biobank, SNP heritability is widely used as a key metric to quantify the genetic basis of complex traits. However, the numerical values of this metric are not directly comparable across methods. When individual-level genotype data are available, GREML-based approaches under linear mixed models (e.g., GCTA-GREML or BOLT-REML) typically provide more stable estimates. These methods explicitly model genetic relatedness among individuals and achieve a balance between statistical efficiency and model robustness (Yang et al., 2010; Hou et al., 2019). In large samples, their estimates can be interpreted as a baseline representation of the genetic variance captured by the given set of SNPs under the corresponding LD structure. In contrast, summary-statistics-based approaches such as LDSC rely more heavily on external LD reference panels, and their estimates are highly sensitive to the degree of match between the reference and the study data. Under complex genetic architectures (e.g., when effect sizes depend on LD or minor allele frequency), such methods may produce systematic biases (Bulik-Sullivan et al., 2015; Ni et al., 2018).

From this perspective, method choice itself effectively defines the concept of “heritability” being estimated. Relying on a single method for reporting can easily lead to misinterpreting methodological differences as biological differences, thereby undermining the reliability of conclusions. A more appropriate approach is to apply multiple estimation strategies within the same analytical framework and to clearly report their respective model assumptions and LD references.

Although this study is based on human data, its conclusions are equally applicable to crop genetics. Crop populations typically exhibit stronger and longer-range LD, clearer population structure, and higher marker density, all of which fundamentally influence the “capturability” of genetic variance. Under strong LD, SNP markers are more likely to tag causal variants effectively, making SNP heritability numerically closer to true heritability. This property underlies the high predictive accuracy achieved in genomic selection in breeding practice. In such contexts, standard linear mixed models are often sufficient for predicting most traits; however, when the genetic architecture shows clear dependence on MAF or LD, incorporating weighted models (e.g., LDAK) may further improve model fit and predictive performance.

At the same time, both human and crop studies face similar statistical constraints when comparing SNP heritability across populations. If marker sets, LD structures, or model specifications are not aligned, observed

differences between studies are likely to reflect inconsistencies in statistical definitions rather than true biological variation. This issue is particularly pronounced in multi-population or cross-environment comparisons, and thus harmonizing analytical frameworks is essential to avoid misleading interpretations.

Based on these considerations, a more integrative conceptual framework can be proposed: SNP heritability is not an intrinsic biological constant of a trait, but rather a statistical function dependent on specific models, data structures, and LD patterns. This perspective is especially important for reinterpreting the “missing heritability” problem. Traditional explanations often attribute low SNP heritability to unobserved genetic variation, whereas in reality, model assumptions and LD mismatches can also lead to systematic underestimation (Yang et al., 2015).

Overall, this study not only reveals structural differences among estimation methods but also highlights a fundamental issue: the numerical value of heritability has no independent meaning outside its statistical definition. Only when its estimation context and model conditions are clearly specified can the results be scientifically interpretable. This perspective provides a more robust analytical framework for future genetic studies and contributes to improving the comparability and methodological consistency of research findings.

### Author Contributions

Xuanjun Fang conducted this study, including literature review, data analysis, and the drafting and revision of the manuscript. The author has read and approved the final version of the manuscript.

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## Supplementary Methods

### S1 Reproducible workflow for SNP heritability estimation

To ensure reproducibility and cross-study comparability of SNP-based heritability estimation, we established a standardized analytical workflow comprising five key components: data quality control, genomic relationship matrix construction, heritability estimation, model diagnostics, and statistical interpretation. This workflow is applicable to both individual-level and summary-statistics-based analyses and explicitly accounts for differences in statistical estimands across methods.

#### S1.1 Quality control

In this study, to minimize systematic bias as much as possible and enhance the robustness of genetic parameter estimation, comprehensive quality control procedures were first applied to the raw genotype and phenotype data. For genotype data, filtering was conducted primarily from two aspects: the reliability and representativeness of variant sites. On the one hand, a minimum allele frequency (minor allele frequency, MAF) threshold ( $MAF > 0.01$ ) was applied to remove variants with extremely low frequency in the population, thereby avoiding unstable estimates introduced by rare alleles. On the other hand, SNP missingness was controlled (typically limited to within 5%) to reduce the impact of missing data on analytical results. In addition, Hardy-Weinberg equilibrium tests were performed in unrelated individuals to identify potential genotyping errors or sequencing biases from the perspective of population genetic structure, further improving data accuracy and consistency. Through these multiple filtering steps, the interference of low-quality markers in subsequent analyses can be effectively eliminated.

At the individual level, quality control mainly focused on sample completeness and consistency. Specifically, individuals with significantly high missingness rates were excluded to prevent systematic distortion of the overall data structure. Meanwhile, by evaluating the distribution of individual heterozygosity, samples that deviated markedly from the population mean were identified and removed, as such outliers often indicate potential sequencing errors or contamination risks. In addition, consistency between genetically inferred sex and recorded sex was verified, and samples with clear mismatches were excluded. Where necessary, individuals with high levels of relatedness were further identified and removed to ensure that samples satisfy the basic assumption of independence required in statistical analyses, thereby improving the validity of model estimation.

Considering the potential impact of population structure on genetic effect estimation, principal component analysis (principal component analysis, PCA) was further introduced to identify and correct for population stratification. By applying dimensionality reduction to the genotype matrix, principal components reflecting genetic variation within the population were extracted, and the top 10 to 20 principal components were included as covariates in subsequent statistical models. This approach allows explicit control of underlying population structure during analysis, effectively reducing confounding effects caused by stratification and preventing systematic bias in heritability estimation and association results. Overall, these quality control and structural correction procedures provide a reliable data foundation for subsequent genetic analyses.

#### S1.2 Genomic relationship matrix construction

After completing rigorous data quality control, a genomic relationship matrix (GRM) was constructed based on the filtered high-quality SNP set to characterize the genetic similarity structure among individuals. Specifically, the standard GRM is obtained by centering and standardizing the genotype at each locus, and then calculating the weighted average of the genome-wide genetic covariance between individual  $i$  and individual  $j$ , yielding the following form of estimation:

$$G_{ij} = \frac{1}{M} \sum_{k=1}^M \frac{(x_{ik} - 2p_k)(x_{jk} - 2p_k)}{2p_k(1-p_k)}$$

Here,  $x_{ik}$  denotes the genotype coding of individual  $i$  at locus  $k$ ,  $p_k$  represents the allele frequency at that locus, and  $M$  is the total number of SNPs included in the analysis. By standardizing genotypes with respect to allele

frequency, the resulting matrix provides a comparable measure of genetic similarity across loci with different allele frequency scales, thereby establishing a foundation for subsequent decomposition of genetic variance.

However, in real data, genetic effects are typically not uniformly distributed across all variant sites; instead, they are jointly influenced by allele frequency and linkage disequilibrium (LD) structure. Based on this understanding, a stratified GRM construction strategy can be further introduced, in which SNPs are grouped according to minor allele frequency (MAF) intervals or LD levels, and multiple sub-GRMs are constructed accordingly (i.e., the GREML-LDMS framework). This approach allows different classes of variants to contribute heterogeneously to genetic variance. By incorporating a more refined structural representation at the model level, this strategy helps mitigate fitting biases of the standard GRM under complex genetic architectures, thereby improving the interpretability and stability of genetic parameter estimates.

### **S1.3 Estimation procedures**

After the genomic relationship matrix is constructed, the estimation of SNP heritability mainly relies on two methodological pathways: individual-level data and summary statistics. When complete individual-level data are available, linear mixed models (LMMs) are typically used to decompose phenotypic variance, with the basic form given as:

$$y = X\beta + g + \varepsilon$$

Here,  $g$  represents the additive genetic effects captured by genome-wide SNPs and is typically assumed to follow a normal distribution with mean zero and a covariance structure defined by the genomic relationship matrix (GRM), i.e.,  $g \sim N(0, \sigma_g^2 G)$ . The residual term  $\varepsilon$  reflects the random error not explained by the model and satisfies  $\varepsilon \sim N(0, \sigma_e^2 I)$ . Within this framework, the genetic variance and environmental variance can be estimated using the restricted maximum likelihood (REML) method, from which SNP heritability can be further derived:

$$h_{\text{SNP}}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

In practical applications, GCTA-GREML, BOLT-REML, and several closed-form estimation methods can all implement the above estimation process. While they differ in computational efficiency and model approximation, they are theoretically grounded in the same variance decomposition framework.

When individual-level data are unavailable, methods based on GWAS summary statistics provide an alternative approach. Among these, LD score regression (LDSC) estimates heritability by regressing the  $\chi^2$  statistics on LD scores, and its expected form can be expressed as:

$$E[\chi^2] = 1 + \frac{N h^2 l_j}{M}$$

This method utilizes the LD structure to weight summary statistics, thereby enabling heritability inference without requiring individual-level data. Furthermore, the SumHer method extends this framework by introducing weighting schemes that account for dependencies on minor allele frequency (MAF) and LD, allowing genetic effects to be non-uniformly distributed across different frequency ranges and LD regions. As a result, it generally exhibits greater flexibility under complex genetic architectures. It should be noted that the choice of LD reference structure has a substantial impact on estimation results; in practice, one may use external references (such as the 1000 Genomes Project) or, preferably, within-sample LD to improve matching and estimation accuracy.

### **S1.4 Model diagnostics and robustness analysis**

To ensure the reliability of heritability estimates, it is necessary to conduct systematic model diagnostics from multiple perspectives. First, from the standpoint of matrix properties, analyzing the eigenvalue spectrum of the genetic relationship matrix (GRM) can help identify potential numerical instabilities, such as near-singularity or anomalous structures. These issues often indicate the presence of inadequately controlled population structure or sample dependencies within the data. Second, by varying the composition of the SNP set—such as comparing the



full set of SNPs with subsets stratified by minor allele frequency (MAF) or linkage disequilibrium (LD), as well as LD-pruned variant sets-it is possible to assess the sensitivity of heritability estimates to marker selection, thereby determining whether the results depend on specific data-processing strategies.

Furthermore, regional sensitivity analysis involves removing specific high-LD regions (e.g., the MHC region) and re-estimating heritability to examine whether genetic variance is disproportionately concentrated in localized genomic segments. If the estimates change substantially after removal, this suggests that the region plays a dominant role in the genetic architecture of the trait. In addition, consistency comparisons across different methods (such as between GREML and LDSC or SumHer) constitute a critical step. Systematic discrepancies between methods are more likely to reflect differences in model assumptions or LD characterization, rather than being attributable to random estimation error.

### **S1.5 Interpretation framework**

When interpreting SNP heritability, it is necessary to recognize that different methods correspond to different statistical targets (estimands), and therefore their estimates are generally not directly comparable. Specifically, GREML-based approaches rely on the genetic covariance structure among individuals as defined by the genetic relationship matrix (GRM), and essentially estimate the proportion of additive genetic variance captured by this matrix. In contrast, LDSC operates within an LD-weighted regression framework, providing an overall, aggregate-level interpretation of GWAS summary statistics. SumHer further incorporates explicit modeling of minor allele frequency (MAF) and LD, making its estimand dependent on the chosen weighting scheme and assumptions about the genetic architecture.

In this sense, the results  $h_{SNP}^2$  obtained from different methods correspond to distinct statistical definitions. Only under ideal conditions-where the SNP set, LD structure, and model assumptions are completely aligned-can these estimates be expected to converge. Otherwise, the observed differences should be understood as reflecting differences in statistical targets, rather than inconsistencies in underlying biological mechanisms.

### **S1.6 Summary**

In summary, this study establishes a systematic technical framework for SNP heritability analysis. Its core lies in providing a robust foundation for subsequent variance decomposition through standardized data preprocessing and GRM construction. At the same time, by integrating individual-level data methods with summary statistics approaches, it enables multi-path estimation and cross-validation. Furthermore, through comprehensive model diagnostics and sensitivity analyses, the framework ensures the reliability of the results and clearly delineates the boundaries of interpretation. This framework is not only applicable to large-scale human genetic datasets such as the UK Biobank, but also demonstrates strong scalability and can be extended to related research areas, including crop genetics and genomic selection.



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## Research Insight

## Open Access

## Discussion on the Operational Model of Modern Agricultural Service Centers in Socialized Rice Production Services

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**Abstract** Modern agricultural service centers are becoming increasingly important in China's rice sector because they help solve a practical problem that many rural regions now face: rice production still matters, but the traditional household-based way of organizing it is under growing pressure from labor transfer, aging farm populations, fragmented land, tighter operation windows, and rising quality requirements. Focusing on Mashan Agricultural Service Center in Shangyu District, Zhejiang Province, this paper discusses how a modern agricultural service center operates in socialized rice production services and why such a model matters for contemporary agricultural modernization. The study combines policy documents, recent academic literature, and descriptive case materials from Mashan, including project briefs and field-based operational records. Rather than treating the center as a simple site for machinery storage, the paper analyzes it as a regional service platform that links centralized seedling cultivation, machinery dispatch, full-process trusteeship, drying and postharvest handling, technical guidance, and market-oriented branding. The results suggest that the practical value of a modern agricultural service center lies in organizational coordination. Its core contribution is to connect small-scale farmers with standardized, timely, and professionally managed production services without requiring every household to independently invest in costly facilities and equipment. The Mashan case shows that such centers can improve production efficiency, reduce labor burdens and transaction costs, strengthen emergency response under typhoon and harvest pressure, support greener and more standardized production, and create a foundation for regional rice brands. At the same time, their operation still faces constraints, including high capital intensity, shortages of skilled technical personnel, uneven service uptake across different farmer groups, limited digital management capacity, and growing climate risks. The paper argues that future development should focus on stronger regional coordination, useful digital tools, systematic talent training, deeper integration of postharvest and branding functions, and more explicit emergency-service design. In this sense, the modern agricultural service center is not just a service facility. It is an institutional bridge between smallholder farming and a more resilient, efficient, and quality-oriented rice production system.

**Keywords** Modern agricultural service center; Socialized agricultural services; Rice production; operational model; Zhejiang Province

### 1 Introduction

China's rice production is changing in a way that is both familiar and easy to underestimate. Rice remains a strategic staple crop, but the conditions under which it is produced are no longer the same as those that shaped the traditional household farming model. Rural labor has continued to move into non-farm sectors, the average age of those remaining in agriculture has risen, production costs have gone up, and farmers often manage multiple scattered plots rather than one unified operational unit. At the same time, public policy has become more demanding rather than less demanding: local agriculture is now expected not only to keep grain output stable, but also to become more efficient, greener, more standardized, and more responsive to market quality preferences (Tang et al., 2022). In rice farming, where missing a transplanting or harvesting window can have immediate consequences, these structural shifts have made the organization of services almost as important as the organization of land.

This is the context in which agricultural socialized services have expanded. In the Chinese setting, these services usually refer to specialized production services supplied by cooperatives, service organizations, agricultural

enterprises, or village-level operating entities to farm households that still retain land rights and production interests. Recent studies have shown that such services can improve technical efficiency, encourage sustainable practices, raise land productivity, and help smallholders remain connected to modern production systems without being forced into a single large-farm model (Huan et al., 2022; Yang and Li, 2022; Cai et al., 2024). In other words, the key issue is no longer simply whether agriculture will mechanize, but how access to mechanization, agronomic guidance, drying, storage, and postharvest handling can be organized in a way that works for a landscape still dominated by small and medium-scale producers.

