Review

Disease Resistance Gene Analogs (RGAs) in Plants

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Abstract: Plants have developed effective mechanisms to recognize and respond to infections caused by pathogens. Plant resistance gene analogs (RGAs), as resistance (*R*) gene candidates, have conserved domains and motifs that play specific roles in pathogens' resistance. Well-known RGAs are nucleotide binding site leucine rich repeats, receptor like kinases, and receptor like proteins. Others include pentatricopeptide repeats and apoplastic peroxidases. RGAs can be detected using bioinformatics tools based on their conserved structural features. Thousands of RGAs have been identified from sequenced plant genomes. High-density genome-wide RGA genetic maps are useful for designing diagnostic markers and identifying quantitative trait loci (QTL) or markers associated with plant disease resistance. This review focuses on recent advances in structures and mechanisms of RGAs, and their identification from sequenced genomes using bioinformatics tools. Applications in enhancing fine mapping and cloning of plant disease resistance genes are also discussed.

1. Introduction

Many plant-pathogen interactions are determined by the presence of resistance (*R*) genes/alleles that enable plants to recognize pathogens and activate inducible defenses [1]. Plant diseases have been reported to be caused by a wide variety of pathogens, such as *Fusarium* sp. in fusarium head blight (FHB) [2], *Sitodiplosis mosellana* in wheat midge [3], *Blumeria* sp., *Erysiphe Golovinomyces* sp. and *Oidium* in powdery mildew [4], *Puccinia* sp. in rust [5], *Phytophthora infestans* in late blight [6], and *Pseudomonas syringae* in bacterial speck [7]. Plants have developed effective mechanisms to recognize and respond to infections with race non-specific resistance (quantitative) and race-specific resistance (qualitative). Race non-specific resistance involves a number of minor genes that are effective against several pathogens [8]. For example, *NDR1* and three alleles of *rp1*, *rp1-D21*, *rp1-MD19* and *rp-NC3*, have been shown to confer a race non-specific resistance response to rust [9]. In contrast, race-specific resistance is conferred by a single or a few major genes that operate in a gene-for-gene fashion in which plant *R*-genes recognize corresponding pathogen avirulence (*Avr*)-gene effectors to trigger plant defense responses [10]. *Pto* in tomato confers race-specific resistance to *Pseudomonas syringae* pv. tomato (Pst), similarly to *RPS2* in *Arabidopsis* and *N* (mosaic virus resistance gene) in tobacco [11]. On the other hand, the wheat stem rust resistance gene *Sr26* shows resistance to all races of the pathogen *Puccinia graminis* f. sp. *tritici*, thus constituting a race non-specific pathosystem [12].

In the defense system of plants, direct and indirect interactions are two alternative mechanisms to explain the gene-for-gene model. In the direct interaction, pathogen Avr effectors associate directly with plant *R*-genes to trigger signaling. For example, rice *R*-gene *Pi-ta* was shown to directly interact with *Avr-Pita* from *Magnaporthe grisea* [13]. Likewise, a direct interaction was observed between *L* genes (a group of resistant genes to flax rust) and their corresponding rust *Avr* genes in flax [14]. The indirect model is also known as the guard hypothesis [10]. In this model, *R*-gene products act as a "guardee" to monitor the modification of host proteins after associating with the pathogenic effectors, resulting in the initiation of resistance [15]. Examples of guardee proteins are RPM1 interacting 4 (RIN4), flagellin-sensitive 2 (FLS2), Xa21, resistance to pseudomonas syringae 5 (RPS5) and avrPphB susceptible 1 (PBS1). Resistance gene analogs (RGAs) are a large class of potential *R*-genes that have conserved domains and structural features. As such, RGAs can be identified from sequenced genomes using bioinformatics approaches [16–18]. In the last 15 years, more than 50 plant genomes have been sequenced and assembled [19–21]. Despite this great resource, only a relatively small number of *R*-genes have been cloned and fully characterized (Table 1) providing not only information on their structure, function and evolution, but also generating useful genetic resources to create novel resistant cultivars [22]. In comparison, thousands of RGAs have been identified in many plant genomes (Table 2).

Table 1. Cloned *R*-genes from plant species.

Species [a]	*R*-Gene	Accession ID	Domain [b]	Class	Chr	Disease [c]	*Avr* Gene	Pathogen [c]	Reference
T. aestivum (wheat)	*Lr10*	AAQ01784	CNL	NBS	1A	Leaf rust	*AvrLr10*	*P. triticina*	[23]
	Lr1	ABS29034	CNL	NBS	5D	Leaf rust	*Avr1*	*P. triticina*	[24]
	Pm3b	AAQ96158	CNL	NBS	1A	Powdery mildew	*AvrPm3b*	*B. graminis*	[25]
	Sr33	AGQ17390	CNL	NBS	1D	Stem rust		*P. graminis*	[26]
	Sr35	AGP75918	CNL	NBS	3A	Stem rust		*P. graminis*	[27]
	Lr21	AAP74647	NL	NBS	1D	Leaf rust	*AvrLr21*	*P. triticina*	[28]
	Cre3	AAC05834	NL	NBS	2D	Cereal cyst		*H. avenae*	[29]
	Cre1	AAM94164	NL	NBS	2B	Cereal cyst		*H. avenae*	[30]
	Yr10	AAG42168	CNL	NBS	1B	Stripe rust		*P. striiformis*	[31]
	Stpk-V (Pm21)	AEF30547	STK	Oth-R	6V	Powdery mildew		*B. graminis*	[32]
	Lr34	ACN41354	*ABC transporter*	Oth-R	7D	Leaf rust, Powdery mildew, Stripe rust		*P. triticina*; *P. striiformis*; *B. graminis*	[33]
	Yr36	ACF33195	Kinase-START	Oth-R	6B	Stripe rust		*P. striiformis*	[34]
H. vulgare (barley)	*Mla6*	CAC29241	CNL	NBS	1	Powdery mildew	*AvrMla6*	*B. graminis*	[35]
	Mla1	AAG37356	CNL	NBS	1	Powdery mildew	*AvrMla1*	*B. graminis*	[36]
	Mla13	AAO16014	CNL	NBS	1	Powdery mildew	*AvrMla13*	*B. graminis*	[37]
	Rpg1	AAM81980	LRR-PK	RLK	7	Stem rust	*Avr-Rpg1*	*P. graminis*	[38]
	Mlo	CAB06083	TM	Oth-R	4	Powdery mildew		*E. graminis*	[39]
S. lycopersicum (tomato)	*Prf*	AAC49408	CNL	NBS	5	Bacterial speck	*AvrPto*	*P. syringae*	[40]
	Mi	AAC67238	CNL	NBS	6	Root knot		*M. javanica*	[41]
	I2	AAB63274	NL	NBS	11	Fusarium wilt	*Avr1*	*F. oxysporum*	[42]
	Ph-3	AIB02970	CNL	NBS	9	Late blight		*P. infestans*	[43]
	Sw-5	AAG31013	CNL	NBS	9	Tomato spotted wilt		TSWV	[44]
	Tm-2	AAQ10735	CNL	NBS	9	Tobacco mosaic		TMV	[45]
	Bs4	AAR21295	TNL	NBS	5	Bacterial spot	*AvrBs4*	*X. campestris*	[46]
	Hero	CAD29729	CNL	NBS	4	Potato cyst		*G. rostochiensis*	[47]

Table 1. *Cont.*

Species [a]	*R*-Gene	Accession ID	Domain [b]	Class	Chr	Disease [c]	*Avr* Gene	Pathogen [c]	Reference
	Cf-2	2207203A	LRR-TM	RLP	6	Leaf mold	*Avr2*	*C. fulvum*	[48]
	Cf-4	CAA05268	LRR-TM	RLP	1	Leaf mold	*Avr4*	*C. fulvum*	[49]
	Cf-5	AAC78591	LRR-TM	RLP	6	Leaf mold	*Avr5*	*C. fulvum*	[50]
	Cf-9	CAA05274	LRR-TM	RLP	1	Leaf mold	*Avr9*	*C. fulvum*	[51]
	Ve1,2	AAK58681.2	LRR-TM	RLP	9	Verticillium wilt		*V. dahliae*	[52]
	Hcr9-4E	CAA05269	LRR-TM	RLP	1	Leaf mold	*Avr4E*	*C. fulvum*	[49]
	Fen	2115395A	STK	Oth-R	5	Bacterial speck	*AvrPto*	*P. syringae*	[53]
	Pto	A49332	STK	Oth-R	5	Bacterial speck	*AvrPto*	*P. syringae*	[54]
	Pti1	NP_001233803	STK	Oth-R	12	Bacterial speck		*P. syringae*	[55]
	Rx	CAB50786	CNL	NBS	12	PVX		PVX	[56]
S. tuberosum (potato)	*RB*	Q7XBQ9	CNL	NBS	8	Late blight	*Avr1, Ipio, Ipib*	*P. infestans*	[57]
	Rx2	CAB56299	LZ-NL	NBS	5	PVX		PVX	[58]
	R1	AAL39063	LZ-NL	NBS	5	Late blight	*Avr1*	*P. infestans*	[59]
L. sativa (lettuce)	*Rgc2 (Dm3)*	Q9ZSD1	NL	NBS		Downy mildew	*Avr3*	*B. lactucae*	[60]
P. nigrum (black pepper)	*Bs2*	AAF09256	CNL	NBS		Bacterial spot	*AvrBs2*	*X. campestris*	[61]
	Xa1	BAA25068	NL	NBS	4	Bacterial blight	*AvrXoo*	*X. oryzae*	[62]
	Pib	BAA76282	CNL	NBS	2	Blast		*M. grisea*	[63]
	Pi-ta	AAK00132	NL	NBS	12	Blast	*Avr-Pita*	*M. grisea*	[64]
	Pi36	ABI64281	CNL	NBS	8	Blast	*Avr-Pi36*	*M. grisea*	[65]
	Pia	BAK39926	CNL	NBS	11	Blast	*AvrPia*	*M. oryzae*	[66]
	Pi-K[h]	AAY33493	NL	NBS	11	Blast	*Avr-Pik*	*M. oryzae*	[67]
O. sativa (rice)	*Pi37*	ABI94578	NL	NBS	1	Blast		*M. grisea*	[68]
	Xa5	A2XZI2	NL	NBS	5	Bacterial blight	*AvrXa5*	*X. oryzae*	[69]
	Xa13	ABD78944	SET	Oth-R	8	Bacterial blight	*AvrXa13*	*X. oryzae*	[70]
	Pi54	CCD33085	CNL	NBS		Blast	*AvrPi54*	*M. oryzae*	[71]
	Pi9	ABB88855	CNL	NBS	6	Blast	*AvrPi9*	*M. grisea*	[72]
	Piz-t/Pi2	ABC73398	CNL	NBS	6	Blast	*AvrPiz-t*	*M. grisea*	[73]
	Rpr1	BAA75812	CNL	NBS	11	Blast		*M. grisea*	[74]

Table 1. *Cont.*

Species [a]	*R*-Gene	Accession ID	Domain [b]	Class	Chr	Disease [c]	*Avr* gene	Pathogen [c]	Reference
	Pid3	ACN62386	CNL	NBS	6	Blast		*M. grisea*	[75]
	Xa21	AAC49123	LRR-STK	RLK	11	Bacterial blight	*AvrXa21*	*X. oryzae*	[76]
	Xa3/Xa26	ABD36512	LRR-STK	RLK	11	Bacterial blight		*X. oryzae*	[77]
	CEBiP	BAE95828		RLK	3				[78]
	Xa10	AGE45112		Oth-R	11	Bacterial blight	*AvrXa10*	*X. oryzae*	[79]
	Xa25	AGS56390	TM	Oth-R	12	Bacterial blight		*X. oryzae*	[80]
	Xa27	AEW90324	LRR-TM	RLP	6	Bacterial blight	*AvrXa27*	*X. oryzae*	[81]
	Pi-d2	ACR15163	B-lectin, STK	RLK	6	Blast		*M. grisea*	[82]
Z. mays (maize)	*Rp1-D*	AAD47197	NL	NBS	10	Rust		*P. sorghi*	[83]
	Hm1	Q41867			1	Corn leaf blight		*C. carbonum*	[84]
	RPM1	CAA61131	CNL	NBS	3	Downy mildew	*AvrB, AvrRpm1*	*P. syringae*	[85]
	RPS2	AAA21874	NL	NBS	4	Downy mildew	*AvrRpt2*	*P. syringae*	[86]
	RPP8/HRT	AAC83165	CNL	NBS	5	Downy mildew	*AvrRPP8*	*P. parasitica*	[87]
	RPP13	AAF42832	CNL	NBS	3	Downy mildew	*ATR13*	*P. parasitica*	[88]
	RCY1	BAC67706	CNL	NBS	5	Mosaic type		CMV	[89]
	RPP1	AAC72977	TNL	NBS	3	Downy mildew	*ATR1*	*P. parasitica*	[90]
A. thaliana (*Arabidopsis*)	*RPP4*	AAM18462	TNL	NBS	4	Downy mildew		*P. parasitica*	[91]
	RPS4	CAB50708	TNL	NBS	5	Powdery mildew	*AvrRps4*	*P. syringae*	[92]
	RPP5	AAF08790	TNL	NBS	4	Downy mildew	*AvrRp5*	*P. parasitica*	[93]
	RPS5	AAC26126	NL	NBS	1	Downy mildew	*AvrRphB*	*P. syringae*	[94]
	RRS1	ADM88042	WRKY-TNL	NBS	5	Bacterial wilt	*AvrRRS1*	*R. solanacearum*	[95]
	RPP27	CAE51864	LRR-TM	RLP	1	Downy mildew		*P. parasitica*	[96]
	RFO1	AAY86486	LRR-STK	RLK	1	Fusarium wilt		*F. oxysporum*	[97]
	PBS1	AAG38109	STK	Oth-R	5		*AvrPphB*	*P. syringae*	[98]

Table 1. *Cont.*

Species [a]	R-Gene	Accession ID	Domain [b]	Class	Chr	Disease [c]	AVR Gene	Pathogen [c]	Reference
	FLS2	AED95370	LRR-STK	RLK	5	Powdery mildew	*AvrPto, AvrPtoB*	*P. syringae*	[99]
	BAK1	AT4G33430	LRR-STK	RLK	4		*AvrPto, AvrPtoB*	*P. syringae*	[100]
	NDR1	AAB95208	TM	Oth-R	3		*AvrB, AvrRpt2*	*P. syringae*; *P. parasitica*	[101]
	RPW8	AAK09267	RPW8	Oth-R	3	Powdery mildew		*E. cruciferarum*	[102]
L. usitatissimum (flax) [d]	*L6*	AAA91022	TNL	NBS	5	Rust	*AvrL6*	*M. lini*	[103]
	L, L1-L11	AAD25974	TNL	NBS	5	Rust	*AvrBs3*	*M. lini*	[104]
	M	AAB47618	TNL	NBS	8	Rust	*AvrM*	*M. lini*	[105]
	P, P1-4	AAK28806	TNL	NBS	14	Rust		*M. lini*	[106]
B. vulgaris (sugar beet)	*Hs1pro-1*	AAB48305	LRR-TM	RLP	1	Beet cyst		*H. schachtii*	[107]
N. tabacum (tobacco)	*N*	AAA50763	TNL	NBS		Tobacco mosaic		TMV	[108]

[a]: *A. thaliana, Arabidopsis thaliana*; *B. vulgaris, Beta vulgaris*; *H. vulgare, Hordeum vulgare*; *L. sativa, Lactuca sativa*; *L. usitatissimum, Linum usitatissimum*; *N. tabacum, Nicotiana tabacum*; *O. sativa, Oryza sativa*; *P. nigrum, Piper nigrum*; *S. lycopersicum, Solanum lycopersicum*; *S. tuberosum, Solanum tuberosum*; *T. aestivum, Triticum aestivum*; *Z. mayes, Zea mayes*; [b]: SET, sugar efflux transporter; TM, transmembrane; STK, serine/threonine protein kinase; [c]: PVX, potato virus X; *B. graminis, Blumeria graminis*; *B. lactucae, Bremia lactucae*; *C. fulvum, Cladosporium fulvum*; *C. carbonum, Cochliobolus carbonum*; *E. cruciferarum, Erysiphe cruciferarum*; *E. graminis, Erysiphe graminis*; *F. oxysporum, Fusarium oxysporum*; *G. rostochiensis, Globodera rostochiensis*; *H. avenae, Heterodera avenae*; *H. schachtii, Heterodera schachtii*; *M. grisea, Magnaporthe grisea*; *M. oryzae, Magnaporthe oryzae*; *M. lini, Melampsora lini*; *M. javanica, Meloidogyne javanica*; *P. parasitica, Peronospora parasitica*; *P. infestans, Phytophthora infestans*; *P. syringae, Pseudomonas syringae*; *P. graminis, Puccinia graminis*; *P. sorghi, Puccinia sorghi*; *P. striiformis, Puccinia striiformis*; *P. triticina, Puccinia triticina*; *R. solanacearum, Ralstonia solanacearum*; *V. dahliae, Verticillium dahliae*; *X. campestris, Xanthomonas campestris*; *X. oryzae, Xanthomonas oryzae* pv. *oryzae* (Xoo); CMV, cucumber mosaic virus; TMV, tobacco mosaic virus; TSWV, tomato spotted wilt virus; [d]: The chromosome numbers of genes were based on unpublished data.

Table 2. Genome-wide identification of RGAs in plant genomes.

