**FEDERAL UNIVERSITY OF CEARÁ**

**CENTER OF SCIENCES**

**DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY**

**UNDERGRADUATE COURSE IN BIOTECHNOLOGY**

**AYRTON MARKOS DA SILVA**

# *OsPIF14* GENE KNOCKOUT DELAYS SEED GERMINATION, SEEDLING DEVELOPMENT AND GRAIN FILLING IN RICE

**FORTALEZA**

**2020**

AYRTON MARKOS DA SILVA

# *OsPIF14 GENE KNOCKOUT DELAYS SEED GERMINATION, SEEDLING DEVELOPMENT AND GRAIN FILLING IN RICE*

Undergraduate Thesis submitted to the Biotechnology Course of the Center of Sciences of the Federal University of Ceará, as a partial requirement for obtaining the Bachelor Degree in Biotechnology.

Advisor: Prof. Dr. Joaquim Albenísio Gomes da Silveira.
Co-advisor: MSc. Vicente Thiago Candido Barros Alencar.

FORTALEZA
2020

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca Universitária
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

---

S578o Silva, Ayrton Markos da.
OsPIF14 gene knockout delays seed germination, seedling development and grain filling in rice / Ayrton Markos da Silva. – 2020.
65 f. : il. color.

Trabalho de Conclusão de Curso (graduação) – Universidade Federal do Ceará, Centro de Ciências, Curso de Biotecnologia, Fortaleza, 2020.
Orientação: Prof. Dr. Joaquim Albenísio Gomes da Silveira.
Coorientação: Prof. Me. Vicente Thiago Candido Barros Alencar..

1. Phytochrome-interacting factor 14. 2. Morphogenesis. 3. Oryza sativa. I. Título.

CDD 661

AYRTON MARKOS DA SILVA

# *OsPIF14* GENE KNOCKOUT DELAYS SEED GERMINATION, SEEDLING DEVELOPMENT AND GRAIN FILLING IN RICE

Undergraduate Thesis submitted to the Biotechnology Course of the Center of Sciences of the Federal University of Ceará, as a partial requirement for obtaining the Bachelor Degree in Biotechnology.

Approved on: __/__/____.

EXAMINATION BOARD

_____
Prof. Dr. Joaquim Albenísio Gomes da Silveira (Advisor)
Federal University of Ceará (UFC)

_____
MSc. Vicente Thiago Cândido Barros Alencar
Universidade Federal do Ceará (UFC)

_____
Dr. Ana Karla Moreira Lobo
Universidade Estadual Paulista (UNESP)

To the women of my life: my grandmother, mother and sister, for all the companionship, support and love given to me throughout this incredible journey.

## ACKNOWLEDGEMENTS

To the Federal University of Ceará and the Biotechnology Course.

To CNPq, for the financial support with the maintenance of the scholarship.

To Prof. Dr. **Joaquim Albenísio Gomes da Silveira**, for his excellent guidance. For supporting and encouraging me during times of doubt. For being a great example of a professor, researcher and human being. Thank you.

To the MSc. **Vicente Thiago C. B. Alencar**, for his teachings, patience and collaboration throughout these four years of LABPLANT. For being more than a co-advisor, a friend. For being the one who opened the world of science to me and made me the professional I am today. Thank you.

To Dr. **Ana Karla Moreira Lobo** for the patience, collaboration friendship and valuable contributions offered to this work. Thank you.

To Dr. **Fabrício Eulálio Leite Carvalho** for all the collaboration, assistance, friendship and valuable contributions offered to this work. Thank you.

To Dr. **Nelson José Madeira Saibo** for providing the plant material used in this work. For scientific collaboration and valuable contributions. Thank you.

To all my friends at LABPLANT, especially **Rikaely**, **Moaciaria**, **Victor, Dani, Paulo** and **Ana Luiza**, for always being accessible, for collaboration and moments of relaxation. Thank you.

To my beloved mother, **Maria Lucivete**, for always supporting me. For being an example of a mother and father. Thanks to you I could realize at a young age that I should do my part to change the world and that I had to enjoy the most of everything it has to offer. Thank you for taking this journey and dreaming that dream with me. Know that I am forever grateful and that there are not enough words or actions to describe how important you are in my life. Thank you for being my example of struggle, determination and overcoming. You gave me life and the world. Thank you very much.

To my sister, **Ana Luh**, for always giving me reasons to smile and not give up. Thank you for being that perfect human being who, with all simplicity and innocence, kept me going. Perhaps today you may not be able to read or even understand these words, but know that you are one of the reasons why I continue to struggle daily to be a better professional and human being. Thank you very much.

To my grandmother, **Maria Francilina**, for believing in me. For raising me as a son and listening to me even when you didn't understand what I was saying. Your care and

dedication gave me hope to continue and your presence meant security and the certainty that I am on the right track. I am forever grateful for the affection, for the patience and for your ability to bring me peace in the rush of each day. Thank you for making me a worthy man and human being. Thank you very much.

To my friends **Joaquim** and **Thaís**, for believing in me even in the moments that I gave up on myself. For being with me in times when everything seemed impossible and hopeless. For being a safe haven during my tears and happiness every day. For being brothers that the university gave me. For being wonderful friends and incredible professionals that I have as an example. You are an important part of this achievement. Thank you.

To the friends I earned in the course, **Luisa**, **Livia**, **Guilherme**, **Clara** and **Luzia** for the happy moments, carrying out work and so many occasions when we were together.

To my aunt and uncle, **Aurinete** and **Abelardo**, for welcoming me to their home. For taking care of me as a son. For their patience and for always making me feel at home throughout my undergraduation. Thank you.

To my family for the affection, support, encouragement and understanding.

To the Professors of the Biotechnology Course for the teachings and doubts taken during this academic journey. Thank you.

To all those who were in some way close to me, making this life more and more worthwhile. Thank you.

"Knowledge emerges only through invention and reinvention, through the disturbing, impatient, continuous and hopeful investigation that human beings seek in the world, with the world and with each other."

(Paulo Freire)

## ABSTRACT

Phytochrome-Interacting Factors (PIFs) are transcription factors belonging to the basic helix-loop-helix (bHLH) family that regulate essential metabolic processes in plants. However, the physiological role of these proteins is not fully understood in rice. Therefore, this study aimed to understand the role of PIF14 in the photo- and skotomorphogenesis processes. For this, rice seeds knocked out for the *OsPIF14* gene (14-KO1 and 14-KO2 lines) and wild type (WT) were exposed to two growth regimes, 100 μmol photons $m^{-2}$ $s^{-1}$ and dark, for 16 days in a growth chamber and physiological parameters were assessed. It was observed that the germination rate of both mutants was lower than WT and only this latter showed 100% of germination rate in both growth conditions. The root and shoot length of seeds germinated under light were around two times less in the mutants when compared to WT. A similar result was observed in seeds germinated in the dark. However, this response was more prominent in the roots length. The content of chlorophyll *a*, *b* and carotenoids of the transformed light-grown seedlings was lower compared to WT. While in the dark-grown seedlings, only the content of chlorophyll *a* and *b* was lower than WT. The chlorophyll *a*/*b* ratio showed no difference in light-grown genotypes. However, in the dark-grown mutants, the chlorophyll *a*/*b* ratio was higher than WT. The size and length of seeds with shell was lower in the transformed lines when compared to WT seeds. Shelled seeds showed only a trend of lesser value in these parameters. The starch content was lower, while the concentration of total soluble sugars and sucrose were higher in transformed seeds when compared to WT. These results suggest that the transcription factor PIF14 is related to the processes of germination, growth, photosynthetic pigments synthesis/accumulation and carbohydrates content in rice. In addition, the altered carbohydrate content affected germination rate and, consequently, the subsequent developmental processes. Thus, this transcription factor plays an important role in the processes of morphogenesis in rice.



**Keywords**: Phytochrome-interacting factor 14. Morphogenesis. *Oryza sativa*.

## RESUMO

Fatores de interação do fitocromo (PIFs) são fatores de transcrição pertencentes à família da hélice-alça-hélice básica (bHLH) que regulam os processos metabólicos essenciais nas plantas. No entanto, o papel fisiológico dessas proteínas não é totalmente compreendido no arroz. Portanto, este estudo teve como objetivo compreender o papel do PIF14 nos processos de foto e escotomorfogênese. Para isso, sementes de arroz nocauteadas para o gene OsPIF14 (linhas 14-KO1 e 14-KO2) e tipo selvagem (WT) foram expostas a dois regimes de crescimento, 100 μmol fótons $m^{-2}$ $s^{-1}$ e escuro, por 16 dias em uma câmara de crescimento e parâmetros fisiológicos foram avaliados. Observou-se que a taxa de germinação de ambos os mutantes foi menor que a do WT e apenas este último apresentou 100% de taxa de germinação nas duas condições de crescimento. O comprimento da raiz e do caule das sementes germinadas sob luz foi cerca de duas vezes menor nos mutantes quando comparadas ao WT. Resultado semelhante foi observado em sementes germinadas no escuro. No entanto, essa resposta foi mais proeminente no comprimento das raízes. O conteúdo de clorofila *a*, *b* e carotenoides das plântulas crescidas sob luz foi menor em comparação à WT. Enquanto nas plântulas crescidas no escuro, apenas o conteúdo de clorofila *a* e *b* foi menor do que WT. A razão clorofila *a*/*b* não mostrou diferença nos genótipos cultivados na luz. No entanto, nos mutantes crescidos no escuro, a proporção de clorofila *a*/*b* foi maior do que WT. O tamanho e o comprimento das sementes com casca foram menores nas linhas transformadas quando comparadas às sementes WT. Sementes sem casca apresentaram apenas tendência de menor valor nesses parâmetros. O teor de amido foi menor, enquanto a concentração de açúcares solúveis totais e sacarose foram maiores nas sementes transformadas quando comparadas à WT. Esses resultados sugerem que o fator de transcrição PIF14 está relacionado aos processos de germinação, crescimento, síntese acúmulo de pigmentos fotossintéticos e teor de carboidratos no arroz. Além disso, o conteúdo alterado de carboidratos afetou a taxa de germinação e, consequentemente, os processos subsequentes de desenvolvimento. Assim, esse fator de transcrição desempenha um papel importante nos processos de morfogênese do arroz.



**Palavras-chave**: Fator de interação do fitocromo 14. Morfogênese. *Oryza sativa*.

## LIST OF FIGURES

### CHAPTER I

**CHAPTER II**

## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| 14-KO | *OsPIF14* gene knocked out rice lines |
| APA | Active Phytochrome A-binding |
| APB | Active Phytochrome B-binding |
| bHLH | Basic Helix-Loop-Helix |
| CBF | C-repeat Binding Factor |
| Chl | Chlorophyll |
| CUL | Cullin |
| $HClO_4$ | Perchloric Acid |
| DNA | Deoxyribonucleic Acid |
| MCW | Methanol:Chloroform:Water |
| OsDREB1 | Rice Dehydration-Sensitive Element-Binding 1 |
| Osphy | Rice phytochrome |
| OsPIF14 | Rice Phytochrome-Interacting Factor 14 |
| OsPIL | Rice Phytochrome-Interacting Factor-Like |
| Pfr | Active phytochrome |
| Pr | Inactive phytochrome |
| Phy | Phytochrome |
| PIF | Phytochrome-Interacting Factor |
| PIF14 | Phytochrome-Interacting Factor 14 |
| PIL | Phytochrome-Interacting Factor-Like |
| RNA | Ribonucleic Acid |
| RPM | Rotations per minute |
| SLR1 | Slender Rice 1 |
| WT | Wild type |

## LIST OF SYMBOLS

| | |
|---|---|
| ºC | Degree Celsius |
| η | Nano |
| h | Hour |
| µ | Micro |
| % | Percentage |
| ϕ | Phi |
| ® | Trademark |

**CONTENTS**

# CHAPTER I

*PHYTOCHROME-INTERACTING FACTORS: CENTRAL INTEGRATORS IN LIGHT-MEDIATED PLANT DEVELOPMENT*

# 1 INTRODUCTION

Phytochromes (phy) are photoreceptor chromoproteins involved with physiological processes that respond to light stimuli (Figure 1). These proteins are present in every single land plant and in most of green algae lines, except in chlorophytes. They absorb light in the red, far-red and blue spectrum, been able to assume two interconversible forms: one active (Pfr) and other inactive (Pr) (INOUE; NISHIHAMA; KOHCHI, 2017; LI et al., 2015; QUAIL, 2002; ROCKWELL; SU; LAGARIAS, 2006).

When red light hits phy, the PϕD ring rotates resulting in a conformational change from Pr to Pfr (MONTGOMERY; LAGARIAS, 2002). Once in the active form, part of the phy are targeted to the nucleus where they can interact with Phytochrome-Interacting Factors (PIFs) regulating genic expression. Meanwhile, the residual phy stays in the cytosol acting in the regulation of mRNA translation (DAI et al., 2012; PAIK; YANG; CHOI, 2012).

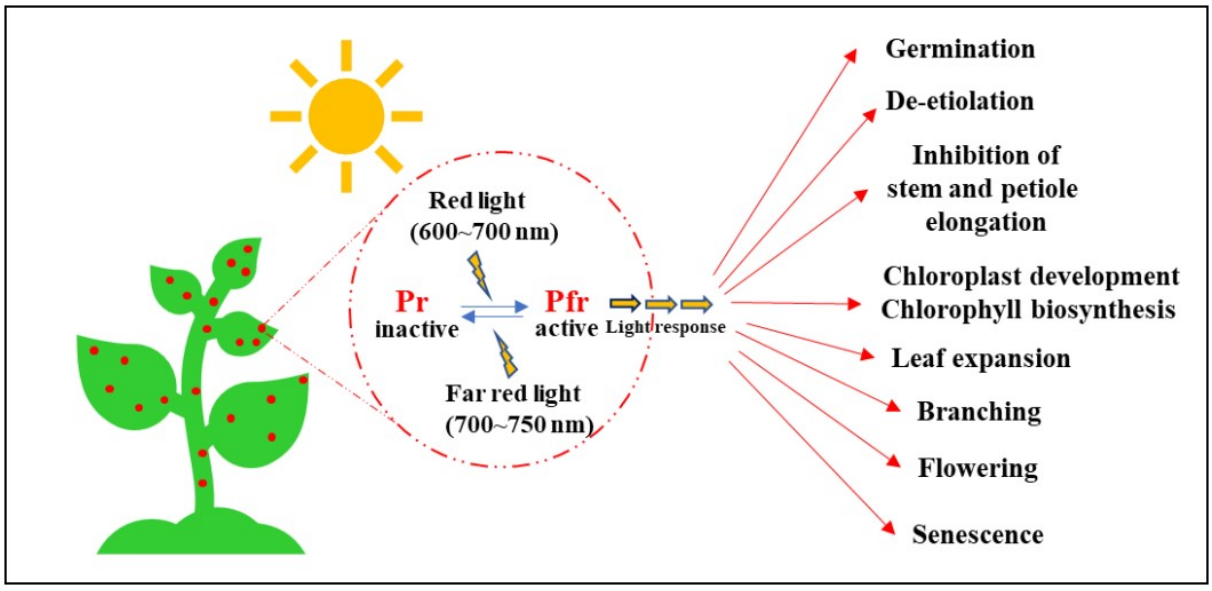


**Figure 1** – A schematic diagram depicting the involvement of phytochromes in different stages of photomorphogenesis. The red dots represent phytochromes that are present ubiquitously in plants. Inactive phytochrome (red light-absorbing Pr form) can be converted to active phytochrome (far-red light-absorbing Pfr form) by absorbing red light. The Pfr form can be converted back to the Pr form upon absorbing far-red light or in the dark (known as dark reversion, or more recently, thermal reversion). The active Pfr form regulates various photomorphogenic development through other downstream components of the phytochrome-mediated light signal. Reproduced from Tripathi et al. (2019) – *International Journal of Molecular Sciences*.

PIFs are basic transcription factors and form a subset of basic-helix-loop-helix (bHLH) transcription factors that usually has the function of repressing seed germination (PAIK et al., 2017). Besides, it acts in seedling skotomorphogenesis promoting shade

avoidance and gene regulation (LEIVAR; QUAIL, 2011). The basic transcription factors represent a family of proteins that contain a bHLH domain, involved in binding to the target DNA. PIFs belong to a subgroup of 15 from 162 bHLH protein family members, a transcription factor superfamily in arabidopsis (TOLEDO-ORTIZ; HUQ; QUAIL, 2003).

Unlike arabidopsis, the functional significance of phy-PIFs interaction is not yet fully understood in crops, such as rice (*Oryza sativa*), an important worldwide commodity. Recently, some studies reported that in rice there is one PIF (*Os*PIF14) and five PIF-Like (PIL) proteins (*Os*PIL11, *Os*PIL12, *Os*PIL13, *Os*PIL15, and *Os*PIL16) with the conserved active phytochrome B-binding (APB) motif; amid these, *Os*PIL15 contains an additional active-phyA-binding (APA) motif (CORDEIRO et al., 2016; NAKAMURA et al., 2007). Among the rice PIF/PILs proteins, only *Os*PIF14 was shown interacting with *Os*phyA, *Os*phyB, *Os*phyC, and preferably with *Os*phyB *in vitro* (CORDEIRO et al., 2016).

