|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | Cultivar | Version | Accession no | Source | Reference |
| *Brachypodium distachyon* | Bd21 | v3.0 | GCA\_000005505.4 | Ensembl Plants | International Brachypodium Initiative, 2010 |
| *Hordeum vulgare* | Morex | v2 | GCA\_901482405.1 | Phytozome v12.1 | Mascher et al., 2017 |
| *Oryza brachyantha* | IRGC101232 | v1.4b | GCA\_000231095.2 | Ensembl Plants | Chen et al., 2013 |
| *Oryza sativa* | Nipponbare | IRGSP-1.0 | GCA\_001433935.1 | Ensembl Plants | Kawahara et al. 2013 |
| *Setaria italica* | Yugu1 | v2.2 | AGNK01000000.1 | Phytozome v12.1 | Bennetzen et al., 2012 |
| *Sorghum bicolor* | BTx623 | v3 | GCA\_000003195.3 | Ensembl Plants | Paterson et al., 2009 |
| *Triticum aestivum* | Chinese Spring | v1.0 | GCA\_900519105.1 | Ensembl Plants | International Wheat Genome Sequencing Consortium et al., 2018 |
| *Zea mays* | B73 | v4 | GCA\_000005005.6 | Ensembl Plants | Zhang et al., 2009 |

**Supplementary file 1A. List of databases used for NLR identification.**

**Supplementary file 1B. List of known and functionally characterized NLR-type resistance proteins from grasses used as reference sequences.**

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Accession number | Species | Reference |
| MLA10 | AY266445.1 | *Hordeum vulgare* | Halterman and Wise, 2004 |
| RGA1-A | KT725812.1 | *Secale cereale* | Mago et al., 2015 |
| Os11gRGA5 | AB604627.1 | *Oryza sativa* | Okuyama et al., 2011 |
| Os11gRGA4 | AB604622.1 | *Oryza sativa* | Okuyama et al., 2011 |
| Piz-t | DQ352040.1 | *Oryza sativa* | Zhou et al., 2006 |
| Pi-ta | AF207842.1 | *Oryza sativa* | Bryan et al., 2000 |
| Rpg5 | EU883792.1 | *Hordeum vulgare* | Brueggeman et al., 2008 |
| LR10 | AY270157.1 | *Triticum aestivum* | Feuillet et al., 2003 |
| Yr10 | AF149112.1 | *Triticum aestivum* | Liu et al., 2014 |
| Pib | AB013448.1 | *Oryza sativa* | Wang et al., 1999 |
| Pi9 | DQ285630.1 | *Oryza sativa* | Qu et al., 2006 |
| Rp1-D | XM\_008664205.2 | *Zea mays* | Collins et al., 1999 |
| Xa1 | AB002266.1 | *Oryza sativa* | Yoshimura et al., 1998 |
| Pm8 | KF572030.1 | *Triticum aestivum* | Hurni et al., 2013 |
| Pm3 | GU230859.1 | *Triticum aestivum* | Bhullar et al., 2010 |
| Rdg2-a | HM124452.1 | *Hordeum vulgare* | Bulgarelli et al., 2010 |
| Lr21 | FJ876280.1 | *Triticum aestivum* | Huang et al., 2009 |
| Pit | AB379815.1 | *Oryza sativa* | Hayashi and Yoshida, 2009 |
| Pi5-1 | EU869185.1 | *Oryza sativa* | Lee et al., 2009 |
| Pi5-2 | EU869186.1 | *Oryza sativa* | Lee et al., 2009 |
| Pid3 | KX791058.1 | *Oryza sativa* | Shang et al., 2009 |
| Sr45 | LN883757.1 | *Triticum aestivum* | Steuernagel et al., 2016 |
| Sr22 | LN883743.1 | *Triticum aestivum* | Steuernagel et al., 2016 |
| Lr22a | KY064064.1 | *Triticum aestivum* | Thind et al., 2017 |
| Pik\*-1 | HM048900\_1 | *Oryza sativa* | Zhai et al., 2011 |
| Pik\*-2 | ADZ48538.1 | *Oryza sativa* | Zhai et al., 2011 |
| Pikh-1 | HQ662330\_1 | *Oryza sativa* | Costanzo and Jia, 2010b |
| Pikh-2 | AET36550.1 | *Oryza sativa* | Costanzo and Jia, 2010b |
| Pikm-1 | AB462324\_1 | *Oryza sativa* | Ashikawa et al., 2008 |
| Pikm-2 | BAG72135.1 | *Oryza sativa* | Ashikawa et al., 2008 |
| Piks-1 | HQ662329\_1 | *Oryza sativa* | Jia et al., 2009 |
| Piks-2 | AET36548.1 | *Oryza sativa* | Jia et al., 2009 |
| Pikp-1 | HM035360.1 | *Oryza sativa* | Yuan et al., 2011 |
| Pikp-2 | ADV58351.1 | *Oryza sativa* | Yuan et al., 2011 |

