



SCIENTIFIC NOTE

Physiological indexes of custard apple as a function of nitrogen fertilization and humic substances

*Índices fisiológicos da pinheira em função de adubação nitrogenada e substâncias húmicas*

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KEYWORDS

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PALAVRAS-CHAVE

*Annona squamosa*  
Clorofila  
Radiação fotossinteticamente ativa  
Fruta tropical

**ABSTRACT:** Custard apple (*Annona squamosa* L.) is native to tropical America and it occurs spontaneously in the Brazilian northeast region, where it is exploited mainly at subsistence level owing to lack of adequate management and selected genetic material. In this study, we aimed to evaluate the physiological characteristics of custard apple as a function of nitrogen fertilization and application of humic substances in the municipality of Bom Jesus, Piauí state, Brazil. We adopted a randomized block experimental design with treatments arranged in a 4 × 2 factorial scheme, referring to four doses of nitrogen and the presence and absence of humic substances (Humitec®). The following variables were evaluated: leaf area index (LAI), chlorophyll (A, B, and Total), and photosynthetic active radiation (PAR). The maximum agronomic efficiency for intercepted photosynthetically active radiation was registered under fertilization with 174.70 g/plant of N.

**RESUMO:** A pinheira (*Annona squamosa* L.) é originária da América tropical, ocorrendo espontaneamente no Nordeste brasileiro, onde é explorada principalmente em nível de subsistência, pela falta de manejo adequado e material genético selecionado. O objetivo do presente trabalho foi avaliar características fisiológicas da pinheira em função de adubação nitrogenada e substâncias húmicas, em Bom Jesus-PI. Adotou-se o delineamento em blocos casualizados com tratamentos distribuídos em esquema fatorial 4 × 2, referentes às quatro doses de nitrogênio e a presença e ausência de substâncias húmicas (Humitec®). Foram avaliadas as seguintes variáveis: índice de área foliar (IAF), clorofila (A, B e Total) e radiação fotossinteticamente ativa (RFA). A máxima eficiência agrônoma para radiação fotossinteticamente ativa interceptada é obtida com a dose de 174,70 g/planta de N.

## 1 Introduction

Custard apple (*Annona squamosa* L.), also known as sweetsop or sugar apple, probably originated in the Caribbean region and is the most widely distributed and also the most commonly grown *Annona* species in the tropical regions of the Americas (including Brazil), as well as in Africa, Asia, and the Pacific (PAULL; DUARTE, 2011).

Fertilizer management is one of the most important agronomic techniques for custard apple plants, which, in general, extract high nutrient contents from soil. Among all nutrients, nitrogen is of fundamental importance because it is part of amino acids, proteins, and nucleic acids, and it directly or indirectly influences many biochemical plant processes (PESSARAKLI, 2002). Nevertheless, it has been poorly studied, especially its association with humic substances and their physiological consequences to custard apple plants.

Humic substances stimulate plant growth by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities (TAHIR et al., 2011), and hormones (TREVISAN et al., 2010). Because of their direct effect on plants, these substances have been used on plant production applied directly on soil or substrate, because they improve soil fertility and nutrient availability (EYHERAGUIBEL; SILVESTRE; MORARD, 2008).

Biological development of cultivated plants can be closely evaluated through physiological variables such as photosynthetically active radiation (PAR), leaf area index (LAI), and leaf chlorophyll. The solar radiation intercepted depends on leaf area, leaf angle, plant architecture, and plant distribution in the field, and the efficient use of radiation is directly related to the canopy potential for PAR (radiation between 390 and 770 nm) interception, considering that PAR is a measure of incident radiation photons with wavelengths useful for photosynthesis (NING et al., 2009).

Accordingly, Dang et al. (1997) reported that the amount of PAR absorbed by a canopy is linearly related to its

photosynthetic capacity, with significant correlations between PAR, light levels, and nitrogen plant supply.

In this sense, the objective of this research was to study the effect of the application of humic substances and nitrogen fertilization on the physiological characteristics of custard apple.