In general, it is known that PIFs have the function of negatively controlling photomorphogenesis and also act as regulators of many other metabolic pathways, including the circadian cycle, hormonal signaling, as well as responses to abiotic stresses (LEIVAR; MONTE, 2014; LEIVAR; QUAIL, 2011; SHOR et al., 2017; HAUVERMALE; ARIIZUMI; STEBER, 2012; QI et al., 2020; PAIK et al., 2017). It occurs through a series of cellular signaling molecules, which is more characterized in arabidopsis plants (KIDOKORO et al., 2009; LARNER; FRANKLIN; WHITELAM, 2018; LEIVAR et al., 2008; LUCYSHYN; WIGGE, 2009; OH et al., 2004; PAIK et al., 2017; STEPHENSON; FANKHAUSER; TERRY, 2009).

Rice is the staple food for nearly half of the world's population. However, its productivity is negatively affected by abiotic stresses such as high light. Light quality and intensity determine plant growth and development, but when too much it causes disturbances in cell metabolism generating reactive oxygen species and oxidative damage. It has been shown in arabidopsis plants that PIFs play and important role in light-induced signaling. Still, little is known about these proteins function, specially PIF14, in cereals. To the best of our knowledge, nothing has been reported of PIF14 function in seed germination. Therefore, it is expected that the present work can provide important information and contribute to the understanding of this protein in rice plants.

## 2 MOLECULAR MECHANISMS OF PIFS REGULATION

PIFs are central players in phytochrome signaling networks. Under dark, PIFs accumulate promoting skotomorphogenesis, while light induce its degradation promoting photomorphogenesis. Is important to note that it has been already shown that PIFs can be degraded under both light and dark conditions. Still, in general, it is known that for the appropriate transition from skoto- to photomorphogenesis, an optimal level of PIFs is required.

Phytochromes induce PIFs degradation in response to light signals via direct interaction (Figure 2). It has been shown that DNA binding is not necessary for PIFs light-induced degradation (AL-SADY et al., 2008; SHEN et al., 2008). However, QIU et al. (2015) showed that the transcriptional activity of HEMERA, a plastid development-related regulator, is required for PIF degradation.

In fact, two biochemical steps precede PIFs light-induced degradation: (1) PIFs phosphorylation in response to light and (2) PIFs ubiquitination followed by quick degradation through the 26S proteasome pathway (LEIVAR; QUAIL, 2011). In the dark, PIF abundance has been shown to be regulated by diverse factors including HLH proteins, kinases, and E3 ubiquitin ligases. Among these, kinases and E3 ubiquitin ligases are the main focus in the study of PIFs degradation.

As shown in figure 2, several kinases have been reported as PIF-phosphorylating enzymes. Among these, phy is probably the best candidate due to its physical interaction with PIFs (HUQ et al., 2004; HUQ; QUAIL, 2002; NI; TEPPERMAN; QUAIL, 1999), that is necessary for PIFs light-induced degradation and phosphorylation (AL-SADY et al., 2008; SHEN et al., 2008). Also, because it acts as a Ser/Thr kinase either *in vitro* (YEH; LAGARIAS, 1998) or *in vivo* (SHIN et al., 2016).

Ni et al. (2017) showed that a protein kinase family called PPK (PPK1-PPK4) is able to phosphorylate PIF3. However, even though this study presents strong evidence of PPKs as protein kinases of PIF3, these enzymes do not appear to function as the first light-regulated kinase of PIF phosphorylation in response to light. Then, PPKs are probably general kinases involved with many pathways. Furthermore, CASEIN KINASE II and BRASSINOSTEROID-INSENSITIVE 2 (BIN2) have also been described as PIF-phosphorylating enzymes (BERNARDO-GARCÍA et al., 2014; BU et al., 2011a; LING et al., 2017).

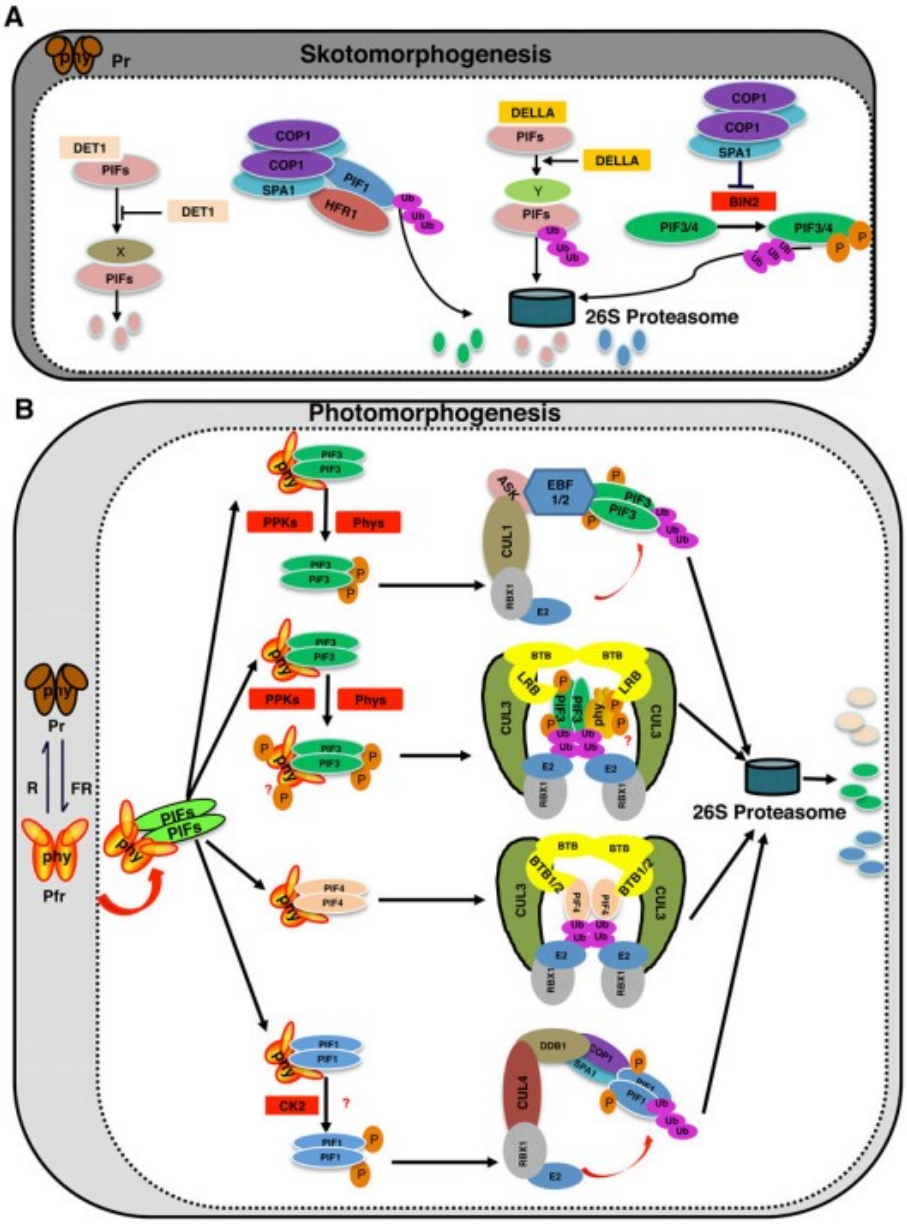


**Figure 2** – Dynamic regulation of PIF levels in dark and light. Model shows the mechanisms of light-dependent phosphorylation and degradation of PIFs by various kinases and E3 ubiquitin ligases. (A) In the dark, DET1 interacts with PIFs and stabilizes them by an unknown mechanism. PIF1 also forms a heterodimer with HFR1, thereby triggering the codegradation of both HFR1 and PIF1. COP1 function is necessary for this codegradation of PIF1 and HFR1. Moreover, DELLAs negatively regulate PIF abundance by promoting PIF degradation through the 26S proteasome-dependent pathway both in dark and light conditions. In addition, the COP1-SPA complex promotes the stability of PIF3 by inhibiting the BIN2-mediated phosphorylation and degradation of PIF3 and PIF4. X and Y indicate unknown factors necessary for DET1- and DELLA-induced degradation of PIFs in the dark, respectively. (B) Upon light exposure, different PIFs are phosphorylated by different protein kinases, including PPKs, phytochromes, and possibly other kinases, which triggers ubiquitination by different E3 ubiquitin ligase complexes followed by degradation through the 26S proteasome pathway. The ubiquitination and degradation of PIF1, PIF3, and PIF4 involve CUL4, CUL1, and CUL3-based E3 ubiquitin ligase complexes, respectively. Reproduced from Pham; Kathare; Huq (2018) – *Plant Physiology*.

COP1 is a highly conserved E3 ubiquitin ligase that acts as a central repressor of photomorphogenesis in plants (PODOLEC; ULM, 2018; YI; DENG, 2005). Besides, COP1 is able to interact with members of SPA family (SPA1-SPA4) that can enhance COP1 activity (HOECKER, 2017). In dark-grown seedlings, PIFs and COP-SPA complexes acts as negative regulators repressing photomorphogenesis by promoting and maintaining etiolated development (HOANG; HAN; KIM, 2019). In Arabidopsis there are three principal classes of CULLIN (CUL) RING UBIQUITIN LIGASE involved in substrate ubiquitination: CUL1, CUL3, and CUL4 (VIERSTRA, 2009).

Even though presenting different substrate specificity components, all three CULs are involved in PIFs light-induced degradation (PHAM; KATHARE; HUQ, 2018; ZHANG et al., 2017; ZHU et al., 2015). As members of the bHLH superfamily, PIFs are foreseen to bind with target DNA. Therefore, several studies have shown that Arabidopsis PIF1-PIF5 and rice PIF14 can bound to either the G-box (CACGTG) and/or the E box (CANNTG) (CORDEIRO et al., 2016; HORNITSCHEK et al., 2009; HUQ et al., 2004; HUQ; QUAIL, 2002; MARTINEZ-GARCIA; HUQ; QUAIL, 2000). Moreover, many proteins can directly interact with PIFs modulating DNA binding and, consequently, the transcriptional activity of PIFs. These proteins can be divided into two classes: factors enhancing or inhibiting PIF DNA-binding activity.

Among the factors enhancing PIF DNA-binding activity, PIFs conserve the bHLH feature of binding to DNA as both homodimers and heterodimers *in vitro* (BU et al., 2011b; PHAM; KATHARE; HUQ, 2018; TOLEDO-ORTIZ; HUQ; QUAIL, 2003). Moreover, they are also able to interact with others transcription factors modulating the PIF-DNA link (CHEN et al., 2013; OH; ZHU; WANG, 2012; ZHANG et al., 2014). Conversely, three groups can inhibit PIFs DNA-binding ability: the HLH classes of transcription factors (HAO et al., 2012; HORNITSCHEK et al., 2009; LORRAIN et al., 2008; SHI et al., 2013; ZHU et al., 2016b), phyB (PARK et al., 2012), and DELLA proteins (DE LUCAS et al., 2008; FENG et al., 2008).

As discussed above, PIFs have expanded their function from central phytochrome signaling components to essential signal integrators of several growth and development-related signaling pathways. It happens through the diverse range of genetic and physical events with components from multiple pathways. In the recent years, a great amount of information concerning to PIFs signal integration has been discovered, allowing a better understanding of physiological aspects. Nevertheless, the molecular details related to these processes still to be investigated. In summary, further studies are necessary to fully

comprehend PIFs complexity as integrators of signaling pathways and to uncover new roles of PIFs in signal integration from unknown pathways.

## 3 PIFs IN ARABIDOPSIS

The role of PIFs (bHLH transcriptions factors) involved with physiological processes has been highly investigated through genetic, biochemical, and physiological aspects in Arabidopsis. To this species has been described eight PIFs (PIF1 to PIF8). While only PIF1 and PIF3 present an APA motif, all Arabidopsis PIFs have an Active Phytochrome B-binding (APB) motif (Figure 3). However, is important to note that many phy-interacting proteins lack APA or APB motifs, thereby these proteins cannot be ruled out as a phyA or phyB binding due to the absence of APA or APB motif (ENDO et al., 2013; HUANG et al., 2016; KAISERLI et al., 2015).

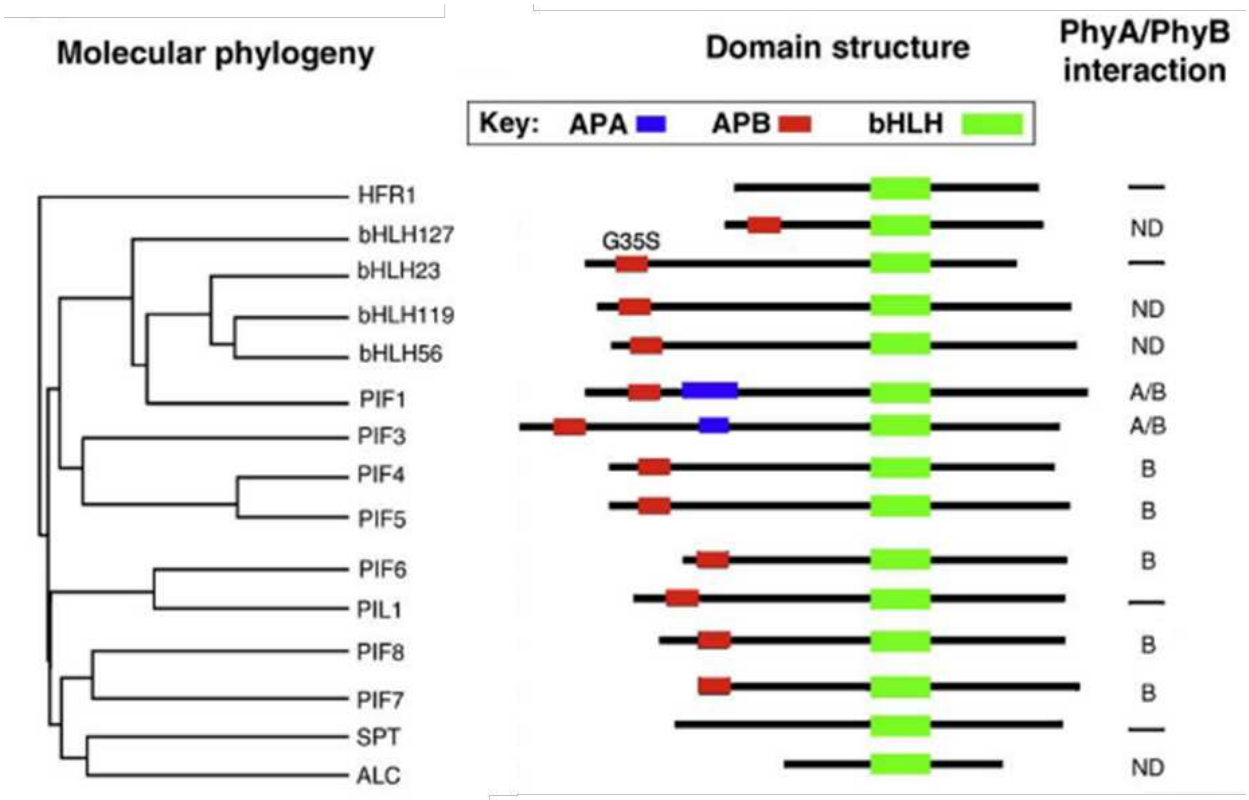


**Figure 3** – The PIF-subfamily of phy-interacting bHLH transcription factors. Pfr-specific interaction with phyA and/or phyB is indicated as A and/or B, respectively. Lack of interaction is indicated as (–) and not determined as (ND). Addapted from Leivar; Quail (2011) – *Cell Press*.

PIF1 (previously named PIL5) has been reported to acts as a negative regulator of photomorphogenesis under blue light (CASTILLON; SHEN; HUQ, 2009) and dark conditions (XU et al., 2014), while light-induced proteolytic degradation stabilizes positively acting transcription factors promoting photomorphogenesis (BU et al., 2011a; SHEN et al., 2008). Among the photomorphogenic processes, this transcription factor was described as an

important regulator of chlorophyll biosynthesis (HUQ et al., 2004; TANG et al., 2012) and hypocotyl elongation (CAPELLA et al., 2015; LI et al., 2014b).

Oh et al. (2004) showed that PIF1 plays a major role in the inhibition of light-dependent seed germination. This negative regulation of seed germination was proposed to be caused by the repression of gibberellin biosynthesis genes (OH et al., 2006). Furthermore, PIF1 is constitutively expressed in short-day conditions, and its abundance oscillation imposed by phy dictates the action of PIF1 during the night (SOY; LEIVAR; MONTE, 2014).

