Indian J. Hort. 65(2), June 2008: 220-225

Influence of light intensity on gas exchange, herbage yield and andrographolide content in *Andrographis paniculata* (Nees.)

R. Saravanan*, Sunil Krishti, N.A. Gajbhiye and S. Maiti
National Research Centre for Medicinal and Aromatic Plants, Boriavi PO, Anand 387 310

ABSTRACT

The medicinal herb *Andrographis paniculata* containing a bitter principle andrographolide is widely used in indigenous system of medicines for treatment of various ailments in south Asian countries including India. It is found to grow as an understory in its natural habitats. With a view to understand the role of shade on herbage yield, a two-year field study was conducted on sandy loam soil. The crop was grown under 25, 50, 70 and 100% incident photosynthetic photon flux density (PFD) by providing artificial shade nets and observations on plant height, fresh and dry weight of leaf, stem and total plant along with leaf gas exchange parameters. Significant differences were observed for all growth characters between open and shaded plants. Leaf photosynthesis increased from 11.56 to 19.70 mmol $m^{-2}s^{-1}$ as PFD increased from 25 to 100% and herbage yield increased from 226.70 to 379.45 g. Andrographolide content was not consistent under different PFD levels and highly influenced by the weather parameters. Since the total herbage and andrographolide yield was the highest under open light condition, it was concluded that *A. paniculata* is suitable for open cultivation.

Key words: Medicinal plant, shade, plant growth, photosynthesis, herbage, andrographolide content.

INTRODUCTION

Andrographis paniculata Nees. (Kalmegh) is widely used as medicinal plant in India and south-east Asian countries for treatment of liver disorder, bowl complaints, malaria, hypertension and general debilities and it is included in Indian Pharmacopoeia (IP). The whole herb contains medicinally active diterpinoid compounds like andrographolide (Handa *et al*., 4), deoxyandrographolide and neoandrographolide with varying proportions in different plant parts. The herb grows well in shaded places as undergrowth in forest. Andrographolide content varies with season, physiology or developmental stage and nutritional status of the plants. The information on the effect of light intensity in the synthesis and accumulation of andrographoloide during the pheno-phases is lacking. There are several studies on the effect of shade on nutritive value of forage crops in the literature (Biscoe and Gallachar, 3; Bierhuizen *et al*., 2). Considerable evidence exists on the effect of shade on the fruit flavour quality of orchard crops, largely from studies of canopy pruning and fruit thinning strategies (Marini *et al*., 6) and orchard design (Wagenmakers and Callesen,12; Pattern and Proebsting, 9). However, information available on the effect of shading on secondary metabolites on medicinal plants is very little particularly in *A. paniculata*. Mapping the response of *A. paniculata* with changes in environmental conditions, including light's is an important step in understanding possible reasons for the variability in the andrographolide content in this crop. This may give growers the potential to manipulate active principal content by managing the growth environment with an aim of producing herb of a more consistent quality through a single crop and also from season to season.

*Corresponding author: E-mail: sarav_nrc@yahoo.com

MATERIALS AND METHODS

The field experiments were carried out at the experimental farm of National Research Centre for Medicinal and Aromatic Plants, Anand (lat. 07° 15'N, long. 78° 14'E.), Gujarat during the rainy season (June-November) in two consecutive years (2002 & 2003). The shade treatments were imposed in the field with artificial shade nets which provided 25, 50 and 70% of incident photosynthetic active photon flux density (PFD) during the growing period. The open light condition received 100% (approx. 1,800-2,000 mmol $m^{-2}s^{-1}$ PAR) of the sun light at noon, whereas 70, 50, and 25% PFD treatments received approximately 1,400-1,500, 1,000-1,100 and 650-700 mmol $m^{-2}s^{-1}$, respectively. The PFD measurements were just above the plant canopy using the external light sensors of portable photosynthesis systems (Li-Cor 6400, Li-Cor, Inc, Lincoln, NE, USA). For each treatment, there were five replications and fifty plants per replications were maintained. Leaf photosynthetic parameters were measured in clear days between 11.00 and 14.00 h using portable, open circuit, infra-red gas analysis system. The youngest fully expanded leaves from the exposed outer layer of the

canopy were used for gas exchange measurement. Leaf respiration was measured on the same leaves in which photosynthesis was recorded by covering the leaf chamber by black cloth until the measurement values stabilized for the gas exchange parameters. Simultaneously, measurements were made on CO_2 and H_2O vapour flux, air (T_{air}) and leaf temperature (Tc), net photosynthesis (P_n), stomatal conductance (g_s), transpiration (E) and intercellular CO_2 partial pressure (C_i). A leaf chamber of 6 cm^2 was used. Artificial illumination was supplied to the leaf from a red-blue light source attached to the sensor head. The irradiation was set according to the prevailing incident PFD. Leaf area was recorded using portable leaf area meter (LI-3000A).

