



Adaptive Reprogramming During Early Seed Germination Requires Temporarily Enhanced Fermentation-A Critical Role for Alternative Oxidase Regulation That Concerns Also Microbiota Effectiveness

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Plants respond to environmental cues *via* adaptive cell reprogramming that can affect whole plant and ecosystem functionality. Microbiota constitutes part of the inner and outer environment of the plant. This *Umwelt* underlies steady dynamics, due to complex local and global biotic and abiotic changes. Hence, adaptive plant holobiont responses are crucial for continuous metabolic adjustment at the systems level. Plants require oxygen-dependent respiration for energy-dependent adaptive morphology, such as germination, root and shoot growth, and formation of adventitious, clonal, and reproductive organs, fruits, and seeds. Fermentative paths can help in acclimation and, to our view, the role of alternative oxidase (AOX) in coordinating complex metabolic and physiological adjustments is underestimated. Cellular levels of sucrose are an important sensor of environmental stress. We explored the role of exogenous sucrose and its interplay with AOX during early seed germination. We found that sucrose-dependent initiation of fermentation during the first 12 h after imbibition (HAI) was beneficial to germination. However, parallel upregulated AOX expression was essential to control negative effects by prolonged sucrose treatment. Early downregulated AOX activity until 12 HAI improved germination efficiency in the absence of sucrose but suppressed early germination in its presence. The results also suggest that seeds

inoculated with arbuscular mycorrhizal fungi (AMF) can buffer sucrose stress during germination to restore normal respiration more efficiently. Following this approach, we propose a simple method to identify organic seeds and low-cost *on-farm* perspectives for early identifying disease tolerance, predicting plant holobiont behavior, and improving germination. Furthermore, the research strengthens the view that AOX can serve as a powerful functional marker source for seed hologenomes.

Keywords: seed quality, ROS, Warburg effect, bacterial endophytes and mycorrhizal fungi, organic seeds, biotic stress, *on-farm* seed selection

INTRODUCTION

Understanding the role of microbiota in adaptive plant robustness is important for crop improvement and developing innovative tools that could allow more efficient plant selection (Arnholdt-Schmitt et al., 2014; Arnholdt-Schmitt et al., 2015, 2018; Nogales et al., 2015). Research on the relevance of endophytic and associated microbiota and usage of microbes as bioinoculants are often hampered by low reproducibility, due to a lack of better understanding of fundamental principles of the plant-microbe interactions (Arnholdt-Schmitt, 2008; Vicente and Arnholdt-Schmitt, 2008; Campos et al., 2015; Mercy et al., 2015, 2017; Bedini et al., 2018; Albornoz et al., 2020) emphasized the need to study mycorrhizal benefits on a case-by-case basis which should consider more holistic and context-dependent views on mycorrhiza functioning at plant family and biome-wide levels. Also, it is widely confirmed that endophyte effects are genotype-specific (Abdelrazek et al., 2020a,b). Furthermore, Durán et al. (2018) identified bacterial endophytes as drivers for soil suppressive take-all disease. Nevertheless, they highlighted that they did not find a relevant correlation between disease suppression and reduced pathogen biomass. In our opinion, these are key observations. They encouraged us to initiate the work on the hypothesis that the competence of individual plant hosts for resilience plays the most critical role for beneficial or non-beneficial plant-microbe interactions, which can be superior to plant families and biome origins.

However, there is a lack of knowledge on traits that aid in (a) early prediction of the plant strength, (b) demonstration of its relevance for plant-microbe interactions, and (c) transformation of such knowledge toward user- and environment-friendly applications for sustainable agriculture. We earnestly aim with the perspective to understand these phenomena and to contribute to the knowledge base toward closing these three gaps.

Seed Germination/Reprogramming as a Model to Study Plant Adaptive Robustness

The capacity for efficient reprogramming as a trait *per se* is recognized as a marker for adaptive plant robustness (Cardoso and Arnholdt-Schmitt, 2013). Seed germination can serve as an experimental *in vitro* tool to study environmental stress-induced reprogramming and to identify early functional markers and tools for predicting plant performance under field conditions (Mohanapriya et al., 2019). Dry seeds are known to respond upon

water imbibition and subsequent penetration of oxygen. Thus, radicle emergence can be seen as an indicator of environmental stress recovery from the dry-to-water imbibed conditions and low-to-high oxygen status.

Efficient seed germination under field conditions is especially required in organic agriculture, where the application of chemical herbicides and pesticides to suppress competitors shall be avoided to support healthy food and feed production and to improve the sustainability of bio-based socioeconomic systems. At the same time, organic agriculture impacts seed quality and the amount of microbiota in seeds (Cope-Selby et al., 2017; Wassermann et al., 2019). Recently, the use of the so-called “organic seeds” vs. conventionally produced seeds is raised as an ethical issue (European Parliament and of the Council, 2018). However, the better quality of organic seeds in terms of their contribution to agriculture sustainability, nutritional quality, and yield performance is under intensive debate (e.g., Bhaskar et al., 2019; Voss-Fels et al., 2019) and requires scientific validation (Simon et al., 2017; Abdelrazek et al., 2020a,b). Appropriate methods and tools are in absolute need to discriminate organic vs. conventional seeds by traits that should allow predicting the superior quality of organic seeds.