Species [a]	Genome Size (Mb) [b]	Total Annotated Genes [b]	Total RGAs (%) [c]	NBS Coding Genes [d]							PPR [e]	RLK [f]	RLP [g]	Other [h]	Identification Method Used [i]	Reference
				CNL	TNL	CN	NL	TN	N	Total						
Dicots																
A. thaliana (*Arabidopsis*)	125	25,498	5.27	51	79	8	20	17	26	201	441	600	56	46	H, P, B	[109–113]
A. lyrata (lyrata)	207	32,670	0.56	21	103	17	14	20	10	185					H, B	[114]
P. trichocarpa (black cottonwood)	485	45,555	3.18	119	64	19	83	13	46	344	600	379		127	MEME, CO, Paircoil2, MC	[111,115–117]
V. vinifera (grape)	475	30,434	3.81	203	97	26	12	14	0	352	600			210	H, B, MEME	[111,118]
L. usitatissimum (flax)	373	43,484	0.34	31	57	10	5	22	7	132				16	MEME/MAST	[119]
S. lycopersicum (tomato)	900	34,727	0.84	118	18	19	43	5	49	252		16	13	13	H, B	[120,121]
C. papaya (papaya)	372	28,629	0.18	4	6				44	54					TBN, MEME, CW, MC, H	[122]
C. sativus (cucumber)	367	26,682	0.26	25	19	1	17	5	3	70					H, CO, ME, CX, SMART, P, B	[123]
S. tuberosum (potato)	844	39,031	1.47	65	37	24	184	12	113	435				142	H, B	[124]
M. truncatula (*Medicago*)	454	62,388	1.20	152	118	25	0	38	328	661				92	B, H	[111]

Table 2. *Cont.*

Species [a]	Genome Size (Mbp) [b]	Total Annotated Genes [b]	Total RGAs (%) [c]	NBS Coding Genes [d]							PPR [e]	RLK [f]	RLP [g]	Other [h]	Identification Method Used [i]	Reference
				CNL	TNL	CN	NL	TN	N	Total						
G. raimondii (cotton)	880	40,976	1.19	35	41	18	96	9	31	230		60	144	56	B, CO, SMART, MC, CW, IPS, ME5,	[125,126]
B. rapa, (chinese cabbage)	485	41,174	0.60	19	93	15	27	23	29	206				42	B, H	[111]
B. oleracea (cabbage)	630	45,758	0.52	6	40	5	24	29	53	157				82	B, H	[111]
F. vesca (strawberry)	240	34,809	0.27		61		16	8	1	86				8	B, MU, ME, MEME	[127]
M. x domestica (apple)	742	57,386	1.86	218	161	54	276	69	182	960				110	H, B, CW, MEME	[17]
L. japonicus (lotus)	472	19,848	0.42	9	8	19	3	16	29	84					BP, CO, P, MEME	[128]
T. cacao (cocoa*)*	430	28,798	1.09	82	8	46	104	4	53	297				17	B, H	[111]
P. patens (moss)	510	35,938	0.46	9	3	2	5	0	1	20	103			45	B, CO, MU, ME	[129,130]
Average	500	37,433	1	69	56	19	55	18	56	263	436	264	71	72		
Monocots																
O. sativa (rice*)*	420	59,855	4.22	159	0	7	40	3	45	254	477	1429	90	281	H, B, MEME, P	[110,130–133]
T. aestivum (wheat)	17,000	94,000	2.37	98		0	555		318	971				1266	H, B, MEME	[134]

Table 2. *Cont.*

| Species [a] | Genome Size (Mbp) [b] | Total Annotated Genes [b] | Total RGAs (%) [c] | NBS Coding Genes [d] | | | | | | | PPR [e] | RLK [f] | RLP [g] | Other [h] | Identification Method Used [i] | Reference |
				CNL	TNL	CN	NL	TN	N	Total						
Z. mayes (maize*)*	2300	32,540	0.90	58	0	21	31	0	69	179		113		2	P, H, B, CO	[135,136]
S. bicolor (sorghum*)*	739	34,496	1.29	36	0	99	133	0	64	332				114	P, H, B, CO, ME, CW	[137,138]
H. vulgare (barley)	5100	30,400	1.38	101		51	145		34	331				89		[139,140]
B. distachyon (*Brachypodium*)	272	25,532	1.23	133	0	28	87	0	34	282				34	P, H, B, CO, CW	[140,141]
T. urartu (Red wild einkorn)	4940	34,879	1.63	235	0	44	218		38	535				35	H	[140,142]
A. tauschii (Tausch's goatgrass)	4360	43,150	1.94	296	0	63	288		81	728				112	H	[140,143]
Average	4391	44,357	2	140	0	39	187	1	85	452	477	771	90	242		

[a]: *A. tauschii, Aegilops tauschii*; *A. lyrata, Arabidopsis lyrata*; *B. distachyon, Brachypodium distachyon*; *B. oleracea, Brassica oleracea*; *B. rapa, Brassica rapa*; *C. papaya, Carica papaya*; *C. sativus, Cucumis sativus*; *F. vesca, Fragaria vesca*; *G. raimondii, Gossypium raimondii*; *L. japonicus, Lotus japonicus*; *M. truncatula, Medicago truncatula*; *M. x domestica, Malus x domestica*; *P. patens, Physcomitrella patens*; *P. trichocarpa, Populus trichocarpa*; *S. bicolor, Sorghum bicolor*; *T. cacao, Theobroma cacao*; *T. urartu, Triticum urartu*; *V. vinifera, Vitis vinifera*; [b]: Most of the information concerning the genome sizes and the total number of annotated genes was obtained from [144]; [c]: The percentages calculated based on present data, not referred from references; [d]: CNL, CC-NBS-LRR; TNL, TIR-NBS-LRR; CN, CC-NBS; NL, NBS-LRR; TN, TIR-NBS; N, NBS; [e]: PPR, pentatricopeptide repeat; [f]: RLK, receptor like kinase; [g]: RLP, receptors like proteins; [h]: Other, includes TIRX, XN, TNLX, TNTNL, TTNL, XTNX, CNX, TX and Partial NBS–LRR; [i]: B, BLAST; CO, COILS; CW, ClustalW; CX, ClustalX; H, HMM; MC, MARCOIL; IPS, InterProScan; ME, MEGA; MU, MUSCLE; P, Pfam.

Though a large number of resistance gene loci have been identified in plants using linkage mapping or association studies, most of them correspond to flanking molecular markers or quantitative trait loci (QTL). Mapped genome-wide RGAs, as *R*-gene candidates, are valuable genomic resources to develop high-density *R*-gene genetic maps, design diagnostic markers and co-localize QTL. The markers designed from RGAs can be used for fine mapping and cloning of *R*-genes and, for breeding purposes. This review focuses on recent advances in studies of the structures and functions of RGAs, their identification using bioinformatics tools and their applications in genetic research and breeding for disease resistance.

2. Structure and Functional Mechanisms of Resistance Gene Analogs (RGA)

RGAs can be grouped as either nucleotide binding site leucine rich repeat (NBS-LRR) or transmembrane leucine rich repeat (TM-LRR) [145]. Recent findings have identified other modes of plant resistance mechanisms including pentatricopeptide repeats (PPRs) and peroxidases. NBS-LRR can be further classified as toll/interleukin receptor (TIR)-NBS-LRR (TNL) or non-TNL/coiled coil-NBS-LRR (CNL) [145]. Both TNL and CNL specifically target pathogenic effector proteins inside the host cell, termed effector triggered immunity (ETI) response [146]. Likewise, TM-LRRs can be subdivided into two classes: receptor like kinases (RLKs) and other receptor like proteins (RLPs) [145]. RLPs and RLKs are pattern recognition receptors (PRRs) that mediate pathogen/microbe associated molecular pattern (PAMP/MAMP) triggered immunity (PTI/MTI) to allow recognition of a broad range of pathogens [146]. PAMP/MAMPs are conserved features of most pathogens, such as chitin, flagella, and lipopolysaccharides.

2.1. Nucleotide Binding Site Leucine Rich Repeat (NBS-LRR) Family

NBS-LRR is the best-known family of RGAs. The two classes of NBS-LRR are distinguished by their N terminal TIR or non-TIR domains. The non-TIR domains are most commonly coiled coil (CC) structures [147]. Another non-TIR domain is the leucine zipper (LZ), with interspersed hydrophobic heptad repeat sequences L-X(6)-L-X(6)-L-X(6)-L [148]. The domain combination refers to LZ-NBS-LRR proteins [148] which are not as common but have been found in agricultural plants such as tomato and potato [40,59]. At the N-terminal region lies the highly irregular and variable LRR domain [149]. This domain is responsible for protein-protein interactions [150]. Between the NBS and LRR domains exists a region called the ARC domain, named so because of its occurrence in <u>A</u>PAF-1, <u>R</u> protein and <u>C</u>ED-4 [10]. This ARC domain can be further divided into ARC1 and ARC2 subdomains. The ARC domain, together with the NBS domain, forms a region for nucleotide binding [151].

Various conserved motifs exist within domains and subdomains of TNL and CNL [152]. The pentapeptide EDVID (EDxxD) motif, denoted as CC$_D$, can be identified in the CC domain [153]. Motifs like CC$_R$ (resembling RPW8 protein) can also be found [154]. Similarly, the TIR domains are composed of four motifs: TIR1, TIR2, TIR3 and TIR4 [155,156]. The NBS domain itself comprises motifs that mainly interact with nucleotides [19], such as the P-loop (also known as Walker A and Kinase-1a), resistance nucleotide binding site-A (RNBS-A), Walker B (Kinase-3a) and RNBS-C. The hhGRExE [157] motif is a linker region attaching the NBS domain to the CC or TIR domains [152]. There are two motifs on ARC1, namely GLPL [109,155] (also called GxP [157]) and Motif VII [158]; both partake in nucleotide binding [159]. Motifs identified in ARC2 are Motif VIII, RNBS-D, Motif X and MHD [109,155,158].

RNBS-D motif is not consistently present between the TIR and CC domains. It likely co-evolved with their N terminal domain to allow interaction with ARC2 [160]. Figure 1 illustrates the various motifs and their structural organization in some of the most common R proteins.
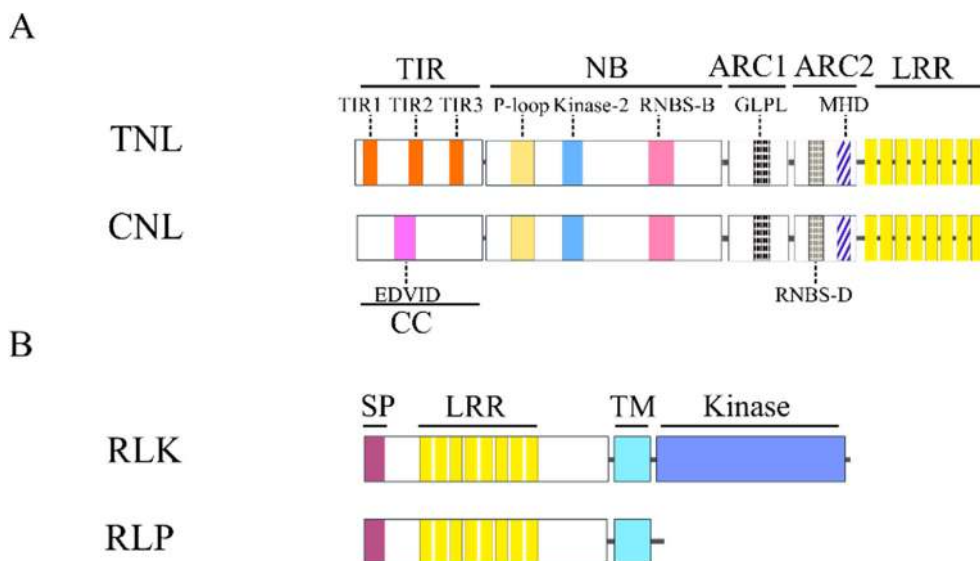

Figure 1. Schematic representation of common structures of four major plant R proteins. Motifs are depicted as colored boxes and labeled under the domain names. Note: the domain lengths are not drawn to scale for ease of visualization. (**A**) Typical domain dissection for TNL and CNL proteins. Only highly conserved motifs are illustrated; (**B**) Domain structures for RLKs and RLPs. The kinase domain is absent in RLPs. Other common domains utilized in our genome-wide identification pipeline are labeled above the colored boxes. TIR: Toll/interleukin-1 receptor; NB: nucleotide-binding site; ARC: abbreviated from Apaf-1, R proteins and CED-4; CC: coiled-coil; SP: signal peptide; TM: transmembrane; LRR: leucine-rich repeats.

NBS-LRR proteins are part of the STAND super family with ATPase activity [157]. In the resting or auto-inhibited state, NB-ARC interacts with both its N-terminal LRR and C-terminal CC/TIR domains to maintain a closed conformation [161]. In order to become activated, the LRR domain must be detached from the NBS domain. After detachment, the NB-ARC domain will switch its ADP nucleotides to an ATP to enable rotations within the NB-ARC domain leading to an open conformation [161] allowing the NBS or the N-terminal regions to be exposed for subsequent downstream signaling [161].

As previously mentioned, TNL and CNL proteins recognize pathogen effectors that are secreted into the cell allowing plants to trigger the ETI response. Mechanisms associated with several characterized R proteins and their related ETI responses are summarized in Figure 2. Host protein RIN4 (RPM1-interacting protein 4), guarded by the NBS-LRR encoded proteins RPM1 and RPS2, is targeted by effectors such as AvrRpm1, AvrB and AvrRpt2 [162]. Similarly, enhanced disease susceptibility 1 (EDS1) is also a common target due to its ability to interact with different NBS-LRR proteins such as resistance to pseudomonas syringae 4 (RPS4), resistance to pseudomonas syringae 6 (RPS6) and suppressor of npr1-1, constitutive 1 (SNC1) during their subsequent ETI downstream response [163,164]. Aside from targeting immune regulatory components, effectors can also target PTI/MTI signaling cascades (Figure 2).

MAP kinase cascade, specifically MPK4, is capable of suppressing NBS-LRR protein SUMM2 in absence of effector HopAl1; however, when MPK4's activity is compromised by HopAl1 effector, SUMM2 is activated and initiates hypersensitive programmed cell death (PCD) [165]. ETI and PTI/MTI responses usually result in the production of calcium and phytohormones, oxidative reactive oxygen species (ROS) burst, activation of MAPK cascade (s) [166] and transcription of defense genes to facilitate the hypersensitive response (HR) in order to limit pathogen expansion [167]. Overlaps between the ETI and PTI/MTI defense pathways exist and are important for immune regulation [168]. However, ETI responses have a more heightened downstream effect than PTI/MTI and may also induce PTI/MTI activation in the presence of effectors [168]. This is crucial because effector presence is a true indication of bacterial inhabitancy whereas PAMP/MAMP recognition in PTI/MTI must discriminate between harmful pathogens and beneficial microorganisms [168].
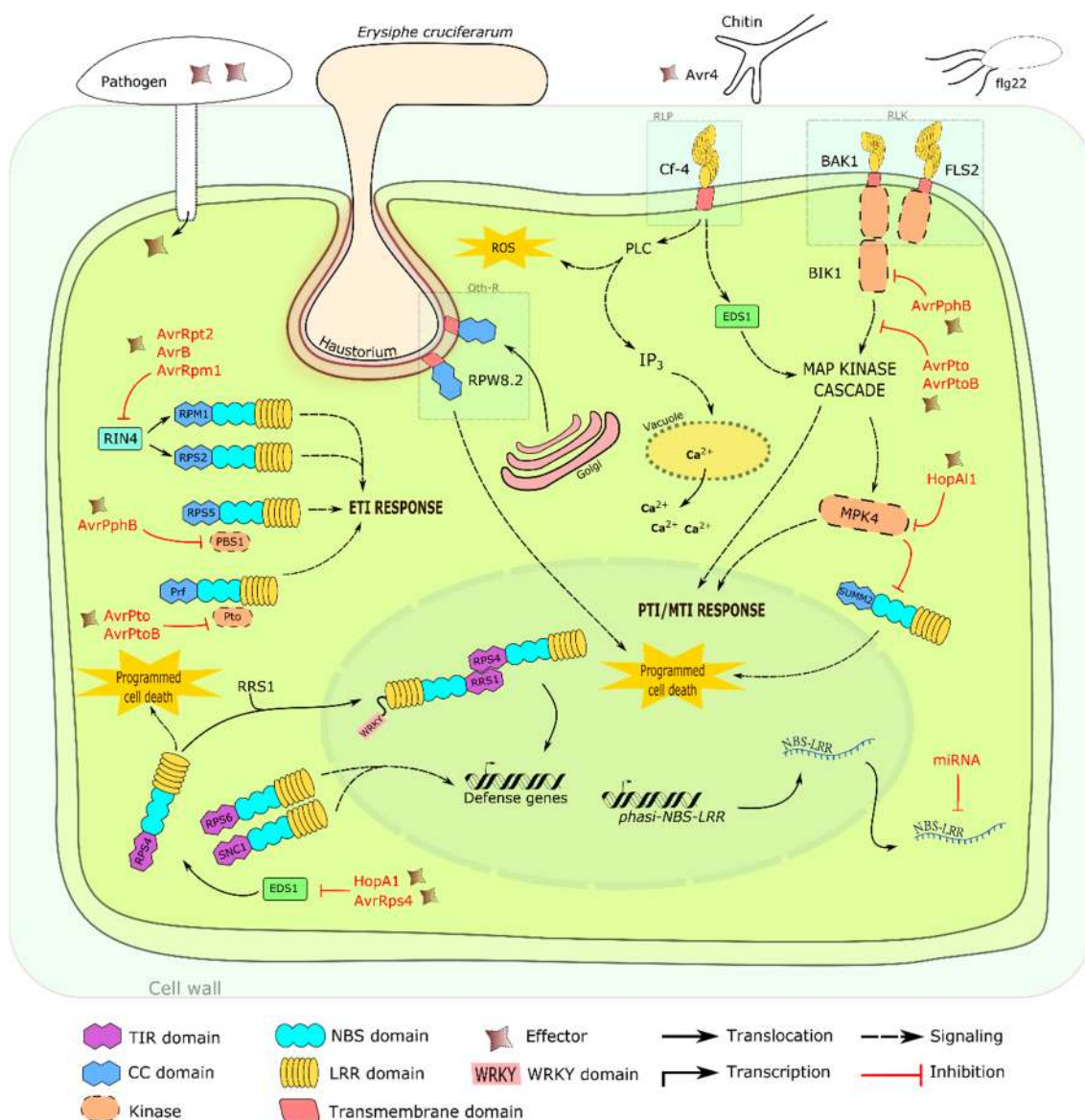

Figure 2. Intracellular signaling mechanisms of RGAs in plant defense. RIN4, PBS1, Pto and EDS1 are targeted and modified by numerous effectors and, as a result, their corresponding TNL or CNL will detect the modification to initiate ETI responses [162–164]. TIR-TIR interactions occur between RPS4 and RRS1 to further activate defense

genes [169]. Flg22, a bacterial PAMP, activates FLS2 and BAK1 RLKs to initiate the MAP kinase cascade that triggers PTI/MTI responses [170]. MAP kinase cascade signaling can be interrupted by pathogenic effectors. When MPK4 is compromised, SUMM2 will not be inactivated and will initiate PCD [165]. Effector Avr4 is recognized by Cf-4 RLP to initiate MAP kinase cascade and ROS production while simultaneously increasing calcium levels in the cytosol [171]. Upon *Erysiphe cruciferarum* infection, RPW8.2 can translocate from the Golgi to the extrahaustorial membrane where the fungal haustorium has penetrated to activate the downstream signaling of PCD [172,173]. Under normal conditions, NBS-LRR transcripts derived from the *PHAS* locus are regulated through transcript degradation by miRNAs [174]. Such miRNAs include, among others, miR1510, miR1507, miR2109, miR482/2118, miR5668, miR5376, miR172 and miR5041 [174–176]. Single arrows may indicate multi-step processes.