|  |  |  |  |
| --- | --- | --- | --- |
| Accession | Country of origin | Used for *Pik-2* cloning | Used for *Pik-1* cloning |
| W0654 | Sierra Leone | Full-length | Full-length |
| W0655 | Sierra Leone | Not sequenced | Full-length |
| W0656 | Guinea | Fragment | Not amplified |
| W1057 | Guinea | Fragment | Not amplified |
| W1401 | Sierra Leone | Fragment | Not amplified |
| W1402 | Sierra Leone | Fragment | Not amplified |
| W1403 | Sierra Leone | Not sequenced | Not amplified |
| W1404 | Sierra Leone | Full-length | Full-length |
| W1405 | Sierra Leone | Full-length | Full-length |
| W1407(B) | Mali | Full-length | Full-length |
| W1703 | Mali | Full-length | Full-length |
| W1705 | Mali | Full-length | Full-length |
| W1706 | Chad | Fragment | Not amplified |
| W1708 | Cameroon | Fragment | Not amplified |
| W1711 | Cameroon | Fragment | Not amplified |
| W1712 | Cameroon | Fragment | Not amplified |

**Supplementary file 1C. List of *Oryza brachyantha* accessions.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Version | Assembly accession number | Source | References |
| *Dactylis glomerata* | v1 | GCA\_007115705.1 | NCBI | Huang et al., 2020 |
| *Leersia perrieri* | v1.4 | GCA\_000325765.3 | Ensembl Plants | Direct submission |
| *Oryza barthii* | v1 | GCA\_003020155.1 | Ensembl Plants | Direct submission |
| *Oryza glaberrima* | v1 | GCA\_000147395.2 | Ensembl Plants | Direct submission |
| *Oryza glumaepatula* | v1.5 | GCA\_000576495.1 | Ensembl Plants | Direct submission |
| *Oryza longistaminata* | v1.0 | GCA\_000789195.1 | Ensembl Plants | Direct submission |
| *Oryza nivara* | v1.0 | GCA\_000576065.1 | Ensembl Plants | Direct submission |
| *Oryza punctata* | v1.2 | GCA\_000573905.1 | Ensembl Plants | Direct submission |
| *Oryza rufipogon* | v1 | GCA\_000817225.1 | Ensembl Plants | Direct submission |
| *Zizania latifolia* | v1 | GCA\_000418225.1 | NCBI | Direct submission |

**Supplementary file 1D. List of plant datasets used for BLASTN search.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *Os* | *Oryza sativa* cv. K60 | Chromosome 11 | 27973977 | 28008157 |
| *Oniv* | *Oryza nivara* | Chromosome 11 | 23864010 | 23917629 |
| *Oglum* | *Oryza glumaepatula* | Chromosome 11 | 26116594 | 26116594 |
| *Ol* | *Oryza longistaminata* | Contig CM003669.1 | 2965866 | 8003128 |
| *Opunc* | *Oryza punctata* | Chromosome 11 | 4972847 | 4983002 |
| *Ob* | *Oryza brachyantha* | Chromosome 11 | 15529280 | 15547390 |
| *Lp* | *Leersia perrieri* | Chromosome 11 | 20337647 | 20286182 |
| *Ta* | *Triticum aestivum* | Chromosome 1D | 33124348 | 31125148 |
| *Dg* | *Dactylis glomerata* | Scaffold QXEO01001682.1 | 1295679 | 1340805 |
| *Si* | *Setaria italica* | Scaffold 8 | 39159743 | 39261506 |
| *Sb* | *Sorghum bicolor* | Chromosome 2 | 6043453 | 6215456 |

**Supplementary file 1E.** **Coordinates of genomic regions used in** **Figure 1B.**

|  |  |  |  |
| --- | --- | --- | --- |
| *Triticum aestivum* | Chromosome 1D | 33124348 | 31125148 |
| *Triticum aestivum* | Chromosome 4A | 739372651 | 742475497 |
| *Triticum aestivum* | Chromosome 7D | 3066514 | 4338541 |
| *Sorghum bicolor* | Chromosome 5 | 70290712 | 70702146 |
| *Sorghum bicolor* | Chromosome 2 | 5911252 | 6248983 |
| *Setaria italica* | Scaffold 8 | 39100454 | 39349775 |