The Complex Role of Sucrose in Adaptive Reprogramming

Cellular reprogramming is an energy intensive phenomenon. Reactive oxygen species (ROS) are known to interact with redox-sensitive protein cysteine thiol groups relevant for energy metabolism and metabolic channeling linked to cell differentiation and cell cycle regulation (Bigarella et al., 2014; Dumont and Rivoal, 2019; Gupta et al., 2020a,b; Pengpeng et al., 2020; Qi et al., 2020). Sugars and sugar phosphates interact with hormone-mediated signal networks to modulate energy metabolism. Auxin-stimulated sugar metabolism is frequently reported (e.g., Zhao et al., 2021); however, only few examples revealed that sucrose can induce new cell programs (Grieb et al., 1994; see in Zavattieri et al., 2010) and also, vice versa, can change auxin metabolism (Lin et al., 2016; Meitzel et al., 2021). In maize, sucrose induced several cell cycle markers during germination than glucose (Lara-Núñez et al., 2017). Downstream of sugars, two important antagonistic protein kinases are involved in energy sensing and physiological adaptation (reviews in Bailey-Serres et al., 2018; Sakr et al., 2018; Schmidt et al., 2018). While sucrose non-fermenting-1-related protein kinase 1 is activated when energy is depleted (Schmidt et al., 2018; Wurzing et al.,

2018; Wang et al., 2020), the target of rapamycin (TOR) is induced under conditions of energy excess to stimulate the cell cycle progression and the cell proliferation (Sangüesa et al., 2019). Sucrose can have various functions: besides its metabolic role, it acts as a signaling component (Baena-González and Hanson, 2017; Sakr et al., 2018), as an osmotic stressor that can disrupt communication within and between cells (Moon et al., 2015), shown to trigger aerobic alcohol fermentation in support of respiration, and biosynthesis of higher molecular weight compounds, such as lipids (Mellema et al., 2002).

Multifunctional Role of AOX as Switch Between Respiration and Fermentation During the Germination Process

Alcohol fermentation has been found to play a critical role in controlling tissue level pyruvate in plants, thereby, adjusting respiration rates to prevailing cellular energy status (Zabalza et al., 2009). Fan et al. (2020) identified hormone and alcohol degradation pathways that were mostly activated during the early stages of somatic embryogenesis (SE), which is a prominent example of *de novo* programming. Ethanol has been shown to reduce ROS levels and led to high induction of *alternative oxidase* (AOX) and *glutathione-S-transferase* transcripts (Nguyen et al., 2017). Transcriptome analyses at 2,4-dichlorophenoxyacetic acid (2,4-D) induced reprogramming indicated that the extent of aerobic fermentation is connected to cell proliferation and is regulated by interacting levels of sucrose and AOX (Costa et al., 2021). Transient upregulation of genes related to alcoholic and lactic acid fermentation is shown to be associated with glycolysis and modified complex stress signaling patterns with enhanced superoxide dismutase (SOD) and decreased transcript levels of nitric oxide (NO) producing *nitrate reductase* (NR). Furthermore, the data signaled activation of cell death-regulating system and arrested cell cycle by reducing alpha-tubulin gene transcription in the earliest step of reprogramming. Considering the generality of these observations, we proposed a reference transcriptome profile to identify virus traits that link to harmful reprogramming (Arnholdt-Schmitt et al., 2021). This approach helped to identify an early trait for combating SARS-CoV-2 that covers ROS/reactive nitrogen species (RNS) balancing, aerobic fermentation regulation, and cell cycle control (Costa et al., 2021).

In seeds, fermentation and alternative respiration (AR) are dominating metabolic reactions (Arnholdt-Schmitt et al., 2018; Mohanapriya et al., 2019). During seed germination, structural and functional acclimation of aerobic respiration is central and determines the temperature-dependent efficiency of germination (Bello and Bradford, 2016; Paszkiewicz et al., 2017). Nevertheless, markers for respiration and oxygen consumption were not superior to simple germination tests for predicting the vigor of single seeds (Powell, 2017). However, it is suggested that AR plays the most critical role during germination (Arnholdt-Schmitt et al., 2018; Mohanapriya et al., 2019). This role requires managing ROS/RNS increase and channeling energy and substance flow from fermentation, when carbohydrate storages are released and enzymes get into action (Saleh and Kalodimos, 2017), but the respiration chain is still structurally restricted

and overloaded by massively incoming oxygen. AOX is mainly regulated by pyruvate (Millar et al., 1996; Hoefnagel et al., 1997; Hakkaart et al., 2006; Albury et al., 2009; Carré et al., 2011; Selinski et al., 2018) and strikingly, Ito et al. (2020) showed in *Arum* that energy-related metabolic regulation can be determined by temperature-dependent switching between AOX polymorphisms in the binding site for AOX-pyruvate. In this scenario, it might be of interest that AOX is essential in ethylene-induced drought tolerance and mediating autophagy *via* balancing ROS levels (Zhu et al., 2018). Also, thermo-inhibition of carrot seed germination could be circumvented by seed priming, which was found to be linked to increased ethylene production at higher temperatures (Nascimento et al., 2013). Ethylene biosynthesis is found to be induced by hydrogen peroxide (H₂O₂) and acted positively on germination, independent of auxin-coordinated hormonal crosstalk linked to abscisic acid suppression and gibberellin activation (Wojtyła et al., 2016). During ethylene biosynthesis, cyanide is generated as a by-product of the pathway and probably shifts cytochrome oxidase (COX)-mediated respiration to AR (Siegień and Bogatek, 2006; Machingura et al., 2016). Eckert et al. (2014) stressed that microbiota has developed ethylene-producing pathways to profit during the invasion and to evade defense responses of the host plants. Additionally, Mercy et al. (2017) observed that treating mycorrhiza-infected seedlings with potassium cyanide promoted local arbuscular formation.

