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Cultivation techniques of yam influence the amount of primary and secondary metabolites

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Introduction: Agriculture is an indispensable practice with a long history that dates back millennia. The cultivation of Chinese yam (*Dioscorea polystachya* Turczaninow) is of social importance but not yet optimized; the process is currently complex and labor-intensive. Notwithstanding, the plant is regarded as a promising supplementation for ensuring food security, even in the face of climate change. This is due to its nutritional value and its diverse contribution to the cultivation of food crops.

Method: The EKO-YAM project, presented in this work, is thus concerned with the implementation and evaluation of four different cultivation methods with regard to the content of primary and secondary compounds, as well as economic factors.

Results: This study shows insights into the scientific monitoring of growing and the impact of different cultivation techniques on starch and secondary metabolites.

Discussion: The superordinate goal was the improvement of the growing of *Dioscorea polystachya* in terms of diet, metabolites, costs, and sustainability.

KEYWORDS

Dioscorea polystachya, cultivation technique, secondary metabolites, starch, yam, dioscoreaceae

1 Introduction

The Chinese yam (*Dioscorea polystachya* Turczaninow), a species of the *Dioscoreaceae* family, is notable for its edibility and importance as a food crop in tropical and subtropical regions worldwide (Padhan and Panda, 2020). According to previous research, the underground tubers of this plant have attracted scientific interest (Epping and Laibach, 2020). A successful cultivation of this species in more temperate climates has the potential to enhance dietary diversity and contribute to a more sustainable food supply. This is particularly relevant in the context of climate change, where greater dietary diversity has been shown to mitigate its consequential effects and contribute to food nutrition diversity, food security, and climate change as well (Hunter et al., 2019; Chen et al., 2025). The

cultivation and promotion of diverse food sources is a key component of Germany's national bioeconomy strategy, aimed at ensuring a sustainable future (Research, F. M. O. E. A, 2020). Tubers are of interest due to their high content of resistant starch, polysaccharides, and secondary metabolites, including saponins (Epping and Laibach, 2020). The latter are a crucial factor in the utilization of tubers not only as a staple food but also in Traditional Chinese Medicine (Amat et al., 2014). For instance, *D. polystachya* is employed in the treatment of hyperglycemia due to its ability to reduce blood sugar, an effect that can be attributed to the presence of the saponin gracillin, among other constituents (Wang et al., 2024). Moreover, their regulatory properties on cholesterol absorption and blood pressure are also demonstrated (Chen et al., 2003; Shujun et al., 2008; Amat et al., 2014). Its positive properties justify its use as a functional food. The health-promoting effects of Chinese yam make them a potential candidate for a healthier, more conscious, and more sustainable lifestyle. The potential of such functional foods to protect humans from the outlined diseases has already been documented (Topolska et al., 2021). To harness the benefits of these nutritional and health-promoting properties, proper cultivation practices are essential.

However, cultivating these plants is challenging and expensive, depending on the region and agro-climatic conditions (Wang et al., 2020; Schmidt and Arnica Montana, 2023). Initially, the plant must demonstrate resilience to the local climatic conditions. Secondly, the intensive labor required due to the long, slender growth form of the tubers constitutes a significant challenge next to the incidental costs. Responsible for that is the growth habit itself with the long vines and necessity of climbing support. These tubers typically grow approximately one meter vertically into the ground, gradually thickening towards the bottom. The tuber's fragility during harvesting, characterized by its susceptibility to breakage, further exacerbates the labor-intensiveness of the process (Cai, 2023). This necessitates the removal of a large volume of soil to expose each individual tuber. Consequently, this process is notably time-consuming. Innovative cultivation techniques have been developed to address these challenges, aiming to enhance efficiency and reduce costs (Sharma et al., 2024). This, in turn, facilitates the establishment of the tuber and enhances its accessibility, thereby contributing to food security (Brink, 2023). The selection of cultivation techniques was a critical component in this regard. For instance, the implementation of aeroponic technology was not a viable solution in this current study. Research on *Dioscorea rotundata* had demonstrated the potential for this technique to enhance crop yields. However, its cultivation was optimal in consistently warm and humid climates (Akomi et al., 2020). In the context of field cultivation in temperate climates, the process of cultivation is characterized by more complexity or necessitating the utilization of greenhouses (Im et al., 2024). This finding extended to the application of hydroponics technology. The utilization of this method in a field setting was also not feasible. However, the influence of fertilizer was fundamentally tested in the cultivation of *Dioscorea alata* using semi-autotrophic hydroponics. Appropriate usage resulted in the cultivation of larger tubers *in vitro* (Rose et al., 2024). As in our presented cultivation systems, a

cultivation method in the primary yam-growing region of Nigeria was vegetative propagation via cuttings. It incorporated regional characteristics, such as the incorporation of rice husks, to address the variation in soil types (Gbadamosi et al., 2019). This regional approach, which took account of local conditions and possibilities in terms of soil variation, was also implemented in the design of the current study. As part of the EKO-YAM project (Establishment of cost-efficient and sustainable cultivation systems for yam), the dependence on the content of primary and secondary metabolites of four different cultivation methods for the field was investigated (Krüger and Weng, 2025). These were the mobile raised bed technique (MRBT), the trench cultivation technique (TCT), the mound bed technique (MBT), and the gutter cultivation technique (GCT). Another possibility to address the challenges is the classical breeding of new genotypes of *D. polystachya* by planting and crossing the male and female plant. Subsequent to this, the cultivars could be sorted and selected according to the desired phenotypic characteristics. These characteristics may include, but are not limited to, easier harvesting due to a special growth habit or improved compound composition that result in better quality. However, it should be noted that traditional breeding methods require a considerable investment of time and labor (Goulet et al., 2016; Spengler, 2019). In comparison, novel cultivation methods are a more efficient and expeditious solution. Evidence has demonstrated the general influence of cultivation methodologies on the quality and quantity of primary and secondary metabolites such as starch, saponins, or flavonoids (Gaja et al., 2023; Nannar and Ahire, 2023). However, in the context of the influence from the cultivation technique in yam cultivation, the available data remains limited. Nevertheless, the positive influence of the *in vitro* hydroponics system on the diosgenin content in *Dioscorea composita* was described (Sánchez-López et al., 2025). Another study showed the difference in secondary metabolites such as allantoin, diosgenin, and dioscin between wild and cultivated yam (*Dioscorea opposita* and *Dioscorea alata*) (Shan et al., 2020). However, the specific impact of the cultivation method remained unexamined. Hence, this article aims to elucidate the effects of cultivation techniques on *Dioscorea polystachya* Turczaninow and its harvest. The necessity to enhance cultivation arises from the significant societal contributions of *D. polystachya*, including its nutritional and medicinal value. Eventually, the findings provide a contribution to the sustainable bioeconomy strategy for yam.

2 Materials and methods

2.1 Cultivation and cultivation techniques

The male plant of *Dioscorea polystachya* Turczaninow has been cultivated (N47.772°, E9.226°) at Andreashof e.V. Überlingen, Germany for a period of over two and a half decades by vegetative reproduction. The cultivation was focused on one specific genotype (Ried et al., 2025). A fact that confers upon the plant a unique characteristic in Europe (Lehnert, 2020). Traditionally, the cultivation of the plant had occurred in wood-

supported raised beds. This method was not only very challenging in terms of cultivation technology, labor-intensity, and costs, but also no longer complies with the new EU Regulation 2018/848 (European Union and Union, E2018). Therefore, four alternative cultivation methods were set up. The objective was to determine a cost-efficient method and at least an equal quality of nutrition. The first method was the mobile raised bed technique (MRBT) with open boxes at the bottom to allow an exchange between the soil and the box contents (Figures 1A, C). This corresponds to the new policy EU 2018/848 (European Union and Union, E2018). A grid was attached to the bottom of the boxes to prevent the soil from slipping out during transportation to the fields.