2.2. Receptor Like Kinase (RLK) and Receptor Like Protein (RLP) Families

RLK and RLP are main components of the first line of plant immune response triggered by microbial elicitors PAMPs or MAMPs, where the interactions between receptor and elicitor usually take place in the extracellular space. The two proteins are structurally similar with (1) a signal peptide (SP) at the beginning of N-terminus; (2) extracellular domains for perception of the microbial pattern through the leucine-rich repeats and (3) a transmembrane helix domain that can anchor RLP and RLK in the plasma membrane. RLPs differ from RLKs by the lack of an intracellular kinase domain; thus RLPs are unable to independently transduce the perceived signal into a downstream cascade. Both RLPs and RLKs are considered PRRs that recognize elicitors such as lipids, proteins, nucleic acids, and carbohydrates [177].

2.2.1. RLK Family

On the cell surface, plant receptors sense invasion of pathogens and transduce this information through activated signaling pathways to trigger innate immune responses. RLKs are involved in plant development and defense [178]. In plants, serine/threonine protein kinases (STKs) can phosphorylate serine and threonine residues [55]; receptor histidine kinases (RHKs) can phosphorylate histidine residues, e.g., ethylene (ETR1) [179] and cytokinin receptors (CRE1) [180]. TGF β family members represent the only known STK members present in animals [181].

The most well-known RLKs are flagellin sensitive 2 (FLS2) and BAK1 that initiate the MAP kinase cascade upon flg22 recognition [162]. *Xa21* in rice encodes an RLK involved in resistance to a bacterial disease caused by *Xanthomonas oryzae* (*Xoo*) [182]. Several Xa21 binding proteins have been characterized; however, early events governing Xa21 signaling have not been fully elucidated. *XIK1* is an RLK gene whose expression is induced rapidly upon infection with *Xoo*. The reduced expression of *XIK1* compromised disease resistance mediated by *Xa21* [183]. Xa21 binds to a WRKY transcription factor OsWRKY62 [184]. In transgenic plants, the *OsWRKY62.1* variant of *OsWRKY62* is overexpressed in basal defense and in *Xa21*-mediated resistance to *Xoo*. Therefore, *OsWRKY62* function was implied to be a negative regulator of innate immunity in rice, which served as a critical mediator of both basal and race-specific defense responses [184].

2.2.2. RLP Family

Following RLKs, RLPs are the second most abundant RGAs. RLPs have a gene structure similar to RLKs but without a kinase domain (Figure 1). Of note, not all RLPs are involved in disease resistance, some play a role in plant development.

Cf-9, the first RLP gene identified, provides resistance against leaf mold induced by the fungus *C. fulvum* [51]. Several *Cf* genes belonging to the RLP family have been isolated from tomato [48–50]. Further studies revealed that Cf-9 functions in the cytoplasm by interacting with STK kinase ACIK1 via the CITRX ligand [185,186]. Cf-4, a similar type of RLP, is induced by Avr4 which is an effector that protects chitinous fungi in tomato [187]. This trigger initiates downstream PTI responses via a MAP kinase cascade, ROS accumulation and by releasing calcium ions from the vacuole (Figure 2) [171]. Another type of RLP gene, *CEBiP* isolated in rice, has no typical N-terminal LRR receptor for the perception of elicitor; however, two LysM motifs were observed to perceive chitin oligosaccharide patterns present in fungal cell walls [78], reminiscent of similar discoveries in legume [188,189]. The LysM motif was also noted in the extracellular domains of two legume RLK genes, *NFR1* and *NFR5*, and was suggested to recognize lipochitin-oligosaccharide molecules acting as a nodulation signal triggering plant organogenic processes [188,189]. Thus, the LysM motifs containing PRRs were suggested to function in perception of chitin signals generated by fungi or bacteria [78]. Additional cloned RLP genes involved in plant-pathogen resistance are listed in Table 1.

With respect to RLPs involved in plant development, two typical RLPs, CLAVATA 2 (CLV2) and too many mouths (TMM), were reported to be involved in maintaining meristematic stem cell population balance and regulating epidermis stomatal distribution in *Arabidopsis*, respectively [190,191]. Several studies indicated that CLV2 needed to form a heterodimer with the RLK protein CLV1 via the extracellular ligand CLV3 [192–194]. Meanwhile, CLV2 was also required for the stabilization and accumulation of kinase CLV1 [190]. TMM was recently found to interact with both ligands, EPIDERMAL PATTERNING FACTORS (EPFs) and RLK ERECTA, to negatively regulate the development of stomata [195]. These RLPs seemed to be irrelevant to the identification of disease resistance related RLPs. However, it was speculated that these development related genes were much more conserved than those of disease related *R*-genes [109,196]. Based on this hypothesis, Fritz-Laylin *et al.* [110] identified 73 rice disease resistance related RLP genes from a total of 90 RLPs by comparing them to their orthologs in *Arabidopsis*. Therefore, a better understanding of the multiple functions of RLPs and of the divergence as well as conservation between the two types of RLPs will facilitate the identification of the most interesting RLPs of this family.

2.3. Oth-R-Genes

The defense system is initiated when extracellular receptors transmit signals to their intracellular partners. To observe and receive these signals, plant cells have complex systems of TM receptors that facilitate communication between the intra- and extracellular environments. However, several reported TM receptors do not contain complete domains as observed in NBS-containing proteins, RLPs or RLKs. Thus, the term oth-R, initially proposed by Walter *et al.*, is used to classify these peculiar RGAs [197]. For example, RPW8.1 and RPW8.2 proteins from *Arabidopsis* contain a putative

N-terminal TM domain and a CC domain but lack common NBS, STK or LRR domains [102,198,199]. In response to powdery mildew infection, RPW8.2 is upregulated and trafficked to the target site, *i.e.*, the extrahaustorial membrane, via the *trans*-golgi network [173]. In contrast, RPW8.1 is involved in an assortment of pathogen-mediated responses and, as a result, may induce a broader resistance spectrum in plants [200]. Defense gene *Mlo* also belongs to the TM class that has an intra- and an extracellular loop [39,201]. *LM1*, conferring resistance to stem canker (*Leptosphaeria maculans*) in *Brassica nigra*, is predicted to have two TM motifs [202]. Tomato *Asc1* is an *R*-gene that provides resistance to *A. alternata* [203] and, it encodes a protein with multiple TM domains and a highly conserved motif designated as the Lag1p motif. Asc1 confers insensitivity to the AAL-toxin that inhibits the enzyme sphinganine *N*-acyltransferase involved in sphingolipid metabolism, leading to PCD [204]. *Xa25* is a bacterial blight disease resistance gene in rice that encodes a protein of the MtN3/saliva family, which contains a region of two TM helices [80]. Another type of *Xa10* gene has been characterized in rice as a transcription activator-like (TAL) effector-dependent *R*-gene for resistance to bacterial blight disease [79]. *Rar1* in barley, and *Rtm1*, *Rwm1*, *EDS1*, *NPR1*, *NDR1* in *Arabidopsis* also do not display typical LRR domains involved in pathogen recognition (Table 1).

Pti1 encodes an STK and acts downstream of *Pto*. It confers resistance to *P. syringae* in tomato, which expresses AvrPto and targets several locations in the cell (Figure 2) [55]. The immune cascade can be initiated through interaction with *Pto* and *Prf* [205,206]. *PBS1* can also be classified as an oth-R gene that has an STK domain and is devoid of any other known functions ([98]; Figure 2). *Fen* is also a member of the STK family that mediates a hypersensitive-like response in tomato plants treated with the organophosphorous pesticide fenthion [53]. *Stpk-V*, cloned from the powdery mildew resistance gene *Pm21* locus [32], is yet another example of STK encoding genes of the Triticeae. Confocal imaging revealed the lack of bias of this protein for its subcellular localization because it was observed in membranes, cytoplasm and nuclei alike [32].

3. Other Defense Related Mechanisms

Aside from the typical NBS-LRR and PPR proteins involved in plant defense, recent discoveries led to the description of other modes of defense. Nomura *et al.* [207] demonstrated that chloroplasts are an important component in activation and signaling of immunity. Upon exposure to flg22, chloroplast calcium-sensing receptor (CAS) dependent immune resistance and PCD are initiated [207]. Likewise, mitochondria play a similar role [208], highlighting the importance of these organelles in the defense pathways activated in response to pathogen attacks.

Chloroplast and mitochondrial transmigrated proteins have been described [207,208]. They belong to the PPR protein class that constitutes one of the largest protein families in plants. Intriguingly, PPR proteins have undergone an evolutionary process similar with the above-mentioned LRR associated proteins [209]. Characteristics such as gene clustering and duplications within clusters have been observed [209]. PPR proteins are nucleus-encoded and will translocate to the chloroplast and mitochondria to perform post transcript processing such as RNA editing, splicing and translation modification [210].

Known PPR genes, such as *RPF2 and RPF3* in *Arabidopsis*, *Rf1* in rice and *Rf2* in maize have been identified ([211–214]; Table 3). In addition, an increase in *PPR1* (At1g06580) and *PPR2* (At1g62720) transcripts was observed in response to pathogen attack [215]. Although their roles were not clearly

illustrated, these proteins were speculated to be involved in mitochondrial ROS metabolism [215]. Likewise, Garcia-Andrade *et al.* [216] identified a negative regulation of PPR protein chlororespiratory reduction21 (CRR21), PPRα (at4g21190) and overexpressor of cationic peroxidase3 (OCP3) in response to chitosan, a fungal PAMP/MAMP. These proteins correspond to chloroplast *NADH dehydrogenase-like complex* (*NDH*) transcript editing. NDH-mediated immune response remains inconclusive; however, ROS production and callose deposition were speculated to contribute to pathogen-mediated resistance [216].

Table 3. Other cloned genes relevant to plant defense.

Species	R-Gene	Accession ID	Domain [a]	Chr	Disease	Avr	Pathogen [b]	Reference
H. vulgare (barley)	*Rar1*	AAF18432	CHORD	2	Powdery mildew		*B. graminis*	[217]
S. lycopersicum (tomato)	*Asc*	AAF67518	TLC	3	Black mold rot; Black shoulder		*A. alternate*	[218]
O. sativa (rice)	*Rf1*	BAC77666	PPR	10				[214]
	LYP4/6		TM				*X. oryzae*; *M. oryzae*	[219]
Z. mays (maize)	*Rf2*	AAC49371	PPR	9				[213]
A. thaliana (*Arabidopsis*)	*RPF2*	NP_176454	PPR	1				[211]
	RPF3	NP_176481	PPR	1				[212]
	Rtm1	AT1G05760	Jacalin like	1	Tobacco etch		TEV	[220]
	Rwm1	AEE33357	PGK	1	Mosaic type		WMV	[221]
	EDS1	AAD20950	Lipase-like	3		*AvrRps4*	*P. syringae*	[222]
	NPR1	AAC49611	Ankyrin	1			*P. syringae*	[223]

[a]: CHORD, cysteine and histidine-rich domain; PGK, phosphoglycerate kinase; TLC, tram-lag1-cln8; TM, transmembrane; [b]: *A. alternata*, *Alternaria alternata*; TEV, Tobacco etch virus; WMV, watermelon mosaic virus.

As mentioned above, chloroplast and mitochondria, involved in the production of ROS, activate defense and constitutively initiate PCD [224]. Various levels of ROS are produced at different concentrations in response to diverse pathogens [224]. It is intriguing to note that aside from these organelles and oxidase enzymes, about half of the ROS level is generated exclusively from peroxidases upon PAMP/MAMP recognition [225]. Apoplastic peroxidases, PRX33 and PRX34, have been well studied in response to flg22 and EF-Tu [225–227]. Aside from generating hydrogen peroxide (H_2O_2), they play roles in callose deposition and MAMP/PAMP-mediated transcription of defense genes as well [225,226]. Therefore, peroxidases are important for plant immunity.

In addition, small RNAs have been found to play a major role in defense, especially in regulating immune components in the cell [174]; however, further studies are needed as their mechanisms remain poorly characterized.

4. Bioinformatics Approaches for RGA Identification and Characterization

To date, genome sequences of more than 50 plant species have been sequenced and assembled to various degrees [19,20,228]; the released sequences were deposited in public databases such as Phytozome [19,20] and EnsemblPlants FTP servers [229]. Advances in next generation sequencing

technologies have made whole genome sequencing one of the most important approaches in modern biological research. Current challenges include the provision of functional annotations for the large number of macromolecules. However, experimental investigations to assign protein functions are costly and time consuming. Alternatively, computational approaches to functional prediction are very attractive to solve this complex task [230]. Mining and characterizing genome-wide plant RGAs using computational approaches are rendered possible due to their significant structural features and conserved domains. Several bioinformatics methods have been applied to identifying RGAs and predicting their functions, including sequence alignment, BLAST search, phylogenetic analysis, and domain and motif analysis [231] using several applications such as Hidden Markov Model (HMM) [232], SMART (http://smart.embl-heidelberg.de/) [233], Prosite (http://prosite.expasy.org/), pfam (http://pfam.xfam.org/), and InterProScan5 (http://www.ebi.ac.uk/Tools/pfa/iprscan5/) which are summarized (Tables 2 and 4).

Based on previously used approaches, the identification and characterization of RGAs usually follow a common procedure of four steps (Figure 3). First, a plant RGA database including all known plant RGA gene and protein sequences is generated. GenBank [234] and PRGdb [235] are two important sources of well curated RGA sequences. Second, BLAST searches against the RGA database are performed using a loose E-value cut-off (from 1e-5 to 1 depending on the genome size) to identify RGA candidates. Third, using the RGA candidates as input, a variety of software tools (Table 4) are employed to detect various conserved domains and motifs and produce alignments. Some programs like pfam_scan.pl (developed by Sanger) and InterproScan can be run in a parallel mode. In the last step, a dedicated sorting script is needed to group the RGA candidates into classes as per their domain and motif structures or a combination thereof. For example, to be classified as a gene encoding a TNL protein, an RGA must have a 5′ TIR and an NB-ARC followed by an LRR domain.

To date, no standardized bioinformatics tools and consistent annotation criteria were employed in individual studies. Also, individual software tools may have their own advantages and limitations in identifying different types of RGA domains. Thus, the results from different studies are not necessarily comparable. A comprehensive pipeline package to seamlessly integrate these individual tools is expected to save biologists' time by facilitating processing, standardizing data organization and providing visualization features. The use of consistent criteria to identify the complete RGA complements would permit their comparative analyses across species.

Table 4. Common software used for RGA domain and motif identification.

Software	Latest Version	Input Type [a]	Required Database	Description	Parallel Support [b]	URL [c]	Reference
HMMER	3.1b2	D/P	HMM model	Protein or DNA sequence homolog search toolkits using profile hidden Markov models and featured by remote homolog identification. The latest version is as fast as BLAST thanks to the underlying mathematical models.	HT/MPI	hmmer.janelia.org	[236]
MEME	4.10	D/P		Discover novel and ungapped motifs from nucleotide or protein sequences without well trained dataset samples.	MPI	meme-suite.org	[237]
mCUDA-MEME	3.0.15	D/P		An ultrafast scalable motif discovery program running on graphics processing unit (GPU). The algorithm is based on MEME using a hybrid combination of CUDA, MPI and OpenMPI parallel programming models.	CUDA/ MPI	bit.ly/18X8LmA	[238]
BLAST+	2.30	D/P	BLAST databases, like nr or nt database	Classical similarity search toolkits for bioinformatics data mining. The latest version significantly improves the speed on CPU and efficiency on RAM for long queries.	HT	blast.ncbi.nlm.nih.gov	[239]
pfam_scan.pl	1.0	P	Pfam-A HMM model	A Perl script for PFAM database search, which invokes "hmmscan" in the HMMER toolkit package to search known HMM models.		bit.ly/1M41KRu	
InterproScan	5.9	P	PFAM, SMART, PANTHER, PROSITE, Superfamily, *etc*.	A tool that combines different protein signature recognition methods native to the InterPro member databases into one resource with lookup of corresponding InterPro and GO annotations.	HT	www.ebi.ac.uk/interpro	[240]
Phobius	1.01	P	HMM model	A HMM based tool for transmembrane (TM) topology and signal peptides (SP) prediction from proteins. A pre-training HMM model has been embedded in the tool.		phobius.sbc.su.se	[241]
TMHMM	2.0	P	HMM model	A HMM based tool with similar functions to Phobius.		www.cbs.dtu.dk/services	[242]
nCOILS	2.2	P	Scoring matrix	A program to detect CC domains by comparing and scoring protein sequences with a known coiled-coils database with the MTK or MTIDK calculation matrix, which reports a probability that the sequence adopts a coiled-coil conformation.		embnet.vital-it.ch	[243]

[a]: D, nucleotide; P, amino acid; [b]: HT, hyper-thread; MPI, message passing interface; CUDA, a computing platform implemented by nVIDIA on GPUs; [c]: abbreviated bitly URL links were used to replace real URL, case sensitive.
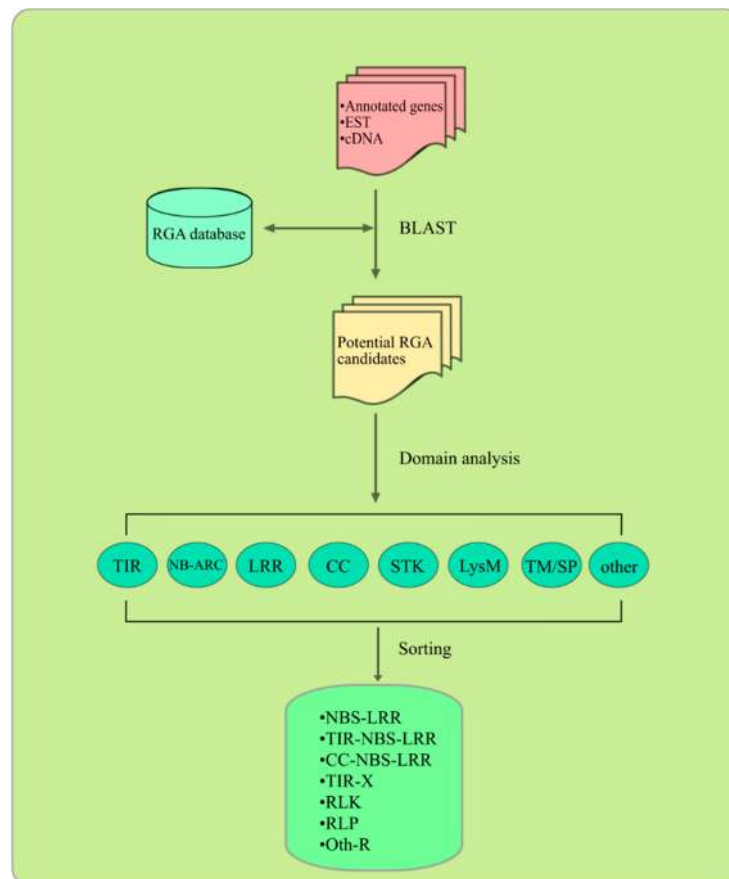
Figure 3. A common procedure for identification and characterization of plant RGAs.