Luo et al. (2014) showed that PIF2 (commonly known as PIL1) interacts with phyB and COP1 *in vivo*. The phyB stabilizes PIF2 in response to light, while COP1 promotes PIF2 degradation in the dark. Contrasting to other PIFs, PIF2 was shown to positively regulates seedling deetiolation in response to blue, red, and far-red lights and regulates its own gene expression (LI et al., 2014a). Besides, it was shown that it interacts with other PIFs, indicating that PIL1 is able to form homo or heterodimers with other PIF proteins *in vivo*. Moreover, it was found that very low fluence light signals, dark, and multiple phys can repress PIL1 gene expression (HWANG; QUAIL, 2008).

PIF3 was the first bHLH protein to be identified as a phytochrome-interacting factor (NI; TEPPERMAN; QUAIL, 1998). Different to the other proteins of PIF family that function predominantly as negative regulators (BAE; CHOI, 2008; DUEK; FANKHAUSER, 2005), PIF3 was reported to act either as a positive or a negative regulator of the photomorphogenic processes. PIF3 was suggested to be a positive regulator of light signals (HALLIDAY et al., 1999), while it could also acts as a negative regulator of hypocotyl elongation (KIM et al., 2003) and chloroplast development (STEPHENSON; FANKHAUSER; TERRY, 2009).

Moreover, PIF3 was found to be related with the regulation of anthocyanin biosynthesis (SHIN; PARK; CHOI, 2007) and ethylene-induced hypocotyl elongation in light condition (ZHONG et al., 2012), while its interaction with TOC1 optimizes diurnal growth regulation of hypocotyl elongation (SOY et al., 2016). Jiang et al. (2017) showed that PIF3 negatively regulates the expression of C-Repeat Binding Factor (CBF) genes, modulating the freezing tolerance, and more recently, it was also reported that CBFs–PIF3-phyB module acts as a molecular hub involved with plant response to cold stress (JIANG et al., 2020).

PIF4 has been reported to function as a negative regulator of phytochrome B signaling (HUQ; QUAIL, 2002) and act as a leaf senescence inductor (SAKURABA et al., 2014) in Arabidopsis. Besides, it was described as a positive regulator of cell elongation (DE LUCAS et al., 2008; OH et al., 2014) and having its activity regulated by several

environmental factors, such as light (JEONG; CHOI, 2013) and hormonal signals (DE LUCAS; PRAT, 2014), acting as a key integrator of multiple signaling pathways (LEIVAR; QUAIL, 2011; LUCYSHYN; WIGGE, 2009; PAIK et al., 2017; QUINT et al., 2016).

It was also showed that PIF4 is an important component of temperature responses (QIU, 2020) by controlling cell elongation (BAI et al., 2013), stomatal development (LAU et al., 2018), flowering (KUMAR et al., 2012), and negatively regulating plant immunity, while promoting growth (GANGAPPA; BERRIRI; KUMAR, 2017). This thermoresponsive growth occurs due to PIF4-TOC1 interaction (ZHU et al., 2016a) and PIF4-BES1 crosstalk that activates brassinosteroid synthesis (MARTÍNEZ et al., 2018).

PIF5 (also known as PIL6) regulates many pathways regulated by PIF4, which seems to be functionally redundant. Along with PIF4, PIF5 regulates leaf senescence through chlorophyll degradation (SAKURABA et al., 2014; ZHANG et al., 2015), anthocyanin (LIU et al., 2015) and hormone (WEI et al., 2017) biosynthesis, as well as hypocotyl elongation (KUNIHIRO; YAMASHINO; MIZUNO, 2010; TAVRIDOU; PIREYRE; ULM, 2020). In collaboration with PIF4 and PIF7 it was shown that PIF5 is involved in the regulation of shade avoidance (HORNITSCHEK et al., 2009; LI et al., 2012a; LORRAIN et al., 2008). Beyond that, PIF5 was reported as a modulator of auxin signaling (NOZUE; HARMER; MALOOF, 2011), ethylene biosynthesis and phy signaling (KHANNA et al., 2007), and functioning at the interface of red light signaling through PIF-TOC1 direct interaction (FUJIMORI et al., 2004).

PIF6 (also known as PIL2) produces two splice variants, α and β; however, only β-form was reported regulating seed dormancy (PENFIELD; JOSSE; HALLIDAY, 2010). Interestingly, PIF6 inhibits hypocotyl elongation in response to light. Besides that, PIF6 is expressed during seed development, but during the water imbibition its expression is dramatically reduced. PIF6 was also described influencing rates of abscisic acid-dependent seed germination (RÜHL et al., 2012). PIF6 also interacts with TOC1; however, the functional importance of this interaction is not yet understood (FUJIMORI et al., 2004). Recently, Tognacca et al. (2019) showed that phyB is involved in the regulation of PIF6 alternative splicing pattern in germinating seeds.

PIF7 acts as a negative regulator of DREB1 expression (KIDOKORO et al., 2009) and seedling deetiolation (LEIVAR et al., 2008). Moreover, PIF7 is involved in early responses to elevated temperature (FIORUCCI et al., 2020), shade avoidance (LI et al., 2012a) and PIF7-ELF3 interaction mediates shade-induced growth (JIANG et al., 2019). Recently, Leivar et al. (2020) showed that PIF7 is required for photoperiodic growth. Finally, it is

known that PIF8 can interact with phyB (LEIVAR; QUAIL, 2011). However, the functional importance of PIF8 is largely unknown. Therefore, further studies are necessary to understand the potential role of this transcription factor in light signaling-related pathways.

## 4 PIFs IN RICE

Nakamura et al. (2007) were the first to characterize a group of bHLH transcription factors subfamily in rice plants. The authors identified that these transcription factors sequence is similar to the arabidopsis PIFs, and were, therefore, considered homologous (*Os*PIL11 – *Os*PIL16). Since then, studies with rice plants have been performed in order to elucidate the role of PIFs in this species (CORDEIRO et al., 2016; JI et al., 2019; LI et al., 2012b; SAKURABA et al., 2017; ZHOU et al., 2014).

It has been reported that *Os*PIL11 overexpression inhibits hypocotyl elongation under red light and its expression is organ-specific and hormone regulated in tobacco mutants (LI et al., 2012b). Concerning to nitrogen nutrition, Piao et al. (2015) raised the possibility of *Os*PIL11 be involved with the leaf senescence cascade in rice. It was also showed that *Os*bHLH107, an *Os*PIL11 homologous, regulates grain size by influencing cell numbers in the longitudinal direction of spikelet hulls (YANG et al., 2018). These authors also found an involvement of OsPIL11 in grain size regulation.

Little is known about *Os*PIL12 functional importance. In the literature, just a few papers show the possible functions and interactions of this proteins in rice plants. It has been reported that *Os*PIL12 is able to interact with the Pseudo-Response Regulator 1 (*Os*PRR1), a probably rice clock component with roles in the flowering time regulation (MURAKAMI et al., 2003, 2005; NAKAMURA et al., 2007). Along with *Os*PIL11, *Os*PIL12 was also raised to be involved in leaf senescence cascade in rice (PIAO et al., 2015).

Todaka et al. (2012) showed by microarray analysis that *Os*PIL13 (also named *Os*PIL1) is a stress-responsive gene. It has also been reported that the overexpression of *Os*PIL1 promotes lengthening of the internodes by increasing cell size, especially under water deficit conditions. Regarding growth and development, overexpressing *Os*PIL1 plants are significantly taller than wild type plants, but transgenic rice plants expressing *Os*PIL1-RD (fused to a transcriptional repression domain) are shorter (TODAKA et al., 2012). Furthermore, Sakuraba et al. (2017) showed that *Os*PIL13 is involved with chlorophyll biosynthesis, and its knockdown cause a pale green phenotype when these plants were grown on a natural rice field.

Rice plants overexpressing *Os*PIL15 exhibited smaller roots and shoots in dark condition, indicating that this transcription factor might be involved in seedling growth and acts as repressor of genes that are involved with skotomorphogenesis (ZHOU et al., 2014). Xie et al. (2019) showed that *Os*PIL15-overexpressing rice plants present smaller tiller angles

as a consequence of negative regulation through light and gravity signals. Regarding grain development, *Os*PIL15 was shown to negatively regulate grain filling and yield through the up-regulation of *Os*PUP7 and OsmiR530 expression (JI et al., 2019; SUN et al., 2020).

Rice bHLH transcription factor OsPIL16 (also named APG) has been reported to acts as a negative regulator of rice grain length and weight (HEANG; SASSA, 2012a). It has been also reported that APG presents an antagonistic function with the Positive Regulator of Grain Length 1 (PGL1) and PGL2 (HEANG; SASSA, 2012a, 2012b). He et al. (2016) revealed that *Os*PIL16 can bind to the N-box region of the rice Dehydration-Sensitive Element-Binding 1B (*Os*DREB1B). It has also been proposed that phyB deficiency could positively regulates *Os*DREB1 expression through *Os*PIL16, thus improving the cold tolerance of phyB mutants.

Cordeiro et al. (2016) characterized the *Os*PIL14 gene, which has been renamed *Os*PIF14 due its preferably interaction with *Os*phyB *in vitro*. During *in vivo* analysis, OsPIF14 gene expression indicates to be modulated by different treatments, such as drought, salt, cold and abscisic acid. It was also identified a possible photoperiod responsive behavior, as previously described (NAKAMURA et al., 2007), and two mRNA bands in agarose gel (α and β isoforms) at low temperature, suggesting that this gene may undergo alternative splicing giving it an importance in rice plants cold tolerance.

Furthermore, OsPIF14 is antagonistically regulated by gibberellic and jasmonic acid, supported by its interaction with Slender Rice 1 (SLR1) (UM et al., 2018), and related to chlorophyll biosynthesis by controlling the activation of the OsFLU1 protein (LI et al., 2019). Recently, Mo et al. (2020) showed that OsPIF14 overexpression promotes mesocotyl and root growth, specifically in the dark and under salt stress. Moreover, it was found that OsPIF14 is negatively regulated by salt and that it physically interacts with SLR1, which negatively regulates its expression.

## REFERENCES

AL-SADY, B. et al. Mechanistic duality of transcription factor function in phytochrome signaling. **Proceedings of the National Academy of Sciences of the United States of America**, v. 105, n. 6, p. 2232–2237, 2008.

BAE, G.; CHOI, G. Decoding of Light Signals by Plant Phytochromes and Their Interacting Proteins. **Annual Review of Plant Biology**, v. 59, n. 1, p. 281–311, 2008.

BAI, M. Y. et al. A triple helix-loop-helix/basic helix-loop-helix cascade controls cell elongation downstream of multiple hormonal and environmental signaling pathways in Arabidopsis. **Plant Cell**, v. 24, n. 12, p. 4917–4929, 2013.

BERNARDO-GARCÍA, S. et al. BR-dependent phosphorylation modulates PIF4 transcriptional activity and shapes diurnal hypocotyl growth. **Genes and Development**, v. 28, n. 15, p. 1681–1694, 2014.

BU, Q. et al. Phosphorylation by CK2 enhances the rapid light-induced degradation of phytochrome interacting factor 1 in arabidopsis. **Journal of Biological Chemistry**, v. 286, n. 14, p. 12066–12074, 2011a.

BU, Q. et al. Dimerization and blue light regulation of PIF1 interacting bHLH proteins in Arabidopsis. **Plant Molecular Biology**, v. 77, n. 4–5, p. 501–511, 2011b.

CAPELLA, M. et al. Arabidopsis thaliana HomeoBox 1 (AtHB1), a Homedomain-Leucine Zipper I (HD-Zip I) transcription factor, is regulated by PHYTOCHROME-INTERACTING FACTOR 1 to promote hypocotyl elongation. **New Phytologist**, v. 207, n. 3, p. 669–682, 2015.

CASTILLON, A.; SHEN, H.; HUQ, E. Blue light induces degradation of the negative regulator phytochrome interacting factor 1 to promote photomorphogenic development of arabidopsis seedlings. **Genetics**, v. 182, n. 1, p. 161–171, 2009.

CHEN, D. et al. Antagonistic basic helix-loop-Helix/bZIP transcription factors form transcriptional modules that integrate light and reactive oxygen species signaling in Arabidopsis. **Plant Cell**, v. 25, n. 5, p. 1657–1673, 2013.

CORDEIRO, A. M. et al. Rice phytochrome-interacting factor protein OsPIF14 represses OsDREB1B gene expression through an extended N-box and interacts preferentially with the active form of phytochrome B ☆. **Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms**, v. 1859, n. 2, p. 393–404, 2016.

DAI, M. et al. Phosphorylation of FAR-RED ELONGATED HYPOCOTYL1 Is a Key Mechanism Defining Signaling Dynamics of Phytochrome A under Red and Far-Red Light in Arabidopsis. **The Plant Cell**, v. 24, n. 5, p. 1907–1920, 2012.

DE LUCAS, M. et al. A molecular framework for light and gibberellin control of cell elongation. **Nature**, v. 451, n. 7177, p. 480–484, 2008.

DE LUCAS, M.; PRAT, S. PIFs get BRright: PHYTOCHROME INTERACTING FACTORs as integrators of light and hormonal signals. **New Phytologist**, v. 202, n. 4, p. 1126–1141, 2014.

DUEK, P. D.; FANKHAUSER, C. bHLH class transcription factors take centre stage in phytochrome signalling. v. 10, n. 2, p. 51–54, 2005.

ENDO, M. et al. Phytochrome-dependent late-flowering accelerates flowering through physical interactions with phytochrome B and CONSTANS. **Proceedings of the National Academy of Sciences of the United States of America**, v. 110, n. 44, p. 18017–18022, 2013.

FENG, S. et al. Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. **Nature**, v. 451, n. 7177, p. 475–479, 2008.

FIORUCCI, A. S. et al. PHYTOCHROME INTERACTING FACTOR 7 is important for early responses to elevated temperature in Arabidopsis seedlings. **New Phytologist**, v. 226, n. 1, p. 50–58, 2020.

FUJIMORI, T. et al. Circadian-controlled basic/helix-loop-helix factor, PIL6, implicated in light-signal transduction in Arabidopsis thaliana. **Plant and Cell Physiology**, v. 45, n. 8, p. 1078–1086, 2004.

GANGAPPA, S. N.; BERRIRI, S.; KUMAR, S. V. PIF4 Coordinates Thermosensory Growth and Immunity in Arabidopsis. **Current Biology**, v. 27, n. 2, p. 243–249, 2017.

HALLIDAY, K. J. et al. poc1: An Arabidopsis mutant perturbed in phytochrome signaling because of a T DNA insertion in the promoter of PIF3, a gene encoding a phytochrome-interacting bHLH protein. **Proceedings of the National Academy of Sciences of the United States of America**, v. 96, n. 10, p. 5832–5837, 1999.

HAO, Y. et al. Interactions between HLH and bHLH factors modulate light-regulated plant development. **Molecular Plant**, v. 5, n. 3, p. 688–697, 2012.

HAUVERMALE, A. L.; ARIIZUMI, T.; STEBER, C. M. Gibberellin signaling: A theme and variations on DELLA repression. **Plant Physiology**, v. 160, n. 1, p. 83–92, 2012.

HE, Y. et al. Phytochrome B negatively affects cold tolerance by regulating OsDREB1 gene expression through phytochrome interacting factor-like protein OsPIL16 in rice. **Frontiers in Plant Science**, v. 7, n. DECEMBER2016, p. 1–12, 2016.

HEANG, D.; SASSA, H. Overexpression of a basic helix-loop-helix gene Antagonist of PGL1 (APG) decreases grain length of rice. **Plant Biotechnology**, v. 29, n. 1, p. 65–69, 2012a.

HEANG, D.; SASSA, H. An atypical bHLH protein encoded by POSITIVE REGULATOR OF GRAIN LENGTH 2 is involved in controlling grain length and weight of rice through interaction with a typical bHLH protein APG. **Breeding Science**, v. 62, n. 2, p. 133–141, 2012b.

HOANG, Q. T. N.; HAN, Y. J.; KIM, J. IL. Plant phytochromes and their phosphorylation. **International Journal of Molecular Sciences**, v. 20, n. 14, 2019.

HOECKER, U. The activities of the E3 ubiquitin ligase COP1/SPA, a key repressor in light signaling. **Current Opinion in Plant Biology**, v. 37, p. 63–69, 2017.

HORNITSCHEK, P. et al. Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. **EMBO Journal**, v. 28, n. 24, p. 3893–3902, 2009.

HUANG, H. et al. PCH1 integrates circadian and light-signaling pathways to control photoperiod-responsive growth in Arabidopsis. **eLife**, v. 5, n. FEBRUARY2016, p. 1–26, 2016.