The second technique was soil-based trench cultivation (TCT). For this method, a trench with a depth of approximately one meter was excavated (Figures 1B, D). The side walls were partially reinforced and filled with soil compositions of varying types. The layer at the base of the trench, measuring 100 centimeters, consisted of quartz sand (TCT-QS), quarry sand (red gravel sand, TCT-RG), or a humus soil mixture (TCT-HU). These layers were then covered with a soil mixture comprising compost and topsoil/humus. Both sands were non-calcareous and pebble-like. The quartz sand was derived from a white commercial sand from the Rhine (Kehl, Germany; N48.583°, E7.803°) and the red gravel was derived from a local stone quarry in the Black Forest (Donauschingen, Germany; N47.994°, E8.319°).

The third method was a mound bed cultivation technique (MBT). For this method, soil was piled up and covered with a perforated tarpaulin (Figures 2A, C). The seed tubers were inserted into the existing holes. A gutter cultivation system (GCT) was developed as the fourth installation (Figures 2B, D). For that purpose, a one-meter-long polyvinyl chloride roof gutter was inserted into the ground at an angle of approximately 5-15° and the seed tubers were placed in it. The half tubes were commonly available at a local hardware store. Due to the phenomenon of geotropism, the tubers naturally exhibited a vertical growth pattern and thus grow diagonally downwards along the length of the tubes in the GCT set-up. The upper portion of the soil was composed of humus, while the lower portion consisted of sand. The sand and gravel were not replaced during the three years but left in the trench. The humus soil was replenished annually and recycled using biodynamic methods for future growing seasons. It was evenly distributed across all cultivation techniques.

The installation of climbing support structures was implemented for *D. polystachya*, facilitating the growth of its elongated vines and shoots. The tubers were planted during the spring season between April and May and the tubers were harvested in the autumn between October and November depending on the weather conditions, respectively. During the winter month, the tubers were labeled and stored in a dark, dry and cool (8-15 °C) environment for the subsequent planting cycle. Generally, the upper

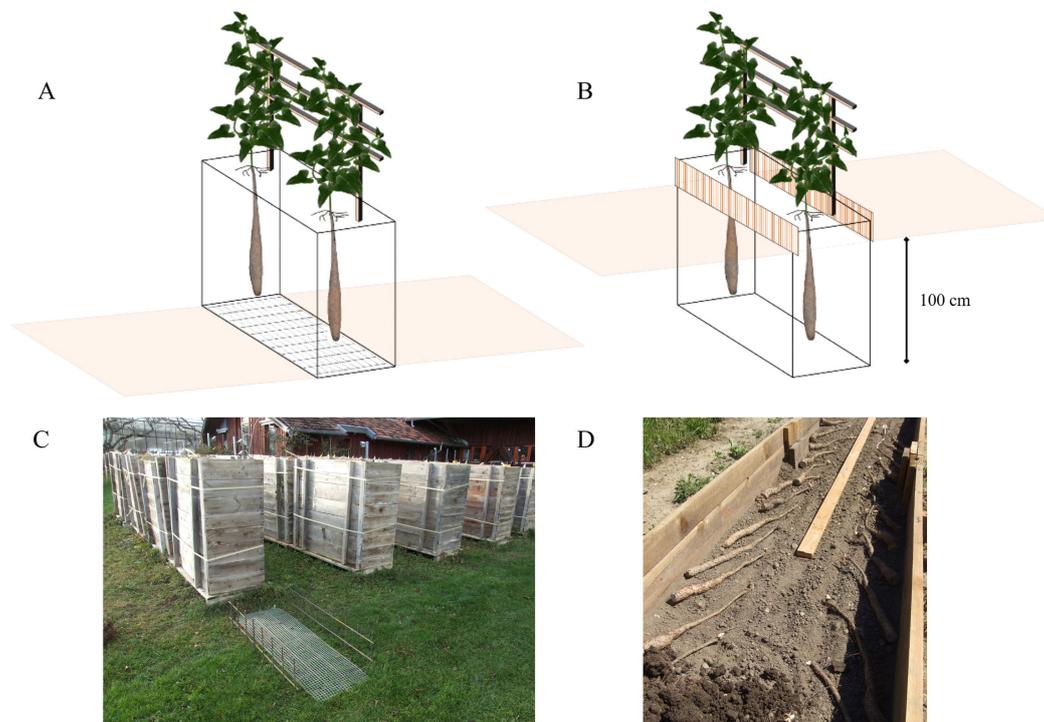
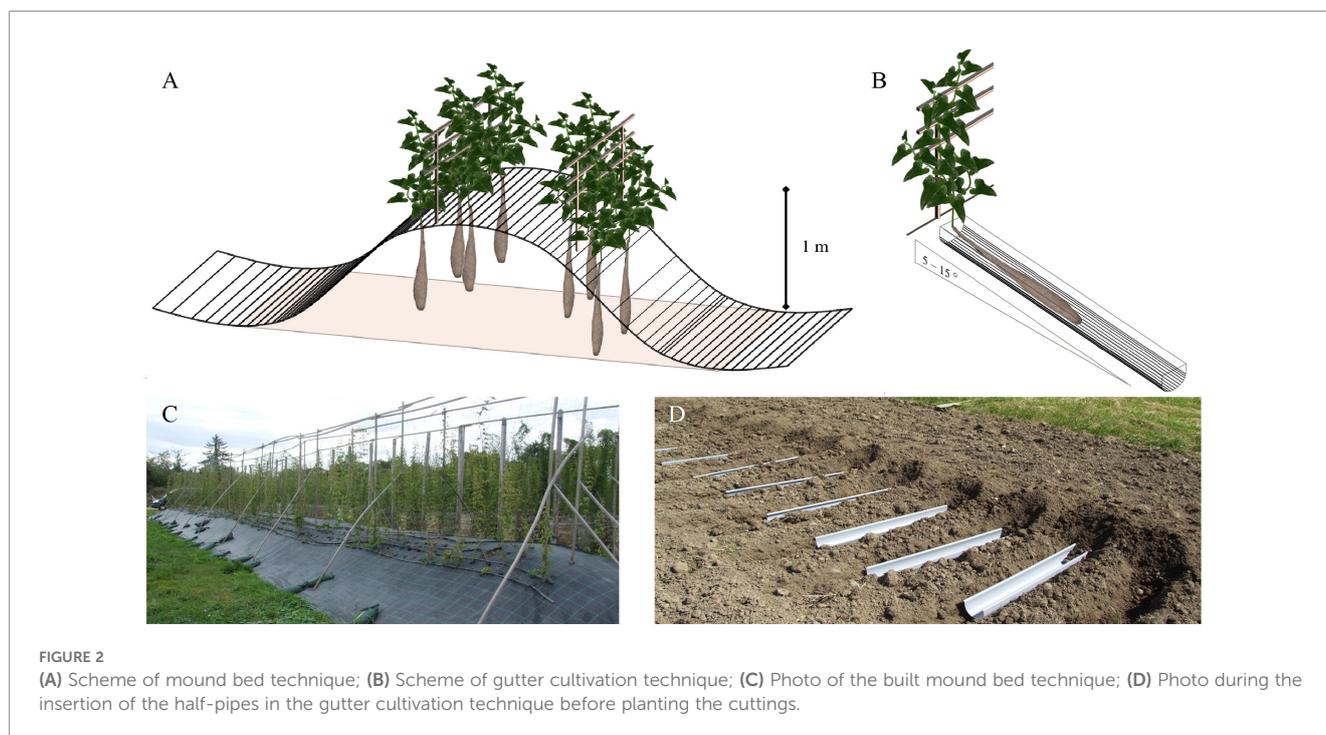


FIGURE 1
(A) Scheme of mobile raised bed technique; **(B)** Scheme of trench cultivation technique; **(C)** Photo of raised beds and an example of a grid for the bottom of boxes for soil exchange; **(D)** Example of one trench during planting the cuttings.



portion of the tubers were cut off in spring (standardized to 150 g) and used for vegetative cultivation. The plantations were monitored over three growing seasons from 2022 to 2024.

