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Sudan University of Science & Technology

College of Graduate Studies

Effect of Laser Radiation on Germination and Growth Rate of Sorghum Vulgar Seeds

تأثير أشعة الليزر على معدل إنبات و نمو بذور الذرة (أبوسبعين)

A Thesis Submitted as Partial Fulfillment of the

Requirements for Master Degree in Laser Applications

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قال تعالى:

صدق الله العظيم

الآيات: (63 - 64) من سورة الواقعة

Dedication

This work is dedicated

To my family

To my Friends, to all people gave me help and support.

Acknowledgements

I am extremely thankful to **Allah** that gave me chance and courage to complete this work

I am thankful to **Dr.S.T.Kafi** for his understanding and continuous support in supervising this work.

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I also thank all my colleagues at the laser institute for listening to me and providing assistance.

Abstract

Laser is one of the sources for inducing biostimulation effect and genetic changes in plants. In this study the IR laser (wavelength 915 nm) was used to stimulate Sorghum vulgar *Spp* seeds. Seeds were divided into two groups, the first was irradiated in dry condition and the other one was irradiated in wet condition (soaked in water for 24 hours). Both dry and wet seeds were irradiated separately with 5 laser doses according to the exposure times to laser (5, 10, 15, 20 and 25 seconds), with different irradiance (4, 8, 12, 16, and 20 W/cm^2). As a control, untreated seeds were used. After sowing, germinated seeds were counted every 48 hours for 8 days. To monitor the growth rate of Sorghum *Spp* plant grown from treated and non-treated seeds, the laser induced chlorophyll fluorescence technique equipped with blue laser (405nm) as excitation source was used.

The obtained results showed that the irradiation of dry seeds at 16 W/cm^2 for 20 seconds significantly affects the germination rate of seeds, but the growth rate was not good, while irradiation of wet seeds at 8 W/cm^2 for 10 seconds enhanced the germination rate and plant growth rate compared to other samples (treated and non-treated seeds).

الملخص

الليزر واحد من المصادر المستخدمة لإحداث التحفيز الحيوي والتغيرات الجينية في النباتات. في هذه الدراسة أُستخدم ليزر الأشعة تحت الحمراء (الطول الموجي 915 نانومتر) لتحفيز بذور الذرة (أبو سبعين). قُسمت البذور الى مجموعتين، المجموعة الأولى شُعِّعت وهي جافة والأخرى شُعِّعت رطبة (بُلّلت بالماء لمدة 24 ساعة). كلا المجموعتين شُعِّعت منفصلة على 5 جرعات ليزر وفقا لزمن التعرض (5، 10، 15، 20 و 25 ثانية)، و شدة اشعاع (4، 8، 12، 16 و 20 واط/سم^2). وأستخدمت بذور غير مشععة كعينة مرجعية. بعد زراعة البذور، رُصد عدد البذور التي نبتت كل 48 ساعة لمدة 8 أيام. لرصد نمو نبات أبوسبعين المزروع من بذور معالجة وغير معالجة بالليزر، أُستخدمت تقنية الليزر المحدث لإنبعاث الكلوروفيل باستخدام الليزر الأزرق (الطول الموجي 405 نانومتر) كمصدر إثارة.

أوضحت النتائج المستخلصة أن تشعيع بذورأبوسبعين الجافة عند 16 واط/سم^2 لـ 20 ثانية يؤثر بشكل واضح على معدل الإنبات، لكن معدل النمو لم يكن جيداً. بينما تشعيع البذور الرطبة عند 8 واط/سم^2 لـ 10 ثواني عزَّز معدل الإنبات ومعدل نمو النبات مقارنة بالبذور الأخرى (المعالجة وغير المعالجة بالليزر).

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CHAPTER ONE

INTRODUCTION

1.1 Overview

Current worldwide problems: climate change, water supply, poverty and the population need for food. For example, one of common worldwide problems is the quantity and quality of agricultural seeds and foods. In developing countries this is more apparent, causing various diseases and malnutrition. There are several techniques that used to biostimlate seeds, such as, chemomutagens, ionizing radiation, flash lamps and lasers, those latter being the source of irradiation which constitutes the focus of the present study. The laser treatment is one of the physical methods that also include electromagnetic treatment that can improve sowing quality, help achieve higher productivity, and at the same time reduce the risk of contamination from soil and water (Hernandez, et al., 2010). Laser biostimulation is a physical phenomenon based on the absorption of light energy by grains, which is then transformed into chemical energy that can be used by the plant at later stages of growth. The energy supply increases the energy potential of seeds, which in turn impacts the physiological processes in germinating seeds (Gładyszewska., 2006).

Laser-induced chlorophyll fluorescence (LICF) have wide range of application in the field of remote assessment for detecting environmental stress induced physiological changes in leaves of plants. The study of laser-induced chlorophyll (*Chl*) fluorescence of green plant leaves provides basic information about the functioning of the photosynthetic apparatus and also regarding the capacity and performance of photosynthesis (Pandey & Gopal., 2011). The development and use of different types of laser sources for excitation of *Chl* molecules, such as CW and pulsed lasers have made *Chl*

fluorescence a very popular tool in plant physiology, environmental studies and agriculture. Studies on *Chl* fluorescence emission spectra and photosynthetic apparatus have indicated an inverse proportional relationship between fluorescence intensity and photosynthetic process performance. Therefore, *Chl* fluorescence emission of dark-adapted leaves can provide considerable information on the organization and function of the photosynthetic apparatus (i.e. the plant growth) (Ombinda-Lemboumba., 2007).

1.2 Literature Review

1.2.1 Laser biostimulation of seeds germination

Previous studies showed that seed treated with low intensity laser improved germination and accelerated plant growth. In 2005 Wilczek, et al., studied the germination of the Polish hybrid alfalfa var when irradiated with He-Ne laser, it was found that the laser treatment of seed significantly increased the percentage of seeds germinating normally and decreased the share of seeds germinating abnormally (Wilczek, et al., 2005). In 2006 Gładyszewska used He-Ne laser pre-sowing biostimulation of cereal grains resulted in an increase in the germination rate of seeds, germinating at T = 20°C, as compared to the control group (Gładyszewska., 2006; Muszyñski and Gladyszewska., 2008). Also Ćwintal, et al., in 2010 presented that Pre-sowing stimulation of seeds with laser light, at different doses, caused a significant increase in the content of specific protein, phosphorus and molybdenum, while decreasing the content of crude fibre in the dry matter of the Alfalfa plants (Ćwintal, et al., 2010). Also in 2014 Maamoun, et al., determined that He-Ne laser enhanced the germination rate of black cumin (dry and wet) seeds with different doses (Maamoun, et al., 2014). In different study found that the exposure of seeds to He-Ne laser light improved the germination rate and uniformity and modified growth stages, which caused acceleration of flowering and ripening of pea plants (Podleśna, et al., 2015).

1.2.2 LICF

Laser induced chlorophyll fluorescence spectroscopy technique has been known for more than half century, and it has been used by physiologists to characterize the status of vegetation. It can be done by using different excitation sources, for example, He-Ne (632nm), argon ion (455nm), and excimer (308nm) lasers which were previously used by Ombinda-Lemboumba in 2007 as excitation sources to investigate *Chl* fluorescence under constant illumination and also to detect green fluorescent protein (Ombinda-Lemboumba., 2007). In 2008 Kancheva, et al., showed that the heavy metal induced stress is detectable from *Chl* fluorescence demonstrating that the analysis of fluorescence spectra may timely and accurately indicate the onset of stress in plants (Kancheva, et al., 2008). In 2011 Gouveia-Neto, et al., used laser induced fluorescence signatures from plants to evaluate the effect of abiotic stresses (water deficit and soil salinity) upon the evolution and characteristics of in vivo chlorophyll emission spectra of leaves of Saccharum officinarum and Jatrophacurcas L. plants (Gouveia-Neto, et al., 2011). In 2011 Pandey and Gopal studied LICF spectra, reflectance spectra and fluorescence induction kinetics (FIK) curves of Triticumaestivum L. plants treated with different concentrations of cadmium. LICF spectra were recorded using violet diode laser (405 nm) and using red diode laser (635 nm) for excitation (Pandey & Gopal., 2011). In this study the LICF were recorded using diode laser (405 nm).

1.3 Thesis problem

The continuing demand for the Sorghum crops over the last fifty years, however, crop productivity has not kept pace with increasing demand, due to a lag in crop improvement efforts in Sorghum. The aim of this study is to enhance the germination and growth rate of Sorghum vulgar plant using laser light.

1.4 Objectives

The objectives of this study were:

1- To verify the effect of pre-sowing IR laser (915 nm) biostimulation on the germination rate of Sorghum vulgar seeds. In addition, to determine the optimal radiation dose that could positively affect the germination.

2- To monitor Sorghum vulgar plant growth using laser induced chlorophyll fluorescence spectroscopy.

3- To correlate between spectroscopic data and vegetative observations.

1.5 Thesis Layout

The introduction, literature review and objectives of this study are outlined in chapter one.

In chapter 2, the background of laser biostimulation of seeds and laser induced chlorophyll fluorescence are presented. First, the description of Sorghum vulgar *Spp* seeds and the interaction and effect of laser radiation on germination of seeds. Second, an overview of laser induced chlorophyll fluorescence, including the basics of fluorescence, photosynthetic and the chlorophyll fluorescence of green plant material.

In chapter 3, the materials and setup used for seeds irradiation and chlorophyll fluorescence spectroscopy are described. First, the seeds material using IR laser as excitation source and the setup used for irradiation are described. In addition, the germination tests and the data collection are described. Second, the materials, setup for fluorescence imaging, using laser and LED, and the method used for the analysis of spectra are described.

In chapter 4, the results of investigations of the enhancement of Sorghum vulgar seeds germination using IR laser and spectroscopic analysis of chlorophyll fluorescence spectra are presented.

In chapter 5, the conclusion is presented, and recommendations for further study are given.

Appendix and a list of references are shown at the end.

