

SUPPORTING INFORMATION

The Structural Characterization and Antipathogenic Activities of Quinoin, a Type 1 Ribosome-Inactivating Protein from Quinoa Seeds

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Fig. S1. Purification of RIPs from *C. quinoa* seeds. (a), Elution profile from the CM-Sepharose chromatography showing three main active peaks. (b) and (c), re-chromatography FPLC elution profiles of peak 1 and peak 2, respectively, on an AKTA Purifier System from cation exchange chromatography using a Source 15S PE 4.6/100 column. Experimental conditions are described in the Material and Methods.

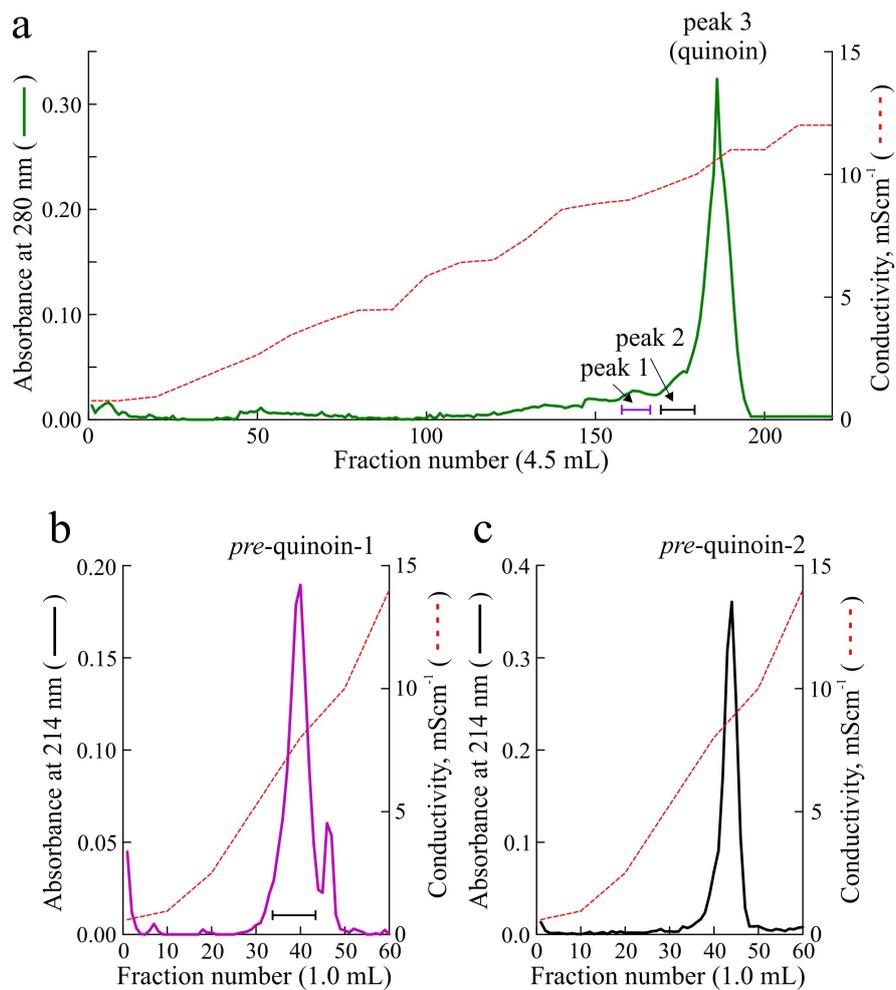


Table S1. Accession numbers, species and common names of ribosome inactivating proteins sequences used in this work for primary structure analyses.

