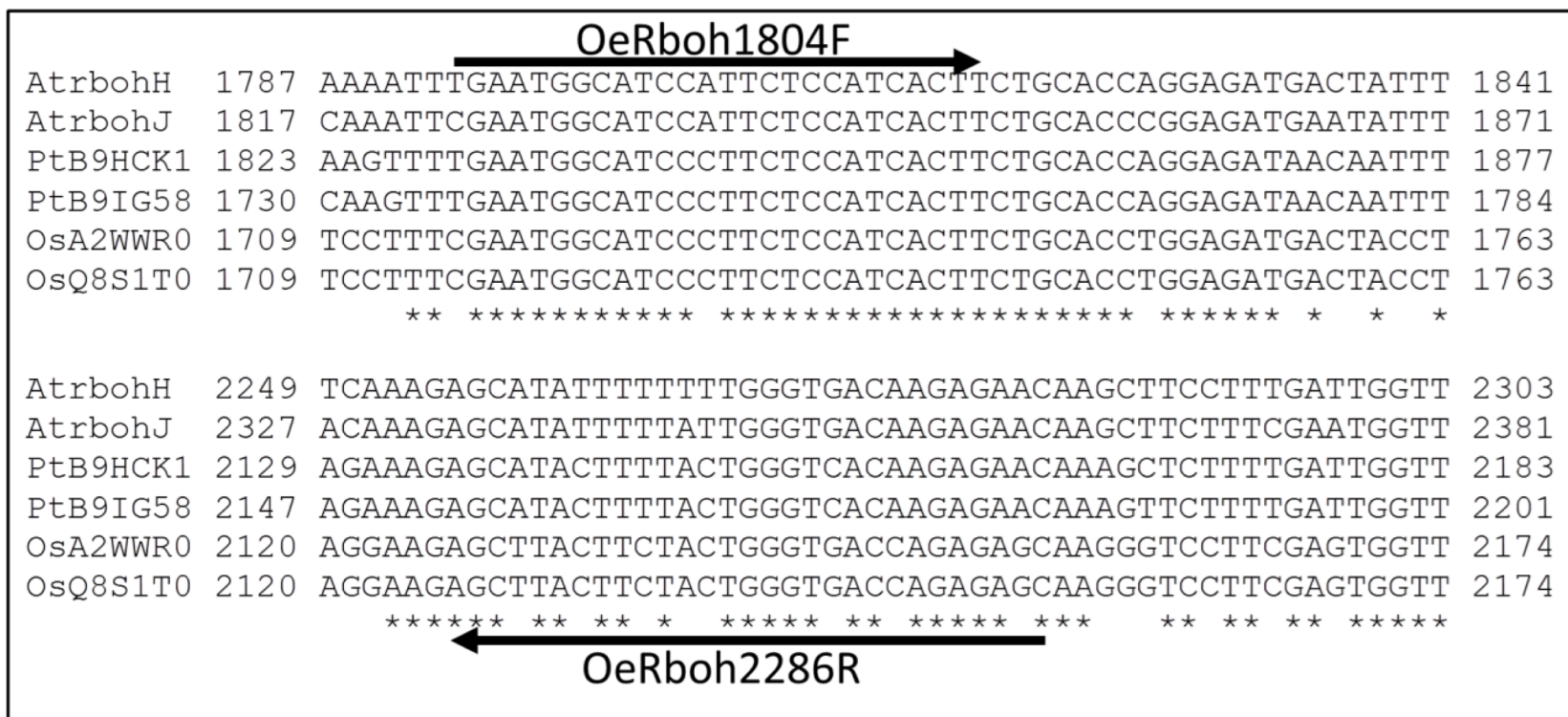


Supplementary Material

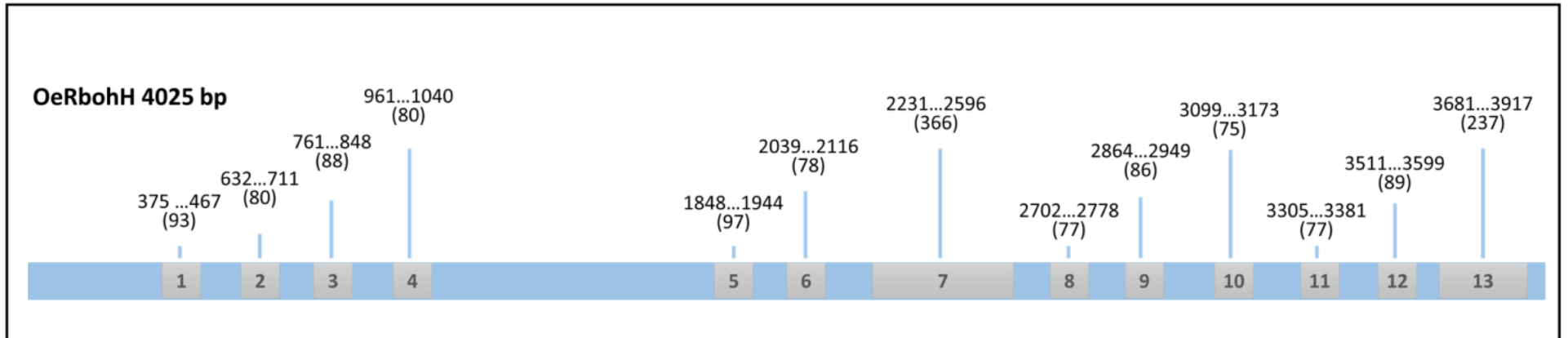
Generation of superoxide by OeRbohH, a NADPH oxidase activity during olive (*Olea europaea* L.) pollen