AOX Is a Key Molecule for Cellular Reprogramming: Toward a Perspective

Recently, we identified AOX as the stress level sensing coordinator for auxin inducible metabolic reprogramming by comparing induction of SE and seed germination (Arnholdt-Schmitt et al., 2018; Mohanapriya et al., 2019). Association of AOX to target cell reprogramming is also observed in other systems such as adventitious root development in olive (Santos Macedo et al., 2009; Porfirio et al., 2016) and elicitor-induced hairy roots (Sircar et al., 2012). Furthermore, our group had contributed to novel functional marker strategies by highlighting AOX as a marker across taxonomic borders that includes “shared” AOX genes in plant holobionts (Arnholdt-Schmitt, 2005a,b, 2008; Arnholdt-Schmitt et al., 2006; Campos et al., 2015; Mercy et al., 2017; Bedini et al., 2018). Based on the role of AOX in carbohydrate metabolism (Vanlerberghe et al., 1994), our approach has been stimulated to understand the role of fermentation and sugars during plant-mycorrhiza interactions (Mercy et al., 2017; Bedini et al., 2018) and had led to a privately explored patent (Lucic and Mercy, 2014). However, the early phase of reprogramming was not sufficiently considered in that research (Mercy et al., 2017) to drive our core functional marker approach (Arnholdt-Schmitt, 2008; Mercy et al., 2015). Recently, Mohanapriya et al. (2019) observed that arbuscular mycorrhizal fungi (AMF) inoculation in carrot seeds interacted with the AOX-inhibitor salicyl hydroxamic acid (SHAM) and palliated negative SHAM effects on early germination. Also, AMF effects in seeds seemed to be modified by non-culturable microbiota. Integrated *in silico* studies on experimental data revealed that endophytes interact with AOX expression in

species-, stress-, and developmental-dependent manner. Also, Costa et al. (2021) highlighted the importance of microbiota-plant genotype interaction and its impact on early carrot seed germination which can be modified by SHAM.

In our earlier work in Mohanapriya et al. (2019), we demonstrated successful prediction of oxycaloric equivalents from germinating seeds at 10 HAI. The present perspective questions the metabolic nature of AOX coordination and provides deeper phenotyping during germination of endophyte-free and microbiota-inoculated seeds focused at early times around 12 HAI. **Figure 1** demonstrates the step-by-step rationale of fundamental insights and deduced practical strategies (methodology of experiments is provided in **Supplementary File 1**).

In our findings, we observed that (a) during *Arabidopsis thaliana* seed germination alcohol dehydrogenase (ADH) transcript levels were increased at 12 h after seed stratification (SL) in water followed by a decline, and the increase in ADH transcript levels is in general accompanied by increased AOX1a transcript accumulation (**Figure 1B2**). (b) In agreement with (a), germinating carrot seeds displayed a higher level of ADH activity at 12 HAI than 24 HAI. In the presence of 3% sucrose, this level was further enhanced (**Figures 1A3,B3**). (c) Two hours short pulses of sucrose before water imbibition enhanced early germination in seeds of two different species, viz., carrot and wheat (**Figures 1A2,E3** and **Supplementary Figure 2**). Additionally, in carrots, we showed that the effectiveness of such early sugar pulse was dependent on sucrose concentration. A short pulse could be substituted by a longer pulse at a lower concentration of sucrose (**Figure 1A2**). (d) On the contrary, SHAM treatment until 6 and 12 HAI suppressed germination in the presence of 3% sucrose. However, it favored early germination in the absence of sucrose (**Figure 1C1**).

(e) Three carrot native bacterial endophytes (EN1, EN2, and EN3) were used for carrot seed inoculations with two cultivars (cv. Kuroda, cv. Early Nantes) and showed a tendency to improve germination (**Figure 1D1**). However, a positive effect is dependent on cultivar-endophyte interaction. SHAM treatment reduced the early germination percentage of endophyte-treated seeds against the respective endophyte-treated controls. This was observed in all cases though to a different degree of inhibition (**Figure 1D2** and **Supplementary Table 2**). (f) Sucrose has displayed different impacts on endophyte-mediated effects on germination and is dependent on cultivar and endophytes. However, in no case did endophytes improved germination of sucrose-treated seeds when compared with endophyte-treated controls without sucrose (**Figure 1D2** and **Supplementary Table 2**). (g) In a good germinating carrot cultivar (cv. Kuroda), the two selected *Rhizophagus* species (*Rhizophagus irregularis* and *Rhizophagus proliferus*) acted negatively on early germination, while in a delayed germinating carrot cultivar (cv. Early Nantes), both *Rhizophagus* species acted positively (**Figure 1D1** and **Supplementary Table 2**). In both cultivars, sucrose could improve *Rhizophagus* effects on early germination to higher levels than the AMF-treated controls. However, this is dependent on cultivar-species interaction. In the presence of sucrose, *R. irregularis* (M1) improved germination of both cultivars compared to M1-treated control seeds in the absence of

sucrose (**Figure 1D2**). (h) In addition, at lower concentrations of SHAM (5 mM), early germination could be improved to higher levels as compared to the AMF-treated controls (**Figure 1D2**), but this is observed only in the cv. Kuroda variety, which did not show positive AMF effects against non-AMF-treated controls (**Figure 1D1** and **Supplementary Table 2**).