**Supplementary file 1F.** **Coordinates of genomic regions used in Figure 1 – Figure Supplement 4.**

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Pik-1 | Pik-2 | Species |
| *Os*Pikp | Pikp-1\_HM035360.1 | Pikp-2\_HM035360.1 | *Oryza sativa* |
| *Os*Pikh | Pikh-1\_HQ662330.1 | Pikh-2\_HQ662330.1 | *Oryza sativa* |
| *Os*Pik\* | Pik\*-1\_HM048900\_1 | Pik\*-2\_HM048900\_1 | *Oryza sativa* |
| *Os*Piks | Piks-1\_HQ662329\_1 | Piks-2\_HQ662329\_1 | *Oryza sativa* |
| *Os*Pikm | Pikm-1\_BAG72135.1 | Pikm-2\_BAG72135.1 | *Oryza sativa* |
| *Obart* Pik | OBART11G23150 | OBART11G23160 | *Oryza barthii* |
| *Olongi* Pik | KN541092.1\_2 | KN541092.1 | *Oryza longistaminata* |
| *Opunc* Pik | OPUNC11G19550.n | OPUNC11G19560 | *Oryza punctata* |
| *Ob*Pik W1703 | ObPik-1\_W1703 | ObPik-2\_W1703 | *Oryza brachyantha* |
| *Ob*Pik W1407 | ObPik-1\_W1407 | ObPik-2\_W1407 | *Oryza brachyantha* |
| *Ob*Pik W1705 | ObPik-1\_W1705 | ObPik-2\_W1705 | *Oryza brachyantha* |
| *Ob*Pik IRGC101232 | OB11G27420.n | OB11G27420 | *Oryza brachyantha* |
| *Ob*Pik W1405 | ObPik-1\_W1405 | ObPik-2\_W1405 | *Oryza brachyantha* |
| *Ob*Pik W1404 | ObPik-1\_W0654 | ObPik-2\_W0654 | *Oryza brachyantha* |
| *Ob*Pik W0654 | ObPik-1\_W1404 | ObPik-2\_W1404 | *Oryza brachyantha* |
| *Oglum* Pik | OGLAB11G20210.1n | ORGLA11G0185700 | *Oryza glaberrima* |
| *Oglab* Pik | OGLUM11G22320.n | OGLUM11G22330 | *Oryza glumaepatula* |
| *Oniv* Pik | ORUFI11G24730 | ORUFI11G24740 | *Oryza rufipogon* |
| *Oruf* Pik | ONIVA11G22690.n | ONIVA11G22700 | *Oryza nivara* |
| *Os*Pik Nipp | Pikm5\_NP Nipp\_DP000010.2 | PIK6\_NP XM\_015762499.2 | *Oryza sativa* cv. Nipponbare |
| *Ta*Pik 1D | TraesCS1D02G051500.1 | TraesCS1D02G051400.1 | *Triticum aestivum* |
| *Sb*Pik 5 | SORBI\_3005G219700 | SORBI\_3005G219900 | *Sorghum bicolor* |
| *Si*Pik 8.1 | Seita.8G239300.n | Seita.8G239400 | *Setaria italica* |
| *Si*Pik 8.2 | Seita.8G238800 | Seita.8G238900 | *Setaria italica* |
| *Dg*Pik | QXEO01001682.1 | QXEO01001682.1\_2 | *Dactylis glomerata* |
| *Ta*Pik 4A.1 | TraesCS4A02G493400.1 | TraesCS7A02G006200.1 | *Triticum aestivum* |
| *Ta*Pik 4A.2 | TraesCS4A02G491000.1 | TraesCS4A02G490900.1 | *Triticum aestivum* |
| *Ta*Pik 7D | TraesCS7D02G007700.1 | TraesCS7D02G007600.1 | *Triticum aestivum* |

**Supplementary file 1G. Genes used for the comparisons of *d*S and rates of Pik-1–Pik-2 presented in** **Figure 1C.**

**Supplementary file 1H.** **Summary of the amplification experiment of the Pik-1–integrated HMA domain from wild rice species.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Accession | Species | Origin | Amplified | Sequence confirmed |
| W0654 | *O. brachyantha* | Sierra Leone | Yes | Yes |
| W0008 | *O. australiensis* | Australia (SE Canberra) | Yes | No |
| W1628 | *O. australiensis* | Australia (N) | No | NA |
| W1643 | *O. barthii* | Botswana | Yes | Yes |
| W1605 | *O. barthii* | Nigeria | Yes | No |
| W0042 | *O. barthii* | unspecified | Yes | Yes |
| W0698 | *O. barthii* | Guinea | Yes | Yes |
| W1526 | *O. eichingeri* | Uganda | No | NA |
| W1171 | *O. glumaepatula* | Cuba | No | NA |
| W2203 | *O. glumaepatula* | Brazil (S) | Yes | No |
| W1480(B) | *O. grandiglumis* | Brazil (N) | Yes | No |
| W0005 | *O. granulata* | Sri Lanka | No | NA |
| W0067(B) | *O. granulata* | Thailand | Yes | Yes |
| W0542 | *O. latifolia / O. alta* | Mexico | No | NA |
| W1539 | *O. latifolia / O. alta* | Argentina (N) | No | NA |
| W1228 | *O. longiglumis* | Singapore (S) | No | NA |
| W1504 | *O. longistaminata* | Tanzania | No | NA |
| W1540 | *O. longistaminata* | Republic of Congo | Yes | Yes |
| W0643 | *O. longistaminata* | The Gambia | Yes | Yes |
| W2081 | *O. meridionalis* | Australia (N) | No | NA |
| W2112 | *O. meridionalis* | Australia (NE) | No | NA |
| W1354 | *O. meyeriana* | Malaysia | Yes | No |
| W1328 | *O. minuta* | Philippines | Yes | Yes |
| W0614 | *O. officinalis* | Myanmar | Yes | Yes |
| W1200 | *O. officinalis* | Philippines | Yes | Yes |
| W1408 | *O. punctata* | Nigeria | Yes | Yes |
| W1514 | *O. punctata* | Kenya | Yes | Yes |
| W1808 | *O. rhizomatis* | Sri Lanka | Yes | No |
| W0001 | *O. ridleyi* | Thailand | Yes | No |
| W2035 | *O. ridleyi* | Philippines | No | NA |
| W2003 | *O. rufipogon* | India (SW) | Yes | Yes |
| W1715 | *O. rufipogon* | Chin (Beijing) | No | NA |
| W2117 | *O. rufipogon/ O. meridionalis* | Australia (NE) | Yes | No |