HUQ, E. et al. Phytochrome-interacting factor 1 is a critical bHLH: Regulator of chlorophyll biosynthesis. **Science**, v. 305, n. 5692, p. 1937–1941, 2004.

HUQ, E.; QUAIL, P. H. PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in Arabidopsis. **EMBO Journal**, v. 21, n. 10, p. 2441–2450, 2002.

HWANG, Y. S.; QUAIL, P. H. Phytochrome-regulated PIL1 derepression is developmentally modulated. **Plant and Cell Physiology**, v. 49, n. 4, p. 501–511, 2008.

INOUE, K.; NISHIHAMA, R.; KOHCHI, T. Evolutionary origin of phytochrome responses and signaling in land plants. **Plant Cell and Environment**, v. 40, n. 11, p. 2502–2508, 2017.

JEONG, J.; CHOI, G. Phytochrome-interacting factors have both shared and distinct biological roles. **Molecules and Cells**, v. 35, n. 5, p. 371–380, 2013.

JI, X. et al. The basic helix-loop-helix transcription factor, OsPIL15, regulates grain size via directly targeting a purine permease gene OsPUP7 in rice. **Plant Biotechnology Journal**, v. 17, n. 8, p. 1527–1537, 2019.

JIANG, B. et al. PIF3 is a negative regulator of the CBF pathway and freezing tolerance in Arabidopsis. **Proceedings of the National Academy of Sciences of the United States of America**, v. 114, n. 32, p. E6695–E6702, 2017.

JIANG, B. et al. Cold-Induced CBF–PIF3 Interaction Enhances Freezing Tolerance by Stabilizing the phyB Thermosensor in Arabidopsis. **Molecular Plant**, v. 13, n. 6, p. 894–906, 2020.

JIANG, Y. et al. The ELF3-PIF7 Interaction Mediates the Circadian Gating of the Shade Response in Arabidopsis. **iScience**, v. 22, p. 288–298, 2019.

KAISERLI, E. et al. Integration of Light and Photoperiodic Signaling in Transcriptional Nuclear Foci. **Developmental Cell**, v. 35, n. 3, p. 311–321, 2015.

KHANNA, R. et al. The basic helix-loop-helix transcription factor PIF5 acts on ethylene biosynthesis and phytochrome signaling by distinct mechanisms. **Plant Cell**, v. 19, n. 12, p. 3915–3929, 2007.

KIDOKORO, S. et al. The phytochrome-interacting factor PIF7 negatively regulates dreb1 expression under circadian control in Arabidopsis. **Plant Physiology**, v. 151, n. 4, p. 2046–2057, 2009.

KIM, J. et al. Functional Characterization of PIF3 in Phytochrome-Mediated The Plant Cell. **The Plant Cell**, v. 15, n. October, p. 1–9, 2003.

KUMAR, S. V. et al. Transcription factor PIF4 controls the thermosensory activation of flowering. **Nature**, v. 484, n. 7393, p. 242–245, 2012.

KUNIHIRO, A.; YAMASHINO, T.; MIZUNO, T. Phytochrome-interacting factors PIF4 and PIF5 Are implicated in the regulation of hypocotyl elongation in response to Blue Light in Arabidopsis thaliana. **Bioscience, Biotechnology and Biochemistry**, v. 74, n. 12, p. 2538–2541, 2010.

LARNER, V. S.; FRANKLIN, K. A.; WHITELAM, G. C. Photoreceptors and Light Signalling Pathways in Plants. **Annual Plant Reviews online**, p. 107–131, 2018.

LAU, O. S. et al. Direct Control of SPEECHLESS by PIF4 in the High-Temperature Response of Stomatal Development. **Current Biology**, v. 28, n. 8, p. 1273- 1280.e3, 2018.

LEIVAR, P. et al. The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. **Plant Cell**, v. 20, n. 2, p. 337–352, 2008.

LEIVAR, P. et al. Phytochrome-imposed inhibition of PIF7 activity shapes photoperiodic growth in Arabidopsis together with PIF1, 3, 4 and 5. **Physiologia Plantarum**, v. 169, n. 3, p. 452–466, 2020.

LEIVAR, P.; MONTE, E. PIFs: Systems integrators in plant development. **Plant Cell**, v. 26, n. 1, p. 56–78, 2014.

LEIVAR, P.; QUAIL, P. H. PIFs: pivotal components in a cellular signaling hub. **Trends in Plant Science**, v. 16, n. 1, p. 19–28, 2011.

LI, F. et al. Phytochrome diversity in green plants and the origin of canonical plant phytochromes. **Nature Communications**, v. 6, n. 1, p. 1–12, 2015.

LI, L. et al. Linking photoreceptor excitation to changes in plant architecture. **Genes and Development**, v. 26, n. 8, p. 785–790, 2012a.

LI, L. et al. Expression Patterns of OsPIL11, a Phytochrome-Interacting Factor in Rice, and Preliminary Analysis of Its Roles in Light Signal Transduction. **Rice Science**, v. 19, n. 4, p. 263–268, 2012b.

LI, L. et al. PIL1 Participates in a Negative Feedback Loop that Regulates Its Own Gene Expression in Response to Shade. **Molecular Plant**, v. 7, n. 12, p. 1805–1806, 2014a.

LI, Y. et al. Arabidopsis VQ MOTIF-CONTAINING PROTEIN29 represses seedling deetiolation by interacting with PHYTOCHROME-INTERACTING FACTOR1. **Plant Physiology**, v. 164, n. 4, p. 2068–2080, 2014b.

LI, Z. et al. Rice FLUORESCENT1 Is Involved in the Regulation of Chlorophyll. **Plant and Cell Physiology**, v. 60, n. 10, p. 2307–2318, 2019.

LING, J. J. et al. Noncanonical role of Arabidopsis COP1/SPA complex in repressing BIN2-mediated PIF3 phosphorylation and degradation in darkness. **Proceedings of the National Academy of Sciences of the United States of America**, v. 114, n. 13, p. 3539–3544, 2017.

LIU, Z. et al. Phytochrome-interacting factors PIF4 and PIF5 negatively regulate anthocyanin biosynthesis under red light in Arabidopsis seedlings. **Plant Science**, v. 238, p. 64–72, 2015.

LORRAIN, S. et al. Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. **Plant Journal**, v. 53, n. 2, p. 312–323, 2008.

LUCYSHYN, D.; WIGGE, P. A. Plant Development: PIF4 Integrates Diverse Environmental Signals. **Current Biology**, v. 19, n. 6, p. R265–R266, 2009.

LUO, Q. et al. COP1 and phyB physically interact with PIL1 to regulate its stability and photomorphogenic development in Arabidopsis. **Plant Cell**, v. 26, n. 6, p. 2441–2456, 2014.

MARTINEZ-GARCIA, J. F.; HUQ, E.; QUAIL, P. H. Direct Targeting of Light Signals to a Promoter Element-Bound Transcription Factor. v. 288, n. May, p. 859–863, 2000.

MARTÍNEZ, C. et al. PIF 4-induced BR synthesis is critical to diurnal and thermomorphogenic growth . **The EMBO Journal**, v. 37, n. 23, p. 1–15, 2018.

MO, W. et al. PHYTOCHROME-INTERACTING FACTOR-LIKE14 and SLENDER RICE1 interaction controls seedling growth under salt stress. **Plant Physiology**, 2020.

MONTGOMERY, B. L.; LAGARIAS, J. C. Phytochrome ancestry: Sensors of bilins and light. **Trends in Plant Science**, v. 7, n. 8, p. 357–366, 2002.

MURAKAMI, M. et al. The Evolutionarily Conserved OsPRR Quintet: Rice Pseudo-Response Regulators Implicated in Circadian Rhythm. **Plant and Cell Physiology**, v. 44, n. 11, p. 1229–1236, 2003.

MURAKAMI, M. et al. Circadian-associated rice pseudo response regulators (OsPRRs): Insight into the control of flowering time. **Bioscience, Biotechnology and Biochemistry**, v. 69, n. 2, p. 410–414, 2005.

NAKAMURA, Y. et al. Characterization of a set of phytochrome-interacting factor-like bHLH proteins in Oryza sativa. **Bioscience, Biotechnology and Biochemistry**, v. 71, n. 5, p. 1183–1191, 2007.

NI, M.; TEPPERMAN, J. M.; QUAIL, P. H. PIF3 , a Phytochrome-Interacting Factor Necessary for Normal Photoinduced Signal Transduction , Is a Novel Basic Helix-Loop-Helix Protein. v. 95, p. 657–667, 1998.

NI, M.; TEPPERMAN, J. M.; QUAIL, P. H. Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. **Nature**, v. 400, n. 6746, p. 781–784, 1999.

NI, W. et al. PPKs mediate direct signal transfer from phytochrome photoreceptors to transcription factor PIF3. n. May, 2017.

NOZUE, K.; HARMER, S. L.; MALOOF, J. N. Genomic analysis of circadian clock-, light-,and growth-correlated genes reveals PHYTOCHROME-INTERACTING FACTOR5 as a modulator of auxin signaling in arabidopsis. **Plant Physiology**, v. 156, n. 1, p. 357–372, 2011.

OH, E. et al. PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in Arabidopsis thaliana. **Plant Cell**, v. 16, n. 11, p. 3045–3058, 2004.

OH, E. et al. Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. **Plant Journal**, v. 47, n. 1, p. 124–139, 2006.

OH, E. et al. Cell elongation is regulated through a central circuit of interacting transcription factors in the Arabidopsis hypocotyl. **eLife**, v. 2014, n. 3, p. 1–19, 2014.

OH, E.; ZHU, J. Y.; WANG, Z. Y. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. **Nature Cell Biology**, v. 14, n. 8, p. 802–809, 2012.

PAIK, I. et al. Expanding Roles of PIFs in Signal Integration from Multiple Processes. **Molecular Plant**, v. 10, n. 8, p. 1035–1046, 2017.

PAIK, I.; YANG, S.; CHOI, G. Phytochrome regulates translation of mRNA in the cytosol. **Proceedings of the National Academy of Sciences of the United States of America**, v. 109, n. 4, p. 1335–1340, 2012.

PARK, E. et al. Phytochrome B inhibits binding of phytochrome-interacting factors to their target promoters. **Plant Journal**, v. 72, n. 4, p. 537–546, 2012.

PENFIELD, S.; JOSSE, E. M.; HALLIDAY, K. J. A role for an alternative splice variant of PIF6 in the control of Arabidopsis primary seed dormancy. **Plant Molecular Biology**, v. 73, n. 1–2, p. 89–95, 2010.

PHAM, V. N.; KATHARE, P. K.; HUQ, E. Phytochromes and Phytochrome Interacting Factors. v. 176, n. February, p. 1025–1038, 2018.

PIAO, W. et al. Rice phytochrome B (OsPhyB) negatively regulates dark- and starvation-induced leaf senescence. **Plants**, v. 4, n. 3, p. 644–663, 2015.

PODOLEC, R.; ULM, R. Photoreceptor-mediated regulation of the COP1/SPA E3 ubiquitin ligase. **Current Opinion in Plant Biology**, v. 45, p. 18–25, 2018.

QI, L. et al. PHYTOCHROME-INTERACTING FACTORS Interact with the ABA Receptors PYL8 and PYL9 to Orchestrate ABA Signaling in Darkness. **Molecular Plant**, v. 13, n. 3, p. 414–430, 2020.

QIU, Y. et al. Hemera couples the proteolysis and transcriptional activity of phytochrome interacting actors in arabidopsis photomorphogenesis. **Plant Cell**, v. 27, n. 5, p. 1409–1427, 2015.

QIU, Y. Regulation of PIF4-mediated thermosensory growth. **Plant Science**, v. 297, n. March, p. 110541, 2020.

QUAIL, P. H. Phytochrome photosensory signalling networks. **Nature Reviews Molecular Cell Biology**, v. 3, n. 2, p. 85–93, 2002.

QUINT, M. et al. Molecular and genetic control of plant thermomorphogenesis. **Nature Plants**, v. 2, n. January, p. 1–9, 2016.

ROCKWELL, N. C.; SU, Y.-S.; LAGARIAS, J. C. Phytochrome Structure and Signaling Mechanisms. **Annual Review of Plant Biology**, v. 57, n. 1, p. 837–858, 2006.

RÜHL, C. et al. Polypyrimidine tract binding protein homologs from arabidopsis are key regulators of alternative splicing with implications in fundamental developmental processesW. **Plant Cell**, v. 24, n. 11, p. 4360–4375, 2012.

SAKURABA, Y. et al. Phytochrome-interacting transcription factors PIF4 and PIF5 induce leaf senescence in Arabidopsis. **Nature Communications**, v. 5, 2014.

SAKURABA, Y. et al. Rice Phytochrome-Interacting Factor-Like1 (OsPIL1) is involved in the promotion of chlorophyll biosynthesis through feed-forward regulatory loops. **Journal of Experimental Botany**, v. 68, n. 15, p. 4103–4114, 2017.

SHEN, H. et al. Light-induced phosphorylation and degradation of the negative regulator phytochrome-interacting factor1 from Arabidopsis depend upon its direct physical interactions with photoactivated phytochromes. **Plant Cell**, v. 20, n. 6, p. 1586–1602, 2008.

SHI, H. et al. HFR1 sequesters PIF1 to govern the transcriptional network underlying light-initiated seed germination in Arabidopsis. **Plant Cell**, v. 25, n. 10, p. 3770–3784, 2013.

SHIN, A. Y. et al. Evidence that phytochrome functions as a protein kinase in plant light signalling. **Nature Communications**, v. 7, n. May, 2016.

SHIN, J.; PARK, E.; CHOI, G. PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in Arabidopsis. **Plant Journal**, v. 49, n. 6, p. 981–994, 2007.

SHOR, E. et al. PHYTOCHROME INTERACTING FACTORS mediate metabolic control of the circadian system in Arabidopsis. **New Phytologist**, v. 215, n. 1, p. 217–228, 2017.

SOY, J. et al. Molecular convergence of clock and photosensory pathways through PIF3-TOC1 interaction and co-occupancy of target promoters. **Proceedings of the National Academy of Sciences of the United States of America**, v. 113, n. 17, p. 4870–4875, 2016.

SOY, J.; LEIVAR, P.; MONTE, E. PIF1 promotes phytochrome-regulated growth under photoperiodic conditions in Arabidopsis together with PIF3, PIF4, and PIF5. **Journal of Experimental Botany**, v. 65, n. 11, p. 2925–2936, 2014.

STEPHENSON, P. G.; FANKHAUSER, C.; TERRY, M. J. PIF3 is a repressor of chloroplast development. **Proceedings of the National Academy of Sciences of the United States of America**, v. 106, n. 18, p. 7654–7659, 2009.

SUN, W. et al. OsmiR530 acts downstream of OsPIL15 to regulate grain yield in rice. **New Phytologist**, v. 226, n. 3, p. 823–837, 2020.

TANG, W. et al. Transposase-derived proteins FHY3/FAR1 interact with PHYTOCHROME-INTERACTING FACTOR1 to regulate chlorophyll biosynthesis by modulating HEMB1 during deetiolation in Arabidopsis. **Plant Cell**, v. 24, n. 5, p. 1984–2000, 2012.

TAVRIDOU, E.; PIREYRE, M.; ULM, R. Degradation of the transcription factors PIF4 and PIF5 under UV-B promotes UVR8-mediated inhibition of hypocotyl growth in Arabidopsis. **Plant Journal**, v. 101, n. 3, p. 507–517, 2020.

TODAKA, D. et al. Rice phytochrome-interacting factor-like protein OsPIL1 functions as a

key regulator of internode elongation and induces a morphological response to drought stress. **Proceedings of the National Academy of Sciences of the United States of America**, v. 109, n. 39, p. 15947–15952, 2012.

TOGNACCA, R. S. et al. Alternative Splicing Regulation During Light-Induced Germination of Arabidopsis thaliana Seeds. **Frontiers in Plant Science**, v. 10, n. September, p. 1–12, 2019.

TOLEDO-ORTIZ, G.; HUQ, E.; QUAIL, P. H. The Arabidopsis Basic/Helix-Loop-Helix Transcription Factor Family. **The Plant Cell**, v. 15, n. 8, p. 1749–1770, 2003.

TRIPATHI, S. et al. Regulation of photomorphogenic development by plant phytochromes. **International Journal of Molecular Sciences**, v. 20, n. 24, p. 1–17, 2019.

UM, T. Y. et al. Jasmonate zim-domain protein 9 interacts with slender rice 1 to mediate the antagonistic interaction between jasmonic and gibberellic acid signals in rice. **Frontiers in Plant Science**, v. 871, n. December, p. 1–11, 2018.

VIERSTRA, R. D. The ubiquitin-26S proteasome system at the nexus of plant biology. **Nature Reviews Molecular Cell Biology**, v. 10, n. 6, p. 385–397, 2009.

WEI, Z. et al. Brassinosteroid biosynthesis is modulated via a transcription factor cascade of COG1, PIF4, and PIF5. **Plant Physiology**, v. 174, n. 2, p. 1260–1273, 2017.