In **Figure 2**, we present a simplified scheme that summarizes our conclusion based on wet-lab experiments, state-of-the-art knowledge, and our hypothetical inferences related to the dynamic metabolic interplay between sucrose, aerobic fermentation, COX-mediated respiration, AOX regulation/AR, and microbiota on cell reprogram functioning. In this scheme, we separated AOX as a macromolecule (gene/protein) from its functional pathway, AR, to highlight the outstanding position of AOX as the key and only enzyme of the pathway that, if present in an organism, is recognized to provide a central metabolism-coordinating function for efficient survival (Mohanapriya et al., 2019; Arnholdt-Schmitt et al., 2021; Costa et al., 2021). We consider that under development- and/or environment-induced conditions of rapid sucrose increase, the COX pathway is stimulated *via* enhanced glycolysis, pyruvate production, and increased tricarboxylic acid (TCA) cycle, in a way that the respiratory chain can get overloaded by electrons followed by enhanced ROS/RNS levels and, on the other hand, restricted due to rapidly consumed oxygen and/or yet low numbers of functional mitochondria concerning the presence of oxygen during germination. In response, aerobic alcoholic and lactic fermentation are stimulated (refer points a, b, and c; Costa et al., 2021). At the same time, AOX is activated [refer point d and in Mohanapriya et al. (2019), Costa et al. (2021)] mainly through AOX gene sequence-dependent pyruvate regulation and ROS/RNS.

Depending on stress level and the amount of sucrose and duration of the situation of high sugar-level, anaerobic glycolysis can reach high turnover during cell reprogramming and high levels of adenosine triphosphate production even corresponding to the Warburg effect. This latter hypothesis is supported by parallel research on auxin-induced callus growth (Costa et al., 2021), where we observed a rapid increase in *ADH1* transcripts of 1,777% and a parallel increase in *LDH* (*Lactate dehydrogenase*) transcripts of 346%. Warburg effects are increasingly recognized also in human systems (Kutschera et al., 2020; Melkonian and Schury, 2020) as being part of normal physiology. However, in plants, they are studied still more concerning photosynthesis (Kutschera et al., 2020) and anaerobic conditions are best explored under flooding conditions and are related to anaerobic tolerance in rice (Narsai et al., 2017). It is shown that AOX plays a beneficial role under low oxygen and especially during reoxygenation (Jayawardhane et al., 2020).

Under increased sucrose, fermentation can escape feedback downregulation with the help of enhanced AR, since AOX-transferred electrons enable the continuation of the TCA cycle for metabolic reorganization though with relatively less energy efficiency. Thus, fermentation and AOX are complementing each other to maintain metabolic and energetic homeostasis thereby avoiding inefficient situations when the respiration chain is overloaded with oxygen availability. As soon as oxidative stress gets sufficiently diminished at equilibrated oxygen availability in the COX pathway, AOX will be downregulated and normal

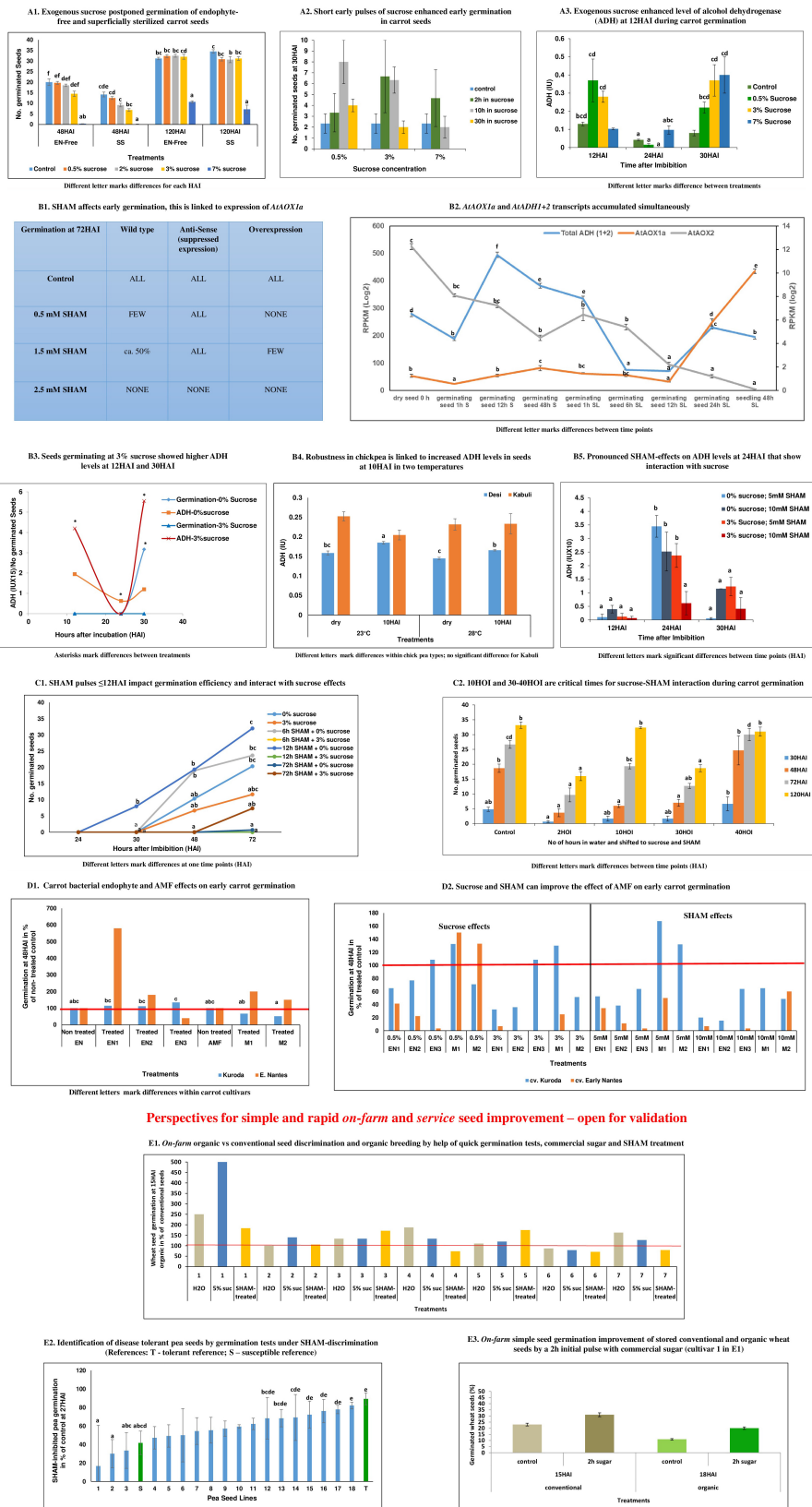


FIGURE 1 | (Continued)