## 2 Materials and Methods

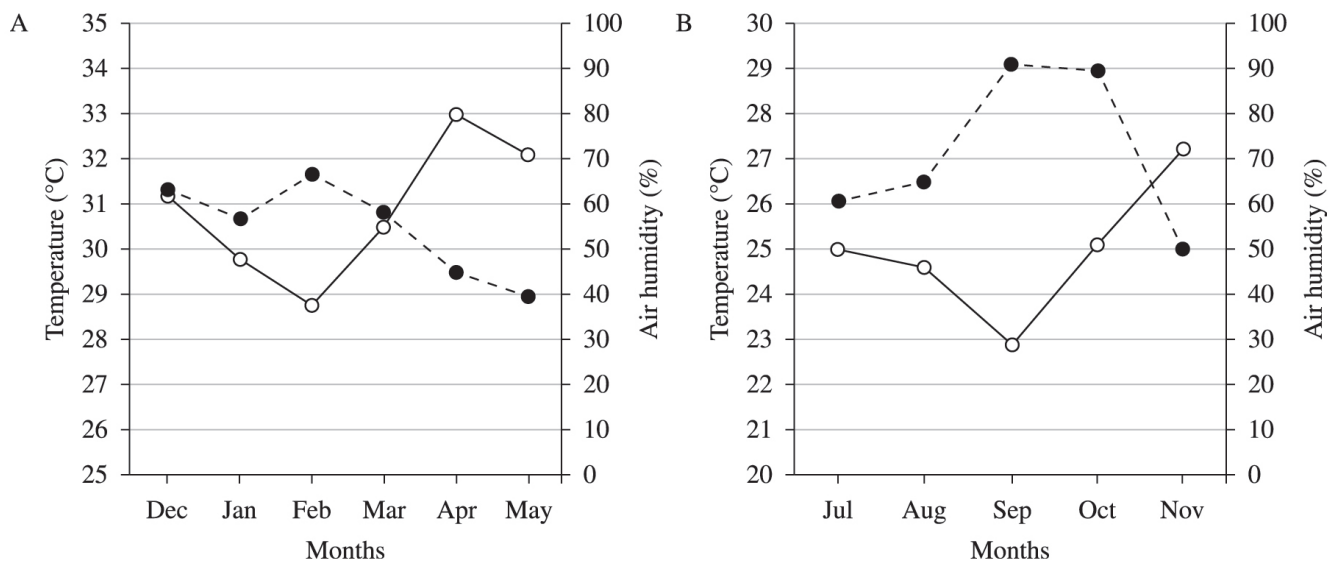
The study was carried out from December 2010 to May 2011 (first trial) and from June 2011 to November 2011 (second trial) on “Campus Profa. Cinobelina Elvas”, Federal University of Piauí, Piauí state, northeastern Brazil.

Five-year-old custard apple (*Annona squamosa* L.) plants propagated by seeds were used in this study.

The soil of the experimental area presented - before the execution of this experiment - the following physical and chemical characteristics: pH = 4.2; O.M. = 1.0 g kg<sup>-1</sup>; P (Mehlich-1) = 5.0 mg dm<sup>-3</sup>; K<sup>+</sup> = 0.13 cmol<sub>c</sub> dm<sup>-3</sup>; Ca<sup>2+</sup> = 1.10 cmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup> = 0.40 cmol<sub>c</sub> dm<sup>-3</sup>; Na<sup>+</sup> = 0.02 cmol<sub>c</sub> dm<sup>-3</sup>; Al<sup>3+</sup> = 0.5 cmol<sub>c</sub> dm<sup>-3</sup>; H<sup>+</sup> + Al<sup>3+</sup> = 3.40 cmol<sub>c</sub> dm<sup>-3</sup>; SB = 1.65 cmol<sub>c</sub> dm<sup>-3</sup>; CEC = 5.05 cmol<sub>c</sub> dm<sup>-3</sup>; V = 32.67%; m = 23.26%; sand = 510.0 g kg<sup>-1</sup>; silt = 100 g kg<sup>-1</sup>; and clay = 390 g kg<sup>-1</sup>.

