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FACULDADE DE CIÊNCIAS AGRONÔMICAS
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DENISE PUNTEL BASSO

CONTRIBUTION TO THE CHARACTERIZATION OF THE ACQUISITION OF THE EMERGENCY VIGOR DURING SEED MATURATION

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Thesis submitted to São Paulo State University (UNESP), School of Agriculture, Botucatu, to obtain the title of Doctor of Agronomy (Agriculture).

Supervisor: Prof. PhD Edvaldo Aparecido Amaral da Silva

Supervisor: Prof. PhD Olivier Leprince

Botucatu

2018

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉCNICA DE AQUISIÇÃO E TRATAMENTO DA INFORMAÇÃO – SERVIÇO TÉCNICO DE BIBLIOTECA E DOCUMENTAÇÃO - UNESP – FCA – LAGEADO – BOTUCATU (SP)

Basso, Denise Puntel, 1984-
B322c Contribuição da caracterização da aquisição do vigor de emergência durante a maturação de semente / Denise Puntel Basso. – Botucatu : [s.n.], 2018
183 p. : fots. color., ils. color., grafs. color., tabs.

Tese (Doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, Botucatu e Agrocampus Ouest, França, 2018
Orientador: Edvaldo Aparecido Amaral da Silva
Coorientador: Olivier Leprince
Inclui bibliografia

1. Maturação. 2. Alongamento. 3. Fator de choque térmico. 4. Vigor. 5. Qualidade de sementes. I. Silva, Edvaldo Aparecido Amaral da. II. Leprince, Olivier. III. Universidade Estadual Paulista "Júlio de Mesquita Filho" (Campus de Botucatu). Faculdade de Ciências Agronômicas. IV. Agrocampus Ouest. V. Título.

Elaborada por Ana Lucia G. Kempinas – CRB-8:7310

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: CONTRIBUTION THE CHARACTERIZATION OF THE ACQUISITION OF THE EMERGENCY VIGOR DURING SEED MATURATION

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ORIENTADOR: EDVALDO APARECIDO AMARAL DA SILVA

Aprovada como parte das exigências para obtenção do Título de Doutora em AGRONOMIA (AGRICULTURA), pela Comissão Examinadora:

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Prof. Dr. JOSÉ BALDIN PINHEIRO
Departamento de Genética / ESALQ/USP

Botucatu, 22 de maio de 2018

AGRADECIMENTOS

Ao meu orientador Dr. Edvaldo A. Amaral da Silva (Brasil), sinto eternamente grata por ter me escolhido e ter me dado a oportunidade de fazer parte do doutorado em Angers-França. Pela valiosa orientação, paciência, e pelo encorajamento entusiasmado.

Gostaria de expressar minha gratidão ao meu orientador Dr. Olivier Leprince (França), por todos ensinamentos, obrigada pelas valiosas contribuições a este trabalho de pesquisa, eu aprendi muito com sua orientação durante meu doutorado.

A Dra. Julia Buitink, muito obrigada por todos seus conselhos, você é o meu exemplo como pesquisadora, sou profundamente grata por ter tido a oportunidade de ter convivido e compartilhado bons momentos ao seu lado.

Dr. Joao Nakagawa, muito obrigada pelas incansáveis discussões sobre ciência, você é uma pessoa admirável, obrigada também por ter contribuído para o meu crescimento pessoal e profissional.

As minhas queridas pós-doutorandas Andrea Akemi e Souha Berriri, pela orientação, pela ajuda em todos os momentos durante o desenvolvimento do projeto, pela amizade. Vocês foram simplesmente maravilhosas.

A todos os professores, funcionários, técnicos do Departamento de Produção e Melhoramento Vegetal – Agricultura, Dr. Maurício Zanotto, Dra. Márcia Sartori, Dorival Arruda, Thiago Gianeti, Eliane, Adelina, obrigada por toda ajuda.

Em especial a técnica do Laboratório de sementes, Valéria Giandoni por todos momentos que passei ao seu lado durante meu doutorado, pelo incentivo diário e motivação e auxilio na execução do projeto.

Aos funcionários da Fazenda Lageado, Célio Mariano, Casimiro Alves, Aparecido da Silva, Mateus, Antônio Camargo e Ciro de Oliveira, pela essencial contribuição nas atividades e experimento no campo.