2.2 Plant collection and extraction

The plant material that was collected was derived from a single cultivar of *Dioscorea polystachya* Turczaninow. This single genotype was reproduced every year of planting and was listed as a registered variety and registered trademark since 2009 as Lichtyam®. The middle portion of the tubers was extracted from plants of every cultivation technique and soil from the tubers harvested by hand on an annual basis. In total 181 samples were received over the three years. Distributed evenly across all techniques, 70 samples were taken in 2022, 43 in 2023, and 68 in 2024 from over 1700 grown plants. For the starch analysis, tuber material was freeze-dried and subsequently pulverized to a fine powder using an A11 basic analytical mill (IKA-Werke, Staufen, Germany) followed by a mixer mill (90 s at 30 s⁻¹). Quantification of soluble and resistant starch was performed using the Resistant Starch Assay Kit (Rapid) in accordance to the manufacturer's instructions (Megazyme, Wicklow, Ireland).

For the analysis of secondary metabolites, these tubers were sliced into small pieces and dried in the air. Thereafter, they were pulverized using a ball mill (30 s at 30 s⁻¹) and mixed with 80% (v/v) ethanol in a 1:10 ratio. The extract was vortexed for one minute and extracted for another 15 minutes in an ultrasonic bath. Following this extraction process, the extract was subjected to centrifugation and subsequently analyzed using high-performance liquid chromatography (HPLC).

2.3 HPLC

A program with a gradient of water and acetonitrile over 33 minutes was developed (see Table 1). In each instance, 7 µL of the sample was injected. The evaluation was conducted at 210 nm (example in Supplementary Figure S1).

TABLE 1 Gradient program of the HPLC analysis.

Minute	0	2	25	28	29	33
Water proportion [%]	95	40	10	10	95	95
Acetonitrile proportion [%]	5	60	90	90	5	5

2.4 Methodology

The tubers were labeled over a period of three years and cultivated under four different types of cultivation and three different types of soil. The upper part of the tubers, standardized to 150 g pieces, was used as cuttings for vegetative reproduction in the following growing season. Immediately following the harvest in late autumn, the tubers were thoroughly washed and a segment was extracted from the middle portion of the tuber (excluding the end piece). The rest was stored for the upcoming season at temperatures ranging from 6 to 8°C or otherwise processed, for example sliced or processed into flour. Following this procedure, the excised piece was divided into slices by hand, then dried (secondary metabolites: either by air-drying at room temperature or by oven-drying at 38°C; starch analysis: freeze-drying), and ground into a fine powder using

a ball mill. Finally for starch analysis, the aforementioned assay was performed and in case of secondary metabolites extraction, the powder was extracted three times per sample, as previously described, and measured by HPLC. The evaluation was based on the peak areas of the chromatograms at the different wavelengths (Supplementary Figure S1). Using a constant ratio of powder to extraction agent, a direct comparison could be made between the chromatograms and the average peak areas at 210 nm. In order to obtain the content of extracted plant constituents per kilogram of plant, the peak areas determined were set in relation to the average dry residue. To this end, usually two times 0.5 mL of extract was subjected to evaporation to dryness (drying furnace, 60°C), after which the residue was weighed and the average was calculated (Supplementary Table S1).

Some pieces of tuber were also peeled with a hand peeler and then dried. Both the peel of yam tubers and the pulp were then ground, extracted and measured in the same way.

All samples were compared with a reference powder consisting of samples of *Dioscorea polystachya* Turczaninow tubers from different batches. Therefore, ground tuber material was used which was harvested from plants grown at Andreashof in a self-contained box cultivation with a closed bottom. This cultivation method was the conventional technique before the implementation of the four new techniques.

2.5 Chemicals

Chemicals for starch quantifications that were not provided by the Resistant Starch Assay Kit (Rapid) included: maleic acid (Carl Roth, Karlsruhe, Germany), calcium chloride dihydrate (Carl Roth, Karlsruhe, Germany), acetic acid (Carl Roth, Karlsruhe, Germany), sodium hydroxide (AppliChem, Darmstadt, Germany) and ethanol absolute (AppliChem, Darmstadt, Germany). The extraction process for secondary metabolites utilized ethanol puriss (Merck, Darmstadt, Germany), diluted with ultrapure water (Siemens LaboStar, Günzburg, Germany). The solvents employed for the high-performance liquid chromatography (HPLC) were obtained from Fisher Scientific (Schwerte, Germany) and were mixed with 0.1% formic acid (Carl Roth, Karlsruhe, Germany). The reference substances diosgenin, dioscin, gracillin, and trillin, were purchased from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany) and protodioscin and pseudoprotodioscin were procured from Biomol GmbH (Hamburg, Germany).

2.6 Devices and software

Drying in the oven of tubers was carried out in the drying oven Modell 800 from Memmert GmbH & Co. KG (Schwabach, Germany). The ball mill TissueLyser MM300–85220 from RETSCH GmbH (Haan, Germany) and a proper stainless steel ball of 2.7 cm in diameter was used for grinding the samples to a fine powder. For the subsequent extraction in tubes (Eppendorf AG, Hamburg, Germany), the mixing proceeded in a vortexer MS1

Minishaker from IKA-Werke GmbH & CO. KG (Staufen, Germany), an ultrasonic bath from Bandelin electronic (Berlin, Germany), and a centrifuge Heraeus Megafuge 1.0 from Thermo Electron LED GmbH (Osterode, Germany). The solvent was added with pipettes Research[®] plus from Eppendorf AG (Hamburg, Germany). The measurements of the chromatograms were done in the HPLC system 1260 Infinity II from Agilent (Santa Clara, CA, United States) and analyzed with the Agilent software Data Analysis (Version 2.7, Santa Clara, CA, United States). The drying cabinet Heraeus T6060 (Hanau, Germany) and the scale Mettler Toledo XS205 DualRange (Greifensee, Switzerland) were used to determine the dry mass and for all other weighing activities. For sample preparation of the starch quantification, a mixer mill MM400 from RETSCH GmbH (Haan, Germany) and a 10 ml grinding jar containing a stainless steel ball of 1.2 cm in diameter were used. Enzymatic digestion of non-resistant starch was performed in Supelco 22 mL clear glass vials with a screw top (Merck, Darmstadt, Germany) in a SW-20C shaking water bath from JULABO GmbH (Seelbach, Germany). Centrifugation of samples were performed in a SX4750 swinging-bucket rotor in an Allegra X-15R centrifuge (Beckman Coulter, Brea, CA, USA). For non-resistant and resistant starch quantification, 4 mL ROTILABO sample vials with threads ND13 (Carl Roth, Karlsruhe, Germany) were used.

3 Results

The objective of this project was to assess and compare sustainability, yield, labor and material costs between different cultivation methods, as well as quality of the yam in terms of ingredients and phenotypic parameters. The four techniques yielded a variety of tuber shapes, ranging from straight (Figures 3A–C) to strongly branched (Figure 3D).