The climatic data of air temperature and air humidity (thermo-Hygrometer Instrutemp®, Brazil), which were collected during the execution of the experiments, are shown in Figure 1.

Plants, spaced 4 m between rows and 3 m along the row, were drip-irrigated daily by self-regulating emitters positioned every 0.5 m for a flow of 2.8 L h<sup>-1</sup>. Pruning was performed following instructions by Paull and Duarte (2011). Liming with 1.500 kg ha<sup>-1</sup> (1.80 kg/plant) was performed 60 days before the beginning of the experiment, and all plants were fertilized with 120 g of K<sub>2</sub>O (potassium chloride, 60% of K<sub>2</sub>O) at 30, 60 and 90 days after pruning and 120 g of P<sub>2</sub>O<sub>5</sub> (triple superphosphate, 42% of P<sub>2</sub>O<sub>5</sub>) at 30 days after pruning, according to instructions by Fernandes (1993).



**Figure 1.** Average air temperature and air humidity during the execution of the experiments. (A - first trial; B - second trial).

Urea (45% of N), monthly applied (four fertilizations) and defined according to Fernandes (1993), was used as nitrogen source, while the humic substances used in the experiment were extracted from leonardite humic acid powder (Humitec®), whose complete composition is as follows: humic extract (16.5%), organic carbon (11.2%), humic acids (13.2%), and fulvic acids (3.3%). The humic substances applied followed the recommendations of the producer, i.e., 30 mL diluted in 3 L of water every 60 days after pruning, reaching two applications for each experiment.

We adopted a randomized block experimental design with treatments distributed in a 4x2 factorial arrangement referring to four doses of nitrogen (0, 100, 175 and 250 g/plant of N) and the presence and absence of humic substances, with four replications.

The following variables were verified 142 days after pruning : i) Leaf Chlorophyll (*a*, *b* and Total Index) was measured using a chlorophyll meter (Falker®, Brazil) in three leaves of each seedling following the methodology by El-Hendawy, Hu and Schimidhalter (2005); and ii) Leaf area index (LAI) and intercepted photosynthetically active radiation (*Int.PAR*) were measured in triplicate using a multi-sensor (80 sensors) PAR ‘Ceptometer’ (AccuPAR, Decagon Devices Inc., USA) in the crop canopy, between 9-10 a.m. of a sunny day. The maximum agronomic and economic efficiencies were also calculated for *Int.PAR* as a function of N fertilization.

Statistical analyses included analysis of variance (ANOVA), mean separation of humic substances using the Tukey test, and regression analysis of nitrogen doses using Sigmaplot software. Values were considered statistically significant at  $p < 0.01$ .

### 3 Results and Discussion

In this study, no significant interactions between nitrogen doses and humic substances were registered, showing that these factors are not interdependent in custard apple cultivation.

As it can be seen in Table 1, nitrogen doses significantly influenced only photosynthetically active radiation (PAR).

The lack of significant influence of nitrogen doses for LAI could be explained by the uniform defoliation performed in all plants and the low capacity of custard apple to vegetate

again, as reported by Paull and Duarte (2011). LAI average values are higher than those reported by Farias et al. (2003) also in study on N doses.

LAI was not significantly affected by humic substances (Table 1), a result that disagrees with those of Dang et al. (1997), who found that LAI and photosynthesis are enhanced by humic substances applied on soil. Independently of nitrogen doses or humic substances, all LAI average values are below 3.38, which strongly promote consequences for commercial crops, especially in relation to defoliation practice. This way, Andriolo (1999) reported that when LAI is higher than 3.5, liquid CO<sub>2</sub> assimilation for fruit species decreases due to the higher energy consumption needed to supply those leaves below compensation level, showing that the LAI values registered in the present work were adequate.

