Applied Physics Research; Vol. 8, No. 4; 2016
ISSN 1916-9639 E-ISSN 1916-9647
Published by Canadian Center of Science and Education

Characterization of Water and Nitrogen Stress of Maize by Laser Induced Fluorescence

Adama Penetjiligue SORO[1], Emma Georgina ZORO-DIAMA[1], Kédro Sidiki DIOMANDE[2], Guy Euloge BANY[1], Yvon BIBILA MAYAYA BISSEYOU[1] & Adjo Viviane ADOHI-KROU[1]

[1] Laboratoire de Cristallographie et Physique Moléculaire (LaCPM), Université Félix Houphouët Boigny, Côte d'Ivoire

[2] Centre National de Recherche Agronomique (CNRA), Côte d'Ivoire

Correspondence: Adama Penetjiligue SORO, Laboratoire de Cristallographie et Physique Moléculaire (LaCPM), Université Félix Houphouët Boigny, Côte d'Ivoire. E-mail: adams.soro@gmail.com

Received: April 21, 2015 Accepted: May 6, 2016 Online Published: July 29, 2016

doi:10.5539/apr.v8n4p64 URL: http://dx.doi.org/10.5539/apr.v8n4p64

Abstract

Water and nitrogen are essential for the optimal development of corn plants. A deficiency of these elements leads to lower crop production. Also, the health status of a plant influences the photosynthesis process. The photosynthetic diagnosis of a plant from the chlorophyll fluorescence spectrum induced by laser is non-destructive to the sample, reliable and fast method. As part of this work, we showed that it is possible to detect the nitrogen and water deficiencies of corn from the chlorophyll fluorescence ratio at 690 nm and 740 nm, when the measurements are performed before the senescence phase.

Indeed, we found that the R fluorescence ratio increases over time, for any stress on the plant. However, R decreases with the nitrogen stress and increases with increasing water loss.

The measures should be performed 51 Days After Planting (DAP) to detect water deficiency and the suitable date for nitrogen deficiency detection is 61 DAP.

Before each of these dates, the plants will be considered water deficient if the fluorescence ratio $R \leq 1.34$ and will be nitrogen stressed if $R > 1.36$.

Keywords: chlorophyll fluorescence, Maize, nitrogen deficiency, water deficiency, hydric stress

1. Introduction

Corn is the most consumed cereal in the world. Indeed, it represents 41 % of world cereal production (Perrier-Brusle, 2010). It is mainly used for feeding cattle in industrialized countries, but in sub- Saharan Africa and Latin America, it is used to feed the population (Bassalet, 2000). In Côte d'Ivoire, the 2014 national production of this cereal exceeded 600 000 tonnes (Réseau Non-Gouvernemental Européen sur l'Agroalimentaire le Commerce l'Environnement et le Développement [RONGEAD], 2014). However, the country is not self-sufficient in this cereal. It is forced to import corn to provide industrial and agro-pastoral needs. Therefore, it is necessary, even imperative to increase the production of this cereal to meet the existing needs and ultimately ensure food security of the Ivorian population. In Côte d'Ivoire, the corn growing areas are mainly located in Savannah, low rainfall area (Yéo, 2011).

Water and nitrogen are essential mineral elements for the optimal development of corn plants (Saccardy, 1997; Plénet, 1995; PNTTA, 1999). A deficiency in these elements leads to lower crop production. In agronomy, detecting the nutritional deficiencies of plants is usually carried out by foliar diagnosis which is a destructive method.

The fluorescence emission is directly related to the photosynthesis process (Papageorgiou & Govindjee, 2004; Stirbet & Govindjee, 2011; Wim & Andrej, 2013) in which are made of many biological exchanges. The health status of a plant influences this process. Thus, the study of the fluorescence spectrum can detect any stress on the plant at leaf and canopy scale (Chappelle, Wood, McMurtrey & Newcomb, 1984; Méthy, Olioso & Trabaud, 1994; Bourrié, 2007). The interest of this photosynthetic diagnosis is that it is non-destructive, fast and reliable. As

part of this work we characterize the water and nitrogen deficiencies of corn plants by chlorophyll method of laser-induced fluorescence.

2. Material

2.1. Experimental Material

Data were collected in vivo and in situ using an USB4000 - type FL fluorescence spectrometer. This device can record plant chlorophyll fluorescence spectra whose wavelengths range is from 360 nm to 1 000 nm in steps of 0.22 nm. The samples were excited by a LED emitting at 450 nm through a bifurcated optical fiber. The acquisition, storage and processing of the collected spectral data were conducted using a laptop. The Figure 1 shows the experimental setup.

