ウェブサイト公

（様式 10）
（海外特別研究員事業）
令和１年１１月１３日

海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

採 用 年 度　平成２９年度
受 付 番 号　０３１２
氏　　名 佐野直人

（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：ベルサイユ(フランス国立農学研究所)　（フランス国)

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
自然変異を利用した種子寿命を制御する分子機構の解明

3. 派遣期間：平成２９年　１１月１日　～　令和１年１０月３１日

4. 受入機関名及び部局名
受入機関：フランス国立農学研究所　部局名：ジャン=ピエール・ブルジャン研究所

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

Background

Seed longevity, the period over which seed remains viable, is an important trait not only for daptation to changing environments, but also, for example, for agriculture and the seed industry. Post-harvest, seeds are often subjected to a pre-sowing treatment termed “priming”. This treatment involves imbibition of seeds in water under controlled conditions to trigger pre-germinative metabolism then drying prior to full germination before they have lost desiccation tolerance. The benefit of priming is that when seeds are subsequently sown, germination is enhanced and better synchronized, however, there is a trade-off as primed seed stocks generally age poorly during dry storage, rapidly losing viability. Other pre-sowing treatments such as coating, coloring and agrochemical application also risk causing similar transient seed imbibition and loss of storability. The shortened storage-life of treated seeds is a disadvantage for commercial distribution of high-quality seeds.

To find solutions to this problem, the molecular mechanisms underlying seed ageing following priming were analyzed using Arabidopsis as a model. We found that the Est-1 accession retained longevity for longer after priming compared to the reference accession Col-0, and QTL analysis using recombinant inbred lines (RILs) derived from the Est-1 × Col-0 detected three QTL regions associated with the loss of seed longevity during priming (Sano et al. 2017). We also analyzed the bulked transcriptome of the RIL population and revealed that genes related to plant hormone brassinosteroid (BR) biosynthesis/signaling and cell wall modification were highly expressed in primed seeds with shorter longevity. After priming, BR-deficient mutants showed significantly longer longevity than the wild type (WT). Moreover, tetrazolium staining indicated that mutant seed coats were less permeable after priming than those of WT. Together these results suggest that the loss of seed longevity in primed seeds is due to increased seed coat permeability, which is positively regulated, at least partly, via BR signaling. Nevertheless, the molecular aspects responsible for changes in seed coat permeability are still largely unknown. In addition, the genes underlying the QTLs for primed seed longevity have not yet been identified.

Purpose

The objective of this project was to identify the genes that determine primed seed longevity and seed coat permeability.

Results

1. Identification of genes underlying QTL for the regulation of seed longevity after priming

The three QTL regions previously identified for primed seed longevity on chr 1, 2 and 3 comprised 985, 2813 and 617 genes, respectively. Based on the expression level of these genes in seeds (FPKM > 1) and SNPs information (at least 1 SNP in gene), the number candidate genes was narrowed down to 302, 953 and 153 genes, respectively. Among these, 13, 21 and 10 genes showed different transcript abundance between short and long-life seeds in the RIL population, and allowed six candidate genes to be prioritized for further validation of

effects on seed ageing using mutants in candidate genes. Three independent mutant alleles were isolated from T-DNA insertion collections for the candidate gene NmrA-like negative transcriptional regulator family protein, however, none showed a significant difference in longevity compared to WT. Isolation and analysis of T-DNA insertion mutant alleles for the other five candidate genes is ongoing.

2. Analysis of flavonols and proanthocyanidins in RIL seeds

Arabidopsis mutants such as *transparent testa*, impaired in the enzymatic steps or transcriptional regulation of flavonoid synthesis, are known to have altered seed coat properties and seed longevity. In order to clarify whether flavonoids are involved in the mechanisms underlying the QTL for primed seed longevity, dry seeds of Est-1, Col-0 and 4 RILs with extreme longevity after priming phenotypes were analyzed for flavonols and proanthocyanidins using LC-MS on the IJPB chemistry platform. No clear link was found between flavonol or proanthocyanidin contents and longevity after priming. Nevertheless, proanthocyanidin levels in Est-1 seeds were much higher than in those of Col-0. In order to explore fully the potential role of these metabolites in longevity after priming, it will be important to analyze their levels in seeds having a simpler genetic context, specifically the T-DNA insertion mutants affected in the candidate genes that have been identified.

