Role of a Respiratory Burst Oxidase of *Lepidium sativum* (Cress) Seedlings in Root Development and Auxin Signaling

Kerstin Müller^{1*}, Ada Linkies^{2*}, Gerhard Leubner-Metzger^{2,3}, and Allison R. Kermode^{1,4}

¹Simon Fraser University, Department of Biological Sciences, 8888 University Drive, Burnaby BC V5A 1S6, Canada

²Albert-Ludwigs-University, Institute for Biology II, Faculty of Biology, University of Freiburg, Schänzlestr. 1, 79104, Freiburg, Germany

³Royal Holloway, University of London, School of Biological Sciences, Egham, Surrey, TW20, 0ZX, UK

*Authors contributed equally to this work

Supplementary Data

⁴Author for correspondence: kermode@sfu.ca; Tel +1-778-782-3982; Fax 778-782-3496

Supplementary Figure S1. Alignment of the deduced amino acid sequences of the AtrbohB and LesarbohB cDNAs. Alignment of the AtrbohB amino acid sequence with that of its putative ortholog LesarbohB. EF hand, transmembrane and NOX/Duox like FAD/NADP binding domains of the two plant species were determined with the algorithm DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST) and are indicated in the alignment. Note that the amino acids are missing at the LesarbohB termini due to missing bases in the sequencing. Green fillings in the top panel and black fillings in the sequences represent identical amino acids between both sequences. The top panel with green filling also shows amino acid numbers.

Supplementary Figure S2. Alignment of the deduced amino acid sequences of the AtrbohF and LesarbohF cDNAs. Alignment of the AtrbohF amino acid sequence with that of its putative ortholog LesarbohF. EF hand, transmembrane and NOX/Duox like FAD/NADP binding domains of the two plant species were determined with the algorithm DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST) and are indicated in the alignment. Green fillings in the top panel and black fillings in the sequences represent identical amino acids between both sequences. The top panel with green filling also shows amino acid numbers.

