An-Najah National University
Faculty of Graduate Studies

Screening for Genetic Variation in the Circadian Clock in Diverse Barley Collection

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Dedication

This thesis is dedicated to my Family and all of my friends for being a great source of support in my life. Also, I dedicated this thesis to Dr. Munqez Shtaya for your continuous help and guidance.

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Firstly, thanks to God Almighty for conciliation and for providing patience to complete my project.

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الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

البحث عن التنوع الجيني في الساعة البيولوجية في مجموعة متنوعة من الشعير

Screening for Genetic Variation in the Circadian Clock in Diverse Barley Collection

اقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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List of Abbreviations

CCA1	: CIRCADIAN CLOCK ASSOCIATED1
LHY	: LATE ELONGATED HYPOCOTYL
TOC1	: TIMING OF CAB EXPRESSION 1
FAO	: Food and Agriculture Organization
ICARDA	: International Center for Agricultural Research in Dry Areas
PRR7	: PSEUDO RESPONSE REGULATOR 7
PRR9	: PSEUDO RESPONSE REGULATOR 9
PRR5	: PSEUDO RESPONSE REGULATOR 5
GI	: GIGANTEA
EC	: Evening Complex
ELF3	: EARLY FLOWERING 3
ELF4	: EARLY FLOWERING 4
Ppd-H1	: PHOTOPERIOD H 1
EAM8	: EARLY MATURITY 8
QTL	: Quantitative Trait Locus
ztl-1	: ZEITLUPE
PSII	: Photosystem II
DF	: Delayed Fluorescence
Chl*	: Singlet-state excited molecules
Qp	: Photochemical quenching
qN	: Non-photochemical quenching
PAM	: Pulse-Amplitude-Modulated
LEDs	: Light Emitting Diodes
PSI	: Photon System Instruments
RAE	: Ratio of Amplitude Error
VDE	: Violaxanthin De-epoxidase

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Abstract

The circadian clock is an endogenous self-sustaining mechanism which regulates a wide variety of rhythmic molecular and physiological process during the 24-h period and thus allows plants to adaptive to different daily and seasonal changes in their environment. However, there is little information about how the circadian clock has an impact on crop performance. In Barley plants *Hordeum vulgare*, the core oscillator mechanism which generates circadian rhythms consist of several components: CIRCADIAN CLOCK ASSOCIATED1 (CCA1) is morning-expressed transcription factor while TIMING OF CAB EXPRESSION 1 (TOC1) which considered an evening-expressed transcription factor. As elements of the core oscillator, regulation of these genes is an important part of the circadian clock, and uncovering those regulatory mechanisms can shed light on how the clock works and how it incorporates environmental feedback.

The main objectives of this study to identify natural variation in the circadian clock in a collection of barley accessions using high-throughput measurements of chlorophyll fluorescence (F) and to determine the variation of circadian parameters in response to environmental variation.

Chlorophyll fluorescence (F) measurements have been used as a tool for studying circadian rhythms in a collection of 84 different barley accessions and by using this technique, the correlation between parameters of the circadian rhythms and geographical differences for example elevation at the site of origin of the accessions have also been analyzed. Results obtained from this study indicated that there are circadian oscillations of ~24 hours of F parameters and these oscillations varied between different barley accessions as a consequence of CCA1 which varied in the regulation of rhythmic oscillations of F parameters. Also, plants showed a strong correlation between parameters of the circadian rhythms of fluorescence and geographical differences at the sites of origin of the barley plants.

Chapter one

Introduction

1.1 Origin of barley

Barley (*Hordeum vulgare* L.) belongs to the order *Poales*, family *Poaceae* (*Gramineae*)-Grass family and genus *Hordeum*. Barley is one of the earliest known cereal crops which have great importance for the mankind worldwide (Dakir et al., 2002). It is one of the most important cereal crops which originated in the Fertile Crescent including historical Palestine (Badr et al., 2000). The area of Fertile Crescent includes several parts of Palestine, Iraq, South-eastern Turkey, Lebanon, Syria, Western Iran and Jordan. Cultivated barley was domesticated from a wild species called *Hordeum spontaneum* about 10,000 years ago (Azhaguvel and Komatsuda, 2007).

1.2 Description and Classification

Barley (*Hordeum vulgare* L.) is a diploid (2n) with 14 chromosomes, self-pollinating species, self –fertilizing species, and has a short growing season (Mayer et al., 2012). leaves of barley are linear and each stem has leaves ranging from 5 to 10 that are 5-15 mm wide and leaf structure consists of glabrous ligule and auricles that envelop the stem (Gomez-Macpherson, 2000). Spikelets of barley are attached to the rachis of the spike by rachillas and the barley inflorescence is indeterminate. The

grains of barley are dry indehiscent fruit that termed as a caryopsis (Evers and Millar 2002).

Barley is classified according to their growth habit as winter or spring cultivar. Winter cultivars are planted in the fall season and mature in early summer and require before flowering and complete their life cycle in the spring season to be exposed to the cold period (vernalization) (Stark 2003b). Spring cultivars are planted in the spring season and mature in mid-summer but these cultivars do not withstand frost temperature and require excess irrigation and less productivity than winter cultivars.

Barley is classified according to the spike morphology as two-row barley (*Hordeum vulgare* L. *distichum*) & six-row barley (*Hordeum vulgare* L. *hexastichum*) (Badr et al., 2000; Kilian et al., 2006). During the process of domestication, six-rowed barley has appeared from the two-rowed wild barley as a result of a mutation that causes differences in spikelets morphology (Komatsuda et al., 2007). The fertility of spikelets plays an important role in determine row number of barley spike. Two-rowed genotype produces less grain (25-30) per spike than a six-rowed genotype (25-60). The majority of the cultivated barley is six-rowed type whereas the wild barley is a two-rowed type (Hordeum, 2012). Two-rowed barley has only the central spikelets are fertile but the lateral are sterile whereas six-rowed barley has all three spikelets (six spikelets in both sides) are fertile (Bonnett, 1966).

Barley also classified according to their hull adherence as hulless or naked (lemma and palea are separated or not attached to caryopsis) and hulled or husked barley (at maturity, palea and lemma stay attached to caryopsis). Hulled barley has the hull that protects embryo during and after harvesting from destruction and has the characteristic of higher-yielding and it is used to feed the animal. But, hulless (naked) barley used directly as a food for humans. The great majority of the cultivated barley called hulled caryopses whereas the remaining genotypes called naked or hulless caryopses (Von Bothmer and Komatsuda, 2011).

1.3 Importance, Uses and Chemical composition

Barley is the fourth economically most important worldwide crop in crop production after wheat, maize, and rice and the average per year over 134 million tons (FAO 2014).

The barley production is used primarily for feeding of animals, mainly pigs and cattle because the barley grains have a higher content of crude protein and fiber in comparison with other cereals and are considered an appropriate source of starch (Verstegen et al., 2014). Both barley grain and straw can be used for feeding in different forms of forages like silage (Heuzé, 2013). Because the barley silage has high nutrition value and digestibility so it is used as feed to beef cattle and dairy cows for the production of milk and meat (Walsh et al., 2008; Wallsten and Martinsson, 2009).

Barley grains also used in a significant percentage for malting, a process that dries germinated cereal grains and which comes back in Egypt and the Middle East to at least 8000 years ago (Ullrich, 2011). Hulled barley production has considered the most economical benefit from the grains of barley. Malt is used to make alcoholic beverages like whiskey and beer through the process of brewing and distilling. Malting barley compared with feed barley, it contains fewer amounts of protein (Verstegen et al., 2014).

A small proportion of barley production is used for food consumption. The barley grains can be cooked for bread making and this use has been important in ancient times and has continued as a major source of food for some culture, mainly in North Africa and Asia (Newman et al., 2008). Barley grains are beneficial for human health because it is a good source of β-glucans and high fiber content (Baik and Ullrich, 2008).

The barley grains comprise of starch about 65-68% that forms the main components, and also protein 10-17%, β-glucan 4-9%, lipids 2-3% and minerals 1.5-2.5% (Quinde et al., 2004). β-glucan has the importance of maintaining human health by lowering the levels of blood cholesterol and glycemic index (Pins and Kaur, 2006). It also contains phenolic compounds, phytic acid, lignin that connected with good health (Kaneko et al., 2003). Also, it rich in vitamin B-complex, vitamin E and a good source of trace elements like selenium, iron, magnesium, zinc, copper (Drugs, 2012).

1.4 Barley in Palestine

Barley is one of the vital crops in Palestine. It covered about 5100 dunums of the total cultivated area (PCBS, 2012). It is grown in temperate regions under different conditions and distributed from a wide geographical range of Palestine. The cultivation of barley is successful in an area with about 300 mm average rainfall due to it more tolerant to drought conditions than the other crops. Barley production is different depending on the cultivar and conditions of cultivation. For example, Irrigated barley yields 400-500 kg/dunum while rain-fed barley yields about 125 kg/dunum. The main producer governorates are as follows Jenin governorate is the highest producer of barley, constituting 46% of total production, followed by Hebron governorate 21%, and Nablus governorate 16% (Challinor et al., 2007).

There are several types of barley cultivar which cultivated in Palestine as ICARDA cultivars, Nabawi, Rihan and Baladi. These cultivars have several differences in their morphological features as well as in their crop quality and quantity. ICARDA group cultivars are the result of breeding programs carried out by ICARDA (Stephensen et al., 2008).

