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Canopy and fruit characters with morphological relationships of European and Asian Water chestnuts (*Trapa* spp.).

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Summary

Seventeen water chestnut lines representing Europe and Asia were studied for their canopy growth and fruit morphology during the summer 2005, in the experimental field, Saga University. Leaf area of a single leaf, SLA, LAI at the beginning of flowering was significantly higher for the Chinese lines compared to other Asian and European lines. European lines had a LAI of less than one and produced thicker leaves. Since all the Asian lines formed compacted canopies, their rosette leaves tend to orient vertically over the water surface, but the rosettes of European lines expanded over the water table. Even though Chinese lines produced heavy, large fruits with 6.5-20.5 g per fruit, a huge variation in fruit shape and size was observed. Multivariate grouping using both vegetative and fruit characters revealed to have four distinct groups of lines.

Key words: aquatic plant, canopy, fruit, *Trapa,* water chestnut

Introduction

Water chestnut (*Trapa* spp.) is a common aquatic herb distributed in various parts of the world. It lengthens a stem from the bottom to the surface of the water and develops floating leaves in the shape of rosette on the tip of stem. It has been commercially cultivated for edible fruits in water bodies of low, flat lands or lakes in India, China and Italy (Kumar et al. 1985, Mazumder 1985). The fruit contains about 80% starch, 5% protein and significant amounts of vitamins (Tulyathan et al. 2005). In Japan, water chestnut is distributed all over the country and locally introduced into the paddy fields as an alternative crop (Arima et al., 1992, 1999). Several studies on water chestnut have been carried out in the field of botany (Agrawal and Mohan, 1995; Groth et al., 1996; Smith, 1995; Unni, 1984) and only few reports on its agronomy and yield characteristics have been found (Arima et al., 1992, 1999; Kusum and Chandra, 1980). Here we discuss the vegetative and reproductive growth characteristics of several water chestnuts collected from Europe and Asia. Using both vegetative and reproductive characteristics, multivariate clustering has been done in order to identify their similarities and relationships. This information can be utilized in the characterization of different ecotypes of water chestnut and for future breeding programs to improve their yield.

Materials and Methods

Seventeen lines of water chestnuts, eight from China (*Trapa acornis* L., *Trapa bicornis* L., *Trapa bispinosa* Roxb, *Trapa quadrispinosa* Roxb), one from France (*T. natans* L.), one from India (*T. bispinosa* Roxb.), three from Italy (*T. natans* L.), three from Japan (*T. incisa* L., *T. japonica* Flerov, *T. natans.* L. var. *Rubeola Makino*) and one from Korea (*T. japonica* Flerov.), were used (Table 1). Plots of 5 m^2 each were arranged in the paddy field of Saga University (located at 34^0 N), Japan, and uniformly grown 40-cm-long seedlings were transplanted to each plot on 7 May 2005. Prior to transplanting, the paddy field was ploughed, puddled, leveled, the plots demarcated; then water was impounded. Water level was maintained at a 15 cm depth throughout the experimental period. The field was arranged in a randomized complete block design (RCBD) with three blocks. N, P_2O_5 and K_2O were applied as a top dressing using compound fertilizers at a rate of 2.4, 2.7, 2.4 g m^{-2}, respectively. All the agronomic practices were the same as those described by Arima et al., (1999).

At the time of flowering, number of rosettes m^{-2}, rosette diameter, leaf area of a single leaf, leaf area index (LAI), leaf dry weight and leaf greenness (SPAD) were estimated. Rosette height from the water level and the rosette diameter were measured when the canopy reached its maximum compactness, in order to estimate the leaf angle and is expressed in degrees from the horizontal axis. The measurement of leaf angle was carried out from 7:00 to 8:00. After the cropping season, the field was drained and the fruits were harvested. Individual fruit characteristics such as fruit weight, edible ratio (ratio of edible part to the total weight), length, height, width and the spin length were also measured (Figure 1).

Data were analyzed using the SAS package (Version 6.1). Least squares estimates were obtained and the variations are expressed as standard errors. Correlations were checked using Pear-

Table 1. Scientific name and collection site of the plant materials used in the experiment and their single leaf characteristics at the beginning of flowering.

