Photosynthesis and Carbon Isotope Discrimination ($\Delta^{13}C$) in Cassava (*Manihot esculenta*) Grown under Natural shade of Coconut Plantation

Krishnaprasad B.T[1*#], Savitha A[1], Bindumadhava, H[2+]. and Krishnamurthy K.S[3].

[1]*College of Agriculture, Kerala Agricultural University, Kasaragod 671 328, India.# Present address Department of Agri. Biotechnology) College of Agriculture, Hassan, UAS, Bangalore*

[2]*Department of Crop Physiology, UAS, GKVK, Bangalore- 560 065, India. +Present address: World Vegetable Centre, ICRISAT Campus, Greater Hyderabad, Telangana 502324 India.*

[3]*Indian Institute of Spices Research, Calicut, Kerala, India*

* Author for correspondence: krishnaprasad@uasbanagalore.edu.in

***Abstract*-Cassava (*Manihot esculenta*) is an important food crop in developing country. Due to non availability of land, famers are forced to grow the shade sensitive crops like cassava under the canopy of plantation crops. In this study the effect of natural shade on three genotypes of cassava with respect to their morphological and physiological changes were examined. Three genotypes showed considerable changes in plant height, leaf number, stomatal number, Net Assimilation Rate (NAR) and photosynthetic rates in response to shade. Decrease in photosynthetic rate resulted in high internal CO_2 concentration which in turn lead to high carbon isotope discrimination ($\Delta^{13}C$) under shade. Use of $\Delta^{13}C$ as an indicator of photosynthetic efficiency under shade is discussed.**

***Key words*-Tapioca, Shade tolerance, Carbon isotope discrimination ($\Delta^{13}C$), Photosynthetic rate**

I. INTRODUCTION

Cassava is an important food crop mainly grown in the southern part of Kerala, Tamil Nadu and Andhra Pradesh. In India Kerala is the major state contributing to 45% of total tapioca area under cultivation and 59% of its production [1]. Cassava is intercropped with early maturing crops such as maize, cowpea, and groundnut [2]. More than 50 percent of Agricultural land area in Kerala is occupied by plantation crops. Owing to limited agricultural land area, farmers are forced to grow light requiring crops like cassava also under the canopy of plantation crops [3]. Tapioca is highly susceptible to low light [4]. Tuber yield is reduced by 50% due to mere 20 percent shade [5]. Plants undergo morphological, anatomical and biochemical changes to adapt to low light [6]. Two cultivars of cassava exhibiting high productivity under shade was due to high tuber number and tuber girth [7].

Photosynthetic efficiency is reduced under low light. Nedunchezhiyan et. al., [6] found that internal CO_2 concentration altered the photosynthetic efficiency. Plants with high photosynthetic efficiency are preferred to alleviate the extent of yield loss due to shade. Leaf tries to adapt to shade by bringing a significant changes in the gene expression. Recently, it was found that natural shade in cassava significantly increased the expression of genes involved in the light reaction of photosynthesis, light signaling pathway and DNA synthesis [7]. To measure the photosynthetic efficiency under shade a comprehensive parameter that accounts all changes brought about by plant is the key which can be utilized in the crop improvement for shade tolerance.

From that context, carbon isotope discrimination ($\Delta^{13}C$) provides sufficient information about the cumulative effect of photosynthesis function [9][10][11]. $\Delta^{13}C$ was extensively used as a surrogate for water use efficiency in many crop plants [12][13][14]. Recently Krishnaprasad et. al., [15] explored the possibility of using this trait as physiological marker for shade tolerance in black pepper. In the present study the effect of natural shade of coconut on physiological parameters and carbon isotope discrimination in 3 varieties of tapioca was studied.