1.5 Adaptation

Barley has a wide range of adaptation. In comparison with wheat, it is highly tolerated to salt and has the ability to thrive in too cold conditions (Badr et al., 2000). Barley is sensitive to acidic soils but highly tolerant to

alkaline soils compared with other cereals and soil PH ranging from 6.0 to 8.5 is agreeable for plant growth. If the calcium in the soil environment is active, the barley can resist the PH that ranging from 5.0 to 6.0 although barley is considered sensitive to acidic soils. In areas where salinity is a major problem, barley is considered a good rotation crop because it can resist salinity very well and it can be planted in soils where salinity is 8-16 mili.mhos (Perveen et al., 2008). Higher yielding of barley is obtained under coarse-textured with low nitrogen, well-drained, and non-acidic soils (Young, 1998).

At barley different stages from seed germination to maturity, drought (water deficit) has a negative effect on the final yield and this effect depends on the duration and severity of the stress (Mohammmad et al., 1996). At the reproductive stage and under high-temperature conditions, drought cause decreasing in the yield by decreasing the number and size of the grain. At the flowering stage, water deficit causes a significant decrease in grain weight but at the tillering stage, it has no significant effect on the grain number per spike (Prasad et al., 2011). At the early growth stage, drought reduces the percentage of seed germination and it affects negatively on the establishment of seedling (Dodig et al., 2014). Drought conditions at the post-anthesis stage reduce the rate of grain filling and duration and this causes shriveled grains. At the pre-anthesis stage, drought causes greater yield reduction compared with post-anthesis stage of growth because it affects yield potential at the level of the sink through reducing the number of fertile spike per unit area at the phases of crop establishment

and tillering, in addition to the grain number per spike (Maccaferri et al., 2011).

Temperature is considered to be one of the most important factors that determine yield as it controls development. For barley growth, optimum temperature varies according to the stage. For instance, at 22°C the rate of leaf emergence reaches optimum while at 20°C the rate of leaf growth reaches optimum (Tamaki et al., 2002). During the post-anthesis stage, high temperatures (>35°C) reduce the weight of grain significantly and cause changes in malting performance (Savin et al. 1996, Wallwork et al. 1998, Passarella et al. 2002). In comparison with drought, the reduction in grain weight was greater under the heat stress and this means, the short season varieties of barley under warmer areas have better yield (Savin and Nicholas, 1999).

1.6 Overall losses

Losses can occur at each stage of cereals grain storage, handling, and processing. These losses can be up to 100% in certain cases and considered highly variable and may be either qualitative or quantitative. The evaluation of quantitative losses is much easier than qualitative losses. Examples of qualitative losses, including contamination caused by mycotoxins development and mould, germination loss, insect infection, nutritional deterioration, physical emergence. Some of these qualitative losses are more difficult to visually detection (Navarro, 1997).

There are two important stages of losses in cereals like barley, rice, wheat and maize that can occur and these include pre-harvest and post-harvest losses. The pre-harvest losses may result from diseases, insect pests, and weeds and are estimated at about 35% of the total production of cereal crops (Schildbach, 1989).

Improper storage conditions like unbalanced temperature, O2 /CO2 ratio or levels, and humidity and improper operation during harvest and/or after harvest that allows insect pests, rodents, and microorganisms to be an infection of stored grains lead to post-harvest losses. In developing countries, FAO's has estimated post-harvest losses of 10-15% during the 1980s (Navarro, 1997).

Hypothesis: Natural clock mutants in barley and other crops such as pea and lentil have been identified and used for breeding genotypes adapted to different environments (Faure et al. 2012, Weller et al. 2012). We postulate that natural genetic variation is common in the barley clock and varies within and between different barley germplasm groups (i.e. wild/cultivated barley).

Changes in light and temperature have been identified as major cues entraining or resetting the clock every day. Preliminary data in barley showed that osmotic stress affects the phase and amplitude of circadian clock genes (Habte et al. 2014). We thus propose that seasonal variation in water availability, temperature and radiation affects circadian clock parameters.

This work proposes to:

1. Perform a comprehensive survey for the extent of natural variation in the circadian clock of wild and domesticated barley accessions adapted to different eco-geographic origins.

2. Examine clock plasticity in response to different environmental cues to understand how input signals change the circadian clock.

Objectives:

The main objectives of this work:

1. Identify natural variation in the circadian clock in a collection of barley accessions by measuring circadian rhythmicity using high-throughput measurements of chlorophyll fluorescence (F).

2. Using the F measurements to determine the variation of circadian parameters in response to environmental variation.

Chapter Two

Literature Review

2.1 Circadian clock: Importance and Properties

Many biochemical and physiological processes in most organisms exhibits an activity pattern with a period that nearly corresponds to the earth rotation. The circadian clock regulates these 24-h rhythms through an internal timing mechanism that enables organisms to anticipate changes that occur in the environment and to coinciding their physiological cases accordingly (Dunlap, 1999; Harmer et al., 2001). In plants such as *Arabidopsis thaliana*, the circadian clock has been intensively studied and shown to regulate various process according to the appropriate time of day and this process includes a stem and hypocotyl growth, leaf movement, stomata opening, photosynthesis, shade avoidance, photoperiodic flowering as well as molecular processes such as about 30% genome expression (Harmer, 2009; Yakir et al., 2007).

The circadian system has several characteristics to their illustrate and definition. Firstly, it is endogenous or self-sustained, which means even in the cases of absence of environmental inputs, the circadian rhythm can be persisted (McClung, 2006). The second characteristic, it is entrainable, which means that the external signals are required to synchronize the clock phase through the process is called entrainment. In this process, variation in temperature changes can adjust the circadian clock phases to correspond to

the environmental rhythm. Finally, it is temperature compensated, which means the most of the biological process changes slightly according to the different temperature, but through the circadian clock, it able to keeping the same rhythm under constant environmental temperature, this indicates the clock has an important role in compensating for different ambient temperature (Roenneberg et al., 2013).

2.2 Plant circadian clock molecular basis

The circadian clock in most organisms is consisting of three sections: input pathway that receives environmental signals (as light and temperature) from the environment and transmits it to the central oscillator, which is a heart of the system and has autoregulatory transcriptional feedback loops and functions to generates circadian rhythms. Finally, this results in the environmental signals connected directly to an output pathway, which creates over rhythms (Millar and Kay, 1997; Bognar et al. 1999; Devlin, 2002).

The majority of studies about circadian clock system were made using *Arabidopsis thaliana* as a model plant because of it has the ability to produce a huge number of seeds and it's considered a selfing species with brief life cycle as well as it can be distributed in a wide geographical region which leads to it has a natural genetic variation that used extensively during studies of gene mapping (Shindo et al., 2007). in *Arabidopsis thaliana*, the general framework of the circadian clock is consists of several interlocked feedback loops that have been determined as the core or central oscillator

of the circadian clock (Locke et al., 2006; Harmer, 2010; Pokhilko et al., 2010). The core oscillator is comprised of *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*) and an evening-expressed transcription factor *TIMING OF CAB EXPRESSION 1* (*TOC1*) (Alabadi et al., 2001). *CCA1* and *LHY* are known as two morning-expressed transcription factors that accumulate at dawn and they are expressed in the morning and inhibit the expression of *TOC1* through it is binding to the *TOC1* promoter region. The levels of *CCA1* and *LHY* are decreases in the evening while the expression of *TOC1* is rise. In these cases, *TOC1* then stimulates the expression of *CCA1* and *LHY* through an unknown mechanism (Alabadi et al., 2001). Recent studies have shown that *TOC1* is responsible for limiting the expression of *CCA1* and *LHY* (Gendron et al., 2012; Huang et al., 2012; Pokhilko et al., 2012). *CCA1* and *LHY* in the morning increase the expression of *PSEUDO RESPONSE REGULATOR 7* (*PRR7*), *PSEUDO RESPONSE REGULATOR 9* (*PRR9*) and *PSEUDO RESPONSE REGULATOR 5* (*PRR5*) and these prohibit the expression of *CCA1* and *LHY*, ending the morning phase through binding to their promoter (Locke et al., 2006; Zeilinger et al., 2006; Nakamichi et al., 2010). In turn, falling levels of two morning-expressed transcription factors (*CCA1* and *LHY*), allow for increasing the expression of *LUX ARRHYTHMO* (*LUX*), *EARLY FLOWERING 3* (*ELF3*), and *EARLY FLOWERING* (*ELF4*) which is termed as evening complex with a peak before dusk. Then, These evening complex prohibit the expressions of *PRR7*, *PRR9* and *PRR5* during the ends of the day phase (Hernando et al.,

2017; Millar, 2016). During the evening, when the expression levels of *CCA1* and *LHY* are decreasing, the levels of *TOC1* expression are rising. *TOC1* then increases the expression levels of *CCA1* and *LHY* through binding to their promoter (Pruneda-Paz et al., 2009).

