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Comparative Characterization of Peanuts Grown by Aquatic Floating Cultivation and Field Cultivation for Seed and Resveratrol Production

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Peanut pods (Tainan 12, a Spanish cultivar, *Arachis hypogaea* L.) have been obtained from peanuts grown in a newly developed aquatic floating cultivation system without artificial aeration or periodic renewal of the solution. The system provided a convenient status for examination of root and pod development. Compared to field-grown peanuts of the same cultivar, the aquatic-cultivated peanut pods and seeds were smaller, whereas seed/pod weight ratios, crude fat and protein contents, and SDS-PAGE protein patterns varied within similar ranges. During cultivation, the highest detected temperature of the aquatic solution was higher than the field-soil temperature. After gas chromatographic analysis of the fatty acid compositions, the oleic acid/linoleic acid ratio of the aquatic-cultivated seeds was higher than that of field-cultivated ones. When the peanut roots were collected, cleaned, dried, weighed, pulverized, and subjected to resveratrol analysis, dry root weights were 4.2 ± 0.1 and 2.2 ± 1.1 g/plant and resveratrol contents were 0.074 ± 0.009 and 0.114 ± 0.212 mg/g for the aquatic- and field-cultivated peanut roots, respectively. This indicates that the aquatic-cultivated peanut roots could be a potent and consistent source of resveratrol.

KEYWORDS: ***Arachis hypogaea*** **L.; aquatic floating cultivation; peanut roots; resveratrol; SDS-PAGE; fatty acid composition**

INTRODUCTION

For aquatic cultivation of most plants, air aeration or artificial circulation of the nutrient solution to enhance oxygen dissolution is generally required. A recirculating hydroponic system using a nutrient film technique to facilitate aeration has been developed for the aquatic cultivation of peanuts (*1*−*3*). In that system, normal peanut growth and pod development have been obtained. Normal peanut pods have also been raised in darkened and artificially aerated nutrient solution (*4*). In the literature, aquatic cultivation of peanuts without artificial aeration or circulation of the nutrient solution has been scarcely investigated. In comparison to a soil-cultivated system, an aquatic-cultivated system is feasible to examine the intact root and pod development of peanuts, and mature pods can be successively harvested over time.

Peanut roots collected in the field after peanut pod harvesting could be a potent source of resveratrol; however, the contents deviated tremendously between growing seasons and among samples (*5*). Resveratrol is a phytoalexin and usually produced after external stimulation. It is obvious that each peanut plant in the field would encounter its own circumstance and accordingly produce different amounts of resveratrol. By an aquatic cultivation system, all plants are more likely to have a similar growing circumstance and consistent products. Nevertheless, peanut is a common crop, and facilities equipped for artificial aeration for aquatic cultivation are costly unless a product with high added value is expected. In this study, an approach was attempted to develop a practical and inexpensive aquatic cultivation system to produce peanut seeds for food use and roots for resveratrol extraction. On the basis of the results of preliminary experiments, Tainan 12 (a Spanish cultivar) was selected and grown through punctures of a round polylon plate floating on an aquatic solution. Concurrently, peanuts of the same cultivar were grown in an experimental field for comparison. During cultivation, peanut pod and root formation in the solution were visually examined. Mature pods were harvested for comparative characterization, and peanut roots were collected for determination of resveratrol.

MATERIALS AND METHODS

Peanut and Cultivation. In an attempt to develop an aquatic cultivation system with which to provide a simplified and controlled situation to avoid daily watering, artificial aeration, and solution renewal, extensive preliminary trials with various cultivars, growing

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Figure 1. Aquatic floating cultivation of peanuts: (A) peanuts grown on round polylon plates floating on Murashige–Skoog solution in china buckets; (B) a polylon plate fluctuated depending on the solution level.

