

Technical Note

# NRD: *Nicotiana* Resistance Database, a Comprehensive Platform of Stress Tolerance in *Nicotiana*

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**Abstract:** *Nicotiana* is one of the most important economic crops and model plants; however, its growth is affected by various biotic and abiotic stresses. In this study, 27,142 potential resistance genes were identified in six *Nicotiana* species, belonging to fourteen gene families and transcription factors related to stress resistance. The results indicate that *Nicotiana* has a potential abundance resistance background to biotic and abiotic stress, and these genes could be used in resistance breeding in the future. Analyzing the genome sequences of 19 pathogens, 5,421,414 Single Nucleotide Polymorphisms and 1958 Simple Sequence Repeats of pathogens have been obtained. The abundance loci show that the biotic pathogens have a high variability and biodiversity. An open-access database, named the *Nicotiana* Resistance Database (NRD), has been developed as a user-friendly resistance research platform for *Nicotiana*. The platform provides theoretical and technical support for the resistance research, including the cultivation of resistant varieties, and the genetics and breeding of *Nicotiana* and relative species.

**Keywords:** *Nicotiana*; pathogens; stress tolerance; resistance genes; database



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## 1. Introduction

Under natural or agricultural conditions, plants grow under various stresses rather than ideal conditions [1]. These stresses can be divided into biotic and abiotic stress [2]. Only by constantly adapting to the environment, adjusting their morphological structure and physiological metabolism, can plants survive and reproduce [3,4]. Plants can resist stresses and maintain their life processes through osmotic adjustment [5,6], biomembrane regulation [7], reactive oxygen metabolism [8], hormones [9,10], stress proteins [11,12], and cross-adaptation [13,14]. *Nicotiana* is commonly used as a model plant in the study of molecular plant–microbe interactions [15,16]; it is also a vital material for the cigarette industry, and thus is of high commercial value all over the world. However, stress conditions cause extensive losses to the application of *Nicotiana* [17,18]. The pathogens of *Nicotiana* diseases cause extensive damage to tobacco, especially various viral diseases, such as tobacco mosaic virus (TMV), potato virus Y (PVY), cucumber mosaic virus (CMV), among others [19,20]. Abiotic stress, such as heat, cold, drought, waterlogging, and freezing, has caused many productivity reductions [21].

Although stress has tremendous damaging effects on *Nicotiana*, it also brings about a series of research studies on *Nicotiana* resistance. The methods of transcriptome and point mutation analysis have been widely used in CMV [22] and TMV [20] research, which could be used to reduce the harm caused by pathogens. In addition, some of the resistance genes

have been found in different plant species, such as *GbRLK* from *Gossypium* [23], *TaWRKY10* from wheat [24], and *TaNAC2a* from tobacco [25]. Furthermore, some vital families and transcription factors, such as protein kinase (PKinase) and v-myb avian myeloblastosis viral oncogene homolog (MYB), have been considered to play an important role in abiotic stress responses [26,27]. Nonetheless, these efforts are far from enough to reduce the occurrence of *Nicotiana* diseases and improve the yield of *Nicotiana*. The expression products of resistance genes from the same family have the same relatively conserved domain, which is also the functional unit and evolutionary unit of this family. The sequences and functions of potential resistance genes can be inferred from known domains. To further study tobacco resistance, comparative genomic studies should be conducted using the genome sequences of *Nicotiana* and its pathogens.

Due to the fast development of sequencing techniques, increasingly more genomes have been sequenced in the past few years. Here, we chose three flue-cured tobacco varieties (*Nicotiana tabacum* cv. K326, *N. tabacum* cv. Basma Xanthi, and *N. tabacum* cv. TN90), two ancestors of *N. tabacum* (*N. sylvestris* and *N. tomentosiformis*), a common model species (*N. benthamiana*), and 19 pathogens of *Nicotiana* [28–32] for this research. Based on these sequences of the hosts and pathogens, a series of bioinformatic and comparative genomic analyses could be performed, and this study commits to the excavation of resistance genes and the variation of pathogens.