Zhejiang Province offers a particularly useful setting for discussing this problem. It is economically developed, highly urbanized, and agriculturally under strong pressure to modernize through quality and organization rather than through simple land expansion. Yet Zhejiang has also moved quickly to build modern agricultural service centers as regional infrastructure for socialized production services. Provincial policy now explicitly encourages service centers to provide integrated support such as full-process mechanized operation, centralized seedling raising, drying, storage, processing, training, and emergency services, while local districts like Shangyu have paired these broader goals with concrete support measures for rice machine transplanting, grain security, and high-quality rice development. This makes Zhejiang a good place to examine not only whether service centers exist, but how they actually function.

Although the literature on mechanization services and agricultural socialized services has grown rapidly, much of it still emphasizes outcomes such as yield, technical efficiency, fertilizer reduction, or machinery use. Those studies are valuable. They show that services matter. Yet they often say less about the concrete operational model of the service center itself: how functions are bundled, how teams are organized, how seedling, harvesting, drying, and training are coordinated, and how a center works as a regional institution rather than as a single business unit.

This study takes Mashan Agricultural Service Center in Shangyu District, Zhejiang Province, as a case to explore the operational model of modern agricultural service centers in socialized rice production services. By combining policy documents, recent academic literature, and practical case materials, the study examines the main service functions, organizational structure, and operational mechanisms of the center. Particular attention is given to how different service links, including seedling cultivation, machinery operation, grain drying, technical guidance, and postharvest management, are integrated into a coordinated service system. The purpose of this study is to summarize the practical experience of modern agricultural service centers, evaluate their role in improving rice production efficiency and supporting agricultural modernization, and identify the main challenges affecting their sustainable development. It is expected that the findings will provide useful references for the improvement of agricultural socialized service systems and the future development of modern rice production in China.

## **2 Development Background of Modern Agricultural Service Centers in Rice Production**

### **2.1 Transformation of traditional rice production modes**

Traditional rice production in China was historically built on household labor, manual coordination, and highly localized experience. That model worked under conditions where rural households had relatively abundant family labor, local production rhythms were strongly village-based, and the gap between production and postharvest treatment was much smaller than it is today. But the present situation is different. Rice still provides an essential foundation for food security, yet the typical household now faces much tighter constraints in labor availability, operation timing, and cost control (Tang et al., 2022).

Three changes stand out. The first is labor restructuring. Rural labor migration has reduced the number of people available for time-sensitive field work, and those who remain are, on average, older than before. Studies from Chinese grain-producing regions show that aging and labor transfer now directly shape technology demand, production organization, and outsourcing behavior in farm operations (Shen et al., 2024; Li et al., 2023). The second change is fragmentation. Even where total household-managed land is not extremely small, it may be split into multiple plots with different road access, irrigation conditions, and operational convenience. This makes coordinated fieldwork more expensive and reduces the efficiency of household-level machinery ownership. Recent analysis from Jiangsu suggests that both land-scale and service-scale operations matter, and that their

interaction can significantly reduce per-unit machinery costs in rice production (Fu and Yang, 2025). The third change is quality pressure. Rice production is no longer judged only by whether grain is harvested. It is increasingly judged by whether the product can meet expectations for stable quality, safe postharvest handling, and recognizable market identity (Tang et al., 2022).

This transformation has made the traditional “each household manages every step for itself” pattern less workable. Timing is especially important in rice. Seedling preparation, transplanting, pest control, harvesting, and drying all have narrow operational windows. Once labor becomes scarce and plots remain scattered, households face higher coordination costs even before actual field work begins. It is one thing to own land; it is another to ensure that all necessary services arrive on time, in sequence, and at an acceptable quality level. That distinction explains why the transformation of rice production is not only about scaling up land, but also about reorganizing services.

## 2.2 Demand for socialized agricultural services in rice farming

The demand for socialized agricultural services in rice farming has grown because these services solve problems that individual households increasingly struggle to solve alone. At its core, socialized service is a way of making modern production inputs and operations available without requiring every farmer to own every machine, dryer, nursery system, diagnostic skill, and postharvest facility. In a fragmented and labor-constrained environment, that is not a marginal convenience. It is often the only realistic path to timely and standardized production.

Recent evidence makes this quite clear. Agricultural socialized services have been shown to improve the technical efficiency of smallholder rice producers, especially where farmers face production bottlenecks in machinery access and management quality (Cai et al., 2024). Other studies show that socialized services can encourage the adoption of sustainable agricultural practices, reduce fertilizer input, promote land protection, and increase land productivity when service supply matches the production needs of farmers (Huan et al., 2022; Cheng et al., 2022; Yang and Li, 2022). The logic is straightforward. When production services become specialized, providers can invest in better equipment, accumulate operational experience, and spread fixed costs across a larger service area. Farmers, in turn, can buy access to capability rather than capability itself.

In rice farming, this demand is particularly strong because production stages are tightly connected. Delays in seedling supply affect transplanting; poor field management affects pest pressure and uniformity; slow harvesting increases losses; and inadequate drying can damage the value of grain already produced. It is therefore misleading to think of rice service demand as being limited to one or two isolated operations. A farmer may begin by demanding harvesting service, but what actually matters is often a chain: seedling, transplanting, spraying, harvesting, drying, and sometimes even marketing. Once this chain perspective is adopted, the value of a coordinated service system becomes obvious.

Another reason demand has increased is the changing economics of fixed investment. High-quality dryers, transplanting equipment, nursery facilities, storage systems, and repair capacity are expensive. Their value depends on scale, but many households cannot justify such investment on their own land area. This is exactly why service-platform models are expanding. They pool demand, reduce duplication, and turn capital-intensive technology into shared regional infrastructure. In that sense, socialized services do not simply help farmers save labor. They reduce the threshold for entry into modern agriculture.

## 2.3 Policy support for modern agricultural service centers in Zhejiang Province

Zhejiang Province has actively formalized this service logic through policy. In 2024, the provincial government issued opinions on accelerating the construction of modern agricultural service centers, making clear that these centers should serve as important platforms for full-process agricultural services, including mechanized operation, centralized seedling raising, postharvest drying, storage, processing, technical guidance, and training. The policy direction is important because it does not treat service centers as isolated infrastructure projects. It treats them as nodes in a regional agricultural service network.

The Zhejiang policy framework is especially notable for two reasons. First, it emphasizes service proximity and regional coverage. The idea of a local service radius is crucial in rice production because time-sensitive operations lose value if machinery, seedlings, or drying capacity arrive too late. Second, the policy ties service center construction to broader goals of agricultural modernization, green development, emergency capacity, and digital management. This means the service center is expected to do more than dispatch machines. It is expected to act as a local platform for coordinated agricultural support.

Shangyu District reflects this provincial direction at the district level. The district's 2024 early-rice machine-transplanting subsidy scheme explicitly aimed to raise machine-transplanting rates, stabilize grain production, and strengthen production capacity through targeted support for transplanting operations. Meanwhile, Shangyu's 2024 development statistics continued to frame grain production, high-standard farmland, and agricultural modernization as strategic tasks rather than residual rural issues. Zhejiang's 2024 notice on leading agricultural varieties and major promoted technologies likewise signals that modernization in the province is expected to combine better varieties, better organization, and better service support.

For rice production specifically, this policy environment matters because it legitimizes service integration. It allows a service center to combine nursery work, machinery operation, drying, and technical assistance within one operational system instead of treating each as a separate administrative line. That is one reason why Zhejiang is a particularly useful province for examining how modern agricultural service centers function in practice.

#### **2.4 Relationship between agricultural service centers and agricultural modernization**

Agricultural service centers are closely tied to agricultural modernization because they provide a way to modernize production without assuming that all producers will become large-scale owner-operators. This is an important institutional point. In many Chinese regions, modernization is not happening through the disappearance of smallholders. It is happening through new arrangements that allow smallholders, cooperatives, and service organizations to operate together within a more professional and standardized system (Li et al., 2024; Zeng et al., 2025).

From a production perspective, service centers support modernization in at least four ways. First, they improve continuity across production stages. Modern agriculture depends not only on advanced inputs, but on correct sequencing. A service center can connect pre-production, in-season management, and postharvest handling more reliably than a set of disconnected providers. Second, service centers make technology more accessible. Farmers who cannot afford their own dryers or nursery systems can still benefit from them through service purchase. Third, service centers reduce coordination failures. A field may not need more theory; it may simply need seedling delivery, machinery dispatch, and drying capacity to arrive in the right order. Fourth, service centers make aggregation possible. By bringing together enough service demand, they make investment in equipment, training, and postharvest infrastructure more economically reasonable.

Modernization also has an organizational side that is sometimes overlooked. A service center does not only modernize the field. It modernizes routines: booking, scheduling, team management, maintenance, training, storage, quality records, and in some cases digital traceability. This is why the institution itself deserves attention. Mechanization without organization can remain partial and unreliable. Organization is what turns individual technologies into a functioning production system.

### **3 Organizational Structure and Operational Functions of Mashan Agricultural Service Center**

#### **3.1 Basic construction and functional layout of the service center**

According to the case materials supplied with the manuscript, Mashan Agricultural Service Center is located in Mashan Village, Shangyu District, and is operated by the Shaoxing Shangyu Mashan Grain Specialized Cooperative. The center occupies 6.73 mu and was designed as a working service platform rather than as a symbolic demonstration building. The reported original construction included a 2,400 m<sup>2</sup> drying center, a 1,888 m<sup>2</sup> seedling cultivation center, and a 200 m<sup>2</sup> machinery shed, with total project investment exceeding RMB 6 million. The internal layout also included repair rooms, storage rooms, dryers, processing areas, and training or meeting space (Figure 1).



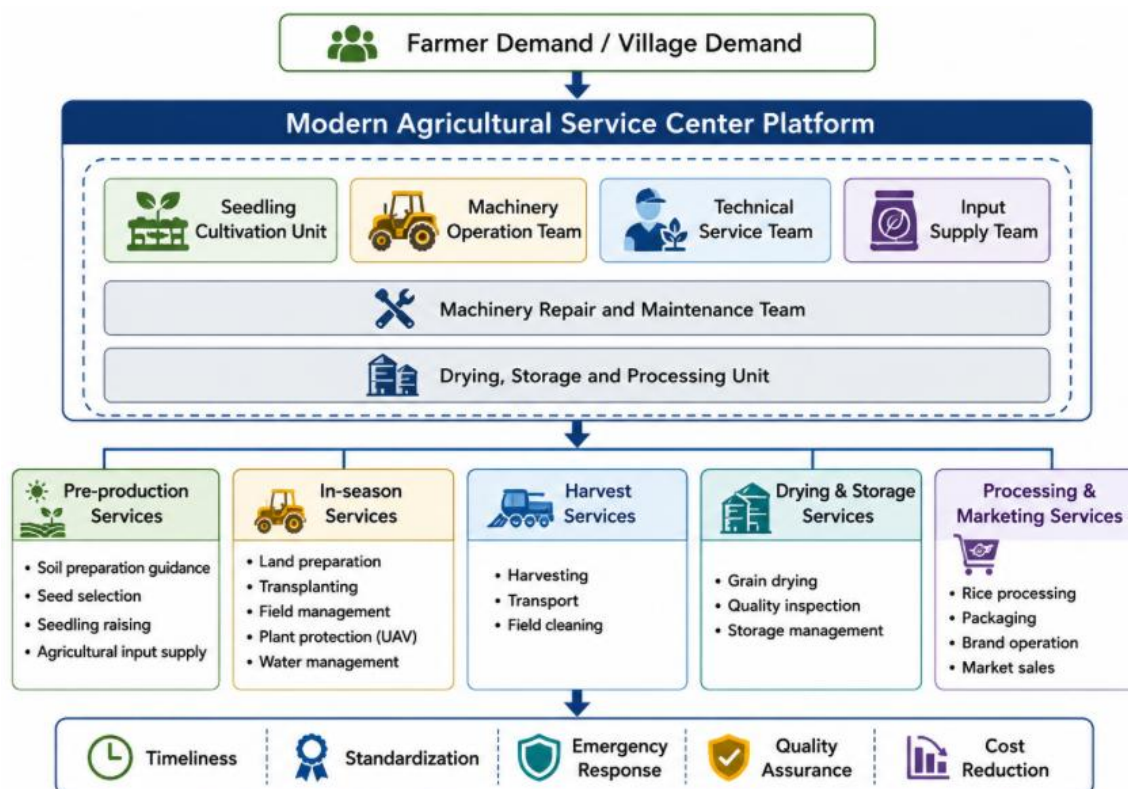


Figure 1 Operational framework of the modern agricultural service center in socialized rice production

What is striking here is the functional logic of the layout. The center does not simply place a few farm machines under one roof. Its facilities correspond to key bottlenecks in rice production: seedlings before planting, machinery during operations, drying after harvest, and training and support around the whole chain. That kind of layout matters because service efficiency depends on adjacency between functions. The seedling center supports transplanting. The dryers support harvesting. The storage and processing spaces support marketization. The repair area supports service continuity. In short, the building layout is an operational design, not just a construction plan.

The materials also indicate that the center later expanded its built area by an additional 805 m<sup>2</sup>, adding eight more dryers, a 750-ton indoor metal granary, a 50-ton rice processing line, and additional support space. This later expansion is revealing. It suggests that postharvest service became more central over time, which is typical of service centers moving from basic operation assistance toward more integrated grain-chain functions.

### 3.2 Organizational management structure and service teams

Mashan's organizational design is just as important as its buildings. The supplied materials state that the center has seven fixed workers, around 100 sets of agricultural machinery and equipment, machinery assets valued at about RMB 8 million, and a 100% licensing rate among machinery operators. On paper, seven fixed workers may not sound like a large number, but the relevant issue is not headcount alone. It is how the center organizes specialized teams and supports seasonal mobilization.

The case materials describe four primary service teams already in operation: a mechanized operation team, an agricultural input delivery team, a technical service team, and a machinery repair team. This arrangement reflects a practical division of labor. The operation team focuses on field work; the technical team supports agronomic decisions and training; the repair team protects service continuity; and the input-delivery team reduces farmer-side coordination burdens. That is a significant shift away from the older model in which a machine operator might be expected to solve any and all problems alone.

This type of team structure is one of the clearest operational features of modern agricultural service centers. It shows that a service center is neither identical to a machinery cooperative nor reducible to a warehouse. It is an

organization that combines tasks with people. In rice farming, this matters because field service quality depends not only on whether the machine arrives, but whether diagnosis, scheduling, repair, and postharvest support are connected to the machine's arrival.

### **3.3 Agricultural machinery and supporting facilities**

Machinery is naturally central to the center's operation, but the case materials suggest that Mashan's core strength lies in the relationship between machinery and supporting facilities. The center reportedly holds around 100 sets of agricultural machinery and equipment, and the later facility expansion significantly increased drying, storage, and processing capacity. This combination is important because many discussions of mechanization still focus too narrowly on field machines while underestimating postharvest infrastructure.

For rice production, drying is not optional support. It is part of the service chain. Harvesting capacity without drying capacity only shifts the bottleneck downstream. The same is true for seedling cultivation. Transplanting service is much easier to standardize when tray seedlings are centrally prepared and delivered in a coordinated manner. The center's facility bundle therefore supports a "field-to-postharvest" model rather than a single-link operation model.

