APPLICATION OF LASER INDUCED FLUORESCENCE TECHNIQUE TO THE STUDY OF POTASSIUM DEFICIENCY OF THE PALM OIL TREE [*Elaeis guineensis* (JACQ)]

K. DIOMANDE[1]., K. BALLO[2], A. KONATE[1], A. ADOHI-KROU[1], P. A. SORO[1], N. EBBY[1]

[1]Laboratoire de Cristallographie et Physique Moléculaire,
Université de Cocody, UFR/SSMT, 22 BP 582 Abidjan 22.

[2]Centre National de Recherche Agronomique (CNRA), Station Expérimentale et de Production Robert Michaux de Dabou, BP 8 Dabou

ABSTRACT

Potassium is the main nutrient element that plays a significant role in palm oil tree (*Elaeis guineensis* (Jacq)) production and its resistance to water deficits during the dry season. One can observe up to 30 % decrease in production when potassium is deficient. Potassium nutrition control of palm oil tree is a very important issue leading to a K^+ fertilisation policy. Laser-Induced Fluorescence (LIF) of chlorophyll **a** is a fast and simple method compared to the usually used Foliar Diagnostic method. In this study, LIF was used to detect water stress in the palm oil tree caused by potassium deficiency by analysing the fluorescence spectrum of chlorophyll **a**. Two groups of palm oil trees (one K^+ -deficient (K0) and the other non K^+ -deficient (K3)), were used in a potassium fertilisation experiment. For each selected leaf, 10 leaflets were collected on the lower side exposed to the sun, leaflets were radiated with a laser beam which was absorbed by the chlorophyll **a** of leaflets to yield a florescence spectrum. The average of the 10 fluorescence spectrum gave the spectral response of the leaf. Results show that leaves of rank 6 and 9 were the best samples for LIF analysis, because of the stability of chlorophyll spectra characteristics such as fluorescence peak ratio and gaussian area ratio. The analysis of the fluorescence spectra of the leaf of rank 9 showed that when the palm oil tree was K^+ deficient, fluorescence peak ratio (R = F690/F735) was superior to 0.45, but less than this value when K^+ was not deficient.

Keywords : Palm oil tree, potassium, laser, fluorescence spectrum, Chlorophyll **a**, Côte d'Ivoire.

RESUME

*APPLICATION DE LA TECHNIQUE DE FLUORESCENCE INDUITE PAR LASER À L'ÉTUDE DE LA DÉFICIENCE DU POTASSIUM CHEZ LE PALMIER À HUILE [*Elaeis guineensis *(JACQ)]*

*Le potassium est un élément nutritif qui joue un rôle prépondérant sur le rendement et la résistance à la sécheresse du palmier à huile (*Elaeis guineensis *(Jacq)). On observe jusqu'à 30 % de baisse de rendement en Côte d'Ivoire lorsqu'il y a déficience en potassium. Le contrôle de la nutrition potassique des plants de palmier à huile est une activité très importante qui permet de conduire la stratégie de fertilisation. La méthode de Fluorescence Induite par Laser (FIL), rapide et simple, comparativement à la méthode classique au Diagnostic Foliaire, a permis de caractériser la déficience en potassium du palmier à huile par l'étude de la fluorescence de la chlorophylle **a**. Les échantillons utilisés sont issus d'une plantation expérimentale de fertilisation en potassium. Des prélèvements ont été faits sur deux types de palmier à huile ; une série d'arbres déficients (K0) et une autre non déficiente (K3) en potassium. Sur chaque arbre, 10 folioles ont été récoltées par feuille sélectionnée. La chlorophylle **a** a* *absorbé la radiation laser envoyée sur la partie inférieure de la face ensoleillée de la foliole pour donner un spectre de fluorescence. Une moyenne de 10 spectres a donné la réponse spectrale de la feuille. Les résultats montrent que les feuilles de rang 6 et 9 ont été les échantillons les plus appropriées pour cette technique à cause de la stabilité des éléments caractéristiques du spectre de la chlorophylle* **a** *qui sont : le rapport d'intensité des pics et celui des aires des gaussiennes. L'analyse des spectres de fluorescence, portée sur les feuilles de rang 9 indique que lorsque le palmier est déficient en potassium, le rapport d'intensité des pics de fluorescence (R = F690/F735) est supérieur à 0,45 mais reste inférieur à cette valeur pour un palmier non déficient en potassium.*

Mot clés *: Palmier à huile,* Elaeis guineensis, *potassium, laser, fluorescence, Côte d'Ivoire.*

INTRODUCTION

In the last decade, several spectroscopic methods have been considered for the characterisation of the physiological state of plants. Among them, the use of a fluorescence signal emitted by plants has been the object of intense research activities. Measurements of chlorophyll **a** fluorescence have become a useful investigation tool in photosynthesis research (Hugo, 1991 ; Chapelle *et al.*, 1984; Subhash *et al.*, 1994 ; Yasunori Saito, 1998). Palm oil is the second source of oil in the world and also the main financial resource for several developing countries. Several research activities have shown that potassium is the main essential nutrient element in palm oil tree production and its resistance to the dry season. One can note 30 % decrease in production, in Côte d'Ivoire, when potassium is deficient (Ballo *et al.*, 1994 ; Ballo *et al.*, 1996 ; Caliman *et al.*, 1995 ; NG Siew Kee, 1986 ; Braconnier et d'Auzac, 1985).

