ORIGINAL ARTICLE

HOW TO CORRECT MILLET (*Pennisetum glaucum* (L.) R. Br.) LEAF AREA INDEX MEASURED WITH LAI-2000 PLANT CANOPY ANALYZER

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ABSTRACT

Background: The leaf area index (LAI) is crucial variable to describe the state of plant development. The measurement of the LAI can be performed directly through destructive methods, and indirectly based on the radiation interception methods (with SunScan device) or gap fraction methods (with LAI-2000 device). Previous studies showed that indirect measurements underestimated LAI values. **Objectives**: The objective of this study was to determine the relationship between the LAI values obtained by the indirect method using the LAI-2000 and those obtained by the direct method. **Methods:** The experiment was conducted during three years comparing direct and indirect LAI measurements of millet (*Pennisetum glaucum* (L.) R. Br.). The experiment layout was a randomized complete block design with variety (SOUNA 3 and SANIO) and sowing density as factors. **Results**: A strong linear relationship was found between LAI values estimated by the LAI-2000 and values of LAI based on direct method. So, correlations have been established between direct and indirect LAI values. This correlation is independent of the varieties and planting density of millet. **Conclusions:** There is a strong correlation between LAI-2000 values and those of the LAI based on direct method. So, the LAI values can be estimated using LAI-2000 which is less laborious and time consuming.
***Keywords**: Millet, optical leave-area-meter, leaf area index, plant canopy analyzer, radiant interception.*

1. INTRODUCTION

The leaf area index (LAI) is crucial variable to describe the state of plant development [1]. LAI is the ratio of the biomass area (the total aboveground surface of the leaves) to the area of the soil on which the plant grows. This variable is a function of the interception of solar radiation and essential in forecasting the productivity of crop biomass [2]. That means that it exists relationship between LAI ratio and the light conditions during the growing season.

Therefore, knowledge and accuracy of LAI measurements are essential for understanding the interactions between crop growth and environment. The measurement of LAI can be done directly through destructive methods which could be long and laborious work, or indirectly based on the measurement of radiation interception by the plant cover [3, 4, 5]. The direct measurement methods apply to a few leaves or to all the leaves of the plant and involve a leaf area measurement using a device or a specific relationship between dimensions (such as length and leaf width) and leaf area by a coefficient. Whereas the indirect measurement methods aim to estimate the LAI of a plant cover. They involve the measurement of LAI from measurements of the interception of solar radiation through the plant cover based on the theory of radiative transfer [6]. These methods are non-destructive and are based on a statistical and probabilistic approach to the distribution and arrangement of leaves in the plant cover [7]. Measuring the fraction of the sky through the canopy at various angles is a particularly powerful approach [8]. Indirect measurement techniques based on the relationship between transmitted radiation and the architecture of the plant cover are good alternatives to direct destructive measurements. Different equipment like LAI-2000 Plant Canopy Analyzer (Model LAI-2000, Li-cor®, Inc, Lincoln, Nebraska 68504, USA) has been developed for this purpose, which is most commonly used in agronomy and ecophysiology research, and which is the subject of our study to estimate the leaf area index. With this device, the measurements are carried out when the sky is uniformly covered, in widespread radiation conditions. The measurement of the scattered radiation not intercepted by the LAI-2000 indicates the fraction of the sky not stopped by the foliage, branches or stem [8]. The sensor does not distinguish living and dead tissue, so it is impossible to separate the green leaves photosynthetic active LAI from senescent leaves. The whole plant is considered as one leaf surface which intercept the radiation [9], opposite to destructive techniques which measure the area of living leaves. The millet (*Pennisetum glaucum* (L.) R. Br.) is a crop with very heterogeneous canopy where indirect measurements are recommended. The spatial heterogeneity of the plant canopy of millet comes from the fact that it is sown at variable spacings according to the zones and ecotypes, ranging from 0.5 m x 0.5 m to almost 1.5 m x 1.5 m in farmer area. This heterogeneity is very strong during the first weeks of its cultivation and can be maintained throughout the cycle if the growth of the plants is weak (LAI weak). In addition, due to its strong cross-pollination character, significant variability in growth within a plot is often observed, especially during tillering and upstream phases with height disproportions between plants. Previous studies, mainly on millet and maize, have shown that indirect measurements obtained by LAI with LAI-2000, underestimate the LAI values of plant canopy compared to measurements obtained by destructive techniques [10, 11, 9]. Nevertheless, it has been shown that there is a positive correlation between the LAI values obtained by the indirect method and those obtained by the direct method [12]. However, there is the problem of correcting the values of LAI obtained by the indirect method, which is not very laborious and which constitutes an alternative to direct measurements. The implementation of a good LAI measurement methodology under different conditions of growth of the plant canopy by the indirect LAI-2000 method in order to correct the data of the interception effects, as well as the variations due to the geometry of the canopy, disruption or defect in the design of the instrument is necessary. The hypothesis is that it is possible to correct the LAI values obtained using this equipment by a correction equation in the case of millet which has heterogeneous architecture.