The machinery-support relationship also changes how capital is used. When dryers, seedling facilities, and storage are attached to machinery service, the whole investment becomes more productive because each function reinforces the value of the others. A harvester becomes more useful when it can deliver grain straight into a regional drying system. A seedling center becomes more useful when it supports machine transplanting across a wider service area. This is precisely the kind of asset coordination that distinguishes a modern service center from a loose network of separate providers.

### **3.4 Main operational functions in rice production services**

The internal project briefs describe Mashan's service structure as a "1+8" model centered on full-process mechanized operation and supported by drying and processing, centralized seedling cultivation, technical services, agricultural input delivery, machinery maintenance, agricultural study and training, product marketing, and storage and preservation. The wording itself is useful because it makes clear that the center is not built around one machine type or one contractual relationship. It is built around coordinated services.

The main functions can be grouped into three layers. The first layer is direct production service: seedling raising, transplanting, field operation, harvesting, drying. The second layer is support service: repair, input delivery, technical guidance, training. The third layer is value-extension service: storage, simple processing, local branding, and marketing. This layered structure matches the way modern rice production actually works. Farmers need one set of services to get grain grown, another set to get it managed well, and a third set to protect or increase its value after harvest.

The materials also indicate that Mashan serves both a core nearby service area and a wider regional production area across seven towns and streets, with "nanny-style" services for around 5,000 mu nearby and more than 50,000 mu-times of full-process mechanized services annually across a broader area. This layered service radius is one of the center's most important operational traits. It suggests that the center combines intensive local support with wider regional dispatch, rather than choosing one scale at the expense of the other.

## **4 Main Operational Models of Socialized Rice Production Services**

Before discussing the five main operational models, it is useful to state a general principle (Table 1). The service models of a modern agricultural service center are not separate in practice. They overlap. Centralized seedling raising feeds transplanting. Machinery scheduling affects trusteeship quality. Drying affects the value of harvest rescue. Training influences the quality of field management and the willingness of farmers to buy services. Still, for analytical clarity, the operational models can be discussed one by one.

Table 1 Main operational models of socialized rice production services

| Operational model                                                    | Core content                                                                                | Main organizational carrier                            | Main practical value                                                                           |
|----------------------------------------------------------------------|---------------------------------------------------------------------------------------------|--------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Centralized seedling cultivation service model                       | Unified nursery preparation, tray seedling production, delivery to farmers or service teams | Seedling center+technical team                         | Improves seedling uniformity, supports machine transplanting, reduces household nursery burden |
| Full-process trusteeship service model for rice production           | Bundled services from pre-production to postharvest, partly or fully entrusted by farmers   | Service center+cooperative+specialized operation teams | Reduces coordination costs and timing failures across stages                                   |
| Agricultural machinery scheduling and cross-regional operation model | Seasonal dispatch of machinery across villages and towns, including emergency tasks         | Operation team+repair team+service booking             | Raises machinery utilization and supports timely peak-season operations                        |
| Grain drying and postharvest service model                           | Centralized drying, temporary storage, grain processing and preservation                    | Drying center+storage+processing line                  | Reduces postharvest loss and supports quality retention and branding                           |
| Technical guidance and farmer training service model                 | Agronomic advice, pest diagnosis, training, production guidance                             | Technical team+training space+external experts         | Improves service quality, green production, and farmer trust in standardized management        |

#### 4.1 Centralized seedling cultivation service model

Centralized seedling cultivation is one of the clearest examples of how service organization can change the economics of rice production (Figure 2). In household-based production, raising seedlings is not only laborious; it is also highly variable. Timing, tray preparation, seed quality, temperature, and management consistency all affect later field performance. Once labor becomes scarce and machine transplanting becomes more common, this stage turns into a serious coordination problem. Centralized seedling services address that problem by moving the work into a specialized facility.

Recent research on machinery rice transplanting and centralized rice seedling cultivation in China argues that these systems do more than save labor. They improve the efficiency of seedling supply, raise the space-use efficiency of nursery land, and even create a “seedling field saving” effect that can release time and land resources under crop rotation systems (Ruan et al., 2025). That finding is conceptually important because it shows that a service center can affect production before the crop even enters the main field.

Mashan’s materials are consistent with that logic. The center reportedly supplies more than 200,000 trays of early- and late-rice seedlings per year and provides associated technical guidance to nearby farmers. In practical terms, such a model does three things. It standardizes the starting point of the crop. It reduces household-level technical unevenness. And it makes machine transplanting much easier to coordinate. When many households receive seedlings from the same center, later operations can be scheduled with much greater confidence.

There is also a subtle institutional effect here. Seedling service often serves as the first point of deeper farmer-center cooperation. A household that begins by purchasing seedlings may later purchase transplanting, harvesting, drying, or even partial trusteeship. In that sense, centralized seedling supply is not a minor entry service. It can be the organizational gateway into the wider service system.

#### 4.2 Full-process trusteeship service model for rice production

The full-process trusteeship service model is perhaps the most important modern form of socialized rice production service. Under this model, farmers do not surrender land rights, but they rely on the service center or related provider to complete part or all of the production chain. The crucial point is not whether every farmer purchases every link. It is that a bundled service option exists, and that the burden of cross-stage coordination shifts away from the household.



Figure 2 Postharvest service process flow in modern agricultural service centers (Photoed by Xinfeng Ren)

This matters because transaction costs in agriculture are often hidden. Farmers do not only “pay for operations.” They spend time finding operators, matching timing, arranging inputs, watching weather, securing drying space, and handling breakdowns. When these transactions are fragmented, production risk remains high even if one or two operations are mechanized. Full-process trusteeship reduces these coordination costs by organizing production as a service package rather than a scattered set of purchases (Li et al., 2024).

The literature supports this interpretation. Agricultural socialized services have been shown to improve rice producers’ technical efficiency not only through machinery access, but through systemic support at different production links (Cai et al., 2024). Studies on grain production behavior also show that socialized service adoption can promote grain cultivation by improving machinery use, encouraging more connected land operation, and increasing the operational attractiveness of grain production for households that might otherwise shift away from it (Li et al., 2024).

Mashan’s “1+8” system fits this trusteeship logic well. Even if not every farmer purchases the entire package, the center is clearly structured to make that possible. The practical value of the model lies in continuity. It reduces the chance that a farmer will solve one production bottleneck only to get stuck at the next. In rice farming, where delays compound quickly, that continuity is one of the strongest arguments for the service-center model.

### 4.3 Agricultural machinery scheduling and cross-regional operation model

Machinery scheduling is often treated as an administrative detail, but in real rice production it is one of the decisive elements of service performance. The usefulness of machinery depends not only on ownership, but on where it can go, when it can get there, how quickly it can be maintained, and whether dispatch can respond to narrow crop windows and bad weather. A modern service center therefore operates as a scheduling hub as much as a machinery owner.

This is especially true in regions like eastern China, where production calendars are dense, weather volatility can compress operation windows, and service demand peaks sharply. Cross-regional operation helps solve a classic utilization problem. A machine that is underused in one village but urgently needed in another becomes much more valuable when it can be moved efficiently across the service area. Research on machinery-based services and land productivity in China suggests that such services can significantly improve land productivity, but with different effects depending on crop conditions, scale, and local context (Yang and Li, 2022).

The policy environment in Shangyu also points in this direction. The district’s 2024 early-rice machine-transplanting subsidy scheme relied on an administrative mechanism designed around actual machine operations, implying that service performance is increasingly tied to measurable operation capacity rather than



informal local arrangements. Even where detailed real-time scheduling data are not publicly available, the institutional direction is clear: machine service is becoming more formalized, more trackable, and more closely integrated with district-level grain goals.

In the Mashan case, the existence of specialized mechanized operation teams and a multi-town service radius suggests that cross-regional machinery dispatch is already part of the center's operating model. This is not only an efficiency question. It is also a resilience question. When a center can move harvesting or drying support quickly across several towns, it functions as a regional buffer against timing shocks.

#### **4.4 Grain drying and postharvest service model**

Postharvest service is where many service-center models prove their real maturity. It is relatively easy to advertise mechanized harvesting. It is much harder to build an integrated system that can handle wet grain, unstable weather, drying queues, storage, and simple processing in a region with many small producers. That is why drying and postharvest service deserve special attention.

Rice quality is highly sensitive after harvest. Grain harvested at the right time can still lose value through delayed or improper drying. Review work on rice harvest losses shows that losses occur across the wider harvest process rather than at cutting alone, and that postharvest handling remains a major source of avoidable loss (Qu et al., 2021). From a wider sustainability perspective, this is a serious issue. Once land, fertilizer, water, labor, and energy have already been used to produce the crop, postharvest avoidable loss becomes a direct efficiency and environmental problem.

The service-center model addresses this by centralizing drying. A center can invest in dedicated dryers, create more stable drying routines, reduce dependence on household sun-drying, and offer grain safety and quality conditions that small farmers cannot easily achieve individually. The broader literature on rice greenhouse gas mitigation also indirectly supports this focus by reminding us that sustainable rice systems are not only about emissions in the field; they are also about reducing waste and improving total system efficiency (Qian et al., 2023).

Mashan's later expansion fits this model closely. The addition of eight dryers, greater single-batch drying capacity, a metal granary, and a processing line suggests that the center evolved from a primarily operations-based unit into a more complete postharvest service platform. This is a major operational shift because it means the center can connect rescue harvesting to stable postharvest management and then to value-added rice products.

#### **4.5 Technical guidance and farmer training service model**

Technical guidance is often treated as a soft or secondary function compared with machinery and drying, but in practice it is one of the most important ways a service center increases the quality and credibility of its services. Farmers do not only need a machine operator. They need advice about timing, seedling quality, pest risk, field management, drying decisions, and sometimes market expectations. If the center lacks a technical layer, it risks becoming a basic contractor rather than a modern agricultural service institution.

This issue has become even more important as digital and green production goals have expanded. Research on digital agricultural technology services in Sichuan shows that such services can increase farmers' willingness to adopt digital production technologies by expanding information channels, improving cognitive understanding, and increasing technology accessibility (Gong et al., 2024). At the same time, studies on sustainable agricultural practice adoption among Chinese smallholders suggest that socialized services can help farmers enter more sustainable production pathways when those services reduce knowledge barriers and improve access to practical support (Huan et al., 2022).

The logic applies directly to rice service centers. Training and expert guidance help in at least three ways. They reduce farmer uncertainty about service quality. They improve the agronomic quality of the operations themselves. And they help align service-center goals with green and standardized production aims. Technical guidance is therefore not separate from the operational model. It is part of how the model builds trust and effectiveness.



In the Mashan case, the internal materials note regular training sessions and field guidance activities, especially during periods of frequent pest occurrence. This suggests that the center's service model already combines machinery with agronomy, which is precisely what a modern operational platform should do.

## **5 Practical Effects of Modern Agricultural Service Centers in Rice Production**

### **5.1 Improvement of rice production efficiency**

The first and most visible practical effect of a modern agricultural service center is improved production efficiency. But efficiency should be understood broadly. It includes labor saving, of course, but it also includes timeliness, continuity across operations, and reduced coordination risk. A well-functioning service center makes it easier to complete production steps within the correct agronomic window and with fewer gaps between stages.

This broader understanding is supported by existing literature. Agricultural socialized services have been found to improve technical efficiency among smallholder rice producers, partly because they replace irregular household arrangements with more standardized and professional support (Cai et al., 2024). Other studies show that mechanization and machinery-based services improve land productivity and production efficiency when machinery access is timely and well matched to field conditions (Yang and Li, 2022; Liu and Li, 2023).

Mashan's organizational structure helps explain why. The center combines centralized seedling supply, dedicated operation teams, drying capacity, machinery repair, technical guidance, and regional dispatch. Each of these reduces a different form of production friction. The result is not only faster operations, but a more stable production chain. A farmer no longer has to negotiate each link separately at peak season. That reduction in coordination failure is a genuine efficiency gain, even if it does not always appear directly in yield statistics.

### **5.2 Reduction of labor pressure and farming costs**

The second major effect is the reduction of labor pressure and farming costs, especially for households facing labor outflow or aging. In current rural China, the question is often not whether labor is expensive, but whether sufficient labor is available at the right moment. Socialized services turn this structural challenge into an organizational problem that can be handled collectively rather than privately.

Several studies are helpful here. Research on aging agricultural labor force and outsourced pest-control services in rice areas of Fujian shows that outsourcing has become an important response to the aging of the agricultural labor force and can influence input use behavior in greener directions (Shen et al., 2024). Work on labor migration and fertilizer use among smallholders further suggests that socialized services can mediate or offset some of the production difficulties created by labor transfer (Li et al., 2023). In practice, a service center lowers the demand for family labor not by replacing the farmer entirely, but by taking over the most time-sensitive, equipment-sensitive, or physically demanding parts of production.

This also affects costs in a deeper way. A household that would otherwise need to purchase or maintain expensive machines, temporary drying arrangements, or separate labor contracts instead buys service when needed. This reduces fixed-cost pressure and allows smallholders to access scale advantages through the service system. For households that still want to remain in grain production but do not want to bear the full capital and management burden, this is one of the strongest benefits of the service-center model.

### **5.3 Enhancement of disaster prevention and emergency response capacity**

The value of a service center becomes especially visible when weather no longer cooperates. Under typhoon threats, heavy rain, or compressed harvest windows, the difference between ordinary service provision and real emergency capacity becomes obvious. A center with machines, drying space, operators, and coordination ability can protect grain already grown. One without postharvest linkage may only move the risk from field to yard.

This is important because climatic risk to rice production is increasing. Recent climate projections for China's rice regions suggest that extreme climate challenges, especially heat stress and some forms of wet-period risk, are likely to intensify (Chen et al., 2025). At the same time, the practical literature on rice harvest losses shows that loss control depends heavily on timing and postharvest handling, both of which become more difficult under abnormal weather (Qu et al., 2021).

Mashan's emergency harvesting case illustrates what a modern service center can contribute under such stress. According to the supplied materials, when the "double rush" period overlapped with typhoon weather, the center organized more than 20 harvester operations, completed emergency early-rice harvesting on more than 12,000 mu, and then dried more than 14,000 tons of grain. Even without experimental comparison, this is persuasive case evidence. It shows that the center's real strength in emergencies is not the harvester alone, but the combination of harvesting and drying in one coordinated system.

#### 5.4 Promotion of standardized and green rice production

Modern agricultural service centers also make standardized and greener rice production more realistic (Figure 3). This does not happen automatically. Machines can be used badly as well as well. But when operations are bundled with technical support, scheduling discipline, and postharvest facilities, the service-center model can reduce some of the variability and waste that come from uncoordinated household production.

Recent studies provide several angles on this. Agricultural socialized services in south China have been found to encourage greener production behavior, particularly in fertilizer-use decisions among smallholder rice farmers (Shi et al., 2023). Socialized services of agricultural green production have also been shown to reduce fertilizer input in rice systems, especially when combined with stronger information and social support (Yang et al., 2022). Meanwhile, rice-focused studies of greenhouse gas mitigation remind us that more sustainable systems depend on management choices across water, organic inputs, tillage, and crop handling rather than on a single technical fix (Qian et al., 2023).

At the operational level, Mashan's model supports standardization in several ways. Centralized seedling cultivation reduces uneven crop establishment. Organized machinery service improves timing. Technical guidance strengthens field management. Centralized drying reduces postharvest instability and household-level weather exposure. If greener plant protection tools are adopted, professionally managed application can also reduce operator exposure and improve targeting relative to improvised field practice. Evidence from UAV-based herbicide application in direct-seeded rice suggests that modern spray systems can provide effective control while reducing labor burden and lowering carrier volume when used under appropriate conditions (Paul et al., 2024). The lesson is not that every center should rush toward any fashionable machine, but that professionalized service can make precision and greener input use more achievable.