development and germination.

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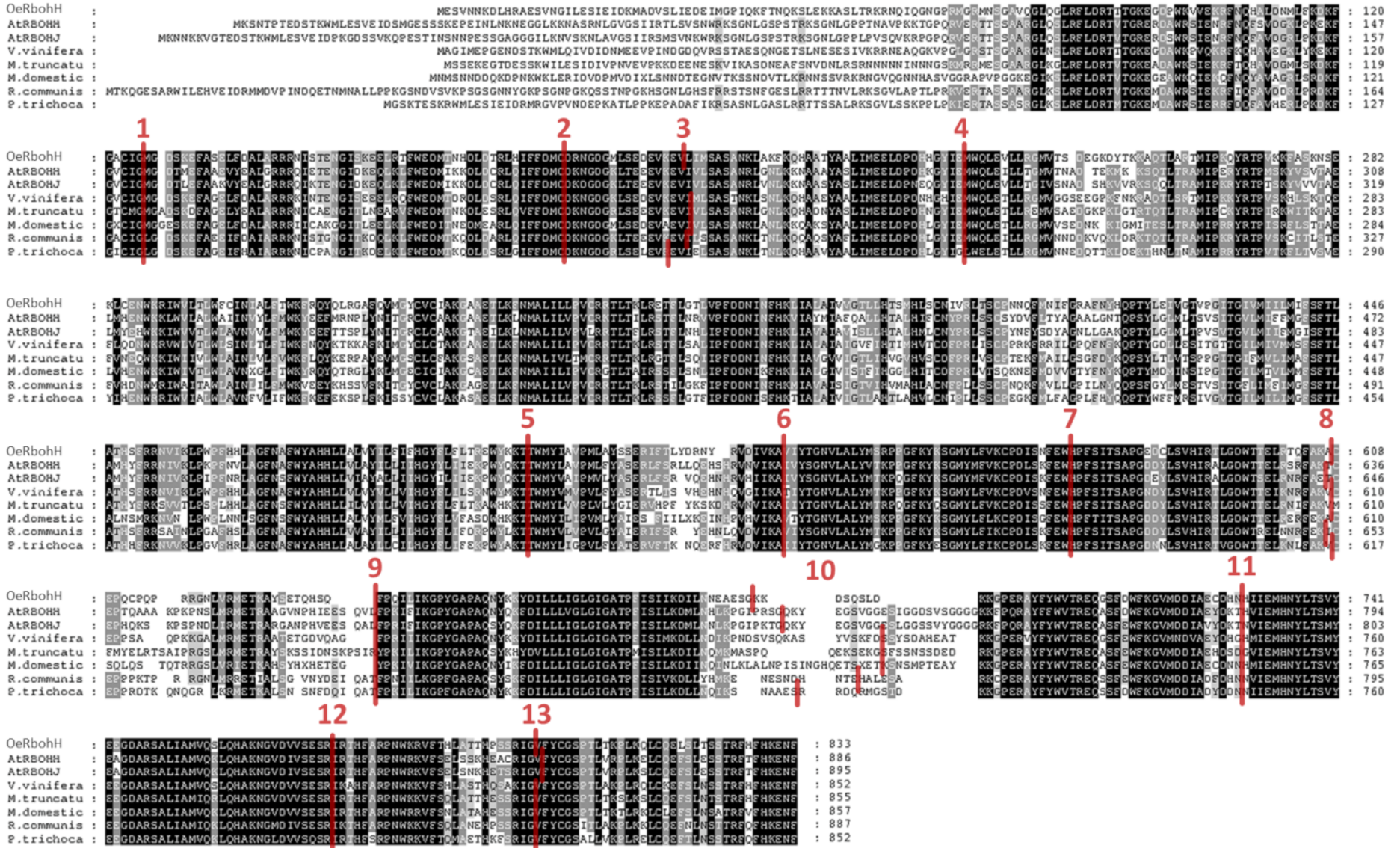
* **Correspondence:** Juan de Dios Alché juandedios.alche@eez.csic.es