## 2. Materials and Methods

### 2.1. Data Sources

Six *Nicotiana* genome sequences and 19 genome sequences of common pathogens were downloaded from the NCBI (<http://www.ncbi.nlm.nih.gov/> (accessed on 28 December 2021)) or the EMBL (<http://www.ebi.ac.uk/> (accessed on 15 December 2021)). The *Nicotiana* genome sequence information contains their source, size, and version; the pathogen genome sequence information includes their length and size (Tables 1 and 2). All information can be found in the *Nicotiana* Resistance Database (NRD, <http://biodb.sdau.edu.cn/NRD/index.html> (accessed on 7 February 2022)). Some preliminary modifications to these data were performed by Perl scripts, i.e., merging their IDs and replacing all the lowercase letters with uppercase letters in the sequences.

**Table 1.** Information of six *Nicotiana* genomes.

Latin Name	Genome Size/Gb	Version	Reference
<i>N. benthamiana</i>	2.63	Niben.v0.4.4	[28]
<i>N. sylvestris</i>	2.22	GCA_000393655	[29]
<i>N. tomentosiformis</i>	1.69	GCA_000390325	[29]
<i>N. tabacum</i> cv. Basma Xanthi	3.74	GCA_000715095	[30]
<i>N. tabacum</i> cv. K326	3.73	GCA_000715075	[30]
<i>N. tabacum</i> cv. TN90	3.64	GCA_000715135	[30]

**Table 2.** Information of 19 *Nicotiana* pathogens.

Pathogens	Genome Number	Total Sequence Length/bp	Pathogens	Genome Number	Total Sequence Length/bp
Alfalfa mosaic virus (AMV)	18	47,450	Tobacco leaf curl virus (TLCV)	7	16,505
Tobacco curly stop virus (BCTV)	3	9025	Tobacco mosaic virus (TMV)	69	435,826
Cucumber mosaic virus (CMV)	149	398,422	Tobacco necrosis virus (TNV)	7	26,095
Chilli veinal mottle virus (CVMV)	7	68,945	Tomato ringspot nepovirus (TomRSV)	6	30,410

Table 2. Cont.

Pathogens	Genome Number	Total Sequence Length/bp	Pathogens	Genome Number	Total Sequence Length/bp
Potato virus X (PVX)	20	127,819	Tobacco rattle virus (TRV)	22	101,787
Potato virus Y (PVY)	110	1,065,081	Tobacco streak virus (TSV)	25	66,613
Tomato aspermy virus (TAV)	11	30,409	Tomato spotted wilt virus (TSWV)	85	340,357
Tomato black ring nepovirus (TBRV)	6	24,870	Tobacco vein-banding mosaic virus (TVBMV)	13	67,964
Tobacco bushy top virus (TBTV)	8	26,472	Tobacco vein distorting virus (TVDV)	2	12,012
Tobacco etch virus (TEV)	6	57,012			

### 2.2. Comparative Analysis of the Pathogen Genomes

A BLAST-Like Alignment Tool (BLAT) alignment [33] was performed to find similar sequences using the sequences of 19 different pathogens. Single Nucleotide Polymorphisms (SNPs) were detected on the identified similarities compared with the reference sequences. Simple Sequence Repeats (SSRs) in all these pathogen sequences were detected by finding the dimer, trimer, tetramer, pentamer, hexamer, and heptamer quantities in at least 12 bps. All the analyses were performed in-house using Perl or Python algorithms.

### 2.3. Potential Resistance Genes in *Nicotiana*

To identify the resistance genes in *Nicotiana*, 16 resistance families and transcription factors were collected from 25 previous reports (Table 3 and [http://biodb.sdau.edu.cn/NRD/host\\_domain.html](http://biodb.sdau.edu.cn/NRD/host_domain.html) (accessed on 7 February 2022)). The Hidden Markov Models of these resistance genes were downloaded from the Pfam database (<http://pfam.xfam.org/> (accessed on 7 February 2022)) [34]. A hmmsearch using HMMER software [35] was performed to get the potential gene members of six *Nicotiana* species. The numbers of the potential resistance genes were counted and analyzed using Perl scripts. The functions of these genes were annotated through comparison with the Gene Ontology database (GO) [36] and Kyoto Encyclopedia of Genes and Genomes database (KEGG) [37]. The candidate genes related to environmental adaptation were identified according to the annotation results.