XIE, C. et al. Phytochrome-interacting factor-like protein OsPIL15 integrates light and gravitropism to regulate tiller angle in rice. **Planta**, v. 250, n. 1, p. 105–114, 2019.

XU, X. et al. PHYTOCHROME INTERACTING FACTOR1 enhances the E3 ligase activity of CONSTITUTIVE PHOTOMORPHOGENIC1 to synergistically repress photomorphogenesis in Arabidopsis. **Plant Cell**, v. 26, n. 5, p. 1992–2006, 2014.

YANG, X. et al. Overexpression of OsbHLH107, a member of the basic helix-loop-helix transcription factor family, enhances grain size in rice (Oryza sativa L.). **Rice**, v. 11, n. 1, p. 1–12, 2018.

YEH, K. C.; LAGARIAS, J. C. Eukaryotic phytochromes: Light-regulated serine/threonine protein kinases with histidine kinase ancestry. **Proceedings of the National Academy of Sciences of the United States of America**, v. 95, n. 23, p. 13976–13981, 1998.

YI, C.; DENG, X. W. COP1 - From plant photomorphogenesis to mammalian tumorigenesis. **Trends in Cell Biology**, v. 15, n. 11, p. 618–625, 2005.

ZHANG, B. et al. BLADE-ON-PETIOLE proteins act in an E3 ubiquitin ligase complex to regulate PHYTOCHROME INTERACTING FACTOR 4 abundance. **eLife**, v. 6, p. 1–19, 2017.

ZHANG, D. et al. The chromatin-remodeling factor PICKLE integrates brassinosteroid and gibberellin signaling during skotomorphogenic growth in Arabidopsis. **Plant Cell**, v. 26, n. 6, p. 2472–2485, 2014.

ZHANG, Y. et al. PHYTOCHROME-INTERACTING FACTOR 5 (PIF5) positively regulates dark-induced senescence and chlorophyll degradation in Arabidopsis. **Plant Science**, v. 237, p. 57–68, 2015.

ZHONG, S. et al. A molecular framework of light-controlled phytohormone action in arabidopsis. **Current Biology**, v. 22, n. 16, p. 1530–1535, 2012.

ZHOU, J. et al. Overexpression of OsPIL15 , a phytochrome - interacting factor - like protein gene , represses etiolated seedling growth in rice. **Journal of integrative plant biology**, v. 56, n. 4, p. 373–387, 2014.

ZHU, J. Y. et al. TOC1-PIF4 interaction mediates the circadian gating of thermoresponsive growth in Arabidopsis. **Nature Communications**, v. 7, p. 1–10, 2016a.

ZHU, L. et al. CUL4 forms an E3 ligase with COP1 and SPA to promote light-induced degradation of PIF1. **Nature Communications**, v. 6, p. 1–10, 2015.

ZHU, L. et al. A negative feedback loop between PHYTOCHROME INTERACTING FACTORs and HECATE proteins fine-tunes photomorphogenesis in Arabidopsis. **Plant Cell**, v. 28, n. 4, p. 855–874, 2016b.

# CHAPTER II

*OsPIF14 GENE KNOCKOUT SEVERELY DELAYS SEED GERMINATION AND SEEDLING ELONGATION*

# 1 INTRODUCTION

## 1.1 Problem characterization

Plant metabolism is directly regulated by light properties (quality, intensity, direction, and photoperiod) detected by specific protein receptors. Then, plants can use this information to cope with diverse light conditions in the external environment. Among the several plant regulatory receptors, phytochromes (phy) play a key role in the perception of light. The phy system regulates a wide range of physiological processes from seed germination to flowering (FRANKLIN; QUAIL, 2010; KONG; OKAJIMA, 2016). In response to red light, phy translocate from the cytoplasm to the nucleus, where it interacts with several light-responsive transcription factors, including Phytochrome-Interacting Factors (PIFs) (GALVÃO; FANKHAUSER, 2015).

Seed germination and seedling establishment are critical phases for plant life cycle (HADAS, 2004). Seed germination is a general expression for illustrating a series of complex metabolic processes that lead to the break of dormancy and use of seed reserves for growth. Therefore, seedling establishment starts with seed germination and is finished when the seedling has become effectively independent of its seed reserves (GOMMERS; MONTE, 2018). Based on the environmental conditions for plant morphogenesis, seedling establishment can be divided in two processes: skotomorphogenesis and photomorphogenesis (PHAM; KATHARE; HUQ, 2018).

Plant growth and development are regulated by the daily cycle of light and dark. In darkness, plants undergo skotomorphogenesis which is characterized by elongated hypocotyls, closed cotyledons, and retarded primary root growth. Conversely, under light, an indispensable environmental factor for photosynthetic organisms, plants are subjected to photomorphogenesis, which is distinguished by short hypocotyls, open, expanded and green cotyledons, and cessation of root elongation (JOSSE; HALLIDAY, 2008; PHAM; KATHARE; HUQ, 2018; PHAM; XU; HUQ, 2018). Both, PIFs abundance and degradation, are described as involved with the processes of skoto- and photomorphogenesis, respectively (PHAM; KATHARE; HUQ, 2018).

PIFs are bHLH proteins involved with several plant morphogenic processes (PAIK et al., 2017; PHAM; KATHARE; HUQ, 2018). These transcription factors are usually described as photomorphogenesis negative regulators; however, some exceptions occurs as in the case of PIF2 in Arabidopsis (LEIVAR; MONTE, 2014; LEIVAR; QUAIL, 2011; LI et al.,

2014). In Arabidopsis, PIFs play essential roles in the integration of light (PAIK et al., 2017) and other signaling pathways, such as hormone (DE LUCAS et al., 2008; FENG et al., 2008; FRANKLIN et al., 2011), reactive oxygen species (CHEN et al., 2013), and temperature (QIU, 2020).

Rice has one PIF and five PIF-Like (PIL) proteins (PIF14, PIL11-PIL16) (CORDEIRO et al., 2016; NAKAMURA et al., 2007). It was demonstrated that, the overexpression of *OsPIL11* inhibits hypocotyl elongation under red light (LI et al., 2012), whereas *OsPIL13* promotes internode growth (TODAKA et al., 2012) and *OsPIL15* represses seedling growth in the dark (ZHOU et al., 2014). *OsPIF14* was reported as antagonistically regulated by gibberellic and jasmonic acid (UM et al., 2018), and related to chlorophyll biosynthesis (LI et al., 2019). Besides, the *OsPIF14* overexpression promotes mesocotyl and root growth, specifically in the dark and under salt stress (MO et al., 2020). Nevertheless, the biological function and regulatory mechanism of these transcription factors in rice are still largely unknown.

## 2 HYPOTHESIS

*OsPIF14* gene is involved with skoto- and photomorphogenesis in rice.

# 3 OBJECTIVES

## 3.1 General objectives

To understand the function of Phytochrome-Interacting Factor 14 (PIF14) in rice germination and seedling developmental processes.

## 3.2 Specific objectives

1. To analyze seed germination of knockout PIF14 rice lines under light and dark stimuli;
2. To assess PIF14 absence responses in rice seedling elongation under light and dark conditions;
3. To quantify the photosynthetic pigments content of PIF14 rice knockout lines grown under different light conditions;
4. To evaluate the sugars content of knockout PIF14 rice quiescent seeds.

# 4 MATERIALS AND METHODS

## 4.1 Plant material and growth conditions

Wild type (WT) and knockout *Os*PIF14 gene (14-KO1 and 14-KO2 lines) rice seeds (*Oryza sativa spp. Japonnica; cv. Nipponbare*) were kindly provided by Dr. Nelson Saibo from ITQB/Nova Lisboa University, Portugal. The seeds were sown in Germitest® paper under controlled conditions (25 ºC, 70% relative humidity and 12 h photoperiod), and exposed to the following treatments during 16 days: light (100 μmol photons $m^{-2}$ $s^{-1}$) and darkness (full dark).

## 4.2 Growth and development assessment

### *4.2.1 Seed germination percentage*

Seed germination was performed 24 h and 48 h after sown, under controlled conditions, using 1 mm coleoptile length as a marker for germination.

The formula used to analyze this parameter was:

**G(%) = (N/A) x 100**, where:

**N** = number of germinated seeds;

**A** = total number of seeds sown.

### *4.2.2 Shoot and root length*

The shoot and root length of the transgenic and WT rice seedlings were performed daily in the same time of seed sown under controlled conditions using a caliper.

### *4.2.3 Pigments content*

To measure the amount of photosynthetic pigments, 50 mg of fresh shoot material from 16-day-old rice seedlings was first homogenized with 2 mL of 80% ice-cold acetone. The homogenate was centrifuged at 16,000 g for 5 min at 4 ºC and the obtained supernatant was used for the spectrophotometric quantification of chlorophyll *a*, *b* and carotenoids. The absorbance was measured in three wavelengths, 663, 646 and 470 ηm. The amount of these

pigments was calculated using the equations proposed by Lichtenthaler and Wellburn (1983). All pigments content was expressed as microgram per gram of fresh weight.

### 4.3 Seed length and width

The seed length and width of the transgenic and WT rice seedlings were performed using 25 seeds of each genotype under controlled conditions with a caliper.

### 4.4 Carbohydrate content measurement

Total soluble sugars were extracted from 20 mg of lyophilized seed material with 1,5 mL of MCW solution (methanol:chloroform:water 12:5:3 v/v/v) and incubated in thermomixer during 30 min at 30 ºC and 400 rpm. The extraction procedure was performed twice. Following, the homogenate was centrifuged at 10,000 g during 10 min at room temperature and the supernatants from the first and second extraction were collected and reunited. The starch content was extracted from the MCW pellet, hydrolyzing it with 1,5 mL of $HClO_4$ (30% v/v) and following the same conditions described by total soluble sugars. The total soluble sugars from MCW and acid extractions were measured by the phenol-sulphuric acid method (DUBOIS et al. 1956) and sucrose was quantified through direct microdetermination (VAN HANDEL 1968).

### 4.5 PIF14 *in silico* analysis

Analyses were carried out using *in silico* bioinformatic tools. Interaction analysis were performed using Cytoscape (v 3.8.0) and STRING database with PIF14 GenBank reference (Os07g0143200). Only proteins with confidence score more than 0.700 and no more than 5 additional interactors were selected. The network module was visualized using Cytoscape.

### 4.6 Statistical analysis

The experiments were arranged in a completely randomized design. In the germination and seedling growth analysis each replicate was represented by 5 individuals. The germinated seeds were divided in three groups (each one composed by 3-10 seedlings)

for the biochemical measurements, which shoot was used to pigments determination. The carbohydrate quantification was performed from non-germinated seeds where each replicate was represented by 8 seeds. The data were subjected to analysis of variance (ANOVA) and averages were compared by Tukey's test or t-test at 5% of probability ($p \leq 0.05$). All statistical analyses were conducted using SigmaPlot 12.0 (Systat Software, San Jose, USA).

# 5 RESULTS

## 5.1 OsPIF14 knockout affects seed germination

In order to test the hypothesis that OsPIF14 gene is involved in light responses, an initial characterization of seed germination and seedling development of PIF14 knockout mutants (14-KO1 and 14-KO2 independent lines) and wild type (WT) were performed. The seeds were sown under two growth conditions: light (100 μmol photons $m^{-2}$ $s^{-1}$) and dark (full darkness) during 16 days. The germination rate of 14-KO1 and 14-KO2, under light condition, were lesser in comparison to WT (84% and 82%, respectively) at 24 h (Fig. 1A). This response was kept at 48 h after sown, which all WT seeds germinated and less than 50% of 14-KO1 and 14-KO2 seeds germinated at this time (Fig. 1C).

As seen under light condition, the germination rate of 14-KO1 and 14-KO2 were also lesser than WT 24 h after sown under dark (Fig. 1B). The difference, however, was less than that observed under light (16% and 12%, respectively). Similarly, 48 h after sown while almost all WT seeds germinated, less than 60% of the transformed seeds were germinated. During the analyses, no statistical difference was observed between 14-KO1 and 14-KO2 rice seed germination. Interestingly, under dark condition, the WT seeds seemed to suffer more than the transformed seeds 24 h after sown. While the difference between WT under light and dark condition were 72%, the difference between 14-KO1 and 14-KO2 under these same conditions were 4% and 2%, respectively. Altogether, these results suggest that the PIF14 transcription factor is involved with rice seed germination processes. Moreover, this transcription factor might have some involvement with skotomorphogenesis.

## 5.2 OsPIF14 knockout delays seedling development

Due to the delayed germination, the KO-PIF14 lines showed a lesser seedling elongation (Fig. 2). Figure 2A and 2B show the shoot length of the three genotypes under both light and dark growth conditions. Under both treatments, 14-KO1 and 14-KO2 presented a lesser shoot length in comparison to WT. No significant difference was observed between the genotypes in the first four days of evaluation under light (Fig 3A). In the dark, however, a significant difference was observed only after eight days of treatment (Fig 3B).

Similarly, the root length of all genotypes under both light and dark growth conditions were lesser when compared to WT seedlings. However, is important to note that

this significant difference among the genotypes was observed only after four days of treatment (Fig 2C and 2D). Interestingly, under dark condition, the transformed seedlings seemed to recover this lesser elongation. While root length of 14-KO1 and 14-KO2 were 3 and 2.6 times lesser than WT at 16 days after sown under light, the difference under dark was 2.5 and 1.9, respectively. Shoot length followed the same pattern with 2.9 and 2.4 times lesser than WT under light versus 1.9 and 1.7 under dark. These results indicate that the delay that were present in the germination phase continues in the seedling stage due to the OsPIF14 gene knockout.

### 5.3 OsPIF14 knockout affects photosynthetic pigments content

The content of all photosynthetic pigments of the mutants were lesser in comparison to WT (Fig. 4). Figure 4A, shows a lesser chlorophyll (Chl) *a* content of 14-KO1 and 14-KO2 lines in comparison do WT under light and dark conditions. The chl *b* content presented a similar response to that seen in Figure 4A (Fig. 4B). The chl *a*/*b* ratio presented no difference among the genotypes under light. Under dark, however, the transformed lines presented a tendency of having a bigger ratio when compared to WT (Fig. 4C). Similar to chl *a* and *b* content, the carotenoid content was lesser in the transformed lines under both light and dark conditions. However, is important to note that the carotenoid content of 14-KO1 and 14-KO2 presented no significant difference between the growth conditions. Together, these results suggest that PIF14 is important for the photosynthetic pigments content in light and dark-grown seedlings.

### 5.4 OsPIF14 knockout affects seed morphology and reserves content

To understand how the *OsPIF14* gene knockout is affecting seed germination, seed morphology and nutrient reserves were analyzed. The length and width of seeds with shell of knockout lines were lower than WT seeds (Fig. 5A and C). This difference was more pronounced in the 14-KO1 line than 14-KO2. Conversely, shelled seeds showed only a slight tendency of minor value in these parameters (Fig. 5B and D). Moreover, to verify if the transformation induces some negative effect in seed filling, the carbohydrate content was measured (Fig 7). KO-PIF14 lines showed lower starch content than the WT seeds (Fig. 8A). The total soluble sugars content, however, were higher in these mutant lines than the WT (Fig.

8B). Bearing in mind that sucrose is one of the main carbohydrates for starch synthesis, its content was evaluated and a lower starch content in the seeds of transformed lines was observed. It confirms that somehow sucrose is not been breaking down to form starch. Together, these results indicate that the KO-PIF14 lines have a lesser nutrient reserve, which may affect seed morphology. Therefore, seed germination rate and seedling elongation and were strongly affected by the knocked out of *OsPIF14* gene.

### 5.5 PIF14 interacts with grain filling-related proteins *in silico*

In order to have a big picture of PIF14 function *in silico* analysis were carried out. During these analysis, two proteins related to starch accumulation were identified (Fig. 8). The first protein AP2-1 (known as APETALA2-like protein 1), is described as a probable transcription factor, and probably involved in spikelet transition. Also, literature data shows that this protein acts as a regulator of starch biosynthesis especially during seed development (e.g. endosperm starch granules), and as a repressor of grain growth and type I starch synthesis genes expression (SEKHAR et al., 2015; FU; XUE, 2010).

The second protein, CYTOKININ-Protein RESPONSIVE GATA TRANSCRIPTION FACTOR 1 (CGA1), is described as a transcriptional regulator that specifically binds 5'-GATA-3' or 5'-GAT-3' motifs within gene promoters. Literature data shows that this protein regulates chloroplast development, modulates plant architecture (e.g. height, length and width of leaf blades, and flowering tillers production). It alsorepresses tillering, probably by modulating number of cells, promotes senescence and chlorophyll accumulation, and is involved in grain filling, panicle development and starch production (HUDSON et al., 2013).