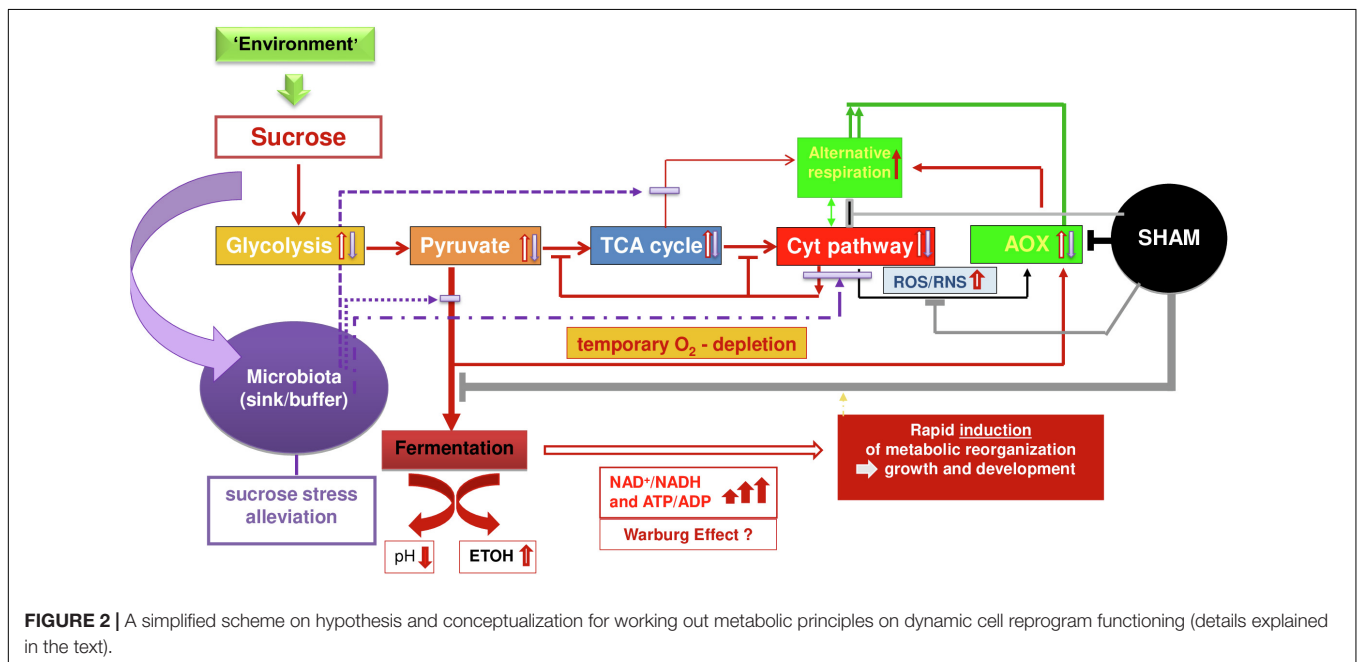
FIGURE 1 | (Continued)

