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# Growth of *Dioscorea membranacae* Pierre ex Prain & Burkill and dioscorealide B content at different harvest times

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## Summary

Rhizomes of *Dioscorea membranacea* Pierre ex Prain & Burkill, popularly known as Hua-Khao-Yen-Tai, have been used to treat cancer in Thai traditional medicine. The bioactive compound isolated from the rhizome is dioscorealide B (DB), a naphthofuranoxepin which reduces inflammation and exhibits cytotoxic properties against human breast cancer cells. In order to produce rhizomes of sufficient quality for medicinal use, optimal harvest times must be identified. The aims of the current study were therefore to examine growth and DB content of *D. membranacea* rhizomes at differing harvest times. Mother rhizomes (MR) 10-16 cm in length were individually cultivated in 50 cm diameter pots. Shoot growth, rhizome development and DB content were examined at 3-monthly intervals to 15 months after planting (MAP). The number of shoots and dry weight increased significantly throughout the experimental period, while the DB content of the MR was 4.0% w/w at commencement. Degradation of the MR was evident at 9 MAP, with eventual shrivelling at 12 MAP. The daughter rhizomes which formed at 3 MAP and progressively developed throughout the experimental period, exhibited 41.8 g dry weight plant$^{-1}$ and 2.7 %w/w DB content at 15 MAP.

Key words: Dioscorea membranacea, rhizome, dioscorealide B, shoot growth, harvest time

## Introduction

*Dioscorea membranacea* Pierre ex Prain & Burkill belongs to the section *Stenophora* in the family Dioscoreaceae, popularly known as Hua-Khao-Yen-Tai in Thailand (1, 2). Plant distribution ranges from mixed deciduous forests to lower mountain evergreen forest regions of South-East Asia, including Thailand, Vietnam, Laos and Myanmar (3). *D. membranacea* is a rhizomatous perennial, while other members of this family are commonly tuberous perennials (1).

*Dioscorea* spp. typically propagates from specialised vegetative structures (tubers or rhizomes) but can also propagate from true-seeds. The underground shoots, the most useful parts of this genus, may be harvested within one year when yellowing of the tops of the plants, with subsequent complete withering, has occurred (4). Rhizomes of *D. membranacea* have been widely used by Thai traditional doctors as ingredients in many medicinal preparations, including those used in the treatment of dermopathy, lymphopathy, inflammation, cancers, venereal diseases and leprosy (5). The bioactive compound isolated from these rhizomes is dioscorealide B (DB), a naphthofuranoxepin which reduces inflammation and exhibits cytotoxic properties against human breast cancer cells (6, 7).

The natural forests which usually provide the raw materials for traditional Thai medicinal use have now been over-exploited, greatly reducing natural reserves of these plants. Rhizome quality has generally been variable, due to uncontrolled harvesting at various stages of plant maturity, resulting in uneven accumulation of bioactive compounds, with consequent hindrance to the development of medicinal products.

Further, production of *D. membranacea* rhizomes has not been adequately reported. The aim of the present study has been to evaluate growth of *D. membranacea* and rhizome DB content in order to determine optimal harvest times for rhizomes intended for medicinal use.

## Methods

*D. membranacea* rhizomes were collected from forests of Chumphon province, Thailand. The experiment was conducted in a greenhouse at the Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University, Rangsit Campus, Pathumthani, Thailand, from August 2008 to December 2009. Seed rhizomes (mother rhizomes, MR) 10-16 cm in length with sprouted shoots 5-6 cm in length were individually cultivated in 50 cm diameter pots containing 35 kg of growing media consisting of decomposed rain tree leave soil, sand, coconut husks, compost and carbonised rice husks in the ratio of 2:2:2:1:1 by volume.

A slow release fertiliser (Osmocote N-P-K; 14-14-14), was applied monthly at the rate of 10 g $pot^{-1}$. Water was added daily to maintain soil moisture levels and promote optimal plant growth. The plants were harvested at three month intervals, from 3 MAP to 15 MAP, and the numbers of green and withered shoots were recorded. Shoot withering was indicated by drying and yellowing of the leaves and stem of the shoot. The dry weight of shoots was determined by drying at 70°C for 72 hours. The MR and daughter rhizomes (DR) were then harvested, washed, thinly sliced, dried at 50°C for 48 hours and powdered for DB and dry weight determination.

DB determination was performed as described by Sirikatitham et al. (8). For extraction, 40 g of dried powdered rhizome were macerated with addition of chloroform. The plant extract was concentrated to dryness under reduced atmospheric pressure. Sample extract solutions were prepared by accurately weighting 10 mg of plant extract and adding acetonitrile. The solution was sonicated for 15 min and allowed to cool at room temperature, then filtered through a 0.45 ìm membrane.