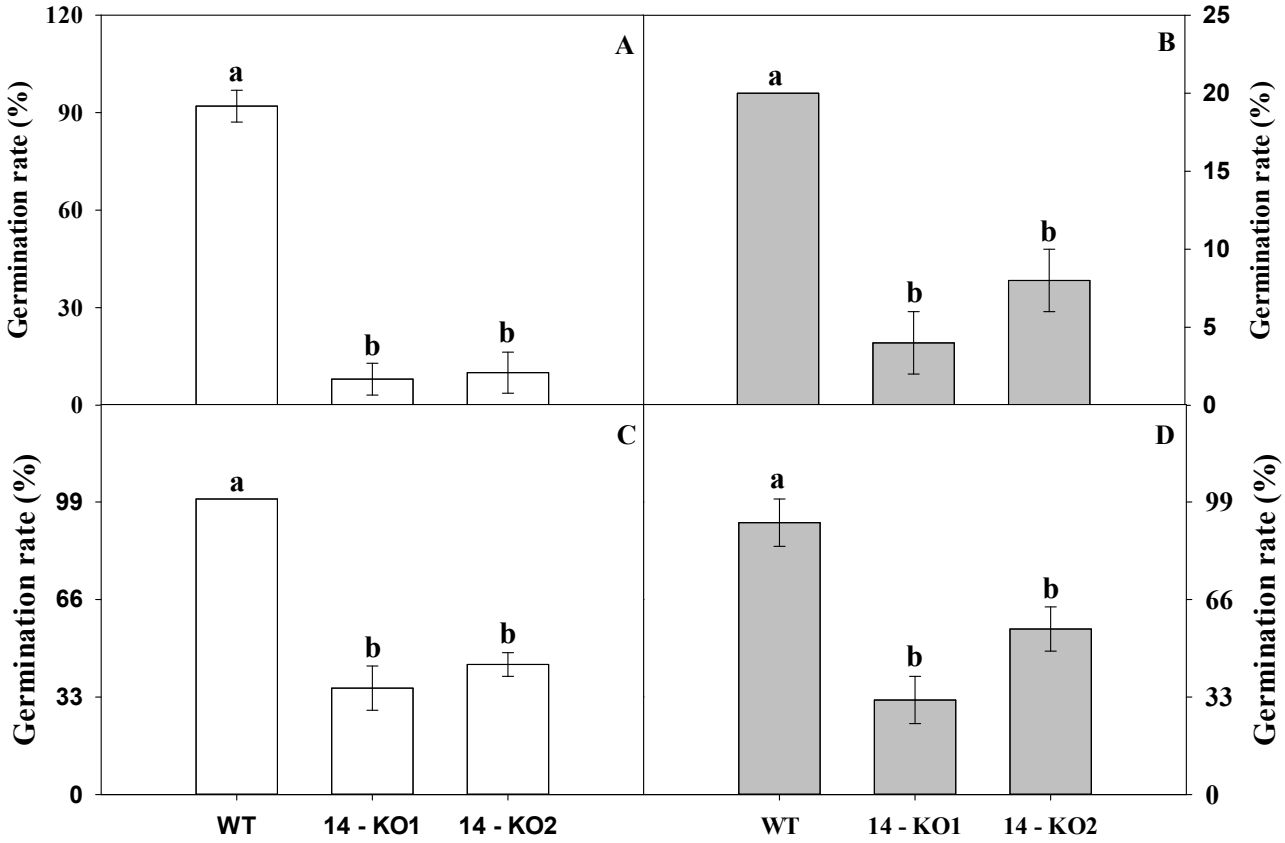


**Figure 1** – Germination rate of WT and transformed rice seeds under light and dark growth conditions. Germination rate after 24 h under light (A) and dark (B), and 48 h under light (C) and dark (D) conditions. Represented values indicate the average of five independent replicates (±SE). Different letters indicate significant differences at ($p \leq 0.05$) between WT and KO-PIF14 lines according to Tukey's test.

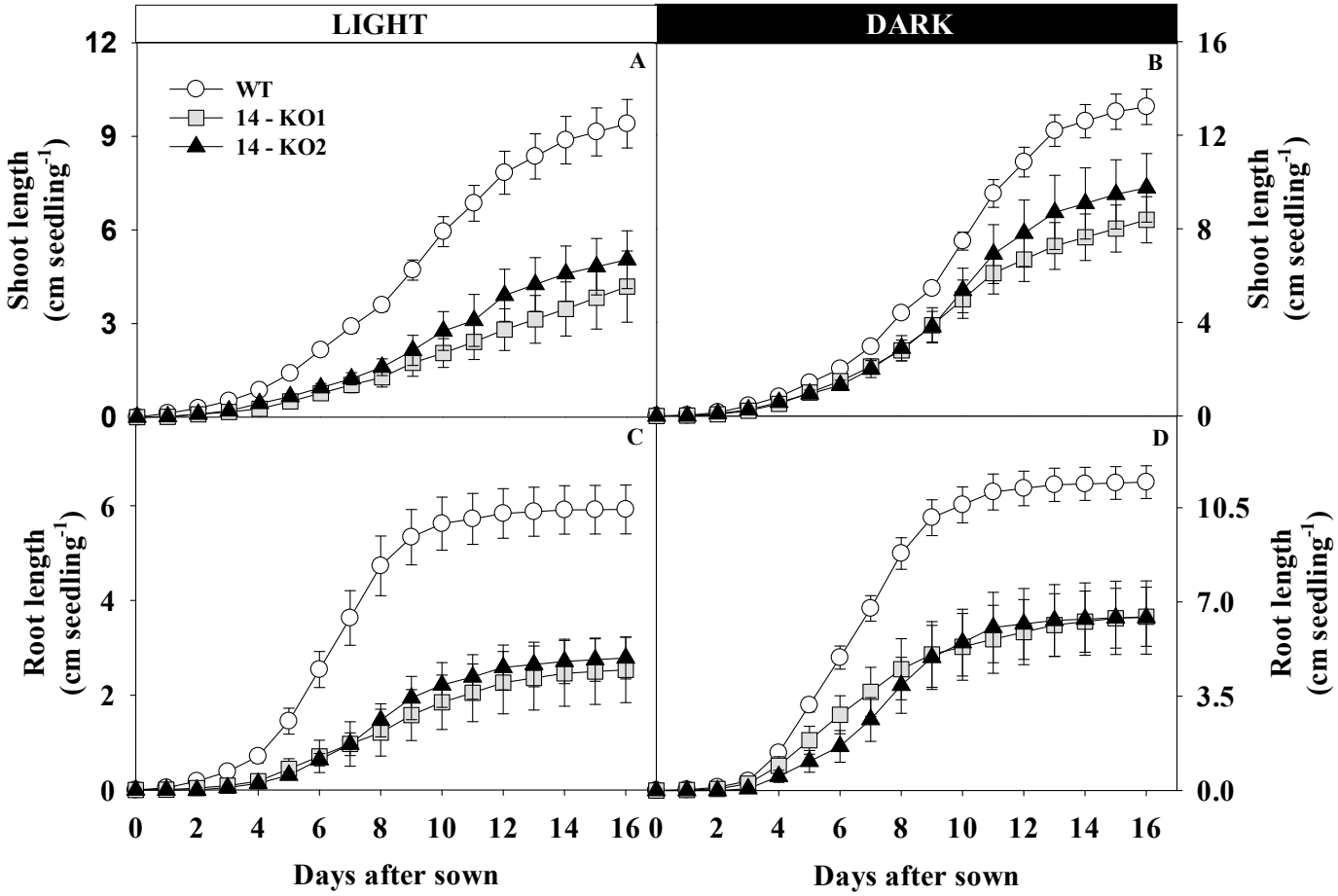


**Figure 2 – Seedling elongation of knockout *OsPIF14* gene (14-KO1 and 14-KO2) and wild type (WT) rice lines.** Shoot (A, B) and root (C, D) length under light (100 μmol $m^{-2}$ $s^{-1}$ - A, C) and dark (full darkness - B, D) growth conditions of 16-days-old WT and transformed rice lines.

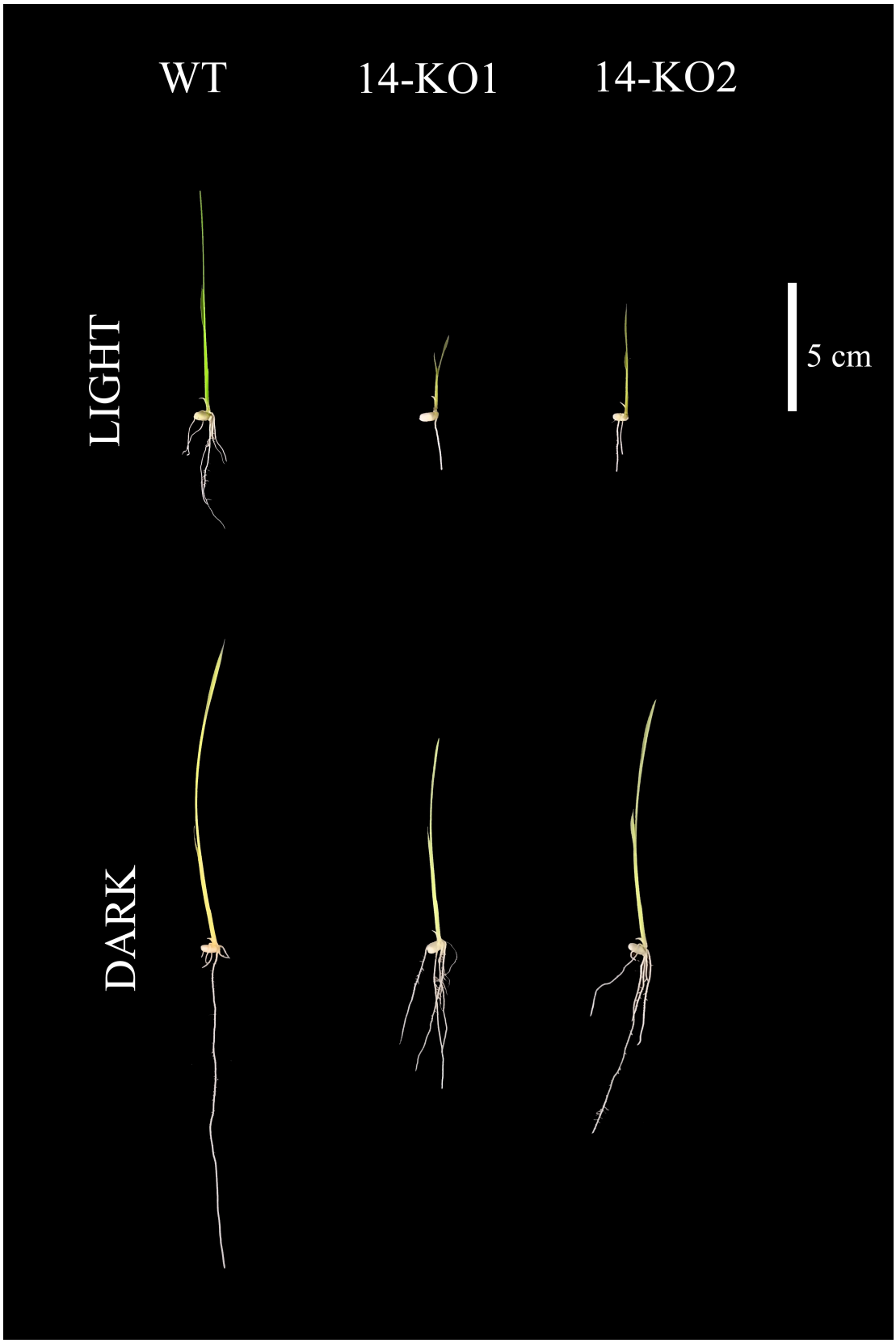


**Figure 3 – Morphological visual aspect of WT, 14-KO1 and 14-KO2 rice seedlings 16 days after sown.** 16-day-old light-grown rice seedlings at stage V2 (WT) and V1 (14-KO1 and 14KO2) (A), and dark-grown seedlings at stage V1 (WT, 14-KO1 and 14-KO2) (B).

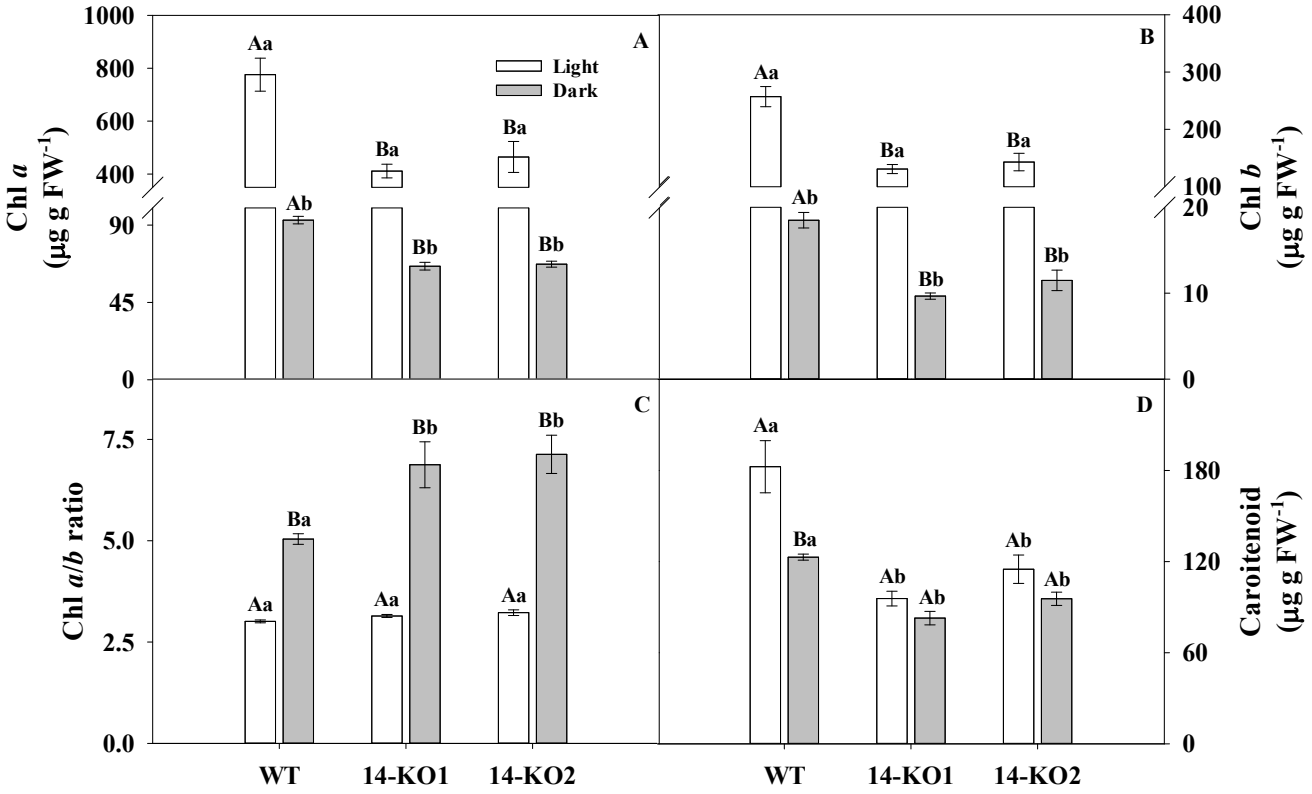


**Figure 4 – Photosynthetic pigments content of knockout PIF14 and WT rice lines.** Chl *a* (A), *b* (B), chl a/b ratio (C), and carotenoid content (D) of 16-days-old WT and transformed rice lines grown under light and dark conditions. Represented values indicate the average of four independent replicates (±SE). Different capital and lower-case letters indicate significant differences among treatment and genotype, respectively, at ($p \leq 0.05$) between WT and KO-PIF14 lines according to Tukey's test.