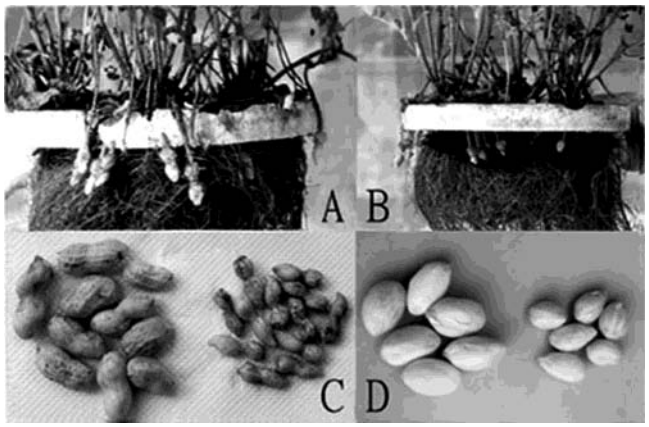

Figure 2. Peanut pod development in aquatic floating-cultivated system and comparison of the aquatic- and field-cultivated peanut pods and seeds: (A) peanut pods originated from pegs passed through clearance between the edge of the polylon plate and the internal wall of the bucket; (B) peanut pods originated from pegs penetrated through the polylon plate; (C) field-cultivated (left) and aquatic-cultivated (right) peanut pods; (D) field-cultivated (left) and aquatic-cultivated (right) peanut seeds.

conditions, and cultivation methods have been done in our laboratory. Accordingly, Tainan 12 (*A. hypogaea* L.; a Spanish cultivar) was selected and cultivated in this study. A series of china buckets (27 cm diameter and 30 cm height) were filled with Murashige−Skoog (MS) solution (1.25% in tap water of the recommended concentration) (*6*). Three 1-week-old peanut seedlings were respectively planted into three punctures of a round polylon plate (2 cm thickness) floating on the MS solution without artificial aeration (**Figure 1**). The buckets were placed in a tunnel-type greenhouse (3 m width, 5 m length, 2 m wall height, and 2.5 m roof height; with a transparent polyethylene plastic roof and white nylon-net walls) for cultivation on February 21, 2000. The solutions were not renewed, and water losses were replenished with tap water weekly. Conductivity of the aquatic solutions was measured every 2 weeks with a conductivity meter (model SC-120, Suntex Instruments Co., Ltd.) and maintained at 1.2−1.5 ohm/cm by addition of a Hyponex powder (no. 1, Hyponex Corp., Marysville, OH). Mature pods, depending on the pod color and hardness checked by fingers, were harvested two times on the 120th and 127th days after cultivation. For comparison, the same cultivar of peanut seeds (Tainan 12) were planted in an experimental field of the National Chiayi University on March 1, 2000, with normal cultivation practice and harvested on the 110th days after cultivation (70 days after flowering) (*7*). The distance between the greenhouse and experimental field was ∼200 m.

For comparison, only the two-seed pods collected from the aquatic- and field-cultivated peanuts were air-dried in the greenhouse for 10 days. Length, width, and weight of each individual pod were measured (**Figure 2C**). After shelling, the seeds (kernels) were weighed and seed/pod ratios were determined (**Figure 2D**). Both peanut seeds were stored at −25 °C for compositional characterization.

Peanut roots were collected, cleaned with tap water, and dried at 40−45°C in a forced-air oven. The dried roots were weighed, pulverized, and stored at −25 °C for resveratrol analysis. The dry matter content of the roots was determined by further heating samples at 105 °C until constant weights were reached.

Temperature of the Aquatic Solutions and Field Soils. The highest and lowest temperatures of the aquatic solutions and field soils in the late stage of cultivation, prior to harvest, were determined in late June 2000. For the aquatic solutions, two maxima−minima thermometers were respectively placed beneath the polylon plates of two cultivation buckets, and another one was placed at a shaded place in the greenhouse. The temperatures were recorded every 2 days and readjusted for the next determination. In the field, three maxima−minima thermometers were buried 10 days prior to harvest in the position of the geocarposphere zone (∼15 cm beneath the surface soil), and one was hung on the peanut vines. The former temperatures were recorded when peanuts were harvested, and the latter was recorded and adjusted as for aquatic solutions.