Table 3. Information of 16 resistance domains.

Pfam No.	Resistance Domains	Sources
PF02045	NFYA	[38,39]
PF00847	AP2, CBF1, NAC, MYB	[40]
PF03242	LEA	[41]
PF01849	NAC (NAM, ATAF and CUC)	[42]
PF04404	ERF	[43]
PF00447	HSF	[44]
PF00170	bZIP	[45]
PF00266	LOS5/ABA3	[46]
PF00069	Pkinase	[23]
PF00808	NFYB	[47]

**Table 3.** *Cont.*

Pfam No.	Resistance Domains	Sources
PF08688	SbpAPX	[48]
PF00036	EF-hand	[49]
PF00249	R2R3-MYB	[50]
PF00230	AQP	[51]
PF03106	WRKY	[24]

#### 2.4. Development of a Web-Based Platform

The LAMP (Linux–Apache–MySQL–PHP/Python) framework was used to build the *Nicotiana* Resistance Database (NRD). HTML (Hyper Text Markup Language) scripts and web pages were constructed through Dreamweaver, and the web interface was run on an Apache webserver. The information on SSRs, SNPs, and the candidate genes were stored in MySQL databases. Furthermore, the PHP scripts and Python scripts were connected to the web pages and MySQL databases to obtain a complete framework. The NRD (<http://biodb.sdau.edu.cn/NRD/index.html> (accessed on 7 February 2022)) has been set up on a World-Wide Web server allowing internet access with a web client. All data in NRD are freely available for all academic and non-commercial users. All the sources and results of *Nicotiana* and pathogens analysis in this research can be found and downloaded at NRD.

### 3. Results

#### 3.1. SNPs and SSRs in *Nicotiana* Pathogens

The pathogen sequences contained a few RNA sequences, and their lengths were generally less than 1 Mb. We identified 5,421,414 potential SNPs in the pathogen sequences, and the number of SNPs ranged from 26.3 (BCTV) to 6709.55 (TMV) per sequence (Table 4). On average, there were 3261.26 SNP loci in each sequence, and most sequences contained over 1000 loci. The PVY contained 33,472.87 SNP loci per sequence, with the highest average number, followed by the CMV and TMV. The results show high distribution abundance in different pathogen sequences. Moreover, common diseases, such as TMV, CMV, PVX, and PVY, had higher variation rates than other pathogens.

We detected 1958 SSRs in the sequences of the 19 pathogens (Table 5). The distribution of the SSRs in these pathogens ranged from 1.3 (TSV) to 5.9 (TMV) per sequence. Most sequences only contained one or two SSR feature element(s). The quantities of dimers and hexamers were higher than the other motifs. The TSWV, PVY, and TMV had over 100 SSRs, whereas the number of SSRs in the other pathogens only ranged from 5 (BCTV) to 56 (PVX).

**Table 4.** SNPs in 19 *Nicotiana* pathogens.

Pathogens	Genome Number	SNP Number	Average
Alfalfa mosaic virus (AMV)	18	5151	286.17
Tobacco curly stop virus (BCTV)	3	79	26.33
Cucumber mosaic virus (CMV)	149	869,705	5836.95
Chilli veinal mottle virus (CVMV)	7	22,082	3154.57
Potato virus X (PVX)	20	86,399	4319.95
Potato virus Y (PVY)	110	3,682,016	33,472.87
Tomato aspermy virus (TAV)	11	834	75.82
Tomato black ring nepovirus (TBRV)	6	234	39.00
Tobacco bushy top virus (TBTV)	8	2307	288.38
Tobacco etch virus (TEV)	6	3054	509.00
Tobacco leaf curl virus (TLCV)	7	1166	166.57
Tobacco mosaic virus (TMV)	69	462,959	6709.55
Tobacco necrosis virus (TNV)	7	3647	521.00

Table 4. Cont.