Sample sizes of 0.5 g dry powder from leaves and stems of three months old plants were extracted exhaustively with a 1:1 mixture of dichloromethane and methanol by cold maceration. The extract was filtered and the solvent was removed on rotary evaporator. The dark green crystalline residue obtained was washed with toluene. After complete removal of toluene, the residue was dissolved in HPLC mobile phase, i.e. 80% methanol. This solution was filtered through 0.45 micron syringe filter and 10 µl was used for HPLC analysis. The HPLC (Shimadzu, Japan) system consisted LC-10AD pumps and SPD-10A UV-VIS Detector. The HPLC column RP-18 (250 mm x 4.6 mm, 5 µm, LiChrospher, Merck) was used for analysis at room temperature. The maximum absorbency of andrographolide was found at 223 nm (Rajani *et al*., 10) and same wavelength was fixed for detection. Standard andrographolide was used (M/s Sigma-Aldrich, USA) for identification and quantification of andrographolide extract. A stock solution of standard andrographolide was prepared in methanol and different known amount of andrographolide was loaded in HPLC for calibration curve. The analysis was carried out on mobile phase 4:1 of methanol and water at a flow rate 1 ml min^{-1} with the known quantity of standard compound. Weather data on monthly rainfall, maximum and minimum temperature, relative humidity (RH) and pan evaporation were collected from Department of Meteorology, Anand Agricultural University, Anand for 2002 and 2003.

RESULTS AND DISCUSSION

Plant height varied significantly under different light intensities. It was reduced up to 32% under 100% photosynthetic photon flux density (PFD) compared to 25% light. The plants were shorter having reduced internodal length and compact under open light (100%) conditions. The total leaf area per plant was the highest under open light condition followed by 25% light. Lowest leaf area was recorded in plants grown under 70% light treatment. The highest fresh and dry weight of leaves and stems were also obtained under open light (Table 1). The fresh weight and dry weight of stems were the least under 25% light levels. The stem biomass was contributing more to the total plant biomass than leaves in all light levels. The whole plant fresh weight and dry weight were recorded higher under open light. However, significant differences were not observed for plants grown under 70 and 50% light levels for biomass production.

Even though changes in fresh weight of leaves, stems and whole plant were not consistent under different PFD, their dry weights significantly differed from control under different light regimes. Watson *et al.* (13) found that *berseem* clover (*Trifolium alexandrium* L.) and 'Nangeela' subclover (*T. subterraneum* L.) yielded nearly as much dry matter (DM) under 50% shade as in full sunlight. Wong *et al*. (14) observed that shade intolerant legumes had reduced leaf/stem ratio and very large increase in shoot/root ratio, whereas shade tolerant legumes maintained a balance between leaf and stem under shaded conditions. The influence of PFD on leaf morphological characteristics of various species have also demonstrated that acclimation to higher PFDs involves thicker leaves with a greater cell size and number for both the palisade and spongy mesophyll layers (Nobel, 8; Lichtenthaler, 5).

There were significant reduction in net photosynthesis (P_n) and leaf dark respiration (R_d) in the plants grown under different light intensities (Fig 1A) and the reduction was more pronounced between control and 70% light (40%) and between 50 and 25% light intensities (25%). Similarly, the leaf respiration was

Table 1. Plant growth parameters of *Andrographis paniculata* grown under different light intensities.