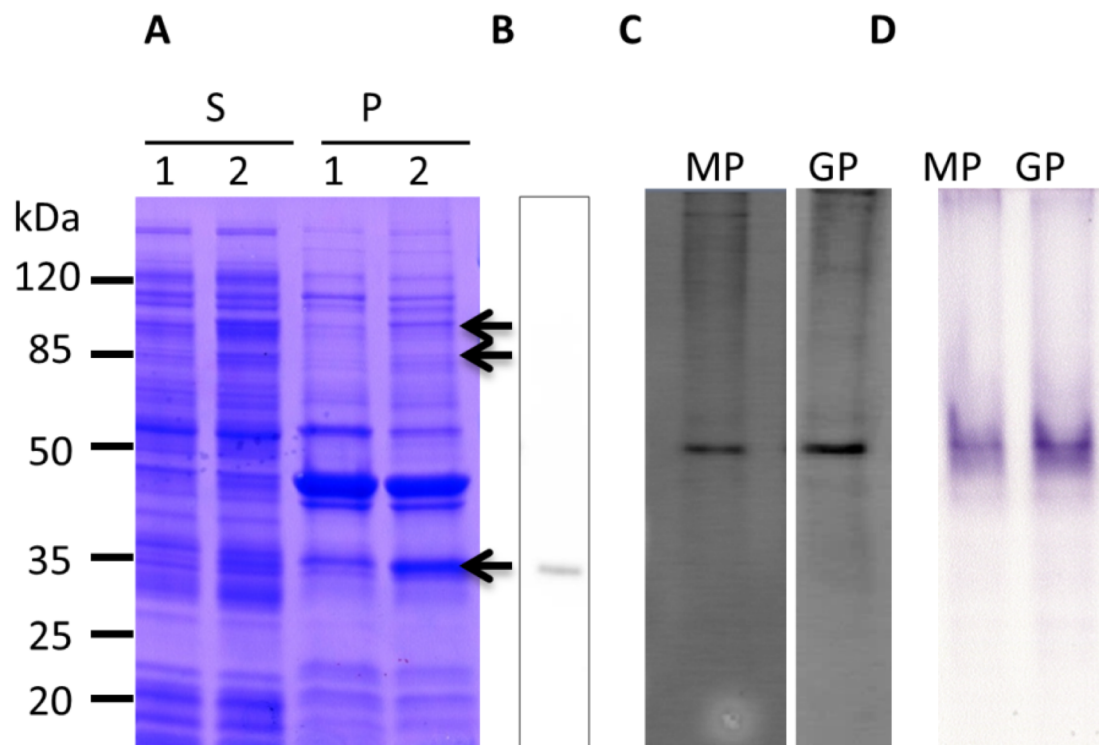
Supplementary Figure 1. Partial ORF alignment of homologous plant NOXs from *A. thaliana* (At5g60010, At3g45810), *P. trichocarpa* (PtB9HCK1, PtB9IGS8) and *O. sativa* (OsA2WWR0, OsQ8S1T0). Arrows indicate the conserved domains used to design degenerated primers forward and reverse.