Starch quantification revealed an overall total starch (TS) content above 50 g per 100 g dry weight (DW), thus being the major constituent in Chinese yam tubers (Figure 4). Over the three cultivation periods, fluctuations in TS contents were observed for each cultivation method. In 2022, peak in TS content was observed for all tested cultivation technique compared to the subsequent years and a significantly higher TS amount was detected in TCT-QS compared to GCT grown yam tubers (average of 70.65 g per 100 g DW and 64.82 g per 100 g DW, respectively), while neither significant difference was detected in the following two years nor between the other cultivation techniques. A decline in TS content was observed for all tested cultivation techniques in 2023 compared to the previous year, followed by an increase in 2024 except for the MBT cultivation method. While the first two cultivation periods revealed a comparable trend of TS content, with highest levels detected in TCT-QS followed by MBT and lowest content in GCT, different results were obtained in 2024, showing the lowest content for MBT cultivated yams. However, combining the results of the three cultivation periods, TS content was less in GCT tubers compared to the other methods (tendency: GCT < MBT < TCT-HU < MRBT < TCT-RG < TCT-QS). No significant difference in



FIGURE 3 Photos of four different tubers. **(A)** tubers from the trench cultivation technique with red gravel (2022); **(B)** part of the 2022 harvest using the mound bed technique; **(C)** up to one-meter-long tubers from the 2022 harvest of the mobile raised bed technique; **(D)** branched tubers obtained from the gutter cultivation technique in 2022.

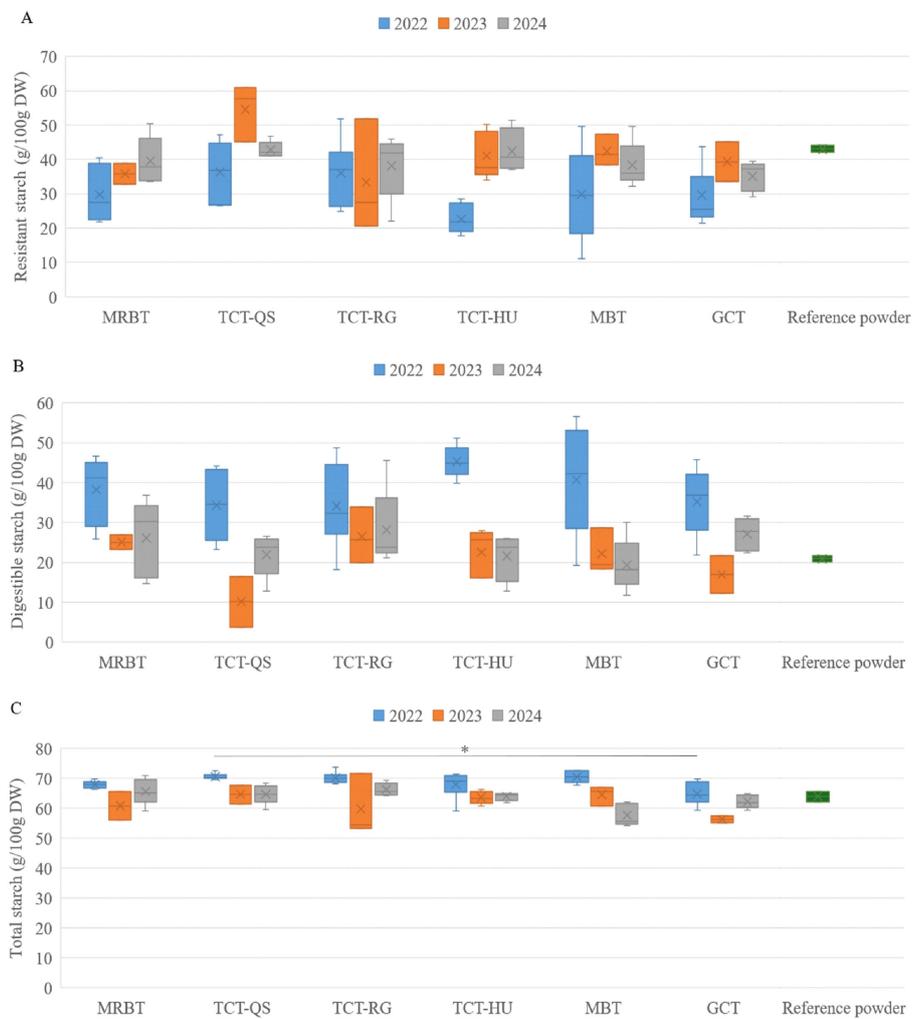


FIGURE 4 The amount of resistant starch **(A)**, digestible starch **(B)** and total starch **(C)** detected in the tubers of the different cultivation methods over the three years (■2022, ■2023, ■2024). Number of biological replicates between n=2 and n=12. The level of significance was determined using the Kruskal-Wallis ANOVA test followed by the *post-hoc* Dunn's test. Asterisks indicate statistical significance: * p value ≤ 0.05. The techniques are: Mobile raised bed technique (MRBT); Trench cultivation technique with quartz sand (TCT-QS), with red gravel (TCT-RG), and with humus soil (TCT-HU); Mound bed technique (MBT); Gutter cultivation technique (GCT). A reference powder (■) of multiple batches of DP was utilized for comparison.

resistant starch (RS) content was quantified between the cultivation methods. A tendency towards higher RS content in tubers of TCT-QS cultivation (average RS content of 42.53 g per 100 g DW) was detected over the three years compared to the other methods, whereas MBT, TCT-HU, TCT-RG, GCT and MRBT tubers contained comparable amounts of RS (35.55 g, 34.05 g, 36.23 g, 32.22 g and 34.44 g, respectively).

Concerning secondary metabolites, the results obtained from the cultivation methods exhibited some variation (Figure 5; Supplementary Tables S2–S4). To arrange the results according to the chronological sequence of the cultivation methods, the GCT demonstrated the highest content of extracted substances in the first year of cultivation, with 140.4 g kg⁻¹ of dry tuber. Phenotypically, the presence of numerous branches and thicker peel on the tubers was evident. The MBT, the TCT-HU, and the MRBT with the modified, open-bottomed raised bed boxes illustrated high similarity in their initial results, with concentrations of 127.3, 125.3, and 124.8 g kg⁻¹ tuber, respectively. The TCT yielded a tuber extract content that was almost half of that observed in the GCT, dependent on the soil composition, in descending order: TCT-QS > TCT-RG.

An analysis of the peels of yam tubers (Figure 6; Supplementary Table S5) in the first year (2022) revealed that GCT exhibited the highest total content, with a value of 471.7 g kg⁻¹. This content was between two and three and a half times higher than that observed in the other cultivation methods. Notably, despite the second highest content observed in the unpeeled tuber of MBT, the peels content was the third highest (262.5 g kg⁻¹), following the content in TCT-HU and before MRBT, which were 313.4 g kg⁻¹ and 249.4 g kg⁻¹ of peel, respectively. For the remaining two cultivation methods, the content decreased in the order TCT-QS > TCT-RG to around one third. All methods demonstrated a distinction in content relative to the same mass of peel (mean 2.5-fold) and pulp (mean 0.8-fold). Without an exception, all peel samples showed a clear difference compared to the unpeeled tuber. When comparing the peeled tuber

with the unpeeled tuber, the variation was smaller than with the peel extracts.

In the second year (2023), the highest average total content was recorded for MBT, followed by TCT-HU and GCT. The amount of the tubers from the MRBT, TCT-RG, and TCT-QS were also measured in a decreasing order. In general, the quantities became more similar in the second year and no longer fluctuated so significantly within each cultivation technique. The content for TCT-RG and TCT-QS showed a moderate increase and generally less variation from the mean value. The average values fell for the four methods with the highest concentrations of the extracted compounds in the first year. However, the results for MBT remained almost unchanged, but MRBT and GCT decreased only slightly.