**Supplementary file 1I. Table of p-values for all pairwise comparisons of SPR binding to AVR-PikD between the HMA mutants.**

| Concentration  (nM) | Sample 1 | Sample 2 | Difference | Lower  confidence level | Upper  confidence level | p-value |
| --- | --- | --- | --- | --- | --- | --- |
| 50 | IAQVV | E230R | 0.7594754 | -6.380544 | 7.899495 | 0.9999725 |
| 50 | LAKIE | E230R | 15.1954147 | 8.055395 | 22.335434 | 0.0000005 |
| 50 | LAKIV | E230R | 0.1973327 | -6.942687 | 7.337352 | 1 |
| 50 | LAKVV | E230R | -1.2876815 | -8.427701 | 5.852338 | 0.9990674 |
| 50 | LAQVV | E230R | -0.2110851 | -7.351104 | 6.928934 | 1 |
| 50 | LVKIE | E230R | 29.9244059 | 22.784387 | 37.064425 | 0 |
| 50 | Pikp-HMA | E230R | 21.3309677 | 14.190948 | 28.470987 | 0 |
| 50 | LAKIE | IAQVV | 14.4359393 | 7.29592 | 21.575959 | 0.0000016 |
| 50 | LAKIV | IAQVV | -0.5621428 | -7.702162 | 6.577877 | 0.9999965 |
| 50 | LAKVV | IAQVV | -2.047157 | -9.187176 | 5.092862 | 0.9838216 |
| 50 | LAQVV | IAQVV | -0.9705606 | -8.11058 | 6.169459 | 0.9998555 |
| 50 | LVKIE | IAQVV | 29.1649305 | 22.024911 | 36.30495 | 0 |
| 50 | Pikp-HMA | IAQVV | 20.5714923 | 13.431473 | 27.711512 | 0 |
| 50 | LAKIV | LAKIE | -14.998082 | -22.138101 | -7.858063 | 0.0000007 |
| 50 | LAKVV | LAKIE | -16.483096 | -23.623116 | -9.343077 | 0.0000001 |
| 50 | LAQVV | LAKIE | -15.4065 | -22.546519 | -8.266481 | 0.0000004 |
| 50 | LVKIE | LAKIE | 14.7289911 | 7.588972 | 21.86901 | 0.000001 |
| 50 | Pikp-HMA | LAKIE | 6.1355529 | -1.004466 | 13.275572 | 0.1407186 |
| 50 | LAKVV | LAKIV | -1.4850142 | -8.625033 | 5.655005 | 0.9976792 |
| 50 | LAQVV | LAKIV | -0.4084178 | -7.548437 | 6.731601 | 0.9999996 |
| 50 | LVKIE | LAKIV | 29.7270732 | 22.587054 | 36.867093 | 0 |
| 50 | Pikp-HMA | LAKIV | 21.133635 | 13.993616 | 28.273654 | 0 |
| 50 | LAQVV | LAKVV | 1.0765964 | -6.063423 | 8.216616 | 0.9997116 |
| 50 | LVKIE | LAKVV | 31.2120874 | 24.072068 | 38.352107 | 0 |
| 50 | Pikp-HMA | LAKVV | 22.6186492 | 15.47863 | 29.758669 | 0 |
| 50 | LVKIE | LAQVV | 30.135491 | 22.995472 | 37.27551 | 0 |
| 50 | Pikp-HMA | LAQVV | 21.5420528 | 14.402034 | 28.682072 | 0 |
| 50 | Pikp-HMA | LVKIE | -8.5934382 | -15.733457 | -1.453419 | 0.008645 |
| 200 | IAQVV | E230R | 8.12408613 | 4.769044 | 11.4791283 | 0 |
| 200 | LAKIE | E230R | 43.4535131 | 40.098471 | 46.8085553 | 0 |
| 200 | LAKIV | E230R | 7.26935912 | 3.914317 | 10.6244013 | 0.0000006 |
| 200 | LAKVV | E230R | -0.0357561 | -3.390798 | 3.3192861 | 1 |
| 200 | LAQVV | E230R | 4.96818917 | 1.613147 | 8.3232314 | 0.0006703 |
| 200 | LVKIE | E230R | 57.0910393 | 53.735997 | 60.4460815 | 0 |
| 200 | Pikp-HMA | E230R | 53.8341313 | 50.479089 | 57.1891735 | 0 |
| 200 | LAKIE | IAQVV | 35.329427 | 31.974385 | 38.6844692 | 0 |
| 200 | LAKIV | IAQVV | -0.854727 | -4.209769 | 2.5003152 | 0.9912868 |
| 200 | LAKVV | IAQVV | -8.1598423 | -11.514884 | -4.8048001 | 0 |
| 200 | LAQVV | IAQVV | -3.155897 | -6.510939 | 0.1991452 | 0.0782897 |
| 200 | LVKIE | IAQVV | 48.9669532 | 45.611911 | 52.3219953 | 0 |