Step-by-step rationale of the perspective. **(A1)** Exogenous sucrose postponed germination of endophyte-free and superficially sterilized carrot seeds: Sucrose inhibited early germination [at 48 h after imbibition (HAI)] depending on increasing sugar concentrations. This trend is the same for endophyte-free seeds that were superficially sterilized. At 120 HAI, the effect of 0.5–3% sucrose could not be noticed anymore, while 7% sucrose inhibited germination for a prolonged time. This observation indicates a critical role of sucrose during the induction of adaptive performance. To confirm, the role of sucrose, in **Supplementary Figure 1**, the effect of sucrose is shown for auxin-dependent early induction of somatic embryogenesis (SE) as the most studied example of *de novo* programming. It demonstrates that (a) sugar is essential for cell reprogramming since SE induction was not observed at around 45 DAJ (days after inoculation) in controls, but only at 2 and 3% sucrose supply and (b) that SE can be optimized with the help of increasing amounts of exogenous sucrose since SE induction efficiency is highest at 3% sucrose (**Supplementary Table 1**). Cell reprogramming competes with cell division. This is a common insight, which got here validated again through the observed delay in embryonic vs. non-embryonic callus emergence at lower sucrose concentrations. As a general tendency, at higher sucrose levels, less number of seeds showed callus growth, which was later demonstrated to be embryogenic, in comparison to the higher number of seeds with (non-embryogenic) callus growth at lower sucrose levels (**Supplementary Figure 1**). **(A2)** Short early pulses of sucrose enhanced early germination in carrot seeds: A total of 3% sucrose applied for 2 or 10 h imbibition enhanced early germination for about the same degree compared to the control and longer pulse of 30 h. A lower sucrose concentration of 0.5% had the highest effect only by a longer pulse of 10 h and, at 7% sucrose, a higher effect against the control was only indicated when given as a short pulse of 2 h. This observation was confirmed with a second carrot cultivar in a rapid *on-farm* check by using a ca. 5% solution of commercial sugar (significant) (**Supplementary Figure 2**). **(A3)** Exogenous sucrose enhanced the level of alcohol-dehydrogenase (ADH) at 12 HAI during carrot germination: At 12 HAI, treatment with 0.5 and 3% sucrose resulted in an increase in ADH activity as compared to the control, while for 7% sucrose no effect is observed. At 24 HAI, the control indicated a decline of ADH values. In the presence of 0.5 and 3% sucrose, this decline is not avoided or might even have been strengthened. However, at 30 HAI, a second phase started, where sucrose enhanced the levels of ADH in a concentration-dependent manner including a positive effect of 7% sucrose. **(B1)** Salicyl hydroxamic acid (SHAM) affects early germination and this links to expression of *AtAOX1a*: In wild-type *Arabidopsis thaliana* seeds, while monitoring germination at 72 HAI, showed that SHAM treatment led to reduced germination rates. This inhibition is dependent on its concentration of 0.5 or 1.5 mM. However, when alternative oxidase (AOX) had been silenced (antisense), SHAM did not affect germination. On the contrary, when AOX was constitutively overexpressed (OE), SHAM indicated stronger inhibition of germination than in the wild type. Nevertheless, the three genotypes germinated with similar efficiency about their respective controls. This latter observation points to the fact that AOX is critically important for germination, if present. However, in case it is not present or activated (antisense) alternative pathways can substitute the functional role of AOX during germination. **(B2)** *AtAOX1a* and *AtADH1 + 2* transcripts accumulated simultaneously: A study on ADH transcript accumulation in wild-type *A. thaliana* confirmed a biphasic activation of *ADH* during germination. A first increase was observed 12 h after stratification (significant), which includes the imbibition of water. The second enhancement occurred from 12 h SL shortly before root emergence was monitored at 24 h SL. In parallel to increased *ADH* transcript accumulation, *AOX1a* transcripts accumulated during both phases, i.e., induction and early initiation of germination. After early induction, *ADH* transcripts showed a high decline (significant) until the end of the dark stratification phase, while *AOX1a* transcript levels remained more stable. During the second phase at the initiation of exponential root length growth in light observed at 48 h SL, *AOX1a* transcript accumulation keeps on enhancing, while the increase of *ADH* transcripts stopped at that time point. This is also indicated in the first phase. *AOX2* transcript accumulation is differentially regulated in comparison to *AOX1a* and showed continuous downregulation during the whole period, which appeared to be stronger in the SL phase. **(B3)** Seeds germinating at 3% sucrose showed higher ADH levels at 12 and 30 HAI: During early germination of carrot seeds, ADH levels follow a parabola, when monitored between 12 and 30 HAI. This is observed in control seeds and seeds germinating at 3% sucrose. Nevertheless, suppressed germination at 3% sucrose is linked to higher levels of ADH at 12 and 30 HAI. This means the more efficient germination in control seeds is linked at these two-time points to lower levels of ADH. Under both conditions, in the absence of exogenous sucrose and at 3% sucrose, 24 HAI displayed a turning point with the lowest ADH activity levels. However, ADH activity at 24 HAI is higher in controls (significant) than under conditions of sucrose-supplementation. **(B4)** Early chickpea plant vigor is critical for plant productivity under terminal drought conditions (Sivasakthi et al., 2017). From the two principle chickpea types, Desi and Kabuli, vast field experience has shown that Desi is superior in terms of multistress tolerance and yield performance (Purushothaman et al., 2014). In former research, we could discriminate both types at 10 HAI by a lower oxycaloric equivalent (Rq/RCO₂; calorespirometric ratio) value due to differential carbon use and, thus, predict *a posteriori* better yield stability of Desi (Mohanapriya et al., 2019). In this study, we show that Desi increased the level of ADH at 10 HAI during germination (significant at 23 and 28°C), while this is not seen in Kabuli. The reached level of ADH is higher at 23°C than at 28°C. **(B5)** Pronounced SHAM effects on ADH levels at 24 HAI that show interaction with sucrose: During the germination of carrot seeds, the most pronounced effect of SHAM treatment on ADH levels is observed at 24 HAI. At that time point, SHAM stimulated ADH levels compared to levels observed at 12 and 30 HAI. This happened independently of the presence of sucrose (3%). However, under both conditions, 5 mM SHAM showed a stronger stimulating effect on ADH levels (significant) at 24 HAI than 10 mM SHAM. But the level of SHAM-enhanced ADH is higher at both tested concentrations of SHAM when sucrose is not present. On the contrary, at time points 12 and 30 HAI, a higher ADH level in the 0% sucrose controls is associated with the higher concentration of 10 mM SHAM versus 5 mM SHAM. In the presence of 3% sucrose, ADH activity is at any time point higher at 5 mM SHAM than at 10 mM SHAM. Together, these observations point to the importance of differential AOX activity regulation for optimized germination during all three time points independently of the presence or absence of exogenous sucrose. **(C1)** SHAM pulses ≤12 HAI impact germination efficiency and interact with sucrose effects: In control seeds, short pulses of SHAM (10 mM) until 12 HAI enhanced germination efficiency and were more effective than pulses until 6 HAI. However, prolonged SHAM treatment of 72 HAI suppressed early germination. In contrast, at 3% exogenous sucrose, early germination efficiency is reduced against 0% sucrose controls (confirming panel **A1**) and SHAM pulses until 6 and 12 HAI led to complete suppression of early germination. However, from 48 HAI onward to 72 HAI, continuous SHAM treatment in the presence of 3% sucrose increased germination, but along with 0% sugar continuous SHAM suppressed germination also at 72 HAI. Collectively, these results show that plastic AOX regulation is critical for the timing of germination in controls and under conditions of sucrose supplementation. **(C2)** 10 and 30–40 HAI are critical times for sucrose–SHAM interaction during carrot germination: A total of 10 h of previous water imbibition reduced the strong negative effects of the combination of exogenous sucrose (3%) and SHAM (5 mM) on germination efficiency that was observed at only 2 h of previous water imbibition (significant). Also, during the phase of initiated root emergence at 30 Hours of imbibition (HOI), a transfer from water to media supplemented with sucrose and SHAM suppressed germination (significant). Water imbibition until 40 h before transfer to sucrose- and SHAM-containing media relieved and even supported germination when monitored at 30 and 48 HAI (significant). However, this increase in germination efficiency seemed to be restricted from 72 HAI onward (significant). **(D)** Sucrose and SHAM can improve the effect of arbuscular mycorrhizal fungi (AMF) on early germination: In panel **D1**, it is shown that carrot seeds treated with native endophytes (EN1—endophyte 1; EN2—endophyte 2; EN3—endophyte 3; isolated from cv. Kuroda) tend to improve early germination at 48 HAI in both the cultivars (not seen for EN3 in cv. Early Nantes). Exogenous sucrose had differential effects depending on endophyte and cultivar (Panel **D2**), but in no case does sucrose enhance early germination rates compared to the respective endophyte-treated controls (as shown also in **Supplementary Table 2**). However, SHAM treatment (Panel **D2**) reduced early germination against endophyte-treated controls in all cases (as shown also in **Supplementary Table 2**). In a separate trial, two AMF species (*R. irregularis*—M1 and *R. proliferus*—M2) were tested and acted negatively on germination in cv. Kuroda, but positively in slowly germinating seeds of cv. Early Nantes (Panel **D1**). Nevertheless, the effect of M1 on early germination could be improved in cv. Kuroda by 0.5 and 3% sucrose (Panel **D2**). However, this was not seen for M2. In the better germinating cv. Kuroda, the lower concentration of 5 mM SHAM (Panel **D2**) improved the effect of both mycorrhiza species on early germination. In later germinating seeds of cv. Early Nantes, 0.5% sucrose improved the already positive effect on germination (Panel **D1**) of *Rhizophagus* species M1 (Panel **D2**). In this cultivar, SHAM decreased