Line	Scientific name	Collection site	Single leaf area (cm^2)	SLA ($cm^2.g^{-1}$)	SPAD
China-1	*Trapa bispinosa*	Shanghai	11.7±1.3	161.1±3.9	57.8±1.4
China-2	*Trapa quadrispinosa*	Fuzhou	10.8±1.2	126.9±4.2	59.6±1.5
China-3	*Trapa acornis*	Hangzho	9.1±1.3	132.7±3.2	56.2±1.2
China-4	*Trapa bispinosa*	Hangzho	9.8±1.5	124.6±3.6	55.9±1.3
China-5	*Trapa bicornis*	Shanghai	12.6±2.0	170.7±4.2	57.4±1.5
China-6	*Trapa bispinosa*	Fuzhou	8.9±0.6	132.5±5.6	56.2±2.8
China-7	*Trapa quadrispinosa*	Hangzho	8.1±0.7	157.9±3.1	60.5±1.1
China-8	*Trapa quadrispinosa*	Hangzho	7.1±2.8	115.7±4.6	56.1±3.9
India	*Trapa bispinosa*	Bhagalpur	14.7±0.0	153.9±3.2	60.4±1.2
France	*Trapa natans*	Rennes	4.4±0.6	121.0±6.1	59.4±3.7
Italy-1	*Trapa natans*	Milano	2.0±0.3	108.1±7.2	58.3±2.6
Italy-2	*Trapa natans*	Milano	5.5±0.7	113.7±4.5	61.6±3.5
Italy-3	*Trapa natans*	Milano	4.7±0.6	95.6±6.8	58.4±2.5
Japan-1	*Trapa natans*	Saga	8.5±1.7	158.9±2.8	46.2±1.0
Japan-2	*Trapa japonica*	Saga	7.1±0.4	138.5±7.5	56.3±2.8
Japan-3	*Trapa incisa*	Kobe	1.1±0.1	132.0±4.7	42.9±1.7
Korea	*Trapa japonica*	Seoul	5.1±0.4	142.8±3.9	48.4±1.4

The values represent the mean±S.E.; n=10 for the first two columns and n=3 for the third column.

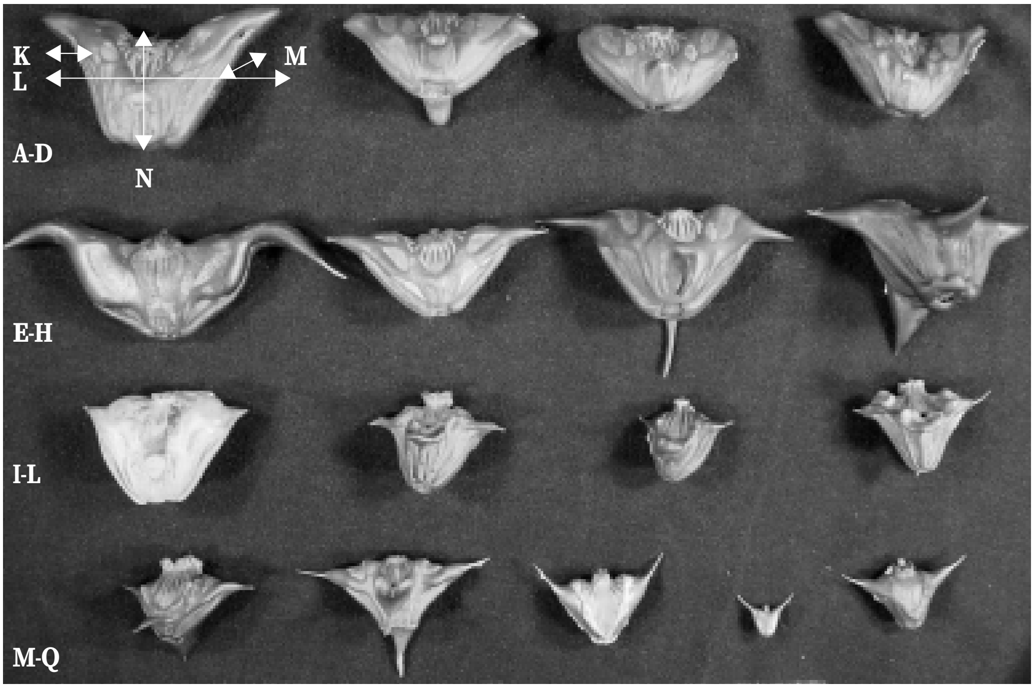

Fig.1. Harvested mature fruits of different water chestnut lines.
The top row, A-D, China1, China2, China3 and China4, the second row, E-H, China-5, China-6, China7 and China8, the third row, I-L, India, France, Italy1 and Italy2, and the bottom row, M-Q, Italy3, Japan1, Japan2, Japan3 and Korean line in this order. K, L, M, N indicates the spin length, fruit length, width and the fruit height, respectively.

