PHOTOSYNTHETICA 45 (2): 312-316, 2007

BRIEF COMMUNICATION

Physiological comparisons of true leaves and phyllodes in *Acacia mangium* seedlings

H. YU* and J.-T. LI**

International Institute for Earth System Science (ESSI), Nanjing University, Hankou Road 22,
*Nanjing 210093, People's Republic of China**
*Mao-Er Shan Forestry Experimental Site, Northeast Forest University, Harbin 150611, People's Republic of China***

Abstract

We found differences between true leaves (TL) and phyllodes (Ph) during ontogeny of *Acacia mangium* plants as reflected in chlorophyll (Chl) and carotenoid contents, gas exchange, Chl fluorescence, and growth. The production of TL enhanced the relative growth rate of the *A. mangium* seedlings, allowing the plants to accumulate enough dry biomass for later growth, while the production of thicker Ph in the later growth stage of *A. mangium* could help plants to cope with higher irradiance in their natural growth conditions.

Additional key words: apparent quantum yield; chlorophyll content and fluorescence; dark respiration rate; growth; net assimilation rate; net photosynthetic rate; relative growth rate; stomatal conductance; transpiration rate.

Acacia mangium is an early succession and important reforestation tree species in tropical and subtropical regions that develops two distinctive leaf types (leaves and phyllodes) during its life history. Upon germination, true compound pinnate leaves (TL) were produced on the *A. mangium* seedlings. Seven to eight weeks after the sowing of the seeds, phyllodes (Ph) [expanded petioles that form simple lamina (Esau 1965, Atkin *et al.* 1998) start to emerge from the top of the seedlings. Later, only Phs are present on older plants.

TL and Ph might have distinct morphological and physiological characteristics; moreover, ontogenetic changes in photosynthesis and growth would likely be magnified in *A. mangium*, due to the replacement of leaves with Ph. This is why we compared the physiological and morphological traits between TL and Ph and analysed the influence of these traits on the growth of *A. mangium* seedlings in the corresponding stage under natural conditions.

Seeds of *A. mangium* were collected from Hainan Province, P. R. China. Seedlings were grown in teaching garden of the Department of Biological Sciences, National University of Singapore. Plants were kept well-watered and fertilized twice a week with Hoagland's solution. The temperature was 29±6 °C. Daily atmospheric relative humidity ranged between 70–100 %. The daily photosynthetic photon flux density (PPFD) ranged from a minimum of 80 μmol m^{-2} s^{-1} at 08:00 h to 1 650 μmol m^{-2} s^{-1} at 12:00 h.

Experiments were conducted at two stages of growth, called TL and Ph stages. The first stage was 60 d after the sowing (DAS) of seeds; during this stage, *A. mangium* plants produce true, compound pinnate leaves. The second stage was 120 DAS, and only phyllodes were present in the 120 DAS plants. Fully expanded compound leaves obtained from 60 DAS seedlings (TL) and mature Ph obtained from 120 DAS seedlings (Ph) were used for comparative determinations.

TL and Ph were ground with 100 % (v/v) acetone and the contents of chlorophylls (Chls) and carotenoids were determined by the methods of Arnon (1949) and Embry and Nothnagel (1988), respectively. Photosynthetic CO_2 exchange rates of attached TL or Ph of 120 DAS seedlings, in response to changes in PPFD, were determined according to Yu and Ong (2000). The responses of Chl fluorescence parameters of attached TL or Ph to different actinic PPFD were determined with a pulse amplitude modulation fluorometer (*PAM 101-103*, *Walz*, Effeltrich, Germany). Following a 30-min dark adaptation of the fully expanded TL or Ph, F_0 was determined

---

Received 16 October 2006, *accepted* 2 January 2007.

*Author for correspondence; fax: (+86)-25-8359-2288, e-mail: yuhua@nju.edu.cn

Acknowledgement: This project was supported by National Science Foundation of China (project number: 30540074).

at irradiance <0.01 μmol m^{-2} s^{-1}. After F_0 was recorded, a 600-ms saturating pulse (5 000 μmol m^{-2} s^{-1}), supplied by a flash lamp (*KL 1500*, *Schott*, Mainz, Germany), was given and F_m was determined. After the measurement of F_0 and F_m, the actinic radiation, supplied by an external source (*Euromex* fiber optic light source, *EK1*, the Netherlands), was switched on. When a steady state had occurred (30 min), actinic radiation was switched off and a far-red radiation was turned on for the accurate measurement of F_0'. The initial actinic PFD was 30 μmol m^{-2} s^{-1} and this was increased in six steps to 1 400 μmol m^{-2} s^{-1}. Each PPFD response was determined at room temperature and repeated six times.