The sample solutions, of 10 µl, were directly injected into a HPLC column and separated under chromatographic conditions. HPLC analysis was performed by means of a ConstaMetric 4100 BioQuarternary pump (TSP, USA) equipped with a

SpectroMonitor 4100 UV-vis detector (LDC analytical, USA). Data acquisition, processing and analysis were facilitated using TSP PC1000 software (TSP, USA).

Chromatographic separations were achieved at room temperature using a reverse-phase analytical column, Luna C18 (250 mm × 4.6 mm i.d., 10 µm) (Phenomenex, USA) with a guard column of the same material. A solvent gradient system comprised of water (A) and acetonitrile (B), was employed according to the following schema: 70-55% A at 0-10 min, 55% A for 5 min, 55-30% A at 15-30 min, 30% A for 5 min, 30-70% A at 35-37 min, 70% A for 8 min. The flow rate of the mobile phase was 1.0 ml/min. All chromatographic separations were achieved at room temperature. The UV detector was used to identify the DB peak at a fixed wavelength of 270 nm.

The data derived from plant cultivation and DB determination were expressed as the average of three replicates.

## Results

### Shoot development

A single shoot with folded leaves appeared 15 days after planting (Figure 1A). This shoot subsequently elongated, climbed and twined to the left, with expanded leaves (Figure 1B). The number of shoots markedly increased from commencement of the experiment, from 2.3 at 3 MAP to 9.7 at 15 MAP. However, the initial single shoot became progressively yellow, withering becoming evident from 6 MAP and the number of green shoots ranging from 2.0 to 3.7 throughout the experimental period (Figure 1C and 2).

Shoot dry weight gradually increased during the first 9 months, after which the plants developed rapidly, achieving a maximum shoot dry weight of 1,654.0 g plant$^{-1}$ at 15 MAP. Total dry weight development showed a pattern similar to that of shoot dry weight (Figure 2).

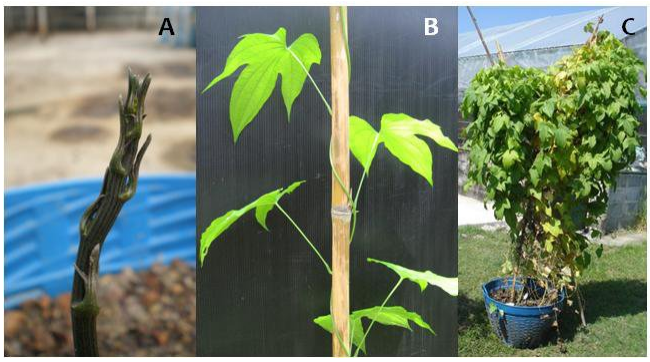


Figure 1 Shoot development of *D. membranacea.* A: beginning of experiment, B: shoot twining to the left and C: plant with withered shoots at 15 months after planting.

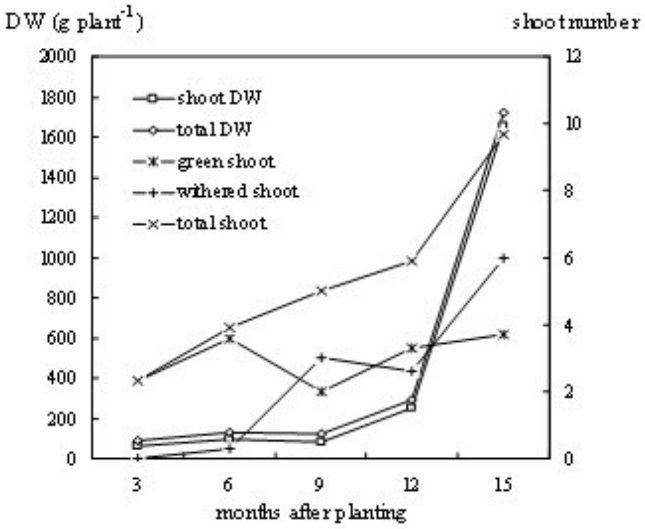


Figure 2 Dry weight (DW) content and number of shootsof *D. membranacea* at different harvest times.

### Rhizome development

DR formed at 3 MAP (Figure 3A) by elongating from the growing point at the terminal end of the MR, with concomitant appearance of roots and shoots. Development of DR was steady during the first 9 months, with each plant being less than 2 g dry weight (Figure 3B, 3C and 4). Subsequently, the DR rapidly enlarged, dry weight increasing to 41.8 g plant$^{-1}$ at 15 MAP. However, the rhizomes remained immature, as indicated by the presence of soft white tissue with light brown skin (Figure 3D). The MR began to degrade at 9 MAP, eventually shrivelling between 12 and 15 MAP (Figure 3C and 3D).