3. Genome-wide association study for primed seed longevity

In parallel, a genome-wide association study (GWAS) was performed using 169 natural accessions for primed seed longevity aiming to detect single nucleotide polymorphisms (SNPs) that correlate with seed aging after priming and compare their position with those of the three QTLs. Eighty-seven SNPs were identified that localized to 11 gene and/or intergenic regions and highlighted 20 genes that were significantly correlated with the trait (Fig. 1), however, none appeared to be co-located with the QTL candidate genes. Furthermore, the most significant SNPs were detected on chr 4 (region #5). Interestingly, they were located in introns or intergenic regions of two genes that function in in the BR signaling pathway. Seeds of T-DNA insertion mutant alleles for these two candidate genes were significantly more resistant to ageing by controlled deterioration treatment (CDT) after priming, suggesting that they may play key roles for the BR signaling pathway involved in seed longevity following priming. We presented these

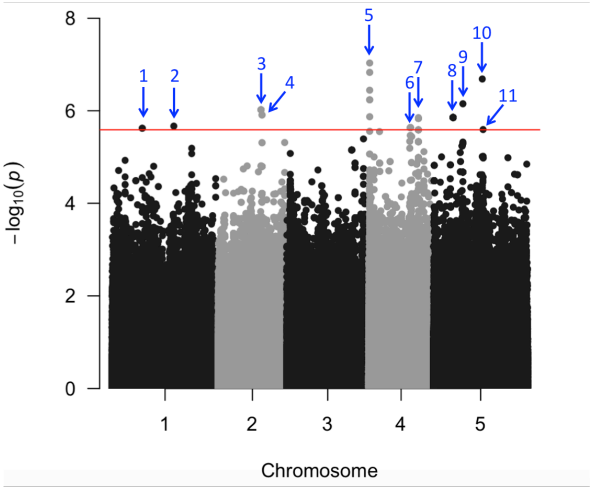


Fig. 1 Manhattan plots of GWAS for primed seed longevity

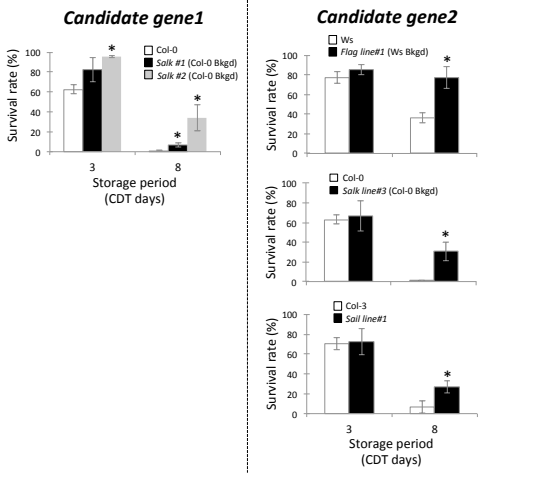


Fig.2 Survival rates of wild type: Col-0, Col-3, Ws and T-DNA insertion lines of two candidate genes after priming and subsequent CDT for 3 or 8 days. (*$p < 0.05$, Tukey-Kramer tests).

results at three international Conferences (International Conferences #1,2,3). Corroboration of the role of these genes is in progress through the functional analysis of mutant lines.