2.3 The role of circadian in controlling of photoperiod response and flowering time

Although the circadian clock plays an important role in the regulation of plant performance and thereby adaptation to various stress in *Arabidopsis thaliana* while in most important cereal crops, the information about its functions much less is known. Barley (*Hordeum vulgare* L.) has a genetic variation that considers extensive compared to other cereals for resistance to abiotic stresses and this reason leads to it considered excellent crop model for studying the effects of the clock on adaptation to various stress and therefore, on performance. Campoli and Colleagues (2012b) have shown that barley has orthologous of circadian clock genes that conserved in *Arabidopsis thaliana* with similar functions. Barley has a single morning-expressed transcription factor *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) ortholog designated as *HvCCA1* orthologous to *AtCCA1* and an evening-expressed transcription factor *TIMING OF CAB EXPRESSION 1* (*TOC1*) ortholog designated as (*HvTOC1*) orthologous to *AtTOC1*. Also, barley has a five *PSEUDO-RESPONSE REGULATOR* (*PRR)* gene orthologs designated as *HvPRR1* orthologous to *AtTOC1*, *HvPRR37/HvPRR73* orthologous to *AtPRR3* and *AtPRR7*, and

HvPRR59/HvPRR95 orthologous to *AtPRR5* and *AtPRR9* (Campoli et al., 2012b). The circadian clock genes in barley have a vital role in controlling of photoperiod response and flowering time. For example, barley adaptation is affected by *PSEUDO-RESPONSE REGULATOR (HvPRR37)* gene, also defined as *Hv PHOTOPERIOD H 1 (HvPpd-H1)* is the major gene which plays important role in controlling of photoperiodic response in barley and under long photoperiods, it induces or enhances early flowering. Mutation in *Ppd-H1* (at which recessive alleles *ppd-H1*) reduces the response to long days (LDs) and causes late flowering in spring barley as well as alters the expression of flowering time-related genes including reduced *HvFT* and *HvCO* gene expression (Turner et al., 2005). Recent studies have revealed that mutation in *EARLY FLOWERING 3* (*HvELF3*) gene and *LUX ARRHYTHMO* (*HvLUX1*) gene caused early flowering through up-regulation of *Ppd-H1* under non-inductive short day (SDs) conditions (Faure et al., 2012; Zakhrabekova et al., 2012; Campoli et al., 2013). The *Hvelf3* mutants allele severely compromised the expression of both clock oscillator and output genes (Faure et al., 2012). Although there was strong evidence about the role of clock genes in controlling photoperiod response and thus adaptation in cereals, not yet reported if allelic variation in the circadian clock has an impact on another physiological trait in most cereals.

The circadian clock gene *EARLY MATURITY 8* (*EAM8*) is a barley ortholog of *HvELF3* genes (Faure et al., 2012). *HvELF3* gene is the candidate gene underlying the eam8 quantitative trait locus (QTL) that

causes early flowering under LDs and SDs. The barley *early maturity 8* (*eam8*) mutation has been used for adaptation of varieties to the environment with short growing season around the world (Lundqvist, 2009; Zakhrabekova et al., 2012). Varieties that have *eam8* alleles flowering early and grown at high latitudes (Borner et al., 2002) and therefore plants that carrying eam8 mutant alleles have high expression in levels of some genes such as *Ppd-H1*, *HvFT1*, and *HvCO*. *eam8* mutant also causes disorder in the expression of the circadian gene which analogous to a*telf3* mutants (Hicks et al., 1996). For example, *eam8* mutant reduced the expression levels of some circadian clock genes such as *HvGI*, *HvTOC1*, and *HvCCA1* as well as under the conditions of LDs, these effects on the expression level of genes become especially apparent (Faure et al., 2012).

The circadian clock gene *EARLY MATURITY 10* (*EAM10*) is a barley ortholog of *HvLUX1* genes. *HvLUX1* gene is the candidate gene underlying the *eam10* locus in barley. The *eam10* mutant causes defect in circadian clock and disrupt the rhythmic expression of core clock genes and therefore output genes (Campoli et al., 2013). *HvELF3* and *HvLUX1* play an important role in regulating the expression of *Ppd-H1* as *AtELF3* and *AtLUX* regulate the expression of *AtPRR9* and *AtPRR7* (Dixon et al., 2011; Helfer et al., 2011). The selection of these evening complex (EC) gene allele which causes early flowering leads to adaptation of different barley varieties to short growing season (Faure et al.,2012).

2.4 The role of circadian in adaptation to environments

The circadian clock is important in the life of plants, there are several reports have shown that the circadian clock investigating the advantage of fitness and higher survival in plants under diurnal conditions (Dodd et al. 2005; Green et al. 2002; Ni et al., 2009). Recent studies have shown that mutation in clock genes may contribute to adaptations of many plant species to the local environments over a wide range of latitudes (Inoue et al, 2017). For example, the period length of the expression of core clock genes in *Capsella bursa-pastoris* is correlated with latitude (Slotte et al. 2007). This ecotype that collected from lower latitudes appeared earlier flowering, explained that the role of clock gene expression mainly *ELF3* in inducing early flowering. Mutation in *ELF3* emerged in Central Asia leads to a short period under light conditions while under dark conditions, it causes severely oscillation, thus indicating the role of mutation in *ELF3* to local adaptation (Anwer et al. 2014).

Recent studies using the model plants *Arabidopsis thaliana* accessions obtained from different locations around the world have shown strong correlations between latitude and length of period, day-length through the main growing season and altitude at the origin site. The results of those studies have revealed that correlation could be complex; Michael et al showed there were positive correlations between the longer period and longer day length and higher latitudes but there was no correlation has observed with altitude (Michael et al., 2003). However, Edwards et al

showed there were positive correlations between the longer period and lower altitudes but there was no correlation has observed with latitude (Edwards et al., 2005). The striking correlation between the period length and day length indicate the adaptive importance of the circadian clock, although the high spread of the Arabidopsis populations and lack of their genetic structure. The accessions also have shown varying patterns in their potential to compensate for variation in ambient temperatures and at a higher temperature, most of the accessions but not all showing faster circadian clock (Kusakina, Gould and Hall, 2014).

2.5 The role of circadian in regulation of photosynthesis

As mentioned above, the circadian clock regulates the various physiological and developmental processes in plants. Photosynthesis is one of the most important processes that is regulated by the circadian clock. The rate of photosynthesis under normal conditions is regulated by the circadian oscillator that consists of auto-regulatory transcriptional feedback loops. Previous studies have revealed that the rate of photosynthesis was altered due to a mutation that alters the function of the oscillator in the circadian clock. For example, over-expression of *CCA1* leads to arrhythmic of CO2 fixation and circadian arrhythmia, as well as a mutation in *ZEITLUPE* (*ztl-1*) (one of the clock genes) leads to a longer period of both the rate of CO2 fixation and the core oscillator (Wang and Tobin, 1998) (Dodd et al., 2004). Several reports about marine algae have demonstrated that there are circadian oscillations of photosynthetic O2 evolution

(Sweeney and Haxo 1961), the concentration of chloroplast ATP (Driessche, 1970), and the electron transport (MacKenzie and Morse 2011). These results demonstrate that there is a circadian rhythm of photosynthetic light-harvesting in marine algae. Other reports have shown that there are circadian oscillations of light-harvesting in higher plants. For example, in three legumes (*Phaseolus vulgaris*, *Pisum sativum*, and *Glycine max*) there are oscillations in the electron transport rat within chloroplasts after it has been isolated at different times (Lonergan, 1981).

In CAM plants, including *Kalanchoe daigremontiana* species, it also found that there are circadian oscillations of the quantum yield of photosystem II (PSII), which has been measured using 'modulated' measuring system or modulated Fluorometer (Wyka et al. 2005). Other plants such as mango, under uniform conditions of light and atmospheric CO2 concentrations, there were no oscillations of the quantum yield of photosystem II while CO2 assimilation continued rhythmic (Allen et al. 2000). These suggest that in some plants the quantum yield of photosystem II is arrhythmic or does not appear to oscillate. Recently, a study has shown that delayed fluorescence (DF) is a basic process, which occurs in all photosynthetic organisms, oscillates with a period of about 24-h as well as is under the circadian clock control in a various collection of plants such as *Hordeum vulgare*, *Arabidopsis thaliana* and *Lactuca sativa* (Gould et al., 2009).

2.6 Chlorophyll fluorescence

Chlorophyll fluorescence or chlorophyll light emission is a key process that occurs in all photosynthetic organisms. When the molecules of chlorophyll in plants absorb light energy, it causes singlet-state excited molecules (Chl*) that can return to the ground state through releasing light energy in one of several pathways: first pathway as re-emission as light (chlorophyll fluorescence), second pathway as photochemistry (photochemical quenching) the energy are transfer to the reaction center to drive photosynthesis process, third pathway as direct converting of light energy to the heat (non-photochemical quenching). These methods of energy dissipation are negatively correlated, which means they are in competition with each other so that increasing the efficiency of one pathway leads to decreasing the yield of the other two pathways). The measurement of chlorophyll fluorescence is extremely easy and the total amount is very small about 1 or 2% of the total light absorbed (Maxwell and Johnson, 2000a; Müller et al., 2001).

2.7 Chlorophyll fluorescence measurements

The measurements of chlorophyll fluorescence are fairly easy and it is commonly used for studying most photosynthetic organisms as plants, cyanobacteria and algae. Chlorophyll fluorescence can be used as an indicator of the photosynthesis yield when it is competing with photochemistry. The relationship between the photosynthetic capacity and fluorescence, indicating that the plants during dark conditions, the

photosynthetic capacity is available at its maximum while the yield of chlorophyll fluorescence is minimal (F_0). However, when the plants are exposed to a strong pulse of light, the photosynthetic capacity reached zero and therefore the yield of chlorophyll fluorescence reached a maximum (F_m) (Papageorgiou, 2004).

The Kautsky effect or chlorophyll fluorescence induction kinetics describes the chlorophyll fluorescence pattern during the transition of photosynthetic material from dark-adapted state to light-adapted state (Govindjee, 1995).

When photosynthetic materials are moved from the dark conditions into light conditions, the yield of fluorescence was increased. The rises in the fluorescence yield due to the reduction of the electron acceptors in the photosynthetic pathway, mainly plastoquinone and in especially (Q_A). When PSII absorbs light, the Q_A has accepted electron but cannot accept another electron until the first electron is passed to the subsequent electron carrier (Q_B) and the reaction center during this period is closed but excess light energy will be scattered, as fluorescence. The presence of the reaction center that is closed at any time leads to a general reduction in photochemistry efficiency and an increase in the fluorescence yield. When chlorophyll fluorescence is reduced due to increased light energy that used for photosynthetic process or for dissipating as heat, the pathway is termed quenching mechanisms. Photochemical quenching (*Qp*) is a term that refers to using more light energy for photochemistry or photochemical reaction.