It was shown that Laser-Induced Fluorescence (LIF) technique, which is faster and simpler to use than the classical foliar diagnosis method and in the case of the palm oil tree stress caused by a potassium deficiency. The ratio of fluorescence curve intensities differ from no potassium deficient trees. Essential parameters for an accurate diagnosis using the LIF technique were also assessed. The aim of this work is to study the stress caused by potassium deficiency on palm oil tree in our experiment site (Chapelle *et al.*, 1984 a & b).

MATERIAL AND METHODS

STUDY SITE

Experiments were conducted at a site of the «Centre National de Recherche Agronomique» (CNRA), in the South of Côte d'Ivoire. At this experiment site, climate is humid intertropical with a bimodal rain fall pattern (1 500 mm/year) a mean temperature of 26 °C and a mean radiation of 14 MJ $m^{-2}d^{-1}$. Soil derived from tertiary sediments, sandy, ferralitic, very highly desaturated and impoverished.

PLANT MATERIAL

The palm oil trees used were LM2TxDA10D type grown since 1974 at La Mé/IDEFOR. Four group of palm oil plants were fertilized with different amount of KCl (60 % of K_2O) in a potassium fertilisation experiment. The following treatment groups were used :

K0 = 0 Kg KCl/ha/year

K1 group = 115 Kg KCl/ha/year

K2 group = 230 Kg KCl/ha/year

K3 group = 345 Kg KCl/ha/year

In our experiment, two groups of four trees (K0 group and K3 group) were selected. For each tree, 10 leaflets of 1, 6, 9, 14, 17 and 23 ranks were selected. Leaf samples cut early in the morning were done 1 h after collection.

METHODS

1-The classical Foliar diagnosis method (Prevot et Ollagnier, 1957), consisting to collecting, transporting the leaf samples from the fields, drying the sample and sending them to the laboratory for chemical analysis was used. To assess the potassium deficiency, K^+ concentration of samples were compared to a control of 0.9 % /dry material. This technique included many processes such as collecting, drying and analyzing leaves.

2-The Laser Induced Fluorescence technique scheme is shown in figure 1. The excitation source was a HeNe laser of 10 mW power emitting at a wavelength of 632 nm. The photomultiplier was XP1011 type from RTC, with a frontal window of 35 mm of useful diameter. The monochromator was a Higler Watt, with a holographic grating of 600 lines/mm. A 632 nm cut filter and a XY recorder for the spectrum plot were used.

For each tree ; 10 leaflets from each leaf rank were collected early before intense photosynthesis activity. Leaves samples were taken to the laboratory and the chlorophyll fluorescence spectra, measured at room temperature. The average of the 10 fluorescence spectra gave the spectral response of the overall leaf. Fluorescence spectra were fitted (Subhash et Mohaman, 1997) with the Origin 3.5 Program. The intensity ratio R (R = F690/ F735), an important stress indicator was calculated with the help of this program.