CHAPTER TWO

BACKGROUND

2.1 Laser Biostimulation of Seeds

Light is the one of the most important environmental factors, which plays a critical role in plants photosynthesis. Moreover, the non-photosynthetic processes involving action of light, like phototropism, photomorphogenesis, photobiosynthesis of carotenoids. These phenomenon are well known and have been the subjects of investigations for last decade. It is clear that also seeds respond to light with a complex variety of reactions that are affected by the exposure time and intensity of light as well as transparency of seed coat which depends on the wavelength of applied light. It is generally accepted that germination process is sensitive to irradiation with various wavelengths of visible and infra-red light (Muszyñski and Gladyszewska., 2008). The laser light of low intensity produces biostimulation when used to irradiate seeds. The basis of the stimulation mechanism in any plant physiological stage is the synergism between the polarized monochromatic laser beam and the photoreceptors when triggered activate numerous biological reactions. There are many facts that indicate the biostimulating action of laser radiation on various organs and tissues in animals and plants. They absorb light *via* their photoreceptors which leads to control all stages of plant development. Laser activation of plants results in an increase of their bioenergetic potential, leading to higher activation at fitochrome, fitohormone and fermentative systems, as a stimulation of their biochemical and physiological processes. The use of laser light at specific wavelengths increases the ratio of excited molecules. Illumination of biological tissue by coherent laser light leads to strong intensity gradients of the radiation in the tissue due to speckle formation, which causes inter- and intracellular gradient forces whose action may significantly influence the paths and speeds of biological processes that

is also one of the important factors in the consideration of laser light as a pre-sowing seed treatment (Hernandez, et al., 2010).

2.2 Sorghum vulgar *Spp* Plant

Sorghum is used as a grain crop by humans and also as a forage crop for poultry and livestock consumption in many developing countries. In world production, Sorghum after wheat, rice and maize ranks the fourth among cereals. Due to its high drought resistance, Sorghum is one of the principle summer crops (Zulfiqar and Asim., 2002).

The use of Sorghum has increased in recent years due to the fact that Sorghums require less water than corn. Sorghums used for forage are typically grouped as: (a) forage sorghum (b) Sudangrass and (c) sorghum-Sudan hybrids. Each of these types has different growth characteristics that influence how they should be used. Even within one type, considerable differences can exist. Typically, forage sorghums are used for silage production or for a one cutting hay crop. Sudangrass is most often used for grazing, multiple hay cuttings, or occasionally green chop. Sorghum- Sudan hybrids are best utilized for single or multiple hay cuttings and grazing (Butler and Bean , 2011).

2.2.1 Sorghum Descriptions

Sorghum plant can best be described as a coarse grass, 0.5 to 4m tall, having nodes and inter-nodes which are generally 10 to 15 in number. Leaves consist of sheath and blade. Stalks of Sorghum are grooved on one side between the nodes and the grooved inter-nodes alternate from side to side and a leaf is born at each node on the grooved side (Zulfiqar and Asim., 2002). Once grain sorghum emerges, the plant develops in a predictable manner characterized by three distinct growth stages — GS I, GS II and GS III. The first growth stage, GS I, is characterized by vegetative growth. The plant develops its vegetative structures, leaves and tillers, which ultimately support grain formation and growth. The second growth stage, GS II, is the period when reproductive structures of the panicle form and the maximum number of

seed per plant are set. It is considered the most critical period for grain production, because seed number per plant accounts for 70 percent of sorghum's final grain yield. The third and final growth stage is grain filling, called GS III. It begins with flowering and continues until dry matter accumulation in the grain stops with the appearance of a black-layer near the point of the seed attachment in the floret (Gerik, et al., 2003).

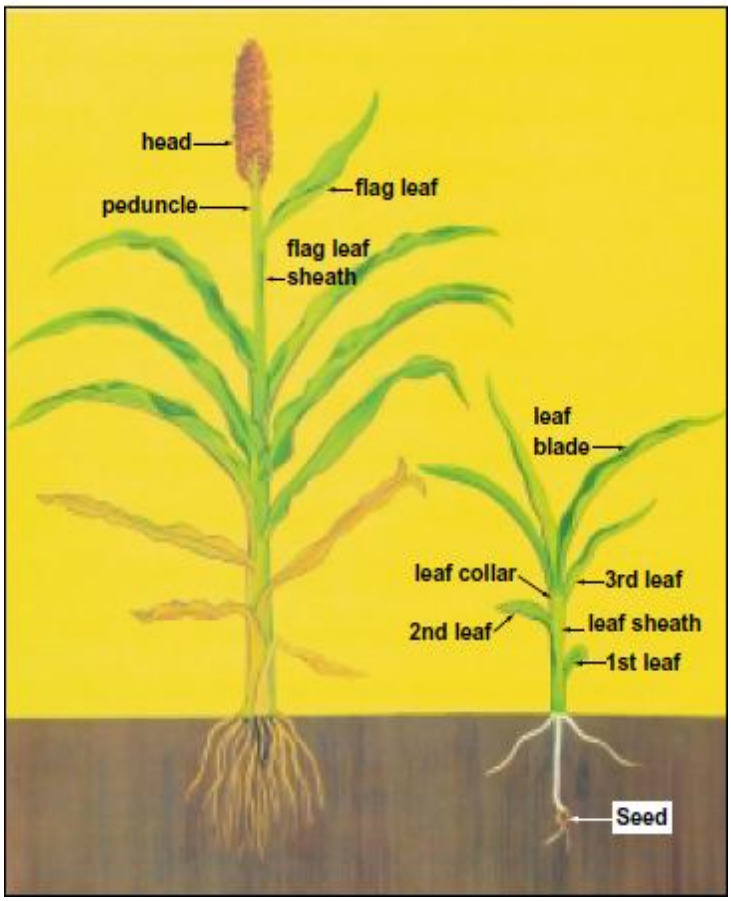

Figure 2-1: Typical grain sorghum plants at physiological maturity (left) and a 3-leaf growth stage (right) (Gerik, et al., 2003).

2.3 Laser Induced Chlorophyll Fluorescence

2.3.1 Fluorescence

Fluorescence is luminescence process in which molecules emit light from electronically excited states created by either a physical, mechanical or chemical mechanism. Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is a phenomenon termed photo luminescence, which is formally divided into two Categories, fluorescence and phosphorescence, depending upon the electronic configuration of the excited state and the emission pathway. In fluorescence, electron de-excitation occurs almost instantly, and emission from a fluorescent substance ceases when the exciting source is removed. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to re-emit light of longer wavelength after brief interval. A molecule exposed to an electromagnetic field absorbs energy in discrete amounts, named quanta, if both resonance and the selection rules of quantum mechanics transition are satisfied. Likewise emission of photon through fluorescence or phosphorescence is also measured in terms of quanta. The energy in a quantum (Plank's law) is expressed by the following equation:

$$\Delta E = E_i - E_j = h\upsilon = hc/\lambda$$

Where Ei and Ej are the energies of the two ground and excited, respectively, υ and λ are the frequency and wavelength of the incoming photon, respectively, h is Plank's Constant, and c is the speed of light (Ombinda-Lemboumba., 2007).

The absorption of photon always occurs from the lowest excited state to one of the higher vibrational states. Owing to the loss of vibrational excitation energy during the excitation/emission cycle fluorescence emission always occurs at lower energy, that is, spectrally red-shifted (the so-called Stokes shift) (Sauer, et al., 2011). This shift is a measure of the relaxation process occurring in the excited state, populated by absorption. The

Stokes shift may come from an environmental effect and also from a change of the geometry of the emitting excited state. This shift is caused by the loss difference between the energy of the absorbed photon and that of the emitted photon. The Stokes shift is measured as the difference between the central wavelengths in the excitation and emission spectra of particular fluorochrome or fluorophore. The size of the shift varies with molecular structure, but can range from just a few nanometers to over several hundred nanometers (Ombinda-Lemboumba., 2007).

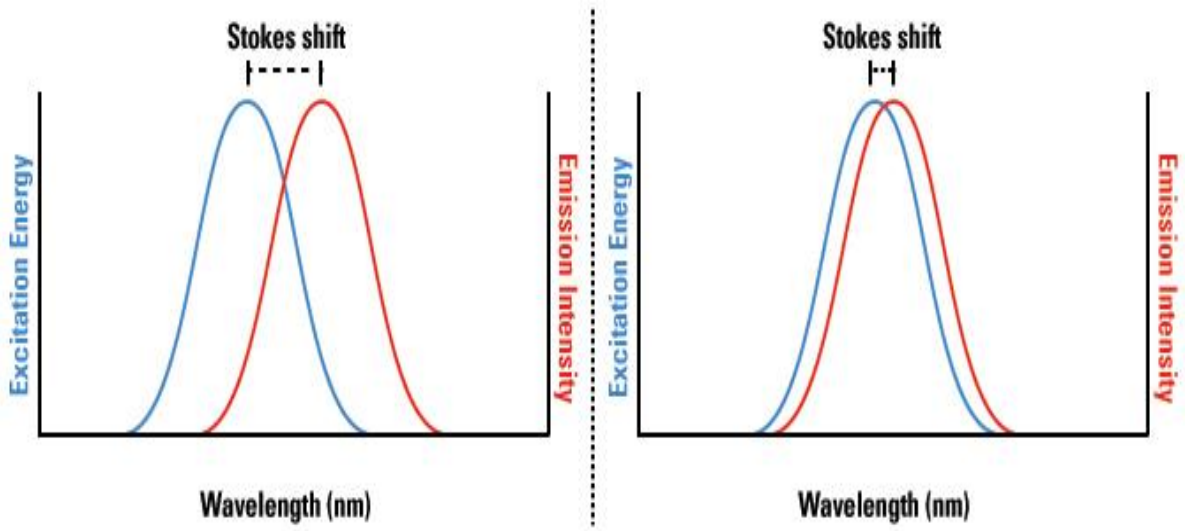

Figure 2-2: The shift between the absorption spectrum and the emission spectrum: Stokes shift ()

2.3.2 Chlorophyll absorption

Chlorophyll is the molecule that captures the sunlight energy and is called a photoreceptor. It is found in the chloroplasts of green plants, and is gives plants their green color. Within the plant tissue, visible and near-infrared (NIR) light is absorbed (>80%) by photosynthetic pigments (Chlorophyll a, b, and carotenoids) and used to drive photosynthetic light reactions and associated electron transport reactions. Chlorophyll molecules organized into two groups of pigments called photosystem I (PSI) and photosystem II (PSII), each containing "antennae" *Chl* molecules and a central chlorophyll molecule (P680 and P700) (Gouveia-Neto, et al., 2011).