In the third year (2024), all cultivation techniques demonstrated a reduction in the total amount of extractable total number of secondary metabolites compared to the second year. The MBT method exhibited the highest content in this harvest (111.0 g kg⁻¹ tuber), with a modest reduction compared to the previous two years. The TCT-HU (92.8 g kg⁻¹), MRBT (91.5 g kg⁻¹), and GCT (86.1 g kg⁻¹) methods yielded slightly lower concentrations. Of the top four methods, all showed a continuous decline over the three-year period. TCT-RG exhibited an increase in the second year, followed by a slight decrease in the third year. A similar trend was observed in TCT-QS. In the third year, both methods revealed the lowest content among all methods. A notable observation was the decline in the content of extracted ingredients in the TCT-HU, which came close to the median level between the first and second year. Furthermore, the TCT-QS demonstrated a level consistent with that of the first year. A general reduction in content was evident in the third year, accompanied by a significant decrease in the standard deviation of the cultivation methods compared to previous years.

The arithmetic mean of all tubers across all cultivation methods corroborated the preceding results. In 2022, the value was 104.7 g kg⁻¹

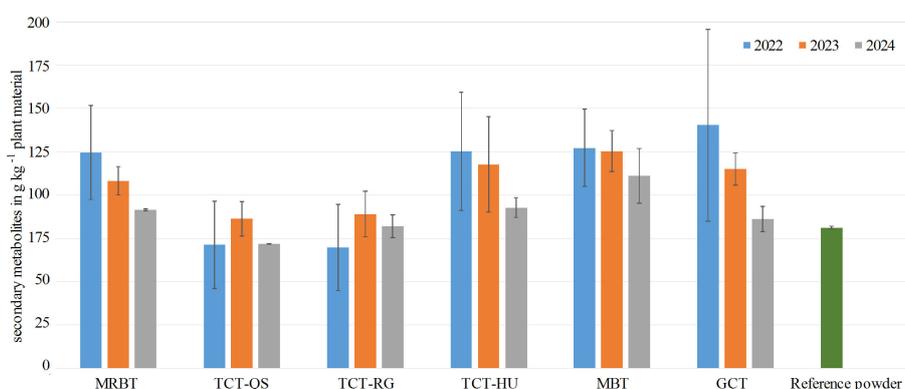


FIGURE 5

The amounts of compounds in g per kg dry plant material of the annual harvest of the different cultivation methods from *Dioscorea polystachya* (DP) over the three years (■2022, ■2023, ■2024). Number of biological replicates between n=6 and n=15. The cultivation techniques were mobile raised bed technique (MRBT); trench cultivation technique with quartz sand (TCT-QS), with red gravel (TCT-RG), and with humus soil (TCT-HU); mound bed technique (MBT); gutter cultivation technique (GCT). A reference powder (■) of many batches of DP was utilized for comparison. The values were determined by integrating the peaks from the HPLC tests at a wavelength of 210 nm and were set in relation to the dry mass of the extract. The level of significance was determined using the Kruskal-Wallis ANOVA test and Tukey *post-hoc*-test.

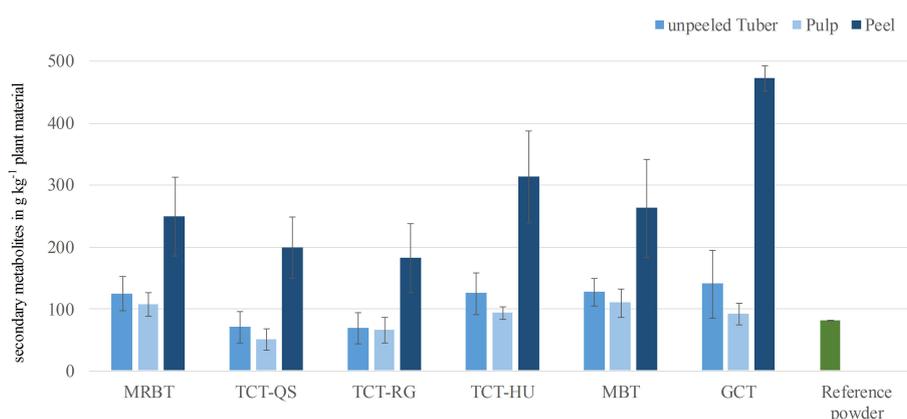


FIGURE 6

The results of the amounts of compounds in g per kg dry plant material in the unpeeled tuber (■), the pulp of the tuber (■), and the peel of the tuber (■) in comparison to the reference powder (■). Number of biological replicates between $n=3$ and $n=4$. The cultivation techniques were mobile raised bed technique (MRBT); trench cultivation technique with quartz sand (TCT-QS), with red gravel (TCT-RG), and with humus soil (TCT-HU); mound bed technique (MBT); gutter cultivation technique (GCT). The values were determined by integrating the peaks from the HPLC tests at a wavelength of 210 nm and were set in relation to the dry mass of the extract. The level of significance was determined using the Kruskal-Wallis ANOVA test and Tukey *post-hoc*-test.

tuber, increased to 107.0 g kg⁻¹ of tuber in the subsequent year, and decreased to 89.2 g kg⁻¹ tuber in the third year. By averaging the yields of each technique over the three years, an order was found: TCT-QS < TCT-RG < MRBT < TCT-HU < GCT < MBT (76.5 g kg⁻¹, 80.3 g kg⁻¹, 108.2 g kg⁻¹, 108.5 g kg⁻¹, 113.9 g kg⁻¹, 121.2 g kg⁻¹, respectively).

In the initial year, further research was conducted on the development of an appropriate drying method for sample preparation. The tubers were subjected to a range of drying conditions, and the impact on the quality and composition of the primary and secondary metabolites was examined. The drying methods evaluated included air drying at room temperature, oven drying at 38°C, and freeze-drying. The results indicated that there were no significant differences between the different drying methods. For starch analysis, freeze-drying was the approved method. For the analysis of secondary metabolites, the different drying methods investigated at the beginning of the project did not show any advantages for one method in terms of quality and maximum yield. Accordingly, the drying method that required the least amount of work and energy was selected for all further sample processing of the annual harvests. This is the air-drying method at room temperature.

A comparison of the results with the reference powder (multiple batches of *D. polystachya*, conventional cultivation technique) revealed a substantial superiority of MBT and TCT-HU over the reference powder across all three years. The MRBT adapted to the conventional method demonstrated comparable performance to the reference powder.

Moreover, the planting duration was also documented, providing further insights into yam cultivation (Figure 7). The longest vegetation period was observed in 2023 for MRBT, with a total of 227 days to harvest, which was 89 days longer than the shortest period recorded for GCT in 2024. The order of planting was dependent on the specific preparation of the respective cultivation type and prevailing weather conditions. However,

harvesting was systematically executed in the sequence TCT, GCT, MBT, and finally MRBT. Overall, the vegetation period, spanning from May to the onset of November, exhibited an average duration of 171, 179, and 169 days in 2022, 2023, and 2024, respectively.

A factor was calculated from the planting duration and the documented hours of sunshine on the aerial part of the plant (Red bars in Figure 7). Together, these factors, in conjunction with the yield, resulted in a determined value in grams per kilogram of tuber material per individual hour of sunshine (Supplementary Table S6) and per planting day. The specific parameters of this phenomenon were subject to variation depending on the cultivation method employed. This value facilitated the comparison and estimation of the efficacy of the method under conditions of equivalent vegetation length and sunshine duration. It was demonstrated that a higher factor corresponded to an enhancement in the yield of secondary plant substances and larger tuber weight. Depending on the cultivation method, it also showed a tendency for one technique to outperform the others. The highest value recorded for MBT was observed in the third year, with an efficiency of 18.64 g kg⁻¹ d h⁻¹. The GCT would have been a valuable resource, if it contained a significant quantity of peel. When the peel proportion was considered normal (second and third year of cultivation), the mean value determined was 14.85 g kg⁻¹ d h⁻¹, which was nearly equivalent to the efficiency of the secondary metabolite yield of the TCT (15.04 g kg⁻¹ d h⁻¹). It was evident that the gutter cultivation in the initial year of planting exhibited a conspicuous deviation. The mean values for the three-year period for each cultivation type (MRBT 16.69 g kg⁻¹ d h⁻¹, TCT 15.04 g kg⁻¹ d h⁻¹; MBT 18.34 g kg⁻¹ d h⁻¹ and GCT 16.02 g kg⁻¹ d h⁻¹) approximated the individual measured values, each with a minimal standard deviation (0.63, 0.60, 0.31, and 1.65 g kg⁻¹ d h⁻¹, respectively).