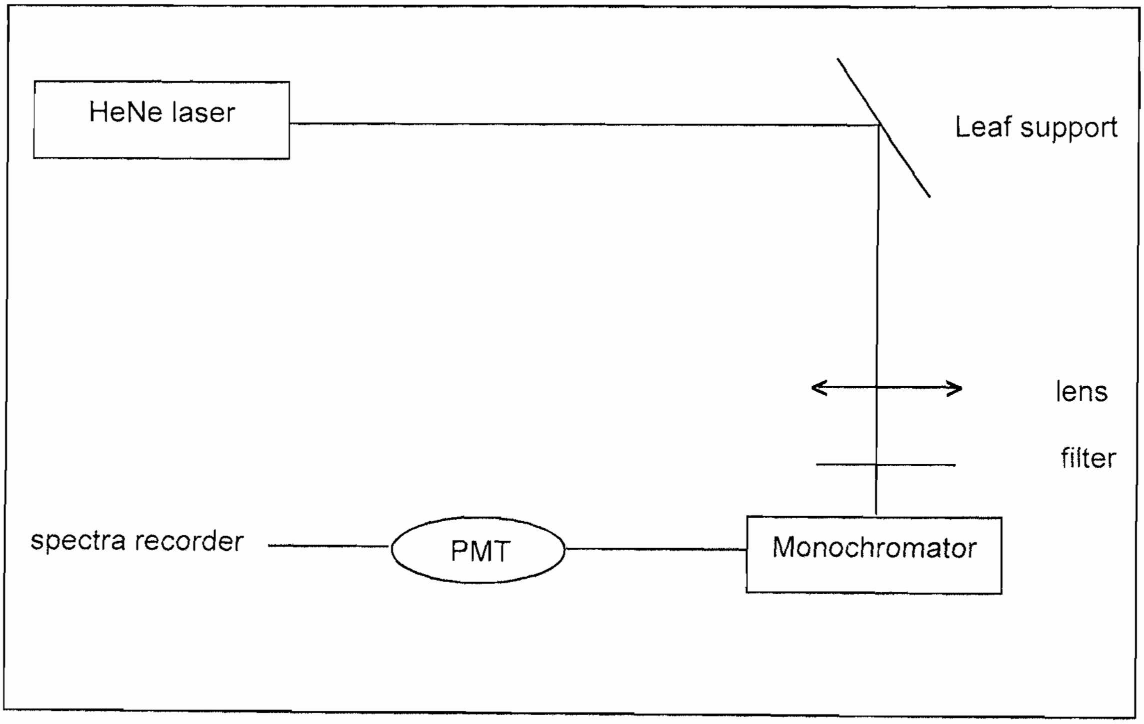

Figure 1 : Scheme of laser-induced fluorescence experiment.

Schéma du dispositif de fluorescence induite par laser.

RESULTS AND DISCUSSION

Figure 2 shows the type of spectrum obtained and its fitted parameters. It is the fluorescence fitted spectrum of leaf of rank 9 for the A2614 K0 tree. *Xc*, *Wy* and *Ai* are the position, the width and the area of relative contribution, of fluorescence curves centred respectively at 690 and 735 nm. *R* represents the ratio of maximum fluorescence near 690 and 735 nm whether the wavelength position of the maxima is slightly shifted toward somewhat shorter or longer wavelength or not.

Figure 3 shows fluorescence spectra of K^+-deficient palm oil tree leaves of rank 9 (A2614 K0) and of non K^+ -deficient leaves of palm oil trees (A2023 K3). The two maxima wavelengths near 690 and 735 nm in leaf fluorescence spectrum for K^+-deficient trees are greater than the non K^+-deficient ones.

Tables 1 and 2 present the fluorescence spectrum parameters of tree A2614 K0 with stress caused by potassium deficiency and tree A2023 K3 without stress, respectively.

A plot of the intensity ratios R versus leaf rank (figure 4), shows that leaves of 6, 9 and 14 ranks had similar closed values. A plot of the ratio of the area of the fitted curves shows that only the value of leaves of 6 and 9 ranks were equal (figure 5). This was confirmed by Ballo *et al.* (1996) who proved that a younger leave from the tree top do not have the same K^+ concentration as older leaves because younger leaves draw potassium from older leaves for their development. In the case of rank 17, the leaves showed visual deficiencies symptoms due to age, so, they were not used in the LIF technique.

One can conclude that the LIF technique gave better results when leaves of rank 6 and 9 were used. In this study, leaves of rank 9 for four trees in each group (K0 and K3) were used from A2615 K0, A2616 K0, A2614 K0, A2514 K0 and A2022 K3, A1923 K3, A1922 K3, A2024 K3 varieties.

Table 3 and 4 present for these samples, the LIF fitted spectrum parameters and the intensity ratios of the fluorescence at the two maxima, an important stress indicator. It was also apparent that all the trees with stress caused by potassium deficiency had their spectrum intensity ratios, $R > 0.45$, while the K3 group of intact trees had $R < 0.45$ (figure 6).

CONCLUSION

Extreme potassium deficiencies are not usually visible on plant, till it is too late. Laser Induced Fluorescence spectrum intensities at specific wavelength can be used successfully to detect early K^+-deficiency. In the case of stress caused by potassium deficiency, the ratio $R > 0.45$ and for non K^+-deficiency stress $R < 0.45$. Laser Induced Fluorescence method should be best for leaf samples of rank 6 and 9. Thus, Laser Induced Fluorescence technique could be an alternative to the classical Foliar Diagnosis method because of its simplicity and efficacy.