Both photosystems contain the two *Chl* pigments (*Chl*-a and -b) that are involved in the photosynthetic mechanism (Ombinda-Lemboumba., 2007).

The pigments antennas absorb much of the visible portion of the electro-magnetic spectrum, mainly in the near UV-blue region, as can be seen in the absorption spectra shown in figure 2-3. There exist a very strong energy transfer mechanism taking place among pigment antenna where the light energy absorbed by carotenoids and *Chl*-b pigments resonantly transfer their energy to neighbours *Chl*-a molecules and the total energy is conveyed to reaction centers in which the migration process will occur as pictured in Figure 2-4.

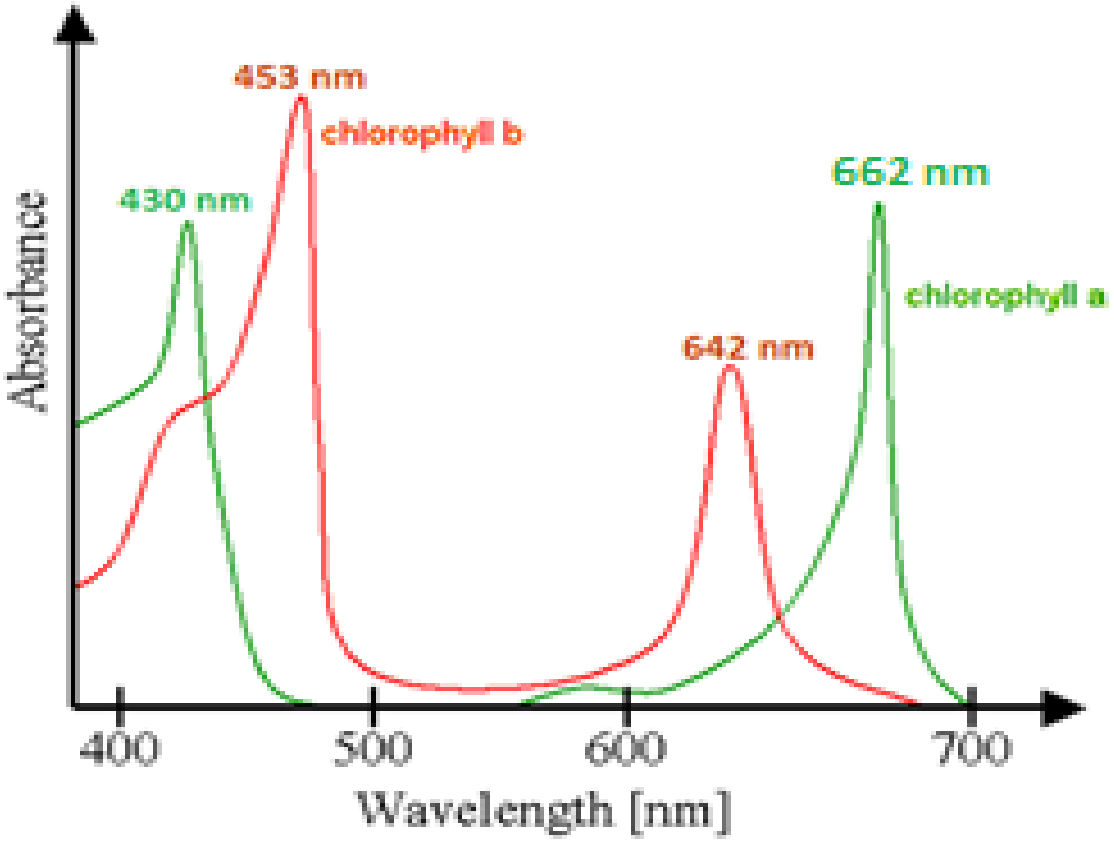

Figure 2-3: Absorption spectrum of pigments antenna of green leaves (Art-xy , 2013)

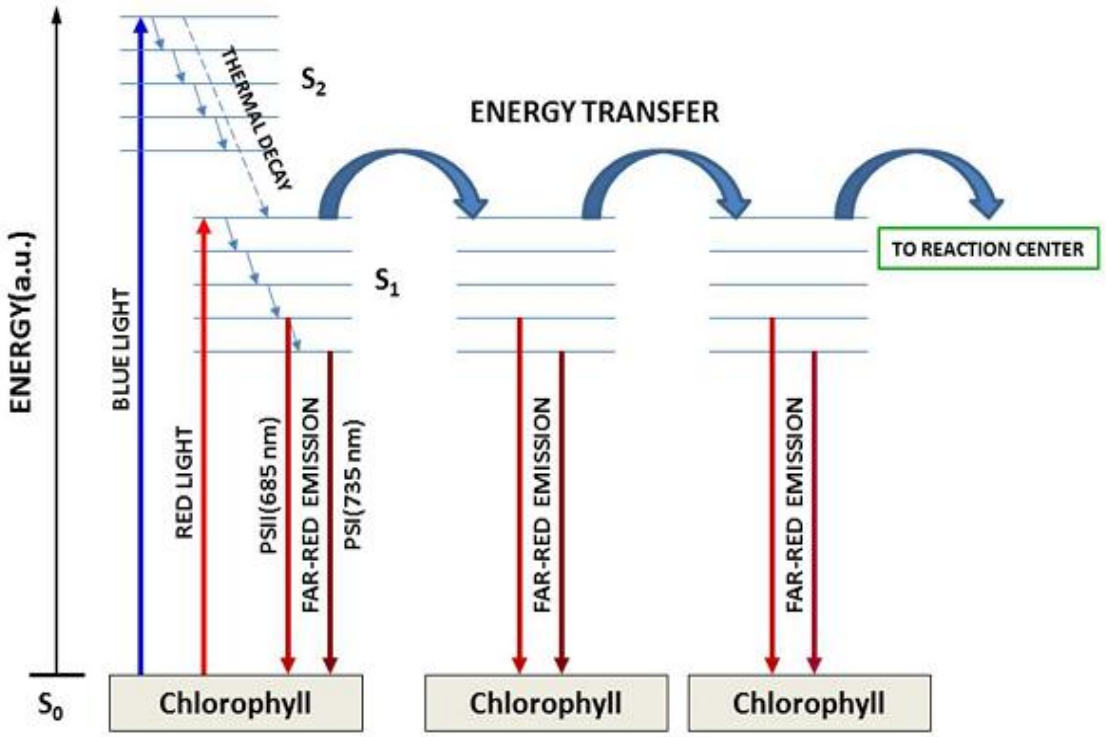

Figure 2-4: Jablonski diagram showing the electronic and vibrational states and energy transfer (Gouveia-Neto, et al., 2011).

2.3.3 Chlorophyll fluorescence (emission)

The chlorophyll fluorescence re-emitted light occurs in the red around 680-690 nm and far-red 730-740 nm spectral regions. When excited with either UV or blue radiation, plants exhibit a fluorescence emission spectrum in two distinct spectral regions blue-green (400-550 nm) and red-far-red (650-800 nm). The red fluorescence is characterized by a maximum in the red region (680-700 nm) which is attributed to the PSII antenna system and referred to as Fr, and one in the far-red (FFr) region (730-740 nm) owing the PSI photosystem (Gouveia-Neto, et al., 2011).

The yield of *Chl*-a fluorescence can be considered as an index of the momentary supply of the singlet *Chl*-a excitation of PSII. At a constant rate of light absorption, this supply is controlled by the following processes:

1. The light induced electron transport across the reaction centers of PSII. This is only significant process from the point of view of photosynthesis, and it consumes the greatest part of PSII excitation.

2. The dissipation of excitation as heat.
3. The emission of *Chl*-a fluorescence.
4. The transfer of excess excitation from the fluorescing *Chl*-a of PSII to the weakly fluorescing chlorophyll-a of PSI. This process is known as the "spillover" of the excitation energy (Papageorgiou, 1975).

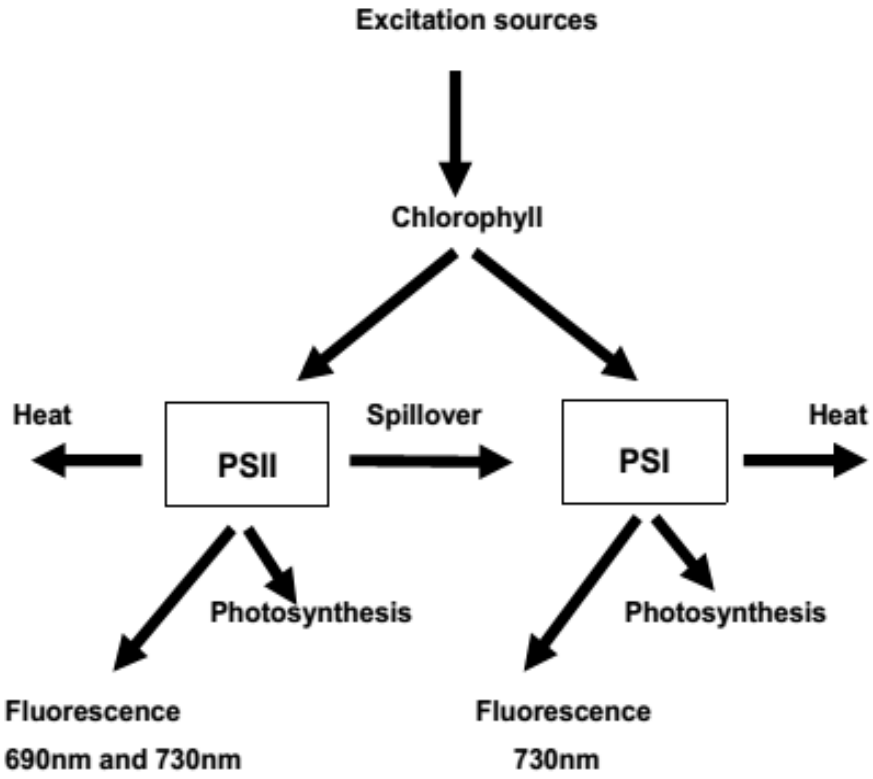

Figure 2-5: A representive diagram of the processes that occurs in photosystems PSII and PSI of green leaves when they absorb energy (Ombinda-Lemboumba, S., 2007).