In relation to chlorophyll *a*, *b* and total, as also observed for LAI data, there was no significant influence of N doses (Table 1) with a short range from the lower (non-fertilized plants) to the higher (250 g/plant of N), which was also reported by Baldotto et al. (2009) in a study on pineapple cultivation in Brazil. The lack of statistical effect could be explained by the pruning principles properly described by Pessaraki (2002), who argued that the more severe the pruning, the greater the vigor of the new sprout, and this effect occurs due to the relative N availability accumulated by plants on older shoots, as well as to the higher N reserves on stem and roots, parts that are not pruned. Therefore, plants only convert into chlorophyll the necessary N, regardless of the N dose applied (HALLIK et al., 2009; WANI et al., 2011).

It is important to note that the leaves used for chlorophyll measurement were randomly chosen and, according to Pessaraki (2002), chlorophyll breakdown is strongly retarded by continuous illumination and light intensity when compared with leaves kept in the dark. Even light quality (in relation to red or far-red components) is relevant because of the participation of phytochrome in light-mediated responses during senescence.

Independently of the nitrogen doses used, humic substance average values for chlorophyll *a*, *b* and total were above those registered by Nogueira and Silva Júnior (2001) in a study on

**Table 1.** Leaf area index (LAI), leaf chlorophyll *a*, *b* and total indexes, and intercepted photosynthetically active radiation (*Int.PAR*) as a function of nitrogen fertilization and humic substances.

Source of variation	LAI	Chlorophyll			<i>Int.PAR</i> μmol m <sup>-2</sup> s <sup>-1</sup>
		<i>a</i>	<i>b</i>	Total chlorophyll Index	
0 g/plant of N	3.37	36.75	15.21	51.97	1,069.2
100 g/plant of N	3.33	39.07	13.80	52.88	1,215.8
175 g/plant of N	3.36	37.95	12.68	50.64	1,476.2
250 g/plant of N	3.34	39.54	13.98	53.53	1,241.4
Without HS	3.37 a	37.83 a	13.07 a	50.90 a	1,160.9 b
With HS	3.33 a	38.83 a	14.77 a	53.60 a	1,340.4 a
SMD	0.06	1.76	2.77	3.26	162.1
Int. (N × SHs)	2.93ns	0.55ns	1.94ns	2.22ns	1.50ns
CV	2.71	6.25	27.06	8.49	17.62

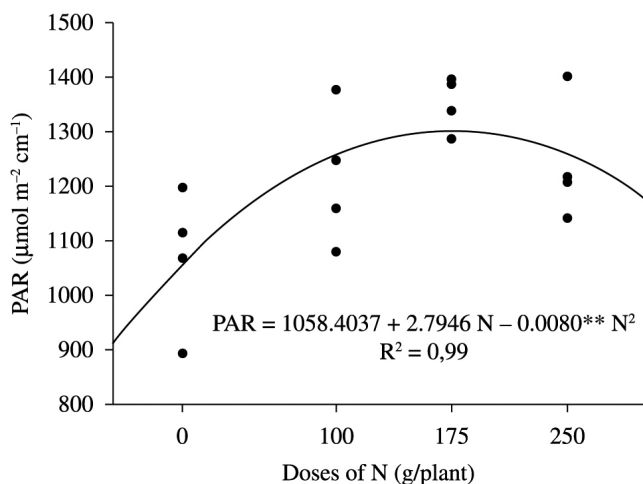
HS = humic substances; SMD = significant minimum difference; CV = coefficient of variation; \*\*significant at  $p < 0.01$  probability error at  $p < 0.01$  probability error; ns: non-significant; means followed by different letters in columns are significantly different according to Tukey test.

soursop (*Annona muricata*), another important fruit species of the *Annonaceae* family. Chlorophyll selectively absorbs 655-665 nm light while transmitting 725-735 nm light.