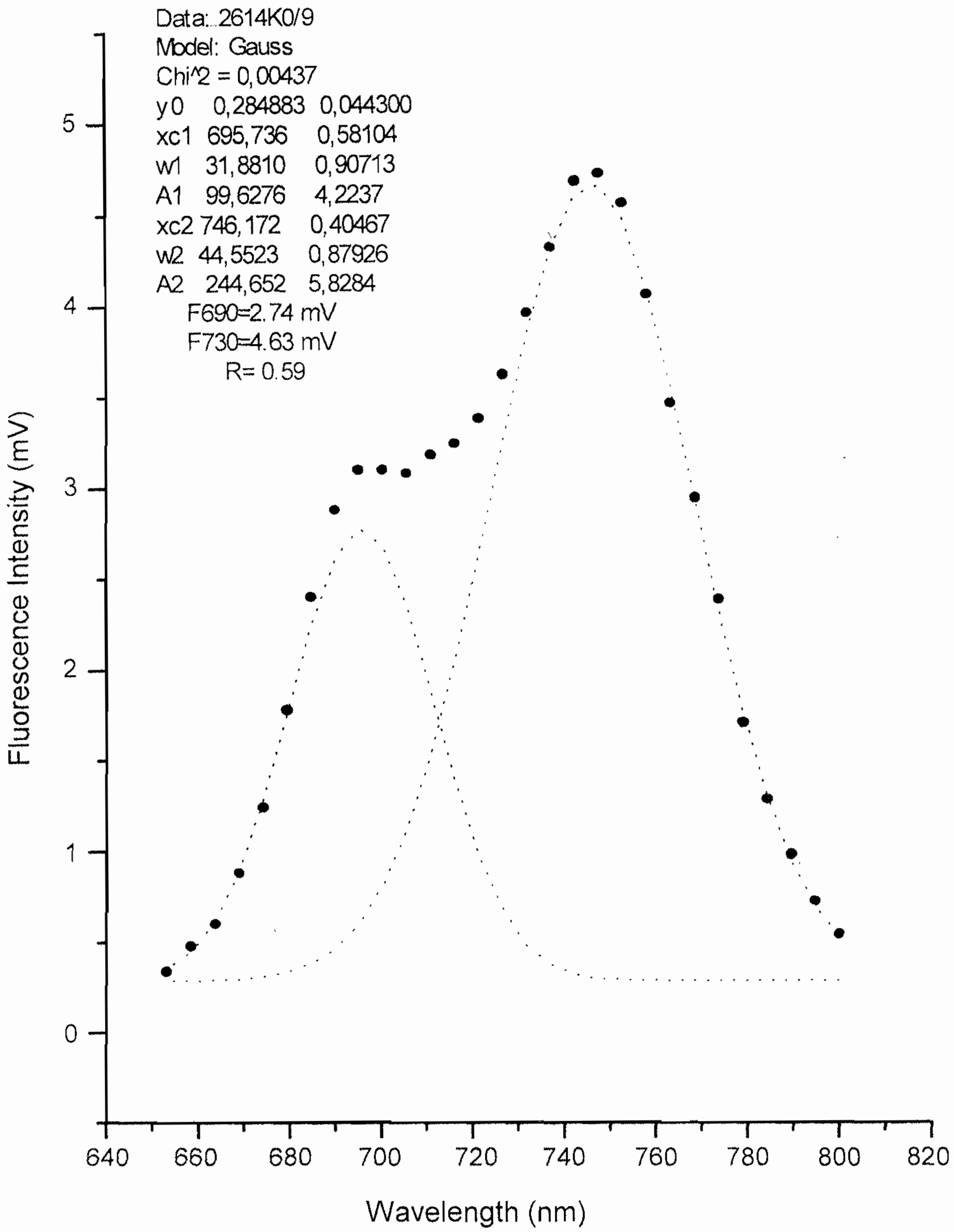

Figure 2 : Fitted florescence spectrum of leaf of rank 9 from a K^+-defficient palm oil tree (A2614 K0).

Spectre normal de feuille de 9è rang foliaire de palmier à huile, deficients en patassium (A2614 K0).