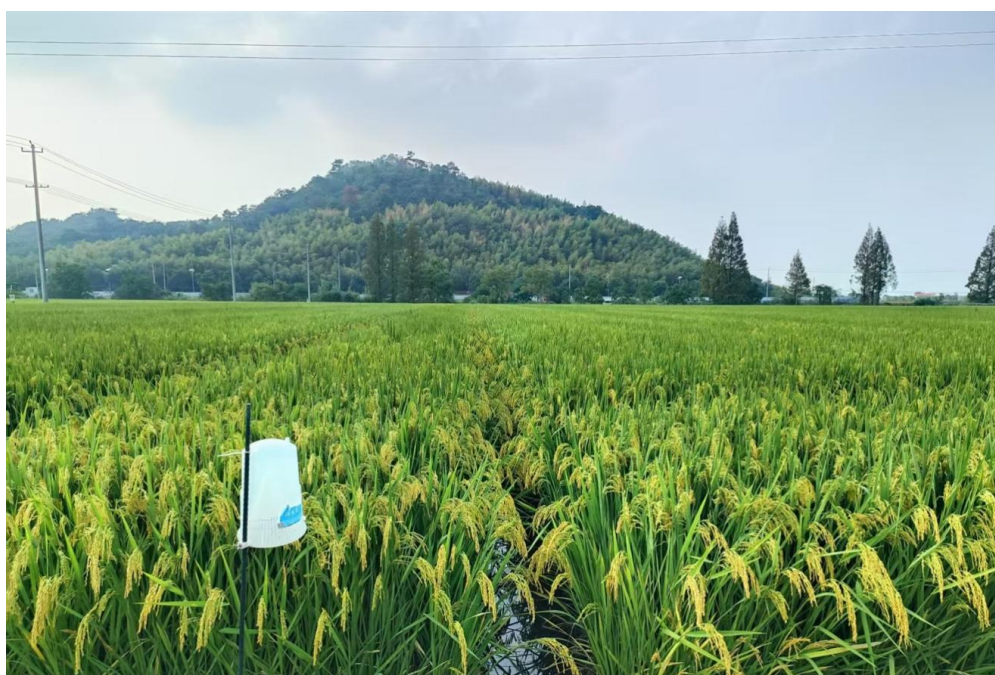


Figure 3 Standardized high-quality rice production field under agricultural service center management

Note: Unified field management supports standardized and green rice production (Photoed by Xinfeng Ren)

## 5.5 Promotion of regional rice brand development

One of the more interesting effects of the modern service center model is that it can help connect production services with local branding. This matters particularly in developed provinces, where agricultural competitiveness increasingly depends on quality recognition, traceability, and regional identity rather than on bulk volume alone. Rice branding is not separate from production organization. It depends on it.

Mashan's case is revealing here. The supplied materials report that the center's registered "Xinfeng" rice brand won the Silver Award in the 2024 "Zhejiang Good Rice" competition (Figure 4). On its own, this is not a scientific production indicator. But in an applied production study, it is meaningful because it shows that service capacity, processing, and branding can be linked. A center that can raise seedlings, coordinate operations, dry grain, store it safely, and process it locally has a much stronger basis for stable branded production than a provider limited to one field-operation link.

This is where modern agricultural service centers begin to resemble local industry-chain hubs rather than narrow service contractors. Their role expands from "helping farmers finish work" to "helping local rice become a differentiated product." That shift is especially important in places like Zhejiang, where agriculture must increasingly earn value through reliability, quality, and regional reputation.

### Postharvest Service Process Flow in Modern Agricultural Service Centers

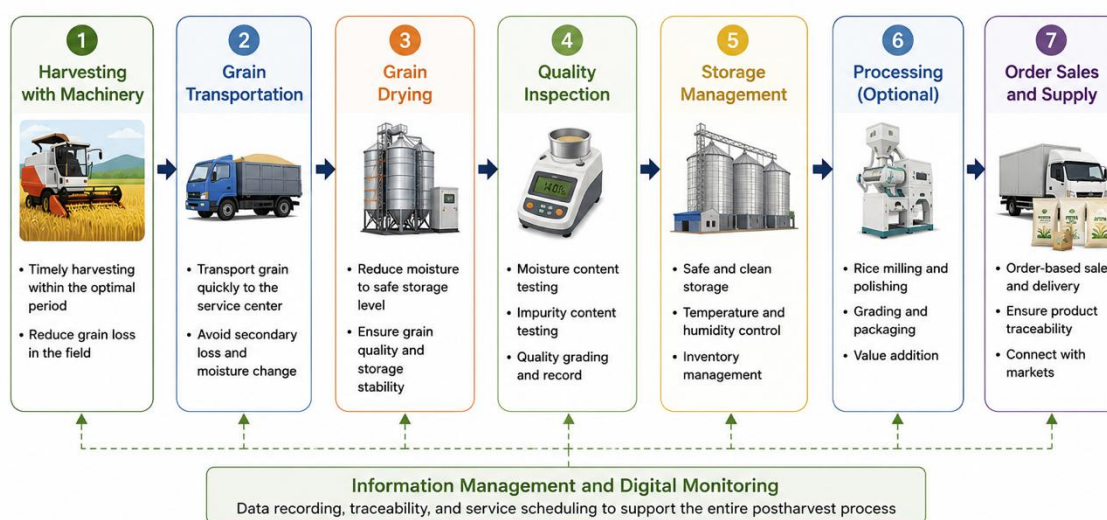


Figure 4 Postharvest service process flow in modern agricultural service centers

## 6 Case Analysis of Socialized Agricultural Services in Mashan Agricultural Service Center

Before discussing the individual cases, one methodological limitation should be stated clearly. The following four cases are based mainly on two internal project briefs supplied with the manuscript materials and related field descriptions. These are operational management materials rather than independently audited datasets. They are therefore used here as descriptive evidence of how the center functions in practice, not as a basis for strict causal inference.

### 6.1 Case of centralized seedling supply for surrounding farmers

The centralized seedling supply case captures the center's role at the earliest and often most fragile stage of rice production. Mashan's seedling cultivation center reportedly supplies more than 200,000 trays of standardized early- and late-rice seedlings each year for nearby farmers and service-linked operations. This volume suggests that seedling cultivation is not a small side activity. It is one of the center's core gateways into the local rice production system.

The practical value of the case lies in standardization. Seedling uniformity influences transplanting quality, later crop establishment, and management stability through the growing season. When seedlings are centrally produced, households no longer need to prepare nursery materials separately, and the timing of seedling availability becomes easier to match with machine transplanting. The project materials also note that technical guidance linked to the center reportedly improved seedling establishment rates for surrounding farmers by around 20%, which reinforces the point that the service is not merely material supply. It combines service products with agronomic support.

This case shows why service centers matter even before field operations begin. In many discussions of modernization, attention goes first to transplanting or harvesting machines. But in practice, the service quality of later links often depends on whether the earliest link was standardized well enough to support them.

### **6.2 Case of emergency agricultural machinery dispatch during typhoon season**

The emergency dispatch case is perhaps the strongest demonstration of Mashan's regional role. According to the project materials, during the overlap of the "double rush" period and typhoon weather, the center mobilized more than 20 harvester operations, completed emergency harvesting of early rice on more than 12,000 mu, and then carried out more than 14,000 tons of drying afterward. These figures show not only operational scale, but operational sequencing.

Analytically, the key point is that harvest rescue and drying rescue occurred together. A harvester without drying support would only provide partial relief. Wet grain rescued from a field can still deteriorate rapidly if postharvest treatment is delayed. The Mashan case therefore illustrates a central feature of the modern service-center model: emergency response depends on linking field capacity with postharvest capacity.

This case also has a wider social meaning. In abnormal weather, the center functions partly like a quasi-public rural infrastructure node. It helps farmers reduce losses that individual households would struggle to prevent on their own. In a climate context where extreme events may become more frequent or more disruptive, this kind of regional protective role may become one of the most important justifications for service-center investment.

### **6.3 Case of grain drying and storage service expansion**

Mashan's drying expansion case shows how a service center evolves beyond basic machinery support. According to the project materials, the center added eight dryers, increased single-batch drying capacity to 400 tons, raised annual drying capacity from 10,000 tons to 18,000 tons, and added a 750-ton indoor metal grain warehouse as well as a 50-ton rice processing line.

This is important because it marks the difference between partial mechanization and integrated service. A center that can harvest but not dry remains incomplete in a rice production setting. The Mashan expansion suggests that the operators recognized this and chose to strengthen the postharvest part of the chain. The expansion also helps explain how the center could respond to emergency harvest demand during bad weather. Without larger drying and storage capacity, emergency dispatch would have had much less value.

From a development perspective, this case suggests a typical upgrading path for rural service centers. They may begin around machinery operation, but long-term effectiveness pushes them toward postharvest control, storage, and processing. In rice systems, where quality and loss control matter greatly after harvest, this transition is logical and necessary.

### **6.4 Case of rice brand-oriented operation and market expansion**

The final case extends the analysis from production support to market development. The internal materials state that Mashan promoted the "Xinfeng" rice brand through standardized production management, processing, and local marketing, and that the brand won the Silver Award in the 2024 "Zhejiang Good Rice" competition.

The significance of this case lies in what it reveals about the center's operational horizon. The modern agricultural service center is not confined to completing field tasks. It can also help stabilize the conditions needed for quality differentiation in the market. Brand-oriented operation depends on more than advertising. It requires some



consistency in production, handling, storage, and output. A center with seedling supply, machinery service, drying, processing, and storage is better positioned to support that consistency than a service provider limited to a single operation link.

This case also suggests a broader development path in eastern China. In higher-income regions, the future of rice production may depend less on low-cost volume and more on the ability to combine service efficiency with local brand value. Mashan's brand case therefore shows a shift from service as production assistance to service as production-plus-market support.

## **7 Current Problems in the Operation of Agricultural Service Centers**

### **7.1 High operational and equipment maintenance costs**

One of the clearest challenges for modern agricultural service centers is the heavy capital and maintenance burden of integrated service provision. Mashan's own case illustrates the point well. Project investment exceeded RMB 6 million; machinery assets were reported at about RMB 8 million; and the center operates drying, nursery, storage, processing, and service-support spaces together. This is precisely why such centers are effective, but it is also why they are financially demanding.

The pressure continues after construction. Equipment depreciation, electricity for drying, fuel, repairs, spare parts, storage management, operator certification, and facility upgrades all create ongoing costs. Research on mechanization and production efficiency confirms that machinery can improve output and efficiency, but it also shows that capital intensity remains a serious barrier where scale or service volume is insufficient to absorb fixed costs (Liu and Li, 2023; Ruan et al., 2025). In practice, service centers succeed only when utilization is high enough and fees, subsidies, or linked business functions together support long-term sustainability.

This means that service centers are vulnerable to underuse. A modern center may look impressive, but if service demand becomes unstable or if newer technologies rapidly raise upgrading pressure, the center can face financial stress. Capital investment is therefore not just a construction issue. It is a continuing operational issue.

### **7.2 Insufficient professional and technical personnel**

Another major problem is the shortage of multi-skilled technical personnel. Mashan's seven fixed workers and specialized teams show organizational capacity, but they also hint at how much is expected from a relatively small technical core. Modern service centers need more than tractor or harvester drivers. They need people who understand scheduling, machinery maintenance, drying control, field conditions, agronomy, safety, records, and increasingly digital tools.

This challenge appears repeatedly in the recent literature. Digital agricultural technology services can only function well when users and service providers have adequate literacy, training, and confidence (Gong et al., 2024). The broader review literature on agricultural socialized services also points out that access and delivery problems often stem not only from equipment shortages, but from measurement problems, coordination gaps, and service-quality inconsistency (Zeng et al., 2025). Technical personnel are therefore not simply labor inputs; they are part of the service model's credibility and adaptability.

In rural areas, attracting and retaining such personnel is not easy. Young people may prefer urban sectors, while older experienced operators may have limited capacity to shift into digital management or more formalized service systems. The more a center expands toward quality-oriented and smart-service functions, the more serious this human-resource constraint becomes.

### **7.3 Difficulties in coordinating services across different farmer groups**

Although service centers are often promoted as bridges between smallholders and modern agriculture, farmers are not identical service users. They differ in plot conditions, road access, irrigation convenience, income expectations, quality preferences, willingness to pay, labor availability, and openness to standardized management. This heterogeneity creates a coordination challenge for service centers.



Research from Jiangsu indicates that the benefits of service-scale and land-scale coordination are real, but they also depend on governance conditions and local operating context (Fu and Yang, 2025). Studies from Jiangxi further show that the benefits of agricultural socialized services vary across farmer type, fragmentation level, region, and digital ability (Liao et al., 2025). That means a service package that works well for one village or household group may be less suitable elsewhere.

For Mashan, which serves both a core nearby area and a wider multi-township region, this problem is very relevant. Intensive “nanny-style” service near the center is easier to deliver than the same depth of service at the outer edge of the service radius. Some households may only want harvesting; others may want bundled operations; still others may care mainly about drying. Coordinating these different needs without overcomplicating management is a difficult but central task.

#### **7.4 Limited digital management capacity in rural areas**

Digital management is increasingly important, but many service centers still operate with relatively traditional management routines. This creates a gap between the complexity of the service chain and the tools available to manage it. A center must handle operation orders, machine schedules, seedling supply, drying queues, repair records, service billing, quality tracking, and sometimes subsidy-related reporting. Without stronger digital management, coordination becomes more difficult as service scale grows.

The recent literature on digital agricultural technology services shows that information channels, technology cognition, and practical accessibility strongly affect technology adoption and service effectiveness (Gong et al., 2024). Yet rural digitalization is uneven. Some farmers adapt quickly to online ordering or digital traceability; others do not. Some service staff can maintain digital records well; others remain more comfortable with phone calls and handwritten lists. As a result, digital capacity often develops more slowly than physical infrastructure.

For centers like Mashan, this is an operational constraint rather than a fashionable concern. Limited digital capacity can increase scheduling errors, reduce traceability, weaken service evaluation, and make regional coordination during peak periods more difficult than it needs to be. The problem is not the absence of advanced artificial intelligence. It is often the absence of stable, usable basic digital management.

#### **7.5 Increasing risks from extreme weather and climate change**

The final major problem is the increasing influence of extreme weather and climate change. Rice production is strongly exposed to heat stress, extreme rainfall, typhoons, and unstable harvesting periods. Modern service centers improve resilience, but they do not remove exposure. In fact, severe weather can test the limits of even a well-equipped center by creating simultaneous spikes in harvesting demand, drying demand, and temporary storage demand.

Recent climate research focused on Chinese rice production regions points toward intensifying extreme climate challenges under future scenarios, especially in relation to heat stress and changing risk patterns across growth stages (Chen et al., 2025). Broader review work on greenhouse gas and climate interactions in rice agriculture also reminds us that climate change is not only a long-term background issue; it is becoming a direct operational concern for rice systems (Qian et al., 2023).

Mashan’s emergency harvest case shows strong response capacity, but it also reveals how dependent regional rice security can become on rapid coordinated intervention. This means future service-center design must treat resilience as a normal design goal, not as an occasional extra function.