Supplementary Figure 2. Genomic sequence of olive pollen *OeRbohH* with introns location (grey boxes). Location and length in bp (in brackets) are indicated.



Supplementary Figure 3. OeRbohH introns are conserved. Amino acid alignment of Rbohs from *A. thaliana*, *V. vinifera*, *M. truncatula*, *M. domestica*, *R. communis*, and *P. trichocarpa*. Introns location is conserved (red boxes).



Supplementary Figure 4. (A) SDS-PAGE of crude protein extracts of *E. coli* after induction of the expression. S: soluble fraction. P: precipitate; 1: negative control (pET51b); 2: expression construct (pET51b-OeRbohH). Arrows indicate the 3 bands which were later identified by LC-ESI-MS as such OeRbohH. (B) Western-blot using an anti-HisTag antibody in the insoluble fraction of the extract. (C) Western-blot using the anti-OeRbohH antibody in olive pollen protein extracts subjected to native-PAGE. The immuno-reactive band in the blot match the NADPH oxidase activity band in parallel in gel activity assays (D). MP, mature pollen; GP: germinated pollen.

Primer name	Sequence	Use
OeRboh1804F	GAATGGCATCCMTTCTCCATCACTTC	Degenerated primer plant Rbohs
OeRboh2286R	TCTCTKGSACCCARWARAARTAWGCTCTT	
UPM long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	Universal Primer for RACE 5' y RACE 3'
UPM short	CTAATACGACTCACTATAGGGC	
PoliT24(A)	TTTTTTTTTTTTTTTTTTTTTTTTTTN	Race 3'
OeRbohFW2	CTAATTGGTTTGGGAATTGGAGCAACCCC	RACE 3'
OeRbohRW2	CTGACTAAGTTTCCCCTTCTGGTTGTGGACTGAGG	RACE 5'
OeXbaRbohF	CGCTCTAGAATGGAATCAGTGAACAATAAAGATC	For coding genomic, pET51b and GFP vector cloning
OeSacRbohR	GCCGAGCTCCAAAGTTTTCTTGTGAAAATG	For coding genomic and pET51b vector cloning
UTRRbohF	ATTGATATTCGTACTAGTATCTCAACAG	Gene expression studies
UTRRbohR	AAAATGCGAGAATTTTACAAGTATTCAC	
Oe18S-F	TTTGATGGTACCTGCTACTCGGATAACC	Olive Housekeeping gene (ribosomal 18S)
Oe18S-R	CTCTCCGGAATCGAACCTAATTCTCC	
OeA-F	TTGCTCTCGACTATGAACAGG	Olive Housekeeping gene (actin)
OeA-R	CTCTCGGCCCAATAGTAATA	
OeqF	AGTGTCACAACCAAGAAGG	For quantitative PCR
OeqR	ATGGGGTTGCTCCAATC	
OeRbohR	ACGTTCTAGAGAAAGTTTTCTTGTGAAAATG	For GFP Vector cloning
OeRboh-Ngo-F	ATAGCCGGCATGGAATCAGTGAACAAT	For YFP Vector cloning
OeRboh-Xma-RS	TATCCCGGGTCAAGCTCCAAAGTTTTC	
OeRboh-Xma-RNS	TATCCCGGGTCCAGCTCCAAAGTTTTC	
OeRbohRW8	CCCATTTACAGATTCTGCTCTATGTAGATCTTTATTGTTT	For OeRbohH promoter isolation
OeRbohRW7	GAAACATCAGCCATTTTATCAATCTCAATGCTCTCC	
OeRbohRW9	ATGAATTGCTCTGTCAAGGTGCTTATTGAGTCTTAGC	
OeRbohRW10	CGTCTCTCAAGATACAGCGAAATCTCCTTGCAGCAG	
pRboh1.8F	ATACTCGAGGCCCGGGGAGGTAT	For pBI101 cloning
pRboh1.5F	TGCACTCGAGCGGGGAGGTAATTCTGCT	
pRboh0.7F	GCGCTCGAGTCCGAATTACGAGT	
pRboh0.2F	TGCACTCGAGGTAAGTTGGGGATCT	
pRbohR	TGCAAGATCTGACTGACTGATTCTCTGTTGAG	