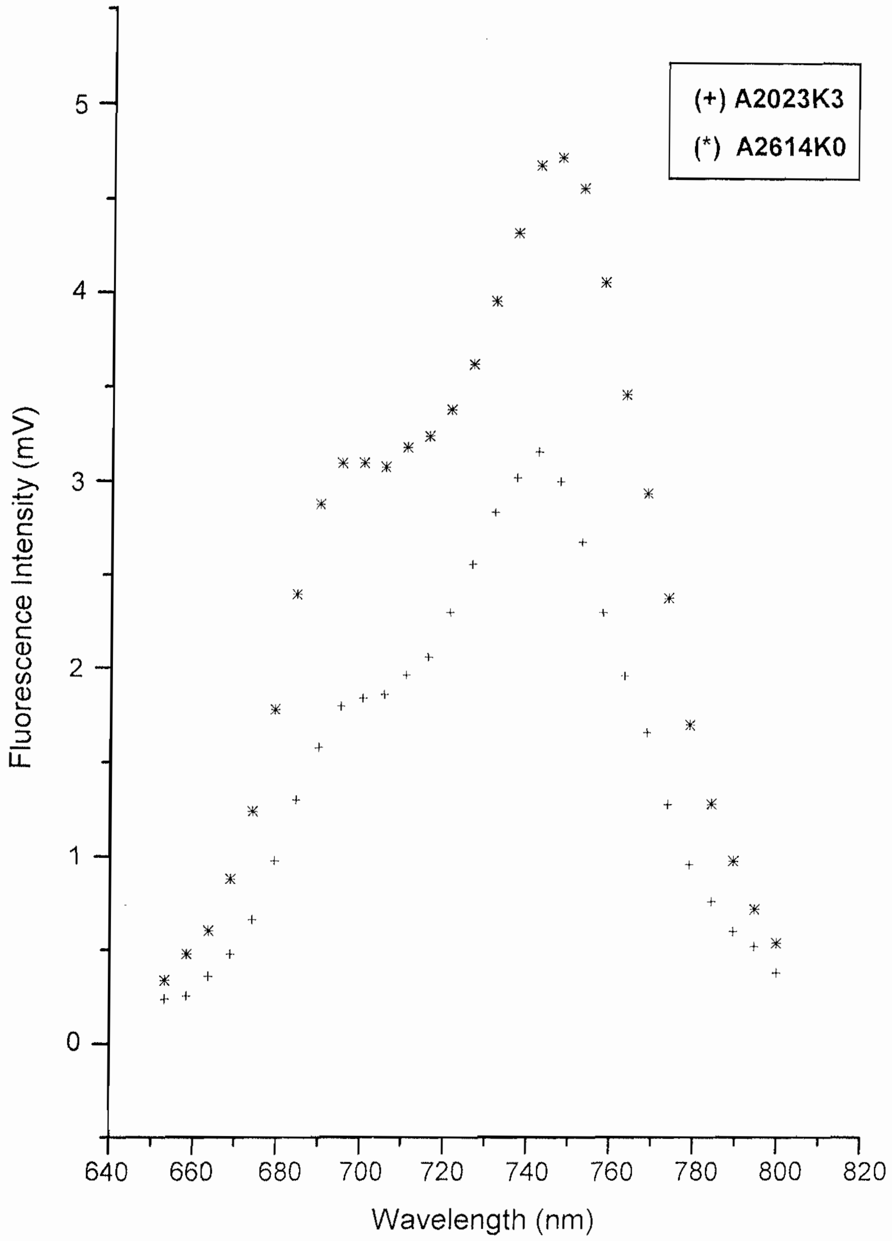

Figure 3 : Florescence spectra for K+-deficient leaves of rank 9 from a K^+-deficient (A2614 K0) and a non K^+-deficient (A2623 K3) palm oil trees.

Spectres de fluorescence des feuilles de rang 9 des palmiers à huile deficient en K^+ (A2614 K0) et non deficients en K^+ (A2023 K3).

Table 1 : Fitted Laser-Induced Fluoresence (LIF) spectrum parameters of rank 1 through 23 leaves from K^+-deficient palm-oil trees (A2614 K0).

Paramètres des spectres de fluorescence induite par laser des feuilles du palmier à huile (A2614 K0) présentant une déficience en potassium.

Parameters	Parameter values of each leaf rank					
	1	6	9	14	17	23
λ_{690} (nm)	695.006	695.786	693.389	693.211	693.775	695.048
λ_{735} (nm)	737.934	746.756	740.748	738.657	739.209	745.975
W_{690} (nm)	27.492	30.5362	27.121	26.152	22.909	30.2538
W_{735} (nm)	46.295	44.7917	45.389	45.469	45.311	45.9969
R= F690/F735	0.53	0.47	0.47	0.48	0.47	0.53
R_a= A690/A735	0.34	0.33	0.33	0.28	0.32	0.36

λ_{690}, λ_{735}, W_{690}, W_{735} are the position and the width of the relative contribution of fluorescence curves centred at 690 nm and 735 nm. R is the intensities ratio of the curves and R_a, the area ratio.

Table 2 : Fitted laser-Induced Fluoresence (LIF) spectrum parameters of rank 1 through 23 leaves from non K^+-deficient (A2023 K3) palm-oil trees.

Paramètres des spectres de fluorescence induite par laser de feuilles intactes de palmier à huile non deficient en K^+ (A2023 K3).

Parameters	Parameter values of each leaf rank					
	1	6	9	14	17	23
λ_{690} (nm)	686.406	694.894	688.538	688.283	688.433	695.068
λ_{735} (nm)	735.395	742.759	735.245	734.235	736.856	744.225
W_{690} (nm)	25.167	28.1725	25.087	23.387	25.134	28.6760
W_{735} (nm)	50.988	44.8676	43.961	43.905	43.701	45.6679
R= F690/F735	0.52	0.41	0.41	0.42	0.45	0.45
R_a= A690/A735	0.38	0.26	0.26	0.27	0.30	0.29

λ_{690}, λ_{735}, W_{690}, W_{735} are the position and the width of the relative contribution of fluorescence curves centred at 690 nm and 735 nm. R is the intensities ratio of the curves and R_a, the area ratio.