Pathogens	Genome Number	SNP Number	Average
Tomato ringspot nepovirus (TomRSV)	6	1712	285.33
Tobacco rattle virus (TRV)	22	10,593	481.50
Tobacco streak virus (TSV)	25	16,888	675.52
Tomato spotted wilt virus (TSWV)	85	219,687	2584.55
Tobacco vein-banding mosaic virus (TVBMV)	13	32,901	2530.85
Tobacco vein distorting virus (TVDV)	2	0	0
Total	574	5,421,414	

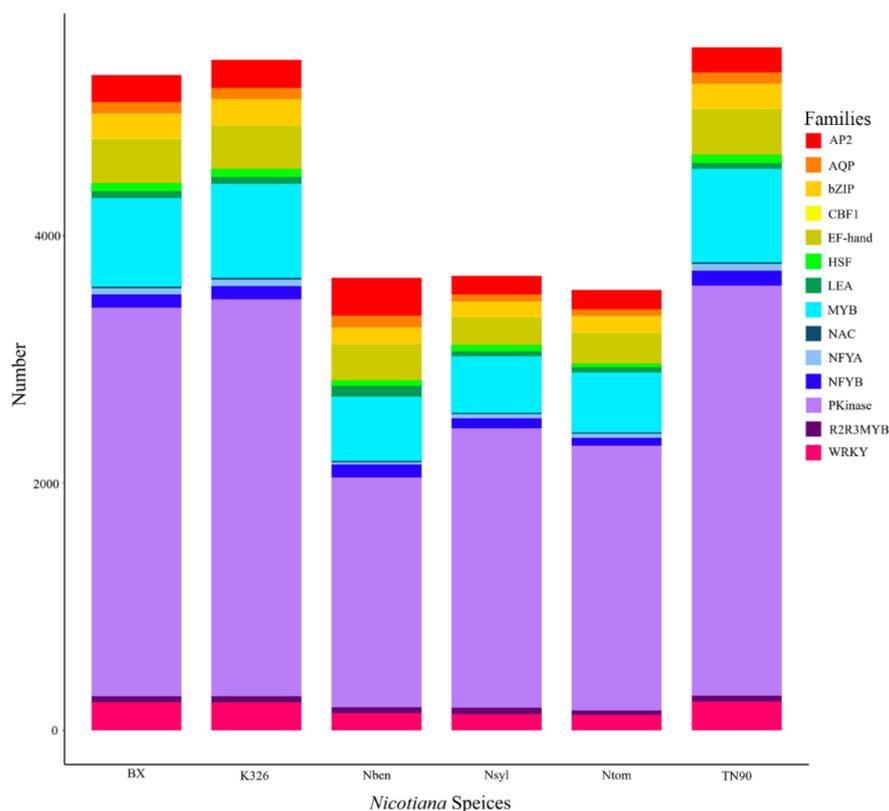
Table 5. SSRs in 19 *Nicotiana* pathogens.

Pathogens	Sequence Number	SSR Number	Average	Feature Element
Alfalfa mosaic virus (AMV)	12	29	2.42	ttttaa tctgag
Tobacco curly stop virus (BCTV)	3	5	1.67	tactgg
Cucumber mosaic virus (CMV)	39	50	1.28	gt tg
Chilli veinal mottle virus (CVMV)	6	35	5.83	attcgcg
Potato virus X (PVX)	19	56	2.95	cttggtg
Potato virus Y (PVY)	110	557	5.06	ttattag
Tomato aspermy virus (TAV)	8	31	3.88	tg
Tomato black ring nepovirus (TBRV)	6	14	2.33	gatttt
Tobacco bushy top virus (TBTV)	6	12	2	gaagtg
Tobacco etch virus (TEV)	6	14	2.33	aaagca
Tobacco leaf curl virus (TLCV)	6	11	1.83	tgttca gtaaatt aag ttttgaa aaattca
Tobacco mosaic virus (TMV)	67	398	5.94	caa
Tobacco necrosis virus (TNV)	7	17	2.43	aac
Tomato ringspot nepovirus (TomRSV)	5	19	3.8	tgtc
Tobacco rattle virus (TRV)	7	15	2.14	tac
Tobacco streak virus (TSV)	18	23	1.28	ta
Tomato spotted wilt virus (TSWV)	139	614	4.42	aaaaca
Tobacco vein-banding mosaic virus (TVBMV)	13	54	4.15	aggaac
Tobacco vein distorting virus (TVDV)	2	4	2	acctca taagga
Total	479	1958		