| | Accession number | Species | name | Identity/Similarity (%) compared to quinoiin | Note |
|----|------------------|-----------------------------------|-----------------|--|---------|
| 1 | Q03464 | <i>Phytolacca americana</i> L. | PAP-alpha | 40.94/48.81 | |
| 2 | P34967 | <i>Phytolacca dioica</i> L. | PD-S2 | 38.97/47.24 | |
| 3 | P84854 | <i>Phytolacca dioica</i> L. | PD-L3/4 | 39.37/48.42 | |
| 4 | P84853 | <i>Phytolacca dioica</i> L. | PD-L1/2 | 38.58/46.06 | |
| 5 | P23339 | <i>Phytolacca americana</i> L. | PAP-S | 38.58/46.45 | |
| 6 | P10297 | <i>Phytolacca americana</i> L. | PAP-I | 36.61/46.06 | |
| 7 | Q6EH50 | <i>Phytolacca heterotepala</i> L. | heterotepalin-4 | 35.82/44.48 | |
| 8 | Q40772 | <i>Phytolacca americana</i> L. | PAP-II | 35.82/42.51 | |
| 9 | P85208 | <i>Phytolacca dioica</i> L. | dioicin-2 | 33.07/42.12 | |
| 10 | P20656 | <i>Saponaria officinalis</i> L. | saporin-6 | 29.24/35.17 | |
| 11 | P27559 | <i>Saponaria officinalis</i> L. | saporin-2 | 27.55/33.85 | |
| 12 | Q41391 | <i>Saponaria officinalis</i> L. | saporin-7 | 28.85/34.78 | |
| 13 | P24476 | <i>Dianthus caryophyllus</i> | dianthin-30 | 28.74/36.61 | |
| 14 | Q41389 | <i>Saponaria officinalis</i> L. | saporin-5 | 27.66/33.59 | |
| 15 | Q7M1Z2 | <i>Saponaria officinalis</i> L. | saporin-9 | 26.87/33.99 | |
| 16 | P85101 | <i>Silene chalcedonica</i> L. | lychnin | 27.77/34.18 | |
| 17 | P27560 | <i>Saponaria officinalis</i> L. | saporin-3 | 26.69/32.20 | |
| 18 | P21326 | <i>Mirabilis jalapa</i> L. | MAP-S | 24.00/31.20 | |
| 19 | P02879 | <i>Ricinus communis</i> L. | ricin | 24.01/33.85 | A-chain |
| 20 | P06750 | <i>Ricinus communis</i> L. | agglutinin | 23.62/32.28 | A-chain |

Fig. S2. Alignment of the amino acid sequences of the six proteins retrieved in *C. quinoa* genome classified as ‘protein synthesis inhibitor like’. A.C.: XP_021750597.1, protein synthesis inhibitor PD-S2-like; A.C.: XP_021750694.1, ribosome inactivating protein PD-L3/PD-L4-like; A.C.: XP_021737780.1, protein synthesis inhibitor PD-S2-like_(2); A.C.: XP_021726635.1, ribosome inactivating protein PD-L3/PD-L4-like; A.C.: XP_021735486.1, antiviral protein MAP-like; A.C.: XP_021750669.1. Identical residues (*), conserved substitutions (:), and semi-conserved substitutions (.) are reported.

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XP_021750597.1 -----
XP_021750694.1 -----
XP_021737780.1 -----
XP_021726635.1 -----
XP_021735486.1 -----
XP_021750669.1 MRAFVVVAISIWLVLEPTFAASDLSFDATSPTTNTYSKLLSRLREWLADPTLRYGNTNV

XP_021750597.1 -----
XP_021750694.1 -----
XP_021737780.1 -----
XP_021726635.1 -----
XP_021735486.1 -----
XP_021750669.1 PVMAATFKKRKYLKSGEGGTITIGKCLKSKTYLISNKMLLLLMMMLWMYCGDGTNYLSKV

XP_021750597.1 -----MQQENKKAWLVLTAIWVVLQQVNAADVTFKLEPKPTQNTYNTFLQSI
XP_021750694.1 -----MNGIQTIMRAVVVAISTWLVILKPTFAASDLSFDATNPAATTYSKLLSDDL
XP_021737780.1 -----MVGIKKFKIVSAVYVVAVAVMMVLEPTLAASDFTFDIEGATDITYSKLLTDV
XP_021726635.1 -----MVGIKVMGACVVVAIAVMMVLEPTLAASDIRISFDVEGATTATYSQLLNDV
XP_021735486.1 --MKLLAAPLSVI IWWCMMVMMAVVPPLTTAQDRETLSYSTVTTFELTNGVTGYSSFLTRL
XP_021750669.1 NKGSMKLLAAPISVIWWCMMMAVVPPLATAQDGETLAYSTVTTFELTNGVTGYQSFLTRL
::: : . * . : * :