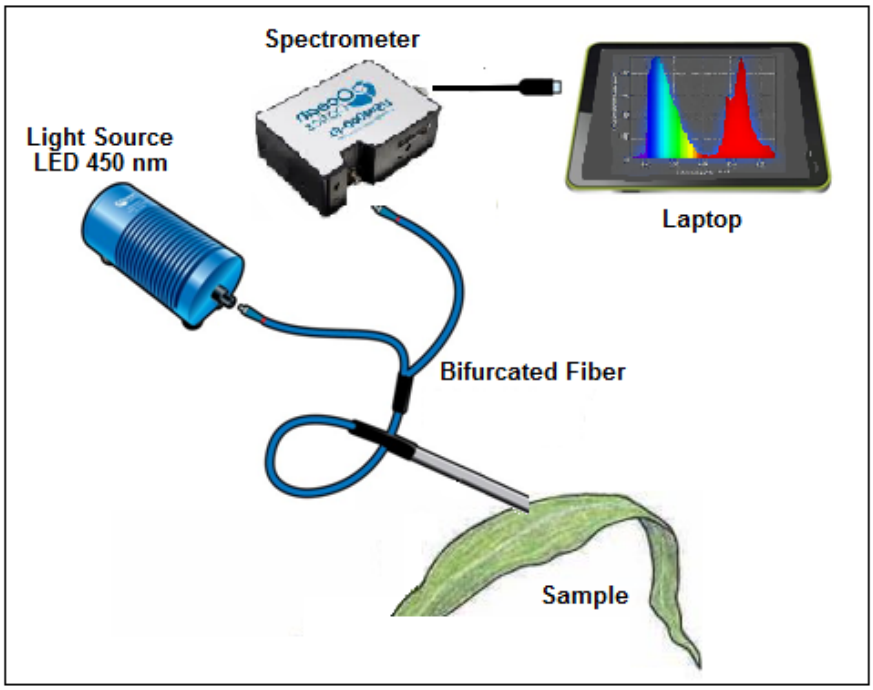

Figure 1. Experimental setup

2.2 Vegetal Material

The corn variety used in this study is called DMRESR-Y. It was provided by the Centre National de Recherche Agronomique (CNRA) in Côte d'Ivoire. Its maturation cycle is 90 to 95 days and its grains are yellow and horny (L. Akanvou , R. Akanvou, Anguété & Diarrassouba, 2006).

3. Methods

The plantations for both studies were divided into blocks. Each block contained the same deficiency levels (nitrogen or water) to ensure that the arrangement of the buckets in the field does not influence the results.

3.1 Water Stress Induction

In the case of the water stress study, the corn planting was made in a greenhouse as we did not want to be dependent on the weather and we had to control the water amount we had to provide to the corn plants.

A mineralogical analysis of the soil used to fill the buckets revealed that it had high content of all essential nutrients for the corn plants development.

We first sought the soil field capacity : it is the amount of water that the ground can retain. Knowing this value allowed us to determine the various doses to apply to the soil to induce the hydric stress. The field capacity of the used ground was 2 liters. We then generated four levels of water stress as listed below:

W12: 12.5 % of the soil field capacity per bucket (0.25 l of water)

W25: 25 % of the soil field capacity per bucket (0.5 l of water)

W50: 50 % of the soil field capacity per bucket (1 l of water)

W100: 100 % of the soil field capacity per bucket (2 l of water)

After a heavy watering the day before, we sowed corn grains the next day. The plantation in the greenhouse consisted of 72 buckets of 20 l capacity, left in 3 blocks (see figure 2).

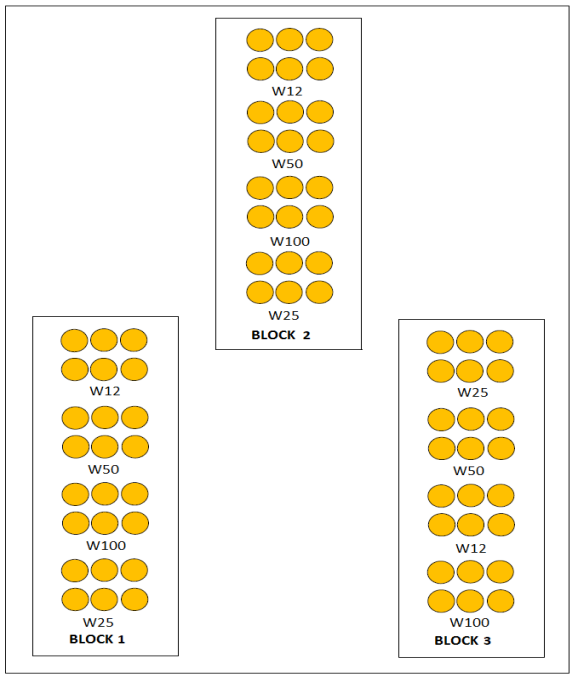

Figure 2. Water stress study planting plan

Every bucket contained two growth pouches at the rate of three grains per pouch. We removed corn plants from the bucket, to have a unique plant in a growth pouch 15 Days After Plantation (DAP). So, every bucket contained two corn plants.