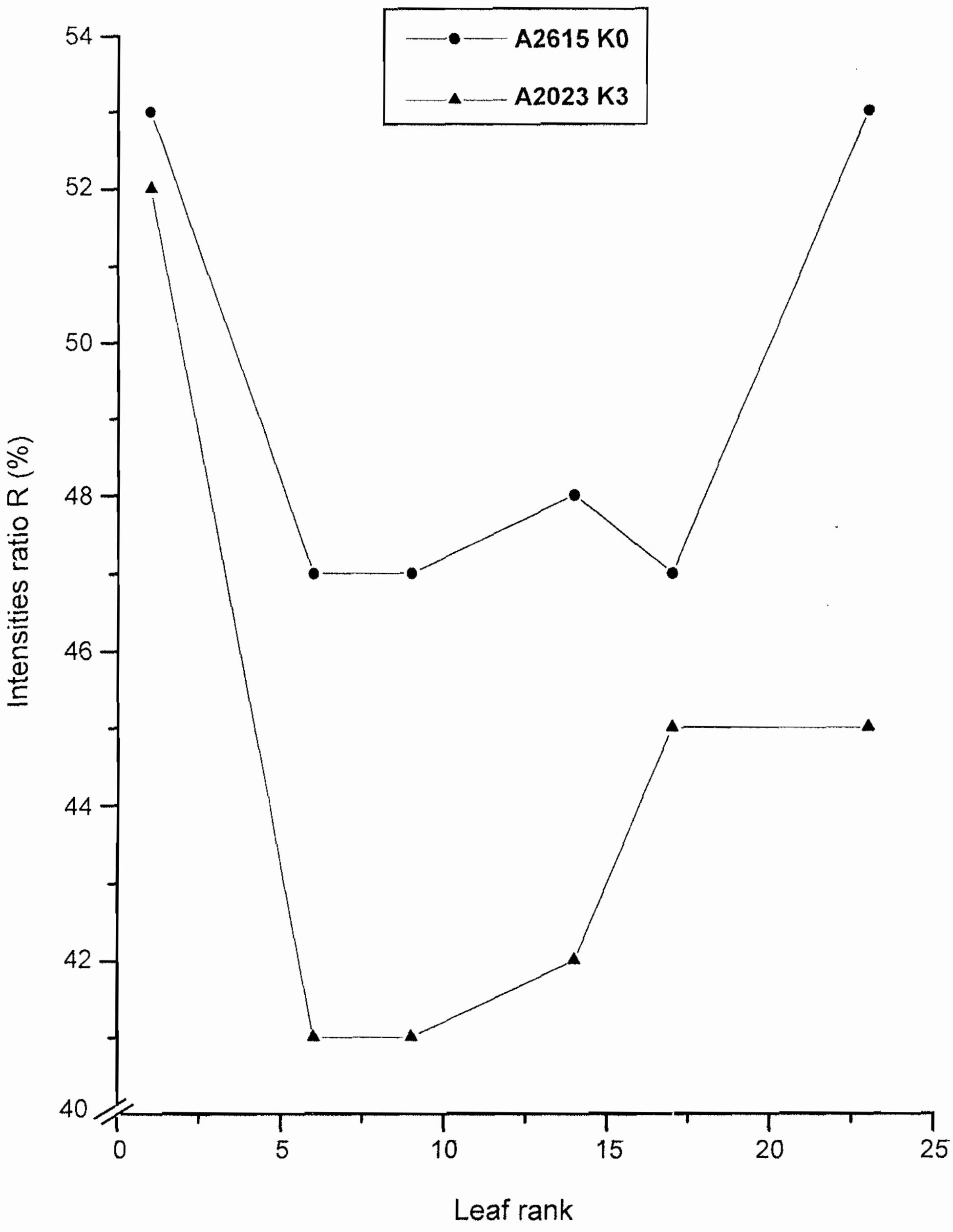

Figure 4 : Fluorescence spectrum intensities ratio versus leaf rank of two palm oil tree groups (K0 and K3).

Rapports des intensités des spectres de fluoresence en fonction du rang des feuilles de deux groupes de palmiers à huile (K0 et K3).