XP_021750597.1 RSQTKDPSLVYEG--IPMIRPPTNPDTYLLVDLESKKDKNEIFVTLALSRNDLYVVAFA
XP_021750694.1 RNLVKDPKLYGG-TNPVMAAT-FKKKYILVDLKGSEGGTITIALNLNN--DIYVVGYL
XP_021737780.1 RNKVKDDKIVYGGRKDI PVMAAPSAAKKYLFVDFTAASEGRTITIAVNLNDFNLVIAYL
XP_021726635.1 RNKVKDKNIVYGGKLDLPVMAAP--SDNYLVVDLTASQKRTITLAVNLNKGFSLYVVIAYL
XP_021735486.1 RNRVEAP---TRACTLQSTRNPPLTGAEYVLVDLKI SNTQWVTLGIDAK---DLYVWAYQ
XP_021750669.1 RNQVESP---TRACTLQSTRNPPLTGAEYVLVDLKI SNTQWVTLGIDAK---DLYVWAYQ
* . : : . . . . * : * : . : : : * : * :

XP_021750597.1 D--KFGGKVRGHFFSNLNI DTIDKAKKVFEVQVFINITYGESYSQIESNAGTN-RLSFP
XP_021750694.1 D--KLNGNFRSHIFKDAPSDAKTDLFPEATGKN-RLTINYKSSYADIESNAGVSSRARVG
XP_021737780.1 D--KINGNFRSHIFKDLPPDVQEGFRDATGAANRLIMNYGSSYAEVEKKAGS--RRTKLG
XP_021726635.1 D--KINGNFRSHIFKDLPNVNDLNFPEAKGKANRLIMNYKSDYASIESKAGS--RNKLG
XP_021735486.1 DNVKYNKYRANFLSDAPQAADRLFPGSTKR---TTRFGGNYNSLEPAAGTT-RKNLV
XP_021750669.1 DNVKYNKYRATFLNDAPQAADRLFPGSTQR---TTRFGGNYNSLEPAAGTT-RKNLV
* * . * : * . : : : . . . . : . * : * * * * .