Durante a labuta na pós-graduação, fui abençoada com um grupo amigável e alegre de colegas (Pós doutores, doutorandos, mestrandos e graduandos) do laboratório de análises de sementes que colaboraram muito na realização desse trabalho, foram exaustivos finais de semanas e feriados coletando sementes, com vocês eu aprendi o verdadeiro valor do trabalho em equipe, vocês sempre me apoiaram nos momentos que mais precisei. Gratidão a todos: Amanda Bedetti

Thiago Batista, Tiago Alexandre, Girlânio Holanda, Matheus Kainan, Vitória Pires, Letícia Moreno, Karina Renostro, Camila Aquino, Maria Rita Gilli, Bárbara Panoff, Julio Maia, Daiane Ajala, Camila Amaro, Hellen Síglia, Rubiana Rossi, Pedro Bento, Juliana Bravo, Gabriel, Alexandre Carneiro, Vitor Maia, Camila Casarini, todos os estagiários e alunos do Colégio Maria Vitória.

Participar do programa de doutorado sanduíche foi um dos melhores momentos da minha vida. Quando cheguei à França fui muito bem recebida por todos da equipe Conserto Benoit Ly Vu, Joseph Ly Vu, Jérôme Verdier, David Lalanne, laeticia Ly Vu, Julia Zinsmeister. Obrigada por sempre me auxilar na minha pesquisa e pela amizade de todos vocês.

Aos meus amigos e colegas de doutorado, vocês não só me permitiram melhorar meu Francês, mas também tornaram minha vida na França muito mais alegre, obrigada por todos pelos momentos que passamos juntos: Julia Courtial, Douae Doua, Justine Colou, lydia Casasni, Elodie Belmas, Quentin Massiquot, Lili, Mathieu Marc, Kevin, Alexis Porcher. Em especial minha colega de escritório Elise Réthoré que esteve comigo durante toda minha estadia na França, sempre me motivando, amei ter conhecido você.

Aos amigos que conheci na França, que tornaram meus dias mais felizes: Pascale Satour, Daniel Beucher, Tanegmart, Hugo Hagmann, Maria Maisto, Giulia Saffirio, Jefferson Cavalcante, Tiago lodi, Agathe, Marlon Gomes, Vanessa Lancon, Sandra Ly Vu, Etienne. Aos amigos que Botucatu me proporcionou, Marina Mouzinho, Priscila Picolé, Alexandre Manzano, Iara Brito, Letícia Mendonca e Andréia.

Finalmente, gostaria de agradecer a minha família, pelo apoio incondicional e encorajamento durante todo o meu estudo.

ACKNOWLEGDGEMENTS

I am eternally grateful to my advisor, Dr. Edvaldo A. Amaral da Silva (Brazil) for having chosen me and given me the opportunity to be a PhD student in Angers-France. Thank you for invaluable guidance, patience and enthusiastic encouragement.

I would like to express my gratitude to my mentor Dr. Olivier Leprince (France), for all of your teachings and patience. Thank you for the valuable contributions to this research. I have improved much with your guidance during my doctorate.

Dr. Julia Buitink, thank you very much for all of your advice, you are my example as a researcher. I am deeply grateful for having the opportunity to live together and share good times by your side.

Dr. Joao Nakagawa, thank you for the untiring discussions on science, you are an admirable person. Thank you also for contributing to my personal and professional growth.

My dear Postdocs Andrea Akemi and Souha Berriri, thanks for your guidance and help during all the development of this work, and for your friendship. You were simply wonderful.

To all the professors, employees, and technicians of the Department of Production and Breeding - Agriculture, Dr. Maurício Zanotto, Dr. Márcia Sartori, Dorival Arruda, Thiago Gianeti, Eliane, Adelina, thank you for all your help.

Especially the technician of the seed laboratory, Valéria Giandoni for all the moments I spent with you during my doctorate, for daily encouragement and motivation, and help in the execution of the project.

To the employees of the Lageado farm, Célio Mariano, Casimiro Alves, Aparecido da Silva, Mateus, Antônio Camargo and Ciro de Oliveira, for the essential contribution in the activities during the field experiments.