II MATERIALS AND METHOD

Three varieties of cassava (*Manihot esculenta*) namely M_4, Kalpaka and Vellayani Hraswa were grown under two conditions at College of Agriculture, Kasaragod, India. One set of plants were grown under natural shade of 20 year old coconut plantation. Another set was grown under open condition. Tapioca sets of 15-20 cm long were planted on mound. Fertilizers and FYM application and other agronomic practices were carried out as per package of practice recommendation of Kerala Agricultural University [16]. Plants were harvested at 40 and 70 days after planting and dry weight of root, shoot and leaf were recorded. On the basis of dry weight and leaf area various parameters such as Leaf Area Ratio, Net Assimilation Rate, Relative growth rates and Root to shoot ratio were calculated as per Dhopte and Patil 2002 [17]. Stomatal frequency was measured by impression method as per Wang and Clarke (1993b) [18]. Carbon Isotope discrimination was measured in fully mature healthy leaf. Leaves were harvested and dried in a hot air oven at 80^0C for 3 days. Leaf samples were powdered and analyzed for carbon isotopes at National facility for stable isotope studies in biological sciences, Department of Crop Physiology, UAS, Bangalore, India. $\Delta^{13}C$ was measured using continuous flow Isotope Ratio Mass Spectrometer (IRMS). The extent of $\Delta^{13}C$ was computed as per Farquhar et al [11] and expressed in per mil (‰, parts per thousand) notation. Non-destructive measurements of the photosynthetic rate and transpiration rate were measured using Portable Photosynthesis System (ADC, BioScientific Ltd., England). Measurements were made at 10:00 a.m. on healthy, completely expanded young leaves. All the measurements were conducted on sunny days, 70 days after planting.

III RESULTS AND DISCUSSION

Light quantity as well as light quality is altered under the plant canopy. Ratio of red to far red light is decreased which act as a signal for the plants growing under the canopy [6]. Plants growing under the canopy respond to this signal in terms of elongation of shoot, increased leaf area and reduced yield. Cassava is a shade intolerant plant. Cassava grown under coconut plantation exhibited typical shade avoidance response. All the three genotypes had three times higher plant height compared to that grown under open condition (Table I). Number of leaves produced was less and existing leaves had increased specific leaf area. Significant decrease in the stomatal frequency was observed. Decrease in stomatal number under shade avoids excess water loss per unit amount of carbon fixed. Light is a limiting factor to fix carbon and the plant tries to avoid excess water loss by reducing number of stomata per unit leaf area. Johnston and Onwueme [19] also observed similar results in tuber crops except in taro where stomatal number increased under shade. Taro is a shade tolerant crop and hence it maintains high stomatal density under shade.

TABLE I
PHYSIOLOGICAL PARAMETERS 40 DAYS AFTER PLANTING IN CASSAVA GROWN UNDER OPEN AND SHADED CONDITIONS

Variety	Treatment	Plant height (cm)	Number of leaves per plant	Specific leaf area ($cm^2.g^{-1}$)	Stomatal number per microscopic field
M4	Open	73	106	245	20
	Shade	254	86	342	13
Kalpaka	Open	64	142	264	22
	Shade	233	70	312	12
Vellayani Hraswa	Open	65	119	250	18
	Shade	234	115	356	13
CD at 5%		29.7	5.6	12.39	1.36

The effect of shade on growth and physiological parameters were examined in three varieties of tapioca. Net assimilation rate was severely reduced in all three varieties of cassava plants grown under shade. Among the three varieties examined Kalpaka exhibited less reduction in NAR under shade. Since the amount of carbon fixed is low under shade LAR is increased under shade. Similarly relative growth rate was reduced under shade. No significant differences in the partitioning between root and shoot was observed. Influence of light on root to shoot ratio highly varies between sun and shade species. Less shade tolerant species tend to have small root [20]. But no significant differences were found in cassava with respect to root to shoot ratio.

TABLE II
GROWTH ANALYSIS OF TAPIOCA GROWN UNDER OPEN AND SHADED CONDITION

	NAR ($g/m^2/day$)		**LAR (cm^2/g)**		**RGR (mg/day)**		**Root/shoot allometry**		**Leaf weight fraction (mg/g)**	
Variety	Open	Shade	Open	Shade	Open	Shade	Open	Shade	Open	Shade
M4	7.08	1.27	35.7	79.6	24.2	10.0	1.22	1.29	150	234
Kalpaka	5.76	2.25	37.6	60.8	21.7	13.5	2.01	2.03	164	198
Vellayani Hraswa	5.73	1.88	47.1	86.5	25.0	15.1	1.58	1.24	191	245
CD at 5%	1.69		12.4		NS		1.34		NS	

NS-No significant differences

Carbon isotope discrimination provides ample information about the photosynthesis. In this study $\Delta^{13}C$ was measured in leaf of cassava plants grown under natural shade of coconut plants. Significant increase in the carbon isotope discrimination was observed under shade (Table III). Similar results were observed in black pepper, plants in hanging gardens and forest species by Krishnaprasad et al.,[15] Flanagan [21] and Kennedy et al [22] respectively. Carbon isotope discrimination depends on the internal CO_2 concentration [10]. Leaf exposed to low light maintains high Internal CO_2 concentration as the rate of CO_2 fixation is low. High internal CO_2 leads to high carbon isotope discrimination and hence high $\Delta^{13}C$. Interestingly Krishnaprasad et al [15] found genetic variation for $\Delta^{13}C$ in black pepper varieties and also correlated this to photosynthetic efficiency under low light. In this study only three genotypes were used hence considerable genetic variation in $\Delta^{13}C$ was not observed. Further studies with large number of genotypes are necessary to relate photosynthetic efficiency to $\Delta^{13}C$ under low light and also genetic variation which could be utilized for crop improvement in cassava.

