

Exploring the involvement of *Alternative respiratory pathway* in *Pisum sativum* L. seed germination



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Introduction

- Organic agriculture, recognized as a more sustainable agricultural system, strongly depends on the use of highly resilient genotypes and seeds. In order to support the needs imposed by organic agriculture, several breeding programs, focused on the development of more resilient varieties/cultivars able to grow under unfavorable natural conditions, have been implemented.
- It is extremely important to develop new phenotyping techniques for selecting useful genotypes, in particular at the seed level. Current research at Uevora, developed in frame of the LIVESEED project, uses calorimetry as a phenotyping tool to investigate the link between seed germination and the alternative oxidase (AOX) respiratory pathway. AOX plays a crucial role in regulating cell reprogramming by controlling metabolic transitions related to the cellular redox state and the variable carbon balance.
- Considering that seed germination involves the activation of several metabolic pathways, including cellular respiration to provide the required energy for embryo germination, the involvement of the AOX was investigated by following transcript, protein and metabolic/respiratory analysis during pea (*Pisum sativum* L.) seeds germination.

Methodology

Calorespirometric measurements

- Calorespirometric measurements were performed in a 4100 Multi-Cell Differential Scanning Calorimeter (TA Instruments, USA) under isothermal mode at 25 °C.
- Four cvs. provided by LIVESEED partners were used: 'Respect-1', 'S134', 'G78' and 'S91'.
- Per cultivar were considered 16 biological samples (16 seeds).
- Seeds were previously soaked in water for 16 h.
- The values of heat rate (μW) emitted by seeds during calorimetric measurements allowed the calculation of the Respiratory heat rate (R_q) and CO_2 production rate (R_{CO_2}).
- Data of germination were collected 6 days after calorimetric measurements.

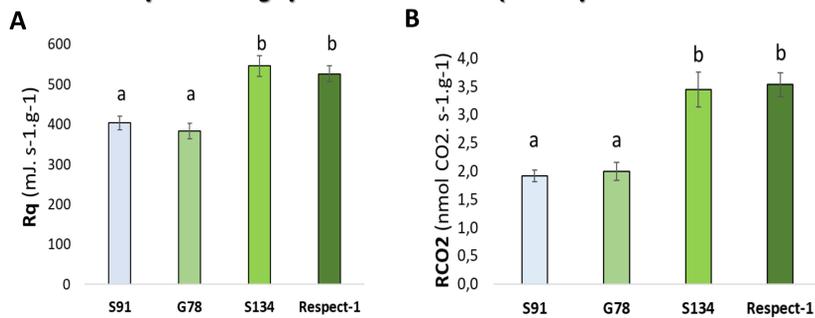


AOX gene and protein expression studies

- Gene expression analysis:** For AOX gene expression studies were considered two cvs. ('Respect-1' and 'S91'). Samples were collected at 16 hours post imbibition and further homogenized using liquid nitrogen. Per cultivar it were considered a total of 12 biological samples, each one consisting in a pull of 4 seeds. Total RNA was extracted using the Maxwell[®] 16 LEV simplyRNA Cells Kit and 1 μg was used for cDNA synthesis. Transcript level accumulation of the three pea AOX genes (*PsAOX1*, *PsAOX2a* and *PsAOX2b*) was assessed by RT-qPCR using the ABI 7500 system. *PsPOB* and *PsSAR1* were used as reference genes for data normalization.
- Protein expression analysis:** For AOX protein expression studies, were considered the four cvs. ('Respect-1', 'S134', 'G78' and 'S91'). Samples were collected at 16 hours post imbibition and further homogenized using liquid nitrogen. Per cultivar a total of 12 biological samples were analysed, each one consisting in a pull of 4 seeds. Total protein content was extracted by phenol precipitation and AOX protein levels were assessed through Western blot using an AOX-specific antibody.