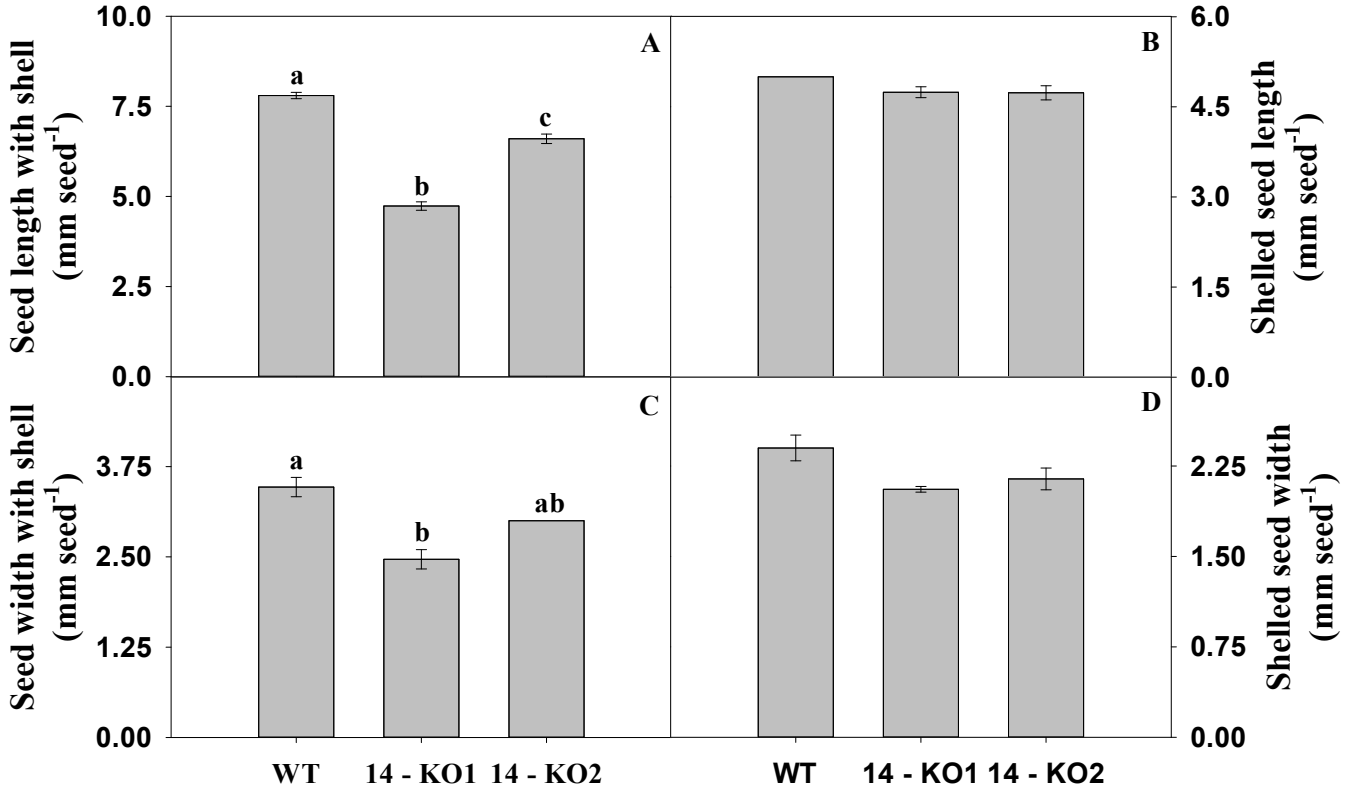


**Figure 5 – Seed morphology of knockout PIF14 rice lines and WT.** Seed length of seeds with shell (A), shelled seeds, and seed width of seeds with shell (C) and shelled seeds (D). Represented values indicate the average of 25 independent replicates (±SE). Different letters indicate significant differences at ($p \leq 0.05$) between WT and KO-PIF14 lines according to Tukey's test.

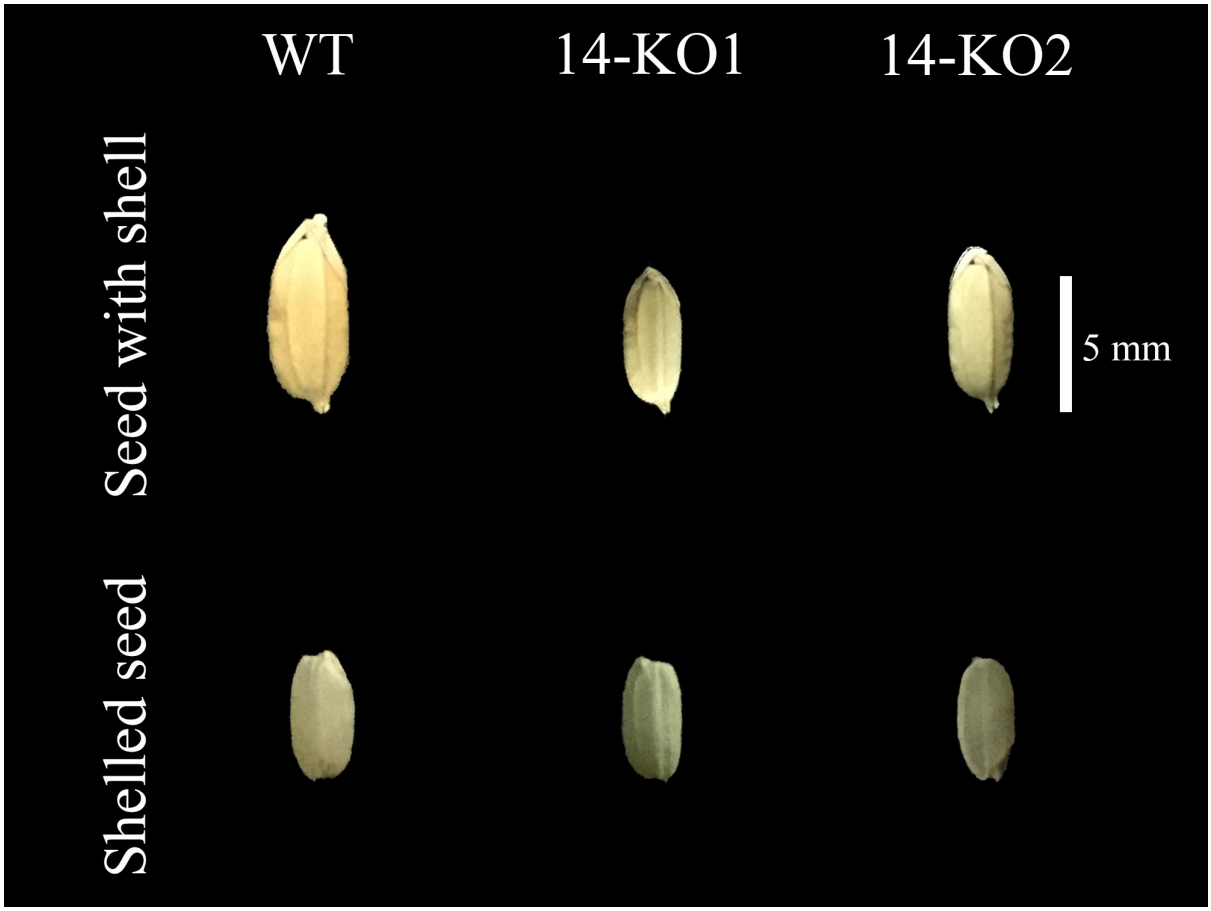


**Figure 6** – **Morphological visual aspect of WT, 14-KO1 and 14-KO2 rice quiescent seeds.** Seeds with shell (A) and shelled seeds (B) of WT and transformed rice lines.

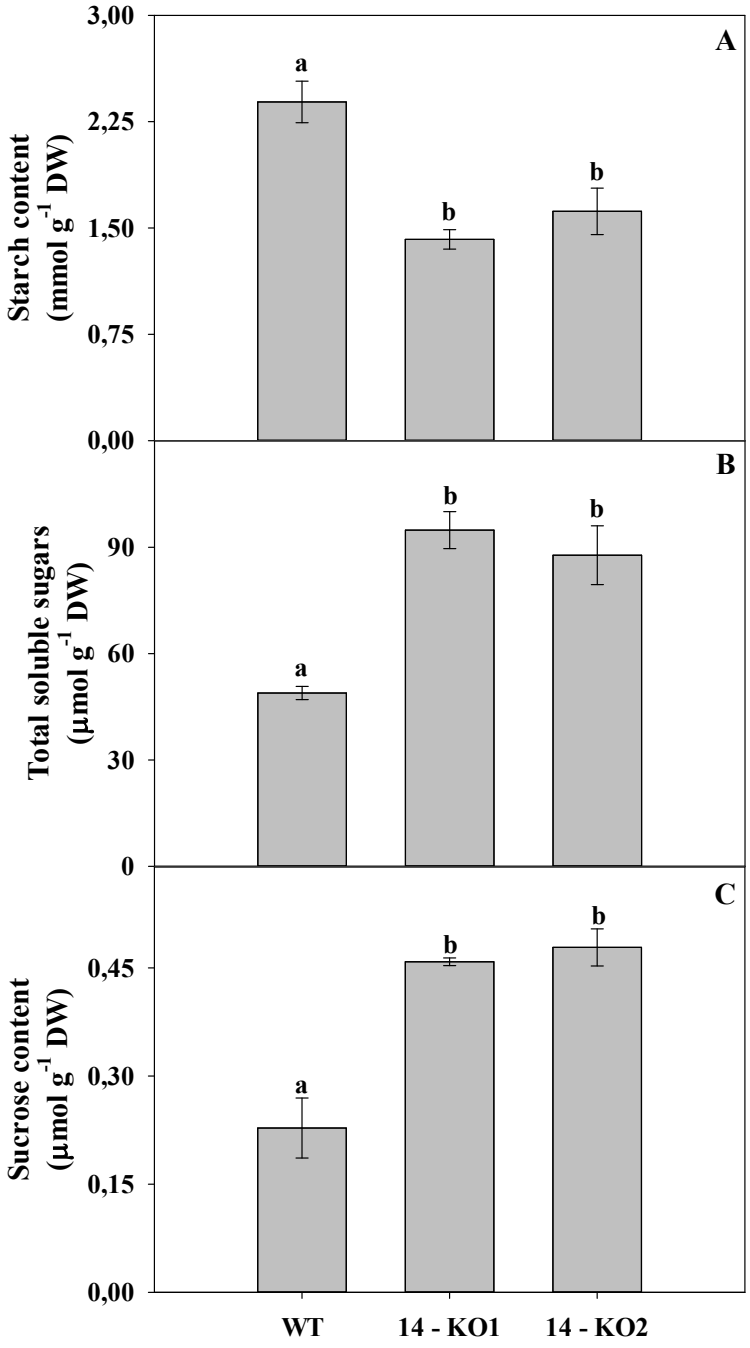


**Figure 7 – Carbohydrate content of PIF14 transformed lines and wild-type rice seeds.** Starch (A), total soluble sugars (B) and sucrose content (C) in PIF14 and wild-type quiescent grains. Represented values indicate the average of four independent replicates (±SE). Different letters indicate significant differences at ($p \leq 0.05$) between WT and KO-PIF14 seeds according to Tukey's test.

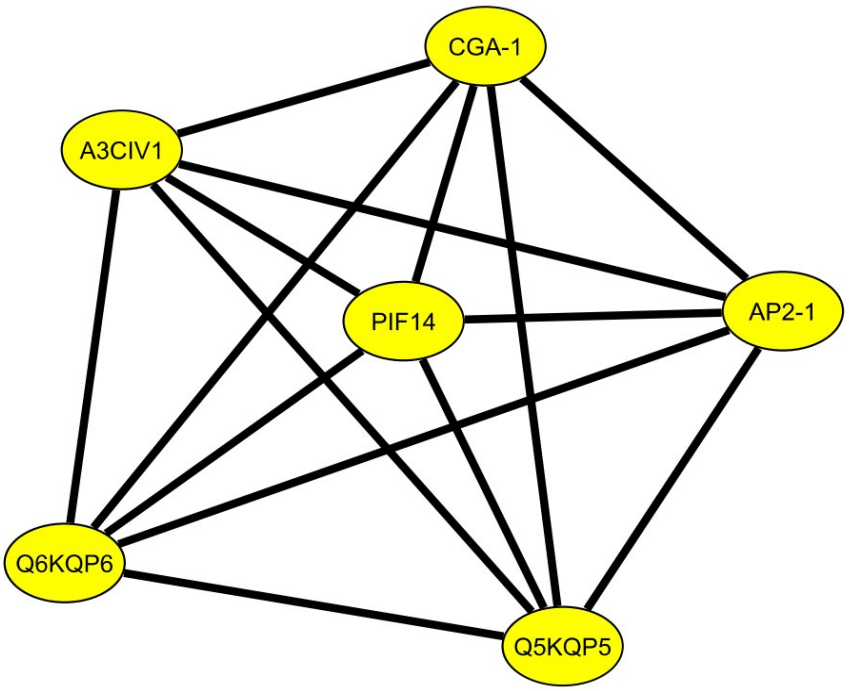


**Figure 8** – **PIF14 interacts with starch accumulation-related proteins *in silico*.** *In silico* protein-protein interaction network of PIF14

# 6 DISCUSSION

Seed dormancy and germination are processes of extreme relevance for a successful seedling establishment in the field. Over time, plants have evolved in a way that they developed a system adapted to the promotion of germination that occurs only under favorable environmental conditions that allows growth and survival. This system is controlled by environmental signals including light intensity, temperature, water availability and nutrient storage (BOTTO; SÁNCHEZ; CASAL, 1998; DERKX; KARSSEN, 1993; STEFANELLO; VIANA; DAS NEVES, 2017; ZUCARELI; HENRIQUE; ONO, 2015). In particular, light is a very important factor in seed germination since it activates light-dependent germination pathways for a successful growth (LEIVAR; MONTE, 2014). During seedling emergence, the seedling finishes its dependence of nutrient storage and starts the photosynthetic active (FORCELLA et al., 2000), being more dependent of light.

As described above, figure 1 shows the germination percentage of WT and OsPIF14 gene knockout rice lines. As expected, WT seeds presented a positively pronounced germination under light, while it was negatively affected by dark. On the other hand, the response observed in 14-KO1 and 14-KO2 lines is very interesting. Even though they have been grown under these two different conditions, no significant difference was observed between the genotypes among the growth conditions.

It is well stablished that starch is the main component of rice grains. The sucrose produced in leaves is transported for heterotrophic organs to be used as a carbon source for starch synthesis in amyloplasts (WEI et al., 2017). In this study, the seed morphology of 14-KO1 and 14-KO2 lines showed different morphology (Fig. 5 and 6). Moreover, the carbohydrate content of the transformed lines showed significant lesser content of starch, while a higher soluble sugars content (Fig. 7). All these observations suggest that KO-PIF14 transformed lines presents a defect in starch synthesis.

Furthermore, *in silico* analysis showed that PIF14 can interact with two grain filling-related proteins (Fig. 8). Both, APETALA and CGA1, are described as involved with starch accumulation (REFERENCE). Altogether, it suggests that PIF14 may regulate grain filling in rice. Since grain filling is a process that occurs during the reproductive stage, the i*n vivo* regulation of these proteins by PIF14 might be stage dependent.

Seed germination is a very important developmental event in plants since it is the first step of plant life cycle. However, for its proper transition from quiescent seed to germinating seed, the environmental factors in which the seed is into might allow it to

germinate (GARCIA; BARRETO; BICALHO, 2020). Besides, intrinsic seed factors, also have its importance in seed germination. The amount of carbohydrate is one of the main intrinsic factors for seed germination, by granting the seed energy for the heterotrophic growth (ZHAO et al., 2018). Hormones also play important roles in germination-related processes, since they are one of the main steps in seed commitment to germination (MIRANSARI; SMITH, 2014; PENFIELD, 2017).

The function of PIF proteins and their interaction in controlling the several plant metabolic processes have been extensively studied. However, the roles of these transcription factors in seed germination, are still largely unknown. Among the rice PIF/PIL proteins, only PIL15 was related to grain filling (JI et al., 2019; SUN et al., 2020). Following this feature, KO-PIF14 lines presented a lesser starch content, which explains the minor germination observed (Fig. 1).

However, other mechanisms, such as hormone regulation, can also be involved in this response. Abscisic acid and gibberellin are main hormones known to be directly related with the seed commitment to germination and reserves utilization (LIU et al., 2016; YANG et al., 2018). Besides, Frey et al., (1999) showed that the manipulation of seed ABA content through the overexpression of a xanthophyll-cycle-related gene increases seed dormancy while the knockout increases seed germination in *Nicotiana plumbaginifolia*.

Arabidopsis PIFs have also been extensively related with hormone regulation (PAIK et al., 2017). Interestingly, Mo et al. (2020) reported that KO-PIF14 lines showed no significant difference in germination when compared to WT seeds. However, different from the present work, the seeds germinated in a supplemented medium, what can explain the difference between our results and the presented by Mo et al. (2020). Besides, they showed that KO-PIF14 phenotype can be recovered with nutrient supplementation.

Nakamura et al., (2007) showed that the heterologous expression of OsPIF/PIL genes represses photomorphogenesis in Arabidopsis. However, the roles of the rice PIF/PIL family can differ. While overexpressing *OsPIL13* lines present an increased internode growth (TODAKA et al., 2012), *OsPIL15* overexpression represses seedling growth in the dark (ZHOU et al., 2014). It is known that cells grow by division or expansion. The cell expansion is explained through the acid-growth hypothesis.

This theory explains that the indole-3-acetic acid, an auxin, induces proton extrusion into the cell apoplast and it activates a range of enzymatic reactions which modifies the extensibility of plant cell walls (ARSUFFI; BRAYBROOK, 2018). The cell division is explained by the division-cell cycle theory, in which a cell leads to duplication of its own

DNA, division of cytoplasm and organelles through a series of cellular processes to produce two descendent cells (RASMUSSEN; BELLINGER, 2018).