Compositional Analyses and Protein Electrophoresis. Percentages of moisture, crude fat, and crude protein (determined by multiplying the total nitrogen content by 5.46) contents of the peanut seeds were determined according to AOAC methods 950.46, 960.39, and 928.08 (*8*). For fatty acid analysis, peanut oil expressed from the deskinned peanut seeds was subjected to gas chromatography following the procedure reported by Chiou et al. (*9*).

For protein electrophoresis, peanut seeds were deskinned and hearts were removed. Cotyledons were ground with a cyclone mill, defatted with precooled hexane (−25 °C) by a homogenizer (5000 rpm, 2 min) (ACE homogenizer, Nihonseiki Kaisha Ltd.), and filtered through a filter paper to prepare defatted powder. The protein content of the powder was quantitated following the determination of nitrogen content described above. Proteins were extracted from the defatted powders (aliquots containing 25 mg of protein) and subjected to SDS-PAGE analysis according to the procedures of Laemmli (*10*) and Chiou et al. (*11*). Resveratrol content in the peanut roots was determined following the procedure reported by Chen et al. (*5*).

Replicates and Statistics. At least duplicate experiments were conducted, and means of the determinations with standard deviation are reported.

RESULTS AND DISCUSSION

When peanuts were cultivated by the aquatic floating system, they grew normally with abundant roots developing in the solution (**Figures 1** and **2**). Because the round polylon plate with peanut plants could be removed from the buckets, it was feasible to examine the process of root and pod development during cultivation. In field cultivation, peanuts flower above the soil, and after fertilization, a peg originates from the base of the ovary and elongates with the ovary at its tip down into the soil to form a pod (*12*, *13*). The literature indicates specific circumstances required for pod enlargement. Light was found to enhance peg elongation while inhibiting pod formation in vitro (*14*, *15*). Darkness and mechanical stimulus are essential to induce pod formation. In particular, a mechanical stimulus is essential in addition to darkness for the normal thickening and diageotropic orientation of peanut pods. In this experiment, some peanut pegs passed through the clearance between the round polylon plate edge and internal wall of the bucket (**Figure 2A**) and some directly penetrated through the tough polylon plate to form normal pods in the solution (**Figure 2B**). This demonstrated that mechanical stimulus was not a prerequisite for peanut pod formation. This was in agreement with the reports of Zharare et al. (*4*), who harvested peanut pods of cv. TMV-1 by cultivation in an aerated nutrient solution, and Wu et al. (*3*), who grew peanuts in hydroponic chambers using a recirculating nutrient film technique.

In general, 8−15 peanut pods including one- and two-seed pods were harvested from each peanut plant. When the harvested

Table 1. Size and Proximate and Fatty Acid Compositions of the Aquatic- and Field-Cultivated Peanut Pods and Seeds

	characteristics and determinations[a]	
	aquatic cultivation	field cultivation
pod length, cm	2.0 ± 0.2	2.6 ± 0.4
pod width, cm	0.4 ± 0.1	1.0 ± 0.2
pod weight, g	0.7 ± 0.1	2.5 ± 0.5
seed/pod weight ratio, %	64.6 ± 0.1	68.4 ± 0.2
seed moisture content, % (wb)[b]	5.0 ± 0.9	5.1 ± 0.5
protein content, % (wb)[b]	27.1 ± 1.1	26.9 ± 0.9
lipid content, % (wb)[b]	35.9 ± 1.2	36.3 ± 0.7
fatty acid composition, %[c]		
palmitic acid (16:0)	10.87 ± 0.70	11.57 ± 0.90
stearic acid (18:0)	4.40 ± 0.62	3.16 ± 0.31
oleic acid (18:1)	48.93 ± 1.55	38.43 ± 0.28
linoleic acid (18:2)	26.64 ± 2.66	36.52 ± 0.38
arachidic acid (20:0)	1.64 ± 0.22	1.20 ± 0.16
eicosenoic acid (20:1)	0.98 ± 0.09	0.80 ± 0.03
behenic acid (22:0)	2.50 ± 0.17	2.16 ± 0.34
lignoceric acid (24:0)	1.30 ± 0.13	1.04 ± 0.03
O/L (oleic/linoleic) ratio	1.84	1.05