Non-photochemical quenching (*NPQ*, *qN*) is the process of disposing or conversion of light energy into heat. In a typical plant, the variations in two quenching process will take 15-20 min to be complete and to reach steady-state, even though the time required to reach this state is very significantly depending on plant species (Maxwell and Johnson, 2000b).

A Pulse-Amplitude-Modulated (PAM) is a technique that has been used for measured chlorophyll fluorescence and is a method for quantum yield (efficiency) analyzed in most photosynthetic organisms. Also, it can be used as an indicator of health by estimating primary productivity and growth. The advantage of the PAM technique includes the measurements of chlorophyll fluorescence are quickly and non-destructive measurements of live individuals (Suggett et al. 2011). Table 1 shows that multiple parameters for chlorophyll fluorescence which can be calculated or measured during various stage of PAM technique measurements. These parameters have shown that they are useful for helping to understand both the vitality and physiology of the plant. For example, during eco-physiological studies, RFD had used as an indicator of the plant's ability to perform photosynthesis processes and for estimating the rate of CO_2 assimilation as well as chlorophyll content. The results about the photosynthetic capacity and the effects of different levels of stresses on the photosynthetic apparatus have been obtained by RFD values (Haitz and Lichtenthaler, 1988; Lichtenthaler and Babani, 2004). *NPQ* is another important parameter; excessive light energy that doesn’t transfer to the reaction center for driving photosynthesis process is dissipated as heat

(Niyogi and Truong, 2013). This helps in the adaptation of most plants and algae to different environments.

Table (1): Multiple parameters for chlorophyll fluorescence

Parameter	Definition	Formula
F_0	Minimum fluorescence yield (dark-adapted state)	Measured
F_m	maximum fluorescence yield (dark-adapted state)	Measured
F_v	Variable fluorescence yield (dark-adapted state)	*FM – F0*
$QY\_^{MAX}$	Optimal PSII quantum yield (dark-adapted state)	*FV / FM*
$Ft\_^{Lss}$	Minimum fluorescence (light-adapted state) or steady-state fluorescence in light	Measured
$QP\_^{Lss}$	photochemical quenching yield in steady-state	(*FM_Lss – Ft_Lss*) / (*FM_Lss –F0_Lss*)
$NPQ\_^{Lss}$	non-photochemical quenching yield in steady-state	(*FM-FM_Lss*)/*FM_Lss*
$RFD\_^{Lss}$	Ratio of fluorescence decline in steady-state	(*FP - Ft_Lss*) / *Ft_Lss*
F_P	peak fluorescence yield during the Kautsky effect initial phase	Measured

Multiple parameters of chlorophyll fluorescence can be calculated or measured using the PAM technique. These parameters can be correlated with the efficiency of photosynthetic systems and therefore with the mechanisms of abiotic stress tolerance for adaptation to environments.

Chapter Three

Materials & Methods

3.1Experimental Setup

3.1.1 Experimental Site

A laboratory experiment was performed At An-Najah National University, Faculty of Agriculture, Tulkarm (Khadouri), Palestine during the growing season 2016-2017.

3.1.2 Plant material

A collection of 84 different accessions of barley from different regions were used (Table 2).

Table (2): Barley accessions used in this study.

Number of accessions	Origin
60	Landraces (ICARDA)
9	Germany
2	Australia
3	Jordan
1	Syria
6	Mutations
3	Wild accessions (ICARDA)

3.2 Experimental Design

Seeds were germinated in Petri dishes containing filter paper in order to optimize germination. After 3 days, the seeds were sown in plastic trays, which composed of a mixture of sand, peat moss, and perlite in the ratio of

1:1:1(v/v). In each plastic tray, we planted five barley accessions and Bowman as control. Each accession was presented by 3 seedlings. After sowing the seeds, each plastic tray was irrigated with water and placed under white fluorescent light (12 h light/12 h dark) at temperature 18–22 ºC. Ten days after sowing, the first leaf of each plant was placed in a horizontal position and it is installed by metal staples (Picture 2). Then immediately, the plastic trays are transferred to the Fytoscope with fluorescence imaging (photon system instruments) for three days (72 h) for measured chlorophyll fluorescence (F) parameters.

3.3 Chlorophyll Fluorescence (F) analysis

The measurements of chlorophyll fluorescence (F) were accomplished using a FytoScope (photon system instruments) fitted with the FluorCam system that measures the chlorophyll fluorescence sequences with a Kautsky effect. The FluorCam system uses the property of a pulse amplitude modulated mode technique to measure the Kautsky effect (Nedbal et al., 2000). Chlorophyll fluorescence measurements were collected every 2 hours for 3 days. At 2-h interval, the barley plants were given a brief period of dark adaptation (15 min) and then the sequence of chlorophyll fluorescence was imaged. photochemistry was driven by blue (450 nm) actinic light (200 µE m^{-2} s^{-1}). The emission of fluorescence (F) was stimulated by using saturating flashes of (2,250 µE m^{-2} s^{-1}) from light-emitting diodes (LEDs). Fluorescence images were taken by a Charge Coupled Device (CCD) camera.

Results were analyzed using the FluorCam7 software package provided by photon system instruments (PSI). Data were imported into the Biological Rhythms Analysis Software System (BRASS) (available from http://www.amillar.org) and the circadian parameter as period, amplitude as well as RAE of the rhythm were estimated using the Fast Fourier Transformation Nonlinear Least Squares (FFT-NLLS) suite of programs, as described previously (Plautz et al., 1997). All period values with relative amplitude error (RAE) < 0.6 were considered. RAE estimates the precision of individual rhythm; values close to 0 indicate perfect rhythm (perfect oscillation) and values close to 1 indicate arrhythmic oscillation.

3.4 Data Management

Barley plants were transferred to the Fytoscope with fluorescence imaging (photon system instruments) for collected the following (F) parameters and analyzed using the FluorCam7 software package:

1- *NPQ* parameter (non-photochemical quenching).

2- *RFD* parameter (Ratio of fluorescence decline).

3- *Fv/Fm* parameter (Optimal PSII quantum yield).

3.5 Experiment Pictures

Picture (1): Sowing of barley accessions in each tray.

Picture (2): Preparation and installation of plants for transfer to photon system instruments.

Chapter Four

Results

Circadian rhythms of chlorophyll fluorescence for collection of barley

Using the FluorCam System, we evaluated a number of chlorophyll fluorescence parameters in a collection of barley during the transition between dark and light adaptation. During this transition, there were several different parameters of chlorophyll fluorescence that were measured and calculated using this system including F_0, F_m, F_p, $F_{v,}$ *NPQ*, *RFD*, and *Fv/Fm*. Some of these parameters indicated weakly oscillated or no oscillations like F_0, F_m, F_p and F_v while other parameters indicated robust oscillations like *NPQ_Lss*, *RFD_Lss*, and *Fv/Fm_Lss* with low RAEs.

From the 84 tested barley accessions, only 14 showed significant rhythms. Results showed that several parameters of fluorescence are rhythmic in Barley (Figs 1, 2 and 3) and indicate a role for the circadian clock in the regulation of photosynthesis. There were clear circadian oscillations of *RFD*, *Fv/Fm* and *NPQ*.

To determine whether these oscillations of fluorescence parameters are controlled by the circadian clock, the spring barley cv Bowman as a control and other different barley accessions were entrained in 12 h of light/12 h of dark at temperature 18–22 ºC for Ten days and then screened for 3 days under continuous light (LL). At 2-h interval, the barley plants

were given a brief period of dark adaptation (15 min) and chlorophyll fluorescence measured. This procedure continued for 3 days. The different barley accessions showed different circadian oscillations of the fluorescence parameters from the Bowman. Gene expression levels that regulating fluorescence parameters were different during light on and light off between different barley accessions. Bowman seedlings showed clear rhythms of *NPQ* and *RFD* with peaks in the middle of the subjective day with periods of 25.98 h and 28.40 h respectively as well as *Fv/Fm* with peaks in the middle of the subjective night with a period of 29.45 h. Clock mutants (loss of function) showed altered oscillations of fluorescence in different accessions compared with Bowman. For example, mutation in clock gene *CCA1* (*cca1*) showed short period Oscillations of *NPQ* (25.83, 25.38, 24.18, 24.76, 24.35, 23.88, 25.57), *RFD* (23.59, 23.81, 28.32, 28.37, 27.48) and *Fv/Fm* (26.48, 24.86, 25.31, 27.08, 24.03, 25.89, 25.30, 26.46, 25.27, 24.90, 25.02, 24.87) in some different accessions. In turn, Over-expression in clock gene *CCA1* (*CCA1-OX*) showed long-period oscillations of *NPQ* (27.36, 28.21, 27.04, 27.91, 26.58), *RFD* (29.01, 30.46, 30.16, 30.67, 29.25, 29.20, 30.19, 30.43) and *Fv/Fm* (30.45) in other different accessions. Differences between circadian parameters as period, amplitude as well as RAE were observed between different barley accessions compared with Bowman plants (Table 3).

Depending on these results, the measurements of fluorescence are considered robust markers for analyzing natural variation in the circadian system in barley plants.