Zhou et al. (2014) showed that the overexpression of *OsPIL15* gene inhibits seedling growth probably by regulating the auxin pathway and negatively regulates cell wall modifying enzymes. Given that, PIF14 is a repressor (CORDEIRO et al., 2016), the lesser shoot and root observed in the transformed lines (Fig. 2 and 3) could be due to the repression of some gene that negatively regulates cell expansion. It is possible, since the absence of it makes the seedlings less capable of growing under both conditions.

However, Mo et al. (2020) showed that overexpressing OsPIL14 lines presented elongated mesocotyls while knockout lines did not show mesocotyl phenotype in rice under dark condition. In accordance with it, they showed that *OsPIL14* regulates the expression of cell elongation-related genes. Therefore, the phenotype observed in the present work is probably caused by the minor carbohydrate content in the seed and consequent lesser seed germination.

Chlorophylls are the main pigments involved in light harvesting for photosynthesis. Given its importance for plant development and surviving, several studies have already shown the steps related to chlorophyll biosynthesis (WANG et al., 2020; ZHANG et al., 2019; ZHOU et al., 2017). Still, little is known about the transcriptional and posttranscriptional regulation of this process. Arabidopsis PIF1 and PIF3 are transcription factors described as negative regulators of chlorophyll biosynthesis. It occurs through the directly binding of PIF1 and PIF3 to the specific promoter sequence of many reactive oxygen species-responsive genes, affecting its expression (CHEN et al., 2013; STEPHENSON; FANKHAUSER; TERRY, 2009).

In rice, Li et al., (2019) showed that PIF14 regulates chlorophyll biosynthesis by directly controlling *OsFLU1* gene expression. Therefore, the lesser chlorophyll content in the transformed lines has precedent (Fig. 4). Concerning to carotenoids, Arabidopsis PIFs were shown related to its biosynthesis (BOU-TORRENT et al., 2015; TOLEDO-ORTIZ; HUQ; RODRÍGUEZ-CONCEPCIÓN, 2010). Moreover, PIF14 was reported as interacting with DELLA proteins (MO et al., 2020). These proteins are known to regulate chlorophyll and carotenoid biosynthesis (CHEMINANT et al., 2011).

# 7 CONCLUSIONS

Overall, KO-PIF14 rice transformed lines displayed similar delays in germination and seedling development under both light and dark conditions. This feature was also observed in pigments content. However, is important to note, in response to light, 14-KO1 was more affected than 14-KO2. Besides, seed morphology and reserves content of the knockout lines were also affected. Our results suggest the presence of PIF14 is important for germination and seedling development, including the photosynthetic pigments content. The impairment in sugars content could lead to the minor germination percentage and culminate in a reduced seedling elongation. It also suggests that PIF14-phyB interaction and its downstream reactions are essential for these processes. However, further studies are necessary to show, in specific, which metabolic mechanisms were affected by the knockout of *Os*PIF14 gene in rice. Based on these results, we propose the following figure, where the knockout of OsPIF14 impairs rice life cycle through different possible processes.

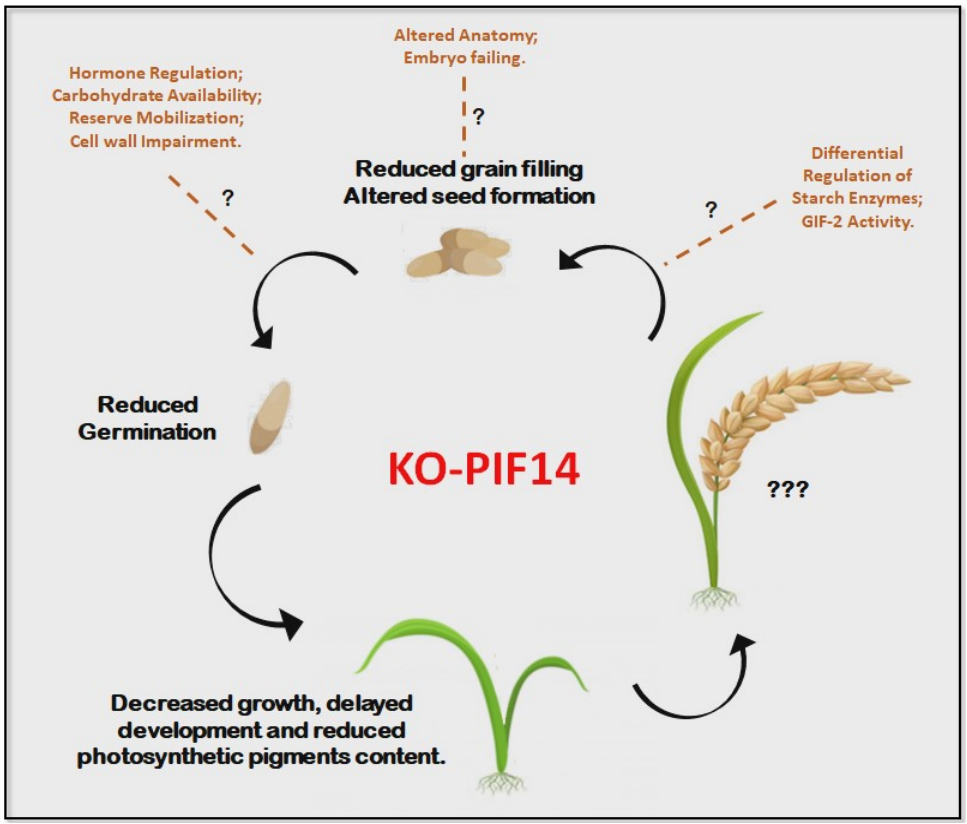


**Figure 9** – **Impaired life cycle of *OsPIF14* gene knockout rice plants** KO-PIF14 rice plants present an altered seed morphology and reduced seed germination rate. It results in a reduced seedling elongation and development. In addition, the knockout seeds presented a minor carbohydrate content in the grain, confirming a seed malformation. This impairment in sugars content could culminate in the minor germination percentage, closing a physiological cycle.

**REFERENCES**

ARSUFFI, G.; BRAYBROOK, S. A. Acid growth: An ongoing trip. **Journal of Experimental Botany**, v. 69, n. 2, p. 137–146, 2018.

BOTTO, J. F.; SÁNCHEZ, R. A.; CASAL, J. J. Burial conditions affect light responses of Datura ferox seeds . **Seed Science Research**, v. 8, n. 4, p. 423–429, 1998.

BOU-TORRENT, J. et al. Regulation of carotenoid biosynthesis by shade relies on specific subsets of antagonistic transcription factors and cofactors. **Plant Physiology**, v. 169, n. 3, p. 1584–1594, 2015.

CHEMINANT, S. et al. DELLAs regulate chlorophyll and carotenoid biosynthesis to prevent photooxidative damage during seedling deetiolation in Arabidopsis. **Plant Cell**, v. 23, n. 5, p. 1849–1860, 2011.

CHEN, D. et al. Antagonistic basic helix-loop-Helix/bZIP transcription factors form transcriptional modules that integrate light and reactive oxygen species signaling in Arabidopsis. **Plant Cell**, v. 25, n. 5, p. 1657–1673, 2013.

CORDEIRO, A. M. et al. Rice phytochrome-interacting factor protein OsPIF14 represses OsDREB1B gene expression through an extended N-box and interacts preferentially with the active form of phytochrome B ☆. **Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms**, v. 1859, n. 2, p. 393–404, 2016.

DE LUCAS, M. et al. A molecular framework for light and gibberellin control of cell elongation. **Nature**, v. 451, n. 7177, p. 480–484, 2008.

DERKX, M. P. M.; KARSSEN, C. M. Changing sensitivity to light and nitrate but not to gibberellins regulates seasonal dormancy patterns in Sisymbrium officinale seeds. **Plant, Cell & Environment**, v. 16, n. 5, p. 469–479, 1993.

FENG, S. et al. Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. **Nature**, v. 451, n. 7177, p. 475–479, 2008.

FORCELLA, F. et al. Modeling seedling emergence. **Field Crops Research**, v. 67, n. 2, p. 123–139, 2000.

FRANKLIN, K. A. et al. regulates auxin biosynthesis at high temperature. v. 108, n. 50, p. 20231–20235, 2011.

FRANKLIN, K. A.; QUAIL, P. H. Phytochrome functions in Arabidopsis development. **Journal of Experimental Botany**, v. 61, n. 1, p. 11–24, 2010.

FREY, A. et al. Engineering seed dormancy by the modification of zeaxanthin epoxidase gene expression. **Plant Molecular Biology**, v. 39, n. 6, p. 1267–1274, 1999.

FU, F. F.; XUE, H. W. Coexpression analysis identifies Rice Starch Regulator1, a rice AP2/EREBP family transcription factor, as a novel rice starch biosynthesis regulator. **Plant Physiology**, v. 154, n. 2, p. 927-938, 2010.

GALVÃO, V. C.; FANKHAUSER, C. Sensing the light environment in plants: Photoreceptors and early signaling steps. **Current Opinion in Neurobiology**, v. 34, n. Figure 1, p. 46–53, 2015.

GARCIA, Q. S.; BARRETO, L. C.; BICALHO, E. M. Environmental factors driving seed dormancy and germination in tropical ecosystems: A perspective from campo rupestre species. **Environmental and Experimental Botany**, v. 178, n. February, p. 104164, 2020.

GOMMERS, C. M. M.; MONTE, E. Seedling establishment: A dimmer switch-regulated process between dark and light signaling. **Plant Physiology**, v. 176, n. 2, p. 1061–1074, 2018.

HADAS, A. Germination and Seedling Establishment. **Encyclopedia of Soils in the Environment**, v. 4, p. 130–137, 2004.

HUDSON, D. et al. Rice cytokinin GATA transcription Factor1 regulates chloroplast development and plant architecture. **Plant physiology**, v. 162, n. 1, p. 132-144, 2013.

JI, X. et al. The basic helix-loop-helix transcription factor, OsPIL15, regulates grain size via directly targeting a purine permease gene OsPUP7 in rice. **Plant Biotechnology Journal**, v. 17, n. 8, p. 1527–1537, 2019.

JOSSE, E. M.; HALLIDAY, K. J. Skotomorphogenesis: The Dark Side of Light Signalling. **Current Biology**, v. 18, n. 24, p. R1144–R1146, 2008.

KONG, S. G.; OKAJIMA, K. Diverse photoreceptors and light responses in plants. **Journal**

**of Plant Research**, v. 129, n. 2, p. 111–114, 2016.

LEIVAR, P.; MONTE, E. PIFs: Systems integrators in plant development. **Plant Cell**, v. 26, n. 1, p. 56–78, 2014.

LEIVAR, P.; QUAIL, P. H. PIFs: pivotal components in a cellular signaling hub. **Trends in Plant Science**, v. 16, n. 1, p. 19–28, 2011.

LI, L. et al. Expression Patterns of OsPIL11, a Phytochrome-Interacting Factor in Rice, and Preliminary Analysis of Its Roles in Light Signal Transduction. **Rice Science**, v. 19, n. 4, p. 263–268, 2012.

LI, L. et al. PIL1 Participates in a Negative Feedback Loop that Regulates Its Own Gene Expression in Response to Shade. **Molecular Plant**, v. 7, n. 12, p. 1805–1806, 2014.

LI, Z. et al. Rice FLUORESCENT1 Is Involved in the Regulation of Chlorophyll. **Plant and Cell Physiology**, v. 60, n. 10, p. 2307–2318, 2019.

LICHTENTHALER, H. K.; WELLBURN, A. R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents . **Biochemical Society Transactions**, v. 11, n. 5, p. 591–592, 1983.

LIU, X. et al. The NF-YC-RGL2 module integrates GA and ABA signalling to regulate seed germination in Arabidopsis. **Nature Communications**, v. 7, 2016.

MIRANSARI, M.; SMITH, D. L. Plant hormones and seed germination. **Environmental and Experimental Botany**, v. 99, p. 110–121, 2014.

MO, W. et al. PHYTOCHROME-INTERACTING FACTOR-LIKE14 and SLENDER RICE1 interaction controls seedling growth under salt stress. **Plant Physiology**, 2020.

NAKAMURA, Y. et al. Characterization of a set of phytochrome-interacting factor-like bHLH proteins in Oryza sativa. **Bioscience, Biotechnology and Biochemistry**, v. 71, n. 5, p. 1183–1191, 2007.

PAIK, I. et al. Expanding Roles of PIFs in Signal Integration from Multiple Processes. **Molecular Plant**, v. 10, n. 8, p. 1035–1046, 2017.

PENFIELD, S. Seed dormancy and germination. **Current Biology**, v. 27, n. 17, p. R874–

R878, 2017.

PHAM, V. N.; KATHARE, P. K.; HUQ, E. Phytochromes and Phytochrome Interacting Factors 1 [ OPEN ]. v. 176, n. February, p. 1025–1038, 2018.

PHAM, V. N.; XU, X.; HUQ, E. Molecular bases for the constitutive photomorphogenic phenotypes in arabidopsis. **Development (Cambridge)**, v. 145, n. 23, 2018.

QIU, Y. Regulation of PIF4-mediated thermosensory growth. **Plant Science**, v. 297, n. March, p. 110541, 2020.

RASMUSSEN, C. G.; BELLINGER, M. An overview of plant division-plane orientation. **New Phytologist**, v. 219, n. 2, p. 505–512, 2018.

SEKHAR, S. et al. Spikelet-specific variation in ethylene production and constitutive expression of ethylene receptors and signal transducers during grain filling of compact-and lax-panicle rice (Oryza sativa) cultivars. **Journal of Plant Physiology**, v. 179, p. 21-34, 2015.

STEFANELLO, R.; VIANA, B. B.; DAS NEVES, L. A. S. Germination and vigor of linseed seeds under different conditions of light, temperature and water stress. **Semina:Ciencias Agrarias**, v. 38, n. 3, p. 1161–1168, 2017.

STEPHENSON, P. G.; FANKHAUSER, C.; TERRY, M. J. PIF3 is a repressor of chloroplast development. **Proceedings of the National Academy of Sciences**, v. 106, n. 18, p. 7654–7659, 2009.

SUN, W. et al. OsmiR530 acts downstream of OsPIL15 to regulate grain yield in rice. **New Phytologist**, v. 226, n. 3, p. 823–837, 2020.

TODAKA, D. et al. Rice phytochrome-interacting factor-like protein OsPIL1 functions as a key regulator of internode elongation and induces a morphological response to drought stress. **Proceedings of the National Academy of Sciences of the United States of America**, v. 109, n. 39, p. 15947–15952, 2012.

TOLEDO-ORTIZ, G.; HUQ, E.; RODRÍGUEZ-CONCEPCIÓN, M. Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors. v. 107, n. 25, p. 11626–11631, 2010.

UM, T. Y. et al. Jasmonate zim-domain protein 9 interacts with slender rice 1 to mediate the antagonistic interaction between jasmonic and gibberellic acid signals in rice. **Frontiers in Plant Science**, v. 871, n. December, p. 1–11, 2018.

WANG, P. et al. Post-translational coordination of chlorophyll biosynthesis and breakdown by BCMs maintains chlorophyll homeostasis during leaf development. **Nature Communications**, v. 11, n. 1, 2020.

WEI, X. et al. GRAIN INCOMPLETE FILLING 2 regulates grain filling and starch synthesis during rice caryopsis development. **Journal of Integrative Plant Biology**, v. 59, n. 2, p. 134–153, 2017.

YANG, N. et al. The inhibited seed germination by ABA and MeJA is associated with the disturbance of reserve utilizations in astragalus membranaceus. **Journal of Plant Interactions**, v. 13, n. 1, p. 388–397, 2018.

ZHANG, S. et al. Structural basis for enzymatic photocatalysis in chlorophyll biosynthesis. **Nature**, v. 574, n. 7780, p. 722–725, 2019.

ZHAO, M. et al. Mobilization and role of starch, protein, and fat reserves during seed germination of six wild grassland species. **Frontiers in Plant Science**, v. 9, n. February, p. 1–11, 2018.

ZHOU, F. et al. A recruiting protein of geranylgeranyl diphosphate synthase controls metabolic flux toward chlorophyll biosynthesis in rice. **Proceedings of the National Academy of Sciences of the United States of America**, v. 114, n. 26, p. 6866–6871, 2017.

ZHOU, J. et al. Overexpression of OsPIL15 , a phytochrome - interacting factor - like protein gene , represses etiolated seedling growth in rice. **Journal of integrative plant biology**, v. 56, n. 4, p. 373–387, 2014.

ZUCARELI, V.; HENRIQUE, L. A. V.; ONO, E. O. Influence of light and temperature on the germination of Passiflora incarnata L. seeds. **Journal of Seed Science**, v. 37, n. 2, p. 162–167, 2015.