During the post-graduate work, I was blessed with a friendly and cheerful group of colleagues (Post Doctors, PhD students, masters and undergraduates) from the seed analysis laboratory who collaborated so much for the development of this work. Thanks for spending holidays collecting seeds. With you I learned the true value of teamwork, you always supported me in the moments that I needed the most. Gratitude to all: Amanda Bedetti Thiago Batista, Tiago Alexandre, Girlânio Holanda, Matheus Kainan, Vitória Pires, Letícia Moreno, Karina Renostro, Camila

Aquino. Maria Rita Gilli, Bárbara Panoff, Julio Maia, Daiane Ajala, Camila Amaro, Hellen Síglia, Rubiana Rossi, Pedro Bento, Juliana Bravo, Gabriel, Alexandre Carneiro, Vitor Maia, Camila Casarini, all the trainees and students of the Colégio Maria Vitória.

Joining the sandwich doctorate program was one of the best moments of my life. When I arrived in France, I was very well received by all of the staff: Benoit Ly Vu, Joseph Ly Vu, Jérôme Verdier, David Lalanne, Laetitia Ly Vu, Julia Zinsmeister. Thank you for always helping me in my research and for your friendship.

To my PhD friends, you have not only allowed me to improve my French but have also made my life in France much more joyful. Thank you for all the moments we spent together: Julia Courtial, Douae Doua, Justine Colou, Elodie Belmas, Quentin Massiquot, Lili, Mathieu Marc, Kevin, Alexis Porcher, Lydia Casasni. Especially my office colleague Elise Réthoré who was with me during my whole stay in France, motivating me, it was lovely meeting you.

To the friends I met in France who have made my days happier: Pascale Satour, Daniel Beucher, Tanegmart, Abdelilah, Hugo Hagmann, Maria Maisto, Giulia Saffirio, Jefferson Cavalcante, Tiago lodi, Agathe, Marlon Gomes, Vanessa Lancon, Sandra Ly Vu, Etienne. To the friends that Botucatu has given to me, Marina Mouzinho, Priscila Picole, Alexandre Manzano, Iara Brito.

Finally, I would like to thank my family for their unconditional support and encouragement throughout my studies.

ABSTRACT

The stable production of high vigorous seeds regardless of the environment is a key lever to increase crop production. Seed vigor is defined as the sum of the physiological properties that lead to homogenous and vigorous seedling establishment. It includes longevity, defined as the capacity to remain viable for long periods during dry storage and the capacity to elongate after germination. However, how these traits are acquired during seed development and how the environment impacts their acquisition remain poorly understood. Yet this information is important to determine the harvest stage corresponding to maximum vigor. Using agronomy and physiological approaches on soybean and *Medicago truncatula*, we confirm that longevity is progressively acquired during seed maturation. In soybean, our data suggest that the climate influenced longevity whereas in Medicago, heat applied during seed maturation had no significant impact. This work also showed that HEAT SHOCK FACTOR A2.2, a homologue of HSFA9 and hub gene involved in seed maturation does not play a role in seed longevity but acts as negative regulator of embryonic dormancy. Longevity is evaluated by the ability to germinate after storage, which represents only a part of the success of crop establishment. How seed maturation affects the loss of seedling establishment capacity during storage was evaluated in soybean using an experimental system set up to assess elongation capacity. The pattern of acquisition of elongation capacity during maturation varied between crop years and growth conditions. The time to 50% loss of elongation capacity during storage was similar to that of loss of germination. Also, it increased steadily during seed maturation after mass maturity and harvest maturity stages, highlighting the importance of the late phase of seed maturation in building seed vigor.

Keywords: Maturation. Elongation. Heat shock factor. Vigor. Seed quality.

RESUMO

A produção de sementes altamente vigorosas, independentemente do ambiente, é uma alavanca fundamental para aumentar a produção agrícola. O vigor das sementes é definido como a soma das propriedades fisiológicas que levam ao estabelecimento homogêneo e vigoroso de plântulas. Inclui a longevidade, definida como a capacidade de permanecer viável por longos períodos durante o armazenamento a seco e a capacidade de alongamento após a germinação. No entanto, como essas características são adquiridas durante o desenvolvimento de sementes e como o ambiente impacta sua aquisição permanecem pouco compreendidas. No entanto, essa informação é importante para determinar o estágio de colheita correspondente ao vigor máximo. Utilizando abordagens agronômicas e fisiológicas em soja e *Medicago truncatula*, confirmamos que a longevidade é progressivamente adquirida durante a maturação das sementes. Em soja, nossos dados sugerem que o clima influenciou a longevidade, enquanto em Medicago o calor aplicado durante a maturação de sementes não teve impacto significativo. Este trabalho também mostrou que o FATOR DE CHOQUE DE TÉRMICO A2.2, um homólogo de HSFA9 que está envolvido na maturação de sementes, não desempenha um papel na longevidade das sementes, mas age como regulador negativo da dormência embrionária. A longevidade é avaliada pela capacidade de germinar após o armazenamento, o que representa apenas uma parte do sucesso do estabelecimento da cultura. Como a maturação de sementes afeta a perda de capacidade de estabelecimento de plântulas durante o armazenamento foi avaliada em soja usando um sistema experimental montado para avaliar a capacidade de alongamento. O padrão de aquisição da capacidade de alongamento durante a maturação variou entre os anos agrícolas e as condições de crescimento. O tempo para perda de 50% da capacidade de alongamento durante o armazenamento foi semelhante ao da perda de germinação. Além disso, aumentou de forma constante durante a maturação das sementes após a maturação em massa e nos estágios de maturação da colheita, destacando a importância da fase tardia da maturação das sementes na construção do vigor das sementes.