The amount of work required per kilogram of harvested tuber for each cultivation technique followed the order MRBT < MBT <

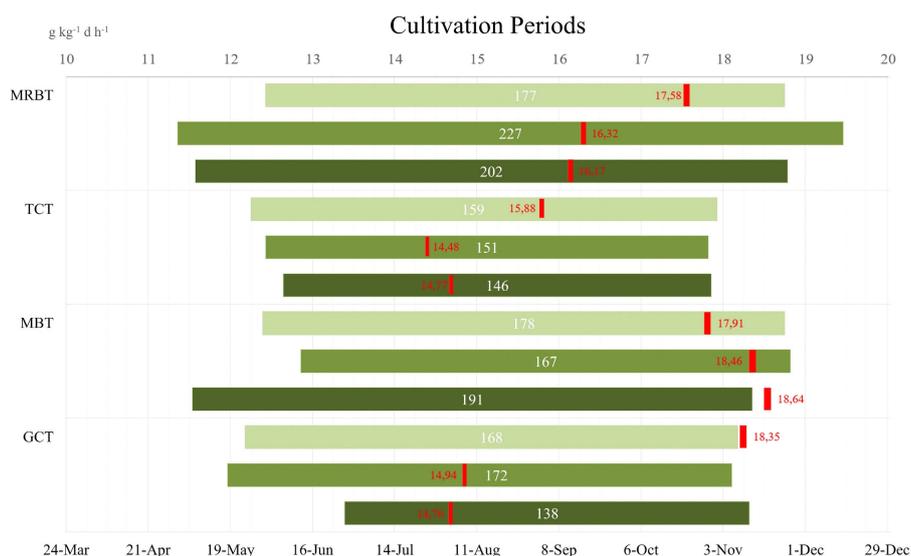


FIGURE 7

The cultivation periods of the four different cultivation techniques: gutter cultivation technique (GCT), mound bed technique (MBT), mobile raised bed technique (MRBT), and trench cultivation technique (TCT) over the three years (2022, 2023, 2024) with the date of planting and harvesting. The white numbers in the bars were the duration of vegetation in days. The red bars describe the correlation between the yield of secondary metabolites (g kg^{-1}) per individual number of hours of sunshine (h) per vegetation period in days (d).

TCT < GCT. Nevertheless, the average tuber weight per cultivation method varied in some cases due to shorter growing periods or influential weather conditions, which than affected the amount of work required per kilogram of tubers. This could have distorted the value for some cultivation techniques, as, for example, harvesting was easier, but the smaller tubers required more work per kilogram of tubers due to the increased working time involved. Hence, the amount of work required per harvested tuber was in absolute numbers in the order of MBT < TCT < MRBT < GCT. The material costs per kilogram of harvested tubers were lowest for MRBT, followed by MBT, TCT, and finally GCT, which was twice as expensive as MERB.

4 Discussion

In the present study, the outcomes derived from the observation period and the innovative cultivation methods employed for *D. polystachya* exhibited variability. The substantial variations documented within the individual cultivation techniques, particularly during the initial cultivation year (2022) were attributed to the inherent biological characteristics of the crop. This fluctuation in the secondary metabolites is a critical factor in the production of herbal tinctures, as evidenced by the pharmacopoeia (Schmiedel and Co., 2023). It is also important to note that environmental conditions changed from year to year throughout the project period and led to different amounts of labor costs, which usually were the main cost factor (Izekor and Olumese, 2010). In 2022, the year was marked by above-average temperatures and ample precipitation during the summer months, with numerous hours of sunshine. This period of mild weather

persisted until October, creating favorable conditions for the cultivation of yam tubers, which typically span from late May to late November. These climatic conditions appeared to be among the most conducive for the growth of Chinese yam plants as well as other yam plants (Yusuf et al., 2020). The subsequent year, 2023, presented a more varied set of weather conditions. The growing season commenced as from May with significant precipitation, including thunderstorms. As the season progressed, there was an increase in temperature and a decrease in precipitation. Toward the end of summer, heat and drought changed into a rise in the frequency of heavy rainfall events, while October was characterized by a golden, warm period. This was followed by again further heavy rainfall events, which delayed the harvest in some cases. Excessive precipitation could also be a critical factor for crop yield (Endo, 2024). The timing of the harvest affected the vegetation period, with a longer or shorter period depending on whether the harvest took place before or after the rainfall. The frost and snow around the beginning of December led to a later harvest of the MRBT tubers. Frosty temperatures tended to have a negative effect on the yield of secondary metabolites. Hence, a study on the influence of cold temperatures on yam cultivation in Japan recommended to cultivate in a greenhouse (Sato et al., 2024). In the final year of the observation period, 2024, the region experienced abundant rainfall, and the summer season was comparatively cooler in relation to the preceding two years. In general, this necessitated the strategic scheduling of planting and harvesting activities in accordance with prevailing environmental conditions. The impact of weather conditions was also investigated in the largest yam-growing region in Nigeria. Moreover, the main challenges were often attributed to the presence of excessive heat and erosion (Jibrin et al., 2024). New adaptation strategies are

equally necessary for future cultivation of yam, both from a nutritional perspective and from a socioeconomic perspective not only in Nigeria.

The influence of the hours of sunshine and the duration of vegetation on the secondary metabolites is demonstrated in Figure 7. The mean values suggested that there was a discrepancy among different cultivation types, contingent on the duration of vegetation and the number of sunlight hours. The latter differed due to the varying planting points over time, which cannot all be realized simultaneously due to operational and weather conditions. The calculation of the factor led to an approximate leveling. It brings the duration, the influence of the weather, and the yield of the secondary metabolites into relation. The observed variations in the contents are presumably attributable to other influencing factors, such as precipitation, potential shade, and other natural factors. Due to previous examinations of *Dioscorea zingiberensis*, they could be considered as an important issue (Hou et al., 2021). On the one hand, the GCT value in the initial year was notably higher than in subsequent years. This occurrence was attributed to the significant presence of peels of yam tubers, a topic that will be elaborated upon subsequently. Nevertheless, the amounts of secondary metabolites in the peel of *D. polystachya* were close to the investigations in the peel of *Dioscorea alata* with 5.2–11.0% and 8.00–11.50%, respectively (Yeh et al., 2013). On the other hand, in the absence of this single value, the mean value of all samples approached equivalence.

The following section will provide a detailed exposition of the cultivation techniques and its impact on primary and secondary metabolites employed.