4. GWAS for seed coat permeability

In seeds, the embryo and endosperm are derived after fertilization, with the embryo going on to form the future plant after dispersal and germination. In contrast, the surrounding seed coat is a transient organ that plays multiple roles and is of maternal origin as it differentiates from the ovule. The resulting seed coat is formed of several overlying cell layers that accumulate large amounts of metabolites prior to undergoing programmed cell death at the end of seed development and being crushed by the growing embryo. Modified permeability for mutant seed coats in Arabidopsis have been characterized with tetrazolium staining, as the tetrazolium salts are metabolically reduced to red colored formazans by NADH-dependent reductases when they reach living cells in the endosperm and embryo. We have performed a GWAS for seed coat permeability using seed lots from 145 Arabidopsis accessions stained with tetrazolium and detected 12 SNPs located on chromosome #2 having a significant correlation with seed coat permeability (Fig. 4A). Among them, 10 SNPs were located in the promoter region of a plant hormone signaling-related gene, which is highly expressed in the endosperm and seed coat during seed development (Fig. 4B). Seed of a T-DNA insertion mutant allele for this gene were significantly less permeable than WT (Fig. 5), indicating that this gene regulates seed development processes that affect permeability. Identification of the causal cis-sequence controlling expression of this gene and thereby affecting permeability is in progress together with histological analyses of seed coat metabolites and/or structure in the mutant.

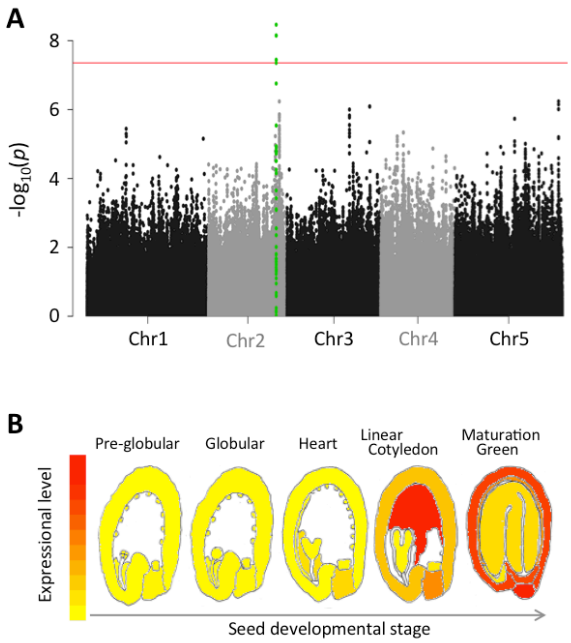
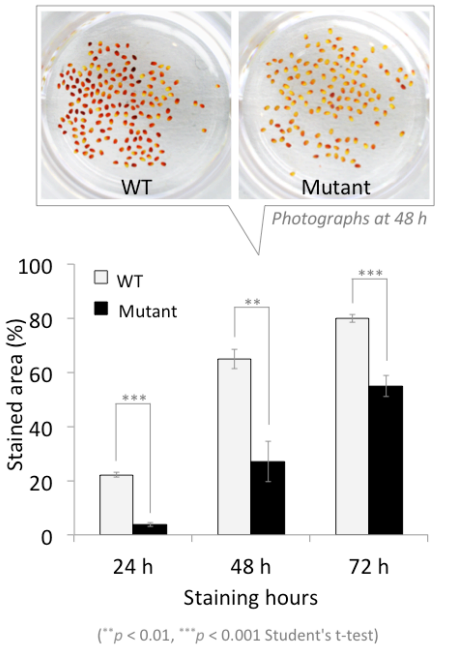


Fig. 4 GWAS for seed coat permeability in 145 accessions



(**$p < 0.01$, ***$p < 0.001$ Student's t-test)

Fig. 5 Seed phenotype of a T-DNA insertion mutant line

Presentation (○Presenter)

International Conferences

#1○Sano N, Seo M, North H. International SPS Conference 2018: Plant Sciences for the Future, Brassinosteroid regulates seed aging following priming in Arabidopsis natural accessions. 24-NMEI (Poster and Oral), Saclay Plant Sciences, France, 2018/07/05

#2○Seo M, Sano N, North H. 6th Plant Dormancy Symposium 2018, Brassinosteroid regulates seed longevity in Arabidopsis. LECTURE-7.4 (Oral), Kyoto Terrsa, Japan, 2018/10/26

#3○Sano N, Seo M, North H. The 23rd International Conference on Plant Growth Substances, Brassinosteroids have a negative effect on seed storability after priming treatment in Arabidopsis. P207 (Poster), Université Paris-Descartes, France, 2019/06/28