Supplementary material

Primers for PpPPO1 (Acc.No. AY904721)

Name	Sequence 5´-3´	T _m	restriction site
cPPO1_forw	GATCCATGGAGTTTACGTGCGTATTG	53.1	Ncol
cPPO1_rev	GCATGTCGACTTTCTCAAGCTTGATC	57.8	Sall

Primers for PpPPO6 (PHYPA_Pp1s90_147V6.1)

Name	Sequence 5´-3´	T _m
cPPO6_forw	ACAACTGGAAAAAGCAGGGC	56.3
cPPO6_rev	CATAGTAACCTCCGGGCTGA	56.1

Table S1: Properties of *PPO* gene family members. Except for PPO9 gene models are derived from the V1.6 genome assembly (https://www.cosmoss.org). For PPO9 an edited version of V1.2 (see below) was used for computation. MW and pl were computed by <u>http://web.expasy.org/cgi-bin/compute_pi/pi_tool</u>.

Gene	Gene model	ORF	MW	Isoelectric	No of
name	number	(aa)	(kDa)	point (pl)	introns in
					CDS
					according
					to gene
					model
PPO1	Pp1s121_25V6.1	536	60.12	9.42	1
PPO2	Pp1s491_22V6.1	550	62.30	9.31	1
PPO3	Pp1s167_107V6.1	559	62.69	7.26	1
PPO4	Pp1s3_280V6.1	575	64.82	6.02	1
PPO5	Pp1s83_207V6.1	569	64.48	9.13	1
PPO6	Pp1s90_147V6.1	552	62.79	8.77	1
PPO7	Pp1s16_226V6.1	546	62.15	5.51	0
PPO8	Pp1s83_200V6.1	541	61.51	5.38	1
PPO9	Pp1s559_8V2.1 plus	544	61.65	5.43	2
	29 N-terminal aa				
PPO10	Pp1s455_4V6.1	549	62.10	6.32	0
PPO11	Pp1s3_617V6.1	623	70.31	7.28	2
PPO12	Pp1s85_167V6.1	546	61.93	5.8	0
PPO13	Pp1s41_46V6.1	573	64.41	7.27	1

Deduced and edited amino acid sequence for PPO9 (Pp1s559_8V2.1 plus 29 N-terminal aa)

>PPO9 V1.2 edited

MGKFQETGSRRELTCCKLGALVLIVSQMLMRITSGTPVPAPILPDNCTSEEGCCMPQPYTGKPARDFEGDL ALPIRIRRPVHKLNESEIARLERGYKLLRELPDLDPRSLSNQANLHCLYCDNGIYYNNMTWPLEIHNHWLF LPWHRMFLYFHERILAKLLDDDTFALPYWNWDNQSSSEEANILPRIYSTNETSYLRDLNRNKCAQPPNLVH LNSIGGCTDKTADELRIENTQVMYTQIVTGAPTPRLFFGEPYSYGDSGGYGPGTFEDNPHGTVHLWVGDPD AATAFNDMGNFGRSARDPVFYTHHSNIDRIWTIWKTLPGKQRTEPTHADFLDSRFTYYDENADQVIVNLSQ IINTPLLRNTRYEYEESPTAWVSRGQKPGHEKNVTACNPLSPSQTNAMIYTTPELAAAGTLDAKPLTFRVT RPERSDVGVEVLEIQGIKVDNTLQSHWGAYLFFPSAELNTSVSCPEFFGTFNFSPHVGQAQVTRDLVWRVG IRQKLIDLGKDDYDDIVVTLVRFGPSIQQLQLGGTQVLYDTSPTTLD



Supplementary figures

Figure S1: Quantification of phenolic compounds in *P. patens* **wild type tissue** (n=6). Error bars represent standard deviation of independent tissue samples. UPLC-MS was used to identify and quantify compounds.



Figure S 2: ESI(-)-MS spectrum of the unknown ester of caffeic acid which was tentatively identified as 2-O-caffeoylthreonic acid, according to data published by Parveen *et al.* (2008).



Figure S3: Characterisation of PPO1 knockout (d|ppo1) plants

(A) Schematic disruption of the genomic *PPO1* locus by insertion of the *nptll* cassette mediated by homologous recombination. The 5' and 3' *nptll* flanking regions originating from the *PPO1* coding sequence are coloured brown; position of the primers used in B are indicated by arrows.

(B) PCR analysis of genomic DNA testing for disruption of the WT locus and 5' and 3' integration of the *PPO1* knockout construct for d|ppo1 lines #1, #3, #5, #6 and #8. Primer combinations are given above each row and their binding positions are displayed in A.

(C) Expression analysis of *PPO1* knockout lines by RT-PCR performed with *PPO1* specific primers (shown as E1/E2 in A). *PPO1* was found to be expressed only in WT, but not in the transgenic *d*/ppo1 lines (upper panel). As a positive control, RT-PCR was carried out with the primers for *ACT3* corresponding to the constitutively expressed *actin3* gene (lower panel).



Figure S4: Protonema growth of *PPO1* knockout plants and wild type. Bright field microscopic images of protonema from 3 to 7 day old liquid cultures grown under standard conditions. The scale bars correspond to 100 µm in A.; 50 µm in B. and 20 µm in C.