### 3.2. Potential Resistance Genes in the *Nicotiana* Genomes

The six *Nicotiana* genomes ranged from 1.69 Gb to 3.74 Gb, in which the potential resistance genes are identified (Table S1). Resistance genes from 16 families were collected as reference sequences in previous research; however, only 14 families and transcription factors have been found in the six *Nicotiana* species. Based on the 14 families and transcription factors, 27,142 genes were detected that might be related to abiotic stress resistance. Among these genes, 15,928 (58.68% of the total number) belong to the PKinase family, followed by the MYB family, containing 3695 genes (Figure 1).

According to our comparison of these genes with the GO and KEGG databases, a majority of the genes were associated with biological processes related to environmental adaptation in all six species. Most of the annotated genes belonged to four families. The PKinase family had 2374 genes, and the MYB, EF-hand, and WRKY families comprised of 1568 genes, 1045 genes, and 1038 genes, respectively. In addition, the three *Nicotiana* species, *N. tabacum* cv. K326, *N. tabacum* cv. Basma Xanthi and *N. tabacum* cv. TN90, had more resistance genes than the other species (Figure 1).



**Figure 1.** Distribution of resistance genes in the following *Nicotiana* species: BX, *N. tabacum* cv. Basma Xanthi; K326, *N. tabacum* cv. K326; Nsyl, *N. sylvestris*; Nben, *N. benthamiana*; Ntom, *N. tomentosiformis*; TN90, *N. tabacum* cv. TN90.

### 3.3. Resistance Platform of *Nicotiana*

The *Nicotiana* Resistance Database (NRD, <http://biodb.sdau.edu.cn/NRD/index.html> (accessed on 7 February 2022)) was developed using seven primary interfaces, identified as HOME, HOST, PATHOGENS, TOOLS, DOWNLOAD, CONTACT, and LINK (Figure 2).

The HOME page gives a brief introduction and shows the overall framework of this platform, with a frame structure and several shortcut entrances.