We induced the water stress 30 DAP. Then, we started to collect spectral data 37 DAP. From this date and once a week, the fluorescence spectrum of every plant was recorded. This operation took place between 09:00 am and 1:00 pm. This phase ended 72 DAP when plants reached the senescence phase.

3.2 Nitrogen Stress Induction

The buckets were filled with poor nitrogenous soil, in order to control the intake of nitrogen fertilizer. Each bucket with a capacity of 30 liters had three growth pouches with three seeds per growth pouch.

We brought an amount of 2.27 g of nitrogen, phosphorus and potassium (NPK) to every hole to allow a good seeding of the corn grain. Then, the corn seedlings were thinned to one plant per hole 15 DAP. So, there were only three plants per pot.

The nitrogenous stress was led 30 DAP by providing various doses of urea. So, we generated five fertilization levels as listed below:

N0: 0 g of urea/plant (no nitrogen provided to the plant)

N1: 0.377 g of urea/plant (1/4 part of the nitrogen recommended dose)

N2: 0.755 g of urea/plant (2/4 part of the nitrogen recommended dose)

N3: 1.133 g of urea/plant (3/4 part of the nitrogen recommended dose)

N4: 1.510 g of urea/plant (nitrogen recommended dose).

This plantation consisted of 80 buckets of 30 l capacity, left in 4 blocks. Every block contained the 5 fertilization levels and each fertilization level included 4 buckets (see figure 3).

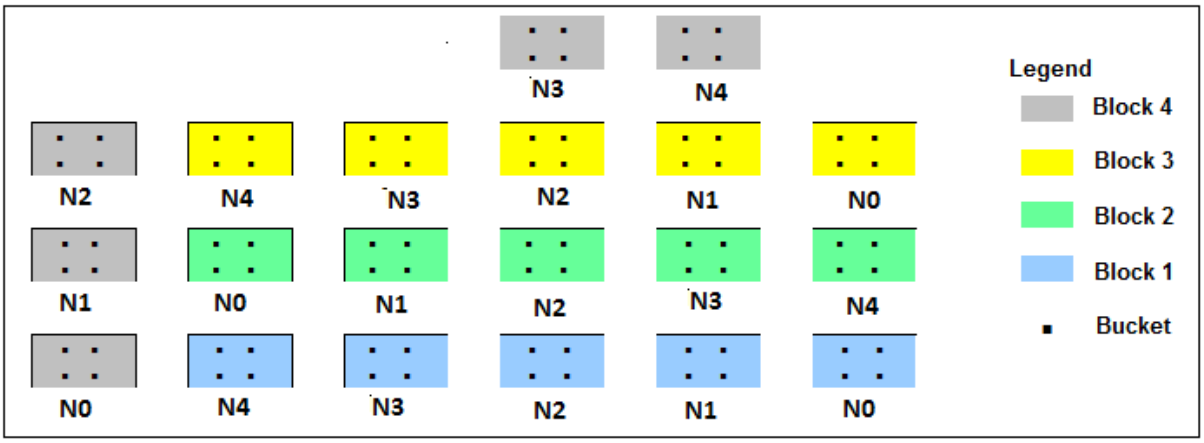

Figure 3. Plantation plan for the nitrogen stress study

Ten days after the application of nitrogen stress; that is 40 DAP, we began collecting spectral data. From that date, once every week, the fluorescence spectrum of each plant was recorded. This operation that ended 82 DAP took place between 09:00 am and 01:00 pm. A total of seven series of measurements were carried out during the different development stages of corn plants.

3.3 Data Processing

During the measures at leaf scale, every deficiency level (hydric or nitrogenous) applied to the plant in the same block, is characterized by the average fluorescence spectrum.

We noticed that for the same applied stress level, there is no significant difference between the measurements performed on the blocks. We then worked with the average values of the ratios R computed on the blocks, for the same given deficiency level.

For all the recorded spectra, the digital data are converted to text files and then imported into the MATLAB software to compute the ratios of intensities of the two characteristic chlorophyll fluorescence peaks ($R = F_{690}/F_{740}$). F_{690} corresponds to the intensity of the fluorescence peak at 690 nm and F_{740} is the intensity of the fluorescence peak at 740 nm. We used the fluorescence ratio in our study, among other fluorescence parameters as it is a pertinent indicator, widely used in plant stress detection (Tremblay, Wang & Cerovic, 2012).