son's correlation coefficient. Significance is expressed at α=0.05. Multivariate clustering was done using the standardized variables and used Euclidean distances with complete linkage.

Results and discussion

In this study, we analyzed the canopy and the fruit characters of 17 water chestnut lines collected from some parts of Europe and Asia and observed their relationships using multivariate statistical techniques.

1. Canopy formation

After transplanting, new rosettes were developed due to stem branching and covered the water surface. When the canopy closure was achieved, the water chestnut begun to emerge the emersed leaves. But the European lines did not achieve a canopy closure and covered only around 20% of the water surface. Further, they are early flowering, therefore, the extensive stem branching was restricted to a shorter inertial vegetative period compared to Asian lines (Arima et al., 2006). Due to the physiological importance, canopy characteristics were investigated at the beginning of flowering.

A large variation in the leaf area of a single leaf was observed. All the Chinese lines produced large leaves ranging from 7.1-12.6 cm^2 and the Indian line had the highest with a 14 cm^2 in the single leaf area (Table 1). European lines produced relatively smaller leaves ranging from 2-

Table 2. Canopy characteristics of different water chestnut lines.

Line	Rosette number (m^{-2})	Rosette diameter (cm)	Leaf angle (degrees)	Rosette height (cm)	LAI
China-1	49±3.1	21.8±0.4	37.9±2.1	8.5±0.5	2.32±0.06
China-2	30±3.2	23.7±2.0	42.7±2.2	10.8±0.6	2.06±0.07
China-3	39±2.6	24.7±1.8	42.4±2.8	11.2±0.2	2.07±0.19
China-4	36±2.7	20.3±1.4	44.7±3.2	10.0±0.3	2.12±0.02
China-5	55±2.8	16.7±0.8	41.9±3.3	7.5±0.3	2.20±0.06
China-6	51±3.4	13.9±0.9	46.8±4.3	7.4±0.7	2.22±0.09
China-7	45±5.0	14.7±1.0	46.4±2.7	7.7±0.4	2.62±0.23
China-8	29±4.3	27.5±1.6	48.6±2.2	11.1±0.7	2.14±0.03
India	24±3.2	27.7±1.5	28.1±0.8	6.2±0.4	2.07±0.04
France	9±0.8	32.4±1.0	0.0±0.0	0.0±0.0	0.43±0.09
Italy-1	10±1.4	24.3±0.7	0.0±0.0	0.0±0.0	0.19±0.05
Italy-2	9±1.1	28.9±1.4	0.0±0.0	0.0±0.0	0.24±0.01
Italy-3	5±0.5	38.3±2.5	0.0±0.0	0.0±0.0	0.21±0.02
Japan-1	33±2.8	27.7±1.0	43.5±2.6	10.5±0.5	1.37±0.08
Japan-2	62±5.6	19.2±0.9	30.8±1.1	6.0±0.3	1.56±0.11
Japan-3	161±16.3	11.3±0.7	26.1±2.2	2.3±0.3	1.15±0.08
Korea	78±8.3	15.3±0.4	39.1±4.4	5.1±0.6	1.42±0.21

Values represent the mean±S.E. of at least 7 measurements.

5.5 cm^2, Japanese and Korean lines produced the middle size leaves ranging 5.1-8.5 cm^2 except for Japan-3. Japan-3 produced the smallest leaves with the leaf area of 1.1 cm^2. Leaves of Asian line had a higher SLA of 120-160 cm^2 g^{-1} and those of the European lines were 95-120 cm^2 g^{-1}. The leaf greenness as expressed by green meter value (SPAD) did not show a huge variation among lines except for Japanese and Korean lines and was ranged from 56-60. As changes in the leaf greenness affect the photosynthesis, the dry matter production of each lines has to be investigated in future study.