Palavras-chave: Maturação. Alongamento. Fator de choque térmico. Vigor. Qualidade de sementes.

RÉSUMÉ

La production de semences vigoureuses est un levier pour augmenter les rendements. La vigueur est définie comme la somme des propriétés physiologiques conduisant à l'établissement homogène et vigoureux du peuplement végétal. Elle comprend la longévité, définie comme la capacité à rester viable pendant le stockage et la capacité de la plantule à s'allonger après germination. Cependant, comment ces caractéristiques sont acquises au cours du développement de la graine et comment l'environnement influence leur acquisition restent mal compris. Ces informations sont importantes pour déterminer le stade de récolte correspondant à une vigueur maximale. En utilisant des approches agronomique et physiologique sur le soja et *Medicago truncatula*, nous montrons que la longévité est progressivement acquise au cours de la maturation. Chez le soja, le climat influence la longévité de manière complexe alors que chez Medicago, la chaleur pendant la maturation ne l'impacte pas significativement. Nous montrons également que HEAT SHOCK FACTOR A2.2, un homologue de HSFA9 impliqué dans la survie à l'état sec ne joue pas de rôle dans la longévité chez Medicago mais agit comme régulateur négatif de la dormance. La longévité se mesure par la perte de la germination pendant le stockage et ne représente qu'une partie du succès de l'établissement de la culture. Donc, l'influence de la maturation sur la perte de capacité d'établir une plantule lors du stockage a été évaluée chez le soja. L'acquisition de la capacité d'élongation pendant la maturation varie entre les années de culture et selon les conditions de croissance des plantules. Le temps nécessaire pour diminuer la capacité d'élongation de 50% pendant le stockage augmente constamment pendant la maturation après la maturité de masse. Ceci démontre l'importance des phases tardives de la maturation dans l'élaboration de la vigueur germinative.

Mots-clés: Maturation. Élongation. Heat shock factor. Vigueur. Qualité des semences.

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LIST OF ABBREVIATION

ABA	Abscisic Acid
ABI3	ABA-insensitive 3
ABI5	ABA-insensitive 5
AWT	Associated wild type.
BR	Brassinosteroids
cDNA	Complementary acid desoxyribonucleic
CK	Cytokinin
CDT	Controlled deterioration treatment
DAF	Days after flowering,
DNA	Acid desoxyribonucleic
DW	Dry weight
DNase	Desoxyribonuclease
ETH	Ethylene
GA	Gibberellin
RH	Relative humidity
HSF	Heat Shock Factor
HSFA9	Heat Shock Factor A9
HSP	Heat Shock Protein
IAA	Indole acid
INRA	Institut de Recherche en Horticulture et Semences
IRHS	Institut de Recherche en Horticulture et Semences
K_2CO_3	Potassium carbonate
LiCl	Lithium chloride
LEA	Late embryogenesis abundant
M	Molar
MeOH	Methanol hydroxide
MM	Maturity mass
NaCl	Sodium chloride
PCA	Principal component analysis
PCR	Polymerase chain reaction
PM	Physiological maturity
RFO	Raffinose family oligosaccharides

RNA	Ribonucleic acid
RNase	Ribonuclease
ROS	Reactive oxygen species
RT-PCR	Reverse transcriptase-PCR
SAM	Shoot apical meristem.
SD	Stardard deviation
SE	Standard error
SUC	Sucrose
TD	Desiccation tolerance
UNESP	University of São Paulo “Júlio de Mesquita Filho’’
UV	Ultra violet
WC	Water content
WT	Wild type