The various graphics were edited with the software ORIGINPro 8. R values given on the charts are the average values for every deficiency level. Indeed, it is recommended to use the mean value of ratio *R* for many measurements for several plants to have reliable results instead of one single measurement (Fedotov, Bullo, Belov & Gorodnichev, 2016).

4. Results and Discussion

4.1 Water Stress Case

The graphs in Figure 4 show for each stress level, the intensities of fluorescence ratios depending on the development stage of corn plants. For all treatments, we notice an increase in the value of R depending on the plant development stage. Thus, the value of R increases with the plant age. From 51DAP to 65DAP each curve compared to other is a function of the nutritional stress: greater the stress is, smaller R is.

From the date 51DAP, we also find that the curves (W12, W25) and (W50, W100) are close to each other. The differences between the ratios values for considered couples treatments are weaker at 65DAP. We can then consider the two extreme curves : W12 represents stressed plants and W100 represents non- stressed ones. At 51DAP the gap between the extreme curves is maximum. It would be the indicated date for the water stress detection. The water deficiency detection measures are efficient in the time interval [51DAP, 65DAP].

As the histograms in Figure 5 show, until 51DAP, all water -deficient plants have a ratio $R \leq 1.34$. But, for non-deficient plants the ratio is still greater than 1.34. In addition, as water is an essential element for plant survival, a lack of water causes early senescence. This is the case from 58DAP. Then, we notice that all plants have $R > 1.34$. So, the appropriate time to make the measures for the hydric stress detection would be 51DAP.

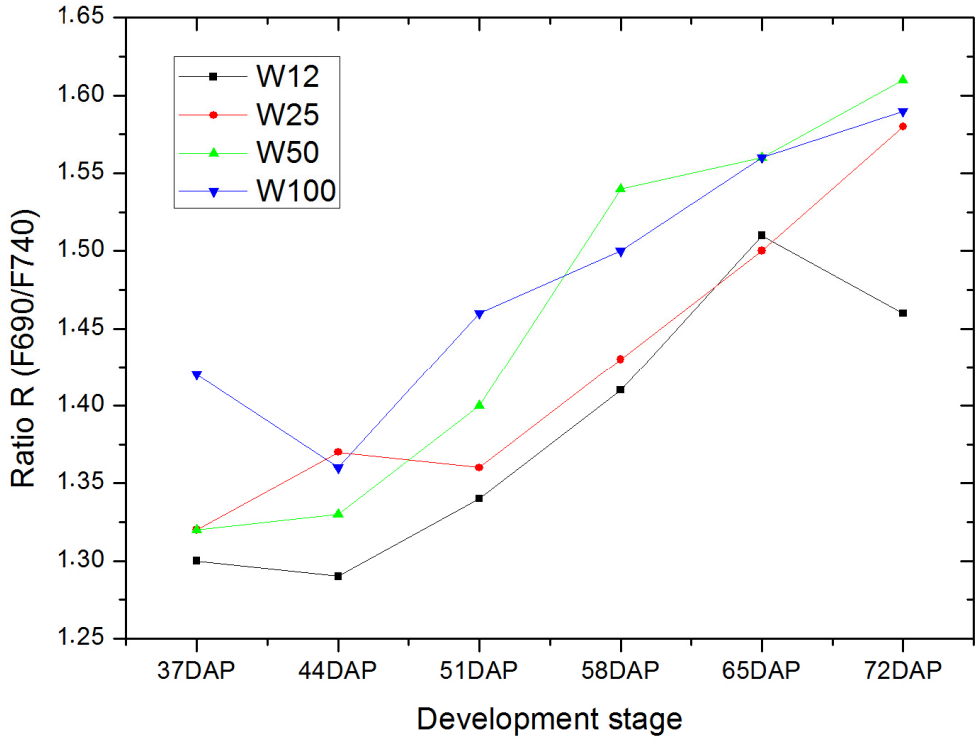

Figure 4. Temporal changes of the fluorescence ratio for the four hydric treatments