Values of various Chl fluorescence parameters were calculated according to Van Kooten and Snel (1990) and Genty *et al.* (1989). Relative linear electron transport rate was calculated as ETR = PPFD × $\Delta F/F_m$' × 0.85 × 0.5 (Krause and Winter 1996).

On 60 and 120 DAS, seedlings were carefully removed from the pots; each seedling was then divided into several portions (laminae of leaves/phyllode blades, stem plus petioles, and roots). To measure the total foliage area of each seedling, the outlines of the TL or Ph were drawn on a graph paper. TL or Ph area was calculated according to an area-mass calibration curve. To determine dry mass (DM) of the different parts of the seedlings, materials were dried in the oven at 80 °C for 7 d until DM was constant, and weighed. To obtain the mean mass of seed reserve, seed mass was determined after the removal of the seed coat. Specific foliage area (SFA; foliage area divided by foliage DM), foliage mass ratio (FMR; foliage DM divided by total plant DM), and foliage area ratio (FAR; foliage area divided by total plant DM) were calculated.

Relative growth rate (RGR) of the seedlings of TL stage was calculated as: RGR = [ln(seedling mass of 60 DAS plant) – ln(seed mass)]/60 d, where ln = natural logarithm. Similarly RGR of the seedlings of Ph stage was calculated using the respective values. Net assimilation rates (NAR) of the seedlings of both stages were calculated as: NAR = RGR/FAR. Total nitrogen (N) contents of TL of whole 60 DAS plants and Ph of whole 120 DAS plants were analyzed calorimetrically by the Kjeldahl method (Walinga *et al.* 1995). Photosynthetic N use efficiency was calculated as photon saturated rate of photosynthetic CO_2 uptake divided by total N content of foliage; foliar N content was expressed per area basis.

All experiments were conducted twice, from February to May in 1999 and 2000, each time with 3 replicates. For simple comparisons between parameters measured on TL and Ph stages, the Student *t*-test ($p \leq 0.05$, $n = 6$) was used.

Total Chl and carotenoid contents per leaf area, Chl *a/b* ratio, and N contents were significantly higher in Ph than in TL (Table 1). The photosynthetic nitrogen use efficiency (PNUE), *i.e.* photon-saturated rate of photosynthetic CO_2 fixation per unit foliar N, was significantly lower in Ph than in TL (Table 1). No significant difference between the apparent quantum yield (Φ) of TL and Ph was observed (Table 1). Similarly, there were also no significant differences between the photon-saturated rate of net photosynthetic CO_2 uptake (on area basis) of Ph and TL. However, when expressed on a DM basis, the TL values were about two times higher those of Ph of 120 DAS plants (Table 1). In addition, the dark respiration rate (R_D) of Ph was significantly higher than that of TL (Table 1). There were no significant differences in stomatal conductance (g_s) and transpiration rate (E) between TL and Ph (Table 1). The compensation irradiance (CI) was significantly lower in TL than in Ph, while no significant differences in saturation irradiance (SI) were found (Table 1).

Table 1. The comparison of chlorophyll (Chl) and carotenoid contents [mg m^{-2}], foliar nitrogen (N) content [mmol(N) m^{-2}], photosynthetic N use efficiency (PNUE) [μmol(CO_2) mol^{-1}($N_{foliage}$) s^{-1}], specific foliage (true leaves, TL/phyllodes, Ph) area [m^2 kg^{-1}(foliage)], apparent quantum yield (Φ) [mmol(CO_2) mol^{-1}(quantum)], net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}], dark respiration rate (R_D) [the same unit], stomatal conductance (g_s) [mmol(H_2O) m^{-2} s^{-1}] , transpiration rate (E) [the same unit], compensation irradiance (CI) [μmol(quantum) m^{-2} s^{-1}], saturation irradiance (SI) [the same unit], foliage mass ratio (FMR) [kg kg^{-1}], foliage area ratio (FAR) [m^2 kg^{-1}(plant)], relative growth rate (RGR) [g kg^{-1} d^{-1}], and net assimilation rate (NAR) [g m^{-2} d^{-1}] of TL *versus* Ph of *A. mangium* seedlings. *Identical letters within the same column* indicate no significant differences between the values ($p \leq 0.05$). Means±SE ($n = 6$).