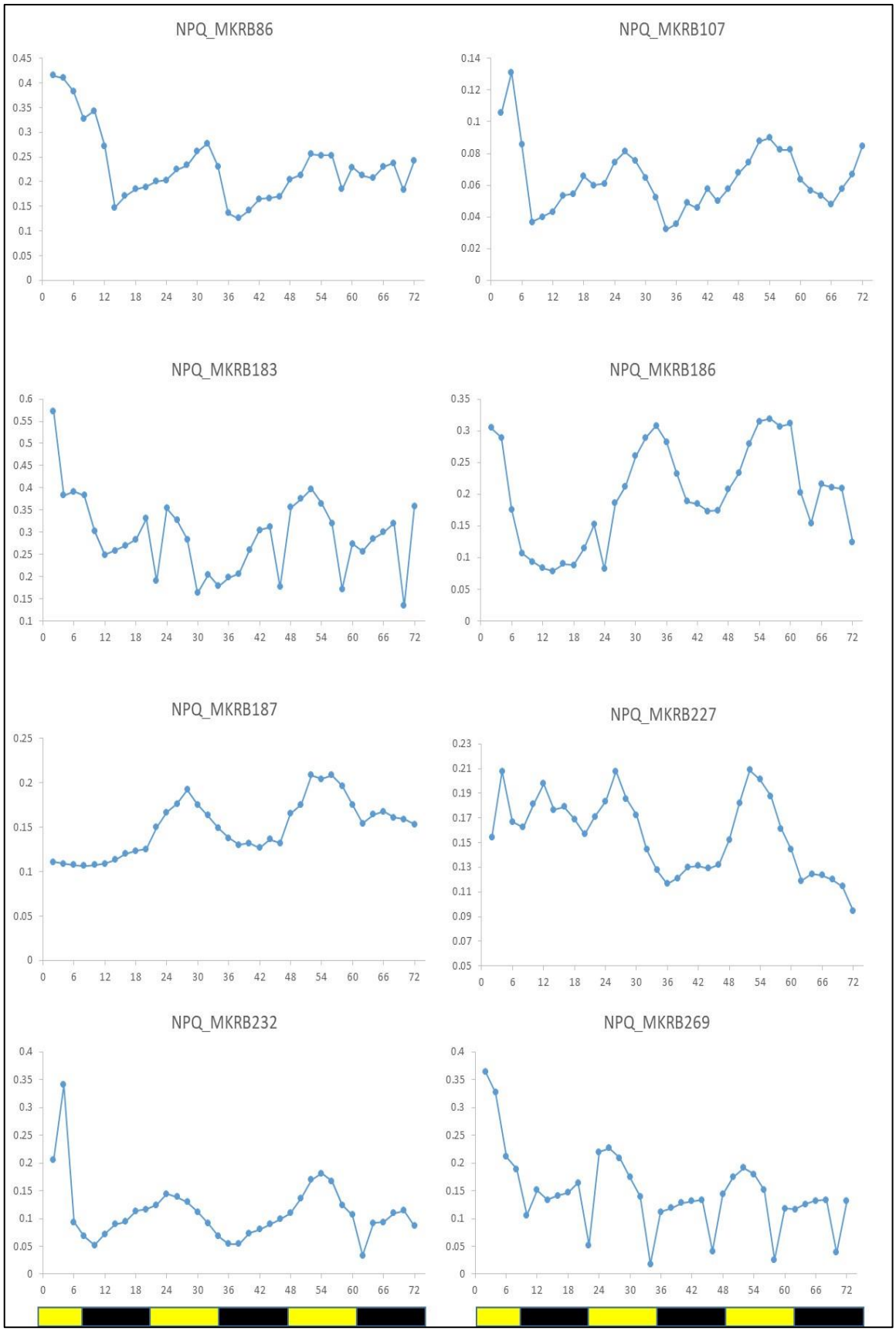

Figure (1a): Fluorescence rhythms in barley accessions for NPQ parameter.

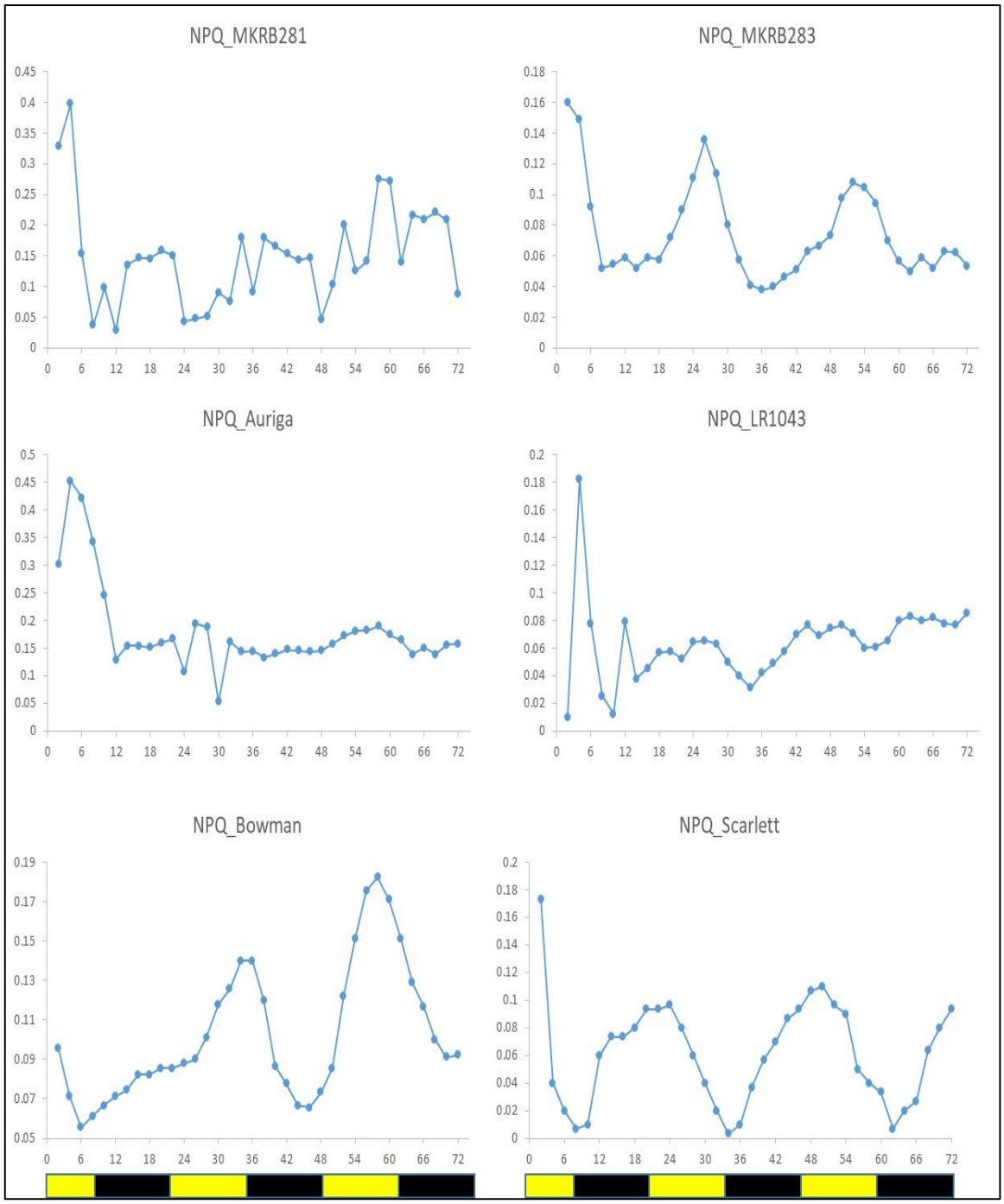

Figure (1b): Fluorescence rhythms in barley accessions for NPQ parameter.