### **8 Optimization Strategies and Future Development Directions**

#### **8.1 Improving regional agricultural service coordination mechanisms**

The first improvement direction is stronger regional coordination. A service center is most effective when it is embedded in a stable service network rather than operating as an isolated provider reacting case by case. For Mashan, this means moving further toward a layered regional model: highly intensive nearby service, scheduled support across surrounding towns, and a clearly organized emergency-response layer for abnormal weather and compressed harvest periods.

This strategy is consistent with Zhejiang's policy emphasis on regional service networks and local service circles (People's Government of Zhejiang Province, 2024). It is also supported by the literature showing that agricultural socialized services work best when they improve operational continuity and reduce the fragmentation of production management (Cai et al., 2024; Li et al., 2024). In practical terms, coordination can be improved through seasonal service agreements with villages, more formal booking systems before peak seasons, better integration with local grain-production plans, and clearer emergency command arrangements for typhoon or heavy-rain periods.

The key is to strengthen the center's role as a regional operating hub. If service demand, machinery dispatch, drying needs, and emergency response are better synchronized at the area level, both farmers and the center gain predictability.

### **8.2 Promoting digital and smart agricultural service platforms**

The second direction is digitalization, but it should be practical rather than decorative. Not every service center needs highly advanced artificial intelligence systems immediately. What many centers need first are reliable digital tools for booking, scheduling, queue management, records, and traceability. These are the digital foundations on which more advanced functions can later be built.

Research on digital agricultural services indicates that such services can raise farmers' willingness to engage with new production technologies by expanding information access and improving technology understanding (Gong et al., 2024). For a service center, digitalization should begin with management pain points: operation orders, machine dispatch, farmer service histories, drying-batch monitoring, storage records, and product traceability. Once those basic layers are stable, more sophisticated functions such as predictive scheduling, weather-linked reminders, or data-assisted quality control can be added.

Mashan is well suited to this direction because its service functions are already diverse. The more activities a center integrates, the more valuable ordinary digital order in management becomes. A modest but well-designed digital platform can improve both service quality and internal efficiency far more than a poorly used "smart" system with weak operational relevance.

### **8.3 Strengthening agricultural talent training and technical guidance**

The third priority is talent development. Infrastructure and machinery can expand quickly; human capacity often cannot. For that reason, centers like Mashan would benefit from a more systematic training structure that distinguishes between different types of personnel: machine operators, repair technicians, dryer operators, agronomic field assistants, digital record managers, and future management staff.

This direction is strongly supported by both practice and research. Digital and green production services depend heavily on human capacity to translate tools into usable service. Studies on sustainable practice adoption and digital production willingness both underline the importance of training and technical assistance in reducing barriers to adoption (Huan et al., 2022; Gong et al., 2024). For a service center, training is also internally valuable because it improves standardization and reduces avoidable operational mistakes.

A useful model would be tiered training. Basic training should focus on safety, standard operating routines, and maintenance. Intermediate training should focus on agronomy-linked service quality and postharvest control. Advanced training should prepare younger staff and team leaders for coordination, digital management, and emergency organization. In the long run, talent may become the main difference between average service centers and exceptional ones.

### **8.4 Expanding integrated rice industry chains and brand development**

The fourth direction is deeper integration of the rice industry chain. Mashan has already moved beyond field services into drying, storage, processing, and branding. That path should be further strengthened because it increases the center's economic resilience and makes better use of postharvest infrastructure.

The logic is strong. If a service center only earns from field operations, its income may remain highly seasonal and vulnerable to competition. If it can also support value retention and local branding, it gains a second source of relevance. Recent research on grain cultivation and food security in China shows that rice production now has to be understood not only as a matter of output, but also of quality structure and stable supply conditions (Tang et al., 2022). A center that can connect standardized production with recognizable local rice products is better positioned for the future than one that remains only an operation contractor.

For Mashan, this means continuing to strengthen the chain from seedling to market: standardized seedling supply, coordinated operations, quality-conscious drying, safe storage, simple processing, local branding, and more stable market channels. The “Xinfeng” rice case suggests that this path is already realistic.

### **8.5 Enhancing emergency agricultural service capacity under climate risks**

Finally, future development should place more explicit emphasis on emergency service capacity under climate risk. The emergency harvesting case shows that Mashan already has a useful base, but climate pressure is likely to persist and perhaps intensify. That means resilience should be formalized rather than treated as an occasional success story.

Several practical steps follow from this. Seasonal emergency plans should be prepared before peak harvest. Machinery, dryers, fuel, and temporary storage arrangements should have reserve capacity. Coordination with township governments, village planners, and possibly agricultural insurance mechanisms should be strengthened. Weather information should be integrated more directly into early service planning. Training for emergency dispatch should be treated as part of ordinary center capability.

The real lesson of recent rice climate research is not just that risks are increasing, but that adaptation must be organized (Chen et al., 2025). Modern agricultural service centers are well placed to become that organizing mechanism at the regional level.

## **9 Conclusion**

This study examined the operational model of modern agricultural service centers in socialized rice production services through the case of Mashan Agricultural Service Center in Shangyu District, Zhejiang Province. The main argument is that the value of such centers lies less in the possession of machinery itself than in the organization of coordinated services. A modern agricultural service center works as a regional platform that links seedling raising, machinery operation, drying, storage, technical guidance, training, and sometimes branding into a connected production-support system.

The discussion shows that Mashan’s operational model has several defining features. It combines physical infrastructure with specialized teams rather than treating mechanization as mere equipment ownership. It operates through layered service radii, with both nearby intensive support and broader regional outreach. It pays serious attention to postharvest functions, which are often the true dividing line between partial and integrated service. And it increasingly connects production service with value-extension functions such as processing and branding.

The practical significance of this model is equally clear. Modern agricultural service centers can improve production efficiency, reduce labor pressure and coordination costs, strengthen emergency response under weather shock, support greener and more standardized rice production, and make it easier for smallholder-based production systems to remain linked with modern agricultural development. In developed provinces such as Zhejiang, where agriculture must often compete through quality, organization, and service rather than through simple expansion of scale, these functions are especially important.

At the same time, the Mashan case also shows that this model is not without pressure. Capital costs remain high. Skilled technical staff are hard to build and keep. Farmers are heterogeneous in demand and capacity. Digital management is still uneven. Climate risk is rising. These are not temporary inconveniences; they are structural issues that will shape whether service centers continue to function well over time.

For that reason, the future direction of modern agricultural service centers should emphasize stronger regional coordination, practical digital management, systematic talent training, wider integration of postharvest and branding functions, and more formalized emergency-service preparation. If these directions are pursued steadily, modern agricultural service centers can become one of the most realistic institutional paths for supporting a rice sector that is efficient, resilient, quality-oriented, and still compatible with the continued presence of large numbers of small-scale farmers.

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## Research Insight

## Open Access

# Application Performance and Promotion Value of Qianjiang 661 in Rice-Rapeseed Rotation Systems

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**Abstract** Rice-rapeseed rotation is one of the most important double-cropping arrangements in the middle and lower reaches of the Yangtze River. It links summer grain production with winter oilseed supply, raises land-use intensity, reduces seasonal fallow, and supports the broader goal of improving domestic edible-oil security. Against the backdrop of labor shortages, tighter sowing windows after late rice harvest, and the continuing push toward mechanized cultivation, the performance of a rapeseed cultivar in this system can no longer be judged by yield alone. Growth duration, stand establishment under paddy-field conditions, maturity uniformity, lodging behavior, and compatibility with mechanized sowing and harvesting have become equally important. This review examines the application value of Qianjiang 661 as a practical rapeseed cultivar for rice-rapeseed rotation systems. Because publicly indexed, variety-specific peer-reviewed data are still limited, the paper integrates three levels of evidence: general research on rice-rapeseed rotation and sustainable crop diversification, publicly accessible reports on rapeseed production and agricultural sustainability, and the case materials specified for this review, including enterprise demonstration information from Longyou Wuguxiang Seed Industry Co., Ltd. and Zhejiang extension-oriented demonstration descriptions. On this basis, the paper evaluates the agronomic fit of Qianjiang 661, its likely contribution to yield formation and system efficiency, and its economic, ecological, and regional strategic significance. The review concludes that the chief value of Qianjiang 661 lies in system fit rather than a single extreme trait. Its promotion value appears strongest in humid eastern rice regions where moderate earliness, stable field performance, and mechanization compatibility matter as much as peak yield. At the same time, broader independent validation, clearer variety-specific cultivation packages, and more transparent multi-location evidence remain necessary before making stronger claims about its regional generalization.

**Keywords** Qianjiang 661; Rice-rapeseed rotation; Rapeseed production; Agricultural sustainability; Promotion value

## 1 Introduction

Rice-rapeseed rotation has long occupied a strategic place in Chinese agriculture because it ties together staple grain production, winter land use, domestic vegetable-oil supply, and the ecological management of farmland in the Yangtze River Basin. In practical terms, the system matters because it enables one field to support both a summer rice crop and a winter oilseed crop within the same annual cycle. In policy terms, it matters because rapeseed remains one of China's major domestically produced oilseed crops, while edible-oil demand continues to place pressure on national supply chains. More broadly, rotation-based intensification is now discussed not simply as a way to produce more per hectare, but as a way to improve the ecological and economic performance of farmland without relying only on expansion or higher external inputs (Godfray et al., 2010; Tilman et al., 2011; Pretty, 2018). Yet the rice-rapeseed rotation system is not automatically efficient. It is constrained by short turnaround time after rice harvest, wet and compacted paddy soils, increasingly scarce labor, and a growing reliance on mechanized sowing and harvesting. These constraints change what breeders and extension workers look for in a rapeseed cultivar. Moderate earliness, strong establishment after rice, reasonably compact and harvestable plant architecture, and synchronized maturity can be just as decisive as theoretical yield potential. The modern discussion therefore moves beyond “high yield” as a single criterion and asks whether a cultivar fits the whole annual production schedule (Lin, 2011; Bommarco et al., 2013; Gurr et al., 2016).

Qianjiang 661 is discussed in this paper from that system-based perspective. A methodological caution is necessary at the outset: compared with nationally prominent rapeseed cultivars that are frequently discussed in indexed papers, the publicly visible, variety-specific documentation for Qianjiang 661 is still limited, especially in English. For that reason, this article does not manufacture numeric superiority claims that cannot be traced. Instead, it uses a review approach that combines broader literature on rice-rapeseed rotation with the case sources specified for this manuscript—especially enterprise demonstration information from Longyou Wuguxiang Seed Industry Co., Ltd. and Zhejiang-oriented demonstration descriptions—to assess the plausible application performance and promotion value of the cultivar under real production conditions. The objective is not to oversell the variety, but to place it accurately within the agronomic and strategic logic of rice-rapeseed rotation in eastern China.

## **2 Development and Significance of Rice-Rapeseed Rotation Systems**

### **2.1 Current development of rice-rapeseed rotation in china**

In China, the agronomic importance of rice-based double-cropping systems rests on geography as much as on policy. The middle and lower Yangtze region combines a warm climate, sufficient moisture, paddy-based farming traditions, and a long enough frost-free season to support rice in summer and rapeseed in winter. This makes the rice-rapeseed sequence especially valuable in provinces such as Hubei, Hunan, Jiangxi, Anhui, Jiangsu, and Zhejiang, where winter fallow represents not only a missed production opportunity but also a missed opportunity for strengthening regional oilseed supply. At the national level, China remains one of the world's leading producers of rapeseed and rapeseed oil, and rapeseed continues to hold a special place in the domestic edible-oil structure even as soybeans dominate the import landscape.

The system has become even more significant because current agricultural strategy increasingly values intensification that works through better system design. The key question is no longer how to increase output through a single crop in isolation, but how to redesign the annual cropping calendar so that land, labor, and seasonal climate are used more effectively. This is exactly the kind of problem addressed in the broader literature on sustainable intensification and crop diversification. Diverse crop sequences often improve ecological functioning, maintain productivity, and reduce some of the vulnerabilities associated with simplified systems. In Chinese rice regions, rapeseed is especially attractive in this role because it occupies the winter gap between rice seasons while contributing to domestic oilseed output (Pretty, 2008; Gurr et al., 2016; Pretty, 2018).

At the same time, the system is under pressure. Delayed rice harvest can narrow the sowing window for rapeseed. Paddy soils may remain wet when field turnover should already be underway. Labor shortages make late, labor-intensive field operations more expensive and less reliable. These changes are pushing rotation systems toward varieties and management packages that are less sensitive to operational delay and more compatible with mechanized production. In that sense, the development of rice-rapeseed rotation in China is not only a question of area, but of technical fit and operational robustness.

### **2.2 Advantages of rice-rapeseed rotation for farmland utilization**

The first and most obvious advantage of rice-rapeseed rotation is that it raises annual land-use efficiency. A field that would otherwise remain idle after rice harvest can continue to produce value during the winter season. This is important in a country where cultivated land is finite and where stable output increasingly depends on making better use of existing farmland rather than adding new land. FAO data show both the global importance of efficient cropland use and the continuing pressure on agricultural land systems; in this context, cropping intensity matters as much as land area itself.

The second advantage is system complementarity. Rice and rapeseed differ in growth season, canopy structure, residue type, nutrient demand pattern, and pest-pathogen spectrum. This does not eliminate production problems, but it can help reduce the ecological rigidity associated with repeated single-crop use of the same land. More generally, crop diversification is often associated with improved ecological intensification because it can strengthen ecosystem services, improve resilience, and distribute production risk across seasons (Lin, 2011; Bommarco et al., 2013; Gurr et al., 2016). In practice, a winter rapeseed crop also means that paddy fields are not

left bare for months, which can reduce erosion risk, support nutrient capture, and improve visual and functional use of farmland in winter.

The third advantage is strategic. Rapeseed rotation in rice areas contributes directly to regional oilseed supply and indirectly to national oil security. China is a major producer of rapeseed oil, but it is also exposed to broader pressures in global edible-oil trade. Under such conditions, domestically integrated oilseed systems become more valuable than their field-scale economics alone might suggest. A hectare of winter rapeseed in a rice region is not just a secondary crop; it is part of a distributed buffer for domestic supply.

### **2.3 Demand for improved rapeseed varieties in rotation systems**

The logic of variety demand in rice-rapeseed systems is straightforward: a rapeseed cultivar must fit the calendar that rice leaves behind. That means it must establish quickly after rice harvest, tolerate the physical legacy of paddy cultivation, survive winter conditions, flower and mature in time for spring harvest, and ideally do so with a plant architecture that is not too difficult to manage mechanically. In other words, breeders are solving a systems problem, not only a single-season yield problem.

This requirement immediately changes the trait portfolio. In rotation systems, breeders and growers emphasize moderate growth duration, stable early vigor, synchronized maturity, lodging resistance, reasonable first-branch/first-pod height for machine harvest, and consistent expression under late sowing. These are classic “fit” traits. They may not produce spectacular headlines, but they often decide whether a variety is actually adopted at scale. The same broader logic appears in studies of sustainable agriculture and land-sparing versus land-sharing debates: system performance depends on how production, ecology, and management constraints are integrated, not on a single maximal trait value (Green et al., 2005; Phalan et al., 2011; Pretty, 2018).

A further point deserves emphasis. In rice-rapeseed areas, variety improvement is increasingly connected to mechanization. A cultivar that yields well in small-plot trials but matures unevenly, lodges badly, or produces excessive harvest loss under machine conditions is unlikely to satisfy commercial farms or service contractors. This is why the demand for improved varieties in rotation systems has shifted toward practical agronomic reliability. Qianjiang 661 should be evaluated in that light.