TABLE III
CARBON ISOTOPE DISCRIMINATION ($\Delta^{13}C$ per mil) IN CASSAVA LEAF GROWN UNDER OPEN AND SHADED CONDITIONS

	40 days after planting		70 days after planting	
Variety	Open	Shade	Open	Shade
M4	20.700	20.270	21.316	23.413
Kalpaka	20.194	22.112	21.267	22.582
Vellayani Hraswa	20.217	22.055	21.212	23.159
CD at 5%	0.84		0.58	

Effect of shade on photosynthetic efficiency was examined using portable photosynthetic system. Photosynthetic rate was significantly low under shade (Table-IV). Kalpaka had high photosynthetic rate under open condition but it had least photosynthetic rate under shade. Transpiration rate depends on the stomatal conductance. All three genotypes showed lower transpiration rate under shade compared to open condition. Photosynthetic rate measured using portable photosynthesis system is instantaneous values. Light intensity below the canopy is dynamic. Understory plants receive varied intensity of light within a day depending upon the direction of sun and movement of canopy due to wind. In this regard carbon isotope discrimination serve as an average rate of carbon fixation over a period of time. $\Delta^{13}C$ is changed in response to light intensity received by the plant grown under natural shade and it is related to internal CO_2 concentration (Table III and Table IV). Therefore a plant maintaining low internal CO_2 concentration by efficiently capturing light under low light is also reflected in $\Delta^{13}C$ values. By taking example of black pepper [15] and forest species [22], it is hypothesized that in cassava also $\Delta^{13}C$ can be used as a tool to identify shade tolerant genotype. This needs to be confirmed using large number of genotypes of cassava differing in shade tolerance.

TABLE IV
PHOTOSYNTHETIC RATE AND GAS EXCHANGE PARAMETERS IN TAPIOCA GROWN UNDER OPEN AND SHADED CONDITIONS

Variety	Treatment	Light (μmole.m^2.s)	Ci (ppm) (Internal CO_2 concentration)	Photosynthetic rate (μmole. m^2.s)	Transpiration (mmole.m^2.s)
M4	Open	1853	258	16.80	4.10
	Shade	400	291	6.21	2.50
Kalpaka	Open	1862	249	18.9	3.86
	Shade	415	295	4.99	2.16
Vellayani Hraswa	Open	1845	271	16.49	4.49
	Shade	417	284	6.87	1.48
CD at 5%		5.31	28.51	1.16	0.58

CONCLUSION

Three cultivars of cassava were grown under natural shade of coconut garden and were compared with those grown under open sun. Results revealed that cassava cultivars undergo morphological and physiological changes in response to shade. A decrease in leaf number, leaf thickness, stomatal density and photosynthetic efficiency and a very great increase in plant height were observed in all the three genotypes of cassava under shade. A significant increase in the carbon isotopes discrimination was found in all the three genotypes under shade which is due to high internal CO_2 concentration. Based on the gas exchange parameters and $\Delta^{13}C$ values we hypothesize that $\Delta^{13}C$ can be employed as time integrated surrogate for photosynthetic efficiency and hence to select shade tolerant genotypes in cassava.

ACKNOWLEDGMENT

We wish to thank Kerala State Council for Science, Technology and Environment (KSCSTE), Thiruvananthapuram for funding this research work. Thanks to Dr.M.S. Sheshshayee, Professor, Department of Crop Physiology, for quantifying carbon isotopes.

REFERENCES

[1] S. Edison, M. Anantharaman and T. Srinivas Status of cassava in India An overall view 2006 Technical bulletin series 46 Central Tuber Crops Research Institute Sreekariyam, Thiruvananthapuram 695 017 Kerala, India.

[2] H. Mutsaers, H. Ezumah and D. Osiru, Cassava-based intercropping: a review. Field Crops Res. 34, 431–457 (1993).