| 200 | Pikp-HMA | IAQVV | 45.7100452 | 42.355003 | 49.0650873 | 0 |
| 200 | LAKIV | LAKIE | -36.184154 | -39.539196 | -32.829112 | 0 |
| 200 | LAKVV | LAKIE | -43.489269 | -46.844311 | -40.134227 | 0 |
| 200 | LAQVV | LAKIE | -38.485324 | -41.840366 | -35.130282 | 0 |
| 200 | LVKIE | LAKIE | 13.6375262 | 10.282484 | 16.9925683 | 0 |
| 200 | Pikp-HMA | LAKIE | 10.3806182 | 7.025576 | 13.7356603 | 0 |
| 200 | LAKVV | LAKIV | -7.3051152 | -10.660157 | -3.9500731 | 0.0000006 |
| 200 | LAQVV | LAKIV | -2.30117 | -5.656212 | 1.0538722 | 0.3776686 |
| 200 | LVKIE | LAKIV | 49.8216802 | 46.466638 | 53.1767223 | 0 |
| 200 | Pikp-HMA | LAKIV | 46.5647722 | 43.20973 | 49.9198144 | 0 |
| 200 | LAQVV | LAKVV | 5.00394529 | 1.648903 | 8.3589875 | 0.0006038 |
| 200 | LVKIE | LAKVV | 57.1267954 | 53.771753 | 60.4818376 | 0 |
| 200 | Pikp-HMA | LAKVV | 53.8698874 | 50.514845 | 57.2249296 | 0 |
| 200 | LVKIE | LAQVV | 52.1228501 | 48.767808 | 55.4778923 | 0 |
| 200 | Pikp-HMA | LAQVV | 48.8659421 | 45.5109 | 52.2209843 | 0 |
| 200 | Pikp-HMA | LVKIE | -3.256908 | -6.61195 | 0.0981342 | 0.0625642 |
| 400 | IAQVV | E230R | 15.4160802 | 10.476265 | 20.3558957 | 0 |
| 400 | LAKIE | E230R | 59.1376719 | 54.197856 | 64.0774874 | 0 |
| 400 | LAKIV | E230R | 17.2208188 | 12.281003 | 22.1606344 | 0 |
| 400 | LAKVV | E230R | -0.6186041 | -5.55842 | 4.3212114 | 0.9999089 |
| 400 | LAQVV | E230R | 10.5836822 | 5.643867 | 15.5234977 | 0.0000008 |
| 400 | LVKIE | E230R | 67.4451738 | 62.505358 | 72.3849893 | 0 |
| 400 | Pikp-HMA | E230R | 66.0719577 | 61.132142 | 71.0117733 | 0 |
| 400 | LAKIE | IAQVV | 43.7215917 | 38.781776 | 48.6614072 | 0 |
| 400 | LAKIV | IAQVV | 1.8047387 | -3.135077 | 6.7445542 | 0.9363629 |
| 400 | LAKVV | IAQVV | -16.034684 | -20.9745 | -11.094869 | 0 |
| 400 | LAQVV | IAQVV | -4.832398 | -9.772213 | 0.1074176 | 0.0591016 |
| 400 | LVKIE | IAQVV | 52.0290936 | 47.089278 | 56.9689091 | 0 |
| 400 | Pikp-HMA | IAQVV | 50.6558776 | 45.716062 | 55.5956931 | 0 |
| 400 | LAKIV | LAKIE | -41.916853 | -46.856669 | -36.977038 | 0 |
| 400 | LAKVV | LAKIE | -59.756276 | -64.696092 | -54.81646 | 0 |
| 400 | LAQVV | LAKIE | -48.55399 | -53.493805 | -43.614174 | 0 |
| 400 | LVKIE | LAKIE | 8.3075019 | 3.367686 | 13.2473174 | 0.0000904 |
| 400 | Pikp-HMA | LAKIE | 6.9342859 | 1.99447 | 11.8741014 | 0.001416 |
| 400 | LAKVV | LAKIV | -17.839423 | -22.779238 | -12.899607 | 0 |
| 400 | LAQVV | LAKIV | -6.6371366 | -11.576952 | -1.6973211 | 0.0025111 |
| 400 | LVKIE | LAKIV | 50.2243549 | 45.284539 | 55.1641705 | 0 |
| 400 | Pikp-HMA | LAKIV | 48.8511389 | 43.911323 | 53.7909544 | 0 |
| 400 | LAQVV | LAKVV | 11.2022863 | 6.262471 | 16.1421019 | 0.0000002 |
| 400 | LVKIE | LAKVV | 68.0637779 | 63.123962 | 73.0035934 | 0 |
| 400 | Pikp-HMA | LAKVV | 66.6905618 | 61.750746 | 71.6303774 | 0 |
| 400 | LVKIE | LAQVV | 56.8614916 | 51.921676 | 61.8013071 | 0 |
| 400 | Pikp-HMA | LAQVV | 55.4882755 | 50.54846 | 60.4280911 | 0 |
| 400 | Pikp-HMA | LVKIE | -1.373216 | -6.313032 | 3.5665995 | 0.9854733 |