SUMMARY

GENERAL INTRODUCTION

Legume crops are important to the global agriculture since it provides a sustainable solution to food and feed protein security. Significant efforts are being made to increase yield and quality of legumes and to identify climate-resilient genotypes with improved grain characteristics (CONSIDINE et al., 2017). Soybean is one of the most important commodities worldwide, being a rich source of protein and oil for a multitude of end-products for feed and food (GUNSTONE, 2011) With the continued increase in world demand for sources of plant oil and proteins, soybean production has spread rapidly to tropical regions. In Brazil the annual growth of soybean production in the last 20 years was 3.5 million tons, representing an increase of 13.4% each year due to an increase in productivity and in the cultivated areas (JUNIOR et al., 2017).

The production of highly vigorous seeds is crucial to achieve crop production efficiency through seed physiological quality. The term "physiological quality" describes the overall value of a seed lot. It is defined as the sum of physiological properties that leads to homogeneous and fast germination, uniform seedling establishment and greater tolerance of stressful conditions during germination (vigor) and remain viable for long periods during storage in dry state (longevity) (BEWLEY et al., 2013). Also, the fast establishment of normal seedlings in the field also allows the efficient control of weeds and avoids the introduction of pathogens. However, a tropical environment such in Brazil is problematic for soybean as seeds have a short lifespan during storage. Knowledge on the acquisition of these different seed quality traits during development is important to determine the ideal harvest point to obtain seeds with maximum physiological potential.

Seed physiological quality is acquired sequentially during seed development and maturation, but in many plant species the different physiological quality traits are not acquired at same time. The capacity to germinate is acquired prior to maximum dry weight. This is followed by the development of desiccation tolerance. Seed longevity is progressively acquired, after seed filling, until the seed reaches a dry state (CHATELAIN et al., 2012, RIGHETTI et al., 2015, LIMA et al., 2017). During this period of development, late embryogenesis abundant (LEA) protein, raffinose family oligosaccharides (RFOs), heat shock proteins (HSP) accumulate, which confer protection against denaturation and deleterious changes in the conformation of macromolecules during drying and in the dry state (ROSNOBLET, 2007; BUITINK and LEPRINCE, 2008; CHATELAIN et al., 2012, VERDIER et al., 2013, RIGHETTI et al., 2015). Some works suggest that HSP can improve seed vigor (KAUR et al., 2015).

Also the content of LEA, RFO serves as an indicative of seed maturity and vigor (JALINK et al., 1998; SINNIAH et al., 1998; VANDECASTEELE et al., 2011; DE SOUZA VIDIGAL et al., 2016). Longevity is also conferred by a range of antioxidants that protect the seeds against oxidative damage during storage, such as glutathione (KRANNER et al., 2006), tocopherols (MÈNE-SAFFRANÉ et al., 2010, VOM DORP et al., 2015), and flavonoids that are present in the seed coat (DEBEAUJON et al., 2000, DE GIORGI et al., 2015). However, the regulatory mechanisms controlling the acquisition and regulation of seed longevity and vigor remains largely unknown.

In parallel with the longevity, seed vigor continues to increase after severing the connection with the mother plant, which occurs at or shortly after mass maturity (maximum seed dry weight) and does not necessarily coincides with harvest maturity (when seeds can be safely harvested without mechanical damage (DEMIR and ELLIS, 1992; ELLIS at al.,1987, reviewed in FINCH-SAVAGE and BASSEL, 2016, BEWLEY et al., 2013). In the same way, how the vigor and seedling establishment is acquired during maturation remains poorly understood.

The position of the seed on the mother appear to be an effect on seed quality both the seed attributes (weight, shape) and physiological quality (vigor, viability, longevity). However, there is no consensus in the literature about this. The differences in quality attributes may be associated with time to pod set, differences in seed maturity or in time left for seed ageing on the plant. In addition, the environment and external conditions around the pod and internal characteristics (e.g. local sink-source relations) may contribute to position effects on physiological seed attributes (ILLIPRONTI et al., 2000).

With climate change, the impact of the maternal environment on the acquisition of seed vigor becomes highly relevant. Climate change is predicted to affect global agricultural production with adverse effects of high temperature and irregular rains, challenging researchers to find out adaptation strategies for crops (VALLIYODAN et al., 2017). Environmental conditions during reproductive growth of soybean can cause differences in seed yield and quality (EGLI et al., 2005; PUTEH et al., 2013) but how they influence acquisition of seed vigor remains poorly understood. To achieve a stable production of high vigor seeds, it is necessary to gain further knowledge into the mechanisms governing seed vigor during seed development by taking into account the maternal effects.