The *Chl* emitting the red (Fr) and far-red (FFr) fluorescence, the red fluorophore has been identified as *Chl*-a. Although isolated *Chl*-b dissolved in an organic solvent exhibits a red fluorescence. At low *Chl* concentrations, the Fr and FFr emission intensity increases with increasing *Chl* concentration. At higher concentrations, the increase of *Chl* fluorescence with increasing *Chl* concentration is mainly detected in the FFr while Fr levels off with rising

content. The re-absorption is caused by the overlapping of the short-wavelength range of *Chl* fluorescence emission spectra with the long-wavelength range of the *Chl* absorption spectrum. The Fr emission is much more affected by the reabsorption than the FFr, leading to the fluorescence ratio Fr/FFr decrease with increasing Chlorophyll content. The simultaneous measure of *Chl* fluorescence in both red and far-red spectral region allows then the approximate determination of the *Chl* content of the leaves in a non-destructive way using the *Chl* fluorescence ratios (Gouveia-Neto, et al., 2011). The intensity of the red and far-red *Chl* fluorescence is inversely related to the photosynthetic activity. When photosynthesis decrease sowing to various stress conditions, the fluorescence intensity ratio (FIR) increases. The increase in *Chl* content in plants results in a decrease in the value of the FIR. The FIR has also been established as an indicator of the *Chl* content in plants (Gopal, et al., 2002). In green leaves, the chlorophylls and carotenoids have a broad absorption band in the 400-500 nm spectral regions and blue light does not penetrate very deeply into the leaf tissue, and as a result the fluorescence associated to blue light excitation is mainly generated in the green mesophyll cells close to the leaf`s surface, therefore little absorption occurs. On the other hand, blue green and orange excitations are not absorbed by carotenoids and penetrates more deeply into the green leaf mesophyll resulting in a *Chl* fluorescence being generated deeper inside the leaf, from where on its way towards the leaf surface, resulting in a longer pathway and hence the re-absorption is stronger, leading to a less intense red emission compared to the far-red one (Gouveia-Neto, et al., 2011).

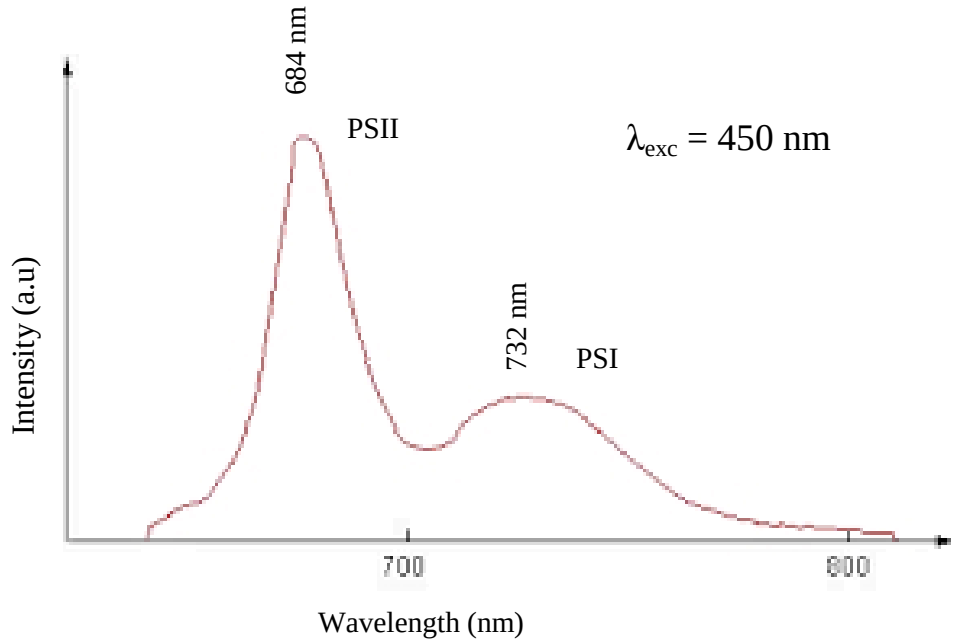

Figure 2-6: Emission spectrum of the chlorophyll (hansatech, 2017)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Seeds

Sorghum varieties (Sorghum vulgar Spp) seeds used in this work were brought from Khartoum grain market (local variety was used in this study). No extra chemical or physiological photosensitization was applied before irradiation.

3.2 Laser source and irradiation procedure

In this method the laser biostimulation of seeds was performed by using the set-up described in figure (3-1). Seeds were divided into two main groups, the first was irradiated in dry condition and the other one was irradiated in wet condition (soaking in water for 24 hours). Both dry and wet seeds were irradiated separately with 5 doses of laser radiation (λ= 915 nm (Omega xp,) with output power of 100 mW (1W peak) and beam diameter of 2 mm) according to the exposure times to laser (5, 10, 15, 20 and 25 second), and irradiance (4, 8, 12, 16, and 20 W/cm^2). As a control, untreated seeds were used, to form 11 treatments of dry and wet seeds. The seeds were irradiated at Institute of Laser, Sudan University of science and technology, Sudan.

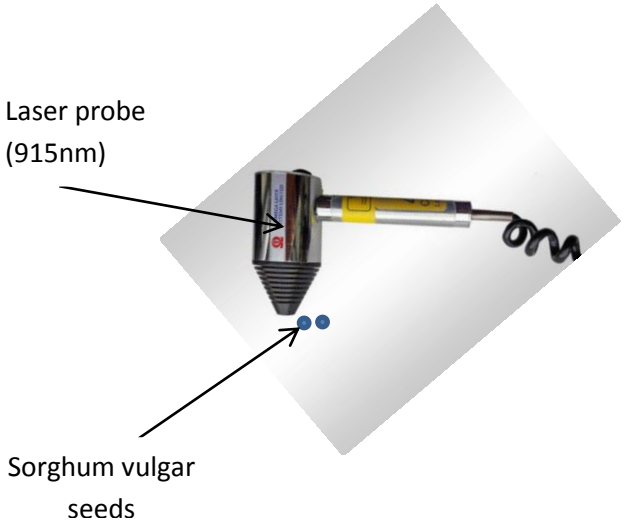

Figure 3-1: Setup of laser seeds irradiation

3.3 Samples Preparation

After the treatments, irradiated seeds were planted in 10 clay pots, 5 pots for dry seeds, 5 pots for soak seeds, and one for control. Each pot consisted of 30 seeds. The pots were filled with clay silt soil mixed with sand and humus to about 10 cm from the top and were irrigated with tap water. The soil was collected from the bank of the Blue Nile in Khartoum. Sorghum seeds were grown in the winter season of 2017. The experiments were carried out at the Botanical Garden of the Faculty of Science, Al Neelain University, Khartoum, Sudan.

3.4 Method

Germinated seeds were counted every 48 hours for 8 days. The following vegetative parameters were recorded: the emergence (germination) rate, stalk height, leaf dimensions (length and width)

The emergence rate index (E_d) was calculated based on the following equation:

$$E_d = \frac{N_e}{N_s} 100\%,$$

Where: N_e – number of emerged plants, N_s – number of sown seeds [Podleśna, et al., 2015].

3.5 LICF Spectroscopy

Spectroscopy measurement of *Chl* fluorescence of plant material were performed by irradiating the leaves with two different excitation sources in the same experimental setup. The two sources were blue diode laser (TCD, model No: 850; wavelength, 405 nm, Power, 200 mW, china), and light emitting diode (LED) operating at a wavelength of 450 nm, the output power of 60 µW. LICF spectra were recorded using high-resolution spectrometer (ocean optic spectrometer, USB 2000/origin lab Dunedin, USA/Northampton, USA), with resolution of 1.34 nm full width at half maximum (FWHM), and equipped with detector covers the wavelength range from 200-1100 nm. A fiber optic was used to guide the collected fluorescence light from the leaf (placed inside a box to provide the background light) to the spectrometer. A computer with dedicated software (by ocean optic) was connected to the spectrometer. These spectra were analyzed using Origin 8.1 software program. To analyze the spectra, the curves were fitting using a combination of Gaussian spectral functions.