2. MATERIELS AND METHODES

2.1 Experimental site

The study was conducted at the experimental station of Bambey (14° 42'N, 16° 28'W, and 20 m above of the sea) in Senegal, , within the Northern Sudanian climatic region. The annual precipitation mean is 450 mm and temperatures range from 20.33°C in December-January to 33.4°C in April-June. The soil is 95% sand, mainly constituted of materials from eolian deposits, classified as leached ferric lixisol [13]. The work was carried out in three rainy conditions on the said station.

2.2 Design and management of experimental plots

The research plots were established with millet crop during rainy season. Three varieties of millet were used: SOUNA 3 and IBMV8402 early varieties (90 days) and SANIO a late variety (120 days). Only IBMV8402 was used in the first year.
The experiment had a randomized complete block design with variety and density as factors. Treatments have been repeated four times. Planting density D 1 (0.5 m x 0.5 m) is the sowing method used by farmers in Senegal and in a large part of the Sahel. D2 (0.9 m x 0.9 m) and D3 (1.3 m x 1.3 m) densities are recommended by research. Prior to sow, seeds have been treated with an anti-fungal (Captafol 10%, Benomyl 10%, Carbofuran 20%) and plants thinned to 3 per hole, 15 days after sowing.

In the first year, plots were fertilized at seed emergence with N-P-K fertilizer (15-10-10) at 1.312 kg/plot; and at tillering and stem elongation phases with urea (0.437 kg/plot). For the second year, manure was spread on all plots before sowing. N-P-K fertilizer as recommended (20-10-10) was applied at 150 kg ha-1 , 11 days after sowing (DAS), corresponding to 1.21 kg, 3.94 kg and 8.21 kg, respectively, for D1, D2 and D3 plots. Two applications of urea at 50 kg ha-1 were added at plants thinning and 37 DAS (corresponding to stem elongation phase for SOUNA 3 and tillering phase for SANIO variety). Urea was added using per pot, 0.405 kg, 1.31 kg and 2.73 kg, respectively, for D1, D2 and D3). In the third year, the same rate of fertilizer using in the second experimentation was spread on all plots. The rains were therefore supplemented by irrigations in order to cover crops water needs.

2.3 Crop leaf area measurements

Direct and indirect measurements of millet leave area index was conducted on the same plants. For all sampling dates, measurements were performed on 6 plants, four replications for each treatment.
LAI-2000, Plant Canopy Analyzer (Model LAI-2000, Li-cor®, Inc, Lincoln, Nebraska 68504, USA) have been used to measure leaf area index in accordance with the manufacturer's prescriptions (Li-cor, 1992). The measurements were carried out weekly between 6:30 and 7:30 a.m and between 6:30 and 7:45 p.m during 3 years. In each plot, LAI-2000 measurements were recorded using 90°, 180° and 360° restriction views in 3 sequences, one above crop canopy and four measurements on ground level, along the diagonals of sowing lines. 90° restriction view was considered as reference in this study.

The direct leaves area measurements were carried out in the laboratory using leave-area-meter, Delta-T MK2 (type AMS, Devices, Ltd, Cambridge, England). The measurements were made on the same 6 millet plants which were used for LAI-2000 measurements. Indeed, after LAI-2000 measurements, plants have been harvested, leaves were collected from stems and green ones were separated from senescent or dry leaves. If more than 25% of leave area was dry, it was considered as senescent or dry. The green leaves were weighed and immediately passed through the leave-area-meter one by one to avoid leaf distortion and rolling due to drought. Measurement was performed on approximately 1/3 of the leaves. Total leaves area was calculated using the following equation.

$$L_A = L_a \times W_A / W_a \qquad (1)$$

Where LA is the total area of leaves sampled, La is the leave area measured using leave-area-meter, WA is the fresh weight of leaves sampled and Wa is the fresh weight of leaves used for direct measurement.