Rosette number is an important yield component. Japan-3 was an exceptional line with the highest, 160 rosettes m^{-2} and European lines produced rosettes less than 10 m^{-2} (Table 2). Other Asian lines produced a moderate number of rosettes ranging from 25-78 m^{-2}. Rosette diameter of European lines was significantly higher than lines, where Italy-3 produced the biggest with a diameter of 38 cm and Japan-3 had the smallest rosettes with a diameter of 11 cm. The leaf angle for the most vertically oriented leaf of a rosette was between (25-50) °for all the Asian lines. But for the European lines leaf angle was always zero, since all the leaves lied over the water surface through-out the life cycle. Therefore the rosette height of European lines was also marked as zero. LAI of the Chinese lines was the highest and was over 2. European lines produced poor canopies with a LAI of less than one; where as Japanese lines had a moderate LAI.

Both the higher number of rosettes m^{-2} and higher single leaf area caused to have a higher LAI for Chinese lines. In European lines, both the rosette number and the leaf area of a single leaf were lower and a poor LAI was resulted. SLA, which describes the leaf thickness, was lower for European lines, suggesting that, European water chestnuts produce thicker leaves compared to all other Asian lines. This is an ideal adaptation by European lines to trap more radiation energy, since they did not produce vertically oriented canopies. For Asian lines SLA was higher and incoming radiation is distributed in vertically oriented leaves in different layers. A significant corre-

Table 3. Fruit morphological variation of different water chestnut lines.

Line	Fresh fruit weight (g)	Length[L] (mm)	Height[N] (mm)	Width[M] (mm)	Spin length[K] (mm)	Edible ratio (%)
China-1	20.6±1.0	41.1±0.9	33.5±0.6	26.1±0.9	10.5±0.3	57.6±0.3
China-2	14.7±0.9	37.4±0.8	27.3±0.6	21.5±0.4	7.7±0.2	59.7±0.2
China-3	9.8±0.9	35.5±2.5	23.1±0.6	19.0±0.8	0.0±0.0	70.2±0.2
China-4	10.6±0.6	35.5±0.9	25.8±0.4	20.5±0.7	2.7±0.1	70.6±0.3
China-5	14.9±0.8	44.6±0.9	28.3±0.3	18.4±0.4	21.3±0.3	61.8±0.2
China-6	6.7±0.4	33.3±0.8	20.6±0.6	16.5±0.4	9.0±0.1	73.5±0.3
China-7	10.9±0.7	37.1±0.7	25.6±0.8	19.9±0.6	12.6±0.3	67.4±0.3
China-8	18.1±0.8	37.9±1.2	30.1±0.9	26.0±0.7	12.6±0.2	57.2±0.2
India	16.3±1.4	36.8±1.3	33.8±1.1	26.5±1.1	5.0±0.1	59.2±0.2
France	5.1±0.4	21.6±0.7	26.8±0.5	19.1±1.2	7.4±0.2	57.4±0.2
Italy-1	2.5±0.2	18.4±0.4	20.5±0.6	13.1±0.5	9.5±0.1	66.8±0.3
Italy-2	4.4±0.5	20.5±1.1	23.6±1.1	16.4±0.8	7.2±0.1	52.1±0.2
Italy-3	4.0±0.2	21.5±1.2	21.1±0.5	14.3±0.3	9.0±0.4	58.2±0.3
Japan-1	4.9±0.3	23.4±0.6	20.0±0.3	17.1±0.5	13.7±0.2	52.7±0.2
Japan-2	1.8±0.2	18.9±0.5	17.0±0.5	9.8±0.4	6.7±0.1	78.2±0.3
Japan-3	0.2±0.0	7.2±0.3	9.4±0.3	3.9±0.2	3.7±0.1	85.9±0.3
Korea	1.9±0.1	18.4±0.2	17.3±0.3	9.5±0.2	9.5±0.1	52.3±0.3

Values represent the mean±S.E. of at least 10 measurements. [(K),(L),(M),(N)] are as described at figure 1.

lation (r=0.71*) of SLA with the leaf angle was also observed. Also a significant correlation (r=−0.82*) was observed for the rosette number m^{-2} and the rosette diameter. Since European lines produced lesser number of rosettes m^{-2}, individual rosette diameter increased and expanded over the water table.