5. Genome-Wide Identification and Characterization of RGAs

Whole genome sequencing of plants has enabled genome-wide identification, mapping and characterization of RGAs. NBS-LRR containing RGAs have been evaluated in numerous plants such as apple, *Arabidopsis*, barley, black cottonwood, grape, maize, *Medicago*, rice and sorghum (Table 2). Hundreds of NBS-LRR encoding genes have been identified from these plant genomes. NBS-LRR genes are a highly duplicated, evolutionarily diverse and clustered gene family [244] and, they represent the major class of *R*-genes contributing to disease resistance in plants [245]. Angiosperms possess NBS-LRR encoding genes but TNL encoded genes are absent from grass genomes [196,246] and other monocots [114]. It has been hypothesized that this absence is likely due to either a loss or the lack of amplification of TNL encoding genes in the monocot lineage [156,247,248]. The loss of TNL-encoding genes in monocots could be due to increased dependence on CNL proteins [156]. In rice, CNL proteins are encoded by many more genes than the total number of CNL and TNL genes in *Arabidopsis* [156]. More NBS-LRR and CNL encoding genes have been identified in most monocots than in dicots (Table 2). CNL and TNL proteins may utilize different downstream signaling components in disease resistance responses [249]. This genomic shift to CNL genes in monocots could have resulted from mutations in genes encoding downstream components of TNL-specific pathways, resulting in a loss of function and conservative selection for TNL genes. Therefore, TNL-encoding genes may have been lost or simply never amplified in monocot genomes due to a lack of selective advantage [156]. In addition, some dicots like *Arabidopsis* contain more TNL than CNL [109,118]. The abundance of TNL likely

results from its older origin than CNL [250]. Besides TNL and CNL, variants of NBS encoding genes such as CN, NL, TN and N vary largely in number (Table 2). Other NBS-LRR like domains such as CTNL and CTN in apple [17], TN-TNL, TTNL, XTNX and SA-CA (signalling commentator with CC domain, e.g., RPW8) in *Arabidopsis* [251], and, CNLX, CNX, CNXL, CXN, NX and NLX in sorghum [138], were reported. Numbers of TIR-X RGAs were also reported such as 126 in cabbages, 46 in *Arabidopsis*, 67 in cottonwood and 92 in *Medicago* [111].

More than 600 members of RLKs were identified in *Arabidopsis* [112] and around 1200 members in rice [132]. They have also been reported in maize, wheat, tomato and cottonwood (Table 2). RLPs with TM domain have also been reported in *Arabidopsis* and tomato (Table 2). Some cloned genes, such as *Hs1pro-1* in sugar beet, *Cf2*, *Cf4-9*, *Ve1* and *Hcr9-4E* in tomato, belong to this class (Table 1).

Some RGAs have been identified as pseudogenes. A total of 49 *R*-pseudogenes in *Medicago* [16], 179 in potato [124], 347 in the rice variety "Nipponbare" and 345 in variety "93-11" [252], 10 in *Arabidopsis* [109], 161 in cottonwood [115] and 62 in lotus [128] have been identified. In tomato, only 10% of the cloned RGAs were classified into pseudogenes [253], but almost half of the identified RGAs were pseudogenes in western white pine (*Pinus monticola*) [254]. Pseudogene paralogs of several *R*-genes such as *Xa21*, *Cf9*, *Pto* and *Dm3*, were also identified [254]. Most identified pseudogenes have strong identity with another NBS protein but their sequences are shortened by premature stop codons or frameshift mutations. Sequence comparisons have suggested that RGA pseudogenes originated from point mutations, like insertion or deletion of nucleotides [254]. Usually, pseudogenes are considered non-functional genes; however, some pseudogenes can be transcribed into mRNA [254,255]. Evidence of expression also exists in species such as rice [256], pine [254] and *Medicago* [16]. In these species, some pseudogenes have 90%–100% identity to ESTs and their functions are ascribed as partial NBS-LRR proteins. Pseudogenes are believed to power *R* gene evolution by driving illegitimate recombination and permitting gene conversion with alleles or paralogs of functional *R*-genes [257].

As noted earlier, there is surprising similarity in the *PPR* genes of *Arabidopsis* and rice supporting their existence prior to the divergence of monocots and dicots [130]. However, massive differences between the numbers of *PPR* genes in higher plants and non-plant organisms are indicative of the expansion of this gene family during the evolution of the plant kingdom [130].

Some RGAs represent a highly divergent defense system in plants, containing a large and diverse family of genes with conserved motifs and domains [254]. To understand the phylogenetic relationship of RGAs, we selected 63 well-characterized RGAs or cloned *R*-genes (Table 1) in seven dicot (*Arabidopsis*, black pepper, flax, lettuce, potato, tobacco and tomato) and four monocot species (barley, maize, rice and wheat) from the NCBI database. These selected RGAs represent the major classes of RGAs: NBS-LRR (CNL, TNL or NL), RLK (LRR-STK) and RLP (LRR-TM). A neighbor-joining tree with 63 RGAs was constructed using MEGA 6 [258] (Figure 4). RGAs were classified into two groups: Clade I for NBS-LRR encoding genes and Clade II for RLK and RLP encoding genes. As RLP and RLK proteins have common domains but differ in presence or absence of a kinase (Figure 1B), LRR-STK and LRR-TM encoding genes clustered into one large group (Clade II) with sub-clusters. CNL and TNL are major subclasses of NBS-LRR encoding genes; thus they grouped into distinct sub-clusters in Clade I (Clades Ia for TNL and Ib for CNL encoding genes, respectively) (Figure 4). We observed that TNL encoding genes were conserved among different dicot species (Clade Ia). In contrast, CNL encoding genes are much more diverse. Some of them are conserved within

angiosperms (Clades Ib-2 and Ib-4), within dicots (Clades Ib-1 and Ib-3) or within monocots (Clade Ib-5), while others have diverged between dicot and monocot species (Clade Ib) or within dicots (between Clades Ib-3 and Ib-5). For example, Clade Ib-3 contains RGAs from dicot species only while Clade Ib-5, from monocot species only. In addition, NL and LZ-NL encoding genes may be more closely related to CNL than to TNL because almost all NL and LZ-NL encoding genes clustered with the CNL clades (Clades Ib-1, Ib-2, Ib-3, Ib-4 and Ib-5), suggesting that the variants of non-TNL, such as NL and LZ-NL, may have evolved from CNL rather than TNL.
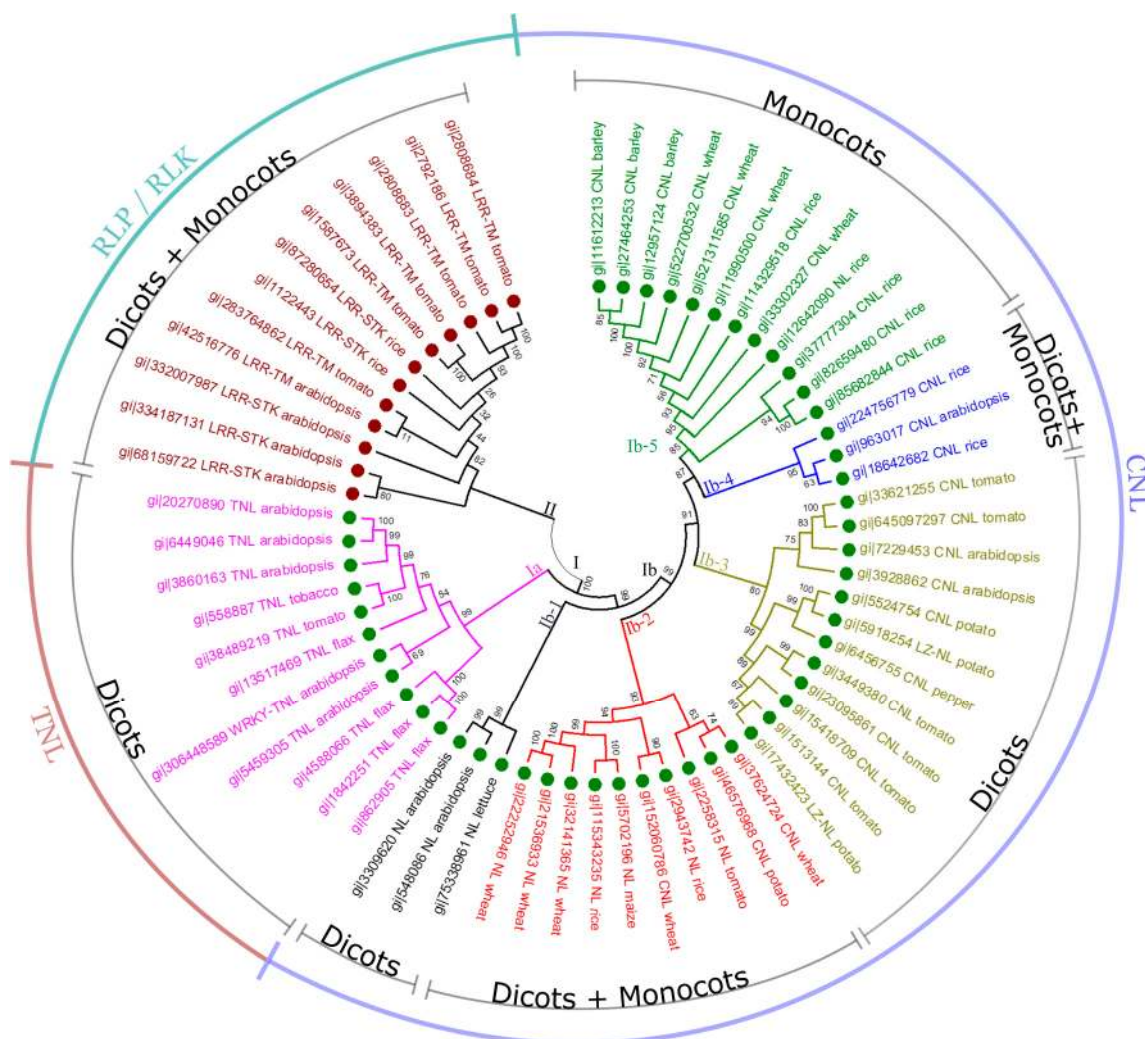
Figure 4. Phylogenetic analysis of RGAs in plants. The protein sequences of 63 RGAs or cloned *R*-genes from eleven plant species were selected for this analysis using MEGA 6 [258]. The protein sequences were aligned using the Muscle algorithm, and then clustered using the neighbor-joining algorithm with the p-distance model, pairwise deletion for gaps or missing data treatment, and 500 bootstrap replicates. The phylogenetic tree consists of two large clades (Clades I and II), representing the NBS-LRR class and the RLK/RLP class of proteins, respectively. Clade I may be divided into two sub-groups (Clades Ia and Ib), containing TNL and CNL proteins, respectively, while Clade Ib may be further split into several diverged CNL clusters. The bootstrap values are labelled on branches.

6. Genome Organization of RGAs

Many studies revealed that RGAs are irregularly distributed in plant genomes and that many reside in clusters. The clustered distribution of RGAs provides a reservoir of genetic variation to drive the evolution of new *R*-gene specificities [257,259,260].

Most RGAs are found in clusters. For example, 38.2% of the mapped NBS genes were located into eight clusters in the lotus genome [128]. Similarly, 50% and 51% of NBS genes were clustered in the rice [261] and *Brachypodium* genome [141], respectively. Higher rates have been found in other species such as potato where 73% of the mapped NBS-LRR genes grouped into 63 clusters [262], while approximately 80% were clustered in *Medicago* [16], 74.3% in the rice cultivar "Nipponbare" and 71.1% and 63.8% in *Arabidopsis* and lyrata, respectively [114]. Many super-clusters were identified for NBS-LRR genes, including a super-cluster with 11 NBS genes on chromosome 4 of *Brachypodium* [141] and one with 82 NBS-encoded genes in *Medicago* [16]. In addition, some *R*-genes appear in complex cluster structures [257] forming a diverse multigene family such as *Cf4/9* in tomato [49], *L* in flax [14], *Mla* in barley [263], *Pi2/9* in rice [260], *Dm3/13* in lettuce [60] and *I2* in tomato [42]. The structures of three haplotypes (linked genes) of the *Pto* cluster in tomato have been defined [264] while a single haplotype of the *Xa21* cluster in rice was partially characterized [265]. Several restorer genes, like the *Rf* gene from various plant species, showed homology to a cluster of *PPR* genes [266]. Genes within a single cluster may determine resistance to different pathogens [257].

RGAs are often irregularly distributed on chromosomes. Gebhardt *et al.* [267] and Lozano *et al.* [124] published genetic maps of potato with resistance traits. Their analyses indicated that large numbers of NBS-LRR genes were found on chromosomes 4 and 11 (approximately 15% of the mapped genes), while only 1% was on chromosome 3. In *Medicago*, chromosomes 6 and 3 encoded approximately 34% and 40% of all TNLs, respectively [16]. In apple, 56% of the 868 identified RGAs were distributed on six chromosomes (Chr 2, 7, 8, 10, 11 and 15) including 25% on Chr 2 while 99% of the non-TNL class was on Chr 11 [268]. Pseudogenes are also distributed and clustered at specific chromosomal locations in the same way as functional NBS-LRR genes [16,124]. Pseudogenes evolve much faster than functional genes and represent a reservoir for evolution of new specificities [269].

In conclusion, genetic and molecular data have shown that disease resistance genes are frequently clustered in plant genomes. Several cloned *R*-genes have common domains which will help to identify disease resistance loci from clusters of paralogs [257]. *R* loci may harbor single genes with multiple alleles, for instance, the *L* locus in flax with 13 alleles or *RPM1* in *Arabidopsis* with two [257]. However, some resistance loci and clusters of resistance genes are inherently unstable, e.g., *Rp1* in maize was described as a fast evolving complex [270].

7. Applications of RGAs

7.1. RGAs Are R-Gene Candidates for Disease Resistance

Map-based cloning remains the major strategy for isolating resistance genes [23,28,29,44,54,65], a strategy that requires high-density genetic maps. Genome-wide RGA identification will facilitate the development of molecular markers towards *R*-gene mapping and cloning. To date, all cloned disease resistance genes in plants belong to several major classes of RGAs (Table 1). Genome-wide RGAs can

be interrogated as *R*-gene candidates. In sorted and assembled genome sequences, the physical location of the identified RGAs can be easily retrieved. Single nucleotide polymorphism (SNP) markers can be designed from RGAs around a target disease gene to construct an RGA genetic map for the specific target region. These mapped genome-wide RGAs and SNP markers in their vicinity are valuable tools to develop high density *R*-gene genetic maps, target *R*-genes, co-localize QTL, design diagnostic markers of *R*-genes for fine mapping and cloning of *R*-genes and for resistance breeding.

7.2. Genome-Wide Association Study (GWAS) with Mapped RGAs Helps Co-Localization of QTL to Resistance Genes

Linkage mapping has been a key tool to identify resistance genes in the past 30 years. However, linkage mapping is sometimes limited by population size or low frequency of recombinant events near the target genes of interest resulting in increased expense and gene cloning difficulties. Under this scenario, association mapping, an application of linkage disequilibrium (LD), was developed to address these issues [271]. Today, modern genotyping techniques such as genotyping by sequencing (GBS) and chip-based genotyping facilitate GWAS for qualitative gene and QTL mapping. The primary advantage of GWAS over linkage mapping is the unprecedented high resolution across the whole genome. Consequently, population size for fine mapping may not need to be as large as for linkage mapping. With the aid of the identified and mapped genome-wide RGAs, more genes or QTL associated with disease resistance are able to be fine-mapped and co-localized.

Along with the development of comprehensive plant haplotype mapping projects in different crops such as *Arabidopsis* (http://1001genomes.org/) [272], flax (http://tufgen.ca), rice (http://ncgr.ac.cn/ricehap3/) [273], wheat (http://wheatgenome.org) [274] and maize (http://panzea.org) [275,276], a large number of crop varieties, ecotypes or breeding lines have been resequenced. The sequencing information of most projects has been released to the research community. These projects provide biologists with an invaluable blueprint to exploit SNP and indel markers, comparable to the prestigious human 1000 Hapmap project [277] (http://1000genomes.org). These plant haplotype maps permit the establishment of relationships between RGAs and plant disease resistance, providing precise sequence information to design diagnostic markers for breeding and to identify *R*-genes.

7.3. RGA Mapping in Plants with Limited Genome Information

In addition to the traditional marker types to construct genetic linkage maps such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), cleaved amplified polymorphic sequence (CAPS), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and SNP markers, specific methods have emerged from the identification of RGAs. NBS profiling is a useful and practical linkage map construction method based on genetic markers that has been implemented in potato [278]. NBS profiling was first used to map RGAs in cauliflower where a genetic linkage map was constructed based on the combined use of AFLP and NBS profiling [279]. Linden *et al.*, have described an advanced NBS profiling approach based on conserved NBS amplification in several crop plants such as potato, tomato, barley and lettuce [278].

Degenerated primers have been designed to clone NBS genes according to their conserved domain structure using PCR. With a properly constructed mapping population such as a doubled haploid (DH)

or a recombinant inbred line (RIL) population, an NBS linkage map can initially sort out these markers by linkage groups or chromosomes. This method is used when the whole genome has yet to be sequenced and assembled because it directly associates the markers with the target gene class. Furthermore, with proper modification of the degenerated primers design, NBS profiling can also be extended to other RGAs as long as the gene family of interest contains substantial members across all chromosomes. Therefore, although many species of interest have already been sequenced and large numbers of SNPs have been identified in these species, NBS profiling remains a powerful tool for the development of markers linked to resistance loci in species with limited genome information. A similar profiling method for other *R*-gene classes, like peroxidase profiling, developed in barley, revealed the resistance of *R*-genes for rusts and mildew [280]. RLK and LRR profiling strategies in potato were also developed [281]. Meanwhile, other non-RGA gene families can also benefit from this idea, like MYB profiling in pot azalea [282].