E230R: Pikp-HMAE230R, IAQVV: ancHMAIAQVV, LAQVV: ancHMALAQVV, LAKVV: ancHMALAKVV, LAKIV: ancHMALAKIV, LAKIE: ancHMALAKIE, LVKIE: ancHMALVKIE

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Comparison | Sd | SN | Total | S | N | pS | pN | *d*S | *d*N | *d*S/*d*N |
| Pikh vs. Pikp | 1 | 2 | 3 | 789.6667 | 2636.3333 | 0.0013 | 0.0008 | 0.0013 | 0.0008 | 1.6698 |
| Pikh vs. Pik\* | 27.5 | 50.5 | 82 | 790.3333 | 2635.6667 | 0.0348 | 0.0192 | 0.0356 | 0.0194 | 1.8356 |
| Pikh vs. Pikm | 27.5 | 55.5 | 87 | 790.1667 | 2635.8333 | 0.0348 | 0.0211 | 0.0356 | 0.0214 | 1.6686 |
| Pikh vs. Piks | 27.5 | 54.5 | 86 | 790.1667 | 2635.8333 | 0.0348 | 0.0207 | 0.0356 | 0.021 | 1.6996 |
| Pikp vs. Pik\* | 28.5 | 52.5 | 85 | 790.3333 | 2635.6667 | 0.0361 | 0.0199 | 0.037 | 0.0202 | 1.8306 |
| Pikp vs. Pikm | 28.5 | 57.5 | 90 | 790.1667 | 2635.8333 | 0.0361 | 0.0218 | 0.037 | 0.0221 | 1.6697 |
| Pikp vs. Piks | 28.5 | 56.5 | 89 | 790.1667 | 2635.8333 | 0.0361 | 0.0214 | 0.037 | 0.0217 | 1.6997 |
| Pik\* vs. Pikm | 0 | 8 | 8 | 791.8333 | 2640.1667 | 0 | 0.003 | 0 | 0.003 | nan |
| Pik\* vs. Piks | 0 | 8 | 8 | 791.8333 | 2640.1667 | 0 | 0.003 | 0 | 0.003 | nan |
| Pikm vs. Piks | 0 | 2 | 2 | 791.6667 | 2640.3333 | 0 | 0.0008 | 0 | 0.0008 | nan |

**Supplementary file 1J.** **Pairwise *d*N and *d*S values between Pik-1 alleles from rice calculated using the method of Nei and Gojobori (1986).**

Sd: the number of observed synonymous substitutions

SN: the number of observed non-synonymous substitutions

S: the number of potential synonymous substitutions (the average for the two compared sequences)

N: the number of potential non-synonymous substitutions (the average for the two compared sequences)

PS: the proportion of observed synonymous substitutions: Sd/S

pN: the proportion of observed non-synonymous substitutions: SN/N

*d*S: the Jukes-Cantor correction for multiple hits of pS

*d*N: the Jukes-Cantor correction for multiple hits of pN

*d*S/*d*N: The ratio of synonymous to non-synonymous substitutions

| Concentration  (nM) | Sample 1 | Sample 2 | Difference | Lower  confidence level | Upper  confidence level | p-value |
| --- | --- | --- | --- | --- | --- | --- |
| 400 | EMANK | E230R | 86.07761 | 62.02624 | 110.12898 | 0 |
| 400 | EMVKE | E230R | 91.209289 | 67.15792 | 115.26066 | 0 |
| 400 | MKANK | E230R | 83.519556 | 60.49213 | 106.54699 | 0 |
| 400 | Pikm | E230R | 77.298931 | 53.24757 | 101.3503 | 0 |
| 400 | EMVKE | EMANK | 5.131679 | -18.91969 | 29.18304 | 0.967442 |
| 400 | MKANK | EMANK | -2.558054 | -25.58548 | 20.46938 | 0.9971982 |
| 400 | Pikm | EMANK | -8.778679 | -32.83004 | 15.27269 | 0.8109631 |
| 400 | MKANK | EMVKE | -7.689733 | -30.71716 | 15.3377 | 0.8547095 |
| 400 | Pikm | EMVKE | -13.910358 | -37.96172 | 10.14101 | 0.4421083 |
| 400 | Pikm | MKANK | -6.220625 | -29.24805 | 16.8068 | 0.9262409 |
| 200 | EMANK | E230R | 65.82556 | 60.553591 | 71.09752 | 0 |
| 200 | EMVKE | E230R | 87.06222 | 81.790259 | 92.334189 | 0 |
| 200 | MKANK | E230R | 53.28956 | 48.017593 | 58.561522 | 0 |
| 200 | Pikm | E230R | 76.73675 | 71.464784 | 82.008713 | 0 |
| 200 | EMVKE | EMANK | 21.23667 | 15.964704 | 26.508633 | 0 |
| 200 | MKANK | EMANK | -12.536 | -17.807963 | -7.264034 | 0.0000209 |
| 200 | Pikm | EMANK | 10.91119 | 5.639228 | 16.183157 | 0.0001023 |
| 200 | MKANK | EMVKE | -33.77267 | -39.044631 | -28.500702 | 0 |
| 200 | Pikm | EMVKE | -10.32548 | -15.59744 | -5.053511 | 0.0001865 |
| 200 | Pikm | MKANK | 23.44719 | 18.175226 | 28.719156 | 0 |
| 50 | EMANK | E230R | 38.83454 | 21.734495 | 55.934593 | 0.0000044 |
| 50 | EMVKE | E230R | 76.07891 | 58.978857 | 93.178955 | 0 |
| 50 | MKANK | E230R | 27.8971 | 10.797048 | 44.997145 | 0.0005356 |
| 50 | Pikm | E230R | 63.94274 | 46.842695 | 81.042793 | 0 |
| 50 | EMVKE | EMANK | 37.24436 | 19.498803 | 54.989921 | 0.0000157 |
| 50 | MKANK | EMANK | -10.93745 | -28.683007 | 6.808111 | 0.3922477 |
| 50 | Pikm | EMANK | 25.1082 | 7.362641 | 42.853759 | 0.0027261 |
| 50 | MKANK | EMVKE | -48.18181 | -65.927369 | -30.436251 | 0.0000002 |
| 50 | Pikm | EMVKE | -12.13616 | -29.881721 | 5.609397 | 0.2926768 |
| 50 | Pikm | MKANK | 36.04565 | 18.300089 | 53.791207 | 0.000026 |

