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Research article

# Seed tolerance to deterioration in arabidopsis is affected by virus infection

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ABSTRACT

Seed longevity is the period during which the plant seed is able to germinate. This property is strongly influenced by environment conditions experienced by seeds during their formation and storage. In the present study we have analyzed how the biotic stress derived from the infection of *Cauliflower mosaic virus* (CaMV), *Turnip mosaic virus* (TuMV), *Cucumber mosaic virus* (CMV) and *Alfalfa mosaic virus* (AMV) affects seed tolerance to deterioration measuring germination rates after an accelerated aging treatment. Arabidopsis wild type plants infected with AMV and CMV rendered seeds with improved tolerance to deterioration when compared to the non-inoculated plants. On the other hand, CaMV infection generated seeds more sensitive to deterioration. No seeds were obtained from TuMV infected plants. Similar pattern of viral effects was observed in the double mutant *athb22 athb25,* which is more sensitive to accelerated seed aging than wild type. However, we observed a significant reduction of the seed germination for CMV (65% vs 55%) and healthy (50% vs 30%) plants in these mutants. The seed quality differences were overcomed using the *A. thaliana athb25-1D* dominant mutant, which over accumulated gibberellic acid (GA), except for TuMV which generated some siliques with low seed tolerance to deterioration. For AMV and TuMV (in *athb25-1D*), the seed quality correlated with the accumulation of the messengers of the gibberellin 3-oxidase family, the mucilage of the seed and the GA1. For CMV and CaMV it was not a good correlation suggesting that other factors are affecting seed viability.



## 1. Introduction

Seed longevity, defined as the period over which seed remains viable, is a crucial property of plants that will affect the maintenance of plant genetic diversity. Many factors have been described to influence seeds longevity and the rate of aging is strongly influenced by environment (moisture content, temperature and initial seed quality) and genetic factors (Sano et al., 2016).

The existing knowledge of molecular mechanisms involved in seed longevity is limited and the few genes studied belong to three different groups. The first one includes seed developmental mutants. The alteration of key regulators of seed maturation (e.g. leafy cotyledon1,_*lec1* or abscisic acid insensitive3, *abi3*) exhibit significantly reduced seed viability upon storage (Sugliani et al., 2009). Next mutant groups are those related to alteration of the seed coat or testa. This natural barrier protects the embryo and seed reserves from biotic and abiotic stresses (Rajjou and Debeaujon, 2008). The third mutant group corresponds to protection and repair systems that prevent seed vigour loss (Sattler et al., 2004) or maintain genome integrity in plants (Waterworth et al., 2010).

Accumulation of defense proteins and specific enzymes associated to protein repair is also related to seed longevity. High accumulation of small heat shock proteins improved tolerance to aging (Prieto-Dapena et al., 2006) while the protein repair enzyme L-isoaspartyl methyltransferase (PIMT) plays a key role in the long-term survival of seeds (Oge et al., 2008). Enzymes playing roles in the detoxification of reactive oxygen species (e.g. glutathione peroxidase and glutathione reductase) and toxic cyanide compounds (e.g. β-mercaptopyruvate sulfurtransferase) are important to extend seed longevity (Rajjou and Debeaujon, 2008).

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Hormones are also associated to seed longevity through their role in the differentiation of the seed coat and in the maturation phase (Braybrook and Harada, 2008; Bueso et al., 2014). The increased level of the stress hormone abscisic acid (ABA) during the maturation phase of seed development prepares the seed to resist desiccation and aging in a dormant state. By the other hand, arabidopsis ABA response mutants are deficient in accumulation of seed proteins protecting against desiccation (Braybrook and Harada, 2008). The growth hormone gibberellin (GA) has been recently associated to seed deterioration (Bueso et al., 2014). Seeds from wild-type plants treated with GA and from a quintuple DELLA mutant (with constitutive GA signalling) are more tolerant to seed deterioration. Also, the overexpression of COG1/DOF1.5 (Park et al., 2003), encoding a DOF ("DNA-binding with One Finger") transcription factor that attenuates light responses mediated by phytochromes, increases GA in the mother plant and reduces the permeability of the seed coat by increasing suberin accumulation in the palisade layer (Bueso et al., 2016).

After being challenged by biotic or abiotic stresses, plants initiate several responses that are modulated by different hormones, which their signalling pathways are internally interconnected to generate an efficient stress response (Navarro et al., 2008; Bari and Jones, 2009; Nishiyama et al., 2013; Verma et al., 2016). The involvement of plant hormones in seed deterioration permits to connect this trait with plan responses to abiotic and biotic stresses (Verma et al., 2016). The main hormones mediating plant defense response to pathogens and abiotic stresses are ABA, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Bari and Jones, 2009; Nakashima and Yamaguchi-Shinozaki, 2013), in which ABA is responsible for plant defence against abiotic stresses while SA, JA and ET are the main components against biotic stress (Bari and Jones, 2009).