8. Conclusions

Plant RGAs are a large group of potential *R*-genes that have conserved domains and structural features which have specific roles in host-pathogen interactions. Bioinformatics software tools and comprehensive pipelines will help in their identification and characterization. Numerous RGAs have been identified from several sequenced plant genomes. These identified genome-wide RGAs with applications in genomics and bioinformatics such as linkage mapping, GWAS, clustering and protein signature profiling will assist traditional methods to enhance marker development, QTL mapping, cloning of plant resistance genes and resistance breeding.

Acknowledgments

Author Contributions

Frank M. You, Sylvie Cloutier and Xiue Wang developed the ideas and did final editing of the paper; Irene Lam drew Figure 2; Manoj Kumar Sekhwal prepared Tables 1–3 and Figure 4; Pingchuan Li prepared Figures 1 and 3 and Table 4. All authors researched literatures, drafted and revised the text and approved the final content.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Grant, M.R.; McDowell, J.M.; Sharpe, A.G.; de Torres Zabala, M.; Lydiate, D.J.; Dangl, J.L. Independent deletions of a pathogen-resistance gene in *Brassica* and *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15843–15848.
2. Buerstmayr, H.; Ban, T.; Anderson, J.A. QTL mapping and marker-assisted selection for fusarium head blight resistance in wheat: A review. *Plant Breed.* **2009**, *128*, 1–26.
3. Doane, J.F.; Olfert, O.; Mukerji, M.K. Extraction precision of sieving and brine floatation for removal of wheat midge, *Sitodiplosis mosellana* (Diptera: *Cecidomyiidae*), cocoons and larvae from soil. *J. Econ. Entomol.* **1986**, *80*, 268–271.
4. Hacquard, S.; Kracher, B.; Maekawa, T.; Vernaldi, S.; Schulze-Lefert, P.; Ver Loren van Themaat, E. Mosaic genome structure of the barley powdery mildew pathogen and conservation of transcriptional programs in divergent hosts. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E2219–E2228.
5. Hooker, A.L. The genetics and expression of resistance in plants to rusts of the genus *Puccinia*. *Annu. Rev. Phytopathol.* **1967**, *5*, 163–182.
6. Nowicki, M.; Foolad, M.R.; Nowakowska, M.; Kozik, E.U. Potato and tomato late blight caused by *Phytophthora infestans*: An overview of pathology and resistance breeding. *Plant Dis.* **2012**, *96*, 4–17.
7. Preston, G.M. *Pseudomonas syringae* pv. tomato: The right pathogen, of the right plant, at the right time. *Mol. Plant Pathol.* **2000**, *1*, 263–275.
8. Burdon, J.J.; Barrett, L.G.; Rebetzke, G.; Thrall, P.H. Guiding deployment of resistance in cereals using evolutionary principles. *Evol. Appl.* **2014**, *7*, 609–624.
9. Hulbert, S.H. Structure and evolution of the *rp1* complex conferring rust resistance in maize. *Annu. Rev. Phytopathol.* **1997**, *35*, 293–310.
10. Van der Biezen, E.A.; Jones, J.D. Plant disease-resistance proteins and the gene-for-gene concept. *Trends Biochem. Sci.* **1998**, *23*, 454–456.
11. Kobe, B.; Deisenhofer, J. The leucine-rich repeat: A versatile binding motif. *Trends Biochem. Sci.* **1994**, *19*, 415–421.
12. McIntosh, R.A.; Wellings, C.R.; Park, R.F. *Wheat Rusts: An Atlas of Resistance Genes*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995; pp. 1–204.
13. Jia, Y.; McAdams, S.A.; Bryan, G.T.; Hershey, H.P.; Valent, B. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO J.* **2000**, *19*, 4004–4014.
14. Dodds, P.N.; Lawrence, G.J.; Catanzariti, A.M.; Teh, T.; Wang, C.I.; Ayliffe, M.A.; Kobe, B.; Ellis, J.G. Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8888–8893.
15. McDowell, J.M.; Woffenden, B.J. Plant disease resistance genes: Recent insights and potential applications. *Trends Biotechnol.* **2003**, *21*, 178–183.
16. Ameline-Torregrosa, C.; Wang, B.B.; O'Bleness, M.S.; Deshpande, S.; Zhu, H.; Roe, B.; Young, N.D.; Cannon, S.B. Identification and characterization of nucleotide-binding site-leucine-rich repeat genes in the model plant *Medicago truncatula*. *Plant Physiol.* **2008**, *146*, 5–21.

17. Arya, P.; Kumar, G.; Acharya, V.; Singh, A.K. Genome-wide identification and expression analysis of NBS-encoding genes in *Malus* x *domestica* and expansion of NBS genes family in Rosaceae. *PLoS ONE* **2014**, *9*, e107987.

18. Dilbirligi, M.; Gill, K.S. Identification and analysis of expressed resistance gene sequences in wheat. *Plant Mol. Biol.* **2003**, *53*, 771–787.

19. Nordberg, H.; Cantor, M.; Dusheyko, S.; Hua, S.; Poliakov, A.; Shabalov, I.; Smirnova, T.; Grigoriev, I.V.; Dubchak, I. The genome portal of the department of energy joint genome institute: 2014 updates. *Nucleic Acids Res.* **2014**, *42*, D26–D31.

20. Goodstein, D.M.; Shu, S.; Howson, R.; Neupane, R.; Hayes, R.D.; Fazo, J.; Mitros, T.; Dirks, W.; Hellsten, U.; Putnam, N.; *et al*. Phytozome: A comparative platform for green plant genomics. *Nucleic Acids Res.* **2012**, *40*, D1178–D1186.

21. Monaco, M.K.; Stein, J.; Naithani, S.; Wei, S.; Dharmawardhana, P.; Kumari, S.; Amarasinghe, V.; Youens-Clark, K.; Thomason, J.; Preece, J.; *et al*. Gramene 2013: Comparative plant genomics resources. *Nucleic Acids Res.* **2014**, *42*, D1193–D1199.

22. Liu, J.; Liu, X.; Dai, L.; Wang, G. Recent progress in elucidating the structure, function and evolution of disease resistance genes in plants. *J. Genet. Genom.* **2007**, *34*, 765–776.

23. Feuillet, C.; Travella, S.; Stein, N.; Albar, L.; Nublat, A.; Keller, B. Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15253–15258.

24. Cloutier, S.; McCallum, B.D.; Loutre, C.; Banks, T.W.; Wicker, T.; Feuillet, C.; Keller, B.; Jordan, M.C. Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large *psr567* gene family. *Plant Mol. Biol.* **2007**, *65*, 93–106.

25. Yahiaoui, N.; Srichumpa, P.; Dudler, R.; Keller, B. Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. *Plant J.* **2004**, *37*, 528–538.

26. Periyannan, S.; Moore, J.; Ayliffe, M.; Bansal, U.; Wang, X.; Huang, L.; Deal, K.; Luo, M.; Kong, X.; Bariana, H.; *et al*. The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. *Science* **2013**, *341*, 786–788.

27. Saintenac, C.; Zhang, W.; Salcedo, A.; Rouse, M.N.; Trick, H.N.; Akhunov, E.; Dubcovsky, J. Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. *Science* **2013**, *341*, 783–786.

28. Huang, L.; Brooks, S.A.; Li, W.; Fellers, J.P.; Trick, H.N.; Gill, B.S. Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. *Genetics* **2003**, *164*, 655–664.

29. Lagudah, E.S.; Moullet, O.; Appels, R. Map-based cloning of a gene sequence encoding a nucleotide-binding domain and a leucine-rich region at the *Cre3* nematode resistance locus of wheat. *Genome* **1997**, *40*, 659–665.

30. De Majnik, J.; Ogbonnaya, F.C.; Moullet, O.; Lagudah, E.S. The *cre1* and *cre3* nematode resistance genes are located at homeologous loci in the wheat genome. *Mol. Plant Microbe Interact.* **2003**, *16*, 1129–1134.

31. Liu, W.; Frick, M.; Huel, R.; Nykiforuk, C.L.; Wang, X.; Gaudet, D.A.; Eudes, F.; Conner, R.L.; Kuzyk, A.; Chen, Q.; *et al*. The stripe rust resistance gene *Yr10* encodes an evolutionary-conserved and unique CC-NBS-LRR sequence in wheat. *Mol. Plant* **2014**, *7*, 1740–1755.

32. Cao, A.; Xing, L.; Wang, X.; Yang, X.; Wang, W.; Sun, Y.; Qian, C.; Ni, J.; Chen, Y.; Liu, D.; *et al*. Serine/threonine kinase gene *Stpk-V*, a key member of powdery mildew resistance gene *Pm21*, confers powdery mildew resistance in wheat. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 7727–7732.

33. Krattinger, S.G.; Lagudah, E.S.; Spielmeyer, W.; Singh, R.P.; Huerta-Espino, J.; McFadden, H.; Bossolini, E.; Selter, L.L.; Keller, B. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* **2009**, *323*, 1360–1363.

34. Fu, D.; Uauy, C.; Distelfeld, A.; Blechl, A.; Epstein, L.; Chen, X.; Sela, H.; Fahima, T.; Dubcovsky, J. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science* **2009**, *323*, 1357–1360.

35. Halterman, D.; Zhou, F.; Wei, F.; Wise, R.P.; Schulze-Lefert, P. The MLA6 coiled-coil, NBS-LRR protein confers *AvrMla6*-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat. *Plant J.* **2001**, *25*, 335–348.

36. Zhou, F.; Kurth, J.; Wei, F.; Elliott, C.; Vale, G.; Yahiaoui, N.; Keller, B.; Somerville, S.; Wise, R.; Schulze-Lefert, P. Cell-autonomous expression of barley *Mla1* confers race-specific resistance to the powdery mildew fungus via a *Rar1*-independent signaling pathway. *Plant Cell* **2001**, *13*, 337–350.

37. Halterman, D.A.; Wei, F.; Wise, R.P. Powdery mildew-induced *Mla* mRNAs are alternatively spliced and contain multiple upstream open reading frames. *Plant Physiol.* **2003**, *131*, 558–567.

38. Brueggeman, R.; Rostoks, N.; Kudrna, D.; Kilian, A.; Han, F.; Chen, J.; Druka, A.; Steffenson, B.; Kleinhofs, A. The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9328–9333.

39. Buschges, R.; Hollricher, K.; Panstruga, R.; Simons, G.; Wolter, M.; Frijters, A.; van Daelen, R.; van der Lee, T.; Diergaarde, P.; Groenendijk, J.; *et al*. The barley *Mlo* gene: A novel control element of plant pathogen resistance. *Cell* **1997**, *88*, 695–705.

40. Salmeron, J.M.; Oldroyd, G.E.; Rommens, C.M.; Scofield, S.R.; Kim, H.S.; Lavelle, D.T.; Dahlbeck, D.; Staskawicz, B.J. Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. *Cell* **1996**, *86*, 123–133.

41. Milligan, S.B.; Bodeau, J.; Yaghoobi, J.; Kaloshian, I.; Zabel, P.; Williamson, V.M. The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* **1998**, *10*, 1307–1319.

42. Ori, N.; Eshed, Y.; Paran, I.; Presting, G.; Aviv, D.; Tanksley, S.; Zamir, D.; Fluhr, R. The I2C family from the wilt disease resistance locus *I2* belongs to the nucleotide binding, leucine-rich repeat superfamily of plant resistance genes. *Plant Cell* **1997**, *9*, 521–532.

43. Zhang, C.; Liu, L.; Wang, X.; Vossen, J.; Li, G.; Li, T.; Zheng, Z.; Gao, J.; Guo, Y.; Visser, R.G.; *et al*. The *Ph-3* gene from *Solanum pimpinellifolium* encodes CC-NBS-LRR protein conferring resistance to *Phytophthora infestans*. *Theor. Appl. Genet.* **2014**, *127*, 1353–1364.

44. Brommonschenkel, S.H.; Tanksley, S.D. Map-based cloning of the tomato genomic region that spans the *Sw-5* tospovirus resistance gene in tomato. *Mol. Gen. Genet.* **1997**, *256*, 121–126.

45. Lanfermeijer, F.C.; Dijkhuis, J.; Sturre, M.J.; de Haan, P.; Hille, J. Cloning and characterization of the durable tomato mosaic virus resistance gene *Tm-2(2)* from *Lycopersicon esculentum*. *Plant Mol. Biol.* **2003**, *52*, 1037–1049.

46. Schornack, S.; Ballvora, A.; Gurlebeck, D.; Peart, J.; Baulcombe, D.; Ganal, M.; Baker, B.; Bonas, U.; Lahaye, T. The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of *AvrBs4* and overexpressed *AvrBs3*. *Plant J.* **2004**, *37*, 46–60.

47. Ernst, K.; Kumar, A.; Kriseleit, D.; Kloos, D.U.; Phillips, M.S.; Ganal, M.W. The broad-spectrum potato cyst nematode resistance gene *Hero* from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. *Plant J.* **2002**, *31*, 127–136.

48. Dixon, M.S.; Jones, D.A.; Keddie, J.S.; Thomas, C.M.; Harrison, K.; Jones, J.D. The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. *Cell* **1996**, *84*, 451–459.

49. Parniske, M.; Hammond-Kosack, K.E.; Golstein, C.; Thomas, C.M.; Jones, D.A.; Harrison, K.; Wulff, B.B.; Jones, J.D. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4/9* locus of tomato. *Cell* **1997**, *91*, 821–832.

50. Dixon, M.S.; Hatzixanthis, K.; Jones, D.A.; Harrison, K.; Jones, J.D. The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. *Plant Cell* **1998**, *10*, 1915–1925.

51. Jones, D.A.; Thomas, C.M.; Hammond-Kosack, K.E.; Balint-Kurti, P.J.; Jones, J.D. Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science* **1994**, *266*, 789–793.

52. Kawchuk, L.M.; Hachey, J.; Lynch, D.R.; Kulcsar, F.; van Rooijen, G.; Waterer, D.R.; Robertson, A.; Kokko, E.; Byers, R.; Howard, R.J.; *et al*. Tomato *Ve* disease resistance genes encode cell surface-like receptors. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 6511–6515.

53. Martin, G.B.; Frary, A.; Wu, T.; Brommonschenkel, S.; Chunwongse, J.; Earle, E.D.; Tanksley, S.D. A member of the tomato *Pto* gene family confers sensitivity to fenthion resulting in rapid cell death. *Plant Cell* **1994**, *6*, 1543–1552.

54. Martin, G.B.; Brommonschenkel, S.H.; Chunwongse, J.; Frary, A.; Ganal, M.W.; Spivey, R.; Wu, T.; Earle, E.D.; Tanksley, S.D. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science* **1993**, *262*, 1432–1436.

55. Zhou, J.; Loh, Y.T.; Bressan, R.A.; Martin, G.B. The tomato gene *Pti1* encodes a serine/threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response. *Cell* **1995**, *83*, 925–935.

56. Bendahmane, A.; Kanyuka, K.; Baulcombe, D.C. The *Rx* gene from potato controls separate virus resistance and cell death responses. *Plant Cell* **1999**, *11*, 781–792.

57. Song, J.; Bradeen, J.M.; Naess, S.K.; Raasch, J.A.; Wielgus, S.M.; Haberlach, G.T.; Kuang, H.; Liu, J.; Austin-Phillips, S.; Buell, C.R.; *et al*. Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9128–9133.

58. Bendahmane, A.; Querci, M.; Kanyuka, K.; Baulcombe, D.C. Agrobacterium transient expression system as a tool for the isolation of disease resistance genes: Application to the *Rx2* locus in potato. *Plant J.* **2000**, *21*, 73–81.

59. Ballvora, A.; Ercolano, M.R.; Weiss, J.; Meksem, K.; Bormann, C.A.; Oberhagemann, P.; Salamini, F.; Gebhardt, C. The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *Plant J.* **2002**, *30*, 361–371.

60. Meyers, B.C.; Shen, K.A.; Rohani, P.; Gaut, B.S.; Michelmore, R.W. Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. *Plant Cell* **1998**, *10*, 1833–1846.

61. Tai, T.H.; Dahlbeck, D.; Clark, E.T.; Gajiwala, P.; Pasion, R.; Whalen, M.C.; Staskawicz, B.J.; Stall, R.E. Expression of the *Bs2* pepper gene confers resistance to bacterial spot disease in tomato. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14153–14158.

62. Yoshimura, S.; Yamanouchi, U.; Katayose, Y.; Toki, S.; Wang, Z.X.; Kono, I.; Kurata, N.; Yano, M.; Iwata, N.; Sasaki, T. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1663–1668.

63. Wang, Z.X.; Yano, M.; Yamanouchi, U.; Iwamoto, M.; Monna, L.; Hayasaka, H.; Katayose, Y.; Sasaki, T. The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *Plant J.* **1999**, *19*, 55–64.

64. Bryan, G.T.; Wu, K.S.; Farrall, L.; Jia, Y.; Hershey, H.P.; McAdams, S.A.; Donaldson, G.K.; Faulk, K.N.; Tarchini, R.; Valent, B. tA single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *Plant Cell* **2000**, *12*, 2033–2046.

65. Liu, X.; Lin, F.; Wang, L.; Pan, Q. The in silico map-based cloning of *Pi36*, a rice coiled-coil nucleotide-binding site leucine-rich repeat gene that confers race-specific resistance to the blast fungus. *Genetics* **2007**, *176*, 2541–2549.

66. Okuyama, Y.; Kanzaki, H.; Abe, A.; Yoshida, K.; Tamiru, M.; Saitoh, H.; Fujibe, T.; Matsumura, H.; Shenton, M.; Galam, D.C.; *et al*. A multifaceted genomics approach allows the isolation of the rice *Pia-blast* resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant J.* **2011**, *66*, 467–479.

67. Sharma, T.R.; Madhav, M.S.; Singh, B.K.; Shanker, P.; Jana, T.K.; Dalal, V.; Pandit, A.; Singh, A.; Gaikwad, K.; Upreti, H.C.; *et al*. High-resolution mapping, cloning and molecular characterization of the *Pi-k^h* gene of rice, which confers resistance to *Magnaporthe grisea*. *Mol. Genet. Genom.* **2005**, *274*, 569–578.

68. Lin, F.; Chen, S.; Que, Z.; Wang, L.; Liu, X.; Pan, Q. The blast resistance gene *Pi37* encodes a nucleotide binding site leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. *Genetics* **2007**, *177*, 1871–1880.

69. Iyer, A.S.; McCouch, S.R. The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. *Mol. Plant Microbe Interact.* **2004**, *17*, 1348–1354.

70. Chu, Z.; Yuan, M.; Yao, J.; Ge, X.; Yuan, B.; Xu, C.; Li, X.; Fu, B.; Li, Z.; Bennetzen, J.L.; *et al*. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev.* **2006**, *20*, 1250–1255.