**Supplementary file 1K. Table of p-values for all pairwise comparisons of SPR binding to AVR-PikD between the HMA mutants.**

E230R: Pikp-HMAE230R, EMVKE: ancHMAEMVKE, EMANK: ancHMAEMANK, MKANK: ancHMA; Pikm: Pikm-HMA

**Supplementary file 1L. Data collection and refinement statistics for the ancHMALVKIE–AVR-PikD co-crystal structure.**

|  |  |  |
| --- | --- | --- |
|  | | Value |
| Data collection statistics | |  |
|  | Beamline | I03 Diamond |
|  | Detector | Pilatus3 6M |
|  | Wavelength (Å) | 0.9700 |
|  | Space group | *P* 41212 |
|  | Cell dimensions (Å) | *a* = *b* = 119.5, c = 36.0 |
|  | Resolution (Å)\* | 59.81–1.32 (1.34–1.32) |
|  | *R*merge# | 0.068 (2.201) |
|  | *R*meas# | 0.070 (2.285) |
|  | Mean *I*/σ(*I)*# | 16.7 (1.3) |
|  | *CC(1/2)#* | 0.999 (0.730) |
|  | Completeness (%)# | 96.3 (94.1) |
|  | Unique reflections# | 59,464 (2,862) |
|  | Multiplicity# | 14.6 (13.9) |
|  | Wilson *B* value (Å2)# | 16.0 |
| Refinement and model statistics | |  |
|  | Resolution (Å) | 59.81-1.32 (1.35-1.32) |
|  | *R*work† | 0.145 (0.271) |
|  | *R*free† | 0.184 (0.295) |
|  | Mean *B* factors: protein/waters/overall (Å2) | 22/35/24 |
|  | R.m.s. bond deviations (Å)† | 0.011 |
|  | R.m.s. angle deviations (°)† | 1.59 |
|  | Ramachandran plot: favoured/allowed/outliers(%)\*\* | 98.2/1.8/0.0 |
|  | MolProbity Score | 1.09 |
| PDB Accession code | | 7BNT |