(Continued)

FIGURE 1 | (Continued)

the germination rate to the level of the untreated controls (**Supplementary Table 2**). **(E1)** *On-farm* organic vs. conventional seed discrimination and organic breeding with the help of quick germination tests, commercial sugar, and SHAM treatment: Seeds from six of seven winter wheat cultivars originated from organic agricultural management could be discriminated at 15 HAI through better germination against conventionally produced seeds when germinated in 5% sugar solution. In water, seeds of only four cultivars showed better germination than organic seeds. When conventionally produced material is compared, seeds of the first cultivar showed poor germination. This was much more pronounced when tested in a 5% sugar solution instead of water. Seeds of the second cultivar demonstrated the highest germination rates among all tested cultivars (as shown in **Supplementary Figure 4**). This was observed for seeds originating from both agricultural conditions, although higher germination in 5% sugar solution indicated the presence of microbiota (Panel **E1**). In contrast to all other cultivars, seeds of the second cultivar did not differ in germination rates for organic vs. conventional production under SHAM treatment when compared to the water control (as shown in also **Supplementary Figure 4**). This signals already low levels of AOX at 15 HAI for this cultivar no matter from which agricultural management system seeds originated. Overall, these observations indicate the interplay between plant genotype, sugar, and AOX activity that impacts differential germination capacities between organic and conventional seeds. **(E2)** Identification of disease tolerant pea seeds by germination tests under SHAM discrimination (T-tolerant reference; S-susceptible reference): Pea lines with differential degrees of root rot disease susceptibility could be ranked by employing SHAM inhibition. The most tolerant line (T) showed the lowest degree of SHAM-related inhibition of germination monitored at 27 HAI. This indicates the reasonability of germination tests under SHAM discrimination for the selection of seed vigor and plant robustness. **(E3)** *On-farm* simple seed germination improvement of stored conventional and organic wheat seeds by a 2 h initial pulse with commercial sugar (first cultivar in 1E1): This figure demonstrates the general potential of improving early germination through a short pulse of sugar its validity across species (in this study winter wheat, but also refer for carrot in Panel **A2** and **Supplementary Figure 2**), agricultural management practices and also related to the aging of seeds.



respiration will reach priority again for driving growth and development. Fermentation and AOX downregulation will again be regulated in adaptation to sucrose- and COX-mediated respiration-transmitted conditions embedded in adaptive hormonal crosstalk and overall complex cellular and apoplastic network signaling. Thus, rapid downregulation indicates efficient adaptation of COX-mediated respiration, a dynamic trait appropriate to mark seed vigor (Mohanapriya et al., 2019).

Sucrose can improve early germination of *Rhizophagus*-treated seeds (as shown in point g) while non-AMF-treated seeds respond upon sucrose typically with a delay in germination (as shown in **Figure 1A1**). This suggests that AMF and its associated bacteria (Pandit et al., under review) can alleviate or buffer the negative effects of sucrose on germination to relevant degrees by providing an additional sink. This is not indicated with the three tested endophytes (f). Also, early germination of endophyte-treated seeds is reduced at 48 HAI through the continuous presence of SHAM when compared to

endophyte-treated controls (e). On the contrary, when seeds from the cv. Kuroda were inoculated with *Rhizophagus*, SHAM treatment (5 mM) could improve early germination to higher levels than observed in AMF-treated controls. This observation is in agreement with the palliating effects observed by *Scutellospora calospora* on negative SHAM effects on carrot germination by using the same cultivar (Mohanapriya et al., 2019). In an overall assessment, it is inferred that AMF treatment might improve early germination by alleviating stress-induced by rapid sucrose excess through two mechanisms: providing an additional sink for sucrose and supplying an enhanced capacity and/or engagement of AR. *Rhizophagus* spores were shown to be a rich source for polymorphic AOX gene sequences (Campos et al., 2015). We believe that there could be an operation of two separate mechanisms since we observed differential effects on early germination of M1-treated seeds upon SHAM treatment in the two selected cultivars (**Figure 1D**). However, M1-treated seeds of both cultivars showed improvement in

early germination when sucrose was provided (**Figure 1D**). We tend to interpret that the isolated native carrot endophytes were already well integrated into the internal host cell habitat. Thus, their reinoculation tended to influence early germination positively, but could not provide a striking new advantage or disadvantage when sucrose is enhanced or SHAM treatment reduced the level of AR. However, we reported that endophytes modulate AOX transcripts in species-, stress-, and development-dependent manner, and endophytes could have modified the effect of AMF inoculation on seed germination efficiency (Mohanapriya et al., 2019).