SA is synthetized by plants in response to a wide range of pathogens, including plant viruses after recognition of viral effectors by resistance (R)-gene products that trigger incompatible interactions. Such interactions generated several responses to limit viral propagation at the infection site, including accumulation of reactive oxygen species (ROS) and pathogenesis-related (PR) proteins, induction of the hypersensitive response (HR), callose deposition and programmed cell death (PCD) (Baebler et al., 2014). JA is involved in resistance to necrotrophic pathogens and insect infestation (Thaler et al., 2004) and ET does not appear to be essential for plant resistance against viruses but can be involved in symptom development (Geri et al., 2004). The mechanisms of stress-response are not restricted to these hormones and recent studies provided substantial evidences for the crosstalk of ABA, SA, JA and ET with auxins (IAA), GAs and cytokinins (CKs) in regulating plant defense response (Navarro et al., 2008; Bari and Jones, 2009; Nishiyama et al., 2013).

To accomplish their life cycle, plant viruses must hijack the functions of different host factors altering, in some instances, the plant physiology and hormonal homeostasis (Pallas and Garcia, 2011; Collum and Culver, 2016). It has been shown that virus infection has a major effect on resource allocation to growth and reproduction, infection resulting in a general reduction of resources allocated to both traits (Pagan et al., 2008) and that some viruses directly affect the viability of seeds (Sastry, 2013). However, how virus infection affects seed deterioration has not been studied yet. In the present study we have analyzed how biotic stress derived from the infection of different plant viruses affect seed quality. We have selected four different viruses differing in genome organization, the host range or the mode of transmission. The *Cauliflower mosaic caulimovirus* (CaMV) has a double-stranded DNA genome whereas the *Turnip mosaic potyvirus* (TuMV), *Cucumber mosaic cucumovirus* (CMV) and *Alfalfa mosaic alfamovirus* (AMV) are single strand RNA (ssRNA) viruses with 1 (TuMV) or 3 (CMV and AMV) RNAs, respectively. In addition, CMV and AMV are seed transmitted whereas CaMV and TuMV are transmitted through aphids. Seeds obtained from infected wild type *Arabidopsis thaliana* Columbia plants and two derived mutants that present improved (*athb25-1D*) or reduced (*athb22 athb25*) seed tolerance to deterioration (Bueso et al., 2014) revealed different results according to the virus used. In addition, we show the impact of virus infection on the hormonal balance.

## 2. Material and methods

### 2.1. Plant inoculations

To produce TuMV, CMV, AMV and CaMV infectious extracts, adequate host plants for each virus were inoculated with an initial infectious material. Thus, to produce the initial TuMV infection, *Nicotiana benthamiana* plants were mechanically inoculated with a plasmid containing a TuMV cDNA (GenBank accession number AF530055.2) corresponding to isolate YC5 from calla lily (Zantedeschia sp.) under the control of CaMV 35S promoter (Chen et al., 2007). A mix of the three 5'-capped transcripts corresponding to the CMV-Fny or AMV-425 strains were produced in vitro and mechanically inoculated in *N. benthamiana* plants. In the case of the AMV, the inoculum contained also few micrograms of purified AMV coat protein. The infection with CaMV was started by mechanical inoculation of Plasmid pCaMVW260 (Scholelz and Shepherd, 1988) in Brassica rapa L. 'Just Right'.At ≈14 days postinoculation (dpi), when infection symptoms were clearly observable, symptomatic tissues were harvested, aliquoted, frozen and stored at −80 °C. Aliquots of the different infected tissues were then ground with a mortar and pestle in the presence of liquid $N_2$ and homogenized in 3–4 vol of inoculation buffer (50 mM potassium phosphate, pH 8.0). The resulting crude extracts were used to mechanically inoculate batches of 3-week-old *A. thaliana* ecotype Col-0 wild type (wt) and *athb25-1D* or *athb22 athb25* mutants. The *A. thaliana athb25-1D* line overexpress the homeobox 25 transcription factor (ATHB25) and presents improved seed tolerance to deterioration under both natural and accelerated aging conditions meanwhile the mutant *athb22-25* has loss function of both *ATHB25* and the most similar zinc finger homeodomain *ATHB22*, that exhibits reduced tolerance to accelerated aging (Bueso et al., 2014).

### 2.2. Dot blot assays

Dot blot analysis was performed using total RNA extracted from inoculated leaves at 7 dpi, as described previously (Sanchez-Navarro et al., 1997). Alternatively, the plants were also analyzed using a fast citrate buffer protocol that does not require any special manipulation (Sanchez-Navarro et al., 1996). The samples were directly applied onto nylon membranes positively charged (Roche Diagnostics GmbH, Manheim, Germany), air-dried and cross-linked by UV crosslinker (700 × 100 μJ/cm2). Hybridization and detection was conducted as previously described (Sanchez-Navarro et al., 1996) using a dig-riboprobe (Roche Mannheim, Germany) complementary to part of the coat protein gene of the corresponding virus. Chemiluminiscent detection using CSPD reagent as substrate was performed as recommended by the manufacturer (Roche Diagnostics GmbH, Manheim, Germany). Films were exposed for 30 min.

### 2.3. Plant material and growth conditions

Arabidopsis (*Arabidopsis thaliana*) plants were grown and germinated on Murashige and Skoog (MS) plates with sucrose as