[a] Mean of at least three determinations with standard deviation. [b] wb, wet basis. [c] Percentage of total peak areas of detected fatty acids in gas chromatography.

mature pods were air-dried and shelled for comparison (**Figure 2C**,**D**) regardless of the lower pod production yield, average length, width, and weight of the aquatic-cultivated peanuts were smaller than those of the field-cultivated ones. However, seed/pod weight ratios of both peanuts varied within similar ranges (**Table 1**). Although the aquatic-cultivated peanut seeds were small, their appearance (no shrinkage) (**Figure 2C**,**D**) indicated that the harvested aquatic-cultivated kernels were mature. In comparison to the growing period when mature peanut pods were harvested, 110 days was required for the field cultivation (70 days after flowering), whereas 120−127 days was needed for the aquatic cultivation. In addition to the fact that a longer cultivation period was required and a lower yield of peanut seeds was obtained by aquatic cultivation, it is obvious that the pods developed under a certain level of stress and consequently produced smaller mature peanut seeds. Apparently, the stress was relevant to the system without artificial aeration, chemical status of the aquatic solution, and the surrounding growth temperature.

Temperatures of the ambient air, aquatic solutions, and field soils in the last 10 days (in late June) prior to harvest are shown in **Figure 3**. In comparison, the highest temperatures of the aquatic solutions (38.2 °C) were close to the ambient air temperatures (38.6 °C) and higher than the field-soil temperatures (33.2 °C). The lowest temperatures of the air, solutions, and soils were 21.9, 25.4, and 26.2 °C, respectively. In comparison, temperatures of the field soils were more stable than other temperatures. In Taiwan, for the spring crop, peanuts are planted in February and March and harvested in June and July (*16*). The peanuts in this experiment were harvested in the hottest season in a year. The temperature difference between the aquatic solutions and field soils should be of importance in affecting the peanut seed development.

Moisture, crude fat, and protein contents and fatty acid compositions of the peanut seeds harvested from the aquatic- and field-cultivated peanuts varied within similar ranges (**Table 1**). In SDS-PAGE analysis, the protein patterns between the peanut seeds were identical (**Figure 4**). Oleic acid (18:1) content was higher and linoleic acid (18:2) content was lower in the oils from the aquatic-cultivated peanut seeds than in oils from the field-cultivated ones. Accordingly, the oleic/linoleic (O/L) ratio of the former was higher than that of the latter. This was

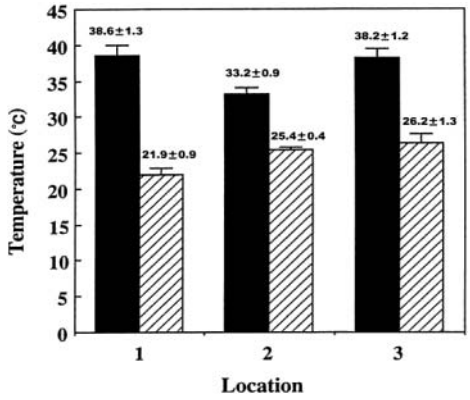

Figure 3. Highest and lowest temperatures of the ambient air, field soils, and aquatic solutions during the period of 10 days prior to harvest of the aquatic- and field-cultivated peanuts: (black bars) average highest temperature; (slashed bars) average lowest temperature; 1, ambient air; 2, field soil; 3, aquatic solution. Error bars represent ±1 SD.