Consensus Identity	1	40 R D
 LesarbohB AtrbohB 	1 2 12 12 RCKATGSHNSEEDCVEITLEA 1 10 20 30 MREEEMESSSEGETNKISRCKATGSDNPDEDYVEITLEV	22 R D 40 R D
Consensus Identity	50 60 70 80	
1. LesarbohB	32 42 52 62 ESISTMKSRAALRSVVSGRLKTMVKSMSFASRKLDRSKS 50 60 70 80	SG
2. AtrbohB Consensus Identity	ETINTMKAKATLRSVLSGRLKTMVKSLSFASRRLDRSKS 90 100 110 120 AXFALRGLRFXAKNDAVGRGWDEVAXRXDXLAXXGKLPK	F G S K
1. LesarbohB	72 82 92 102 A I F A L R G L R F T A K N D A V G R G W D E V A K R L D E L A I G G K L P K	S K
2. AtrbohB Consensus	AMFALRGLRFIAKNDAVGRGWDEVAMRFDKLAVEGKLPK 130 140 150 160 FGXCIGMXESSEFVNELXEALVRRRGTTSSSITKTELXE	S K F W
Identity 1. LesarbohB	112 122 132 142 FGRCIGMAESSEFVNELYEALVRRRGTTSSSITKTELSE 130 140 150 160	F W
2. AtrbohB Consensus	F G H C I G M V E S S E F V N E L F E A L V R R R G T T S S S I T K T E L F E A L V R R R G T T G D E V K E I I A L S	F W A S
Identity 1. LesarbohB	152 162 172 182 EQITGNSFDARLQIFFDMVDKNLDGRITGDEVKEIIALS	A S
2. AtrbohB Consensus	170 180 190 200 E Q I T G N S F D D R L Q I F F D M V D K N L D G R I T G D E V K E I I A L S 210 220 230 240 A N K I S K T K E N V D E Y X A I I M E E L D X D N I G Y I E L H N I E T I I	A S
Identity	192 202 212 222 A N K L S K I K E N V D E Y V A L I M E E L D P D N L G Y I E L H N L E T L L	T O
 LesarbohB AtrbohB 	210 220 240 240 240 250 250 260 270 280	LQ
Consensus Identity	V P S O S N X N S P S S A N K R A L N K M L S O K L I P T K D R N P V K R X A 232 242 252 262	XN
 LesarbohB AtrbohB 	V P S Q S N D N S P S S A N K R A L N K M L S Q K L I P T K D R N P V K R Y A 250 V P S Q S N - N S P S S A N K R A L N K M L S Q K L I P T K D R N P V K R F A 290 300 310 320 X S Y F X I E N W X R I W V I T I W I S T C X X I F T W K F I O Y X R X T V F	R N M N
Consensus Identity	X S Y F X L E N W X R I W V L T L W J S I C X X L F T W K F J Q Y X R X T V F 272 282 292 302	E V
 LesarbohB AtrbohB 	V S Y F L L E N W Q R I W V L T L W L S I C V A L F T W K F I Q Y R R R T V F 289 1 S Y F F L E N W K R I W V L T L W I S I C I T L F T W K F L Q Y K R K T V F	E V
Consensus Identity	X G Y C V X V A K G S Å E T L K F N M A L I L L P V C R N T I T W L R T X S K	JX
 LesarbohB AtrbohB 	LĠYCVSVAKGSÄETLKFNMALİLPVCRNTITWLRTNSK 329 349 349 359 MĠYCVTVAKGSÄETLKFNMALİLLPVCRNTITWLRTKSK	I V
Consensus Identity	370 380 390 400 G S V V P F D D N I N F H K V X A F G I A V G X X L H X I S H L A C D F P R L	410 L H
 LesarbohB AtrbohB 	352 362 372 382 382 379 389 389 389 389 389 389 389 389 389 38	392 LH 409 LH
Consensus Identity	A K N V X X E P X K X F F G X E R P X N Y G W F M K G X D G W T G V X M V V L	450 M L
LesarbohB AtrbohB	402 A K N V A Y E P I K R F F G E E R P D N Y G W F M K G I D G W T G V I M V V L 419 429 439 A K N V E F E P M K K F F G D E R P E N Y G W F M K G T D G W T G V T M V V L	432 M L 449 M T