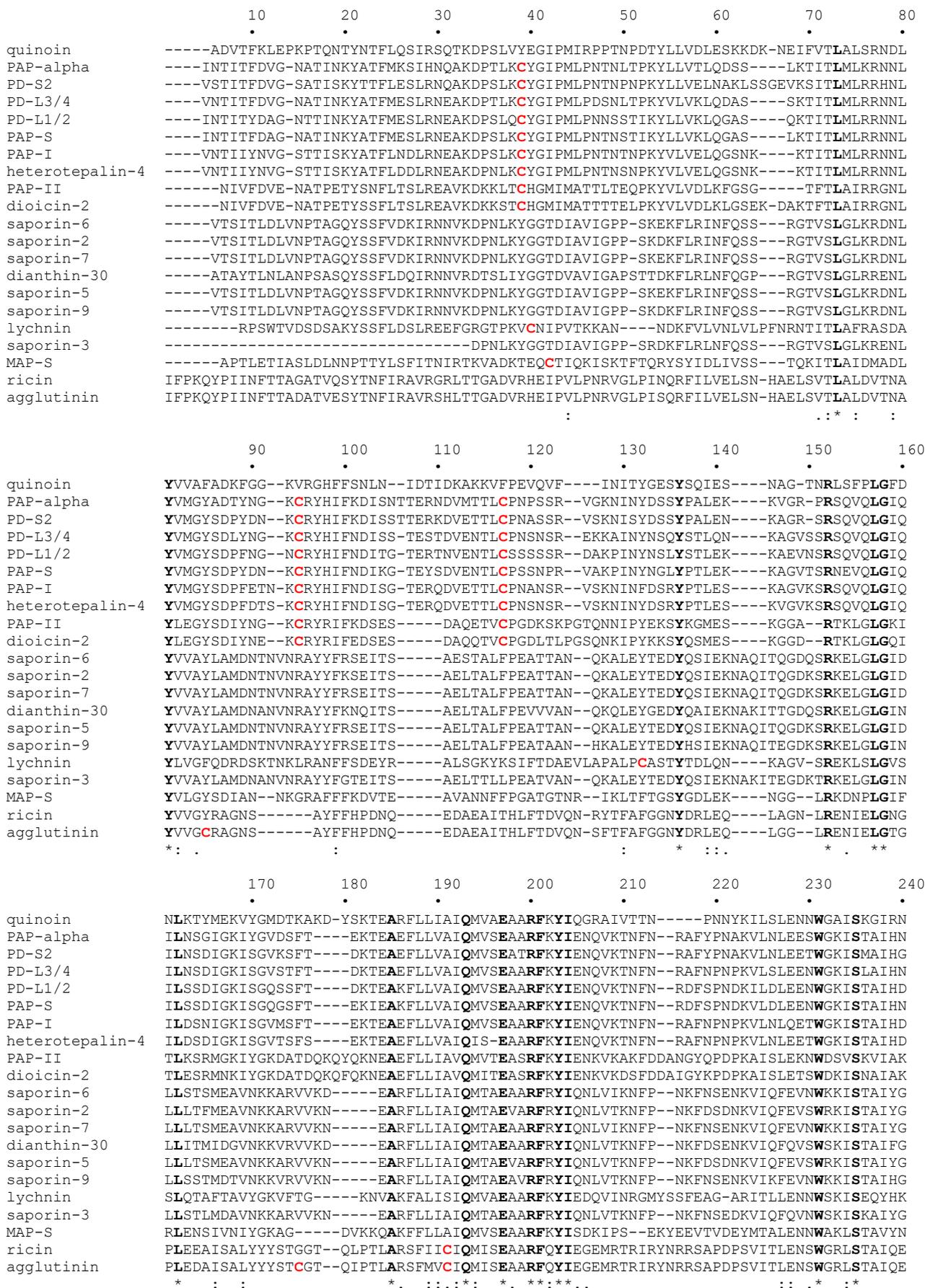
XP_021750597.1 LGFDNLKTYMEKVGMDTKAKDYSKTEARFLLIAIQMVAAARFKYIQGRAIVTTPNPN-
XP_021750694.1 LGVKPLNKFINDVYGKALVVKN---EAQFMLVVIQMVSEATRFKYIESMILEKFADSY-
XP_021737780.1 LGKKPLSKFFDKVYKGLVDNEN---EAKFMLIVMQLSEATRFAYIEQMIVQKFEKEIN
XP_021726635.1 LGKQPLNIFFNKVGKPVDDKN---QAKFMLVVMQMVSEATRFAYIENMIVQKFEQDIN
XP_021735486.1 LGIQNLDGAIRNVYNKQSESELNKGTEARFLLIAIQMVPEAARFKFMEQAIVRGDNSDS-
XP_021750669.1 LGIQNLDGAIRNVYNKQSESELNKGTEARFLLIAIQMVPEAARFRFMEQAIVRGDNSDS-
** . * . : . * . : : : * : * : * : * : * : * :

XP_021750597.1 -----YKILSLENNWGAISKGI RNAVKKV-----INPALILQYPNGTWTVTQVSDIKN
XP_021750694.1 ---NPDPKAMRLEIRWSKISKGIKSDSKKV-----ISPELDLDKVDNKKDVKVTKVEDIVN
XP_021737780.1 ECYKPDLTIELEKSWQKITVGIKNSNGV-----ICPPLKLDQPTGNPWI VSSVNEIAG
XP_021726635.1 ECYKPDFKMI ELEKSWQKITVGIKNSKGGE-----IKPQLDLRGPNNPWPVTRVDQIAK
XP_021735486.1 ---FKKKMVAFQNDWDPISQAIHKAEAAATPKCVTITPTLIISN-VDYRQEVNRVDEIKN
XP_021750669.1 ---FKKKMVAFQNDWDPISQAIHKAEAAATPKCVTITPTLIISN-IDYRQEVNRVDEIKN
* : : : * * : . * : * : * :

XP_021750597.1 DMGLLK YVM-----
XP_021750694.1 DMALLKNFGSSSQSVFTK LKLFMTKFLITNIGEDQAADL-----
XP_021737780.1 DMGLLK YEGPPTANNRVS LALVNVNKLQTHYNQFISFF-----
XP_021726635.1 DMGLLK YEGATKTANKGVYLG PFINKVQRHFNQFLSFLKANNNGNDDEAEQ
XP_021735486.1 DMGLLK YKSTLSVIGSSILDDDI-----
XP_021750669.1 DMGLLK YKSTLSIIGSSILDDDI-----
** . ***