\*the highest resolution shell is shown in parentheses

\*\*as calculated by MolProbity, #as calculated by Aimless

† as calculated by REFMAC5

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Description on the tree | Species | Accession number | Pik-integrated | used for ASR | | |
| I | II | III |
| *O.barthii*\_W0042 | *O. barthii* | MW553215 | y | n | n | y |
| *O.barthii*\_W1643 | *O. barthii* | MW553205 | y | n | n | y |
| *O.punctata*\_W1408 | *O. punctata* | MW553208 | y | n | n | y |
| *O.barthii*\_W0698 | *O. barthii* | MW553211 | y | n | n | y |
| *O.granulata*\_W0067B | *O. granulata* | MW553214 | y | n | n | y |
| *O.longistaminata*\_W0643 | *O. longistaminata* | MW553212 | y | n | n | y |
| *O.officinalis*\_W0614 | *O. officinalis* | MW553213 | y | n | n | y |
| *O.punctata*\_W1514 | *O. punctata* | MW553207 | y | n | n | y |
| *O.rufipogon*\_W2003 | *O. rufipogon* | MW553204 | y | n | n | y |
| *O.minuta*\_W1328 | *O. minuta* | MW553209 | y | n | n | y |
| LOC102699268 | *O. brachyantha* | LOC102699268 | y | y | y | y |
| OBART11G23150 | *O. barthii* | OBART11G23150 | y | n | n | y |
| *Olongi*\_KN541092.1 | *O. longistaminata* | KN541092.1 | y | n | n | y |
| OPUNC11G19550 | *O. punctata* | OPUNC11G19550 | y | n | n | y |
| *Os*Pikp-1 | *O. sativa* | HM035360.1 | y | y | y | y |
| *Os*Pik\*-1 | *O. sativa* | HM048900\_1 | y | y | y | Y |
| *Os*Pikh-1 | *O. sativa* | HQ662330\_1 | y | y | y | Y |
| *Os*Piks-1 | *O. sativa* | HQ662329\_1 | y | y | y | y |
| OsPikm-1 | *O. sativa* | AB462324.1 | y | y | y | y |
| Ob\_LOC102708959 | *O. brachyantha* | LOC102708959 | n | n | y | y |
| Ob\_LOC102709146 | *O. brachyantha* | LOC102709146 | n | n | y | y |
| Ob\_LOC102714171 | *O. brachyantha* | LOC102714171 | n | n | y | y |
| Ob\_LOC102716957 | *O. brachyantha* | LOC102716957 | n | n | y | y |
| Ob\_LOC102717220 | *O. brachyantha* | LOC102717220 | n | n | y | y |
| Os\_LOC\_Os04g39360 | *O. sativa* | LOC\_Os04g39360 | n | y | y | y |
| Os\_LOC\_Os04g39370 | *O. sativa* | LOC\_Os04g39370 | n | y | y | y |
| Os04g0469000\_01 | *O. sativa* | Os04g0469000\_01 | n | y | y | y |
| Os02g0585200 | *O. sativa* | Os02g0585200 | n | y | y | y |
| Os02g0584800\_01 | *O. sativa* | Os02g0584800\_01 | n | y | y | y |
| Os02g0584700\_01 | *O. sativa* | Os02g0584700\_01 | n | y | y | y |
| Os04g0469300\_01 | *O. sativa* | Os04g0469300\_01 | n | y | y | y |
| Os02g0585100 | *O. sativa* | Os02g0585100 | n | y | y | y |
| Os02g0584600 | *O. sativa* | Os02g0584600 | n | y | y | y |
| OSJNBa0060P14.7\_01 | *O. sativa* | OSJNBa0060P14.7\_01 | n | y | y | y |
| Os04g0464100\_01 | *O. sativa* | Os04g0464100\_01 | n | y | y | y |
| Os02g0582600 | *O. sativa* | Os02g0582600 | n | y | y | y |

**Supplementary file 1M. HMA sequences used for building phylogenetic trees and ancestral sequence reconstruction (ASR).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Protein name | Tag | Vector backbone | Concentration (OD600) | Reference |
| Pikp-1 | 6×HA (N-term) | pICH77732 | 0.15 | This study |
| Pikp-1+ | 6×HA (N-term) | pICH77732 | 0.15 | This study |
| Pikp-2 | 3×Myc (C-term) | pCambia | 0.15 | Maqbool et al., 2015 |
| AVR-PikD | 3×FLAG (C-term) | pTRBO | 0.15 | This study |
| AVRblb2 | 3×FLAG (C-term) | pTRBO | 0.15 | Bozkurt et al., 2011 |
| Pikp-1 | 6×His/ 3×FLAG (HF) (C-term) | pICH47742 | 0.4 | De la Concepcion et al., 2018 |
| Pikp-1 | 6×His/ 3×FLAG (HF) (C-term) | pICH47732 | 0.4 | This study |
| Pikp-2 | 6×HA (C-term) | pICH47751 | 0.4 | De la Concepcion et al., 2018 |
| AVR-PikD | 4×Myc (N-term) | pICH47732 | 0.6 | De la Concepcion et al., 2018 |
| P19 | NA | pCB301 | 0.1 | Win and Kamoun, 2003 |
| Pikm-1 | 6×His/ 3×FLAG (C-term) | pICH47742 | 0.4 | De la Concepcion et al., 2018 |
| Pikm-1+ | 6×His/ 3×FLAG (C-term) | pICH47732 | 0.4 | This study |
| Pikm-2 | 6×HA (C-term) | pICH47751 | 0.4 | De la Concepcion et al., 2018 |

**Supplementary file 1N. List of constructs used in cell death assays.**

+including mutants and fusions

NA: not applicable

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annotation:, Genetic maps and mapping:, BAC libraries and chromosome sorting:, BAC pooling, BAC library repository, and access:, IWGSC sequence and data repository and access:, Physical maps and BAC-based sequences:, 1A BAC sequencing and assembly:, 1B BAC sequencing and assembly:, 1D, 4D, and 6D physical mapping:, 2AL physical mapping:, 2AS physical mapping:, 2B, 2D, 4B, 5BL, and 5DL IWGSC–Bayer Whole-Genome Profiling (WGP) physical maps:, 3AL physical mapping:, 3DS physical mapping and BAC sequencing and assembly:, 3DL BAC sequencing and assembly:, 4A physical mapping, BAC sequencing, assembly, and annotation:, 5BS BAC sequencing and assembly:, 6B BAC sequencing and assembly:, 7A physical mapping and BAC sequencing:, 7B physical mapping, BAC sequencing, and assembly:, 7DS BAC sequencing and assembly:, 7DL physical mapping and BAC sequencing:, Figures:, Manuscript writing team:, Appels R, Eversole K, Feuillet C, Keller B, Rogers J, Stein N, Pozniak CJ, Stein N, Choulet F, Distelfeld 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