Consensus Identity	460 V A Y V L A Q S W F X R N R A N L P K S X K R L X G F N A F W Y S H H L F V I	V Y
LesarbohB AtrbohB	442	VY
Consensus Identity	500 510 520 530 V L L I V H G Y F X Y L S K E W Y H K T T W M Y L A V P V L L Y A X E R L I R	A F
 LesarbohB AtrbohB 	482 492 502 512 V L L I V H G Y F I Y L S K E W Y H K T T W M Y L A V P V L L Y A C E R L I R 499 509 509 519 529 V L I, I V H G Y F V Y L S K E W Y H K T T W M Y I, A V P V I, I, Y A F E R I, I R	A F
Consensus Identity	540 550 560 570	X N
1. LesarbohB	522 532 542 552 R P G S K A V R V L K V A V Y P G N V L S L Y M S K P K G F K Y T S G O Y I Y 539 569 569	VN
2. AtrbohB Consensus Identity	RPGAKAVKVLKVAVYPGNVLSLYMSKPKGFKYTSGQYIY 580 590 600 610 CSDVSPXQWHPFSITSASGDDYLSXHIRTLGDWTSQLKS	LX
1. LesarbohB	C S D V S P F Q W H P F S I T S A S G D D Y L S I H I R T L G D W T S Q L K S 589 599 609	L F
2. AtrbohB Consensus Identity	C S D V S P L Q W H P F S I T S A S G D D Y L S V H I R T L G D W T S Q L K S 620 630 650 650 650 8 K V C Q L X S X S Q S G L F X X X X X X A N B I T R F P R L L I D G P Y G A	L Y P A
1. LesarbohB	602 612 617 627 SKVC O ? LSRSOSGLFM ANDITRFPRLLIDGPYGA	PΑ
2. AtrbohB Consensus	SKVC Q L P S T S Q S G L F I A D I G Q A N N I T R F P R L L I D G P Y G A 660 Q D Y R N Y B V L L L V G L G I G A T P L I S I I R D V L N X I K N Q X S I E	P A
Identity 1. LesarbohB	637 Q D Y R N Y N V L L L V G L G I G A T P L I S I I R D V L N I I K N Q K S I E 659 679 689	QN
2. AtrbohBConsensus	Q D Y R N Y D V L L L V G L G I G A T P L I S I I R D V L N N I K N Q N S I E 700 710 720 730 730 T N X H X X X X K X Y V A T K R A Y F Y W V T R E Q G S L E W F S Z V M N E V	
Identity 1. LesarbohB	677 687 697 707 T N N H N V S T K S Y V A T K R A Y F Y W V T R E Q G S L E W F S Q V M N E V 716 726	A E
2. AtrbohB Consensus	T N Q H I K N Y V A T K R A Y F Y W V T R E Q G S L E W F S E V M N E V 740	A E I D
Identity	717 727 737 747 Y D S E G M I E L H N Y C T S V Y E E G D A R S A L I T M L Q S L H H A K S G	I D
2. AtrbohB	736 746 756 766 Y D S E G M I E L H N Y C T S V Y E E G D A R S A L I T M L Q S L H H A K S G 780 800 810 I V S G T R V R T H F A R P B W R S V F K H V A V N H V N O R V G V F Y C G N	I D 820 X C
Consensus Identity 1. LesarbohB	757 767 TVSGTRVRTHFARPBWRSVFKHVAVNHVNQRVGVFYCGN 1 VSGTRVRTHFARPBWRSVFKHVAVNHVNQRVGVFYCGN 1 VSGTRVRTHFARPBWRSVFKHVAVNHVNQRVGVFYCGN	797 A C
2. AtrbohB	776 786 796 806 I V S G T R V R T H F A R P N W R S V F K H V A V N H V N Q R V G V F Y C G N 830 840 847	A C 816 T C
Consensus Identity	810	
 LesarbohB AtrbohB 	LIAELKRLAODFS 826 843 IIGELKRLAODFSRKTTTKFEFHKENF	