Supplementary Table 1. List of primers used in this study.

	PROTEIN HITS	NAME [ORGANISM]	PEPTIDE
1	gi 56201942	Putative respiratory burst oxidase [<i>Oryza sativa Japonica</i> Group]	R.IGVFYCGSPTLTK.Q R.SALIAMVQSLQHAK.N
2	gi 225435144	PREDICTED: putative respiratory burst oxidase homolog protein H-like [<i>Vitis vinifera</i>]	K.NGVDVVSESR.I K.FNMALILIPVCR.R + Oxidation (M) R.SALIAMVQSLQHAK.N R.SALIAMVQSLQHAK.N + Oxidation (M)
			gi 171903618
3	gi 109631188	NOX1 [<i>Striga asiatica</i>]	K.NGVDVVSESR.I R.EQGSFDWFK.G R.IGVFYCGSPTLTKPLK.K
	gi 225435144	PREDICTED: putative respiratory burst oxidase homolog protein H-like [<i>Vitis vinifera</i>]	R.FHFHK.E K.NGVDVVSESR.I R.SALIAMVQSLQHAK.N
	gi 357131247	PREDICTED: putative respiratory burst oxidase homolog protein H-like [<i>Brachypodium distachyon</i>]	R.FHFHK.E R.EQGSFDWFK.G R.SALIAMVQSLQHAK.N
	gi 357505521	Respiratory burst oxidase-like protein [<i>Medicago truncatula</i>]	R.FHFHK.E K.NGVDVVSESR.I R.THFARNWK.K
	gi 294462658	Unknown [<i>Picea sitchensis</i>]	R.EEGSFDWFK.G R.SALIAMLQALNHAK.H + Oxidation (M) K.GIIEHNYCTSVYEEGDA R.S
	gi 18389310	Respiratory burst oxidase protein F [<i>Solanum tuberosum</i>]	R.EQGSFDWFK.G R.THFARNWK.K
	gi 166199744	RecName: Full=Respiratory burst oxidase homolog protein D; AltName: Full=NADPH oxidase RBOHD; AltName: Full=StRBOHD	R.EQGSFDWFK.G R.SALIAMLQSLNHAK.N
	gi 356527324	PREDICTED: respiratory burst oxidase homolog protein C-like isoform 1 [<i>Glycine max</i>]	R.EQGSFDWFK.G R.SALIAMLQSLNHAK.N
	gi 326491643	Predicted protein [<i>Hordeum vulgare subsp. vulgare</i>]	R.SALIAMLQSLNHAK.H K.GIIEHNYCTSVYEEGDA R.S
	gi 168011242	Predicted protein [<i>Physcomitrella patens subsp. patens</i>]	K.AYFYWMTR.E R.THFARNWK.S

Supplementary Table 2. Identification from samples 1, 2 and 3 and peptide summary obtained by LC-ESI-MS/MS. Taxonomy: Viridiplantae (Green Plants).