Treatments	Plant height (cm)	Leaf area (cm^2/m^{-2})	Fresh weight of leaf (g^{-2})	Dry weight of leaf (g^{-2})	Fresh weight of stem (g^{-2})	Dry weight of stem (gm^{-2})	Fresh weight plant (g^{-2})	Dry weight plant (g^{-2})
Open	60.67	97.99	140.02	67.30	729.25	312.10	872.25	379.45
70% light	65.22	55.48	96.38	47.26	526.75	218.20	640.70	265.45
50% light	77.20	67.22	108.61	48.12	466.20	182.90	574.90	230.95
25% light	78.22	78.16	121.28	49.25	522.80	177.45	637.10	226.70
CD at 5%	7.495	15.72	17.27	9.73	76.28	35.26	87.09	39.74

also the highest under open conditions and considerably reduced with reduction in light intensities. Reduction in dark respiration was 21% in 25% light intensities compared to open light condition. Interestingly, the reduction in leaf dark respiration was not reduced in proportionate to the reduction in P_n. This indicated that the irradiance level least affected the respiration as reported in cotton (Zhao and Oosterhuis, 15). Leaf conductance and transpiration were significantly affected in plants under all light intensity levels (Fig. 1B). In 25% light level, the conductance was reduced to 40% of the control, whilst the reduction in leaf transpiration was up to 30%. However, leaf transpiration was 70% of open light in plants receiving 25% light intensities. Murchie *et al.* (7) examined rice for photosynthetic acclimation as a factor controlling photosynthetic activity of lower leaves in a field canopy. They observed that leaf age was dominant in determining P_{max} (maximum photosynthesis) in upper leaves, whereas acclimation to irradiance levels was of greater importance in the lower leaves. The reduction in P_n appeared to be dependent mainly on the factors related to stomatal closure. Bauer *et al*. (1) reported that under short-term shade conditions, leaf stomatal conductance of field grown cotton was reduced by 35 to 42%. They used higher level of shade (up to 85% of unshaded plants) similar to our present study in which we used a maximum of 75% shade. Low PFD showed to decrease photosynthetic electron transport as well as the activities of Calvin Cycle enzymes (Sage and Reid, 11) resulting in low leaf carbon assimilation.

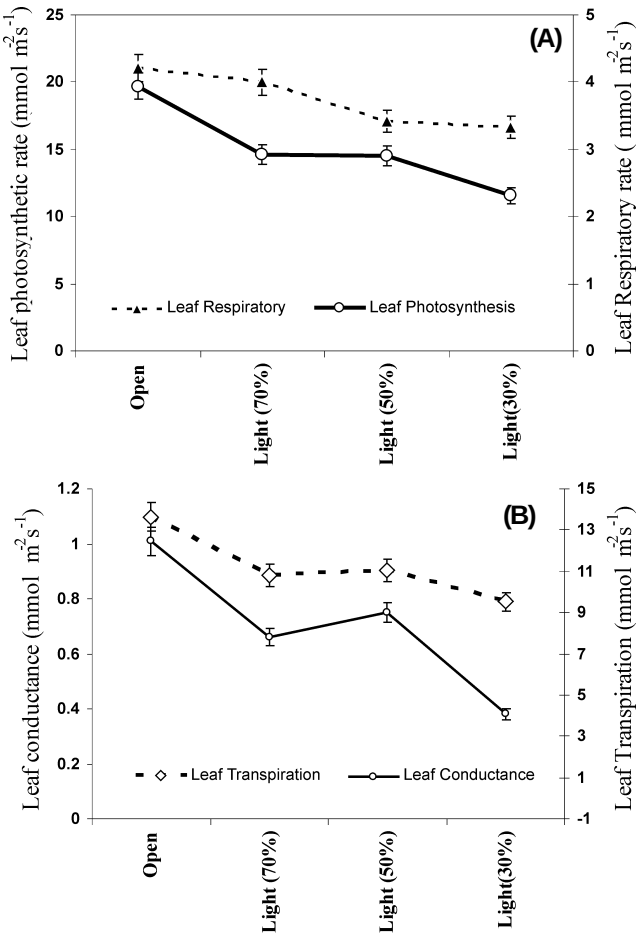

Fig. 1. Effect of different light intensities on photosynthesis and gas exchange of *Andrographis paniculata* . A. Leaf photosynthesis and leaf respiration, B. Leaf conductance and transpiration.

Andrographolide content in leaf varied in full and different reduced light intensities. The highest andrographolide content (1.028%) was recorded in control. There was a reduction up to 64% in andrographolide content in the 25% light condition in the first year. However, higher andrographolide content was recorded in the 50 and 25% light levels respectively in the subsequent year (Fig. 2A). Andrographolide content in leaf did not follow similar pattern in the two years albeit the influence of light on the andrographolide accumulation in leaves. The amount of andrographolide present in the stems was lower than leaves (Fig. 2B). These results imply that PFD is not a major factor influencing the andrographolide content in the leaf and stem.