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Fig. S3. Sequence alignment of ribosome inactivating proteins performed using the ClustalW tool (<https://embnet.vital-it.ch/software/ClustalW.html>) with default parameters. Asterisk, colon and dot are identical, conserved and semi-conserved amino acid residues, respectively. For protein information see **Table S1**. Cysteiny residues are highlighted in red.



| | 250 | 260 | 270 | 280 | 290 |
|-----------------|--|-----|-----|-----|-----|
| quinoiin | A---VKKVINPALILQYPNGTTWTVTQVSDIKNDMG-LLKYVM----- | | | | |
| PAP-alpha | A---KNGALTSPLELKNANGSKWIVLRVDDIEPDVG-LLKYVNGT C QAT----- | | | | |
| PD-S2 | A---KNGAFSKPLELQNADGSKWIVLRVDEIKPDVA-LLKYVSGS C QAT----- | | | | |
| PD-L3/4 | A---KNGALTSPLELKNADDTKWIVLRVDEIKPDMG-LLNYVSGT C QTT----- | | | | |
| PD-L1/2 | A---TNGALPKPLELKNADGTKWIVLRVDEIKPDMG-LLNYVNGT C QTT----- | | | | |
| PAP-S | S---KNGALPKPLELKNADGTKWIVLRVDEIKPDVG-LLNYVNGT C QAT----- | | | | |
| PAP-I | A---KNGVLPKPLELVDASGAKWIVLRVDEIKPDVA-LLNYVSGS C QTTY----- | | | | |
| heterotepalin-4 | A---KNGVLPKPLELVDASGAKWIVLRVDEIKPDVA-LLNYVSGS C QTT----- | | | | |
| PAP-II | VGTSGDSTVTLPGDLKDENNKPWTTATMNDLKNDIMALLTHVT C KVKSSMFPEI | | | | |
| dioicin-2 | VNTPGNSIVTLPKGLLDENKKPWTATMDELKNDIMGLLTHVT C KIK----- | | | | |
| saporin-6 | D--AKNGVFNKDYDFGFGKVRQVKDLQMGLLMLYLKPK----- | | | | |
| saporin-2 | D--AKNGVFNKDYDFGFGKVRQVKDLQMGLLMLYLKPKSNEANSTAYATTVL- | | | | |
| saporin-7 | D--AKNGVFNKDYDFGFGKVRQVKDLQMGLLMLYLKPK----- | | | | |
| dianthin-30 | D-- C KNGVFNKDYDFGFGKVRQAKDLQMGLLKYLRPKSSSIEANSTDDTADV- | | | | |
| saporin-5 | D--AKNGVFNKDYDFGFGKVRQVKDLQMGLLMLYLKPK----- | | | | |
| saporin-9 | D--AKNGVFNKDYDFGFGKVRQVKDLQMGLLMLYLKPK----- | | | | |
| lychnin | S--- C KLGGGQFTEEMKLG-----LLLYN----- | | | | |
| saporin-3 | D--AKNGVFNKDYDFGFGKVRQVKDLQMGLLMLYLGTTPNNAADRYRAEL----- | | | | |
| MAP-S | S---KPSTTTAT C QLATSPVTISPWIFKTVEEIKLVMGLLKSS----- | | | | |
| ricin | S---NQGAFASPIQLQRRNGSKFVYDVSILIPIALMVYR C APPSSQF----- | | | | |
| agglutinin | S---NQGAFASPIQLQRRNGSKFVYDVSILIPIALMVYR C APPSSQF----- | | | | |

Fig. S4. Western blot analysis of quinoin (q1 and q2, 100 and 50 ng, respectively) and PD-S2, PD-L1 and PD-L4 (.1 and .2, 100 and 50 ng, respectively). Purified proteins were separated by SDS-PAGE and subjected to immunoblot analysis using anti-quinoin or anti-PD-S2 (type 1 RIP from seeds of *P. dioica*) rabbit polyclonal antibody.