## **3 Biological and Agronomic Characteristics of Qianjiang 661**

### **3.1 Growth duration and maturity characteristics**

Publicly indexed, variety-specific descriptor sheets for Qianjiang 661 remain scarce, so caution is needed. On the basis of the case materials specified for this review, Qianjiang 661 is treated as a rapeseed cultivar designed for practical deployment in rice-rapeseed rotation environments, especially in Zhejiang and comparable eastern rice areas. Within that production logic, its most important biological feature is not absolute earliness in a vacuum, but maturity that is early enough to fit the annual calendar without losing the biomass and reproductive development needed for commercial seed yield.

This distinction matters. A cultivar can be too late for the next rice crop, but it can also be so early that it loses winter growth potential and seed-yield expression. The agronomic value of a rotation cultivar therefore lies in calibrated duration. Evidence from rapeseed adaptation studies shows that climate and growing season strongly shape where a cultivar remains suitable, and climate change itself may shift the geographical range of rapeseed production (Jaime et al., 2018). In farming terms, then, growth duration is a system trait. A suitable duration means more than “short duration”; it means a duration that fits regional operational reality.

In the materials used for this review, Qianjiang 661 is repeatedly associated with favorable schedule matching in local rice-rapeseed systems. Even without a fully documented public descriptor package, that pattern supports a cautious but useful conclusion: the cultivar’s maturity timing is one of its main arguments for adoption. For a rotation system, that alone is a substantial advantage.

### **3.2 Plant architecture and yield-related traits**

Plant architecture is where variety performance becomes visible to growers. In rapeseed, architecture shapes light interception, lodging behavior, machine harvestability, and ultimately the reliability with which yield components

are translated into harvested seed. A practical rotation cultivar generally needs a stand that is neither too sparse nor excessively sprawling, with adequate branching, sufficient pod-bearing potential, and a canopy that does not collapse under humid spring conditions.

The source materials used for this review describe Qianjiang 661 as having relatively good field uniformity and favorable population growth under production conditions (Figure 1). In a review paper, these descriptions should not be overstated as if they were a fully replicated multi-environment dataset. Still, they do align with the kind of plant architecture that growers need in rice-based systems. Uniform plants help synchronize flowering and maturity; synchronized maturity reduces harvest loss; and manageable architecture reduces the friction between biological potential and mechanical implementation.



Figure 1 Field population performance of Qianjiang 661 at the pod-filling and maturity stage in a rice-rapeseed rotation production environment (Photoed by Geyang Zhan)

From a yield-formation standpoint, rapeseed yield depends on stand establishment, branches or pod-bearing sites, siliques per plant or per unit area, seeds per silique, and seed weight. Under rice-rapeseed rotation, the most vulnerable steps are often the early ones: poor establishment after rice can limit canopy recovery and yield long before flowering begins. A cultivar that protects stand quality under these conditions can therefore create a very real yield advantage even if its intrinsic single-plant productivity is not exceptional.

### 3.3 Adaptability to rice-rapeseed rotation calendars

Adaptability in this context means more than climatic adaptation. It means calendar adaptation, soil adaptation, and management adaptation at the same time. The rice-rapeseed interface is often the most difficult stage of the annual production cycle. Rice harvest can be delayed by weather. Straw and post-harvest residue can interfere with seedbed preparation. Paddy fields may need drainage before rapeseed sowing. Under these conditions, a variety's adaptability is tested by how well it tolerates imperfect sowing windows and field turnover.

The practical case for Qianjiang 661, as it emerges from the review materials, lies largely here. The cultivar is positioned not as a laboratory curiosity or a showcase genotype, but as a field-fit variety that can be integrated into the local rice-rapeseed sequence with relatively little disturbance to the annual plan. That is a meaningful trait in eastern China, where the difference between a promotable cultivar and a merely acceptable one often lies in how well it survives real-world scheduling pressure.



### **3.4 Suitability for mechanized cultivation**

Mechanized cultivation has become one of the clearest filters in modern variety evaluation. For rapeseed in rotation with rice, mechanization includes not only combine harvest but also sowing, drainage coordination, residue management, and the possibility of large-scale, service-based operations. A variety suited to mechanization typically needs reasonably consistent emergence, a canopy architecture that does not collapse easily, synchronized ripening, and a harvest window that is not excessively narrow.

The enterprise and demonstration descriptions associated with Qianjiang 661 present it as compatible with mechanized production conditions. That wording should be interpreted carefully. It does not mean that the variety solves all machine-harvest problems or that it has been publicly benchmarked against every competing cultivar across regions. It means that, in the demonstration contexts cited for this review, it has shown the kind of performance that makes mechanized production feasible and commercially relevant.

This is an important distinction because mechanization is not just a labor-saving convenience. In many parts of eastern China, it has become the condition for maintaining winter rapeseed at all. Varieties that cannot enter mechanized systems risk remaining marginal regardless of their theoretical potential. In that respect, the reported mechanization fit of Qianjiang 661 is one of its strongest practical selling points.

## **4 Application Effects of Qianjiang 661 in Rice-Rapeseed Rotation Systems**

### **4.1 Effects on rapeseed yield formation**

Yield in rice-rapeseed systems is best understood as an outcome of fit between genotype, calendar, and field conditions. In such systems, yield loss often begins before the crop's reproductive phase. Late sowing, poor drainage, uneven seedling establishment, and soil structural problems inherited from paddy management can all reduce the number of effective plants and productive branches. That is why the practical effect of a variety like Qianjiang 661 should be evaluated through the whole sequence of yield formation rather than only through final seed weight per hectare.

The evidence base used in this review suggests that Qianjiang 661 performs well in terms of population uniformity and synchronized maturity under production conditions. Those observations matter because uniform populations generally support more even canopy development and more coherent reproductive progression. In a mechanized farming environment, they also reduce the mismatch between biological maturity and harvesting time. Put plainly, a crop that grows together is more likely to finish together and be harvested efficiently.

Because public multi-location yield tables for Qianjiang 661 are not widely available in indexed literature, this paper avoids inventing a numeric yield ranking. The more defensible conclusion is qualitative: Qianjiang 661 appears to support yield formation by protecting the field-level conditions that matter most in rice-rapeseed systems—namely establishment quality, uniform stand development, and operationally manageable maturity.

### **4.2 Effects on cropping system efficiency**

Cropping system efficiency is wider than rapeseed yield alone. It includes how much land time is productively occupied, how smoothly one crop hands over to the next, and how much management friction accumulates across the year. In this sense, a rapeseed cultivar can improve a system even when its individual yield advantage is moderate, provided it reduces delays and uncertainty in the annual sequence.

For Qianjiang 661, the main efficiency effect likely lies in reducing schedule conflict. A rotation-compatible rapeseed crop helps convert the post-rice period into productive winter use without imposing a late spring penalty on the next rice crop. This is a classic example of system value rather than single-crop value. It reflects the insight from sustainable intensification research that production gains can come from redesigning the use of time, land, and ecological processes, not only from increasing input intensity (Gurr et al., 2016; Pretty, 2018).

### **4.3 Effects on farmland resource utilization**

Winter rapeseed can improve farmland resource utilization in at least three ways. First, it uses solar radiation, water, and residual soil nutrients during a season that might otherwise remain underused. Second, it maintains a



vegetative cover over the field, reducing the ecological cost of leaving paddy land bare through the winter. Third, it increases the output generated per unit of cultivated land over the yearly cycle.

These advantages are especially important in regions where farmland is limited and where annual productivity depends more on multiple cropping than on land expansion. FAO reporting on land use reinforces this broader point: agricultural sustainability is increasingly a question of how existing cropland is used through time, not merely how much cropland exists. For Qianjiang 661, the implication is practical rather than abstract. If the cultivar can be sown after rice, establish reliably under paddy-derived conditions, and mature in time for the next rice season, then it directly improves winter resource capture on the same land base. This is one reason its promotion value should be interpreted at the system level.

#### 4.4 Performance under different ecological conditions

No responsible review should imply that one cultivar performs identically across all ecological zones unless strong multi-environment evidence exists. For Qianjiang 661, that level of public evidence is not yet widely visible. The strongest application case at present is therefore regional rather than national: Zhejiang and comparable eastern rice areas with humid conditions, established rice-based annual calendars, and a growing need for mechanized winter rapeseed cultivation.

This does not mean the cultivar has no wider potential. It means the present evidence is strongest where the demonstration network is strongest. In applied agronomy, that is a common situation. Enterprise and extension materials often accumulate first in the regions where commercialization begins, and only later does a broader independent evidence base develop. For that reason, the most scientifically careful position is that Qianjiang 661 appears promising under eastern Chinese rice-rapeseed conditions, but broader ecological generalization still requires more systematic public validation (Figure 2).



Figure 2 Mechanism by which Qianjiang 661 contributes to rice-rapeseed rotation system performance

## 5 Economic, Ecological and Social Benefits of Qianjiang 661

### 5.1 Contribution to farmers' income

For farmers, the most immediate question is simple: does winter rapeseed add dependable value after rice? In many rice regions, the answer depends less on a spectacular rapeseed yield ceiling than on whether the crop can be planted on time, harvested with manageable cost, and sold without forcing major disruptions in the next rice cycle. A practical cultivar therefore contributes to income not only through seed output, but through lowering operational risk.

Qianjiang 661 appears to fit this income logic. The variety's reported value lies in stable field performance, compatibility with local rotation calendars, and suitability for large-scale or service-based operations. These features matter because they protect the realizable income of the farm. A cultivar that is biologically promising but too risky to manage under labor scarcity or tight seasonal timing may underperform economically even when its plot potential looks strong.

Income diversification also matters. Rotation with rapeseed spreads production across seasons and products, softening the dependence of farm cash flow on a single crop. In production systems where rice remains the main anchor crop, a competent winter rapeseed cultivar can serve as an additive profit source rather than a competitor.

### 5.2 Contribution to winter farmland utilization

The economic and agronomic significance of winter land use is often underestimated because it is easy to focus only on the main summer crop. Yet winter utilization is one of the clearest ways to improve the annual productivity of a field. In regions suitable for double cropping, leaving land idle after rice means accepting both a missed production opportunity and a lower annual return on land, machinery, and management capacity.

Qianjiang 661 contributes to winter utilization if it can be deployed without requiring excessively complex field preparation or labor-intensive management. In that regard, its promotion value comes from being operationally usable, not just biologically interesting. For local governments and extension services, this matters because winter land-use improvement is often a policy objective tied to food and oilseed security as well as to rural efficiency.

### 5.3 Ecological benefits in rotation systems

The ecological benefits of rice-rapeseed rotation do not mean the system is automatically low-input or problem-free. Rather, compared with prolonged winter fallow or overly simplified annual sequences, it can improve the ecological functioning of farmland. Crop diversification is widely linked to ecological intensification, especially where system redesign allows farms to make better use of biological processes and temporal complementarity (Bommarco et al., 2013; Gurr et al., 2016). In practical terms, winter rapeseed cover can reduce the bare-soil period, help capture residual nutrients, and contribute residues that differ from those produced by rice. These processes do not automatically guarantee higher soil fertility, but they can strengthen the ecological basis of annual production. More broadly, research on agricultural sustainability repeatedly shows that diversified systems are often more resilient than simplified ones, especially under environmental stress and management uncertainty (Pretty, 2008; Lin, 2011).

For Qianjiang 661, the ecological value is therefore inseparable from system fit. A rapeseed cultivar that fails under local conditions cannot deliver ecological benefits at scale. A cultivar that establishes reliably and is actually planted can.

### 5.4 Contribution to regional oilseed security

Regional oilseed security is an easily overlooked but important dimension of rotation systems. Rapeseed is not just another winter crop in China; it is one of the few large-scale edible-oil crops that can be widely integrated into existing paddy landscapes in the Yangtze region. That gives it policy significance beyond farm-level profit. China remains a major rapeseed and rapeseed-oil producer, and the stability of domestic oilseed production continues to matter in a global market marked by trade volatility and structural import dependence in vegetable oils.

A variety such as Qianjiang 661 contributes to this objective when it makes rapeseed production easier to maintain in real rice-farming environments. The contribution may look small at plot scale, but it becomes more meaningful when repeated across many winter fields in a region. That is precisely how rotation-based oilseed security works: through distributed, seasonally integrated production rather than through a small number of specialized large plantations.

## 6 Typical Application Cases of Qianjiang 661

### 6.1 Demonstration application at Longyou Wuguxiang Seed Industry Co., Ltd.

According to the demonstration description supplied for this manuscript, Qianjiang 661 was planted on a relatively large scale at the enterprise demonstration base and integrated into the local rice-rapeseed rotation sequence (Figure 3). The key observations emphasized in those materials are not flashy but practical: uniform stand development, reasonably strong growth vigor, orderly field phenotype, and synchronized maturity. These are exactly the traits that matter when a rotation cultivar is moved from breeding logic into commercial operation. From a scientific writing perspective, the importance of this case lies less in isolated visual appeal and more in the fact that enterprise-based demonstrations expose a cultivar to the operational conditions that determine adoption-field turnover, seed multiplication, extension communication, and commercial confidence.

The enterprise case is also important because it shows how seed companies function as bridges between breeding outcome and field uptake. A good cultivar without multiplication capacity, field service, and demonstration support may remain marginal. By contrast, a cultivar embedded in an active seed enterprise has a much higher chance of entering real farm decision-making.

Enterprise demonstration programs are often underestimated in scholarly discussion, but they are crucial for variety diffusion. They test whether a cultivar can move through seed multiplication, field service, grower communication, and mechanized operation at the same time. In that sense, the Longyou Wuguxiang case supports the argument that the promotion value of Qianjiang 661 depends not only on agronomy but also on the institutional platform behind it.



Figure 3 Demonstration planting of Qianjiang 661 at Longyou Wuguxiang Seed Industry Co., Ltd. during the flowering stage (Photoed by Geyang Zhan)

### 6.2 Regional demonstration case in Zhejiang Province

The regional Zhejiang case emphasizes adaptability more than novelty. Across multiple demonstration contexts, Qianjiang 661 is described as matching local rice-rapeseed schedules and maintaining stable field performance. The significance of this case is that it shifts the variety from enterprise-centered observation into a broader extension environment. Once a cultivar performs well at more than one demonstration point, the conversation changes from “can it work?” to “under what conditions should it be promoted?”

This matters particularly in Zhejiang, where paddy-based agriculture, fragmented farm structure in some areas, and the expansion of mechanized service systems make practical fit especially important. Regional demonstration therefore functions as a form of applied screening, separating varieties that are merely promising from those that are operationally promotable.

Regional demonstrations provide field-based evidence for extension workers, growers, and seed suppliers. For Qianjiang 661, the main implication is that its value appears strongest in places where the cropping calendar is tight and local production benefits from a rapeseed cultivar with moderate earliness and manageable field behavior.

### **6.3 Mechanized production application case**

The mechanized production case focuses on whether Qianjiang 661 performs adequately under machine-oriented sowing and harvesting conditions. In practical terms, this means the variety must show sufficiently even emergence, acceptable canopy behavior, and synchronized maturity. The review materials identify these as positive features for Qianjiang 661. That does not eliminate the need for optimized local machinery settings and cultivation techniques, but it does suggest that the cultivar belongs to the category of varieties that can enter commercial mechanized systems without obvious structural mismatch.