2. Fruit characters

The large fruit bearing lines have high quantitative advantage. Single fresh fruit weight of all the Chinese lines was over 6 g per fruit (Table 3). For all other Asian and European lines, it was less than 5 g per fruit. Japan-3 produced exceptionally smaller fruits with a lighter weight of 0.2 g per fruit. In a previous study, it is reported that Chinese water chestnuts produced larger fruits than the Japanese lines (Arima et al., 1999). Apart from the weight, a huge morphological variation in fruit shape was also observed (Figure 1), and is expressed as fruit length, height, width and the spin length (Table 3). Lines with sharp, pointed, large spins cause constant severe problems when cultivate, harvest and process. Therefore, large fruit lines with small blunt spins and higher edible ratio, such a China-1 to China-4, would be ideal selections for cultivation and breeding. The edible ratio for all the lines was over 50%.

3. Cluster analysis

Multivariate grouping using both vegetative and fruit characteristics revealed the existence of distinct groups (Figure 2). We used similarity level 50 for the groupings and subsequent analysis revealed the actual existence of four groups. The dendogram still shows the ecological relationships. All the Chinese lines and the Indian line perform similarly and the European lines formed a different distinct group. Small fruit bearing Japan-3 and the Korean line grouped together, while Japan-1 and Japan-2 formed their own cluster. These results resembled closely with a classification by the RAPD marker (Hoque, 2005).

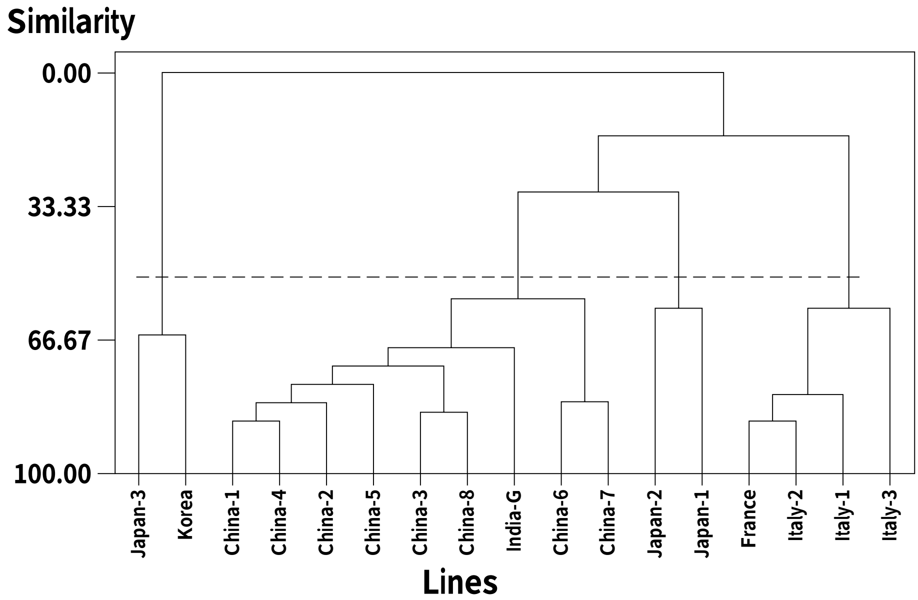

Fig 2. Dendogram of water chestnut lines. Chinese lines with the Indian line group together. European lines formed the second group. Japan-1 and Japan-2 group together and Japan-3 and Korean line formed the last group.

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アジアとヨーロッパから採集したヒシの葉群と果実の形態的特徴

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摘　　要

アジアとヨーロッパから採集した17種類のヒシを2005年に佐賀大学において試験栽培し，葉群と果実の形状を比較して，その形態的特徴により分類した．その結果，開花始期における単葉葉面積，比葉面積，葉面積指数等で中国種がヨーロッパ種や日本種，韓国種に優り，高い生産性を示した．ヨーロッパ種は極早生であり，十分な栄養生長を待たずに開花するために，葉群の発達が低緯度地域のヒシに比べて劣り，葉面積指数が低かった．果実の形状は種・産地により様々であり，中国種が最も大粒であった．各部位の形状により分類したところ，供試した種類は大きく４つのグループに分別された．