At plot scale, LAI is equal to the ratio between the total area of the leaves in a plot and the corresponding area of the plot (D1= 0.25 m²; D2=0.81 m² and D3=1.69 m²).

2.4 Statistics

Correlation tests were performed by Bravais–Pearson's table to determine the significance level of correlation coefficients (r) between parameters. Line 1:1 was also used to show the underestimation by the LAI-2000, of LAI values obtained by leave-area-meter.

3. RESULTS

3.1 Comparison of Leaf Area Index at 3 views restrictor

Regression equations derived from measurement data show significant linear correlation ($p<0.05$) between values of LAI-2000 measured at 90° restriction view and the two other restriction view (180° and 360°). The regression line drawn on scatter diagram relating measures at 90° and 180° restriction view is very close to the 1:1 line (Figure 1). This shows that LAI of millet can be estimated in the field using one of restriction view (slope = 1.0497). However, at 360° restriction view, LAI measurements are underestimated (slope = 0.9729).

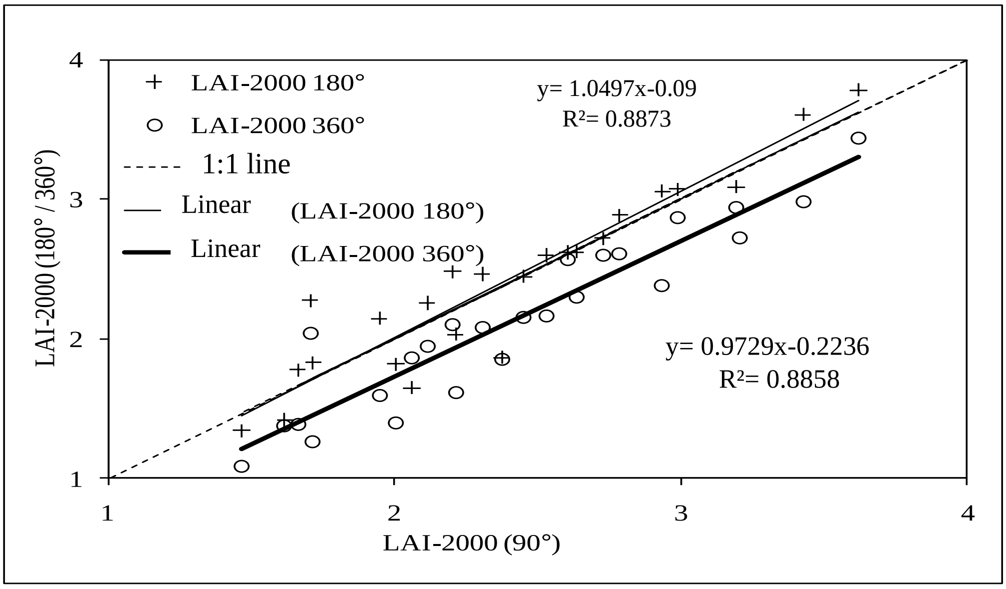

Figure 1 : Comparison of LAI values obtained with 3 restrictors view: 90 °, 180 °, 360 ° (Regressions at α = 0.05)

LAI values obtained using LAI-2000 under-estimated LAI values recorded with area-leaf-meter (real LAI: RL), (Figure 3 a). However, there is a high correlation between the values of LAI-2000 and those of area-leaf-meter (Figure 3 b). In addition, the relationship between LAI-2000 and direct LAI measurements (real LAI: RL) was not affected by millet variety and density.