Compatibility with mechanized production increases commercial value for three reasons. It lowers labor dependence, improves the chances of scaling up, and makes the cultivar more attractive to service-based agriculture. In current eastern Chinese rapeseed production, these are not peripheral advantages; they are central to whether winter rapeseed remains viable as a widespread crop.

## **7 Constraints and Future Development Directions**

### **7.1 Existing limitations in production application**

The most important limitation in the current evaluation of Qianjiang 661 is the evidence base itself. Publicly visible, independent, variety-specific agronomic data remain limited in indexed literature. As a result, the present review can say with more confidence that Qianjiang 661 is promising under certain applied conditions than that it is definitively superior across broad ecological zones. This is not a flaw in the cultivar; it is a limit in public documentation.

A second limitation is the common gap between demonstration success and broad deployment. A cultivar may perform well in carefully managed enterprise or extension plots but show more variable results under heterogeneous farm management. That is particularly true in rotation systems, where drainage conditions, residue handling, sowing timeliness, and machinery quality can differ sharply from place to place.

A third limitation concerns trait transparency. Public discussion of Qianjiang 661 would be stronger if more standardized information were available on oil content, disease reaction, lodging resistance, optimal density range, and performance under delayed sowing. Without that level of disclosure, promotion arguments remain more qualitative than many growers and researchers would prefer.

### **7.2 Optimization of cultivation techniques**

Even a promising cultivar needs a well-matched cultivation package. In rice-rapeseed systems, the first priority is field turnover after rice. Timely drainage, residue handling, and realistic sowing windows likely determine far more of the final outcome than marginal adjustments later in the season. For any cultivar promoted in paddy-derived winter environments, establishment management is therefore central.

The second priority is density and nutrition management. A mechanization-friendly rapeseed stand needs enough population to compensate for uneven emergence, but not so much that canopy overcrowding increases lodging risk or complicates harvest. Balanced fertilization is equally important, especially where high-output breeding has changed nutrient sensitivity patterns. The well-known history of sulfur deficiency symptoms in modern rapeseed underscores how variety improvement and nutrient management can interact in unexpected ways (Schnug and Haneklaus, 2005). The third priority is harvest loss control. In commercial fields, the effective value of a variety is



strongly influenced by how much seed reaches the bin rather than how much biological yield is formed before harvest. This makes synchrony of maturity, pod shatter management, and machine adjustment part of the variety's practical cultivation package.

### **7.3 Future improvement of variety performance**

Future breeding priorities for cultivars like Qianjiang 661 are relatively clear even without exhaustive current data. First, stronger stability under delayed sowing would be especially valuable in rice-based systems, where the rapeseed season often begins under time pressure. Second, improved lodging resistance and a more machine-friendly canopy structure would further reduce operational loss. Third, clearer advances in oil quality and disease robustness would strengthen both market value and agronomic confidence.

It is also likely that future improvement will increasingly connect conventional field selection with molecular breeding tools. In rapeseed more broadly, genomic and SNP-based resources have already become important in breeding research and cultivar development. Even so, the practical target in a system like rice-rapeseed rotation remains the same: a variety that behaves reliably under operational constraints, not merely one that performs impressively in a controlled setting.

### **7.4 Future development of rice-rapeseed rotation systems**

The future of rice-rapeseed rotation will depend on system integration. Variety improvement alone is not enough. Successful large-scale rotation requires aligned sowing and harvesting machinery, better drainage and field-turnover logistics, region-specific extension guidance, and seed systems capable of maintaining varietal purity while scaling supply.

The broader literature on sustainable agriculture suggests the same conclusion in more general terms: crop diversification and sustainable intensification work best when system elements are redesigned together rather than adjusted piecemeal (Gurr et al., 2016; Pretty, 2018). In the Chinese context, this means that the future of winter rapeseed in rice areas will likely be shaped by how well breeding, mechanization, extension, and regional policy are coordinated.

## **8 Conclusions and Perspectives**

### **8.1 Main application advantages of Qianjiang 661**

Qianjiang 661 can be understood as a practical rapeseed cultivar whose main value lies in agronomic balance and production fit rather than in any single extreme trait. Based on the evidence assembled in this review, its most important advantages are its apparent compatibility with rice-rapeseed calendars, its orderly field phenotype, its relevance to mechanized cultivation, and its usefulness in converting winter land from fallow to productive use.

### **8.2 Overall evaluation of promotion value**

The promotion value of Qianjiang 661 appears strongest where rotation systems are tight, mechanization is increasingly necessary, and farmers need a cultivar that is dependable rather than merely impressive in isolated trials. The present evidence supports a positive but cautious evaluation. The variety looks commercially meaningful in Zhejiang and similar eastern Chinese rice areas, but the current public record is still stronger for applied demonstration than for broad independent comparative proof.

### **8.3 Future prospects for large-scale application**

The future prospects of Qianjiang 661 are therefore promising under a realistic condition: promotion should continue to be matched with region-specific management guidance, stronger multi-location validation, and transparent performance data. If these elements develop together, the variety could become a useful component of rice-rapeseed rotation systems that aim to raise winter land-use efficiency, support farmers' income, and contribute to regional oilseed security.

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## Research Report

## Open Access

## Tea Seed Oil Restores Blood Pressure, Redox Balance and Lipid Homeostasis in L-NAME-Induced Hypertensive Rats

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**Background:** Hypertension remains a leading cause of cardiovascular disease and mortality worldwide. This study explores the potential of tea seed oil (TSO) in mitigating hypertension-related complications in a rat model induced with N(G)-nitro-L-arginine methyl ester (L-NAME). TSO was extracted following standard protocols, and experimental groups (A - E) were administered L-NAME along with varying doses of TSO or Enalapril Maleate (as a positive control) for 28 days. Blood pressure indices were measured non-invasively, and blood and tissue samples were collected for biochemical analyses.

**Results:** Groups B and E showed significant changes in systolic blood pressure (SBP) compared to the control (Group A). Additionally, SBP decreased notably in groups C, D, and E compared to hypertensive group B. The administration of TSO at the highest dose (0.6 ml/kg) caused a reduction in diastolic blood pressure (DBP) in group D, similar to the effect of enalapril maleate in group E. Group B, subjected only to L-NAME administration, exhibited notable increases in LDL-C. Liver function markers like AST, ALT, and ALP showed significant changes across groups, with TSO administration leading to reductions in AST in groups C and D, and in group E compared to group B. Group B rats showed a significant rise in malondialdehyde (MDA) compared to Group A. TSO administration in Groups C and D slightly reduced MDA towards Group A levels. In Group E, MDA significantly differed from both groups A and B. Catalase (CAT) decreased significantly in group B but remained unchanged in groups C, D, and E compared to A or B. Superoxide dismutase (SOD) decreased significantly in group B compared to A but increased in C, D, and E compared to B. Glutathione peroxidase (GPx) decreased in groups B and C compared to A but increased in C compared to B. Groups D and E showed no significant difference in GPx values compared to either A or B. Reduced glutathione (GSH) increased significantly in TSO-treated Groups C and D and in the standard drug group compared to A or B.

**Conclusions:** These findings highlight the therapeutic potential of TSO as a natural adjunct in the management of hypertension and its related complications.

**Keywords** Hypertension; Blood Pressure; Tea seed oil; Oxidative stress; Antioxidant enzymes

### 1 Background

Hypertension remains the leading risk factor for cardiovascular diseases, and its annihilative effect is felt in the form of reduced life expectancy and premature death. The escalating global incidence of hypertension is linked to factors such as excessive salt intake, physical inactivity, tobacco and alcohol consumption, and the aging process (Wang et al., 2023). An individual is considered hypertensive if their systolic blood pressure (SBP) reaches 130 mm Hg or higher and/or if their diastolic blood pressure (DBP) exceeds 80 mm Hg (Muntner et al., 2019).

In developed nations, hypertension ranks as the fourth contributor to premature deaths and seventh in developing countries. Globally, hypertension is responsible for nearly 16.5% of annual deaths, with projections estimating that by 2030, 23.5 million people will succumb to hypertension-related complications (Kibret and Mesfin, 2015; Oliveira et al., 2021). The prevalence of hypertension in Africa is particularly alarming, with current projections indicating that 150 million people will be affected by 2025, with Nigeria alone accounting for 40 million of this estimate (Akindele, 2014).

Throughout history, plants have been explored as an indispensable source of medicine due to their easy affordability and lack of common side effects associated with synthetic drugs (Parasuraman, 2018). Tea (*Camellia*

*sinensis*), native to China, stands out as one of the most widely consumed beverages globally. In terms of chemical composition, it contains polyphenols, alkaloids, amino acids, polysaccharides, lipids, proteins, vitamins and trace elements (Wang et al., 2022). Notably, polyphenols, particularly flavonoids and catechins, which make up approximately 33% of tea's composition, have been consistently highlighted in various studies as the primary contributors to its significant health benefits (Khan and Mukhtar, 2018). Several studies have demonstrated the diverse therapeutic potential of tea, including its ability to restore sperm quality in heat-exposed mice (Mahmoudi et al., 2018), its dual anti- $\beta$ -secretase and anti-cholinesterase activities relevant for dementia treatment (Suttisansanee et al., 2019), and its protective role in preventing DNA oxidative damage and inhibiting colorectal cancer cell proliferation (Wang et al., 2020).

This study aims to explore the potential therapeutic effects of tea seed oil (TSO) in modulating blood pressure, oxidative stress, and lipid profiles in an L-NAME-induced hypertensive rat model.

## 2 Methods

### 2.1 Plant material and sample preparation

Tea seeds were gathered from the Mambilla substation, Cocoa Research Institute of Nigeria (CRIN), Kusuku, Taraba state, Nigeria. The seeds were thoroughly cleaned under running tap water for 3 minutes to eliminate any adhering dirt. Subsequently, the seeds were sun-dried until a consistent weight was achieved, mechanically crushed, and ground to increase the surface area. A 100 g sample of the finely ground tea seeds was introduced into the thimble of a Soxhlet apparatus and then extracted with 500 ml of n-hexane for 8 hours, following the AOCS guidelines (Irving, 1958). After the extraction, the solvent was eliminated through rotary evaporation at 60°C under a nitrogen stream. The resulting TSO extract was then oven-dried until a solvent free extract was achieved.

### 2.2 Drugs and chemicals

Enalapril Maleate and NG-nitro-L-arginine methyl ester (L-NAME) were obtained from Honeywell Research Chemicals (Morris Plains, New Jersey). All other chemicals used were obtained from local suppliers and were of analytical grade.

### 2.3 Animals and experimental design

A total of forty (40) male Wistar albino rats, with weights ranging from 140 to 200 g, were sourced from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. All experimental protocols were carried out in accordance with the guidelines approved by the Animal Care and Use Research Ethics Committee (ACUREC), University of Ibadan, Nigeria. These rats were accommodated in adequately ventilated plastic cages and allowed to acclimatize for 14 days prior to the initiation of the experiment. Throughout this acclimatization phase, the rats were provided with commercial rat feed and had unrestricted access to water.

The rats were distributed into five (5) groups of eight rats each. Group A, designated as the control, received 5 ml/kg of normal saline. Groups B-E were subjected to oral administration of 40 mg/kg L-NAME (Metchi Donfack et al., 2021) once daily. Furthermore, animals in groups C and D were concurrently administered 0.45 and 0.6 ml/kg of TSO, respectively, while group E received 2mg/kg Enalapril Maleate (Tawfeek et al., 2018) for a duration of 28 days.

### 2.4 Blood pressure measurement

Before blood pressure measurements, each animal was placed in a restraining holder for 10-15 minutes to allow for proper acclimatization. Systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) were non-invasively assessed using tail-cuff plethysmography with an electrospigmomanometer (CODA, Kent Scientific, USA). At least nine readings were taken per animal, and the average was calculated.

### 2.5 Blood and tissue samples collection

Following an overnight fasting period, the rats were euthanized using cervical dislocation. Blood was then obtained from each rat via cardiac puncture and collected in sterile plain tubes. The collected blood samples were

allowed to clot and subsequently centrifuged at 4,000 rpm for 10 minutes. The resulting serum was meticulously separated into another sterile plain tube and stored at 4°C until required. The heart and liver of each rat were then excised, rinsed with a saline solution at a low temperature. The tissues were immersed in liquid nitrogen and promptly preserved at -80°C for subsequent analyses.

## 2.6 Measurement of lipid profile

The collected sera were used to assess various lipid components, including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The quantification of TC and TGs followed the methods of (Roeschlau et al., 1974), and (Biggs et al., 1975), respectively. The HDL-C level was determined using the method outlined by (Warnick et al., 2001), while the estimation of LDL-C employed the Friedewald formula (Krishnaveni and Gowda, 2015):

$$\text{LDL-C} = (\text{TC}) - (\text{HDL-C}) - (\text{TGs}/5)$$

## 2.7 Estimation of liver function indices

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed following the procedure outlined by (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) activity was determined using the method described by (Babson et al., 1966). Total protein (TP) was quantified using the biuret reaction, as described by (Linne and Ringsurd, 1979). The concentration of albumin (ALB) was determined using the bromocresol green dye-binding method (Doumas et al., 1971). Serum total and conjugated bilirubins were determined according to the method described by (Malloy and Evelyn, 1937) and modified by (Nwanjo and Alumanah, 2006).

## 2.8 Assay of tissue oxidative stress and antioxidant enzyme markers

The heart and liver were individually sectioned into smaller fragments with a sterile scalpel and homogenized in an aqueous solution of 0.1M potassium buffer (pH 7.4). Subsequently, the homogenates underwent centrifugation at 10,000 rpm (4°C) for 10 minutes, and the resulting supernatants were employed for the antioxidant assays. Catalase (CAT) activity was assessed using the method developed by (Goth, 1991). The activity of superoxide dismutase (SOD) was estimated through the pyrogallol autoxidation method as outlined by (Marklund and Marklund, 1974). Reduced glutathione (GSH) was determined using the 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) recycling method described by (Banerjee et al., 1999). Glutathione peroxidase (GPx) activity was determined according to the method of Kinoshita et al. (1996). The level of malondialdehyde (MDA) was measured using the method described by (Okhawa et al., 1979).

## 2.9 Statistical analysis

Data were expressed as mean  $\pm$  standard deviation, using the statistical software SPSS version 27. The data were analysed by one way analysis of variance (ANOVA) followed by a post-hoc Tukey test at  $P < 0.05$ .

## 3 Results

As shown in Table 1, L-NAME administration significantly increased SBP and MAP compared with the control group. Treatment with TSO attenuated these elevations, with the higher dose producing effects comparable to those of enalapril. Significant changes in DBP were observed only in the high-dose TSO and enalapril-treated groups.