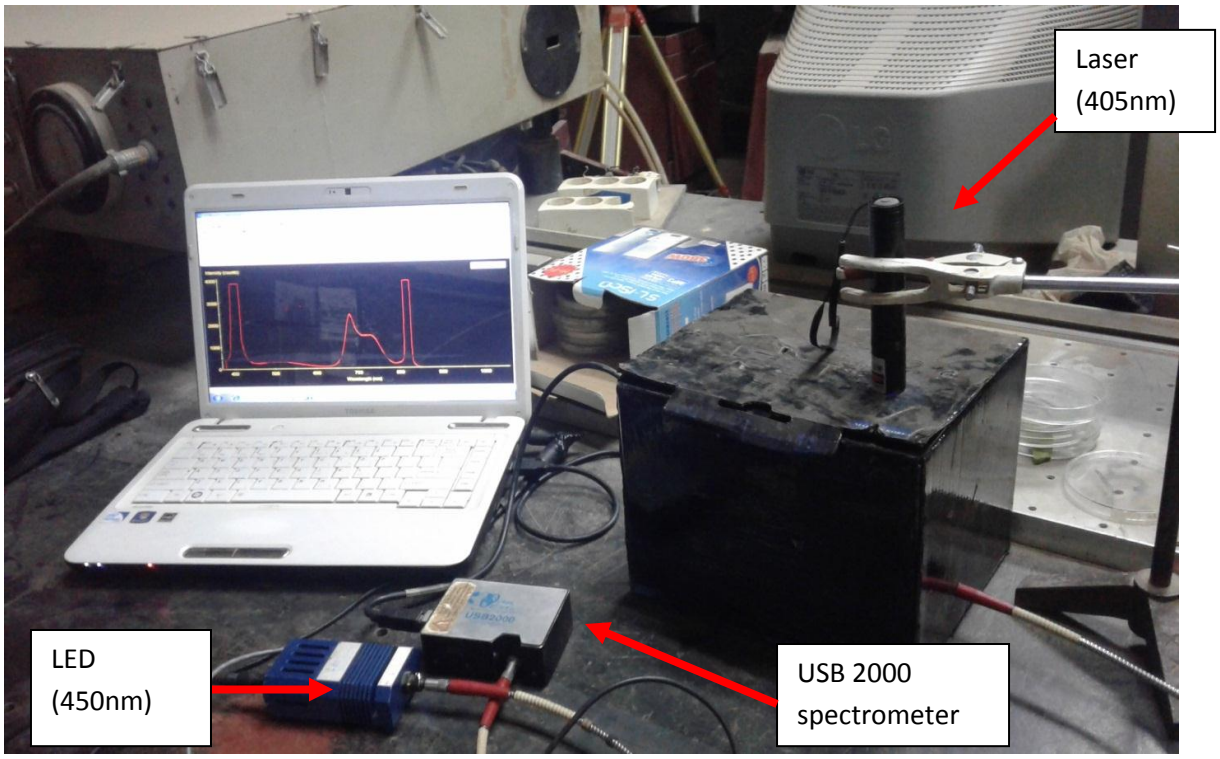

Figure 3-2: *Chl* fluorescence setup using laser (405nm) or LED (450nm).

The measurements of the fluorescence were started from the third week after sowing, and were taken from different leaves in each pot. The laser source and the spectrometer were setup as shown in figure (3-2). A leaf was placed at a distance between source of excitation and the spectrometer inside the box. The fluorescence was obtained from the upper side of leaf. The *Chl* fluorescence spectra were done for a period of four weeks by taking the average of each week. Interactive non-linear curve fitting was done using algorithm method. After choosing the Gaussian spectral function, the individual component peaks were selected. Peak widths were adjusted so as to match approximately the line shapes of the spectrum as shown in figure (3-3). It provides a reasonable matching fit of the spectral data with good F-statistics, standard error for peak amplitude, peak center, the area under the Gaussian fit and bandwidth (full width at half maximum). The peak intensity ratio (P.I.R) and area ratio (A.R.) were then calculated and plotted against time as growth factors. Then the slopes of linear P.I.R and A.R ratio were found using linear fitting.

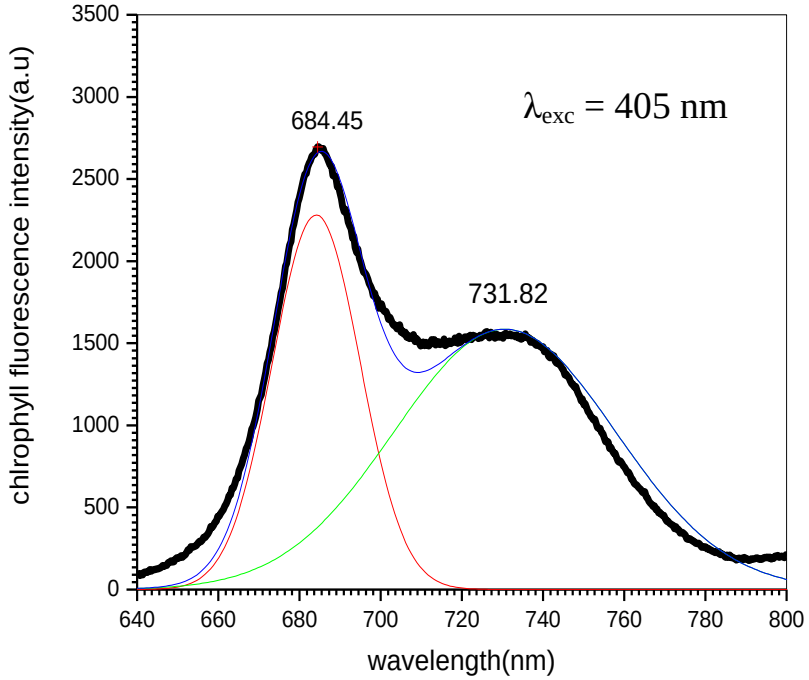

Figure 3-3: Gaussian fit of the *Chl* fluorescence spectrum used for the analysis of the spectrum.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

Results obtained during this work were summarized in tables (table 4-1 to 4-12) and spectra (figure 4-2 to 4-5) shown in this chapter. There include vegetative data (germination, stalk height and leaf dimensions), and spectroscopic data (*Chl* fluorescence spectra and the Fr/FFr ratio). This was done for all sets of plants (plants grown from dry and wet seeds).

The variations of the chlorophyll fluorescence spectra of Sorghum vulgar leaves were plotted and analyzed. One of the most important parameters in the analysis of the chlorophyll fluorescence is the ratio of the red and far red fluorescence Fr/FFr to study the chlorophyll content. Data obtained from the Gaussian fit of the two spectral profiles of fluorescence emission were extracted and tabulated for red fluorescence (Fr) and far red fluorescence (FFr).Data included week order, peak intensity wavelength (λ_{max}), the spectral bandwidth ($\Delta\lambda$), the fluorescence emission intensity (I_F) and the area under the curve (A).The peak intensity ratio (P.I.R) and area ratio (A.R.) were then calculated and plotted against time as growth factors.

4.1 Vegetative data

Table 4-1: Effect of pre-sowing laser radiation seed treatments on emergence rate index (germination) of Sorghum vulgar Spp

Laser doses (W/cm^2)	Emergence rate index (%)							
	2^{nd} day		4^{th} day		6^{th} day		8^{th} day	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Control 0	46.7		76.7		86.7		86.7	
4	30	43.3	76.7	66.7	76.7	70	76.7	70
8	40	53.3	86.7	76.7	86.7	83.3	86.7	90
12	16.7	46.7	66.7	63.3	66.7	63.3	66.7	63.3
16	30	50	96.7	80	96.7	80	96.7	80
20	40	43.3	63.3	56.7	63.3	63.3	66.7	66.7

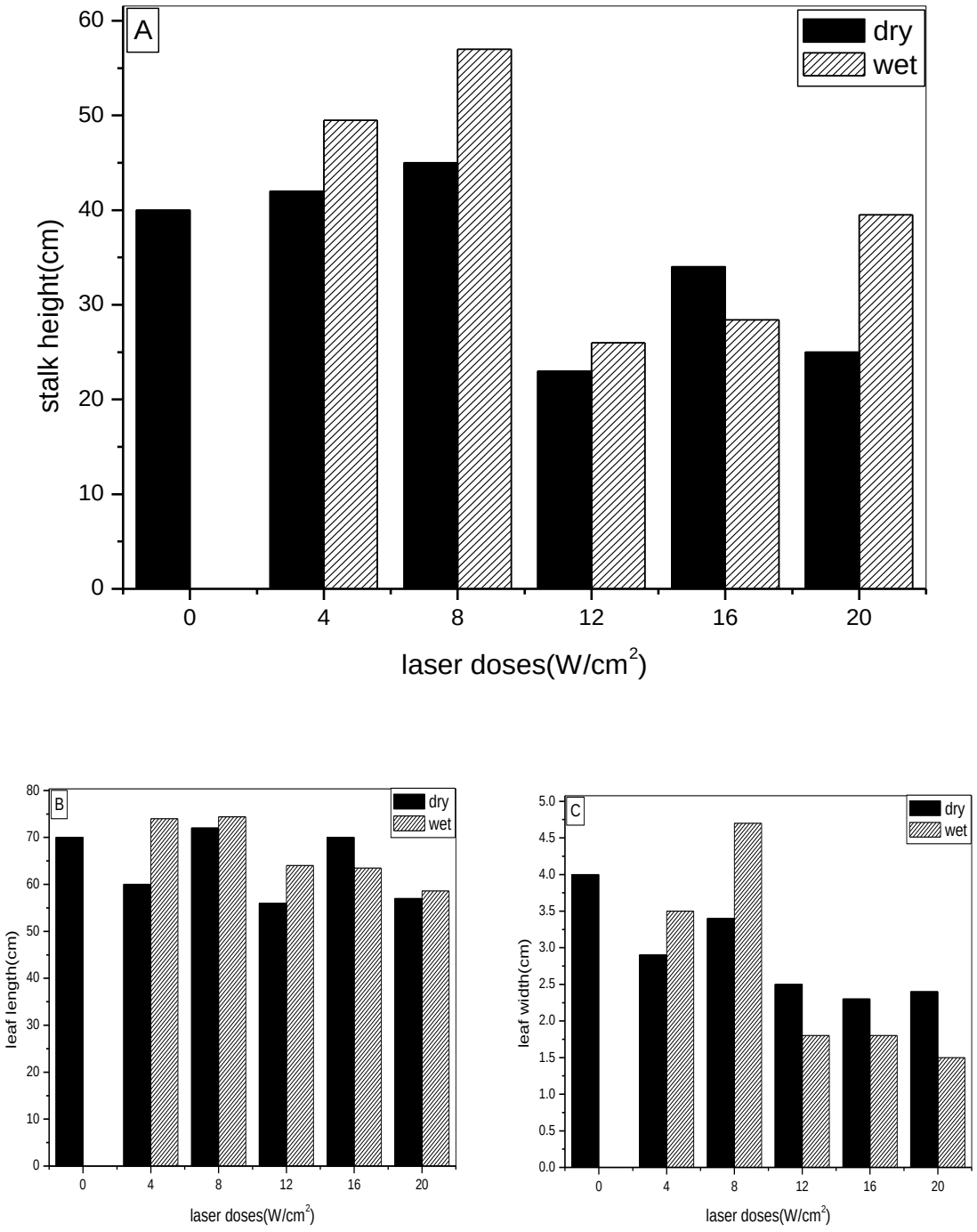

Figure 4-1: The vegetative parameters of Sorghum vulgar plant grown from dry and wet seeds treated and not treated with laser; (A) stalk height, (B) leaf length and (C) leaf width.