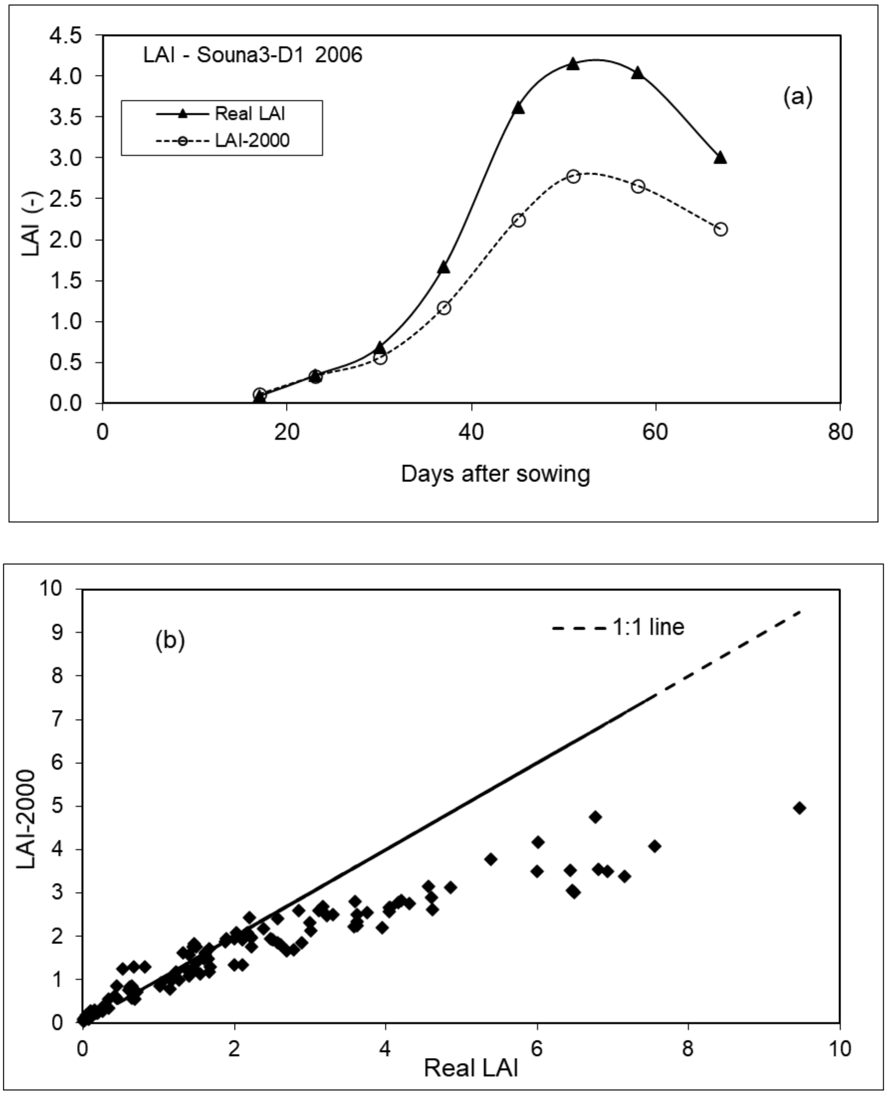

Figure 2 : Comparison LAI-2000 curves and real LAI (a) and linear regression between values of LAI obtained with LAI-2000 and real LAI (b)

3.2 Comparison of Leaf Area Index measures at different times of the day

Figure 2 represents LAI values measured at different moments of the day. Values of LAI measured in the morning and in afternoon appeared not different according to the regression performed. High linear correlation ($p<0.05$) between values of LAI measured at the two moments was observed and the regression line is closed to 1:1 line (slope = 1.0162).