71. Das, A.; Soubam, D.; Singh, P.K.; Thakur, S.; Singh, N.K.; Sharma, T.R. A novel blast resistance gene, *Pi54rh* cloned from wild species of rice, *Oryza rhizomatis* confers broad spectrum resistance to *Magnaporthe oryzae*. *Funct. Integr. Genom.* **2012**, *12*, 215–228.

72. Liu, G.; Lu, G.; Zeng, L.; Wang, G.L. Two broad-spectrum blast resistance genes, *Pi9(t)* and *Pi2(t)*, are physically linked on rice chromosome 6. *Mol. Genet. Genom.* **2002**, *267*, 472–480.

73. Zhou, B.; Qu, S.; Liu, G.; Dolan, M.; Sakai, H.; Lu, G.; Bellizzi, M.; Wang, G.L. The eight amino-acid differences within three leucine-rich repeats between Pi2 and Piz-t resistance proteins determine the resistance specificity to *Magnaporthe grisea*. *Mol. Plant Microbe Interact.* **2006**, *19*, 1216–1228.

74. Sakamoto, K.; Tada, Y.; Yokozeki, Y.; Akagi, H.; Hayashi, N.; Fujimura, T.; Ichikawa, N. Chemical induction of disease resistance in rice is correlated with the expression of a gene encoding a nucleotide binding site and leucine-rich repeats. *Plant Mol. Biol.* **1999,** *40*, 847–855.

75. Shang, J.; Tao, Y.; Chen, X.; Zou, Y.; Lei, C.; Wang, J.; Li, X.; Zhao, X.; Zhang, M.; Lu, Z.; *et al.* Identification of a new rice blast resistance gene, *Pid3*, by genomewide comparison of paired nucleotide-binding site-leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* **2009**, *182*, 1303–1311.

76. Song, W.Y.; Wang, G.L.; Chen, L.L.; Kim, H.S.; Pi, L.Y.; Holsten, T.; Gardner, J.; Wang, B.; Zhai, W.X.; Zhu, L.H.; *et al*. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21. Science* **1995**, *270*, 1804–1806.

77. Sun, X.; Cao, Y.; Wang, S. Point mutations with positive selection were a major force during the evolution of a receptor-kinase resistance gene family of rice. *Plant Physiol.* **2006**, *140*, 998–1008.

78. Kaku, H.; Nishizawa, Y.; Ishii-Minami, N.; Akimoto-Tomiyama, C.; Dohmae, N.; Takio, K.; Minami, E.; Shibuya, N. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11086–11091.

79. Tian, D.; Wang, J.; Zeng, X.; Gu, K.; Qiu, C.; Yang, X.; Zhou, Z.; Goh, M.; Murata-Hori, M.; Luo, Y.; *et al*. The rice TAL effector-dependent resistance protein XA10 triggers cell death and calcium depletion in the endoplasmic reticulum. *Plant Cell* **2014**, *26*, 497–515.

80. Liu, Q.; Yuan, M.; Zhou, Y.; Li, X.; Xiao, J.; Wang, S. A paralog of the MtN3/saliva family recessively confers race-specific resistance to *Xanthomonas oryzae* in rice. *Plant Cell Environ.* **2011**, *34*, 1958–1969.

81. Bimolata, W.; Kumar, A.; Sundaram, R.M.; Laha, G.S.; Qureshi, I.A.; Reddy, G.A.; Ghazi, I.A. Analysis of nucleotide diversity among alleles of the major bacterial blight resistance gene *Xa27* in cultivars of rice (*Oryza sativa*) and its wild relatives. *Planta* **2013**, *238*, 293–305.

82. Chen, X.; Shang, J.; Chen, D.; Lei, C.; Zou, Y.; Zhai, W.; Liu, G.; Xu, J.; Ling, Z.; Cao, G.; *et al*. A B-lectin receptor kinase gene conferring rice blast resistance. *Plant J.* **2006**, *46*, 794–804.

83. Collins, N.; Drake, J.; Ayliffe, M.; Sun, Q.; Ellis, J.; Hulbert, S.; Pryor, T. Molecular characterization of the maize *Rp1-D* rust resistance haplotype and its mutants. *Plant Cell* **1999**, *11*, 1365–1376.

84. Johal, G.S.; Briggs, S.P. Reductase activity encoded by the *Hm1* disease resistance gene in maize. *Science* **1992**, *258*, 985–987.

85. Grant, M.R.; Godiard, L.; Straube, E.; Ashfield, T.; Lewald, J.; Sattler, A.; Innes, R.W.; Dangl, J.L. Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. *Science* **1995**, *269*, 843–846.

86. Bent, A.F.; Kunkel, B.N.; Dahlbeck, D.; Brown, K.L.; Schmidt, R.; Giraudat, J.; Staskawicz, B.J.; Leung, J. *RPS2* of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. *Science* **1994**, *265*, 1856–1860.

87. McDowell, J.M.; Dhandaydham, M.; Long, T.A.; Aarts, M.G.; Goff, S.; Holub, E.B.; Dangl, J.L. Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the *RPP8* locus of *Arabidopsis*. *Plant Cell* **1998**, *10*, 1861–1874.

88. Bittner-Eddy, P.D.; Crute, I.R.; Holub, E.B.; Beynon, J.L. *RPP13* is a simple locus in *Arabidopsis thaliana* for alleles that specify downy mildew resistance to different avirulence determinants in *Peronospora parasitica*. *Plant J.* **2000**, *21*, 177–188.

89. Takahashi, H.; Miller, J.; Nozaki, Y.; Takeda, M.; Shah, J.; Hase, S.; Ikegami, M.; Ehara, Y.; Dinesh-Kumar, S.P.; Sukamto. *RCY1*, an *Arabidopsis thaliana* RPP8/HRT family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. *Plant J.* **2002**, *32*, 655–667.

90. Botella, M.A.; Parker, J.E.; Frost, L.N.; Bittner-Eddy, P.D.; Beynon, J.L.; Daniels, M.J.; Holub, E.B.; Jones, J.D. Three genes of the *Arabidopsis RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. *Plant Cell* **1998**, *10*, 1847–1860.

91. Van der Biezen, E.A.; Freddie, C.T.; Kahn, K.; Parker, J.E.; Jones, J.D. *Arabidopsis RPP4* is a member of the *RPP5* multigene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signalling components. *Plant J.* **2002**, *29*, 439–451.

92. Gassmann, W.; Hinsch, M.E.; Staskawicz, B.J. The *Arabidopsis RPS4* bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. *Plant J.* **1999**, *20*, 265–277.

93. Noel, L.; Moores, T.L.; van Der Biezen, E.A.; Parniske, M.; Daniels, M.J.; Parker, J.E.; Jones, J.D. Pronounced intraspecific haplotype divergence at the *RPP5* complex disease resistance locus of *Arabidopsis*. *Plant Cell* **1999**, *11*, 2099–2112.

94. Warren, R.F.; Henk, A.; Mowery, P.; Holub, E.; Innes, R.W. A mutation within the leucine-rich repeat domain of the *Arabidopsis* disease resistance gene *RPS5* partially suppresses multiple bacterial and downy mildew resistance genes. *Plant Cell* **1998**, *10*, 1439–1452.

95. Deslandes, L.; Olivier, J.; Theulieres, F.; Hirsch, J.; Feng, D.X.; Bittner-Eddy, P.; Beynon, J.; Marco, Y. Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive *RRS1 R* gene, a member of a novel family of resistance genes. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2404–2409.

96. Tor, M.; Brown, D.; Cooper, A.; Woods-Tor, A.; Sjolander, K.; Jones, J.D.; Holub, E.B. *Arabidopsis* downy mildew resistance gene *RPP27* encodes a receptor-like protein similar to CLAVATA2 and tomato Cf-9. *Plant Physiol.* **2004**, *135*, 1100–1112.

97. Diener, A.C.; Ausubel, F.M. Resistance to fusarium oxysporum1, a dominant *Arabidopsis* disease-resistance gene, is not race specific. *Genetics* **2005**, *171*, 305–321.

98. Swiderski, M.R.; Innes, R.W. The *Arabidopsis PBS1* resistance gene encodes a member of a novel protein kinase subfamily. *Plant J.* **2001**, *26*, 101–112.

99. Tabata, S.; Kaneko, T.; Nakamura, Y.; Kotani, H.; Kato, T.; Asamizu, E.; Miyajima, N.; Sasamoto, S.; Kimura, T.; Hosouchi, T.; *et al.* Sequence and analysis of chromosome 5 of the plant *Arabidopsis thaliana*. *Nature* **2000**, *408*, 823–826.

100. Kim, M.H.; Kim, Y.; Kim, J.W.; Lee, H.S.; Lee, W.S.; Kim, S.K.; Wang, Z.Y.; Kim, S.H. Identification of *Arabidopsis* BAK1-associating receptor-like kinase 1 (BARK1) and characterization

of its gene expression and brassinosteroid-regulated root phenotypes. *Plant Cell Physiol.* **2013**, *54*, 1620–1634.

101. Century, K.S.; Shapiro, A.D.; Repetti, P.P.; Dahlbeck, D.; Holub, E.; Staskawicz, B.J. NDR1, a pathogen-induced component required for *Arabidopsis* disease resistance. *Science* **1997**, *278*, 1963–1965.

102. Xiao, S.; Ellwood, S.; Calis, O.; Patrick, E.; Li, T.; Coleman, M.; Turner, J.G. Broad-spectrum mildew resistance in *Arabidopsis thaliana* mediated by RPW8. *Science* **2001**, *291*, 118–120.

103. Lawrence, G.J.; Finnegan, E.J.; Ayliffe, M.A.; Ellis, J.G. The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. *Plant Cell* **1995**, *7*, 1195–1206.

104. Ellis, J.G.; Lawrence, G.J.; Luck, J.E.; Dodds, P.N. Identification of regions in alleles of the flax rust resistance gene *L* that determine differences in gene-for-gene specificity. *Plant Cell* **1999**, *11*, 495–506.

105. Anderson, P.A.; Lawrence, G.J.; Morrish, B.C.; Ayliffe, M.A.; Finnegan, E.J.; Ellis, J.G. Inactivation of the flax rust resistance gene *M* associated with loss of a repeated unit within the leucine-rich repeat coding region. *Plant Cell* **1997**, *9*, 641–651.

106. Dodds, P.N.; Lawrence, G.J.; Ellis, J.G. Six amino acid changes confined to the leucine-rich repeat beta-strand/beta-turn motif determine the difference between the *P* and *P2* rust resistance specificities in flax. *Plant Cell* **2001**, *13*, 163–178.

107. Cai, D.; Kleine, M.; Kifle, S.; Harloff, H.J.; Sandal, N.N.; Marcker, K.A.; Klein-Lankhorst, R.M.; Salentijn, E.M.; Lange, W.; Stiekema, W.J.; *et al*. Positional cloning of a gene for nematode resistance in sugar beet. *Science* **1997**, *275*, 832–834.

108. Whitham, S.; Dinesh-Kumar, S.P.; Choi, D.; Hehl, R.; Corr, C.; Baker, B. The product of the tobacco mosaic virus resistance gene *N*: Similarity to toll and the interleukin-1 receptor. *Cell* **1994**, *78*, 1101–1115.

109. Meyers, B.C. Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. *Plant Cell* **2003**, *15*, 809–834.

110. Fritz-Laylin, L.K.; Krishnamurthy, N.; Tor, M.; Sjolander, K.V.; Jones, J.D. Phylogenomic analysis of the receptor-like proteins of rice and *Arabidopsis*. *Plant Physiol.* **2005**, *138*, 611–623.

111. Yu, J.; Tehrim, S.; Zhang, F.; Tong, C.; Huang, J.; Cheng, X.; Dong, C.; Zhou, Y.; Qin, R.; Hua, W.; *et al*. Genome-wide comparative analysis of NBS-encoding genes between *Brassica* species and *Arabidopsis thaliana*. *BMC Genom.* **2014**, *15*, 3–21.

112. Shiu, S.H.; Karlowski, W.M.; Pan, R.; Tzeng, Y.H.; Mayer, K.F.; Li, W.H. Comparative analysis of the receptor-like kinase family in *Arabidopsis* and rice. *Plant Cell* **2004**, *16*, 1220–1234.

113. Lurin, C.; Andres, C.; Aubourg, S.; Bellaoui, M.; Bitton, F.; Bruyere, C.; Caboche, M.; Debast, C.; Gualberto, J.; Hoffmann, B.; *et al*. Genome-wide analysis of *Arabidopsis* pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. *Plant Cell* **2004**, *16*, 2089–2103.

114. Guo, Y.L.; Fitz, J.; Schneeberger, K.; Ossowski, S.; Cao, J.; Weigel, D. Genome-wide comparison of nucleotide-binding site-leucine-rich repeat-encoding genes in *Arabidopsis*. *Plant Physiol.* **2011**, *157*, 757–769.

115. Kohler, A.; Rinaldi, C.; Duplessis, S.; Baucher, M.; Geelen, D.; Duchaussoy, F.; Meyers, B.C.; Boerjan, W.; Martin, F. Genome-wide identification of NBS resistance genes in *Populus trichocarpa*. *Plant Mol. Biol.* **2008**, *66*, 619–636.

116. Zan, Y.; Ji, Y.; Zhang, Y.; Yang, S.; Song, Y.; Wang, J. Genome-wide identification, characterization and expression analysis of populus leucine-rich repeat receptor-like protein kinase genes. *BMC Genom.* **2013**, *14*, 318–331.

117. Tuskan, G.A.; Difazio, S.; Jansson, S.; Bohlmann, J.; Grigoriev, I.; Hellsten, U.; Putnam, N.; Ralph, S.; Rombauts, S.; Salamov, A.; *et al*. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **2006**, *313*, 1596–1604.

118. Yang, S.; Zhang, X.; Yue, J.X.; Tian, D.; Chen, J.Q. Recent duplications dominate NBS-encoding gene expansion in two woody species. *Mol. Genet. Genom.* **2008**, *280*, 187–198.

119. Kale, S.M.; Pardeshi, V.C.; Barvkar, V.T.; Gupta, V.S.; Kadoo, N.Y. Genome-wide identification and characterization of nucleotide binding site leucine-rich repeat genes in linseed reveal distinct patterns of gene structure. *Genome* **2013**, *56*, 91–99.

120. Andolfo, G.; Sanseverino, W.; Aversano, R.; Frusciante, L.; Ercolano, M.R. Genome-wide identification and analysis of candidate genes for disease resistance in tomato. *Mol. Breed.* **2013**, *33*, 227–233.

121. Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **2012**, *485*, 635–641.

122. Ming, R.; Hou, S.; Feng, Y.; Yu, Q.; Dionne-Laporte, A.; Saw, J.H.; Senin, P.; Wang, W.; Ly, B.V.; Lewis, K.L.; *et al*. The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). *Nature* **2008**, *452*, 991–996.

123. Yang, L.; Li, D.; Li, Y.; Gu, X.; Huang, S.; Garcia-Mas, J.; Weng, Y. A 1,681-locus consensus genetic map of cultivated cucumber including 67 NB-LRR resistance gene homolog and ten gene loci. *BMC Plant Biol.* **2013**, *13*, 53.

124. Lozano, R.; Ponce, O.; Ramirez, M.; Mostajo, N.; Orjeda, G. Genome-wide identification and mapping of NBS-encoding resistance genes in *Solanum tuberosum* group phureja. *PLoS ONE* **2012**, *7*, e34775.

125. Wei, H.; Li, W.; Sun, X.; Zhu, S.; Zhu, J. Systematic analysis and comparison of nucleotide-binding site disease resistance genes in a diploid cotton *Gossypium raimondii*. *PLoS ONE* **2013**, *8*, e68435.

126. Chen, J.Y.; Huang, J.Q.; Li, N.Y.; Ma, X.F.; Wang, J.L.; Liu, C.; Liu, Y.F.; Liang, Y.; Bao, Y.M.; Dai, X.F. Genome-wide analysis of the gene families of resistance gene analogues in cotton and their response to Verticillium wilt. *BMC Plant Biol.* **2015**, *15*, 148.

127. Li, J.; Zhang, Q.Y.; Gao, Z.H.; Wang, F.; Duan, K.; Ye, Z.W.; Gao, Q.H. Genome-wide identification and comparative expression analysis of NBS-LRR-encoding genes upon *Colletotrichum gloeosporioides* infection in two ecotypes of *Fragaria vesca*. *Gene* **2013**, *527*, 215–227.

128. Li, X.; Cheng, Y.; Ma, W.; Zhao, Y.; Jiang, H.; Zhang, M. Identification and characterization of NBS-encoding disease resistance genes in *Lotus japonicus*. *Plant Syst. Evol.* **2010**, *289*, 101–110.

129. Xue, J.Y.; Wang, Y.; Wu, P.; Wang, Q.; Yang, L.T.; Pan, X.H.; Wang, B.; Chen, J.Q. A primary survey on bryophyte species reveals two novel classes of nucleotide-binding site (NBS) genes. *PLoS ONE* **2012**, *7*, e36700.

130. O'Toole, N.; Hattori, M.; Andres, C.; Iida, K.; Lurin, C.; Schmitz-Linneweber, C.; Sugita, M.; Small, I. On the expansion of the pentatricopeptide repeat gene family in plants. *Mol. Biol. Evol.* **2008**, *25*, 1120–1128.

131. Zhou, T.; Wang, Y.; Chen, J.Q.; Araki, H.; Jing, Z.; Jiang, K.; Shen, J.; Tian, D. Genome-wide identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR NBS-LRR genes. *Mol. Genet. Genom.* **2004**, *271*, 402–415.

132. Dardick, C.; Chen, J.; Richter, T.; Ouyang, S.; Ronald, P. The rice kinase database. A phylogenomic database for the rice kinome. *Plant Physiol.* **2007**, *143*, 579–586.

133. Singh, S.; Chand, S.; Singh, N.K.; Sharma, T.R. Genome-wide distribution, organisation and functional characterization of disease resistance and defence response genes across rice species. *PLoS ONE* **2015**, *10*, e0125964.

134. Bouktila, D.; Khalfallah, Y.; Habachi-Houimli, Y.; Mezghani-Khemakhem, M.; Makni, M.; Makni, H. Large-scale analysis of NBS domain-encoding resistance gene analogs in Triticeae. *Genet. Mol. Biol.* **2014**, *37*, 598–610.

135. Wang, S.S.; Wang, F.; Tan, S.J.; Wang, M.X.; Sui, N.; Zhang, X.S. Transcript profiles of maize embryo sacs and preliminary identification of genes involved in the embryo sac-pollen tube interaction. *Front. Plant Sci.* **2014**, *5*, 702–716.