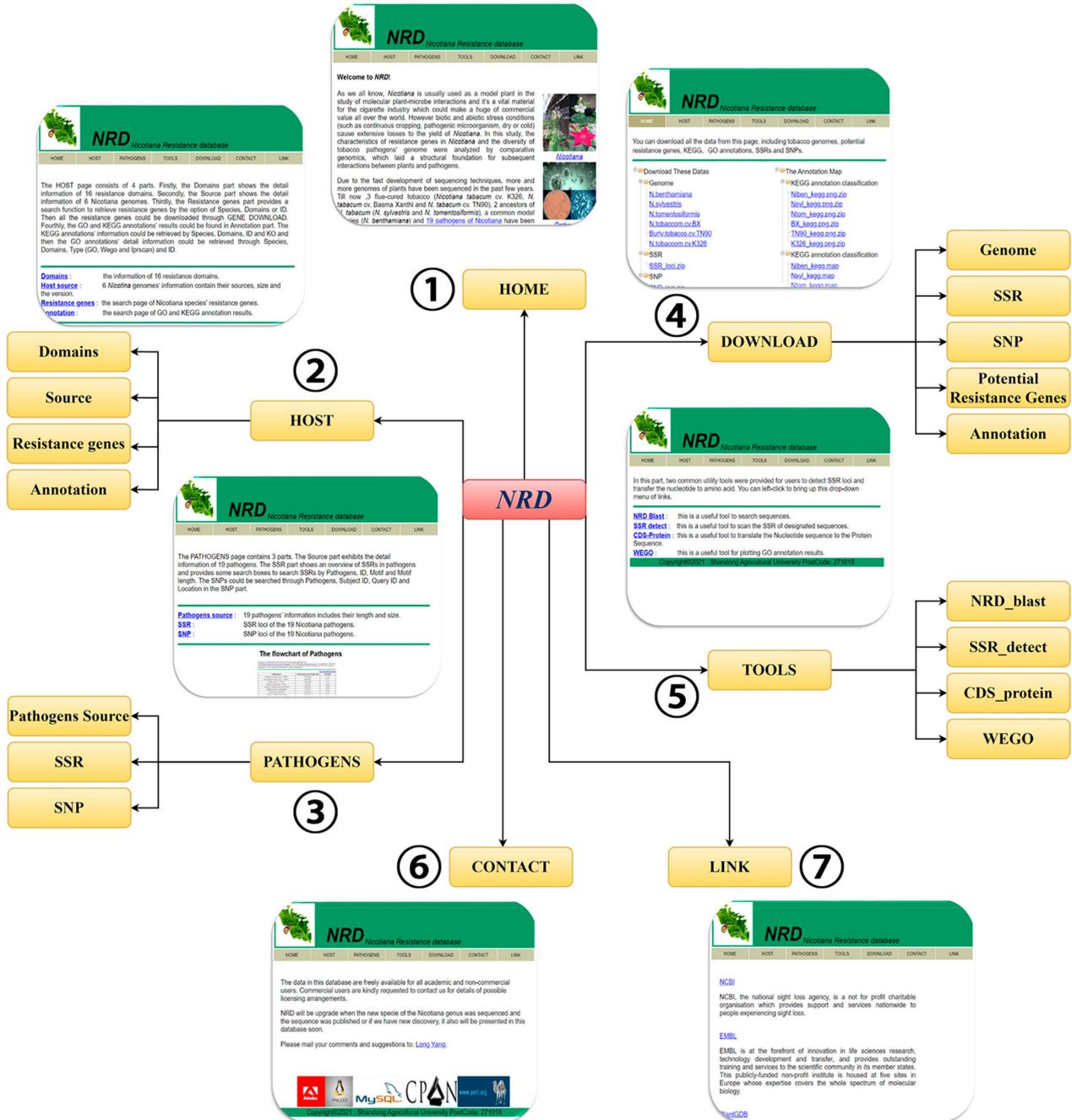
The HOST page comprises four sections. Firstly, the Domains section shows the detailed information of the 16 resistance domains of the resistance families. Secondly, the Source section shows the detailed information of the six *Nicotiana* genomes used in this research. Thirdly, the Resistance genes section provides a search function to retrieve resistance genes via the option of Species, Domains, or ID. Subsequently, all the resistance genes can be downloaded through GENE DOWNLOAD. Fourthly, the results of GO and KEGG annotations can be found in the Annotation section. The KEGG annotation information can be obtained via the Species, Domains, ID, or KO option; alternatively, the GO annotation information can be retrieved through the Species, Domains, Type (GO, WEGO and InterProScan), or ID option.

The PATHOGENS page contains three sections. The Source section exhibits the detailed information of 19 pathogens. The SSR section provides an overview of the SSRs in the pathogen sequences, and provides search boxes to search for SSR loci via the Pathogens, ID, Motif, and Motif length options. The SNP loci can be searched through the Pathogens, Subject ID, Query ID, and Location options in the SNP section.

There are four common utility tools offered for the users on the TOOLS page. The SSR\_detect model helps the user detect SSR loci from any sequence, and the CDS\_protein model can transfer the nucleotide to an amino acid. In addition, the NRD\_blast model provides a sequence alignment platform against the resistance genes in *Nicotiana* or the

plant sequence derived from the NR database (8 October 2021). An outside tool, WEGO, also provides access.

The CONTACT page provides the authors' mailbox for users and the LINK page offers some valuable links to the relative research.



**Figure 2.** The framework of *Nicotiana* Resistance Database (NRD). ① Home: introduction of the study and the frame-chart of NRD. ② Host: provides the information of six *Nicotiana* genomes and 16 resistance families. Users can search the resistance genes in the drop-down menu. ③ Pathogens: provides the information of 19 pathogens and provides the users with search boxes for the Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs) loci. ④ Download: the data can be downloaded on the page. ⑤ Tools: utility tools are offered to users to detect SSRs loci and transfer the nucleotide to an amino acid. ⑥ Contact: mailbox of the authors. ⑦ Link: some useful links.