Figure 3 Mother rhizomes (arrowed) and daughter rhizomes of *D. membranacea*.
A: 3 months after planting, B: 6 months after planting, C: degradation of mother rhizomes (arrowed) at 9 months after planting, D: shrivelled mother rhizomes (arrowed) and daughter rhizomes at 15 months after planting (roots removed).

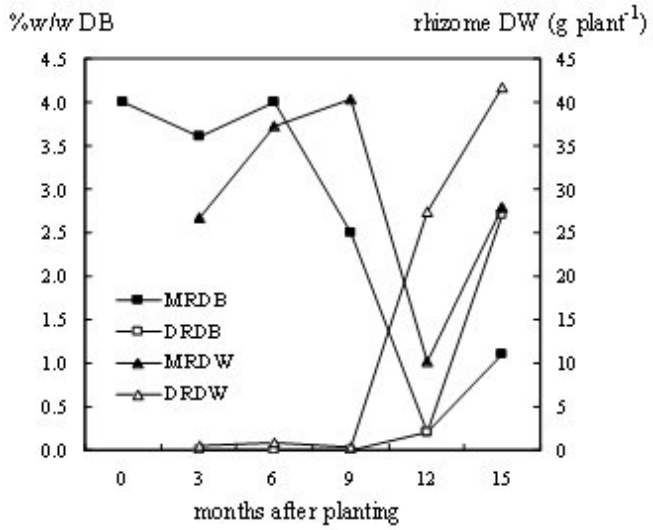


Figure 4 Dioscorealide B (DB) content and dry weight (DW) in mother rhizomes (MR) and daughter rhizomes (DR) of *D. membranacea* at different harvest times.

### DB content

The DB content of the MR was found to be 4.0 % w/w at the commencement of the experiment, it was consistently during 6 MAP as 3.6 to 4.0% w/w were observed at 3 and 6 MAP, respectively. However, these values subsequently sharply declined to 0.2 and 1.1 % w/w at 12 and 15 MAP, respectively. The DB value of the DR could not be determined from 3 to 9 MAP because of slow plant development, resulting in a minimal dry weight value. The DR dry weight then increased, while retaining a low DB content of 0.2 and 2.7 %w/w at 12 and 15 MAP, respectively (Figure 4).

## Discussion

Plants generally exhibit sigmoidal growth patterns. With respect to specialised vegetative structures such as bulbs, corms, tubers and rhizomes, plants exhibit a period of slow growth during establishment, followed by a phase of rapid (exponential) growth, finally declining in growth rate as canopy senescence approaches and underground stems attain maximum weight and volume (4, 9). The growth of *D. membranacea* conformed to this expected pattern, with shoot and DR dry weight slowly increasing during the first 9 months and more rapidly during the next 6 months, with dry weights increasing predominantly in shoots at 15 MAP (Figure 2 and 4). In this study, the final phase of decline in growth was not reached for either shoot or DR dry weight. Although green shoots were successively initiated from 3 MAP, they progressively withered and dried from 6 MAP. In tropical regions, shoots of rhizomatous plants usually die down during the dry season, when harvesting normally occurs (10). In this study, at least two shoots of each plant remained green throughout the experimental period, perhaps as a consequence of the frequent irrigation, promoting further shoot development. The rapid shoot growth after 9 MAP resulted in a higher accumulation of photosynthetic products, which may have subsequently migrated to the DR, significantly increasing their dry weight, while the MR simultaneously shrivelled and decayed. However, the DR remained immature, as indicated by the appearance of soft white tissue with light brown skin at 15 MAP (Figure 3D).

While these results suggest that *D. membranacea* has a growth cycle for mature rhizome development of longer than 15 MAP, they are not, however, consistent with reported growth cycles of other members of the genus *Dioscorea,* in which under-

ground stems are typically harvested within one year after the tops of the plants die off. Harvest times of *D. rotunda,* for example, vary from 140 to >250 days, depending upon plant variety (4, 11) while those of *D. alata* vary from 93 to 176 days (12). Cultivated species of these plants were grown for their tubers (4, 13), whereas the ground organs of *D. membranacea* were rhizomes (1). Consideration of such data, together with the fact that DB content in the DR remained low as 2.7 % w/w at 15 MAP compared to 4.0 %w/w DB (Figure 4) in the MR, suggests that different species and sections of these plants may require unique optimal harvest times.

## Conclusion

*Dioscorea membranacea* Pierre ex Prain & Burkill exhibited a characteristic perennial habit, with a growth cycle exceeding one year. Dioscorealide B content was low and the rhizomes remained immature at the final harvest. The results suggested that the optimal time for harvesting mature rhizomes of *D. membranacea* for medicinal use should be done beyond 15 months after planting.

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