136. Cheng, Y.; Li, X.; Jiang, H.; Ma, W.; Miao, W.; Yamada, T.; Zhang, M. Systematic analysis and comparison of nucleotide-binding site disease resistance genes in maize. *FEBS J.* **2012**, *279*, 2431–2443.

137. Mace, E.; Tai, S.; Innes, D.; Godwin, I.; Hu, W.; Campbell, B.; Gilding, E.; Cruickshank, A.; Prentis, P.; Wang, J.; *et al*. The plasticity of NBS resistance genes in sorghum is driven by multiple evolutionary processes. *BMC Plant Biol.* **2014**, *14*, 253–266.

138. Cheng, X.; Jiang, H.; Zhao, Y.; Qian, Y.; Zhu, S.; Cheng, B. A genomic analysis of disease-resistance genes encoding nucleotide binding sites in *Sorghum bicolor*. *Genet. Mol. Biol.* **2010**, *33*, 292–297.

139. International Barley Genome Sequencing Consortium. A physical, genetic and functional sequence assembly of the barley genome. *Nature* **2012**, *491*, 711–716.

140. Gu, L.; Si, W.; Zhao, L.; Yang, S.; Zhang, X. Dynamic evolution of NBS-LRR genes in bread wheat and its progenitors. *Mol. Genet. Genom.* **2015**, *290*, 727–738.

141. Tan, S.; Wu, S. Genome wide analysis of nucleotide-binding site disease resistance genes in *Brachypodium distachyon*. *Comp. Funct. Genom.* **2012**, *2012*, 418208–418220.

142. Ling, H.Q.; Zhao, S.; Liu, D.; Wang, J.; Sun, H.; Zhang, C.; Fan, H.; Li, D.; Dong, L.; Tao, Y.; *et al*. Draft genome of the wheat A-genome progenitor *Triticum urartu*. *Nature* **2013**, *496*, 87–90.

143. Jia, J.; Zhao, S.; Kong, X.; Zhao, G.; He, W.; Appels, R.; Pfeifer, M.; Tao, Y.; Zhang, X.; Li, Y.; *et al*. *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation. *Nature* **2013**, *496*, 91–95.

144. Michael, T.P.; Jackson, S. The first 50 plant genomes. *Plant Gen.* **2013**, *6*, doi:10.3835/plantgenome2013.3803.0001in.

145. Hammond-Kosack, K.E.; Jones, J.D. Plant disease resistance genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1997**, *48*, 575–607.

146. Chisholm, S.T.; Coaker, G.; Day, B.; Staskawicz, B.J. Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell* **2006**, *124*, 803–814.

147. Maekawa, T.; Cheng, W.; Spiridon, L.N.; Toller, A.; Lukasik, E.; Saijo, Y.; Liu, P.; Shen, Q.H.; Micluta, M.A.; Somssich, I.E.; *et al.* Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors defines a minimal functional module for triggering cell death. *Cell Host Microbe* **2011**, *9*, 187–199.

148. Alber, T. Structure of the leucine zipper. *Curr. Opin. Genet. Dev.* **1992**, *2*, 205–210.

149. Bent, A.F. Plant disease resistance genes: Function meets structure. *Plant Cell* **1996**, *8*, 1757–1771.

150. Martin, G.B.; Bogdanove, A.J.; Sessa, G. Understanding the functions of plant disease resistance proteins. *Annu. Rev. Plant Biol.* **2003**, *54*, 23–61.

151. Tameling, W.I.; Elzinga, S.D.; Darmin, P.S.; Vossen, J.H.; Takken, F.L.; Haring, M.A.; Cornelissen, B.J. The tomato *R* gene products I-2 and MI-1 are functional ATP binding proteins with ATPase activity. *Plant Cell* **2002**, *14*, 2929–2939.

152. Van Ooijen, G.; van den Burg, H.A.; Cornelissen, B.J.; Takken, F.L. Structure and function of resistance proteins in solanaceous plants. *Annu. Rev. Phytopathol.* **2007**, *45*, 43–72.

153. Rairdan, G.J.; Collier, S.M.; Sacco, M.A.; Baldwin, T.T.; Boettrich, T.; Moffett, P. The coiled-coil and nucleotide binding domains of the Potato Rx disease resistance protein function in pathogen recognition and signaling. *Plant Cell* **2008**, *20*, 739–751.

154. Collier, S.M.; Hamel, L.P.; Moffett, P. Cell death mediated by the N-terminal domains of a unique and highly conserved class of NB-LRR protein. *Mol. Plant Microbe Interact.* **2011**, *24*, 918–931.

155. Meyers, B.C.; Dickerman, A.W.; Michelmore, R.W.; Sivaramakrishnan, S.; Sobral, B.W.; Young, N.D. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. *Plant J.* **1999**, *20*, 317–332.

156. Meyers, B.C.; Morgante, M.; Michelmore, R.W. TIR-X and TIR-NBS proteins: Two new families related to disease resistance TIR-NBS-LRR proteins encoded in *Arabidopsis* and other plant genomes. *Plant J.* **2002**, *32*, 77–92.

157. Leipe, D.D.; Koonin, E.V.; Aravind, L. STAND, a class of P-loop NTPases including animal and plant regulators of programmed cell death: Multiple, complex domain architectures, unusual phyletic patterns, and evolution by horizontal gene transfer. *J. Mol. Biol.* **2004**, *343*, 1–28.

158. Pan, Q.; Wendel, J.; Fluhr, R. Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes. *J. Mol. Evol.* **2000**, *50*, 203–213.

159. Takken, F.L.; Albrecht, M.; Tameling, W.I. Resistance proteins: Molecular switches of plant defence. *Curr. Opin. Plant Biol.* **2006**, *9*, 383–390.

160. Lukasik, E.; Takken, F.L. STANDing strong, resistance proteins instigators of plant defence. *Curr. Opin. Plant Biol.* **2009**, *12*, 427–436.

161. Takken, F.L.; Goverse, A. How to build a pathogen detector: Structural basis of NB-LRR function. *Curr. Opin. Plant Biol.* **2012**, *15*, 375–384.

162. Deslandes, L.; Rivas, S. Catch me if you can: Bacterial effectors and plant targets. *Trends Plant Sci.* **2012**, *17*, 644–655.

163. Heidrich, K.; Wirthmueller, L.; Tasset, C.; Pouzet, C.; Deslandes, L.; Parker, J.E. *Arabidopsis* EDS1 connects pathogen effector recognition to cell compartment-specific immune responses. *Science* **2011**, *334*, 1401–1404.

164. Bhattacharjee, S.; Halane, M.K.; Kim, S.H.; Gassmann, W. Pathogen effectors target *Arabidopsis* EDS1 and alter its interactions with immune regulators. *Science* **2011**, *334*, 1405–1408.

165. Zhang, Z.; Wu, Y.; Gao, M.; Zhang, J.; Kong, Q.; Liu, Y.; Ba, H.; Zhou, J.; Zhang, Y. Disruption of PAMP-induced MAP kinase cascade by a *Pseudomonas syringae* effector activates plant immunity mediated by the NB-LRR protein SUMM2. *Cell Host Microbe* **2012**, *11*, 253–263.

166. Meng, X.; Zhang, S. MAPK cascades in plant disease resistance signaling. *Annu. Rev. Phytopathol.* **2013**, *51*, 245–266.

167. Buscaill, P.; Rivas, S. Transcriptional control of plant defence responses. *Curr. Opin. Plant Biol.* **2014**, *20*, 35–46.

168. Cui, H.; Tsuda, K.; Parker, J.E. Effector-triggered immunity: From pathogen perception to robust defense. *Annu. Rev. Plant Biol.* **2015**, *66*, 487–511.

169. Williams, S.J.; Sohn, K.H.; Wan, L.; Bernoux, M.; Sarris, P.F.; Segonzac, C.; Ve, T.; Ma, Y.; Saucet, S.B.; Ericsson, D.J.; *et al*. Structural basis for assembly and function of a heterodimeric plant immune receptor. *Science* **2014**, *344*, 299–303.

170. Chinchilla, D.; Zipfel, C.; Robatzek, S.; Kemmerling, B.; Nurnberger, T.; Jones, J.D.; Felix, G.; Boller, T. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* **2007**, *448*, 497–500.

171. Stergiopoulos, I.; de Wit, P.J. Fungal effector proteins. *Annu. Rev. Phytopathol.* **2009**, *47*, 233–263.

172. Panstruga, R.; Dodds, P.N. Terrific protein traffic: The mystery of effector protein delivery by filamentous plant pathogens. *Science* **2009**, *324*, 748–750.

173. Wang, W.; Zhang, Y.; Wen, Y.; Berkey, R.; Ma, X.; Pan, Z.; Bendigeri, D.; King, H.; Zhang, Q.; Xiao, S. A comprehensive mutational analysis of the *Arabidopsis* resistance protein RPW8.2 reveals key amino acids for defense activation and protein targeting. *Plant Cell* **2013**, *25*, 4242–4261.

174. Zhai, J.; Jeong, D.H.; de Paoli, E.; Park, S.; Rosen, B.D.; Li, Y.; Gonzalez, A.J.; Yan, Z.; Kitto, S.L.; Grusak, M.A.; *et al*. MicroRNAs as master regulators of the plant NB-LRR defense gene family via the production of phased, trans-acting siRNAs. *Genes Dev.* **2011**, *25*, 2540–2553.

175. Zhao, M.; Cai, C.; Zhai, J.; Lin, F.; Li, L.; Shreve, J.; Thimmapuram, J.; Hughes, T.J.; Meyers, B.C.; Ma, J. Coordination of microRNAs, phasiRNAs, and *NB-LRR* genes in response to a plant pathogen: Insights from analyses of a set of soybean Rps gene near isogenic lines. *Plant Gen.* **2015**, *8*, doi:10.3835/plantgenome2014.3809.0044.

176. Shivaprasad, P.V.; Chen, H.M.; Patel, K.; Bond, D.M.; Santos, B.A.; Baulcombe, D.C. A microRNA superfamily regulates nucleotide binding site-leucine-rich repeats and other mRNAs. *Plant Cell* **2012**, *24*, 859–874.

177. Liu, W.; Liu, J.; Triplett, L.; Leach, J.E.; Wang, G.L. Novel insights into rice innate immunity against bacterial and fungal pathogens. *Annu. Rev. Phytopathol.* **2014**, *52*, 213–241.

178. Afzal, A.J.; Wood, A.J.; Lightfoot, D.A. Plant receptor-like serine threonine kinases: Roles in signaling and plant defense. *Mol. Plant Microbe Interact.* **2008**, *21*, 507–517.

179. Bleecker, A.B.; Kende, H. Ethylene: A gaseous signal molecule in plants. *Annu. Rev. Cell Dev. Biol.* **2000**, *16*, 1–18.

180. Inoue, T.; Higuchi, M.; Hashimoto, Y.; Seki, M.; Kobayashi, M.; Kato, T.; Tabata, S.; Shinozaki, K.; Kakimoto, T. Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. *Nature* **2001**, *409*, 1060–1063.

181. Becraft, P.W. Receptor kinase signaling in plant development. *Annu. Rev. Cell Dev. Biol.* **2002**, *18*, 163–192.

182. Lee, S.W.; Han, S.W.; Sririyanum, M.; Park, C.J.; Seo, Y.S.; Ronald, P.C. A type I-secreted, sulfated peptide triggers *Xa21*-mediated innate immunity. *Science* **2009**, *326*, 850–853.

183. Hu, H.; Wang, J.; Shi, C.; Yuan, C.; Peng, C.; Yin, J.; Li, W.; He, M.; Wang, J.; Ma, B. A receptor like kinase gene with expressional responsiveness on *Xanthomonas oryzae* pv. *oryzae* is essential for *Xa21*-mediated disease resistance. *Rice* **2015**, *8*, 1–9.

184. Peng, Y.; Bartley, L.E.; Chen, X.; Dardick, C.; Chern, M.; Ruan, R.; Canlas, P.E.; Ronald, P.C. OsWRKY62 is a negative regulator of basal and *Xa21*-mediated defense against *Xanthomonas oryzae* pv. *oryzae* in rice. *Mol. Plant* **2008**, *1*, 446–458.

185. Nekrasov, V.; Ludwig, A.A.; Jones, J.D. CITRX thioredoxin is a putative adaptor protein connecting Cf-9 and the ACIK1 protein kinase during the *Cf-9/Avr9* induced defence response. *FEBS Lett.* **2006**, *580*, 4236–4241.

186. Rivas, S.; Rougon-Cardoso, A.; Smoker, M.; Schauser, L.; Yoshioka, H.; Jones, J.D. CITRX thioredoxin interacts with the tomato Cf-9 resistance protein and negatively regulates defence. *EMBO J.* **2004**, *23*, 2156–2165.

187. Gabriels, S.H.; Ekengren, S.K.; van Ooijen, G.; Abd-El-Haliem, A.M.; van den Berg, G.C.; Vossen, J.H.; Rainey, D.Y.; Martin, G.B.; Takken, F.L.; de Wit, P.J.; *et al.* An NB-LRR protein required for HR signalling mediated by both extra and intracellular resistance proteins. *Plant J.* **2007**, *50*, 14–28.

188. Radutoiu, S.; Madsen, L.H.; Madsen, E.B.; Felle, H.H.; Umehara, Y.; Gronlund, M.; Sato, S.; Nakamura, Y.; Tabata, S.; Sandal, N.; *et al.* Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* **2003**, *425*, 585–592.

189. Madsen, E.B.; Madsen, L.H.; Radutoiu, S.; Olbryt, M.; Rakwalska, M.; Szczyglowski, K.; Sato, S.; Kaneko, T.; Tabata, S.; Sandal, N.; *et al.* A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. *Nature* **2003**, *425*, 637–640.

190. Jeong, S.; Trotochaud, A.E.; Clark, S.E. The *Arabidopsis CLAVATA2* gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. *Plant Cell* **1999**, *11*, 1925–1934.

191. Nadeau, J.A.; Sack, F.D. Control of stomatal distribution on the *Arabidopsis* leaf surface. *Science* **2002**, *296*, 1697–1700.

192. Ogawa, M.; Shinohara, H.; Sakagami, Y.; Matsubayashi, Y. *Arabidopsis* CLV3 peptide directly binds CLV1 ectodomain. *Science* **2008**, *319*, 294.

193. Rojo, E.; Sharma, V.K.; Kovaleva, V.; Raikhel, N.V.; Fletcher, J.C. CLV3 is localized to the extracellular space, where it activates the *Arabidopsis* CLAVATA stem cell signaling pathway. *Plant Cell* **2002**, *14*, 969–977.

194. Trotochaud, A.E.; Hao, T.; Wu, G.; Yang, Z.; Clark, S.E. The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein. *Plant Cell* **1999**, *11*, 393–406.

195. Lee, J.S.; Kuroha, T.; Hnilova, M.; Khatayevich, D.; Kanaoka, M.M.; McAbee, J.M.; Sarikaya, M.; Tamerler, C.; Torii, K.U. Direct interaction of ligand-receptor pairs specifying stomatal patterning. *Genes Dev.* **2012**, *26*, 126–136.

196. Bai, J.; Pennill, L.A.; Ning, J.; Lee, S.W.; Ramalingam, J.; Webb, C.A.; Zhao, B.; Nelson, J.C.; Sun, Q.; Leach, J.E.; *et al*. Diversity in nucleotide binding site-leucine-rich repeat genes in cereals. *Genome Res.* **2002**, *12*, 1871–1884.

197. Sanseverino, W.; Ercolano, M.R. In silico approach to predict candidate R proteins and to define their domain architecture. *BMC Res. Notes* **2012**, *5*, 678–688.

198. Gururani, M.A.; Venkatesh, J.; Upadhyaya, C.P.; Nookaraju, A.; Pandey, S.K.; Park, S.W. Plant disease resistance genes: Current status and future directions. *Physiol. Mol. Plant Pathol.* **2012**, *78*, 51–65.

199. Xiao, S.; Emerson, B.; Ratanasut, K.; Patrick, E.; O'Neill, C.; Bancroft, I.; Turner, J.G. Origin and maintenance of a broad-spectrum disease resistance locus in *Arabidopsis*. *Mol. Biol. Evol.* **2004**, *21*, 1661–1672.

200. Ma, X.F.; Li, Y.; Sun, J.L.; Wang, T.T.; Fan, J.; Lei, Y.; Huang, Y.Y.; Xu, Y.J.; Zhao, J.Q.; Xiao, S.; *et al*. Ectopic expression of resistance to powdery mildew8.1 confers resistance to fungal and oomycete pathogens in *Arabidopsis*. *Plant Cell Physiol.* **2014**, *55*, 1484–1496.

201. Panstruga, R. Discovery of novel conserved peptide domains by ortholog comparison within plant multi-protein families. *Plant Mol. Biol.* **2005**, *59*, 485–500.

202. Wretblad, S.; Bohman, S.; Dixelius, C. Overexpression of a *Brassica nigra* cDNA gives enhanced resistance to *Leptosphaeria maculans* in *B. napus*. *Mol. Plant Microbe Interact.* **2003**, *16*, 477–484.

203. Brandwagt, B.F.; Kneppers, T.J.; Nijkamp, H.J.; Hille, J. Overexpression of the tomato *Asc-1* gene mediates high insensitivity to AAL toxins and fumonisin B1 in tomato hairy roots and confers resistance to *Alternaria alternata* f. sp. *lycopersici* in *Nicotiana umbratica* plants. *Mol. Plant Microbe Interact.* **2002**, *15*, 35–42.

204. Spassieva, S.D.; Markham, J.E.; Hille, J. The plant disease resistance gene *Asc-1* prevents disruption of sphingolipid metabolism during AAL-toxin-induced programmed cell death. *Plant J.* **2002**, *32*, 561–572.

205. Scofield, S.R.; Tobias, C.M.; Rathjen, J.P.; Chang, J.H.; Lavelle, D.T.; Michelmore, R.W.; Staskawicz, B.J. Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science* **1996**, *274*, 2063–2065.

206. Tang, X.; Frederick, R.D.; Zhou, J.; Halterman, D.A.; Jia, Y.; Martin, G.B. Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. *Science* **1996**, *274*, 2060–2063.

207. Nomura, H.; Komori, T.; Uemura, S.; Kanda, Y.; Shimotani, K.; Nakai, K.; Furuichi, T.; Takebayashi, K.; Sugimoto, T.; Sano, S.; *et al*. Chloroplast-mediated activation of plant immune signalling in *Arabidopsis*. *Nat. Commun.* **2012**, *3*, 926–925.

208. Amirsadeghi, S.; Robson, C.A.; Vanlerberghe, G.C. The role of the mitochondrion in plant responses to biotic stress. *Physiol. Plant.* **2007**, *129*, 253–266.