Consensus Identity	MXPFSKXD	10 RRRWSFDSV	20 7 S A X X X A X G S	30 SASTSPGTEY	SXXGXXZEFVE	50 VTIDLQDDDTI
1. AtrbohF	MK PFSKND	10 RRRWSFDSV	20 7 S A G K T A V G S 20	30 SASTSPGTEY	SING-DQEFVE	49 CVTIDLODDTI 50
2. LesarbohF Consensus	MRPFSK H D 60 VLRSVEPA	RRRWSFDSV 70 TAINVXXDI	SAERNAIGS 80 ISDXXXXXXX	SASTSPGTEY 90 XX <mark>GX</mark> MTPVSI	SNGGYGEEFVE 100 SRSPTMKRTSS	VTIDLQDDDTI 110 SNRXROFSQELK
Identity 1. AtrbohF	59 VLRSVEPA	69 TAINV IGDI	 ISDDNT	79 84 G IMTPVSI	94 S R S P T M K R T S S	104 SNRFROFSOELK
2. LesarbohF	VLRSVEPA	70 TAINVDI	78 [SDETASTA(14	SGGMMTPVSI	SRSPTMKRTSS 150 16	
Consensus Identity	AEAVAKAK	QLSQELKRE	F S W S R S F S G >	K LTTT X X X X X X	Ö X G G X X G G L V N	
 AtrbohF LesarbohF 	AEAVAKAK 118 AEAVAKAK	QLSQELKRE 128 QLSQELKRE	FSWSRSFSG		OSGGAGGGLVN 145 15 ONGGGGLVN	ISALEARA LRKO 163 ISALEARA LRKO
Consensus Identity	RAQLDRTR	180 SSAQRALRO	190 G L R F I S N K X X	200 KNXDGWNDVQ	210 SNFEKXXKNGY	220 YIYRSDFAQCIG
1. AtrbohF	RAQLDRTR	174 SSAORALRG 173	184 G L R F I S N K Q F 183	194 (N V D G W N D V Q 193	204 SNFEKFEKNGY 203	214 YIYRSDFAQCIG 213
LesarbohFConsensus Identity	RAQLDRTR 230 MKDSXEFA	SSAQRALRO 240 LELFDALSF	G L R F I S N K R 7 250 R R R R X X V E K I	INFDGWNDVQ 260 INHDELYEYW	SNFEK LSKNGY 270 SQINDESFDSF	IYRSDFAQCIG 280 RLQIFFDIVDKN
1. AtrbohF	224 MK DSK EFA	234 LE LFDA LSF	244 RRRRLKVEKI	254 N H D E L Y E Y W	264 SQINDESFDSF	274 R LQIFFDIVDK N
2. LesarbohF	223 MK DSNEFA 290	LELFDALSF	243 RRRRQRVEKI 310	INHDE LYEYW		273 RLQIFFDIVDKN 340
Consensus Identity	EDGRITEE 284	294	304	LK X O A E E Y A A	4 324	334
 AtrbohF LesarbohF 	EDGRÍTEE ²⁸³ EDGRÍTEE	EVKEIIMLS 293 EVQEIIMLS	SASANK LSRI 303 SASANK LSRI	LK E O A E E Y A A 31 LK D O A E E Y A A	LIMEE LDPERI 3 323 LIMEE LDPERI	LGYIELWQLĖTL 333 LGYIELWQLĖTL
Consensus Identity	350 LLQKDTYL	NYSQALSYT	360 SQALSQNL	370 GLRXXSRIH	380 RMSSDXVYXXQ	390 ENWKRIWVLX L
1. AtrbohF	344 LLQKDTYL 343	NYSQALSYT	354 FSQALSQNL 353	364 QGLRGKSRIH 363	374 RMSSDFVYIMQ 373	384 DENWKRIWVLS L 383
LesarbohFConsensus	LLOKDTYL ⁴⁰⁰ WXMIMJGL	NYSQALSYT 410 FLWKFFQYK	ISQALSONLI 420 KOKDÁFHVMO	H G LR Q N S R I H 430 G Y C L L T A K G A	RMSSDCVYFIC 440 AETLKFNMALI	ENWKRIWVLFL 450 LFPVCRNTITW
Identity 1 AtroopE	394 WIMIMIG L	404 F LWK F F Q Y K	414 (O K D Å F H V M (424 S Y C T. I. T A K G A	434 . A F. T I.K F N M A I. I	444 TEDVCDNTTTW
 AtrbohF LesarbohF 	WIMIMIGL 393 WVMIMLGL 460	FLWKFFOIR 403 FLWKFFOYK 470	413 (QKDAFHVMC 480	423	433	LFPVCRNTITW 443 LFPVCRNTITW 510
Consensus Identity	LRSTRLSY	FVPFDDNIN	NFHKTIAĠAI	IVVXVILH XG	DHUACDFPRIV	510 RATEYDYNRY L