The emergence rate of seedlings (E_d) in the object with laser irradiation began to grow from the second day after sowing and a dynamic increase in the emergence rate was observed up to 8 days after sowing. Effect of different

doses of laser radiation on the time-course changes in germination percentage of Sorghum vulgar seeds were presented in table 4-1. For dry seeds, there was a significant increase in germination percentage (96.7 %) when the radiation dose was (16 W/cm^2) laser radiation when compared with control sample as well as other treated samples. The doses (12 and 20 W/cm^2) laser radiation gave the lowest germination percentage 66.7 %. While in wet seeds there was a significant increase in germination percentage (90 %) at 8 W/cm^2 laser radiation when compared with control and other treated samples. Also, in both dry and wet seeds, higher dose (20 W/cm^2) gave the lowest germination percentage when compared to other samples (see appendix A).

Some differences in growth and development were observed between Sorghum vulgar plants grown from irradiated and control samples. Plants that developed from irradiated seeds were taller and produced larger leaf dimensions as shown in figure 4-1. The larger leaf dimensions during vegetative growth of plants that developed from the irradiated seeds resulted probably from a faster growth rate in comparison with the control plants. The above results showed that Sorghum vulgar grown from seeds exposed with 8 W/cm^2 for 10 seconds gave the best vegetative growth parameters compared to those grown from other treated seeds and control seeds (see appendix B).

4.2 Spectroscopic data (LICF)

4.2.1 Sorghum vulgar plant developed from dry seeds

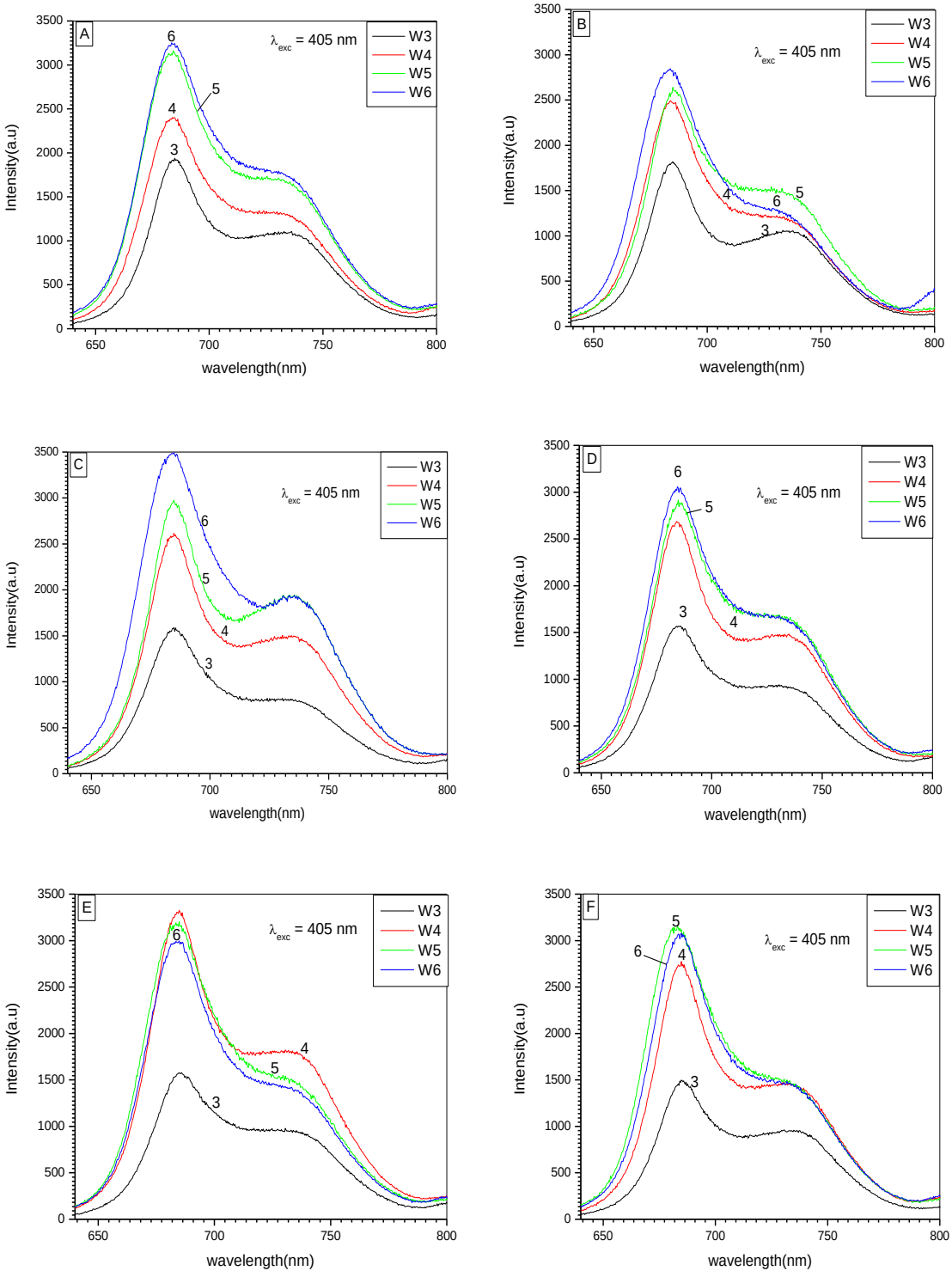

Figure 4-2: Fluorescence spectra for the six groups of Sorghum vulgar plant developed from dry seeds: (A) control, (B) 4W/cm^2, (C) 8 W/cm^2, (D) 12 W/cm^2 , (E) 16 W/cm^2 , (F) 20 W/cm^2; (Wn: order of weeks)

Table 4-2: Parameters obtained from the Gaussian fitting curves for the control plant.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I_F (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I_F (a.u)	A (cm^2)		
W_3	684.6	26.5	1931	4.75	732.5	62.6	1084	7.34	1.78	0.65
W_4	683.3	28.4	2389	6.39	732.9	63.2	1323	9.13	1.81	0.70
W_5	683.3	29.5	3140	8.84	728.2	60.7	1712	11.39	1.83	0.78
W_6	683.3	32.4	3248	9.85	729.8	61.6	1794	11.80	1.81	0.83

Tables 4-3: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 4 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I_F (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I_F (a.u)	A (cm^2)		
W_3	684.7	25.2	1817	4.53	733.9	56	1051	6.45	1.72	0.70
W_4	684.1	26.4	2086	5.34	732.1	60.6	1178	7.88	1.77	0.68
W_5	684.1	26.9	2619	6.36	730.1	63.5	1503	10.55	1.74	0.60
W_6	682.9	32.4	2837	8.81	728.6	62.2	1282	8.64	2.21	1.02

Table 4-4: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 8 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	684.9	26.5	1583	4.11	732.1	60.5	809	5.41	1.96	0.76
W_4	684.5	25.9	2605	6.47	732.5	60.4	1496	9.81	1.75	0.66
W_5	684.5	25.1	2964	6.99	732.5	61.0	1938	12.61	1.53	0.55
W_6	683.8	27.9	3481	10.40	731.2	62.7	1966	16.02	1.77	0.65

Table 4-5: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 12 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	683.7	22.8	1567	3.62	730.3	61.2	930	7.17	1.68	0.50
W_4	684.1	27.3	2669	6.73	730.5	64.6	1482	10.17	1.80	0.66
W_5	684.9	28.6	2878	7.44	729.0	63.5	1664	11.56	1.72	0.64
W_6	684.9	30.6	3037	8.68	730.5	63.2	1665	11.08	1.82	0.78

Table 4-6: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 16 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	684.2	21.6	1581	3.39	729.3	61.5	965	7.56	1.64	0.48
W_4	683.8	21.5	3319	7.44	729.3	61.3	1823	14.08	1.83	0.52
W_5	682.7	26.6	3182	9.09	731.2	61.2	1518	11.51	2.10	0.79
W_6	684.1	31.7	2990	9.07	730.5	63.6	1422	9.52	2.11	0.95

Table 4-7: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 20 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	684.8	22.3	1490	3.35	731.5	58.6	958	6.93	1.56	0.48
W_4	684.2	22.6	2760	6.83	731.2	54.8	1458	10.11	1.90	0.68
W_5	682.2	27.6	3146	9.45	728.4	54.8	1504	10.46	2.10	0.90
W_6	684.5	30.9	3076	9.03	730.5	62.1	1476	9.69	2.09	0.93