209. Dahan, J.; Mireau, H. The Rf and Rf-like PPR in higher plants, a fast-evolving subclass of *PPR* genes. *RNA Biol.* **2013**, *10*, 1469–1476.

210. Schmitz-Linneweber, C.; Small, I. Pentatricopeptide repeat proteins: A socket set for organelle gene expression. *Trends Plant Sci.* **2008**, *13*, 663–670.

211. Jonietz, C.; Forner, J.; Holzle, A.; Thuss, S.; Binder, S. RNA processing factors2 is required for 5′ end processing of nad9 and cox3 mRNAs in mitochondria of *Arabidopsis thaliana*. *Plant Cell* **2010**, *22*, 443–453.

212. Jonietz, C.; Forner, J.; Hildebrandt, T.; Binder, S. RNA processing factor3 is crucial for the accumulation of mature ccmC transcripts in mitochondria of *Arabidopsis* accession columbia. *Plant Physiol.* **2011**, *157*, 1430–1439.

213. Cui, X.; Wise, R.P.; Schnable, P.S. The *rf2* nuclear restorer gene of male-sterile T-cytoplasm maize. *Science* **1996**, *272*, 1334–1336.

214. Wang, Z.; Zou, Y.; Li, X.; Zhang, Q.; Chen, L.; Wu, H.; Su, D.; Chen, Y.; Guo, J.; Luo, D.; *et al*. Cytoplasmic male sterility of rice with boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related *PPR* motif genes via distinct modes of mRNA silencing. *Plant Cell* **2006**, *18*, 676–687.

215. Park, Y.J.; Lee, H.J.; Kwak, K.J.; Lee, K.; Hong, S.W.; Kang, H. MicroRNA400-guided cleavage of pentatricopeptide repeat protein mRNAs renders *Arabidopsis thaliana* more susceptible to pathogenic bacteria and fungi. *Plant Cell Physiol.* **2014**, *55*, 1660–1668.

216. Garcia-Andrade, J.; Ramirez, V.; Lopez, A.; Vera, P. Mediated plastid RNA editing in plant immunity. *PLoS Pathog.* **2013**, *9*, e1003713.

217. Shirasu, K.; Lahaye, T.; Tan, M.W.; Zhou, F.; Azevedo, C.; Schulze-Lefert, P. A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in *C. elegans*. *Cell* **1999**, *99*, 355–366.

218. Brandwagt, B.F.; Mesbah, L.A.; Takken, F.L.; Laurent, P.L.; Kneppers, T.J.; Hille, J.; Nijkamp, H.J. A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B1. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 4961–4966.

219. Liu, B.; Li, J.F.; Ao, Y.; Qu, J.; Li, Z.; Su, J.; Zhang, Y.; Liu, J.; Feng, D.; Qi, K.; *et al*. Lysin motif-containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity. *Plant Cell* **2012**, *24*, 3406–3419.

220. Chisholm, S.T.; Mahajan, S.K.; Whitham, S.A.; Yamamoto, M.L.; Carrington, J.C. Cloning of the *Arabidopsis RTM1* gene, which controls restriction of long-distance movement of tobacco etch virus. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 489–494.

221. Ouibrahim, L.; Mazier, M.; Estevan, J.; Pagny, G.; Decroocq, V.; Desbiez, C.; Moretti, A.; Gallois, J.L.; Caranta, C. Cloning of the *Arabidopsis rwm1* gene for resistance to watermelon mosaic virus points to a new function for natural virus resistance genes. *Plant J.* **2014**, *79*, 705–716.

222. Falk, A.; Feys, B.J.; Frost, L.N.; Jones, J.D.; Daniels, M.J.; Parker, J.E. EDS1, an essential component of *R* gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3292–3297.

223. Cao, H.; Glazebrook, J.; Clarke, J.D.; Volko, S.; Dong, X. The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* **1997**, *88*, 57–63.

224. Dickman, M.B.; Fluhr, R. Centrality of host cell death in plant-microbe interactions. *Annu. Rev. Phytopathol.* **2013**, *51*, 543–570.

225. O'Brien, J.A.; Daudi, A.; Finch, P.; Butt, V.S.; Whitelegge, J.P.; Souda, P.; Ausubel, F.M.; Bolwell, G.P. A peroxidase-dependent apoplastic oxidative burst in cultured *Arabidopsis* cells functions in MAMP-elicited defense. *Plant Physiol.* **2012**, *158*, 2013–2027.

226. Daudi, A.; Cheng, Z.; O'Brien, J.A.; Mammarella, N.; Khan, S.; Ausubel, F.M.; Bolwell, G.P. The apoplastic oxidative burst peroxidase in *Arabidopsis* is a major component of pattern-triggered immunity. *Plant Cell* **2012**, *24*, 275–287.

227. Bindschedler, L.V.; Dewdney, J.; Blee, K.A.; Stone, J.M.; Asai, T.; Plotnikov, J.; Denoux, C.; Hayes, T.; Gerrish, C.; Davies, D.R.; *et al*. Peroxidase-dependent apoplastic oxidative burst in *Arabidopsis* required for pathogen resistance. *Plant J.* **2006**, *47*, 851–863.

228. Mohanta, T.K.; Arora, P.K.; Mohanta, N.; Parida, P.; Bae, H. Identification of new members of the MAPK gene family in plants shows diverse conserved domains and novel activation loop variants. *BMC Genom.* **2015**, *16*, 58–77.

229. Bolser, D.M.; Kerhornou, A.; Walts, B.; Kersey, P. Triticeae resources in Ensembl Plants. *Plant Cell Physiol.* **2015**, *56*, e3.

230. Peng, W.; Wang, J.; Cai, J.; Chen, L.; Li, M.; Wu, F.X. Improving protein function prediction using domain and protein complexes in PPI networks. *BMC Syst. Biol.* **2014**, *8*, 35–47.

231. Bailey, T.L.; Williams, N.; Misleh, C.; Li, W.W. MEME: Discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res.* **2006**, *34*, W369–W373.

232. Yoon, B.J. Hidden markov models and their applications in biological sequence analysis. *Curr. Genom.* **2009**, *10*, 402–415.

233. Schultz, J.; Copley, R.R.; Doerks, T.; Ponting, C.P.; Bork, P. SMART: A web-based tool for the study of genetically mobile domains. *Nucleic Acids Res.* **2000**, *28*, 231–234.

234. Pruitt, K.D.; Tatusova, T.; Klimke, W.; Maglott, D.R. NCBI reference sequences: Current status, policy and new initiatives. *Nucleic Acids Res.* **2009**, *37*, D32–D36.

235. Sanseverino, W.; Roma, G.; de Simone, M.; Faino, L.; Melito, S.; Stupka, E.; Frusciante, L.; Ercolano, M.R. PRGdb: A bioinformatics platform for plant resistance gene analysis. *Nucleic Acids Res.* **2010**, *38*, D814–D821.

236. Eddy, S.R. Accelerated Profile HMM Searches. *PLoS Comput. Biol.* **2011**, *7*, e1002195.

237. Bailey, T.L.; Elkan, C. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **1994**, *2*, 28–36.

238. Liu, Y.; Schmidt, B.; Liu, W.; Maskell, D.L. CUDA-MEME: Accelerating motif discovery in biological sequences using CUDA-enabled graphics processing units. *Pattern Recogn. Lett.* **2010**, *31*, 2170–2177.

239. Camacho, C.; Coulouris, G.; Avagyan, V.; Ma, N.; Papadopoulos, J.; Bealer, K.; Madden, T.L. BLAST+: Architecture and applications. *BMC Bioinform.* **2009**, *10*, 421.

240. Zdobnov, E.M.; Apweiler, R. InterProScan-an integration platform for the signature-recognition methods in InterPro. *Bioinformatics* **2001**, *17*, 847–848.

241. Kall, L.; Krogh, A.; Sonnhammer, E.L. A combined transmembrane topology and signal peptide prediction method. *J. Mol. Biol.* **2004**, *338*, 1027–1036.

242. Krogh, A.; Larsson, B.; von Heijne, G.; Sonnhammer, E.L. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *J. Mol. Biol.* **2001**, *305*, 567–580.

243. Lupas, A.; van Dyke, M.; Stock, J. Predicting coiled coils from protein sequences. *Science* **1991**, *252*, 1162–1164.

244. Radwan, O.; Gandhi, S.; Heesacker, A.; Whitaker, B.; Taylor, C.; Plocik, A.; Kesseli, R.; Kozik, A.; Michelmore, R.W.; Knapp, S.J. Genetic diversity and genomic distribution of homologs encoding NBS-LRR disease resistance proteins in sunflower. *Mol. Genet. Genom.* **2008**, *280*, 111–125.

245. Porter, B.W.; Paidi, M.; Ming, R.; Alam, M.; Nishijima, W.T.; Zhu, Y.J. Genome-wide analysis of *Carica papaya* reveals a small NBS resistance gene family. *Mol. Genet. Genom.* **2009**, *281*, 609–626.

246. McDowell, J.M.; Simon, S.A. Recent insights into *R* gene evolution. *Mol. Plant Pathol.* **2006**, *7*, 437–448.

247. Tarr, D.E.; Alexander, H.M. TIR-NBS-LRR genes are rare in monocots: Evidence from diverse monocot orders. *BMC Res. Notes* **2009**, *2*, 197–206.

248. Kim, J.; Lim, C.J.; Lee, B.W.; Choi, J.P.; Oh, S.K.; Ahmad, R.; Kwon, S.Y.; Ahn, J.; Hur, C.G. A genome-wide comparison of NB-LRR type of resistance gene analogs (RGA) in the plant kingdom. *Mol. Cells* **2012**, *33*, 385–392.

249. Glazebrook, J. Genes controlling expression of defense responses in *Arabidopsis*—2001 status. *Curr. Opin. Plant Biol.* **2001**, *4*, 301–308.

250. Yue, J.X.; Meyers, B.C.; Chen, J.Q.; Tian, D.; Yang, S. Tracing the origin and evolutionary history of plant nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes. *New Phytol.* **2012**, *193*, 1049–1063.

251. Chelkowski, J.; Koczyk, G. Resistance gene analogues of *Arabidopsis thaliana*: Recognition by structure. *J. Appl. Genet.* **2003**, *44*, 311–321.

252. Luo, S.; Zhang, Y.; Hu, Q.; Chen, J.; Li, K.; Lu, C.; Liu, H.; Wang, W.; Kuang, H. Dynamic nucleotide-binding site and leucine-rich repeat-encoding genes in the grass family. *Plant Physiol.* **2012**, *159*, 197–210.

253. Pan, Q.; Liu, Y.S.; Budai-Hadrian, O.; Sela, M.; Carmel-Goren, L.; Zamir, D.; Fluhr, R. Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologues in the genomes of two dicotyledons: Tomato and arabidopsis. *Genetics* **2000**, *155*, 309–322.

254. Liu, J.J.; Ekramoddoullah, A.K. Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (*Pinus monticola* Dougl. ex. D. Don.). *Mol. Genet. Genom.* **2003**, *270*, 432–441.

255. Marone, D.; Russo, M.A.; Laido, G.; de Leonardis, A.M.; Mastrangelo, A.M. Plant nucleotide binding site-leucine-rich repeat (NBS-LRR) genes: Active guardians in host defense responses. *Int. J. Mol. Sci.* **2013**, *14*, 7302–7326.

256. Monosi, B.; Wisser, R.J.; Pennill, L.; Hulbert, S.H. Full-genome analysis of resistance gene homologues in rice. *Theor. Appl. Genet.* **2004**, *109*, 1434–1447.

257. Michelmore, R.W.; Meyers, B.C. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Res.* **1998**, *8*, 1113–1130.

258. Hall, B.G. Building phylogenetic trees from molecular data with MEGA. *Mol. Biol. Evol.* **2013**, *30*, 1229–1235.

259. Young, N.D. The genetic architecture of resistance. *Curr. Opin. Plant Biol.* **2000**, *3*, 285–290.

260. Zhou, B.; Dolan, M.; Sakai, H.; Wang, G.L. The genomic dynamics and evolutionary mechanism of the *Pi2/9* locus in rice. *Mol. Plant Microbe Interact.* **2007**, *20*, 63–71.

261. Yang, S.; Feng, Z.; Zhang, X.; Jiang, K.; Jin, X.; Hang, Y.; Chen, J.Q.; Tian, D. Genome-wide investigation on the genetic variations of rice disease resistance genes. *Plant Mol. Biol.* **2006**, *62*, 181–193.

262. Jupe, F.; Pritchard, L.; Etherington, G.J.; Mackenzie, K.; Cock, P.J.; Wright, F.; Sharma, S.K.; Bolser, D.; Bryan, G.J.; Jones, J.D.; *et al*. Identification and localisation of the NB-LRR gene family within the potato genome. *BMC Genom.* **2012**, *13*, 75–88.

263. Wei, F.; Wing, R.A.; Wise, R.P. Genome dynamics and evolution of the *Mla* (powdery mildew) resistance locus in barley. *Plant Cell* **2002**, *14*, 1903–1917.

264. Bernal, A.J.; Pan, Q.; Pollack, J.; Rose, L.; Kozik, A.; Willits, N.; Luo, Y.; Kochetkova, E.; Guittet, M.; Michelmore, R.W. Functional analysis of the plant disease resistance gene *Pto* using DNA shuffling. *J. Biol. Chem.* **2005**, *280*, 23073–23083.

265. Song, W.Y.; Pi, L.Y.; Wang, G.L.; Gardner, J.; Holsten, T.; Ronald, P.C. Evolution of the rice *Xa21* disease resistance gene family. *Plant Cell* **1997**, *9*, 1279–1287.

266. Geddy, R.; Brown, G.G. Genes encoding pentatricopeptide repeat (PPR) proteins are not conserved in location in plant genomes and may be subject to diversifying selection. *BMC Genom.* **2007**, *8*, 130.

267. Gebhardt, C.; Valkonen, J.P. Organization of genes controlling disease resistance in the potato genome. *Annu. Rev. Phytopathol.* **2001**, *39*, 79–102.

268. Perazzolli, M.; Malacarne, G.; Baldo, A.; Righetti, L.; Bailey, A.; Fontana, P.; Velasco, R.; Malnoy, M. Characterization of resistance gene analogues (RGAs) in apple (*Malus* x *domestica* Borkh.) and their evolutionary history of the Rosaceae family. *PLoS ONE* **2014**, *9*, e83844.

269. Ota, T.; Nei, M. Divergent evolution and evolution by the birth-and-death process in the immunoglobulin *VH* gene family. *Mol. Biol. Evol.* **1994**, *11*, 469–482.

270. Sudupak, M.A.; Bennetzen, J.L.; Hulbert, S.H. Unequal exchange and meiotic instability of disease-resistance genes in the *Rp1* region of maize. *Genetics* **1993**, *133*, 119–125.

271. Bar-Hen, A.; Charcosset, A.; Bourgoin, M.; Guiard, J. Relationship between genetic markers and morphological traits in a maize inbred line collection. *Euphytica* **1995**, *84*, 145–154.

272. Cao, J.; Schneeberger, K.; Ossowski, S.; Gunther, T.; Bender, S.; Fitz, J.; Koenig, D.; Lanz, C.; Stegle, O.; Lippert, C.; *et al*. Whole-genome sequencing of multiple *Arabidopsis thaliana* populations. *Nat. Genet.* **2011**, *43*, 956–963.

273. Huang, X.; Kurata, N.; Wei, X.; Wang, Z.X.; Wang, A.; Zhao, Q.; Zhao, Y.; Liu, K.; Lu, H.; Li, W.; *et al*. A map of rice genome variation reveals the origin of cultivated rice. *Nature* **2012**, *490*, 497–501.

274. Jordan, K.W.; Wang, S.; Lun, Y.; Gardiner, L.; MacLachlan, R.; Hucl, P.; Wiebe, K.; Wong, D.; Forrest, K.L.; Sharpe, A.G.; *et al*. A haplotype map of allohexaploid wheat reveals distinct patterns of selection on homoeologous genomes. *Genome Biol.* **2015**, *16*, doi:10.1186/s13059-13015-10606-13054.

275. Gore, M.A.; Chia, J.M.; Elshire, R.J.; Ersoz, E.S.; Hurwitz, B.L.; Peiffer, J.A.; McMullen, M.D.; Sun, Q.; Grills, G.S.; Ross-Ibarra, J.; *et al*. A first-generation haplotype map of maize. *Science* **2009**, *326*, 1115–1117.

276. Chia, J.M.; Song, C.; Bradbury, P.J.; Costich, D.; de Leon, N.; Doebley, J.; Elshire, R.J.; Gaut, B.; Geller, L.; Glaubitz, J.C.; *et al*. Maize HapMap2 identifies extant variation from a genome in flux. *Nat. Genet.* **2012**, *44*, 803–807.

277. Genomes Project, C.; Abecasis, G.R.; Altshuler, D.; Auton, A.; Brooks, L.D.; Durbin, R.M.; Gibbs, R.A.; Hurles, M.E.; McVean, G.A. A map of human genome variation from population-scale sequencing. *Nature* **2010**, *467*, 1061–1073.

278. Van der Linden, C.G.; Wouters, D.C.; Mihalka, V.; Kochieva, E.Z.; Smulders, M.J.; Vosman, B. Efficient targeting of plant disease resistance loci using NBS profiling. *Theor. Appl. Genet.* **2004**, *109*, 384–393.

279. Gu, Y.; Zhao, Q.; Sun, D.; Song, W. A genetic linkage map based on AFLP and NBS markers in cauliflower (*Brassica oleracea* var. *botrytis*). *Bot. Stud.* **2008**, *49*, 93–99.

280. Gonzalez, A.M.; Marcel, T.C.; Kohutova, Z.; Stam, P.; van der Linden, C.G.; Niks, R.E. Peroxidase profiling reveals genetic linkage between peroxidase gene clusters and basal host and non-host resistance to rusts and mildew in barley. *PLoS ONE* **2010**, *5*, e10495.

281. Vossen, J.H.; Dezhsetan, S.; Esselink, D.; Arens, M.; Sanz, M.J.; Verweij, W.; Verzaux, E.; van der Linden, C.G. Novel applications of motif-directed profiling to identify disease resistance genes in plants. *Plant Methods* **2013**, *9*, 37.

282. De Keyser, E.; Shu, Q.Y.; van Bockstaele, E.; de Riek, J. Multipoint-likelihood maximization mapping on 4 segregating populations to achieve an integrated framework map for QTL analysis in pot azalea (*Rhododendron simsii* hybrids). *BMC Mol. Biol.* **2010**, *11*, 1–19.