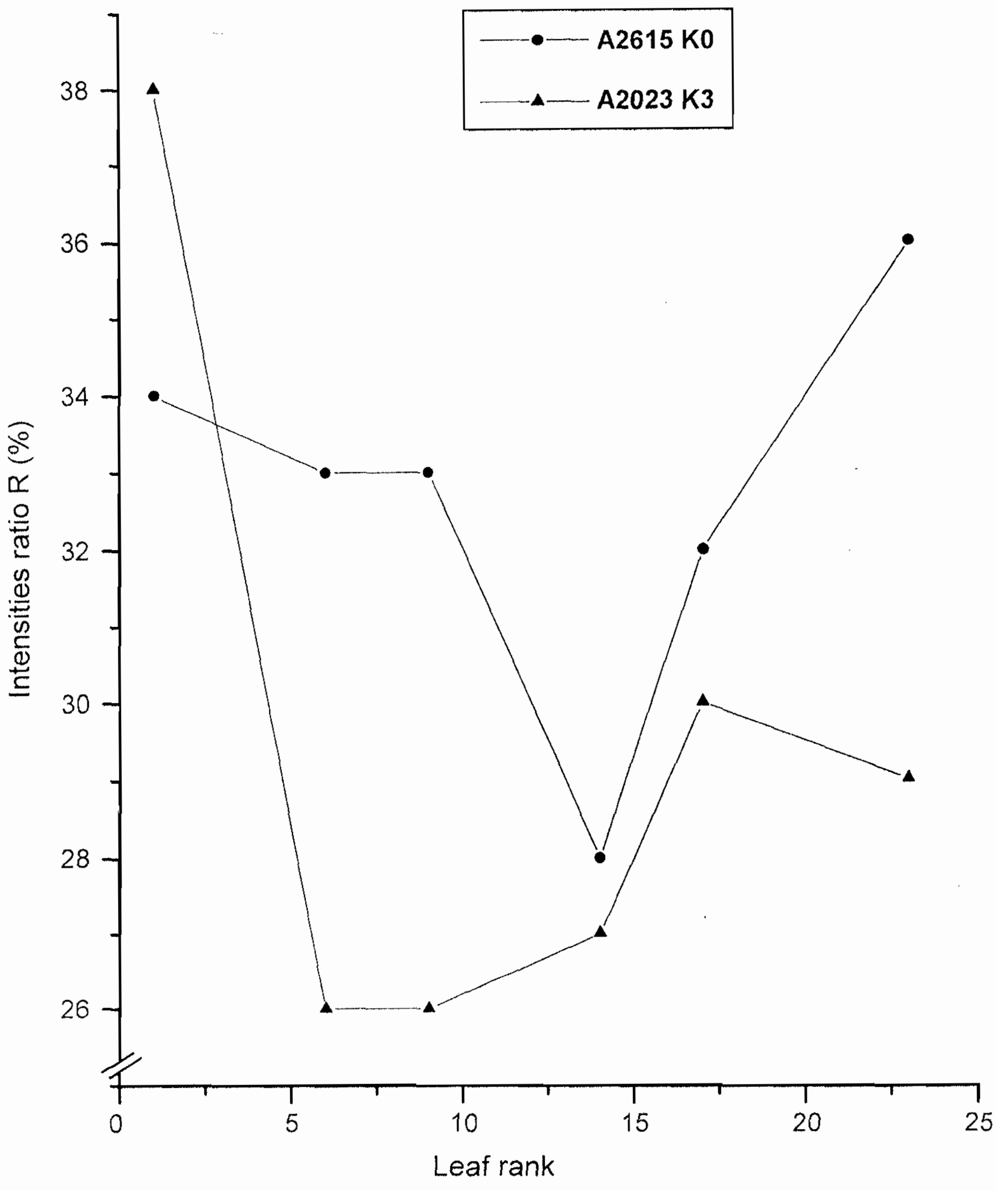

Figure 5 : Ratio of the area of the fitted curves versus leaf rank of two palm oil tree groups (K0 and K3).

Rapports des aires des courbes convenables en fonction du rang des feuilles de deux groupes de palmiers à huile (K0 et K3).

Table 3 : Fitted laser-induced fluorescence (LIF) spectrum parameters of rank 9 leaves from K^+-deficient palm oil tree groups (K0).

Paramètres des spectres de fluorescence induite par laser des feuilles de rang 9 du groupe de palmiers présentant une déficience en potassium (K0).

Parameters	Parameter values of K0 groups			
	A 2615	**A 2616**	**A 2614**	**A 2514**
λ_{690} (nm)	687.46 (0.47)	687.90 (0.55)	686.04 (0.80)	693.39 (0.91)
λ_{735} (nm)	733.23 (0.41)	735.88 (0.31)	734.45 (0.69)	740.75 (0.34)
W_{690} (nm)	26.31 (1.13)	26.45 (0.90)	24.58 (2.31)	27.12 (0.78)
W_{735} (nm)	45.16 (1.05)	42.82 (0.86)	52.59 (1.37)	45.39 (0.94)
R= F690/F735	**0.55**	**0.55**	**0.65**	**0.62**

λ_{690}, λ_{735}, W_{690}, W_{735} are the position and the width of the relative contribution of fluorescence curves centred at 690 nm and 735 nm. R is the intensities ratio of the curves.

Table 4 : Fitted laser-induced fluorescence (LIF) spectrum parameters for rank 9 leaves from non K^+-deficient palm oil tree groups (K3).

Paramètres des spectres de fluorescence induite par laser des feuilles de rang 9 du groupe de palmiers K3.