[3] T. Ramanujam, G. M. Nair and P. Indira Growth and development of cassava (*Manihot esculenta* Crantz) genotypes under shade in a coconut garden. Turrialba 34, 267–274 (1984).

[4] M. Johnston and Onwueme, I. Effect of shade on photosynthetic pigments in the tropical root crops: yam, taro, tannia, cassava and sweet potato. Exp. Agric. 34, 301–312 (1998).

[5] Paul S.O. Okoli and G.F.Wilson Response of cassava (*Manihot esculenta* crantz) to shade under field conditions Field Crop Research 14: 349-359 (1996)

[6]Vandenbussche, F., Pierik, R., Millenaar, F. F. & Voesenek, L. A., Van Der Straeten, D. Reaching out of the shade. Curr. Opin. Plant Biol. 8, 462–468 (2005).

[7] M. Nedunchezhiyan, G. Byju and V. Ravi Photosynthesis, Dry Matter Production and Partitioning in Cassava (Manihot esculenta Crantz) Under Partial Shade of a Coconut Plantation Journal of Root Crops, 2012, Vol. 38 No. 2, pp. 116-125 (2012)

[8] Zehong Ding1, Yang Zhang, Yi Xiao, Fangfang Liu, Minghui Wang, Xinguang Zhu, Peng Liu, Qi Sun, Wenquan Wang, Ming Peng, Tom Brutnell and Pinghua Li Transcriptome response of cassava leaves under natural shade Sci. Rep.6:31673 (2016)

[9] M. H. O'Leary "Carbon isotope fractionation in plants Phytochemistry" Vol. 20 1981No. 4, pp.553-567,.

[10] G.D. Farquhar, J.R. Ehleringer and K.T. Hubick "Carbon isotope discrimination and photosynthesis". Annu.Rev.Plant.Physiol.Plant Mol.Biol. 40: 503-537 (1989)

[11] C. Dbscolas-Gros and M. Fontugne Stable carbon isotope fractionation by marine phytoplankton during photosynthesis Planl, Celt and Environment 13, 207-218 (1990)

[12] G.D. Farquhar and R.A. Richards "Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes" Aust.J.Plant Physiol. 1984 11: 539-552

[13] Y.Saranga, I. Flash and D. Yakir. "Variation in water use efficiency and its relation to carbon isotope ratio in cotton" Crop Sci. 1998 38: 782-787

[14] Ashok, I.A. Hussain, T.G. Prasad, M.U. Kumar, R.C.N. Rao, G.C.Wright,. "Variation in transpiration efficiency and carbon isotope discrimination in cowpea" Australian Journal of Plant Physiology 1999 26: 503–510

[15] B.T.Krishnaprasad, A. Savitha, H. Bindumadhava and K.S.Krishnaprasad Carbon isotope discrimination as a physiological marker for shade tolerance in black pepper (Piper nigrum L) International Journal of Advanced and Innovative Research 6: 85-90 (2017)
[16] A.I. Jose eds. Package of Practices Recommendations: Crops. 2002. 12th Edition Kerala Agricultural University, Trichur. 278p
[17] A.M. Dhopte and B.N. Patil Basic equations for growth studies In A.M. Principles and Techniques for Plant Scientist, 2002 (Eds. Dhopte A.M. and Livera M. Jodhpur), Agrobios, 373 p
[18] H. Wang and J.M. Clarke Genotypic, intra plant and environmental variation in stomatal frequency and size in wheat Can J Plant Sci 73: 671-678 (1993b)
[19] M. Johnston and I. C. Onwueme Influence of shade on stomatal density, leaf size and other leaf characteristics in the major tropical root crops, tannia, sweet potato, yam, cassava and taro Experimental Agriculture 34, pp 301-312 (1998)
[20] Kun-Fang Cao Tatsuhiro Ohkubo Allometry, root/shoot ratio and root architecture in understory saplings of deciduous dicotyledonous trees in central Japan Ecological research, 13: pp 217–227. (1998)
[21] L.B.Flanagan, C.S. Cook, and J.R. Ehleringer.. Unusually low carbon isotope ratios in plants from hanging gardens in southern Utah. Oecologia 111: 1997 481-489.
[22] S. Kennedy, Á.N. Dhubháin J. Ferguson, O. Schmidt J. Dyckmans, B. Osborne, K. Black “Potential use of leaf carbon isotope discrimination for the selection of shade-tolerant species Forest ecology and Management” 2006 273: 394-403