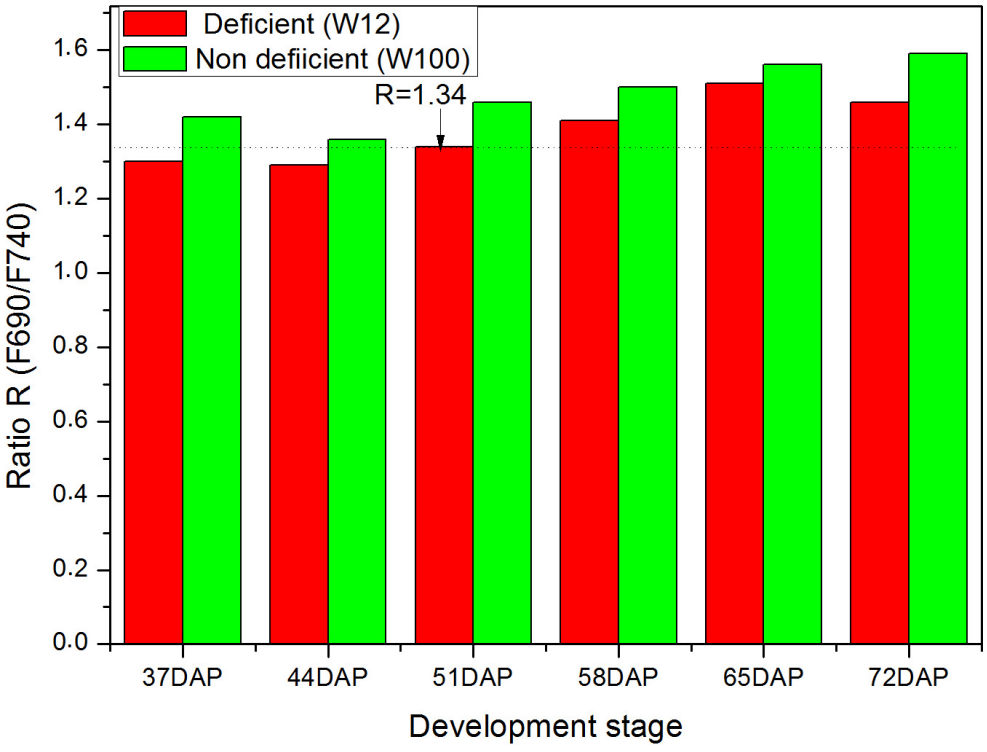

Figure 5. Histograms for the E12 and E100 water stress levels as a function of development stage

4.2 Nitrogen Stress Case

The charts in figure 6 show the fluorescence ratio over the treatment applied to corn plants. We find that at any plant development stage, the ratio R decreases when stress decreases.

According to Méthy, Olioso & Trabaud (1994), the amount of chlorophyll in the plant is proportional to the photosynthetic activity. However, the nitrogen deficiency causes the decrease of the activity. The ratio R therefore increases when the nitrogen stress decreases.

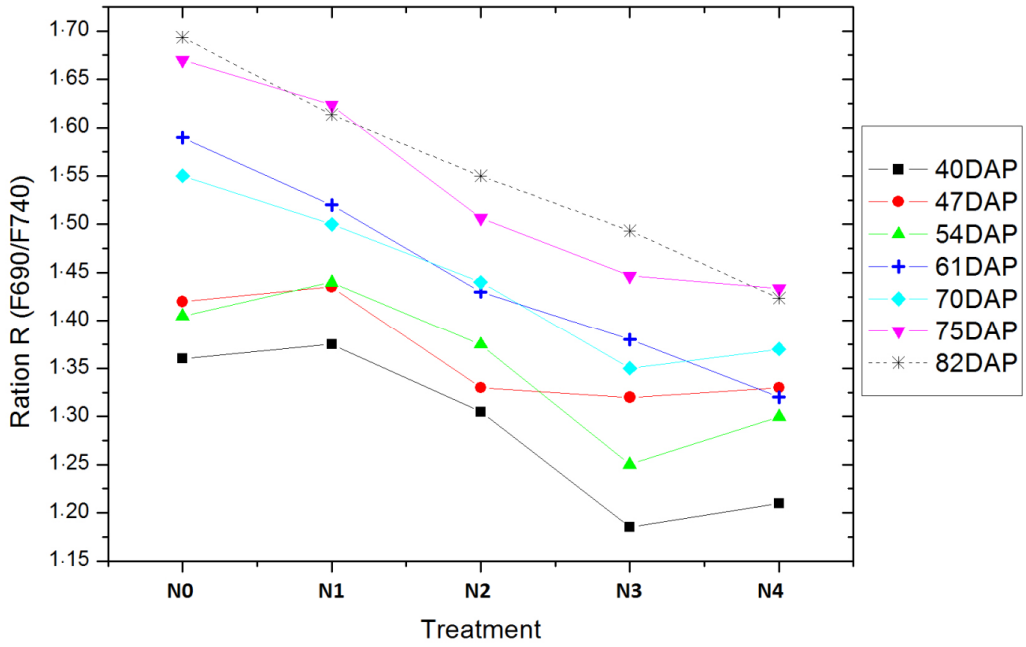

Figure 6. Changes in the relationship between the fluorescence ratio and the nitrogen treatment

The Figure 7 displays the fluorescence ratios over the plant development stage, for each nitrogenous stress level. Despite serrated evolution of certain values, we observe for all treatments, an increase of the R value depending on the plant development stage. Thus, the R value increases with the plant age. This increase is more pronounced for N0 and N1 treatments than for those of N3 and N4.