## 4. Discussion

### 4.1. High Variation Rate of Pathogens Became a Big Challenge

The results show that the pathogen sequences contain a large number of SNPs, and they are discrepant in different species. The high variation rate in the *Nicotiana* pathogens has led to the aggravation of *Nicotiana* diseases in recent years, and makes it very difficult to cultivate new resistant varieties [52,53]. Therefore, it is extremely necessary to combine bioinformatic analysis with experiments in the future. Meanwhile, the innovation of the *Nicotiana* variety resources requires the timely intervention of high-throughput sequencing technology.

### 4.2. Abundance Potential Resistance Genes in *Nicotiana* Species

A huge number of potential genes related to resistance were identified in the six *Nicotiana* species, which indicated there was a good genetic basis for resistance in tobacco, and these genes could be used in tobacco stress resistance breeding. The tobacco varieties *N. tabacum* cv. K326, *N. tabacum* cv. Basma Xanthi, and *N. tabacum* cv. TN90 exhibited more resistance genes than the others. This may be because *N. tabacum* is an allotetraploid, and this species deserves more research attention. Furthermore, the resistance genes in the wild species could also improve resistance in existing cultivation varieties.

PKinase is a family of ABA-activated protein kinases that plays an essential role in signal transduction and environmental adaptation [54]. The PKinase family has a high number of proteins/genes than other resistance gene families. This means the PKinase family provides a large number of resistance genes for *Nicotiana* breeding, and also suggests that the resistance of *Nicotiana* may be mediated by ABA. In addition, the EF-hand, MYB, and WRYK families also provide a lot of resistance sources for the genetic improvement of *Nicotiana*. The number of potential genes identified is far greater than the number of genes annotated, which is possibly due to the quality of the assembly affecting the subsequent annotation.

### 4.3. Significance and Future Perspective

The NRD is a user-friendly database, including HOST, PATHOGENS, TOOLS, and other modules, convenient for users to search and download. The database can summarize and display the resistance data of *Nicotiana* plants, and identify potential resistance genes and domains, as well as specific molecular markers in pathogens, all of which can help accelerate the research progress on the resistance of *Nicotiana*. Based on the NRD, the SSR and SNP markers could be used for gene location, genetic mapping, species diversity, and molecular assistant selection [55]. Especially, the NRD paves the way for resistance breeding in *Nicotiana*. The resistance domains and the resistance genes can be applied to many areas of biological research. The NRD will be upgraded when a new species of the *Nicotiana* genus is sequenced, and the sequence is published.

## 5. Conclusions

The stresses experienced during plant growth can cause enormous losses in yield and quality. *Nicotiana*, one of the most important economic crops and model plants, is no exception. In this study, 27,142 potential resistance genes were identified from six *Nicotiana* species by comparison with 16 resistance gene families and transcription factors, indicating that *Nicotiana* has a potentially rich background of resistance to biotic and abiotic stresses. Meanwhile, the genomic sequence markers of 19 pathogens were analyzed in this study, from which 5,421,414 SNPs and 1958 SSRs were obtained. Abundance loci show high variability and biodiversity in biological pathogens. On this basis, the *Nicotiana* Resistance Database (NRD, <http://biodb.sdau.edu.cn/NRD/index.html> (accessed on 7 February 2022)) has been developed into an open-access and user-friendly tobacco resistance research platform, and contains the genetic sequences of hosts and pathogens. This study provided a theoretical and technical support platform for resistance research, resistant variety cropping and the genetic breeding of *Nicotiana* and related species.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12020508/s1>. Table S1. Potential resistance genes identified in six *Nicotiana*.

**Author Contributions:** Conceptualization, L.Y.; methodology, S.Y.; software, J.L.; validation, S.Y. and L.Y.; formal analysis, Y.C.; investigation, Y.C.; resources, Y.C.; data curation, S.Y. and J.L.; writing—original draft preparation, Y.C. and S.Y.; writing—review and editing, Y.C., S.Y. and K.W.; visualization, S.Y. and J.L.; supervision, L.Y.; project administration, L.Y.; funding acquisition, L.Y. and S.Y. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The datasets analyzed for this study can be found in the NRD (<http://biodb.sdau.edu.cn/NRD/index.html> (accessed on 7 February 2022)).

**Conflicts of Interest:** The authors declare no conflict of interest.

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