So, in order to overcome challenges in agriculture, studies about molecular and physiological mechanisms of seed maturation, dormancy and longevity, and seed vigor can serve as a model to explain how these mechanisms work in many species, therefore, helping breeding programs to increase their chances to improve quality to crops worldwide.

The objective of my PhD is to study the main events related to the acquisition of seed quality (longevity and vigor) that occur during late seed maturation in legumes when the developing seeds prepare for the dry stage. For this purpose, we used soybean seeds grown in two consecutive years under field conditions in Brazil and *Medicago truncatula* grown under controlled optimal conditions and at high temperature.

The work presented here is divided into five parts. A first chapter of bibliographical synthesis exposes current knowledge of seed maturation events and its regulation. In the chapter 2, using soybean we evaluated the impact of the maternal environment on seed quality by comparing the effect of slow and fast drying on the survival in the dry state (desiccation tolerance and seed longevity) at different stages of maturation. Taking advantage of the contrasting climate data of 2015 and 2016, we also evaluated the impact of the environmental conditions on the acquisition of seed longevity and the impact of maturation on the thermal dependence of germination.

In chapter 3 using soybean, the work documents how and when organ elongation during seedling establishment is acquired during seed maturation. We studied whether the progress of maturation, the seed position on the mother plant has an influence on hypocotyl elongation and whether elongation capacity is affected during storage.

The aim of chapter 4 was to assess the role of HSFA2.2 from *Medicago truncatula* and HSFA9 from Arabidopsis in the regulation of seed vigor. HSFA.2.2 is the homologue of the sunflower HSFA9 (PRIETO-DAPENA et al., 2006) whose role in seed vigor has been suggested. For this purpose, we obtained and characterized mutant seeds from both species. Since the role of HSF and their targets, HSP are central to heat response of cells. In Medicago, heat stress reduces seed longevity (RIGHETTI et al., 2015). Therefore, the effect of high temperatures on seed maturation was also studied in *Mthsfa2.2.* seeds. The corresponding mutants were characterized for seed quality characteristics including dormancy and longevity as well as response to phytohormones.

GENERAL DISCUSSION AND PERSPECTIVES

With the prospects of climate changes, the need to produce high vigor seeds that withstand the negative impact of environmental conditions during production and processing and are adapted during subsequent sowing has become a challenge for researchers and the seed industry. This is partly because the impact of the maternal environment on the acquisition of seed vigor remains poorly understood. Therefore, this work contributes to the knowledge of the main events related to seed quality acquisition that occur during seed maturation in legumes when the developing seeds prepare for the dry stage. Seed maturation is a very important developmental stage as desiccation tolerance, vigor and longevity are acquired.

We confirmed that longevity in soybean is progressively acquired during seed maturation after seed filling (Figure 7, chapter 2). Further work is necessary to understand the mechanisms triggering the embryo into a seed longevity program and which protective factors are implicated. There is increasing evidence that synthesis of protective mechanisms that allows the acquisition of longevity are induced sequentially and increases progressively during late seed maturation (PROBERT et al., 2007; RIGHETTI et al., 2015; LEPRINCE et al., 2017). In the legume *Medicago truncatula*, LEA genes are induced during seed filling, but a specific set of LEA polypeptides accumulate later in conjunction with longevity (CHATELAIN et al., 2012). Therefore, it is likely that developing seeds of soybean harvested at different maturity stages are endowed with different amounts of protective compounds. Lima et al. (2017) showed that the longevity increases concomitantly with increased level in transcripts encoding heat shock proteins and heat shock factors and increased content of raffinose family oligosaccharides (RFO), both contributing to resistance against accelerated ageing (TEJEDOR-CANO et al., 2010). The question whether heat shock proteins are required for seed longevity is debatable since in Medicago, seeds of *hsfa2.2* mutants had a longevity similar to wild type (Figure 4-chapter 4). Whether there is a redundant mechanism that allows the synthesis of key HSP during maturation remains to be assessed. In contrast, dormancy was increased in *Mthsfa2.2* seeds, suggesting a role of HSP during seed imbibition (Figure 8 -chapter 4). Using different drying rates, we suggest that the acquisition of longevity is an autonomous embryonic

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