1. AtrbohF	454 LRSTRLSY 453	FVPFDDNIN 463	474 NFHKTIAGAI 473	484 IVVAVILHI 483	494 DHLACDFPRIV 493	504 7 RATEYDYNRY L 503
LesarbohFConsensus	LRSTRLSY 520 FHYFOTKO	53	NFHKTIAGAI 30 GPEGITGILN	IVVGVILHVG 540 <u>4VILM</u> X <u>ISF</u> X	DHIACDFPRIV 550 LATRWFRRNLV	RATEYDYNRY L 560 570 KLPXPFDRLTG
Identity 1. AtrbohF	5 ₁ 4 F H Y F O T K O		24 FPEGITGILN	534 4 V I LM I I S F T	544 LATRWFRRNLV	554 564 K LPK PFD R LT G
2. LesarbohF	513 FHYFOTKO	PTYWDLVK C	23 EPEGITGILN 590	533 4VILM T ISF T 600	543 LATRWFRRNLV 610	553 563 KLPRPFDRLTG 620
Consensus Identity	FNAFWYSH	H L F X I V Y X I	LLILHGUFLY 584	Y F A K P W Y V X T	TWMYLAVPVLI 604	YGGĖRTLRYFR 614
 AtrbohF LesarbohF 	FNAFWYSH FNAFWYSH	HLFVIVYII 573 HTFTTVYVI	LILHGIFLY 583	7 F A K P W Y V R T 593 7 F A K P W Y V H T	TWMYLAVPVLI 603 TWMYLAVPVLI	LYGGERTLRYFR 613 .YGGERTLRYFR
Consensus Identity	630	640 LKVAIYPGN	650 IVLTLQMSKI	PXQFRYKSGQ	670 YMFVQCPÅVSP	680 PFEWHPFSITSA
1. AtrbohF	624 S G S Y S V R L 623	634 LKVAIYPGN 633	644 NVLTLOMSKI 643	654 PTOFRYKSGO 653	664 YMFVQCPAVSE 663	674 PFEWHPFSITSA 673
2. LesarbohF Consensus	SGSYSVRL 690 PEDBYISI	LKVAIYPGN 700 HIRZLGDWI	IV LT LOMSK F 7' TOE LK XX F S F		YMFVQCPAVSE 720 73 SGLLRADEXTK	
Identity	684	694	70)4	714 72	
 AtrbohF LesarbohF 	PEDDYISI 683 PEDNYISI		TQELK K KFSE	D3 EVCQPPVAGK	SGLLRADETTK 713 72 SGLLRADESTK	TSLPKLLIDGP
Consensus Identity		750 X <mark>KYDVLLL</mark>	760 7 G LG I G A T P I	770 FISILKDLLN	780 NIVKMEEHADS	790 SISDFSRSSEXS
1. AtrbohF	YGAPAQDY	744 R <u>KYDVLLL</u> 743	754 7 G LG I GATP E 753	764 FISILKDLLN 763	774 NIVKMEEHADS 773	784 S I S D F S R S S E Y S 783
2. LesarbohFConsensus	YGAPAQDY 800 TGSNXXTP	MKYDVLLLV 810 RRKXILKTT	/G LG I GAT P I 820 [NAYF YWV T I	FISİLKDLLN 830 REQGSFDWFK	NIVKMEEHADS 840 GVMNEVÄELDQ	SISDFSRSSE H S 850 ORGVIEMHNYLT
Identity	794	804	814	824	834	844 RGVIEMHNYLT
 AtrbohF LesarbohF 	793 TGSNSETP	RRKRILKTI 803 RRKKILKTI	TNAYFÝWVTE 813 TNAYFÝWVTE	REQGSFDWFK 823 REQGSFDWFK	833 GVMNEVAE LDÇ	843 RGVIEMHNYLT
Consensus Identity	860 SVYEEGDA	RSALITMVÇ	880 OALNHAKNG V	89 VDIVSGTRVR	THFARPNWKKV	910 7 LXK LSSK HXN A
1. AtrbohF	854 SVYEEGDA 853	RSALITMVO 863	873	88	THFARPNWKK V 3 893	903
2. LesarbohFConsensus	SVYEĖGDA 920 RIGVFYCG	RSALITMVQ	OA LNHAKNĠV 930 SK LCNTFNOF	940	THFARPNWKKV 950 EHF	'L <mark>S</mark> K LSSK HÏNA
Identity	914 RIGVFYCG		924 3 8 J. C. N. T. F. N. O. F.	934 (GSTK FEFHK	944 E. H. Z	
AtrbohF LesarbohF	RIGVFYCG 913 RIGVFYCG		SK LCNTFNOE 923 SK LCNTFNOE	GSTKFEFHK 933 GSTKFEFHK	<u>Fr. H. Fr.</u> 943 Fr. H. Fr.	