	TL	Ph
Chl (*a*+*b*) per area	417.96±18.92 ^{a}	533.82±16.10 ^{b}
Carotenoids per area	55.11±4.02 ^{a}	70.64±1.96 ^{b}
Chl *a/b*	2.43±0.11 ^{b}	2.80±0.04 ^{b}
Foliar N per area	90.12±1.91^{a}	128.12±0.48 ^{b}
PNUE	244.62±6.07 ^{a}	171.67±4.25 ^{b}
Φ	70.45±1.93 ^{a}	70.53±2.30 ^{a}
P_N per area	20.68±2.06 ^{a}	21.18±0.78 a
P_N per DM	671.89±66.93 ^{a}	346.72±31.28 ^{b}
R_D per area	1.75±0.08 ^{a}	3.01±0.12 ^{b}
g_s	388.83±72.35 ^{a}	324.25±24.28 ^{a}
E	6.63±0.93 ^{a}	5.05±0.30 ^{a}
CI	24.83±1.14 ^{a}	31.50±1.81 ^{b}
SI	1383.33±10.54 ^{a}	1362.50±34.86 ^{a}
SFA	32.49±1.18 ^{a}	16.37±0.33 ^{b}
FMR	0.50±0.00 ^{a}	0.39±0.01 ^{b}
FAR	15.53±0.36 ^{a}	6.43±0.06 ^{a}
RGR	86.43±0.42 ^{a}	61.47±0.57 ^{b}
NAR	5.57±0.11 ^{a}	9,56±0.12 ^{b}

For both TL and Ph, the photochemical efficiency of open photosystem 2 (PS2) centres ($\Delta F/F_m$') declined as PPFD increased (Fig. 1*A*). Moreover, the values of $\Delta F/F_m$' of TL were higher than those of Ph in response to high PPFD (800–1 500 μmol m^{-2} s^{-1}). The photosynthetic non-cyclic electron transport rate (ETR) of TL and Ph increased with increasing PPFD (Fig. 1*B*). At lower PPFD (<350 μmol m^{-2} s^{-1}), there was no significant difference in ETR value between TL and Ph (Fig. 1*B*); while ETR of TL was higher than that of Ph at high PPFD (800–1 500 μmol m^{-2} s^{-1}). Furthermore, the ETR of both TL and Ph did not exhibit any saturation at PPFD less than or equal to 1 500 μmol m^{-2} s^{-1} (Fig. 1*B*), and hence non-cyclic electron transport was not a limiting factor for photosynthesis in both leaf types of *A. mangium* seedlings.

With increasing PPFD, non-photochemical quenching *via* xanthophyll cycle (NPQ) of TL and Ph increased steadily (Fig. 1*C*). In addition, the magnitude of increase was much greater in Ph (from 0.33 to 2.41) than in TL (from 0.40 to 1.43).