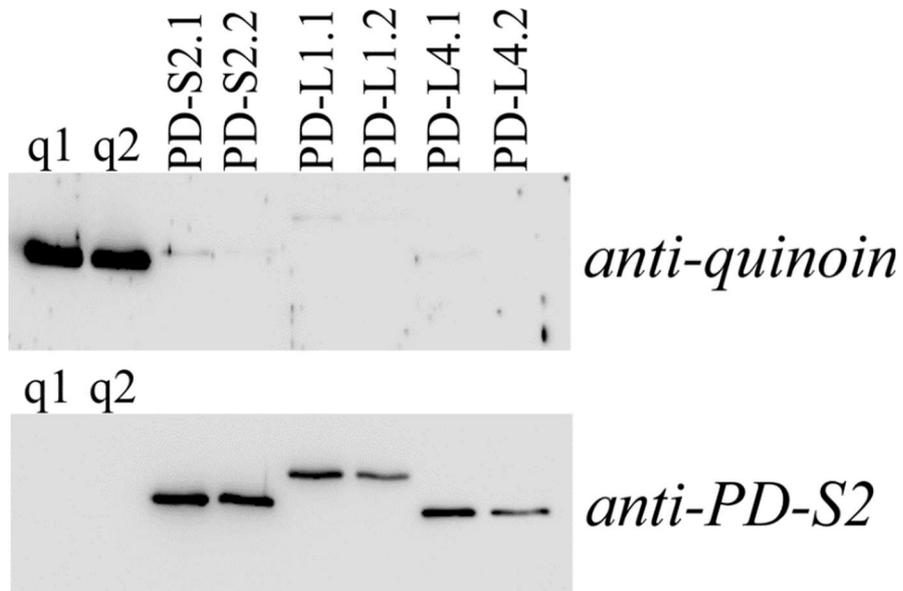


Fig. S5. Open Reading Frame (ORF) organization of quinoin derived from the genomic sequence NW_018745569.1.

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atg caa caa gaa aat aag aag gca tgg ctt gtt ctc acg ata gct ata tgg gtc gtc ctt
M  Q  Q  E  N  K  K  A  W  L  V  L  T  I  A  I  W  V  V  L
      ▼ mature quinoin
caa cag gtt aat gca gct gat gta acc ttc aaa ctc gaa cca aaa cct aca cag aat act
  Q  Q  V  N  A  A  D  V  T  F  K  L  E  P  K  P  T  Q  N  T
tat aac acc ttt ctg caa agt ata cgc agc caa act aag gat cca agc tta gtg tat gaa
Y  N  T  F  L  Q  S  I  R  S  Q  T  K  D  P  S  L  V  Y  E
gga atc cca atg atc cga cca cca acc aac cca gat aca tat ctt ttg gtt gac ctt gaa
G  I  P  M  I  R  P  P  T  N  P  D  T  Y  L  L  V  D  L  E
tct aaa aag gat aag aat gag ata ttc gtt aca ctt gct tta agt aga aac gac ttg tat
S  K  K  D  K  N  E  I  F  V  T  L  A  L  S  R  N  D  L  Y
gta gtg gcc ttt gct gat aaa ttt gga ggc aaa gtt cgc ggc cat ttc ttt tcc aat ctt
V  V  A  F  A  D  K  F  G  G  K  V  R  G  H  F  F  S  N  L
aac att gac acc att gat aag gca aag aaa gtt ttt cca gaa gtt cag gtt ttc ata aat
N  I  D  T  I  D  K  A  K  K  V  F  P  E  V  Q  V  F  I  N
ata acg tac ggg gaa agt tac agt caa atc gaa agc aat gct ggt aca aac cga tta agt
I  T  Y  G  E  S  Y  S  Q  I  E  S  N  A  G  T  N  R  L  S
ttt ccg ttg ggg ttt gat aac ctt aaa act tac atg gaa aag gtc tat ggg atg gat act
F  P  L  G  F  D  N  L  K  T  Y  M  E  K  V  Y  G  M  D  T
aag gcc aaa gat tat agc aaa act gaa gct cgg ttc cta ctc atc gcg att caa atg gtt
K  A  K  D  Y  S  K  T  E  A  R  F  L  L  I  A  I  Q  M  V
gca gag gcc gca cgt ttc aag tac atc cag ggg aga gct atc gtt act aca aat cct aac
A  E  A  A  R  F  K  Y  I  Q  G  R  A  I  V  T  T  N  P  N
aat tat aaa ata ctg tcg tta gag aac aac tgg gga gct att tcg aaa ggc att cgg aac
N  Y  K  I  L  S  L  E  N  N  W  G  A  I  S  K  G  I  R  N
gct gtt aag aaa gtc atc aat cct gcg tta att tta caa tac cct aat ggt aca aca tgg
A  V  K  K  V  I  N  P  A  L  I  L  Q  Y  P  N  G  T  T  W
aca gtt aca caa gtg agt gat ata aaa aat gat atg gga ctc ctt aag tat gtt atg taa
T  V  T  Q  V  S  D  I  K  N  D  M  G  L  L  K  Y  V  M  -