4.1 MRBT

The MRBT exhibited a substantial workload. The logistics of setting up and filling the boxes with soil were challenging, time-consuming, and expensive, partly due to the amount of driving with tractors. The boxes, which were opened downwards, were sometimes with and sometimes without a grid on the (meadow) ground. In some instances, the tubers exhibited minimal penetration, growing only a few centimeters into the underlying soil. The overall effort required over the three-year period was substantial, surpassing that of the traditional closed boxes. The cultivated tubers exhibited a decline in the content of extracted metabolites over the observation period. The environmental conditions previously described, particularly in the third planting year (2024), appeared to have exerted a significant influence on these observations. The growing season in 2023 had a duration of 227 days; although this was longer than the previous year, the impact on the production of secondary metabolites was not substantial. This was probably due to the cold temperatures at the end of the year (Sato et al., 2024). Nevertheless, the onset of the vegetation was likely to have commenced at the end of May since the temperatures increased (>20°C), when all the other techniques were planted. Additionally, it is plausible that the occurrence of frost at the end of the year contributed to the reduced secondary

metabolite concentration post-harvest. It is generally known that Chinese yam from the tropical and subtropical *Dioscoreaceae* family exhibit greater resilience than other *Dioscorea* species (Srivastava et al., 2012; Srivastava et al., 2016). However, it is possible that the temperature fell below a critical level that impaired the synthesis of metabolites (Nievola et al., 2017). When these two factors are considered, the actual vegetation period and production of plant constituents were significantly shorter, thereby making them plausible in relation to the others. This was also shown by the value of yield per hours of sunshine and per vegetation days, which was nearly equal in the last two years. The same effect was observed in 2024 and also with other *Dioscorea* species (Okongor et al., 2021). The environmental conditions were agreeable at the end of May, whereby the vegetation period of 202 days did not correlate with the amounts of secondary metabolites. The realistic vegetation duration was apparently shorter. The yield of compounds was shown to be dependent on a number of factors, as illustrated by the available data and the previously described meteorological information. Furthermore, it can be hypothesized that the constrained space and the greater resistance of the firmer ground compared to the loose soil led to elevated levels of stress for the plants, thereby prompting the increased formation of secondary plant substances. These phenomenon were previously documented in other underground-growing organisms, such as Korean ginseng (Hwang et al., 2024).

4.2 TCT

The findings of the present study demonstrated that quartz sand and quarry sand had a deleterious effect on the secondary metabolite content. In all three years, the yield of ethanol-soluble plant substances was lowest in the presence of quartz sand and quarry sand. Whereas opposite results were observed regarding total and resistant starch content. Phenotypically, the leaves of these techniques exhibited a reduced intensity of green coloration. Despite augmented fertilization in the second and third year, the content declined once more in the third year following a modest uptick in the second vegetation period. This decline was exacerbated by the wet environmental conditions observed in 2024. The uptake of possible trace elements and nitrogenous substances appeared to be reduced because of lower naturally amount of these in sandy soils (Guo et al., 2023). The lowest amount of ethanol-soluble plant constituents was extracted from the red quarry sand. All three methods were generally analogous, and the range of standard deviations from the mean value were nearly equivalent. Moreover, it was the lowest in absolute terms for the three methods described. Nevertheless, the implementation of these techniques facilitated yielding, as the harvest process was limited to the removal of the top layer, enabling the subsequent harvesting of the tubers. However, it is imperative to note that this step must be executed with the required degree of caution as tubers are fragile. In other regions such as Japan or China, deep, stone-free soils are typical, as they were artificially replicated here in the TCT. In such areas, it was possible to use machines for the planting and

harvesting, which could significantly reduce labor costs and time (Nair, 2023; Xiao et al., 2023). The remodeled soil in the current study was one reason why the amount of work involved was lower than with the conventional method, but still relatively high due to the manual labor. The three types of soil that were tested accounted for the majority of the material costs. If the soil is deep but very sandy, it is easy to work but requires more irrigation and fertilization and thus labor time (Endo, 2024). The third variant of TCT with humus soil (TCT-HU) showed another trend. It was observed that the content of secondary metabolites was significantly higher in the first season and, as similar with all other tubers from the other techniques tested, the content leveled off in the second and third year. One potential explanation for this decline is the substantial impact of summer precipitation in 2024 on secondary metabolic activity, attributable to increased growth patterns involving thickness and length (Alemayehu et al., 2023). The hypothesis that the increased nutrient and nitrogen content of TCT-HU soil led to elevated biosynthesis of secondary plant compounds was supported by evidence from corresponding studies with other plants (Bustamante et al., 2020). Although the vegetation periods were quite similar over the three years, a decreasing tendency towards a shorter planting period was observed, but correlated just indirectly with the amount of secondary plant ingredients. It had also been stated that high nitrogen as well as potassium and phosphorus levels negatively affected starch concentration in fresh potatoes and sweet potato cultivars (Liu et al., 2017; Koch et al., 2020). Therefore, the putative lower macronutrients could be responsible for higher TS content in tubers grown in TCT with quartz sand and red quarry sand, respectively. Soil samples were taken and their test results supported the assumption of lower macronutrient concentration in quartz sand and red quarry sand than in humus soil. The consistent values of the correlation between vegetation period, sunshine hours and yield of secondary metabolites showed the equality within the technique. However, more relevant was the influence of the construction of the trench. The installation in the soil was advantageous due to the fact that the temperature and other environmental conditions played a lesser role. Depending on the depth, the amplitude of the temperature was reduced. This is particularly important for the compounds and growth of *D. polystachya*, especially in cold temperatures (Horn, 2016).

4.3 MBT

The installation of the MBT were accompanied by difficulties in 2022. The soil was very compact and firm, which were not conducive to optimal conditions for the proliferation of thick tubers and consequently impeded the processes of planting and harvesting. Nevertheless, the results for secondary plant metabolites were the second highest in the first year. One reason for this could be the increased stress for the plant, which then reacted with increased production of secondary plant metabolites (Sharma et al., 2022). In principle, building a mound was less time-consuming than filling the boxes, for example. However, it is

imperative to consider logistics in order to ensure the efficacy of the process. Conventionally, this method of building small mounds was employed for yam cultivation, especially for personal consumption. Preferably, species exhibiting smaller tubers than those observed in *D. polystachya* were more widely utilized (Martin, 1972). This had historically posed a challenge in terms of achieving optimal effectiveness, as the distance between plants had to be adjusted in accordance with the size of the tuber with consequence to planting and harvesting. For this purpose, a single mound was piled up per plant by the farmers, especially for species with large tubers (Wumbei et al., 2022). This configuration is less economical and more labor intensive than an elongated mound, as evidenced in the present study, although initial difficulties occurred in installation. From the second year onward, the mound structural integrity was enhanced to ensure an even and less compacted soil mixture. Despite these modifications, the total content remained relatively constant, exhibiting only a modest decline of 1.5% in 2023. The plastic film covering the mound and its soil had a positive effect on this increased secondary metabolite production. This polypropylene film was permeable to air, nutrients and water, but kept the soil moist and prevents weed growth. As a result, more nutrients were available to the yam plant. The increased production of secondary metabolites mentioned above was partly due to the higher soil temperatures caused by the black film and had also been described for other crops (Chen et al., 2024). The increased soil moisture and reduced risk of erosion were good for soil health. This was a considerable problem in conventional cultivation to date (Wumbei et al., 2022). On one hand, mulching with a plastic film was costly and a traditional mulching labor intensive, particularly for single mounds. Based on the physical appearance, traditional mulch was subject to wind and could be washed away on the slope. On the other hand, mulching protected the soil against erosion. This approach was addressed in the design of the mound bed in the current study. That led into less costs due to soil protection and easier harvesting. The latter was feasible after furling the film and the use of engaged machines, which removed the soil until the planting line of the tubers. A sustainable increase may be achieved by switching from traditional agricultural film to a jute or hemp material (Marasovic et al., 2024). The vegetation period exerted minimal influence on this cultivation technique. The fluctuations in the three distinct planting times did not translate into variations in the yield of secondary constituents. As demonstrated in the third period of MRBT, the conditions in the first weeks of vegetation in 2024 were suboptimal for growth and, consequently, the production of secondary metabolites. Thus, the long cultivation time did not align with the quantity of plant compounds, except when the initial days were subtracted. In the first and second year of cultivation, the planting time correlated with the amounts of secondary metabolites produced such as allantoin and gracillin. However, looking at the quotient from the correlation of yield, sunshine hours and vegetation days, the MBT had a constantly high value. A study on the productivity of yam tuber-based systems indicated that the effort was worthwhile despite weather dependencies and higher labor costs compared to other crops (Maliki et al., 2012). This cost analysis provided a useful frame of reference, particularly given that

the expenses incurred were among the lowest for MBT, although it should be noted that the initial material costs were marginally higher due to the plastic film. At the same time, MBT had the highest yield of secondary metabolites.