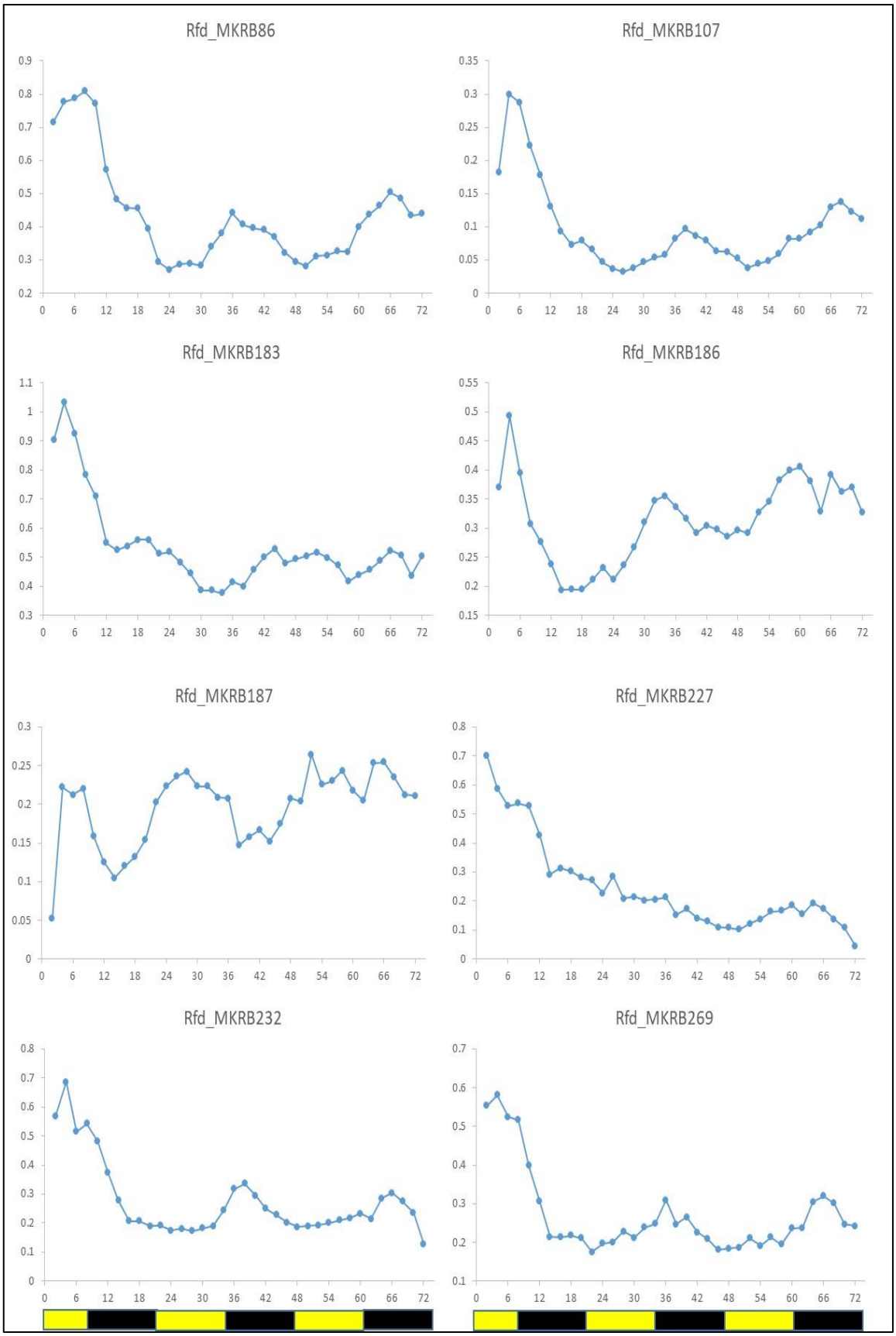

Figure (2a): Fluorescence rhythms in barley accessions for RFD parameter.

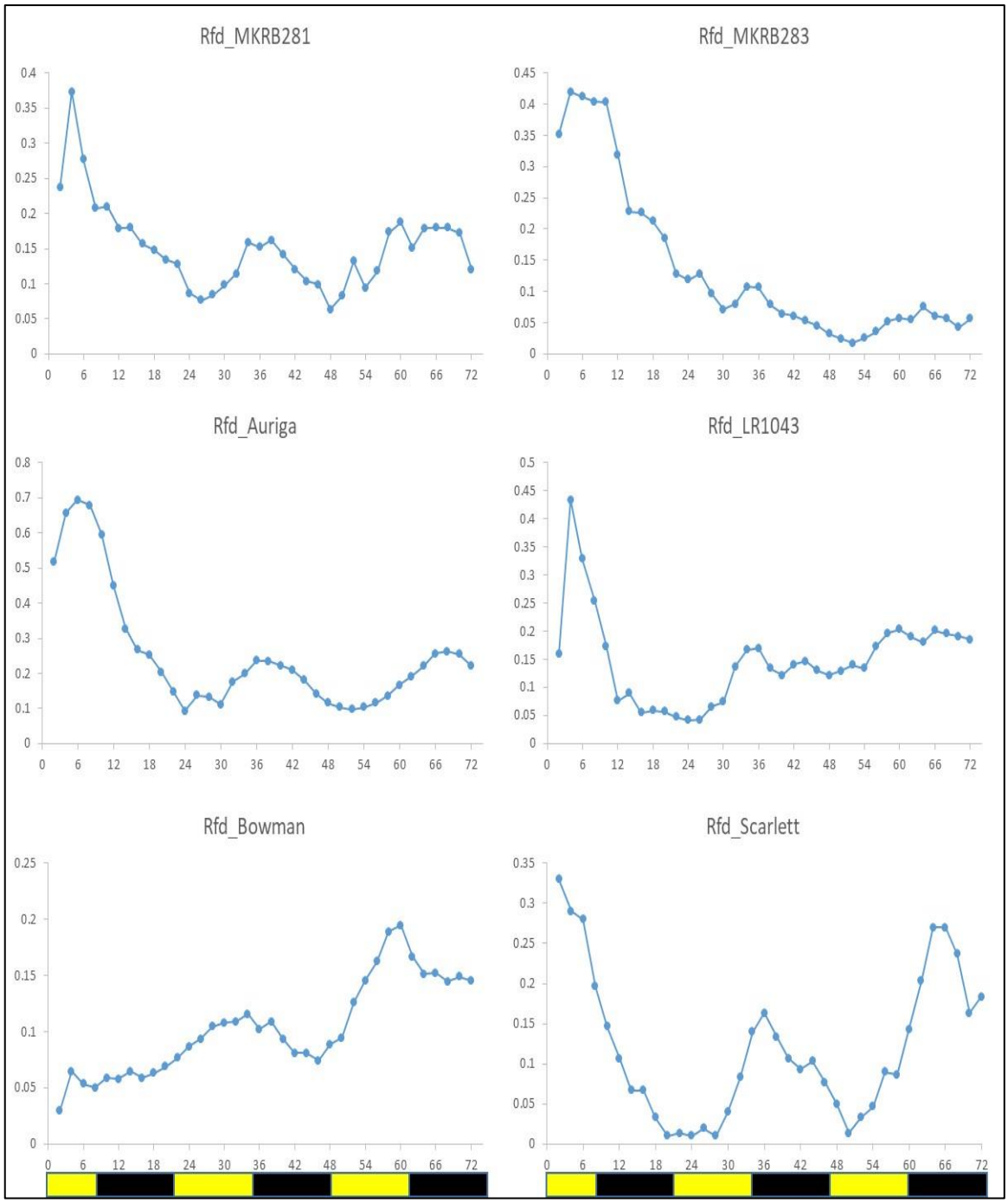

Figure (2b): Fluorescence rhythms in barley accessions for RFD parameter.

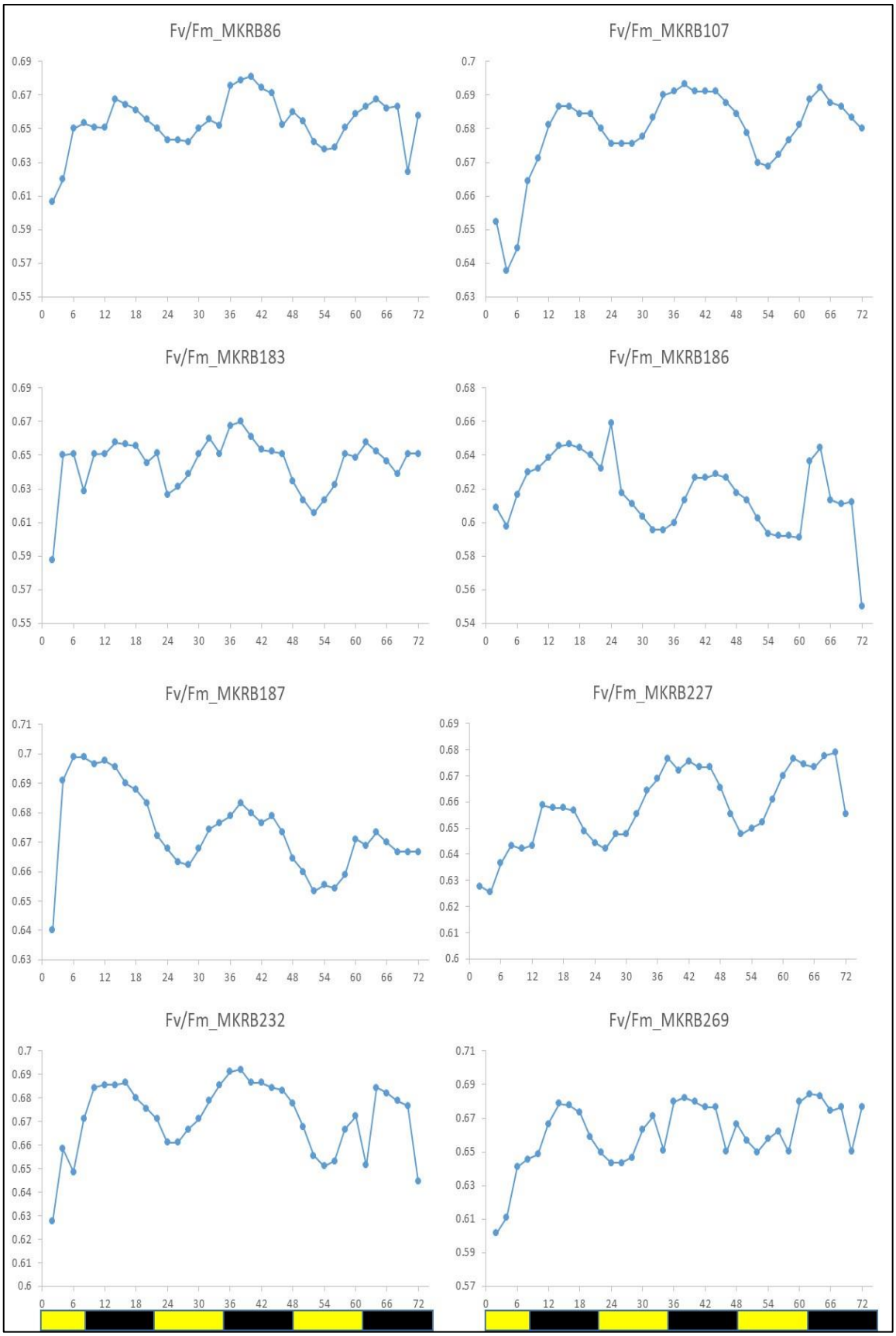

Figure (3a): Fluorescence rhythms in barley accessions for Fv/Fm parameter.