Table 1 Influence of TSO on blood pressure indices

| Blood Pressure Parameters (mm Hg) | Group A (Control) | Group B                        | Group C                         | Group D                         | Group E                          |
|-----------------------------------|-------------------|--------------------------------|---------------------------------|---------------------------------|----------------------------------|
| SBP                               | 119.57 $\pm$ 4.75 | 124.83 $\pm$ 2.63 <sup>a</sup> | 119.00 $\pm$ 13.42 <sup>b</sup> | 119.42 $\pm$ 11.87 <sup>b</sup> | 108.29 $\pm$ 4.03 <sup>a,b</sup> |
| DBP                               | 82.67 $\pm$ 6.09  | 81.00 $\pm$ 8.20               | 80.83 $\pm$ 11.75               | 77.60 $\pm$ 9.75 <sup>a,b</sup> | 73.57 $\pm$ 3.10 <sup>a,b</sup>  |
| MAP                               | 93.50 $\pm$ 3.87  | 97.75 $\pm$ 5.00 <sup>a</sup>  | 93.00 $\pm$ 13.68               | 88.50 $\pm$ 11.22               | 84.86 $\pm$ 2.61 <sup>a,b</sup>  |

Values are expressed as mean  $\pm$  SD (n = 8). A, B, C, D and E represent control, 40 mg/kg L-NAME, 40 mg/kg L-NAME + 0.45 mL/kg TSO, 40 mg/kg L-NAME + 0.6 mL/kg TSO and 40 mg/kg L-NAME + 2 mg/kg Enalapril Maleate, respectively. <sup>a</sup>P < 0.05 significantly different compared with control (A); <sup>b</sup>P < 0.05 significantly different compared with group B

Table 2 shows that TSO improved the L-NAME-induced changes in lipid profile, with lipid levels approaching those of the control group.

Table 2 Effects of TSO on serum lipid profile

| Parameters (mg/dL) | Group A (Control) | Group B                   | Group C      | Group D                     | Group E      |
|--------------------|-------------------|---------------------------|--------------|-----------------------------|--------------|
| TC                 | 158.00±13.64      | 162.40±13.70              | 161.60±15.31 | 152.80±8.64 <sup>a,b</sup>  | 158.20±6.76  |
| TG                 | 58.80±9.47        | 62.80±8.80                | 58.80±4.15   | 79.40±14.27 <sup>a,b</sup>  | 62.80±9.01   |
| HDL-C              | 42.60±5.94        | 35.80±6.38                | 39.84±5.81   | 49.40±4.22 <sup>a,b</sup>   | 45.80±6.57   |
| LDL-C              | 132.40±13.94      | 140.00±17.73 <sup>a</sup> | 130.00±13.93 | 122.80±12.54 <sup>a,b</sup> | 129.80±13.04 |

Values are presented as mean ± SD (n = 8). A = Control; B = 40 mg/kg L-NAME; C = 40 mg/kg L-NAME + 0.45 mL/kg TSO; D = 40 mg/kg L-NAME + 0.6 mL/kg TSO; E = 40 mg/kg L-NAME + 2 mg/kg enalapril maleate. <sup>a</sup>P < 0.05 vs. A; <sup>b</sup>P < 0.05 vs. B

L-NAME induced marked changes in liver function indices, including elevations in AST, ALT, and ALP activities, whereas TSO treatment attenuated these effects (Table 3).

Table 3 Effects of TSO on serum liver enzyme activities

| Parameters | Group A (Control) | Group B                  | Group C                  | Group D                   | Group E                    |
|------------|-------------------|--------------------------|--------------------------|---------------------------|----------------------------|
| AST (U/L)  | 156.20±1.30       | 259.20±2.59 <sup>a</sup> | 168.20±1.79 <sup>b</sup> | 165.00±1.58 <sup>b</sup>  | 161.40±1.14 <sup>b</sup>   |
| ALT (U/L)  | 88.60±1.14        | 184.60±1.95 <sup>a</sup> | 80.60±2.07 <sup>b</sup>  | 82.31±1.82 <sup>b</sup>   | 78.20±0.84 <sup>b</sup>    |
| ALP (U/L)  | 175.20±5.89       | 266.52±6.89 <sup>a</sup> | 184.00±7.07 <sup>b</sup> | 182.00±10.61 <sup>b</sup> | 165.60±7.57 <sup>a,b</sup> |
| TP (g/dL)  | 7.14±0.13         | 6.70±0.21 <sup>a</sup>   | 7.10±0.19                | 7.10±0.21                 | 7.58±0.16                  |
| ALB (g/dL) | 4.02±0.15         | 3.62±0.24                | 3.98±0.23                | 3.96±0.25                 | 3.52±1.23 <sup>a</sup>     |
| TB (mg/dL) | 0.52±0.16         | 0.56±0.15                | 0.54±0.26                | 0.70±0.21 <sup>a,b</sup>  | 0.42±0.13 <sup>a,b</sup>   |
| CB (mg/dL) | 0.30±0.07         | 0.30±0.10                | 0.26±0.11                | 0.34±0.17                 | 0.25±0.09                  |

Values are expressed as mean ± SD (n = 8). A = Control; B = 40 mg/kg L-NAME; C = 40 mg/kg L-NAME + 0.45 mL/kg TSO; D = 40 mg/kg L-NAME + 0.6 mL/kg TSO; E = 40 mg/kg L-NAME + 2 mg/kg enalapril maleate. <sup>a</sup>P < 0.05 vs. A; <sup>b</sup>P < 0.05 vs. B

There was an increase in cardiac MDA levels and impairment of antioxidant defenses, as reflected by reductions in CAT, SOD, GPx, and GSH activities. TSO treatment mitigated these effects and improved cardiac antioxidant status (Table 4).

Table 4 TSO effects on heart MDA levels and antioxidant enzymes

| Parameters            | Group A (Control) | Group B                   | Group C                     | Group D                    | Group E                   |
|-----------------------|-------------------|---------------------------|-----------------------------|----------------------------|---------------------------|
| MDA (nmol/mg protein) | 13.31 ± 5.13      | 26.11 ± 2.30 <sup>a</sup> | 12.32 ± 5.21                | 12.62 ± 4.81               | 11.23 ± 1.37              |
| CAT (U/mg protein)    | 60.18 ± 7.24      | 38.57 ± 8.25 <sup>a</sup> | 49.80 ± 4.15 <sup>a,b</sup> | 59.38 ± 38.16              | 64.12 ± 2.83              |
| SOD (U/mg protein)    | 10.27 ± 2.62      | 4.42 ± 5.70 <sup>a</sup>  | 12.89 ± 0.94 <sup>b</sup>   | 10.26 ± 16.35 <sup>b</sup> | 14.00 ± 1.24 <sup>b</sup> |
| GPx (U/mg protein)    | 20.31 ± 2.75      | 10.21 ± 8.02 <sup>a</sup> | 18.16 ± 2.82 <sup>b</sup>   | 20.14 ± 13.36 <sup>b</sup> | 22.46 ± 3.63 <sup>b</sup> |
| GSH (μmol/g protein)  | 7.71 ± 2.92       | 3.28 ± 4.39 <sup>a</sup>  | 6.87 ± 1.46 <sup>b</sup>    | 7.37 ± 1.29 <sup>b</sup>   | 7.11 ± 3.16 <sup>b</sup>  |

Values are expressed as mean ± SD (n = 8). A, Control; B, L-NAME (40 mg/kg); C, L-NAME + TSO (0.45 mL/kg); D, L-NAME + TSO (0.6 mL/kg); E, L-NAME + Enalapril Maleate (2 mg/kg). <sup>a</sup>P < 0.05 vs. A; <sup>b</sup>P < 0.05 vs. B

Similarly, Table 5 demonstrates that L-NAME administration caused oxidative imbalance in the liver, characterized by elevated MDA levels and altered antioxidant markers. TSO supplementation partially restored these changes toward normal levels.

Table 5 Effects of TSO on liver MDA and antioxidant enzymes

| Parameters            | Group A      | Group B                   | Group C                    | Group D                    | Group E                     |
|-----------------------|--------------|---------------------------|----------------------------|----------------------------|-----------------------------|
| MDA (nmol/mg protein) | 10.13 ± 0.71 | 22.61 ± 4.43 <sup>a</sup> | 13.32 ± 2.26               | 14.45 ± 5.11               | 12.21 ± 6.39 <sup>a,b</sup> |
| CAT (U/mg protein)    | 60.31 ± 5.11 | 30.42 ± 3.37 <sup>a</sup> | 55.38 ± 6.28               | 58.82 ± 8.32               | 67.12 ± 4.53                |
| SOD (U/mg protein)    | 13.72 ± 0.73 | 8.42 ± 1.14 <sup>a</sup>  | 10.62 ± 2.47 <sup>b</sup>  | 15.18 ± 3.42 <sup>b</sup>  | 12.00 ± 2.71 <sup>b</sup>   |
| GPx (U/mg protein)    | 16.48 ± 7.01 | 9.43 ± 6.38 <sup>a</sup>  | 12.00±6.75 <sup>a,b</sup>  | 17.19 ± 2.76               | 19.56 ± 7.38                |
| GSH (μmol/g protein)  | 5.84 ± 9.20  | 5.14 ± 0.12               | 8.50 ± 5.11 <sup>a,b</sup> | 8.87 ± 7.45 <sup>a,b</sup> | 7.85 ± 0.67 <sup>a,b</sup>  |

Values are expressed as mean ± SD (n = 8). A, Control; B, L-NAME (40 mg/kg); C, L-NAME + TSO (0.45 mL/kg); D, L-NAME + TSO (0.6 mL/kg); E, L-NAME + Enalapril Maleate (2 mg/kg). <sup>a</sup>P < 0.05 vs. A; <sup>b</sup>P < 0.05 vs. B



## 4 Discussion

Tea is widely consumed not only as a beverage but also for its numerous health benefits, which have been attributed to its abundance of polyphenols, catechins, and other antioxidant compounds (Musial et al., 2020). In the present study, we employed a well-established model of experimental hypertension induced by chronic administration of L-NAME, a non-selective nitric oxide synthase (NOS) inhibitor (Krasnylenko et al., 2019; Adedapo et al., 2020). By competitively inhibiting NOS, L-NAME reduces nitric oxide (NO) bioavailability, leading to endothelial dysfunction and elevated blood pressure (Zhao et al., 2015).

The co-administration of TSO alongside L-NAME appeared to mitigate the hypertensive effects caused by L-NAME. This normalization of blood pressure parameters may be attributed to the vasodilatory properties of TSO. This finding aligns with the report of Fuchs et al. (2014), who demonstrated enhanced microcirculation following catechin and theaflavin supplementation in healthy individuals, supporting the beneficial effects of tea-derived compounds on vascular function.

Hypertension is intricately associated with disturbances in lipid metabolism, with dyslipidemia frequently occurring as a coexisting condition. Elevated blood pressure is often accompanied by increased serum lipid levels, including TC, LDL-C, and TGs (Lee and Siddiqui, 2019). Among these lipid fractions, elevated LDL-C plays a major role in the development of atherosclerosis through the accumulation of cholesterol within arterial walls, thereby impairing vascular function and increasing cardiovascular risk. Conversely, HDL-C plays a protective role by mediating reverse cholesterol transport, facilitating the removal of cholesterol from peripheral tissues to the liver for excretion. Reduced HDL-C levels in hypertensive individuals may diminish this protective mechanism, thereby accelerating the progression of atherosclerotic disease (Ben-Aicha et al., 2020; Khatana et al., 2020). Furthermore, elevated triglyceride concentrations have been implicated in the development of cardiovascular complications and are considered an independent risk factor for cardiovascular disease (Packard et al., 2020). In the present study, L-NAME administration altered the lipid profile of the experimental animals, while concurrent treatment with TSO restored these parameters toward normal values. This finding is consistent with previous reports demonstrating the lipid-lowering effects of tea-derived products. For example, Samavat et al. (2016) reported significant reductions in TC and LDL-C following long-term supplementation with green tea catechin extract.

The growing recognition of the link between hypertension and hepatic dysfunction shows the importance of evaluating liver function in hypertensive models. Liver enzymes such as ALT, AST, GGT, ALP serve as established biomarkers of hepatic integrity and function (Rahman et al., 2020). Elevations in these enzymes are often associated with systemic inflammation and oxidative stress, which are known contributors to endothelial dysfunction and the pathogenesis of hypertension (Guzik and Touyz, 2017). The increased activities of these enzymes observed following L-NAME administration indicate hepatic stress, whereas their reduction in the TSO-treated groups suggests a hepatoprotective effect of TSO. The reduction in total protein observed in the hypertensive group may reflect impaired hepatic synthetic function or increased protein catabolism under conditions of oxidative stress (Li et al., 2020). A notable reduction in albumin (ALB) concentration in the Enalapril Maleate-treated group raises important considerations regarding the effects of this drug on hepatic protein metabolism. Although angiotensin-converting enzyme (ACE) inhibitors are generally regarded as hepatoprotective, the mechanism underlying this observed decrease warrants further investigation, particularly in the context of long-term administration or possible interaction with hypertensive states.

Oxidative stress is a key mechanism underlying L-NAME-induced hypertension (Tan et al., 2018). In the present study, elevated MDA levels together with reductions in antioxidant defenses confirmed the presence of oxidative imbalance following NOS inhibition. Also, decreased activities of CAT, SOD, GPx, and reduced levels of GSH in the L-NAME-treated group indicate impairment of endogenous antioxidant systems responsible for neutralizing reactive oxygen species and maintaining cellular redox homeostasis (Panday et al., 2020; Maurya and Namdeo, 2021; Vitolo, 2021). The improvement in these antioxidant markers following TSO administration suggests that TSO enhances endogenous antioxidant capacity and limits oxidative damage. These findings further support the

antioxidant potential of the phytoconstituents present in tea seed oil, which may suppress reactive oxygen species generation and promote the restoration of redox balance (Yan et al., 2020).

## 5 Conclusions

This study affirms the therapeutic potential of TSO in effectively reducing blood pressure, enhancing antioxidant defenses and improving lipid profiles. Although further investigation is warranted to elucidate the underlying mechanisms, the study provides valuable insights into the multifaceted benefits of TSO, positioning it as a natural and potentially effective agent in the management of hypertension and its associated complications.

### List of abbreviations

| Abbreviation | Full name                            |
|--------------|--------------------------------------|
| TSO          | Tea Seed Oil                         |
| L-NAME       | NG-nitro-L-arginine Methyl Ester     |
| SBP          | Systolic Blood Pressure              |
| DBP          | Diastolic Blood Pressure             |
| MAP          | Mean Arterial Pressure               |
| ALT          | Alanine Aminotransferase             |
| AST          | Aspartate Aminotransferase           |
| ALP          | Alkaline Phosphatase                 |
| HDL-C        | High-Density Lipoprotein Cholesterol |
| LDL-C        | Low-Density Lipoprotein Cholesterol  |
| TC           | Total Cholesterol                    |
| TGs          | Triglycerides                        |
| GSH          | Glutathione                          |
| GPx          | Glutathione Peroxidase               |
| SOD          | Superoxide Dismutase                 |
| MDA          | Malondialdehyde                      |
| NO           | Nitric Oxide                         |
| NOS          | Nitric Oxide Synthase                |
| GGT          | $\gamma$ -glutamyltransferase        |
| ACE          | Angiotensin-Converting Enzyme        |
| TP           | Total Protein                        |
| ALB          | Albumin                              |
| TB           | Total Bilirubin                      |
| ANOVA        | Analysis of Variance                 |

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare no conflict of interest.

### Ethics approval and consent to participate

Authors declared that all experiments were carried out with strict compliance to the “Principle of Laboratory Animal Care” and ethical guidelines for investigation of experimental pain in conscious animals. The project was approved by the University of Ibadan Animal Care and Use Research Ethics Committee.

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### Authors' Contributions

LEY: Conceptualization, supervision and final correction of draft. JFA and AAO: Methodology, data collection, writing-original draft preparation. LEY and JFA: Editing. All authors have read and approved the final manuscript.

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