Increasing nitrogen doses promoted a significant average enhancement of nearly 28% on PAR from the lower to the 175 g/plant of N, followed by a consecutive decay with N dose increase (Figure 2), possibly promoted by nitrogen enhancement in plant, since Pessarakli (2002) explains that higher nitrogen content in plants increases leaf angle with direct effect on leaf light interception, and consequently on PAR. The maximum agronomic efficiency calculated was under 174.70 g/plant of N, which promoted a maximum intercepted PAR of almost 1300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , while the maximum economic efficiency, i.e., 90% of the maximum agronomic efficiency, was estimated in 157.23 g/plant of N.

Accordingly, more erect leaves get a better light distribution profile into the canopy, reducing the light-saturated wasteful leaves on the upper positions and increasing light availability for the unsaturated lower leaves (REYNOLDS et al., 2009); this is directly related to PAR, because canopy architecture associated with light distribution into the canopy promotes higher PAR values (CARRETERO et al., 2010). Fallovo et al. (2009) found that medium PAR levels are associated with improved plant growth and plant N concentration as a function of higher water requirements.

The application of humic substances significantly influenced PAR with an enhancement of almost 14% (Table 1). The positive effect of humic substances on plant PAR could be explained by their positive action on plants, as reported by Tahir et al. (2011), who argued that the direct effects of humic substances are biochemical actions exerted at the wall, membrane, or cytoplasm of cells, and are mainly of hormonal nature, acting similarly to plant growth substances (YASSEN; HELLAL; ABO-BASHA, 2011) and agricultural humic substances, which are reputed to enhance nutrient uptake, drought tolerance, seed germination, and overall plant performance.



**Figure 2.** Intercepted photosynthetically active radiation (Int.PAR) in custard apple as a function of nitrogen doses.

## 4 Conclusions

Both nitrogen fertilization and the application of humic substances positively affect the intercepted photosynthetically active radiation on custard apple orchard;

The maximum agronomic efficiency for intercepted photosynthetically active radiation is registered under fertilization with 174.70 g/plant of N.

## References

- ANDRIOLO, J. L. *Fisiologia das culturas protegidas*. Santa Maria: UFSM, 1999. 120 p.
- BALDOTTO, L. E. B.; BALDOTTO, M. A.; GIRO V. B.; CANELLAS, L. P.; OLIVARES, F. L.; BRESSAN-SMITH, R. Desempenho do abacaxizeiro 'vitória' em resposta à aplicação de ácidos húmicos durante a aclimação. *Revista Brasileira Ciência do Solo*, v. 33, n. 4, p. 979-990, 2009. <http://dx.doi.org/10.1590/S0100-06832009000400022>
- CARRETERO, R.; SERRAGO, R.A.; BANCAL, M. O.; PERELLÓ, A. E.; MIRALLES, D. J. Absorbed radiation and radiation use efficiency as affected by foliar diseases in relation to their vertical position into the canopy in wheat. *Field Crops Research*, v. 116, n. 2, p. 184-195, 2010. <http://dx.doi.org/10.1016/j.fcr.2009.12.009>
- DANG, Q. L.; MARGOLIS, H. A.; SY, M.; COYEA, M. R.; COLLATZ, G. J.; WALTHALL, C. L. Profiles of photosynthetically active radiation, nitrogen and photosynthetic capacity in the boreal forest: implications for scaling from leaf to canopy. *Journal of Geophysical Research*, v. 102, n. 24, p. 28845-28859, 1997. <http://dx.doi.org/10.1029/97JD00194>
- EL-HENDAWY, S.; HU, Y.; SCHIMIDHALTER, U. Growth, ion content, gas exchange, and water relations of wheat genotypes differing in salt tolerances. *Australian Journal of Agricultural Research*, v. 56, p. 123-134. 2005. <http://dx.doi.org/10.1071/AR04019>
- EYHERAGUIBEL, B.; SILVESTRE, J.; MORARD, P. Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. *Bioresource Technology*, v. 99, n. 10, p. 4202-4212, 2008. <http://dx.doi.org/10.1016/j.biortech.2007.08.082>
- FALLOVO, C.; COLLA, G.; SCHREINER, M.; KRUMBEIN, A.; SCHWARZ, D. Effect of nitrogen form and radiation on growth and mineral concentration of two Brassica species. *Scientia Horticulturae*, v. 123, n. 2, p. 170-177, 2009. <http://dx.doi.org/10.1016/j.scienta.2009.09.003>
- FARIAS, C. H. A.; SOBRINHO, J. E.; MEDEIROS, J. F.; COSTA, M. C.; NASCIMENTO, I. B.; SILVA, M. C. C. Crescimento e desenvolvimento da cultura do melão sob diferentes lâminas de irrigação e salinidade da água. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v. 7, n. 3, p. 445-450, 2003. <http://dx.doi.org/10.1590/S1415-43662003000300006>
- FERNANDES, U. L. B. *Manual de Recomendação de Adubação e Calagem para o Estado do Ceará*. Fortaleza: Banco do Nordeste do Brasil, 1993. 248 p.
- HALLIK, L.; KULL, O.; NIINEMET, U.; AAN, A. Contrasting correlation networks between leaf structure, nitrogen and chlorophyll in herbaceous and woody canopies. *Basic and Applied Ecology*, v. 10, n. 4, p. 309-318, 2009. <http://dx.doi.org/10.1016/j.baae.2008.08.001>