4.4 GCT

The GCT exhibited the highest content in the initial year, attributable to the substantial thicker peel. Although only one genotype was used, conceivably the cultivation technique caused this phenotypic change. As evidenced by the other trials, the majority yield of secondary metabolites was presented in and beneath the peel (Yeh et al., 2013; Liu et al., 2016; Poirier et al., 2018). Additionally, the optimal slope was not a subject of investigation in 2022 during the initial trials. The approximate 5° planting angle, which was determined to be inadequate for the diagonal downwards growing mechanism through the geotropism exhibited by the plants, likely contributed to the observed branching pattern in the harvested tubers, with some branches exhibiting upward growth. While this distribution of growth was advantageous in terms of enhancing the yield of secondary metabolites, it presented challenges in marketing as well as consumer preferences and first of all during processing. This was due to the complexity introduced by numerous branches and the resultant interstices. The complexity hindered the efficient cleaning of the soil and sand, as well as the effective processing of the tubers. Therefore, beginning in 2023, the angle was adjusted to a steeper inclination of 10–15°. Thereby restricted the growth of the tubers to a single direction. A similar approach was taken in a field trial in China (Xiao et al., 2023). However, the authors of the study did not encounter any problems with tuber branching due to an initial angle of 10–15° of planting the tubers. They reported higher water consumption and lower temperatures with this technique, as the tubers grew very close to the surface. These limitations were not observed in our study. Their comparison with the trench cultivation method, which they chose as a reference, was also different. While they concluded that GCT cultivation saves time and labor, on the presented study, the labor required per kilogram of harvested tuber was sometimes twice as high with GCT as with TCT. The phenotypic change, whereby branching above the tuber on the shoot axis decreased when planting was almost horizontal compared to vertical growth, could not be observed. The results are consistent with the experiments on *Dioscorea opposita* (Kawasaki et al., 2014). In contrast, a change was noted in the tuberous plants *Ipomoea batatas* and *Manihot esculenta* (Dlamini et al., 2021; Nair, 2023).

Due to the high percentage of peel in the first year, which was a direct result of the many branches in the tuber, the yield could not be interpreted in relation to the growing period. Nevertheless, a comparison of the second year with the third cultivation year demonstrated a correlation between the production of secondary metabolites and the duration of the planting season. Both exhibited a similar quotient of 0.62 and 0.67 g kg⁻¹ d⁻¹, respectively. The same was further substantiated by the quotient of yield per sunshine hours per vegetation day. In the most recent cultivation year,

planting could only be initiated at a significantly delayed schedule. This delay was attributed to the persistent precipitation and the consequent saturation of the soil, which impeded the preparation of the slope for the installation of the gutters. Consequently, the vegetation period was shortest, and because of this and among others the yields were lower than in previous years. Moreover, this was also applicable to the starch content.

The highest time expenditure per kilogram of harvest was determined for a single value at GCT. However, in comparison with the conventional method, this approach resulted in a slightly elevated workload. The findings of the *Dioscorea persimilis* study diverged from the aforementioned results (Xiao et al., 2023). In this instance, an advantage over the conventional method was observed, primarily attributable to the potential utilization of machinery. Due to the growth of the tuber close to the surface, the use of machines could also be considered in the future (Martin, 1972). Excluding the initial year in the current study, during which the processes were still being established, both methods necessitated nearly equivalent levels of effort. With the difference that GCT resulted in a slightly higher content of secondary metabolites.

All of the techniques were based on a slightly modified version of the “minisett” technique. This had proven to be particularly efficient and saved money and resources (Eytayo et al., 2010). One small difference was that, due to earlier trials at Andreashof, the cuttings were calibrated in such a way that it was not necessary to wait a second planting year before harvesting. Therefore, there was still sufficient tuber material to use. As described in literature, for yam, the predominant cost driver was labor expenses, which were highest on average per tuber for GCT, while the yield of secondary metabolites was marginally lower than that of MBT on average (Izekor and Olumese, 2010). In sum, the cultivation of yam could be a worthwhile endeavor from a socioeconomic and monetary perspective, not only in subtropical regions, but also in Germany (Idumah and Owombo, 2020).

A comparison of the new cultivation techniques with the reference powder revealed significant advancements, particularly the MBT that exhibited the greatest superiority across all three years. Specifically, it exhibited a 56.6% increase in the first year, a 54.1% increase in the second year, and a 36.5% increase in the third year compared to the reference method. GCT demonstrated considerable promise as well. The highest individual values of ethanol-soluble secondary metabolites were measured in this samples, as the peel was thicker due to the guideway in the soil and the use of just one genotype (Pérez et al., 2011). The precise angle and potentially the correct gutter must be examined in greater detail in this instance. In the context of its application in Traditional Chinese Medicine, there is a potential to enhance the health-promoting compounds and perhaps isolate them sufficiently.

The calculated ratio of the amounts of secondary metabolites, sunshine hours and vegetation days allowed an attenuated comparison of the different cultivation methods in order to take into account the non-obvious differences of the individual planting and harvesting points. It showed the equality of the respective method. The variations were due to the absence of other influences such as rainfall, fertilization, temperatures and others (Pant et al., 2021).

5 Conclusion

DP is a plant of pharmaceutical and nutritional interest. Its utilization as a potential food plant is being promoted in Germany by the Federal Ministry of Education and Research (BMBF), among other entities. In contrast to the extensive optimization of most food crops, the cultivation of Chinese yam remains underutilized. This is of critical importance, as the process is arduous, laborious and costly, not least due to its unique growth characteristics. A scientific approach was adopted for the purpose of monitoring the cultivation process over a period of three years. In addition, four different cultivation methods and three soil types were subjected to examination. This scientific monitoring and testing program yielded important findings. The content of primary metabolites, including resistant starch and soluble starch, as well as the content of ethanol-soluble secondary metabolites, was determined. While there was a tendency towards higher total starch and resistant starch in TCT-QS followed by TCT-RG, the opposite was true for the content of ethanol-soluble secondary metabolites. The two cultivation techniques demonstrated the lowest contents of secondary metabolites over the observation period. MBT exhibited the highest secondary metabolites content, followed by GCT, which was positioned at the lowest level in terms of total starch content. It appears that the high nutritional value of these cultivation methods is in competition with the production of secondary plant compounds.

It is evident that weather conditions exert a substantial influence on the cultivation of all food crops, including the four aforementioned techniques. MBT appeared to be the least affected by this, as the annual results fluctuated only slightly. Of particular interest is GCT, whose potential can be further improved, primarily with regard to the utilization of secondary metabolites.

The study suggests that, depending on the objective pursued in cultivation, these results have laid the foundation for improving yam cultivation, which will advance its use as a pharmaceutical, but above all as a food crop. The implementation of this initiative has the potential to enhance food security, particularly in contexts characterized by climate change, by means of biodiversity enhancement.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

Author contributions

DK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. PD: Data curation, Formal analysis, Investigation, Writing – review & editing. JR: Data curation, Formal analysis, Writing – original draft. JA: Data curation, Investigation, Writing – review & editing. RW: Data curation, Investigation, Writing –

review & editing. MB: Data curation, Funding acquisition, Investigation, Resources, Writing – review & editing. JE: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

JA, RW, and MB were employed by Andreashof e.V.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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