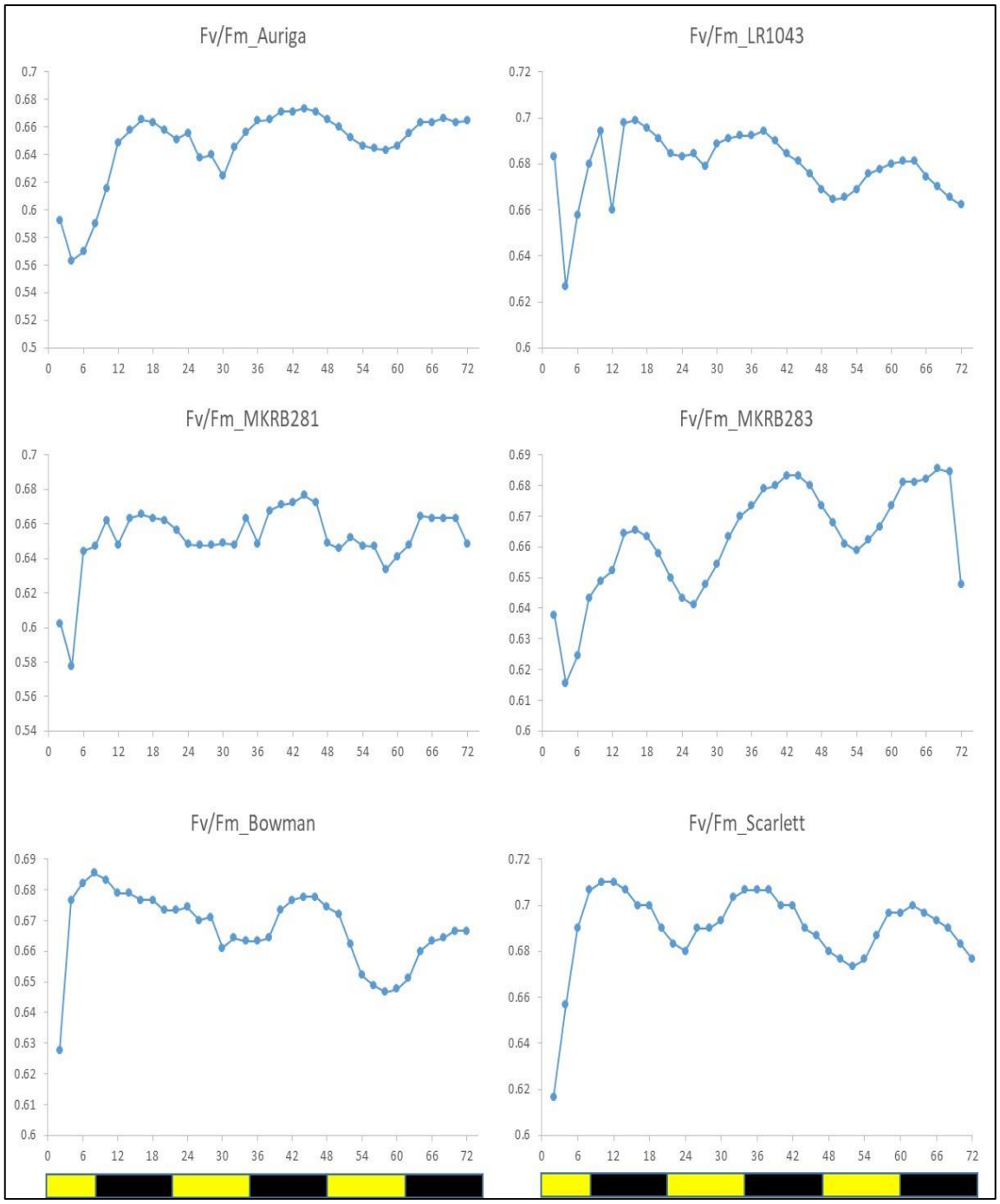

Figure (3b): Fluorescence rhythms in barley accessions for Fv/Fm parameter.

Table (3) showed that the accessions had a range in circadian parameters as period, amplitude, and RAE of *NPQ*, *RFD*, and *Fv/Fm*. *NPQ* period varied from 23.88 to 28.21 h, while *RFD* period varied from 23.59 to 30.67 h. *Fv/Fm* period varied from 24.03 to 30.45 h. MK_RB_187 had a longer period of *NPQ* while Auriga showed the shorter period of *NPQ* (28.21and 23.88 hrs respectively). MK_RB_186 showed significantly longer period of *Fv/Fm*, and MK_RB_227 showed the shorter period (30.45 and 24.03 hrs respectively). MK_RB_227 showed the longest *RFD* period, while MK_RB_183 showed the shorter period (30.67 and 23.59 hrs respectively).

Table (3): Circadian period and amplitude for different barley accessions

Accession	NPQ Period (h)	NPQ Amplitude	NPQ RAE	Fv/Fm Period (h)	Fv/Fm Amplitude	Fv/Fm RAE	RFD Period (h)	RFD Amplitude	RFD RAE
MK_RB_86	27.36	0.08	0.31	26.48	0.03	0.34	29.01	0.26	0.42
MK_RB_107	25.83	0.06	0.57	24.86	0.02	0.52	30.46	0.08	0.44
MK_RB_183	25.38	0.07	0.58	25.31	0.03	0.33	23.59	0.20	0.49
MK_RB_186	24.18	0.11	0.58	30.45	0.02	0.54	30.16	0.09	0.46
MK_RB_187	28.21	0.06	0.42	27.08	0.03	0.21	23.81	0.04	0.55
MK_RB_227	24.76	0.08	0.29	24.03	0.02	0.41	30.67	0.15	0.56
MK_RB_232	27.04	0.07	0.50	25.89	0.02	0.47	28.32	0.16	0.36
MK_RB_269	–[a]	0.11	0.55	25.30	0.03	0.37	29.25	0.12	0.61
MK_RB_281	27.91	0.11	0.49	26.46	0.06	0.57	28.37	0.05	0.57
MK_RB_283	24.35	0.07	0.49	25.27	0.02	0.53	29.20	0.10	0.51
Auriga	23.88	0.07	0.53	24.90	0.02	0.43	30.19	0.16	0.44
LR1043	25.57	0.03	0.41	25.02	0.02	0.39	27.48	0.07	0.47
Bowman	25.98	0.05	0.43	29.45	0.02	0.37	28.40	0.04	0.55
Scarlett	26.58	0.05	0.38	24.87	0.02	0.53	30.43	0.11	0.41

[a]Datamissing.

Geographic differences in the sites of origin of the accessions in the collection may act as drivers of selection affecting circadian traits. Using the fluorescence data and the geographical data, the correlations between the conditions at the site of origin of the accessions, for example elevation, was compared with parameters of the circadian rhythms of fluorescence. Consistent with the idea that the barley rhythms are adapted to their environment, Fig 4 shows correlations, especially of *NPQ*, *Fv/Fm* and *RFD_lss* with elevation; plants from higher elevations have shorter periods.

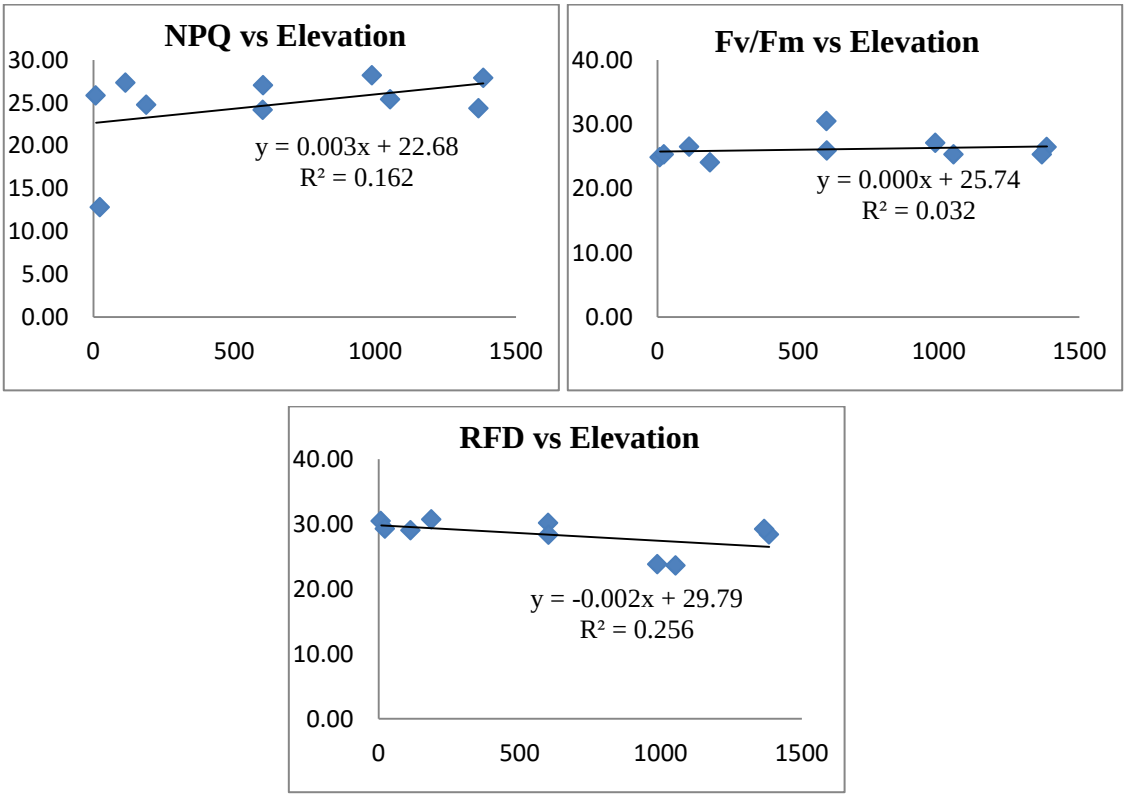

Fig (4): Correlations between elevation (in meters above sea level) and circadian rhythms for NPQ, Fv/Fm and RFD parameter in barley accessions.