We also notice on Figure 7 that charts (N0, N1) and (N3, N4) are close to each other.

The median position of the N2 treatment chart compared to curves treatments couples (N0, N1) and (N3, N4) illustrates the average fertilization rate that we applied. Furthermore, the arrangement of each chart compared to other highlights a parallelism with different fertilization levels we generated.

The table 1 shows the differences between the pairs of curves (N0, N1); (N3, N4) and (N0, N4).

Table 1. Differences between R values for the pairs of curves (N0, N1); (N3, N4) and (N0, N4)

DAP	ΔR (N0-N1)	ΔR (N3-N4)	ΔR (N0-N4)
40 DAP	0.01	0.02	0.15
47 DAP	0.01	0.01	0.10
54 DAP	0.03	0.05	0.11
61 DAP	0.07	0.06	0.27
70 DAP	0.01	0.02	0.18
75 DAP	0.05	0.01	0.24
82 DAP	0.08	0.07	0.27

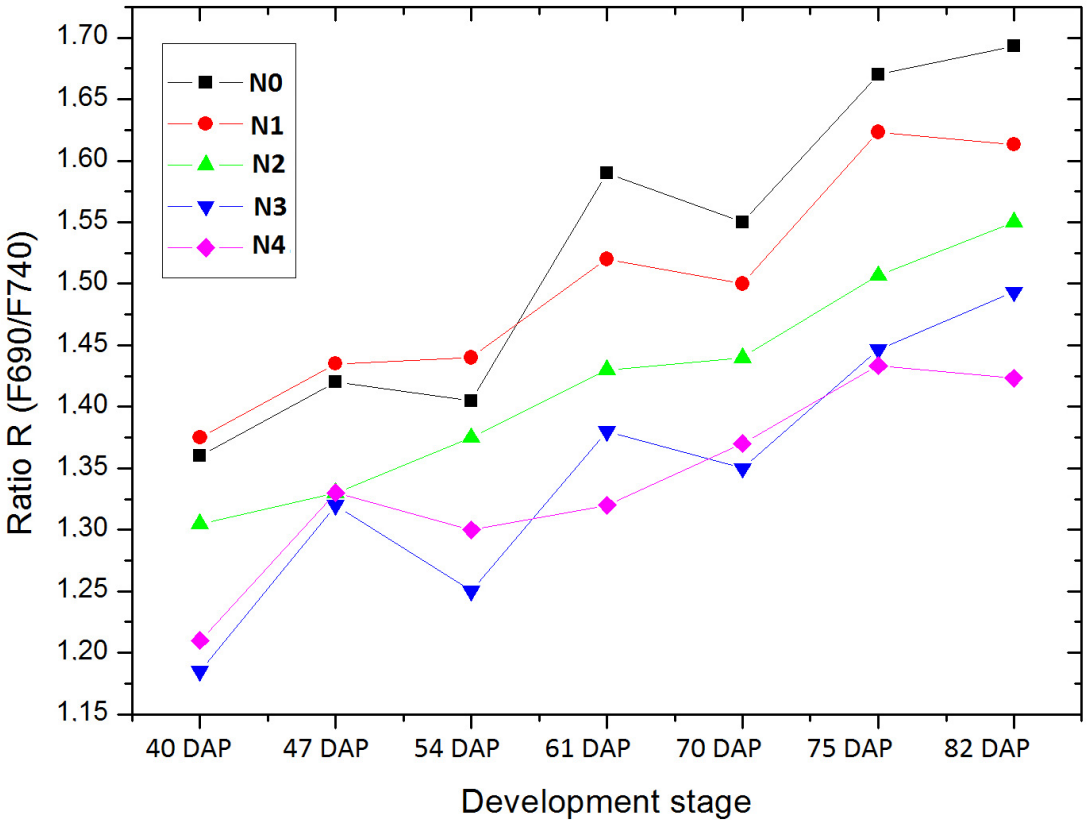

Figure 7. Temporal changes of the fluorescence ratio for the five nitrogen treatments

Table 1 shows that the greatest differences between the fertilization levels during the plant development, are obtained at 61 DAP and 82 DAP. We cannot consider the second date because it is already in the plant senescence phase. Thus, the best period to conduct early nitrogen stress detection measurements would be 61 DAP.