OUTLOOK

The observations offer new perspectives for low-cost prediction of plant holobiont behavior of seeds and for providing simple and rapid *on-farm* support toward sustainable agriculture. We propose three tools for validation:

(A) Seed selection with the help of short germination tests under SHAM discrimination. This tool provides modalities to identify seeds with higher seed vigor, general adaptive plant robustness, and superior internal seed quality related to the content of secondary metabolites (**Figures 1E1,E2** and **Supplementary Figures 3, 4**).

(B) Discrimination of organic vs. conventionally produced seeds with the help of short duration germination tests in water solutions with 5% commercial sugar (**Figure 1E1**).

(C) Germination improvement by 2-h pulses of commercial sugar (**Figures 1A1,E3** and **Supplementary Figure 1**).

Furthermore, we encourage developing novel tests for AMF functionality in germinating seeds in the presence of sucrose. This approach targets compatibility between selected plants and AMF strains to support plant holobiont plasticity.

Our results suggest that polymorphic AOX gene sequences of symbiotic partners can impact plant–AMF compatibility. Therefore, we want to accomplish wider screening of major AOX polymorphisms in species-specific target cells for evaluating plant performance (Abe et al. 2002; Arnholdt-Schmitt, 2015; Nogales et al., 2016) and in AMF sources (Arnholdt-Schmitt, 2008; Vicente and Arnholdt-Schmitt, 2008; Campos et al., 2015). Such a strategy needs to also include near neighboring polymorphisms in conserved functional sites that can discriminate differentially regulated AOX1 and AOX2 (Costa et al., 2009). This approach would include a screening of compatible AOX polymorphisms from both partners in the proposed functional tests to identify the best plant–AMF combinations.

We hypothesize that the observed integration of bacterial endophytes into host plants with similar sensitivity against SHAM effects might point to synchronized AOX regulation in plant holobionts. Into this derivation would fit that we observed the same tendency of inhibiting sucrose effects on endophyte-free and superficially sterilized seeds (**Figure 1A1**), which we noticed also for SE induction (unpublished). Vicente et al. (2015) highlighted a “provocative” lack of interest in bacterial AOX. They anticipated that bacteria-harboring AOX could facilitate

adaptation to extreme conditions, which could also be of interest when thinking about plant endophytes and AMF-associated bacteria (Pandit et al., under review).

This present perspective is complementing Mohanapriya et al. (2019) and Costa et al. (2021). Joining the central figures of these publications is thought of as one teaching tool that can help to explain a straightforward way from fundamental interdisciplinary research to the application that might support sustainable socioeconomies because of the diversity of emergent environmental changes.

DEDICATION

The authors want to dedicate their work to FREEDOM and ETHICS.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

RB performed lab analyses on carrot germination, endophyte isolation, and inoculation trials related to **Figures 1A1–3,B3,4,5, C1,2,D**. JHC coordinated transcriptome analyses supported by KT. JHC, RS, and CN discussed initially the approach of this manuscript with BA-S. GM carried out work on **Supplementary Figure 1** and **Supplementary Table 1**. SS was responsible for AMF inoculation trials under the head of AA and EM performed pea studies for **Figure 1E2** under the responsibility of BA-S. Under the supervision of KG and EM together with AK performed germination analyses of transgenic *Arabidopsis*, and AK carried out the ADH analyses on chickpea. BA-S performed *on-farm* analyses (**Figures 1E1,E3**). CN was responsible for statistics and was in part supported by MO. RB and IV helped BA-S in the literature search. DS contributed with **Supplementary Figure 3**. BA-S initiated the scientific approach, coordinated overall research, and discussion, and wrote the manuscript. All the co-authors commented on research and manuscript during its development and agreed to manuscript submission. RB organized manuscript submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.686274/full#supplementary-material>

Supplementary Figure 1 | Exogenous sucrose delayed callus emergence and was necessary for SE.

Supplementary Figure 2 | A total of 2-h pulse with commercial sugar improved carrot germination efficiency monitored at 40 and 50 HAI.

Supplementary Figure 3 | Effect of SHAM treatment on accumulation of soluble and wall-bound phenolics (A) and flavonoids and lignin (B) in elicitor-treated hairy roots of *Daucus carota*. Values obtained in only elicitor-treated root were considered as 100% and results were expressed in terms of percentage of maximum. The terms E and NE in the x-axis legend denote -with and -without elicitor, respectively. * Soluble phenolics. Values are mean of three independent experiments ± SD.

Supplementary Figure 4 | Rapid germination check of organic and conventional seeds from seven cultivars in water (control) or under SHAM (5 mM) treatment.

Supplementary Table 1 | Effect of exogenous sucrose concentration on carrot SE callus induction.

Supplementary Table 2 | Microbiota effect on the carrot seed germination at different sucrose and SHAM concentrations.

Supplementary File 1 | Materials and methods.

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