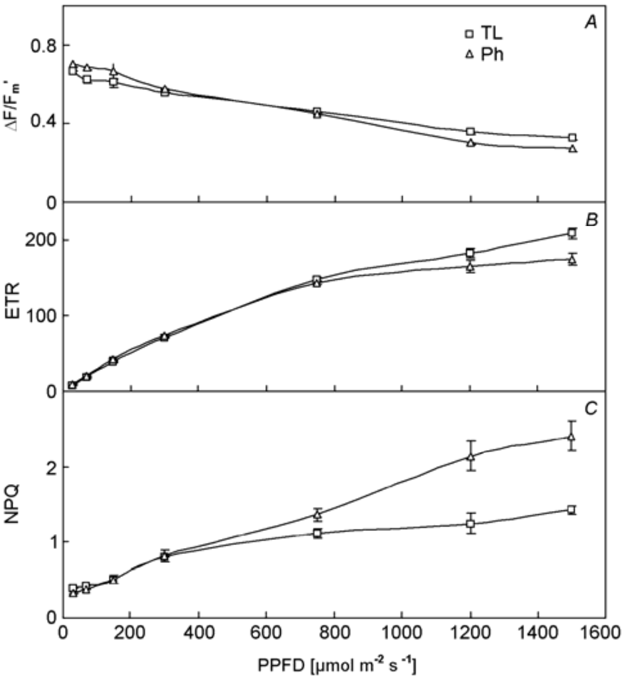

Fig. 1. Relationships between photosynthetic photon flux density (PPFD) and (*A*) the photochemical efficiency of open photosystem 2 (PS2) centres ($\Delta F/F_m$'), (*B*) electron transport rate through PS2 (ETR), (*C*) non-photochemical quenching (NPQ) in true leaves (TL) and phyllodes (Ph) of *A. mangium* plants grown under natural condition. *Vertical bars* indicate SE (*n* = 6).

SFA was much higher in TL than in Ph (Table 1), indicating that Ph was much thicker than TL. For seedlings of TL stage, 50 % of plant biomass was allocated to foliage; this was evident from the observation that the value of FMR was 0.50 (Table 1). With increasing plant age and size, higher proportion of biomass was needed to allocate to structure (stem and root), thus the lower FMR (0.39) was observed in seedlings of Ph stage (Table 1).

FAR is the function of SFA and FMR, and FAR = SFA×FMR. Thus, the higher SFA and FMR of *A. mangium* seedlings in TL stage resulted in a higher FAR. These results indicated that *A. mangium* seedlings in the TL stage pursued the option of increasing photon interception by spreading a given leaf mass over a larger area (*i.e.* higher SFA) and allocating a big proportion of plant biomass to leaf (*i.e.* higher FMR), as the amount of photon interception was crucial for the survival and development of *A. mangium* plants during their early growth stage. The RGR of *A. mangium* seedlings in TL stage was 86.43 g kg^{-1} d^{-1}, while in the Ph stage it was 61.41 g kg^{-1} d^{-1} (Table 1). Thus, RGR was significantly higher in the TL stage than in the Ph stage.

By contrast, NAR in the TL stage (9.56±0.12 g m^{-2} d^{-1}) was significantly higher than that in the Ph stage (5.57±0.11 g m^{-2} d^{-1}) (Table 1). Seedlings in the Ph stage had a slightly higher photosynthetic capacity and far larger foliage areas than seedlings in the TL stage. This is why we concluded that the high photosynthetic CO_2 fixation ability together with larger foliage area resulted in high daily integral of photosynthesis, that eventually lead to high NAR of *A. mangium* plants in the Ph stage.

Significant differences of morphological and physiological properties existed in TL and Ph of *A. mangium* seedlings. Morphologically, TL was much smaller than Ph. Moreover, Ph was thicker than TL, as the value of SLA of Ph (16.37±0.33 m^{-2} kg^{-1}) was significantly lower than that of TL (32.49±1.18 m^{-2} kg^{-1}). Previous studies showed that Ph was much thicker than TL in *A. koa* (Walters and Bartholomew 1984, 1990). The thicker *A. mangium* Ph could be due to the presence of a thick, central parenchymatous mesophyll layer that was absent in TL, and also because Ph had two palisade layers, one on each side of the central mesophyll (Yu 1996). In contrast, TL of *A. mangium* showed only a single palisade layer on the adaxial surface (Yu 1996). Similar Ph and TL structures were also observed in *A. koa* (Walters and Bartholomew 1984). Moreover, Boughton (1986) reported the presence of a thick, central parenchymatous mesophyll layer in all of the 144 Australian *Acacia* species sampled in the field. Physiologically, the photon-saturated rate of photosynthetic CO_2 uptake per DM unit of TL was *ca.* 90 % higher than that of Ph. This was consistent with the comparative studies on the rate of photosynthetic CO_2 uptake of TL and Ph of *A. melanoxylon* (Brodribb and Hill 1993) and *A. koa* (Hansen 1996). Ph of *A. mangium* was much thicker than the TL. Thus, it was the increased thickness of the Ph, relative to the TL, that resulted in a lower photon-saturated rate of photosynthetic CO_2 uptake per unit DM in Ph than TL of *A. mangium*. Consequently, the amount of photosynthetic carbon fixed per unit of foliar DM was much higher in TL than in Ph of *A. mangium*. This might contribute to the higher RGR (86.43 g kg^{-1} d^{-1}) of *A. mangium* plants during their early growth stage, when the plants had only TL (60 DAS) than in the later growth stage (RGR was 61.41 g $kg^{-1}d^{-1}$), when the plants had Ph (120 DAS). Furthermore, higher PNUE in TL than in Ph suggested that TL used N in photosynthesis more efficiently than Ph did.