The yield of andrographolide was higher in leaves under open light condition than other shaded plants in the first year. In the subsequent year, the leaf andrographolide yield did not vary among the light levels (Fig. 2C). Whereas yield of andrographolide from stems of different light levels varied in both the years and low amount of andrographolide yield in shade might be due to the reduction in stem biomass in shaded plants. In the stems, highest andrographolide yield was obtained from stems of 70% light level followed by open and 50% light treatment (Fig. 2D) in 2002. However, highest yield of andrographolide from stems was recorded in the open condition in the second year followed by 70% light level. Plants grown in open light condition (control) yielded higher whole plant andrographolide in both the years (Fig. 2E) and was at par with plants grown under 70% light level in first year. The reduction of leaf dry matter was less pronounced in the shaded plants in both the years and thus compensated for the reduction in whole plant andrographolide yield.

The weather data during the crop growth season of both years are presented in the fig. 3. Comparatively lower rainfalls of 60.6, 0.8 and 92.0 mm were received for June, July and September in the first year than second year. The corresponding rainfall data for these months were 197.0, 460.3 and 157.9 mm respectively in second year (Fig. 3). Consequently, the minimum and maximum RH recorded for these months were

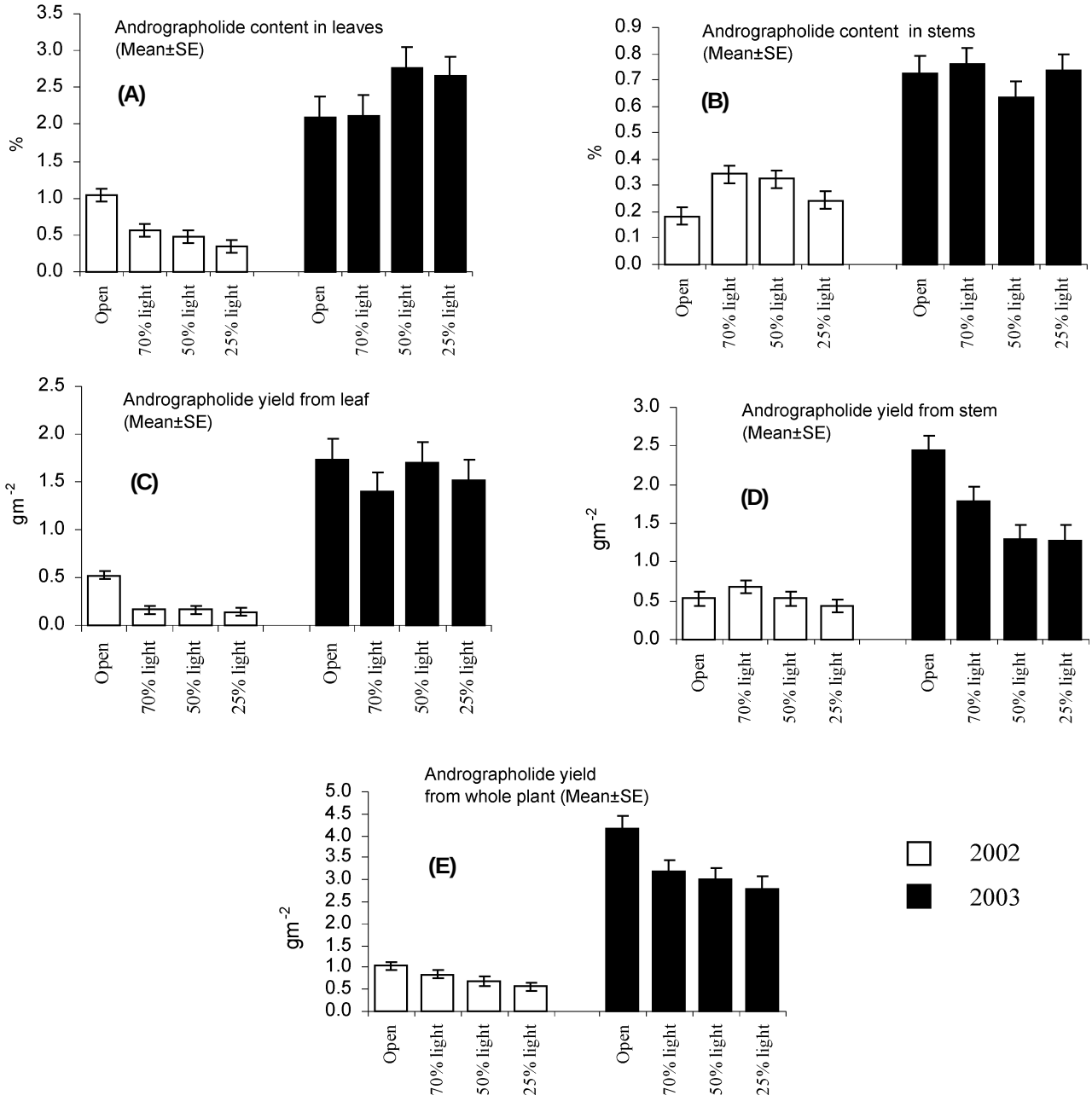

Fig. 2. Effect of different light intensities on andrographolide content (%) and yield in *Andrographis paniculata*. A. content in leaf, B. content in stem, C. yield from leaf, D. yield from stem, E. total yield.

lower in the first year compared to second year. The temperature data also showed lower monthly mean temperatures for second year for the crop growth period. Higher mean evaporation was recorded for June, July and September in first year compared to second year.