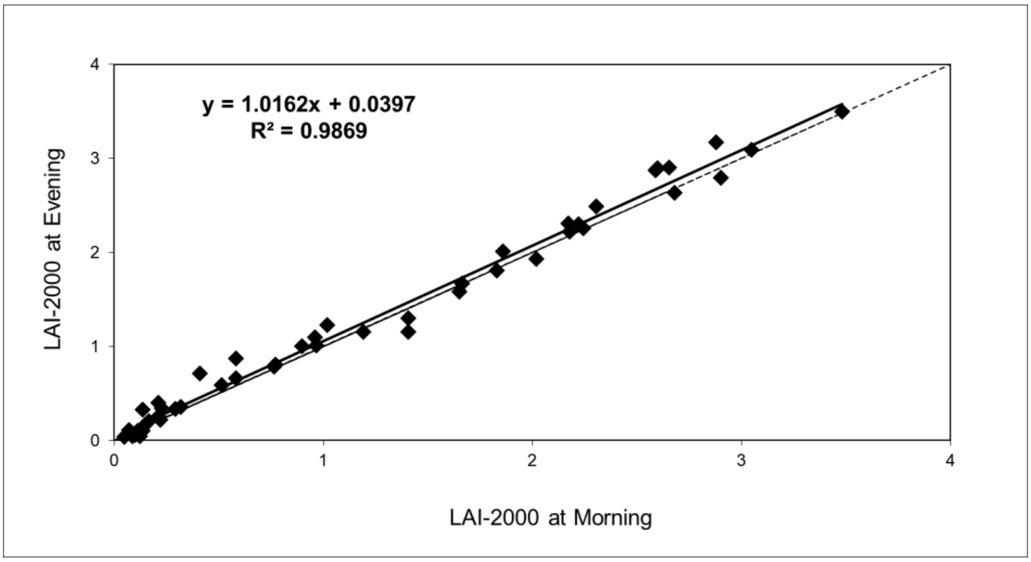

Figure 3 : comparison of LAI-2000 measured at the Morning and at Evening.

3.3 Correction of LAI-2000 values

The relationship between LAI values recorded using the two methods has been established before and after panicle stage. Results showed that this relationship is affected by the presence or not of the panicle (Figure 4). The LAI-2000 values assigned to panicles ranged from 0.4 to 1 m²/m²; approximatively 20% of LAI values. An empirical correction factor (0.8) of LAI-2000 values at flowering stage of millet allowed to overlay data of LAI-2000 in the absence of panicle (equation 2 and 3).

$$LAI\ 2000cor = 0.8\ \times LAI\ 2000 \qquad (2)\ ; \qquad \text{after panicle stage}$$

$$LAI\ 2000cor = LAI\ 2000 \qquad (3)\ ; \qquad \text{before panicle stage}$$

Where, LAI 2000cor is leaf area index corrected, LAI 2000 is leaf area index from indirect measurements.

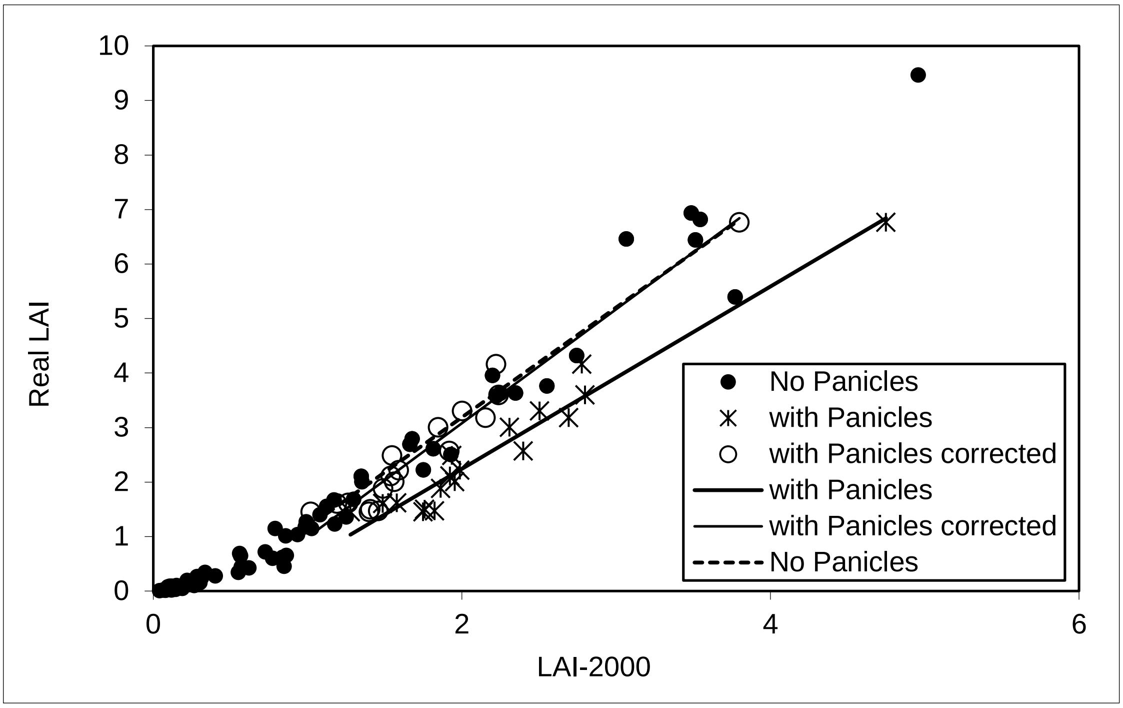

Figure 4 : Correlations LAI-2000/real LAI in relation in presence or absence of panicles.