Chapter Five

Discussion

F measuring protocol was optimized for analyzing circadian rhythms in a range of plant species. The various chlorophyll fluorescence parameters which can be calculated or measured show how the circadian clock has an influence on plant status and regulates the photosynthesis process. *NPQ* (non-photochemical quenching) is an important mechanism that measures the efficiency of heat dissipation of excess energy from light absorption. *NPQ* was measured during the subjective day and showed the highest level of this parameter during the middle of the subjective day when the adverse effects of high light intensity are most likely to be experienced by plants (Ishida et al., 2014).

In barley plants, the mechanism for the role of circadian in the regulation of *NPQ* is unclear while the key features of *NPQ* parameter appear to be conserved in all plant species and involve mechanisms called xanthophyll cycle (Demmig-Adams et al., 2012). Altering the level of key regulator protein which regulating the *NPQ* parameter was shown to have several effects on the productivity of plants (Kromdijk et al., 2016). Covington et al showed that Violaxanthin De-epoxidase (VDE), a key regulator of the xanthophyll cycle is under the circadian control in Arabidopsis plants and thus the circadian system regulates *NPQ* parameter at the gene expression level (Covington et al., 2008). *RFD* reflects the potential photosynthetic activity of the higher plants and used as a measure

for estimating the rate of CO_2 assimilation and chlorophyll content (Lichtenthaler and Babani, 2004). *RFD* like the other chlorophyll fluorescence parameter is affected by the level of chlorophyll pigments. Pan et al reported that both chlorophyll a and b have been shown to be under circadian control in soybean plants (Pan et al., 2015), suggesting that this may be a potential mechanism for the circadian rhythms of *Fv/Fm* and *RFD* parameter that we observed.

In the present study, 84 barley accessions were screened based on changes in chlorophyll fluorescence (F) parameter using the FluorCam System. From 84 accessions only 14 showed significant rhythms. My results showed several chlorophyll fluorescence parameters including *NPQ*, *Fv/Fm* and *RFD* are rhythmic (Figs 1, 2 and 3) and under circadian control. These results indicated that most of the physiological processes as photosynthesis are regulated by the circadian clock.

Our results showed that parameters including period, amplitude, and RAE of the circadian rhythm of fluorescence varied between different barley accessions (Table 3). These differences in circadian parameters as a consequence of changing gene expression levels during light on and light off which regulating F parameters. The changes in gene expression levels cause different oscillations of fluorescence rhythms. These results suggest that the mechanism of the circadian core oscillator varies according to the environment where the plants originated from. The barley accessions showed clear and different circadian oscillation of *NPQ*, *Fv/Fm* and *RFD*

with a different period, amplitude, and RAE. The circadian period parameter is defined as the time to complete one full cycle and it is commonly measured from peak to peak while amplitude parameter is defined as one-half the peak to trough distance and among the results, there are differences in genes expression levels which regulating F parameter. In comparison with Bowman, plants carrying a mutation in *CCA1* (*cca1-1*) showed short period rhythm of F parameters; in previous studies it was observed that *cca1-1* has short period rhythm (Green and Tobin, 1999) and plants carrying over-expression of *CCA1* (*CCA1-OX*) showed long period rhythm of F parameters in different barley accessions. This result is surprising as previous studies have shown that over-expression of *CCA1* affects the most physiological process which is regulated by the circadian clock (Green et al., 2002). RAE refers to rhythmic robustness and depending on results, only 14 accessions showed significant rhythm (RAE < 0.6).

The previous study on both Arabidopsis and barley plants collected from a broad geographic range showed that *NPQ*, *RFD*, *QP*, and *Fp* are rhythmic. There were clear circadian oscillations of *Fp*, *QP*, *RFD*, and *NPQ* in Arabidopsis. Mutations in *CCA1* caused different oscillations of fluorescence compared with WT plants. For example, The *cca1-1* had short period rhythms of *RFD*, *QY* and *NPQ*. In turn, The *CCA1-OX* showed a long-period oscillations of *QP* (26.4 h) and *QY* (27.4 h) (Hussien, 2014).

Another study on B1K collection, one of the few collections of wild-barley which originated from the south-western part of the Fertile Crescent, Dakhiya et al showed that there are differences in period, amplitude, and RAE of the circadian rhythm of *NPQ*, *Fv/Fm*, and *RFD*. For example, *NPQ* periods varied between 24 and 27.1 h, *Fv/Fm* period varied between 26.6 and 24.2 h, and *RFD* periods varied from 23.3 to 28.1 h (Dakhiya et al., 2017).

The differences in circadian parameters suggest there is variability in gene expression levels in the different barley accessions. My results appear the measurement of chlorophyll fluorescence (*F*) could be used as a method for assaying the function of the circadian clock in plants. In the future, it will be interesting to determine what factors are involved in the circadian control of fluorescence, for example which genes are responsible and how they are regulated.

In the present study, we examined the correlations between parameters of the circadian rhythms of fluorescence with the geographical data, for example elevation at the site of origins of the accessions (Fig 4). In the different barley accessions, circadian period length has been shown to be strongly correlated with environmental variation. For example, barley plants from higher elevation (more rain and low temperature) were strongly correlated with a shorter periods of fluorescence parameters including *NPQ, Fv/Fm, RFD* while plants from lower elevation (less rain and high temperature) were strongly correlated with a longer period. Results suggest

which areas at the site of origins of the accessions with higher elevation (more rain and low temperature) produce plants with shorter periods. Clearly, the mechanism of the circadian clock is influenced by the environment, thus allowing the plants to adapt to different conditions as elevation, temperature and local rainfall. These results are in agreement with the previous results in a collection of wild barley accessions (Hussien, 2014).

The previous study also has tested the correlation between circadian period, growth rate, and the environment in *Boechera stricta* plants, Salmela et al showed plants from higher elevations have shorter period rhythms which are associated with more rapid growth (Salmela et al., 2016). Other studies also have tested the correlations between circadian period length and growth under different conditions of temperatures in both Arabidopsis and *Boechera stricta* accessions and showed at higher temperatures, the circadian period rhythm was shorter (Lou et al., 2011; Kusakina et al., 2014).

Chapter Six

Conclusion

The following are the main conclusions of this study:

1. Chlorophyll fluorescence parameters can now be easily measured and used as a tool for analyzing natural variation which occurs in the circadian system in barley plants.

2. The different barley accessions showed different rhythmic oscillations of the F parameter as a consequence of variability in the core circadian clock gene (*CCA1*) expression levels during both lights on and off. This suggests that the circadian parameters varied in these different accessions.

3. We observed correlations between circadian trait, for example circadian period length and the conditions at the site of origin of the accessions (elevation). This suggests that Conditions at the site of origins of accessions affect the mechanisms of the circadian system and thus allow plants to adapt to different conditions including elevation, temperature and rainfall.

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جامعة النجاح الوطنية
كلية الدراسات العليا

البحث عن التنوع الجيني في الساعة البيولوجية في مجموعة متنوعة من الشعير

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قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الإنتاج النباتي بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.
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الملخص

الساعة البيولوجية هي آلية متكيفة ذاتيا التي تنظم مجموعة واسعة من العمليات الفسيولوجية والجزيئية خلال فترة 24 ساعة وبالتالي تتيح للنباتات خاصية التكيف مع مختلف التغيرات اليومية والموسمية في بيئتها. ومع ذالك هناك معلومات محدودة حول كيفية تأثير الساعة البيولوجية على أداء المحاصيل. في نبات الشعير (*Hordeum vulgare*), آلية المذبذب الأساسية تتكون من عدة عناصر هي: معامل النسخ في الصباح (CCA1) و معامل النسخ في المساء (TOC1). وتنظيم هذه الجينات هو جزء مهم من الساعة البيولوجية, والكشف عن تلك الآليات التنظيمية يمكن أن يلقي الضوء على كيفية عمل الساعة واقترانها بالبيئة المحيطة.

الأهداف الرئيسية لهذه الدراسة هي التعرف على التباين الطبيعي في الساعة البيولوجية في مجموعة متنوعة من الشعير باستخدام تقنية قياس الفلورسنس بالإضافة إلى تحديد اختلاف المعاملات الإيقاعية استجابة للتغيرات البيئية.

تم استخدام تقنية قياس الفلورسنس كأداة لدراسة الإيقاعات البيولوجية في مجموعة مختلفة من الشعير, وأيضا باستخدام هذه التقنية تم تحليل العلاقة بين المعاملات الإيقاعية والاختلافات الجغرافية (الارتفاع) في موقع منشأ هذه النباتات. وأشارت النتائج التي تم الحصول عليها من هذه الدراسة إلى أن هناك تذبذبات واضحة ومختلفة بين مختلف نباتات الشعير نتيجة CCA1 التي أحدثت تغيير في تنظيم التذبذبات الإيقاعية للفلورسنس. كما أظهرت النباتات وجود ارتباطا قويا بين المعاملات الإيقاعية للفلورسنس والاختلافات الجغرافية (الارتفاع) في موقع منشأ النباتات.