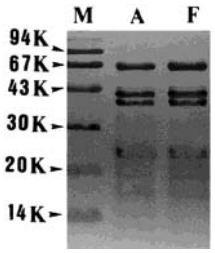

Figure 4. SDS-PAGE protein patterns of the aquatic- and field-cultivated peanut seeds: (A) aquatic floating-cultivation; (F) field cultivation; (M) standard protein markers.

not in agreement with the report of Wu et al. (*3*), who grew peanuts in hydroponic chambers controlled at 22−28 °C. Holaday and Pearson (*17*) reported that monounsaturated fatty acids increase while polyunsaturated fatty acids decrease with an increase of soil temperature. They explained that a higher soil temperature leads to lower polyunsaturation and higher monosaturation due to a higher metabolic rate recorded at elevated temperatures and to decreased availability of oxygen for reoxidizing the desaturase enzyme system required to synthesize linoleic acid. Brown et al. (*18*) also reported that monosaturated fatty acids increase and polyunsaturated fatty acids decrease with increasing temperature. Young et al. (*19*) stated that oleic acid content increases while linoleic acid content decreases with peanut seed maturity. In this study, the temperature of the aquatic solutions in the late stage of cultivation ranged from 25.4 to 38.2°C, which was higher than that of field soil, which ranged from 26.2 to 33.2 °C (**Figure 3**). In addition, the cultivation period of the former was longer than that of the latter. The higher growing temperature, longer cultivation period, and cultivation without artificial aeration might result in a higher O/L ratio in the aquatic-cultivated peanut oil.

Resveratrol is a new functional phytochemical that has been highlighted in recent years for its antioxidant and anti-inflammation activities and its benefits in decreasing the risk of vascular diseases and in the chemoprevention of cancer (*20*−*22*). In our laboratory, resveratrol has been extracted and identified in the field-cultivated peanut roots (*5*). We also observed a potent antioxidant activity in the peanut sprout roots (*23*). In this experiment, the collected dry root weights were 4.2 ± 0.1 and 2.2 ± 1.1 g/plant and resveratrol contents were 0.074 ± 0.009 and 0.114 ± 0.212 mg/g for the aquatic- and field-cultivated peanut roots, respectively (**Table 2**). Apparently, for the field-cultivated peanuts, both collected root weights and

Table 2. Average Dry Weight and Resveratrol Contents of the Aquatic- and Field-Cultivated Peanut Roots

	determinations[a]	
	aquatic cultivation	field cultivation
dry weight, g/plant	4.2 ± 0.1	2.2 ± 1.1
resveratrol, mg/g of dry root	0.074 ± 0.009	0.114 ± 0.212

[a] Mean of at least four determinations with standard deviation.

resveratrol contents deviated considerably from plant to plant. However, for the aquatic-cultivated peanuts, the collected root weights were much higher than field-cultivated roots and resveratrol contents varied in a limited range. From a practical viewpoint in the production of peanut roots as a resveratrol source, it would be of merit to harvest partial roots from the peanut plants and let them continue to grow in an aquatic-cultivated system.

In conclusion, mature peanut pods have been harvested by the newly developed aquatic floating system. The system did not have artificial aeration or periodic renewal of the nutrient solution. Obviously, in the system, peanuts grew under stress, which resulted in a low yield of peanut seed. In comparison to field-cultivated peanuts, the aquatic-cultivated peanut pods and seeds were smaller, whereas seed/pod weight ratios, crude fat and protein contents, and protein patterns of both peanuts varied within similar ranges. The O/L ratio of the aquatic-cultivated kernels was higher than that of field-cultivated kernels. The difference in O/L ratio between the peanut seeds was relevant to the varied temperatures encountered between the aquatic and field cultivations. When peanut roots were collected and subjected to resveratrol analysis, dry root weights were 4.2 ± 0.1 and 2.2 ± 1.1 g/plant and resveratrol contents were 0.074 ± 0.009 and 0.114 ± 0.212 mg/g for the aquatic- and field-cultivated peanut roots, respectively. On the basis of the fact that root weights were much higher in aquatic cultivation than in field cultivation and resveratrol contents of the roots varied in a very limited range, methodology could be used to produce resveratrol if the cultivation condition could be further optimized to enhance resveratrol synthesis. For root production, multiple harvests of the roots in the aquatic-cultivated system are also possible.

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