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Fig. S6. Representative images of bean cotyledonary leaves infected with TNV alone (a) or TNV mixed to 2.0 or 0.2 $\mu\text{g/mL}$ of quinoin (b, c, respectively) on the adaxial leaf surface, or when inoculated separately from quinoin, the latter applied on the abaxial leaf surface (d).

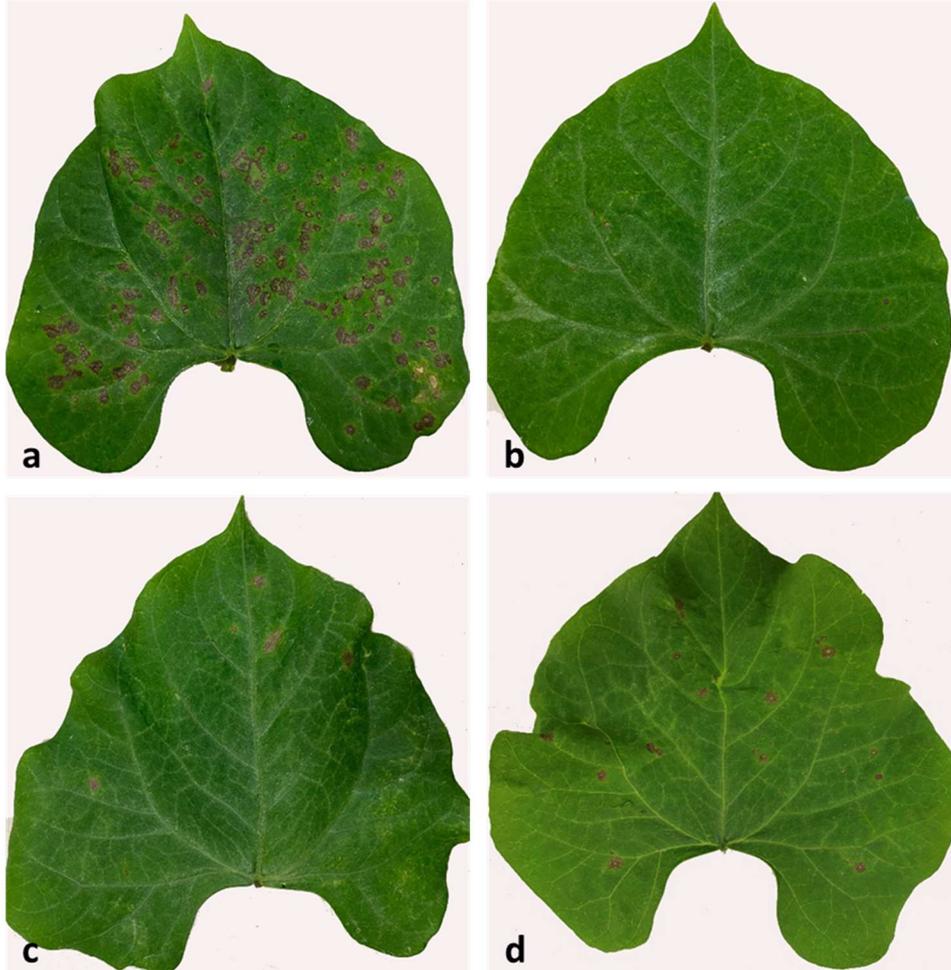


Fig. S7. Stereo microscopy analyses in order to evidence the antifungal effect of quinoin at different concentrations against the plant pathogenic fungi *Cryponectria parasitica* strain E4 (a) and *C. parasitica* strain E13 (b). Water, negative control.