The higher andrographolide content and yield in leaf, stem and whole plant was higher in the second year (2003) than the first year (Fig. 2) might be due the influence of weather factors (Fig. 3), i.e. well distributed higher rainfall in 2003 with higher relative humidity, lower evaporation rate and lower maximum and minimum temperature. All these favourable weather parameters had played a decisive role in crop growth and the andrographolide content in the second year. Bare minimum rainfall (0.8 mm) in July 2002 adversely affected growth and development of the crop at the early stage.

Interestingly, the shade treatments (25, 50 and 70% shade levels) did not increase the andrographolide

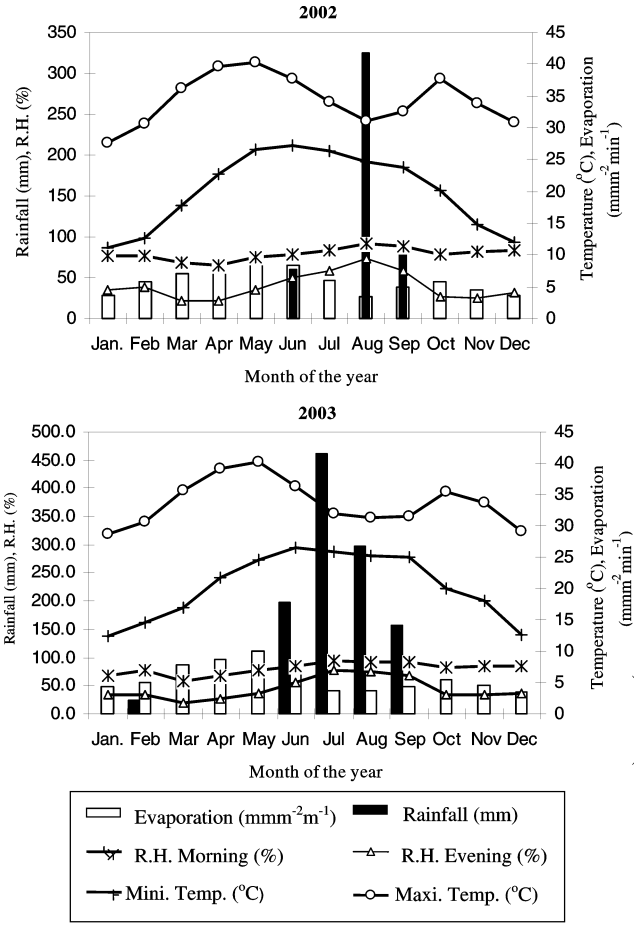

Fig. 3. Mean monthly rainfall (mm), mean maximum temperature (°C), mean minimum temperature (°C), R.H. morning (%), R.H. evening and mean evaporation ($mmm^{-2}min^{-1}$) for 2002 and 2003 at Anand.

yield from *A. paniculata* even though plants grown under different shade levels had increased andrographolide content. The reason for lower andrographolide yield is attributable to lower dry matter production in shade treatments compared to open light condition. The role of light in biosynthesis of andrographolide and the metabolism control in not yet understood. However, light indirectly plays a role by altering the basic processes like photosynthesis and respiration and thereby changing the flux of metabolites and reducing power generated through the light reaction which may in turn, result modification in synthesis and accumulation of andrographolide. Furthermore, the precursor availability in the light or dark periods for the production of andrographolide and the involvement of light in activation of specific enzymes of andrographolide synthesis could be determined to understand the effect of low light on the production of andrographolide.