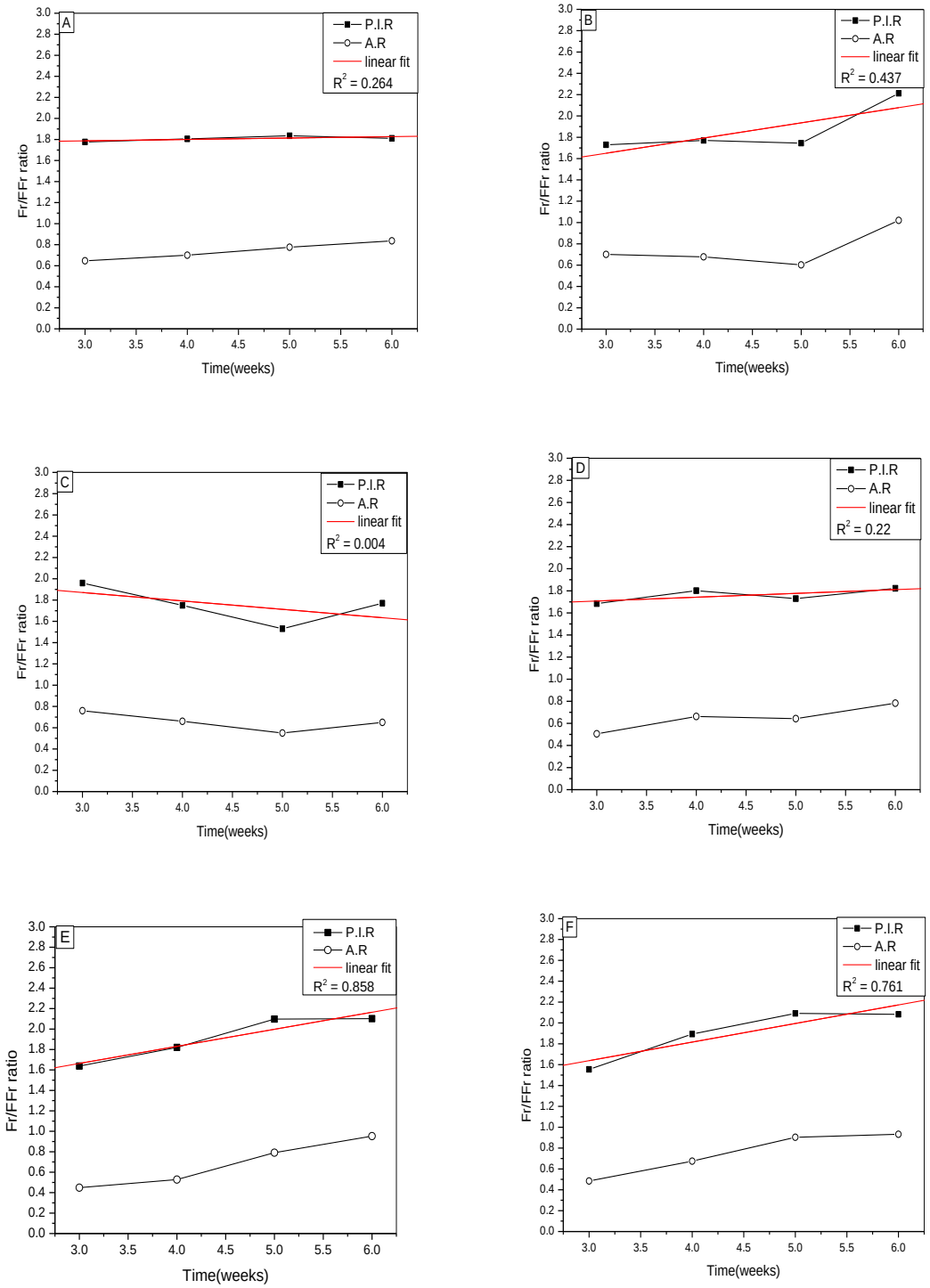

Figure 4-3: Chlorophyll fluorescence ratio as a function of time for: (A) control, (B) 4 W/cm^2, (C) 8 W/cm^2, (D) 12 W/cm^2, (E) 16 W/cm^2, (F) 20 W/cm^2.

From data of fitting and tables the peak wavelength (λ_{max}) for PSII is localized at684 nm and for PSI it is at 732 nm approximately, however there is a blue shift of λ_{max} for PSII and PSI. A shift in the λ_{max} could be happen due to mutation or deformation in *Chl* structure.

For plants that developed from irradiated dry seeds of Sorghum vulgar, as the weeks advanced from the time of measurement of fluorescence for control plant the fluorescence emission spectra showed a regular stepping of the intensity, which mean an increase of the Fr/FFr ratio with time, while for (8 W/cm^2) laser seeds treatment there is an increase of FFr intensity that gave a decrease in the ratio with time as shown in figure (4-2) and (4-3).

4.2.2 Sorghum vulgar plant developed from wet seeds

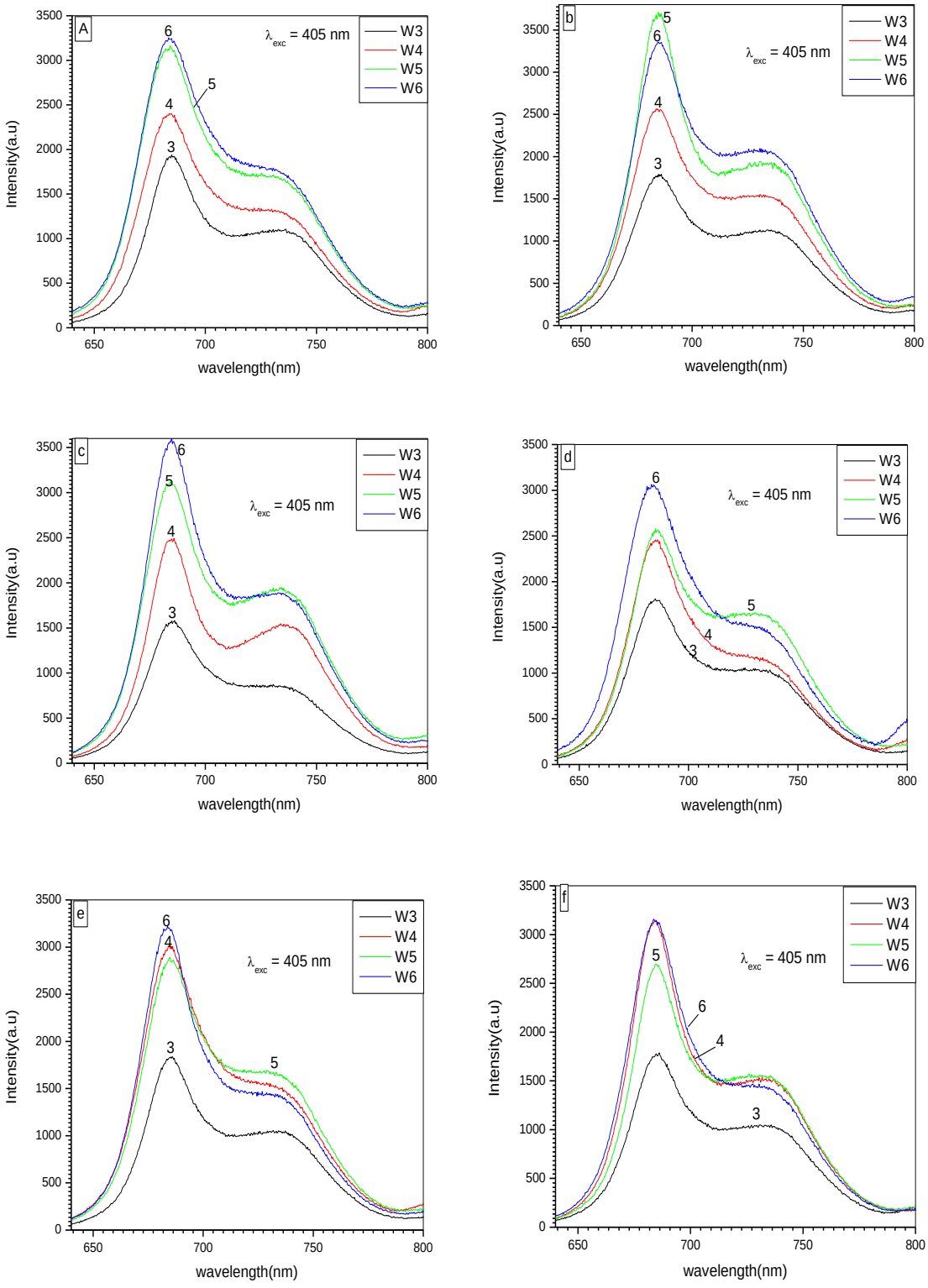

Figure 4-4: Fluorescence spectra for the six groups of Sorghum vulgar plant developed from wet seeds: (A) control, (b) 4 W/cm^2, (c) 8 W/cm^2, (d) 12 W/cm^2 , (e) 16 W/cm^2 , (f) 20 W/cm^2; (Wn: order of weeks)

Table 4-8: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 4 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	684.9	27.4	1781	4.63	733.3	63.6	1126	7.63	1.59	0.61
W_4	684.1	27.3	2555	6.40	730.1	64.8	1532	10.80	1.67	0.59
W_5	684.5	25.7	3695	8.75	731.0	63.7	1937	13.12	1.91	0.67
W_6	684.9	28.5	3357	8.66	730.5	62.6	2092	13.99	1.61	0.62

Table 4-9: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 8 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	684.9	26.5	1575	3.86	730.1	64.2	862	6.05	1.83	0.64
W_4	684.5	25.5	2489	6.1	733.7	59.3	1541	9.68	1.62	0.63
W_5	684.1	27.3	3109	8.03	732.5	63.6	1947	13.03	1.60	0.61
W_6	684.1	26.8	3594	8.51	731.3	62.5	2428	14.42	1.48	0.59

Table 4-10: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 12 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	684.1	28.3	1822	4.69	731.0	64.7	1055	7.27	1.73	0.65
W_4	683.7	28.8	2471	6.51	729.8	64.9	1206	8.36	2.05	0.78
W_5	684.5	27.4	2590	6.48	732.1	62.9	1671	11.23	1.55	0.58
W_6	683.3	32.0	3070	9.18	728.6	62.5	1523	10.19	2.02	0.90

Table 4-11: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 16 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	684.5	26.7	1855	4.51	732.1	64.1	1066	7.22	1.75	0.62
W_4	684.1	29.4	3044	8.21	729.8	63.9	1563	10.68	1.95	0.77
W_5	684.9	28.3	2892	7.43	729.8	63.7	1693	11.50	1.71	0.65
W_6	683.7	27.5	3227	8.57	731.3	62.6	1455	9.72	2.22	0.88

Table 4-12: Parameters obtained from the Gaussian fitting curves for the plant grown from irradiated seeds of Sorghum vulgar Spp, at 20 W/cm^2.