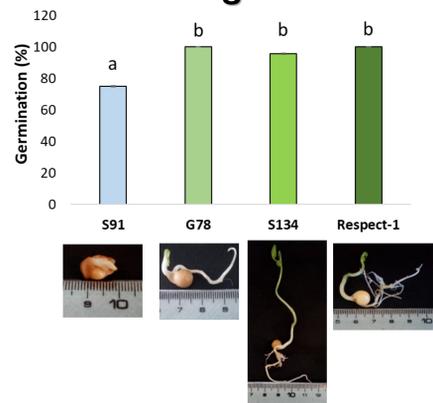
Results

Respiratory parameters (16h post imbibition)



- R_q and R_{CO_2} (graphs A and B, respectively) were lower in cvs. 'S91' and 'G78', compared to cvs. 'Respect-1' and 'S134'.

Seed germination (6 days post imbibition)

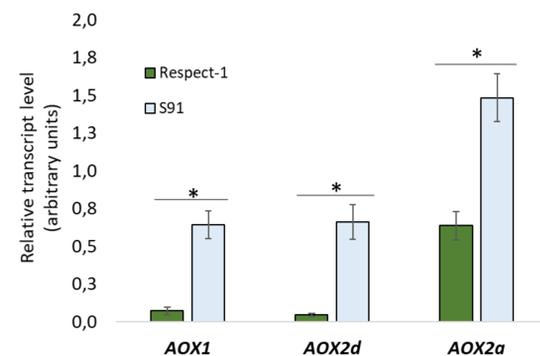


- The cv. 'S91' shows low seed germination, compared to the cvs. 'G78', 'Respect-1' and 'S134'.

Lower values of heat (R_q) and CO_2 emission (R_{CO_2}) rates could be related with lower germination rate.

In general, higher values of metabolic activity are related to higher germination rates.

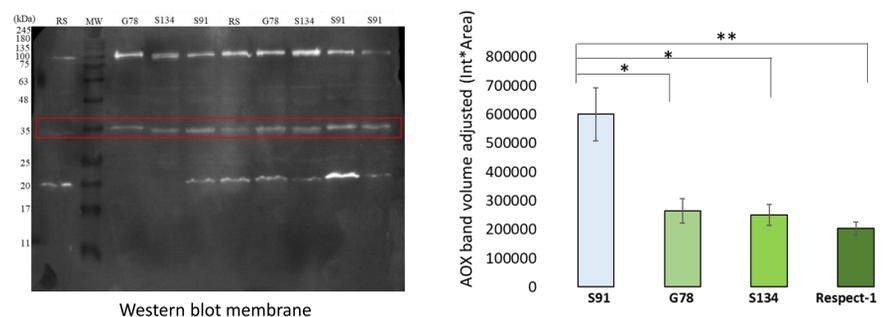
PsAOX gene expression (16h post imbibition)



- Transcript level of *PsAOX1*, *PsAOX2a* and *PsAOX2d* was higher in cvs. 'S91', comparing with the cv. 'Respect-1'.

PsAOX protein levels (16h post imbibition)

- The band correspondent to the *PsAOX* protein was detected in all cvs (see image of Western blot membrane below). However, higher levels of protein were observed in cv. 'S91' when compared to cvs. 'Respect-1' (RS), 'S134' and 'G78'.



Results at protein level confirm the results observed at transcript analysis.

Conclusions

- After 16h imbibition, the cultivar characterized by low seed viability (cv. 'S91') exhibited lower R_q and R_{CO_2} , coincident with significantly higher expression of the *PsAOX* genes (*PsAOX1*, *PsAOX2a* and *PsAOX2d*), and *PsAOX* protein levels.
- These results allowed us to hypothesize that higher levels of AOX (transcript and protein) are linked to low metabolic efficiency, being the energy not used for embryo growth but lost in the form of heat.
- The involvement of AOX and the alternative respiratory pathway during seed germination was demonstrated.

Aknowledgments

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