This high efficiency N resource use into photosynthesis in *A. mangium* seedlings of TL stage could result in their higher growth rate.

RGR is the function of physiological component (NAR, the daily biomass increment of whole plant per unit total leaf area) and morphological component (FAR, the total foliage area per unit total plant DM), and RGR = NAR×FAR (Lambers *et al.* 1998). Moreover, FAR = SFA×FMR. Therefore, RGR = NAR×SFA×FMR. The higher FMR and SFA in seedlings of TL stage than in plants of Ph stage indicated that morphological component contributed to the high RGR observed in seedlings of TL stage. This was an important strategy for young *A. mangium* seedlings to establish as quickly as possible.

We found that leaf production (prior to Ph production) enhanced the RGR of *A. mangium* seedlings, thus enabling the plants to accumulate more biomass for later growth and development. Previous studies on other *Acacia* species demonstrated also that TL allowed faster plant growth than Ph (Brodribb and Hill 1993, Hansen 1996, Atkin *et al.* 1998).

With the increasing plant size, the priority of resource use in leaves for a pioneer species like *A. mangium* gradually changes from enhancement of photosynthesis to adaptation to high PPFD in nature. We found that Phs are better adapted to high irradiance than TLs. This was evident from the observation that carotenoid content and Chl *a/b* ratio was higher in Ph than in TL, and these physiological traits were important for protecting Ph from photoinhibition (Anderson *et al.* 1988, Demmig-Adams *et al.* 1992, Jiang *et al.* 1999). Another physiological trait that helped Ph to cope with high irradiance was the observed higher NPQ value of Ph. NPQ is a photoprotective mechanism that prevents the over-reduction of the photosynthetic electron transport chain by dissipating excess absorbed photon energy in the PS2 antenna system as heat (Demmig-Adams 1990). The ability of plant to direct excitation energy to photochemistry or non-photochemical quenching is very important for the adaptation of plant to high irradiance (Königer 1995). Therefore, high NPQ value observed in Ph might have helped to protect their photosystems against irreversible damage associated with high PPFD and high temperature in the tropics. This was consistent with the reports that NPQ directly contributes to the mechanism that prevents photoinhibition in sun-adapted tropical plants (Thiele *et al.* 1998, Ishida *et al.* 1999). At the same time, a lower value of SFA in Ph than in TL was also an adaptation to high irradiance environment experienced by this early-succession tree species. Moreover, the production of thick Ph will prevent *A. mangium* plant from reducing biomass by mechanical disturbance and herbivory. In addition, the Ph was able to attain slightly higher maximal rates of photosynthesis at high PPFD. Similar results were reported in the comparison of TL and Ph of *A. melanoxylon* (Brodribb and Hill 1993). Therefore, morphological and physiological traits like lower SFA, higher Chl *a/b* ratio, and high thermal energy dissipation (high NPQ) enable Ph to cope with the high amount of incident photons in their growth condition and in the canopy.

Chl fluorescence data showed that the effective quantum yield of PS2 ($\Delta F/F_m$') and photosynthetic non-cyclic electron transport rate (ETR), in response to PPFD lower than 350 μmol m^{-2} s^{-1}, was similar in TL and Ph. While under PPFD>350 μmol m^{-2} s^{-1}, it was higher in TL than in Ph. Hence photochemical activity was higher in TL than in Ph, thus photon energy absorbed by TL was more efficiently utilized in photosynthesis. Besides, the enhanced photochemical activity of TL contributed to its relatively higher photosynthetic capacity [20.68 μmol(CO_2) m^{-2} s^{-1}]. In comparison, it was the higher Chl content and higher N content (*i.e.* higher amount of photosynthetic enzyme) of Ph that played a part in its higher photosynthetic capacity [21.18 μmol(CO_2) m^{-2} s^{-1}].