3.4 Model of correction of LAI-2000 Values

The correlation between direct LAI values (RL) and LAI-2000 measurements, was established (Figure 5) using a polynomial regression that better fits data (equation 4) with a high determination coefficient (R^2 adjusted = 0.97).

$$RL = 0.1389X^2 + 1.3362X + 0.22178 \qquad (4)$$

Where RL is direct measurement values from leaf-area-meter, X is LAI values recorded with LAI-2000.

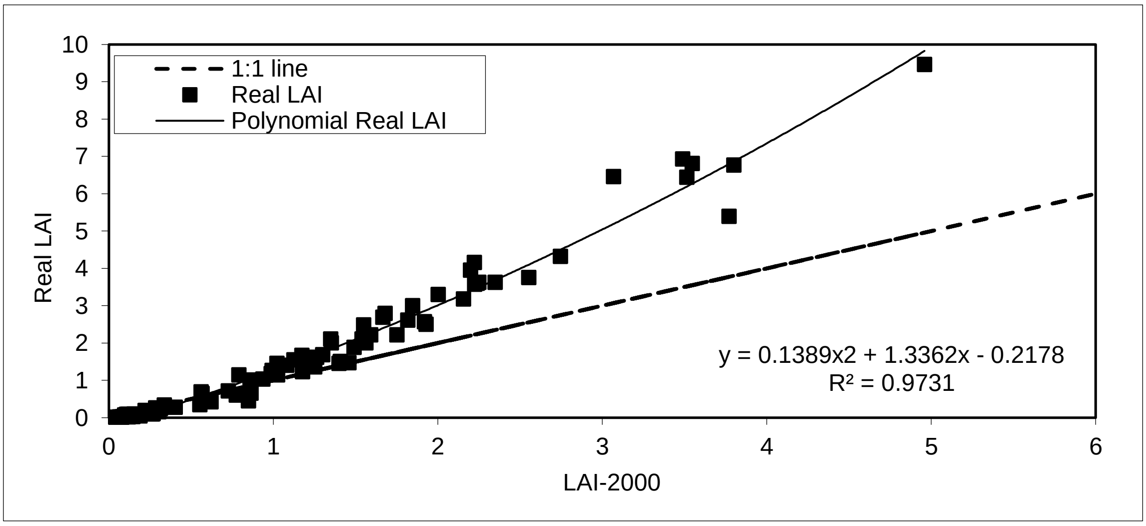

Figure 5 : Correlation between LAI-2000 values and direct measurement values (real LAI).

As shown in Figure 6, there is a strong linear correlation ($P<0.05$) between the corrected values of LAI-2000 using equation 4 and the direct measurements (RL). The corrected values were not different from the real values (RL). All LAI values up to 4 can be correctly predict using equation 4.