W_n	Fr				FFr				Fr/FFr P.I.R	Fr/FFr A.R
	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)	λ_{max} (nm)	$\Delta\lambda$ (nm)	I (a.u)	A (cm^2)		
W_3	684.5	27.5	1801	4.47	731.7	63.9	1066	7.20	1.69	0.62
W_4	683.7	25.4	3163	7.56	731.3	63.9	1553	10.51	2.04	0.72
W_5	684.1	25.4	2709	6.19	730.5	64.2	1574	10.85	1.73	0.57
W_6	683.7	28.1	3163	8.43	731.0	63.2	1466	9.91	2.16	0.85

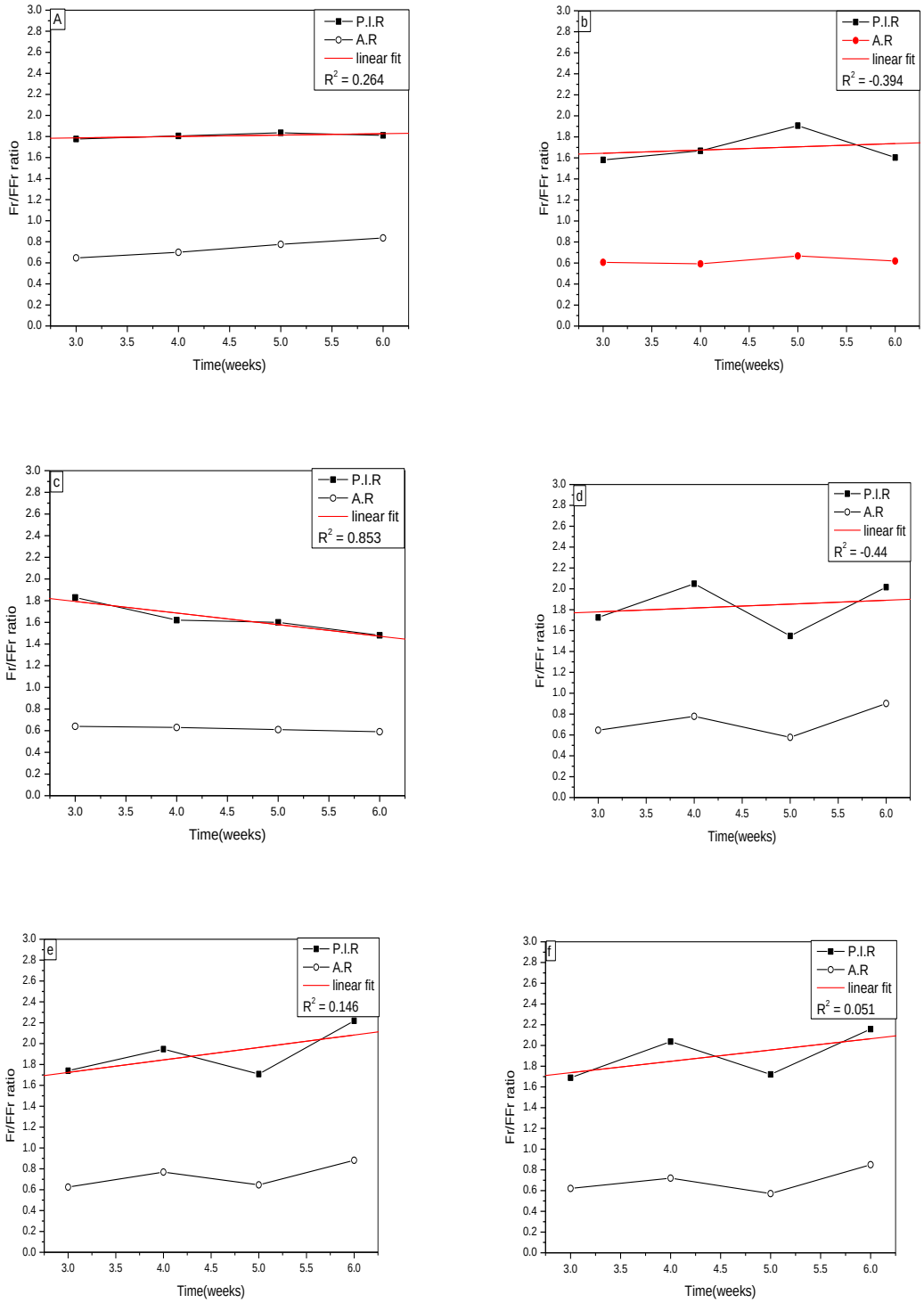

Figure 4-5: Chlorophyll fluorescence ratio as a function of time for: (A) control, (B) 4 W/cm^2, (C) 8 W/cm^2, (D) 12 W/cm^2, (E) 16 W/cm^2, (F) 20 W/cm^2.

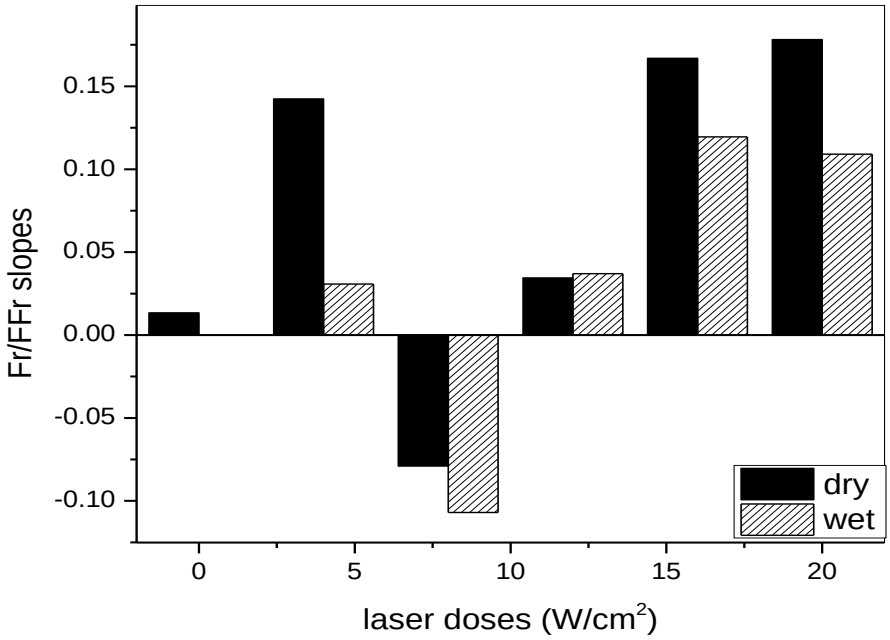

Figure 4-6: Variations of P.I.R slopes with different laser doses as an indicator for Sorghum vulgar plant growth

The slopes in case of the peak intensity ratio (P.I.R) or (A.R) under the laser treatment (dry and wet seeds) and control plant were calculated using linear fitting function. The growth factor Fr/FFr ratio (i.e P.I.R and A.R) when plotted against the time of growth as in figure (4-3) and figure (4-5). It was observed that for the Sorghum vulgar plant grown from seeds irradiated by IR laser (915 nm) for 10 second (8 W/cm^2) the relation between Fr/FFr ratio and time of growth is linear, when fitted, with a negative slope. While for control and other treated seeds, observations showed an increase of Fr/FFr ratio, with positive slopes. The lower slope could be an indicator for the better growth of plant. These values showed that Sorghum vulgar grown from seeds exposed to 8 W/cm^2 laser radiation gave the lowest slope, and hence, the best growth compared to those grown from other treated seeds and control as shown in figure (4-6). These results coincide with Gopal observations, which shows that fluorescence ratio Fr/FFr decreased with increasing *Chl* content of developing leaves (Gopal., 2002).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The exposure of Sorghum vulgar seeds to near-IR laser radiation (915 nm) significantly accelerated the seedling emergence. The radiation doses 16 W/cm^2 for 20 sec of dry seeds, and 8 W/cm^2 for 10 sec of wet seeds gave the highest germination percentages. Also the 8 W/cm^2 for 10 sec laser treatment for dry and wet seeds gave the highest vegetative performance.

The chlorophyll fluorescence data indicated that the Sorghum vulgar plants that developed from irradiated wet seeds were better than those from dry seeds.

The spectroscopic data and the vegetative observations showed that the laser dose 8 W/cm^2 for 10 sec of wet and dry seeds gave the best growth.

5.2 Recommendations

Due to the inconclusive results concerning the impact of a laser beam with a power density of P = 0.8 W/cm^2 on the cereals tested in this experiment, further research is necessary to determine the optimal doses of radiation that could positively affect the germination of Sorghum seeds.

Appendix

A. Sorghum vulgar Seeds Germination

Figure A-1: Germination of non-irradiated Sorghum vulgar seeds (control).

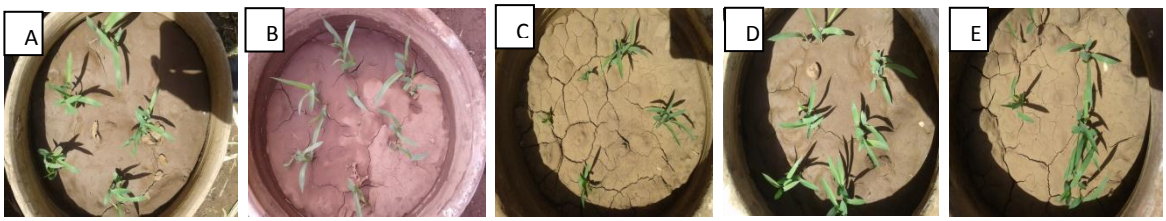

Figure A-2: Germination of irradiated Sorghum vulgar (dry) seeds: (A) 4 W/cm^2, (B) 8 W/cm^2, (C) 12 W/cm^2 , (D) 16 W/cm^2 , (E) 20 W/cm^2.

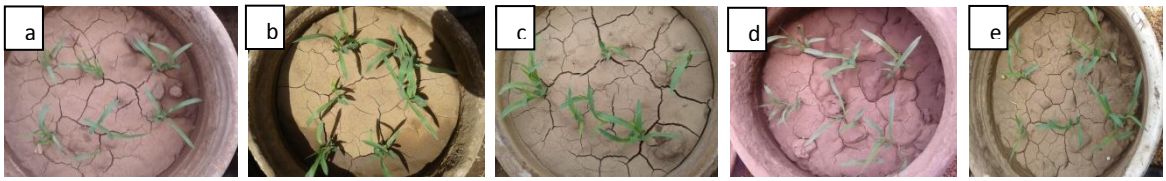

Figure A-3: Germination of irradiated Sorghum vulgar (wet) seeds: (a) 4 W/cm^2, (b) 8 W/cm^2, (c) 12 W/cm^2 , (d) 16 W/cm^2 , (e) 20 W/cm^2.

B. Sorghum vulgar Growth

Figure B-1: Vegetation of Sorghum vulgar plant after six weeks.

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