Parameters	Parameter values of K3 groups			
	A 2022	**A 1923**	**A 1922**	**A 2024**
λ_{690} (nm)	690.63 (0.64)	688.54 (0.51)	688.05 (0.49)	688.27 (0.41)
λ_{735} (nm)	738.73 (0.35)	735.25 (0.31)	734.68 (0.33)	735.48 (0.22)
W_{690} (nm)	25.25 (1.29)	25.09 (0.92)	25.48 (1.13)	24.22 (0.74)
W_{735} (nm)	43.33 (1.06)	43.96 (0.86)	44.91 (10.89)	43.14 (0.63)
R= F690/F735	**0.42**	**0.43**	**0.44**	**0.40**

λ_{690}, λ_{735}, W_{690}, W_{735} are the position and the width of the relative contribution of fluorescence curves centred at 690 nm and 735 nm. R is the intensities ratio of the curves.

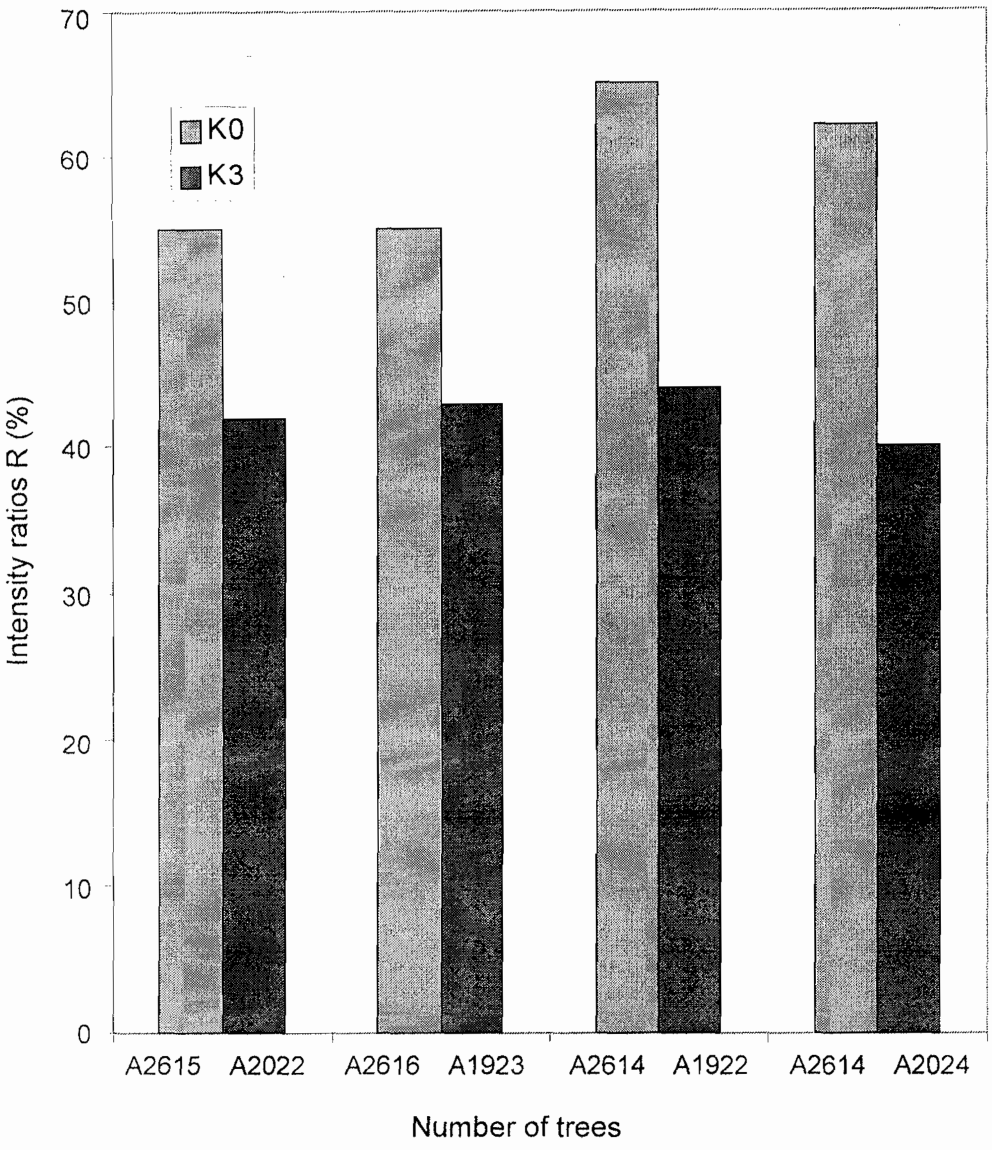

Figure 6 : The intensities ratios of fluorescence curves versus K^{+}-deficient (K0) and non K^{+}-deficient (K3) palm oil trees.

Rapports des intensités des courbes de fluorescence en fonction des groupes K0 et K3 de palmiers.

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