ACKNOWLEDGEMENTS

The authors thank Indian Council of Agriculture Research (ICAR), New Delhi for the financial support through AP-Cess fund Scheme for this present study and Dr P. Manivel, Principal Scientist, NRCMAP, Anand for critically going through the manuscript.

REFERENCES

1. Bauer, P.J., Sadler E.J. and Frederick, J.R. 1997. Intermittent shade on gas exchange of cotton leaves in the humid southeastern USA. *Agron. J.* **89**: 163-66.

2. Bierhuizen, J.F., Ebbens L.J. and Koomen, N.C.A. 1973. Effect of temperature and radiation on lettuce growing. *Netherlands J. Agri. Sci.* **21**: 110-16.

3. Biscoe, P.V. and Gallachar, J.N. 1977. Weather, dry matter production and yield. **In**: *Environmental Effect on Crop Physiology* (Eds., Landberg, J.J. and Cutting, C.V.). Academic Press, London, U.K. pp. 75-100.

4. Handa, S.S., Sharma, A. and Chakraborthi, K.K. 1986. Natural products and plants as liver protecting drugs. *Fitoterapia,* **LVII**: 347-51.

5. Lichtenthaler, H.K. 1985. Differences in morphology and chemical composition of leaves grown at different light intensities and qualities. **In:** *Control of Leaf Growth* (Eds., Baker, N.R., Davies, W.J. and Ong, C.K.). Cambridge University Press, Cambridge. pp. 201-21.

6. Marini, R.P., Sowers, D. and Marini, M.C. 1991. Peach fruit quality is affected by shade during final swell of fruit growth. *J. Amer. Soc. Hort. Sci.* **116**: 383-89.

7. Murchie, E.H., Hubbart, S., Chen, Y., Peng, S. and Horton, P. 2002. Acclimation of rice photosynthesis to irradiance under field condition. *Plant Physiol.* **130**: 1999-2010.

8. Nobel, P.S. 1976. Photosynthetic rates of sun versus shade leaves of *Hyptis emoryi* Torr. *Plant Physiol.* **58**: 218-23.

9. Pattern, K.D. and Proebsting, E.L. 1986. Effect of different artificial shading times and natural light intensities on the fruit quality of 'Bing' sweet cherry. *J. Amer. Soc. Hort. Sci.* **111**: 360-63.

10. Rajani, M., Neeta Shrivastava and Ravishankara, M.N. 2000. A rapid method for isolation of andrographolide from *Andrographis paniculata* Nees. (Kalmegh). *Pharm. Biol.* **39**: 204-9.

11. Sage, R.F. and Reid, C.D. 1994. Photosynthetic response mechanism to environment change to C_3 plants. **In:** *Plant-Environment Interaction* (Ed., Wilkinson, R.E.). Dekkar, New York. USA. pp. 413-99.

12. Wagenmakers, P.S. and Callesen, O. 1995. Light distribution in apple orchard systems in relation to production and fruit quality. *J. Hort. Sci.* **70**: 935-48.

13. Watson, J.H., Hagedorn, C., Knight W.E. and Pearson, H.A. 1984. Shade tolerance of grass and legume germplasm for use in the southern forest range. *J. Range Manag.* **37**: 229-32.

14. Wong, C.C., Sharudin, M.A.M. and Rahim, H. 1985. Shade tolerance of some tropical forages for integration with plantations: II. Legumes. *MARDI Res. Bull.* **13**: 249-69.

15. Zhao, D. and Oosterhuis, D. 1998. Cotton responses to shade at different growth stages on nonstructural carbohydrate composition. *Crop Sci.* **38**: 1196-1203.

(Received: September, 2007; Revised: December, 2007; Accepted: January, 2008)