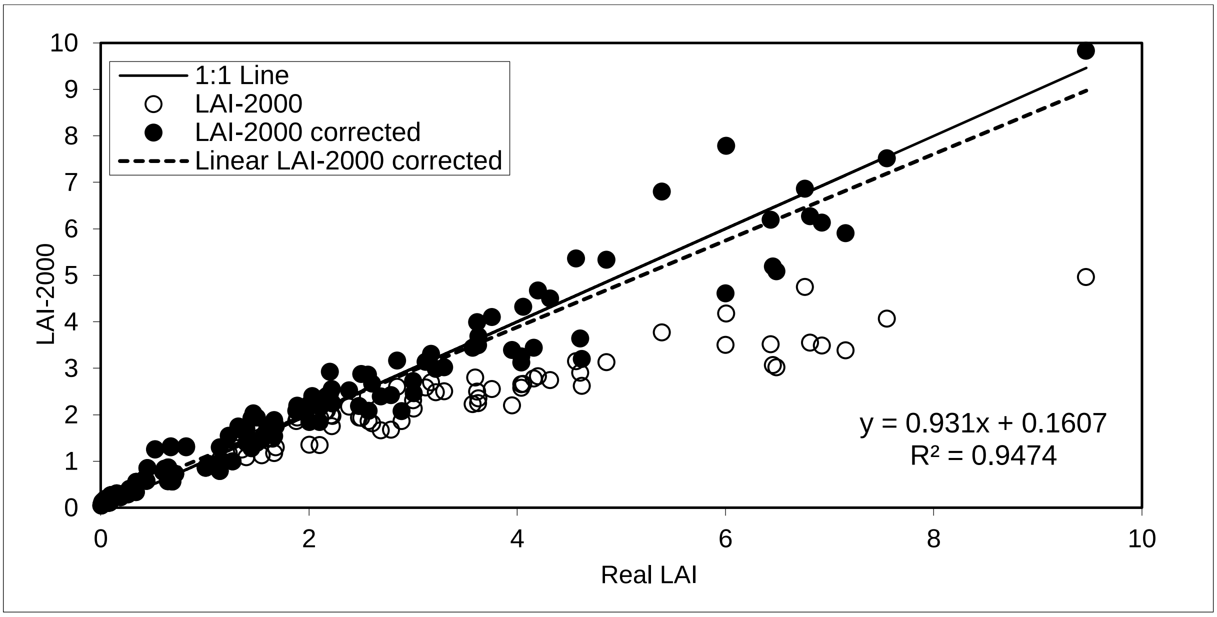

Figure 6 : Linear regression between corrected values and LAI measured values at the test threshold of 5%

4. DISCUSSION

Views restrictor of 90° and 180° during the measurements had high LAI values compared to the 360° angle. This result suggests that it is better to use mask to prevent direct sunlight on the sensor, in case of plants with lower canopy, to avoid a tendency to underestimate LAI [14, 11, 15]. The relationship between indirect and direct measurements is not related to density or variety. This assumes that the measurement accuracy of the LAI-2000 is not influenced by the difference architectures of the millet varieties. The linear regression performed between the values of LAI obtained with LAI-2000 and RL had shown that LAI-2000, significantly underestimates the value of LAI of millet. This was probably due to the heterogeneity of the millet canopy structure. This is consistent with observations reported on maize, trees and vegetables [9, 16, 17]. This is against all odds, because the LAI-2000 measurements include all opaque objects (stem, panicles, leaves), so plant area index should be the highest values. The indirect measurements with LAI-2000 was accurate when the millet leaf area index exceed 3. The best correlation was found at early growth of plants is due to the uniformity of canopy (leaves color, and structure) which might explain the decrease of sources of errors. On the other hand, the lower correlation was obtained from the scattering error introduced by decline in leaf area as the older leaves begin to senesce and the variability in crown structure, light interception of plant canopy [18, 19, 20].

The effect of panicles on LAI-2000 measurements had been empirically quantified. This is consistent with the operating principle of LAI-2000 [15]. Indeed, panicles also intercept sunlight and contribute to LAI measurements as highlighted in previous studies [21, 22]. In a plant canopy, any element that can intercept light influences LAI readings.

The correction of the effect of panicles on the LAI values made possible to obtain a good positive correlation between the LAI values obtained by the indirect method and those obtained by the direct method as reported by [12]. The existence of such correlation between these two methods of LAI measurements had also been shown on maize by [9]. The polynomial equation established to correct LAI-2000 measurements in this study is a good method to estimate real LAI. Many explanations could be put forward: first of all, the adjusted correction method could itself contain shortcomings, however, this cannot justify significant gap observed between the corrected and measured values. Thus, the errors could be due either to a device manipulation of the user of LAI-2000 or leave-area-meter. Also, especially wrong choice of older leaves for direct measurements which is tedious.

5. CONCLUSION

The analysis of the correlation between LAI indirect and direct measurements showed that measurement time, plant density and variety had no effect on the accuracy of LAI-2000 measurements. There was a good correlation after the correction with the presence of panicle on LAI values that allowed to find a polynomial function. This correction showed globally satisfactory results in simulation of the real LAI. Attempts to verify the validity of our correction method on other independent datasets would be successful. There is need for further research on the occurrence of senescent leaves appearance and its effects on plants LAI-2000 measurements.

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