This table also shows that the differences between N0 and N1 on one hand and N3 and N4 on the other hand are very low compared to the differences between N0 and N4. N1 and N0 Fertilizations produce the same effect of stress on the plant. It would therefore be useless to bring 25 % of the nitrogen needs to the plant. However, it would be economical for the farmer to provide 75 % of the nitrogen needs of the plant because the N4 and N3 treatments produce the same stress effect.

We then considered both extreme fertilization levels :

- nitrogen deficient level for N0 treatment plants;

- nitrogen fertilized level for plants that have undergone the N4 treatment.

For both generated fertilization levels, figure 8 shows the fluorescence ratio over the development stage.

The maize variety used in this study has a short-cycle production (90-95 days). At 70 DAP, the culture is in the senescence process. Figure 8 shows that the fertilized plants have a ratio R = 1.36 on that date. All measurements performed before the senescence phase are such that:

- $R \leq 1.36$ for fertilized corn plants;
- $R > 1.36$ for deficient corn plants.

Moreover, the measures we took during the senescence phase provide R values greater than 1.36 whatever studied plants. In addition, the R value for fertilized plants is still lower than deficient plants.

Figure 8 also confirms that the date 61 DAP is convenient to perform nitrogen stress detection measures. These results are similar to those obtained by Soro, Adohi-Krou, Diomandé & Ebby (2004) in a study on oil palm trees.

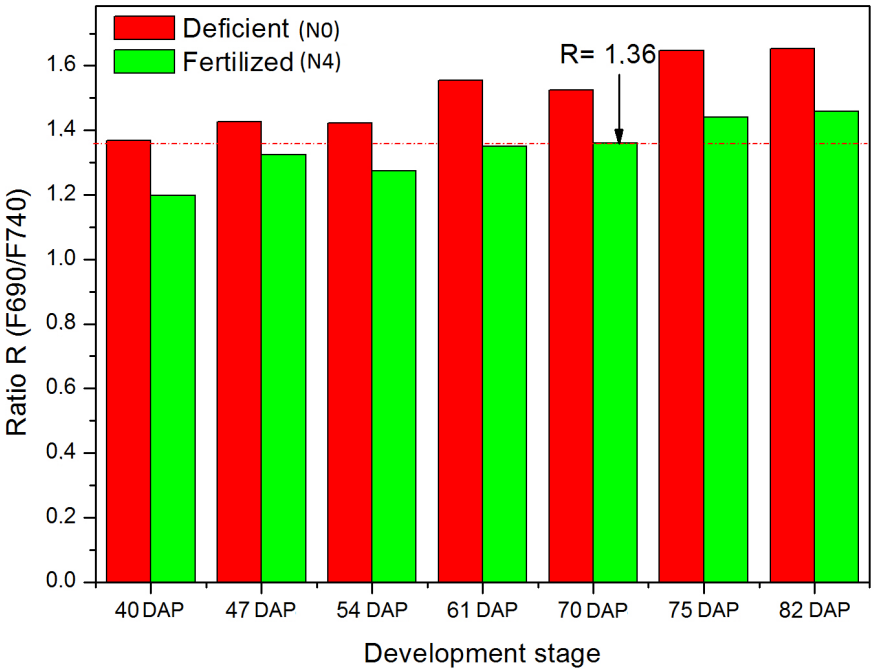

Figure 8. Fluorescence intensity ratios for N0 and N4 treatments over the development stage

5. Conclusion

This study allowed to show that it is possible to detect the nitrogen deficiency and the water deficiency of corn plants from the ratio of chlorophyll fluorescence intensities of at 690 nm and 740 nm, when the measurements are performed before the senescence phase. The senescence phase occurs earlier in plants for water stress:

- to detect water deficiency, the favorable date is DAP 51. Corn plants will be considered water deficient if $R \leq 1.34$ and water unstressed if $R > 1.34$ before the date specified for detection
- While to detect nitrogen deficiency, the convenient day would be 61 DAP. Corn plants will be considered nitrogen fertilized plants if $R \leq 1.36$ and nitrogen deficient plants if $R > 1.36$ before this date.

We find that the fluorescence ratio R increases over time, for any stress on the plant. However, R decreases with the nitrogen stress and increases with increasing water deficiency.

However, further experiments must be led to determine the amount of nitrogen and water to provide to corn plants to correct any detected deficiency.

Acknowledgements

Research reported in this paper was supported by the Programme d'Appui Stratégique à la REcherche Scientifique (PASRES): We also thank the Centre National de Recherche Agronomique (CNRA) and the Centre National de Floristique (CNF) for their scientific collaboration.