References

Anderson, J.M., Chow, W.S., Goodchild, P.J.: Thylakoid membrane organization in sun/shade acclimation. – Aust. J. Plant Physiol. **15**: 11-26, 1988.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-14, 1949.

Atkin, O.K., Schortemeyer, M., McFarlane, N., Evans, J.R.: Variation in the components of relative growth rate in ten *Acacia* species from contrasting environments. – Plant Cell Environ. **21**: 1007-1017, 1998.

Boughton, V.H.: Phyllode structure, taxonomy and distribution in some Australian Acacias. – Aust. J. Bot. **34**: 663-674, 1986.

Brodribb, T., Hill, R.S.: A physiological comparison of leaves and phyllodes in *Acacia melanoxylo*n. – Aust. J. Bot. **41**: 293-305, 1993.

Demmig-Adams, B.: Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. – Biochim. biophys. Acta **1020**: 1-24, 1990.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Embry, J.L., Nothnagel, E.A.: Leaf development and senescence in *Panicum miliaceum* L., a cereal with a short seed-to-seed cycle. – Plant Sci. **55**: 129-136, 1988.

Esau, K.: Plant Anatomy. – John Wiley & Sons, New York – London – Sydney 1965.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Hansen, D.: Establishment and persistence characteristics in juvenile leaves and phyllodes of *Acacia koa* (Leguminosae) in Hawaii. – Int. J. Plant Sci. **157**: 123-128, 1996.

Ishida, A., Nakano, T., Matsumoto, Y., Sakoda, M., Ang, L.H.: Diurnal changes in leaf gas exchange and chlorophyll fluorescence in tropical tree species with contrasting light requirements. – Ecol. Res. **14**: 77-88, 1999.

Jiang, C.-Z., Ishihara, K., Satoh, K., Katoh, S.: Loss of the photosynthetic capacity and proteins in senescing leaves at top positions of two cultivars of rice in relation to the source capacities of the leaves for carbon and nitrogen. – Plant Cell Physiol. **40**: 496-503, 1999.

Königer, M., Harris, G.C., Virgo, A., Winter, K.: Xanthophyll-cycle pigments and photosynthetic capacity in tropical forest species: a comparative field study on canopy, gap and understory plants. – Oecologia **104**: 280-290, 1995.

Krause, G.H., Winter, K.: Photoinhibition of photosynthesis in plants growing in natural tropical forest gaps. A chlorophyll fluorescence study. – Bot. Acta **109**: 456-462, 1996.

Lambers, H., Chapin, F.S., Pons, T.L.: Plant Physiological Ecology. – Springer-Verlag, New York 1998.

Thiele, A., Krause, G.H., Winter, K.: *In situ* study of photoinhibition of photosynthesis and xanthophyll cycle activity in plants growing in natural gaps of the tropical forest. – Aust. J. Plant Physiol. **25**: 189-195, 1998.

Van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.

Walinga, I., Van Der Lee J.J., Houba, V.J.G. Van Vark, W., Novozamsky, I.: Plant Analysis Manual. – Kluwer Academic Publ., Dordrecht – Boston – London 1995.

Walters, G.A., Bartholomew, D.P.: *Acacia koa* leaves and phyllodes: gas exchange, morphological, anatomical, and biochemical characteristics. – Bot. Gaz. **145**: 351-357, 1984.

Walters, G.A., Bartholomew, D.P.: Adaptation of *Acacia koa* leaves and phyllodes to changes in photosynthetic photon flux density. – Forest Sci. **36**: 1050-1060, 1990.

Yu, H.: A Preliminary Study on the Phytoecological Anatomy of Lowland Subtropical Dominant Plant Populations, Guangdong, China. – MSc. Thesis. South China Institute of Botany, Chinese Academy of Science, Guangzhou 1996.

Yu, H., Ong, B.-L.: Photosynthesis and antioxidant enzymes of phyllodes of *Acacia mangium*. – Plant Sci. 159: 107-115, 2000.