- NING, H.; CHUAN-GEN, L.; KE-MIN, Y.; JIANG-SHI, Z. Simulation on Distribution of Photosynthetically Active radiation in canopy and optimum leaf rolling index in rice with rolling leaves. *Rice Science*, v. 16, n. 3, p. 217-225, 2009. [http://dx.doi.org/10.1016/S1672-6308\(08\)60082-7](http://dx.doi.org/10.1016/S1672-6308(08)60082-7)
- NOGUEIRA, R. J. M. C.; SILVA JÚNIOR, J. F. Resistência estomática, tensão de água no xilema e teor de clorofila em genótipos de gravioleira. *Scientia Agricola*, v. 58, n. 3, p. 491-495, 2001. <http://dx.doi.org/10.1590/S0103-90162001000300009>
- PAULL, R. E.; DUARTE, O. *Tropical fruits*. 2nd ed. London: CAB International, 2011. 408 p.
- PESSARAKLI, M. *Handbook of Plant and Crop Physiology*. New York: CRC Press, 2002. 973 p.
- REYNOLDS, M.; FOULKES, M. J.; SLAFER, G. A.; BERRY, P.; PARRY, M. A. J.; SNAPE, J.W.; ANGUS, W. J. Raising yield potential in wheat. *Journal of Experimental Botany*, v. 60, n. 7, p. 1899-1918, 2009. <http://dx.doi.org/10.1093/jxb/erp016>
- TAHIR, M. M.; KHURSHID, M.; KHAN, M. Z.; ABBASI, M. K.; KAZMI, M. H. Lignite-derived humic acid effect on growth of wheat plants in different soils. *Pedosphere*, v. 21, n. 1, p. 124-131, 2011. [http://dx.doi.org/10.1016/S1002-0160\(10\)60087-2](http://dx.doi.org/10.1016/S1002-0160(10)60087-2)
- TREVISAN, S.; PIZZEGHELLO, D.; RUPERTI, B.; FRANCIOSO, O.; SASSI, A. humic substances induce lateral root formation and expression of the early auxin-responsive IAA19 gene and DR5 syntetic element in *Arabidopsis*. *Plant Biology*, v. 12, n. 4, p. 604-614, 2010. PMID:20636903
- WANI, B. A.; RAM, M.; YASIN, A.; SINGH, E. Physiological traits in integral with yield and yield components in wheat (*Triticum aestivum* L.) study of their genetic variability and correlation. *Asian Journal of Agricultural Research*, v. 6, n. 3, p. 194-200, 2011. <http://dx.doi.org/10.3923/ajar.2011.194.200>
- YASSEN, A. A.; HELLAL, F. A.; ABO-BASHA, D. M. Influence of organic materials and foliar application of zinc on yield and nutrient uptake by wheat plants. *Journal of Applied Sciences Research*, v. 7, n. 12, p. 2056-2062, 2011.