References

Akanvou, L., Akanvou, R., Anguété, K., & Diarrassouba, L. (2006). *Bien cultiver le maïs en Côte d'Ivoire*. fiche technique Centre National Recherche Agronomique, 4p. Retrieved from http://www.erails.net/images/cote-divoire/cnra/cnra/file/cultiver_mais.pdf

Bassalet, N. (2000). *Le maïs et ses avenirs*, Cahiers du LIPS, N°13, 47p. Retrieved from http://www.laprospective.fr/dyn:francais/:memoire/cahier_num13.pdf

Bourrié, B. (2007). *La fluorescence chlorophyllienne comme outil de diagnostic,* 8èmes Journées de la fertilisation raisonnée et de l'analyse de terre, GEMAS-COMIFER, Blois, 20-21 novembre 2007, 11p. Retrieved from http://www.comifer.asso.fr/images/pdf/8emes_rencontres/15%20-%20expos%20-%20%20la%20fluorescence%20chlorophyllienne%20comme%20outil%20.pdf

Chappelle, E. W., Wood F. M. Mcmurtrey J. E. & Newcomb W. W. (1984). Laser-induced fluorescence of green plants. 1:A technique for the remote detection of plant stress and species differenciation. *Applied Optics*, 23, 134-138. http://dx.doi.org/10.1364/AO.23.000134

Fedotov, Y., Bullo, O., Belov, M., & Gorodnichev, V. (2016). Experimental Research of Reliability of Plant Stress State Detection by Laser-Induced Fluorescence Method. *Int J Optics, 2016*, 1-6. http://dx.doi.org/10.1155/2016/4543094

Méthy, M., Olioso, A., & Trabaud, L. (1994). Chlorophyll fluorescence as a tool for management of plant ressources. *Remote Sensing of Environment, 47*, 2-9. http://dx.doi.org/10.1016/0034-4257(94)90121-X

Papageorgiou, G. C., & Govindjee. (2004). Chlorophyll a fluorescence: A signature of photosynthesis. *Advances in photosynthesis and respiration* (vol 19. Springer, Dordrecht, 795 p).

Perrier-Brusle, L. (2010). *Le maïs dans le monde: production, consommation et tension sur les marchés*. IRD France, 31p. Retrieved from http://laeti.perrierbrusle.free.fr/scpo_seances5_expose.pdf

Plénet, D. (1995). *Fonctionnement des cultures de maïs sous contrainte azotée - Détermination et application d'un indice de nutrition*. Thèse de doctorat, Institut National Polytechnique de Lorraine, Académie de Nancy-Metz, 247p.

PNTTA (Programme National de Transfert de Technologie en Agriculture). (1999). *Fertilisation azotée des céréales : cas des blés en Bour et en irrigué*. Bulletin mensuel d'information et de liaison du PNTTA, N° 62, 4p. Retrieved from http://agrimaroc.net/bul62.htm

RONGEAD – ONG CHIGATA. (2014). *Diagnostic de la filière maïs en Côte d'Ivoire*, 59p. Retrieved from http://www.rongead.org/IMG/pdf/Diagnostic_de_la_Filiere_Mais_en_Cote_d_Ivoire_RONGEAD_2014.pdf

Saccardy, K. (1997). *Effets de la contrainte hydrique sur le mécanisme photosynthétique des plantes en C4 ; interactions avec les fortes lumières*. Thèse, Université de Paris 11, Paris, 166 p.

Soro, P. A., Adohi-Krou, A., Diomandé, K., & Ebby, N. (2004). Caractérisation de la déficience en potassium des plants de palmier à huile par fluorescence induite par laser. *REVIST, 5*, 23-34.

Stirbet, A., & Govindjee. (2011). On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient, *Journal of Photochemistry and Photobiology B, 104*(1-2), 236-257. http://dx.doi.org/10.1016/j.jphotobiol.2010.12.010

Tremblay, N., Wang Z., & Cerovic, Z. G. (2012). Sensing crop nitrogen status with fluorescence indicators- A review, *Agronomy Sust Developm, 32*(2), 451-464. http://dx.doi.org/10.1007/s13593-011-0041-1

Wim, V., & Andrej, P. (2013). Chlorophyll *a* fluorescence induction (Kautsky curve) in a Venus flytrap (*Dionaea muscipula*) leaf after mechanical trigger hair irritation, *Journal of Plant Physiology, 170*, 242–250. http://dx.doi.org/10.1016/j.jplph.2012.09.009

Yéo, Y. A. (2011). *Analyse de la compétitivité de la filière maïs en Côte d'Ivoire*, Programme de Renforcement et de Recherche sur la Sécurité Alimentaire en Afrique de l'Ouest (PRESAO), Composante SRAI, Michigan State University. *Rapport Final N°2-2011-11, 80p*. Retrieved from http://fsg.afre.msu.edu/srai/RCI_Rapport_competitivite_mais_RCI.pdf