Genome-wide analysis of lectin receptor-like kinases family from potato (*Solanum tuberosum* L.)

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Lectin receptor-like kinases (LecRLKs) are involved in response to diverse environmental stresses and pathogenic microbes. A comprehensive acknowledgment of the family members in potato (*Solanum tuberosum*) genome is largely limited until now. In total, 113 potato LecRLKs (*St*LecRLKs) were first identified, including 85 G-type, 26 L-type and 2 C-type members. Based on phylogenetic analysis, *St*LecRLKs were sub-grouped into seven clades, including C-type, L-type, G-I, G-II, G-III G-IV and G-V. Chromosomal distribution and gene duplication analysis revealed the expansion of *St*LecRLKs occurred majorly through tandem duplication although the whole-genome duplication (WGD)/segmental duplication events were found. *Cis*-elements in the *St*LecRLKs promoter region responded mainly to signals of defense and stress, phytohormone, biotic or abiotic stress. Moreover, expressional investigations indicated that the family members of the clades L-type, G-I, G-IV and G-V were responsive to both bacterial and fungal infection. Based on qRT-PCR analysis, the expressions of PGSC0003DMP400055136 and PGSC0003DMP400067047 were strongly induced in all treatments by both *Fusarium sulphureum* (*Fs*) and *Phytophthora infestans* (*Pi*) inoculation. The present study provides valuable information for LecRLKs gene family in potato genome, and establishes a foundation for further research into the functional analysis.

1 **Genome-wide analysis of lectin receptor-like kinases family from**

2 **potato (*Solanum tuberosum* L.)**

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11 **Abstract**

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13 microbes. A comprehensive acknowledgment of the family members in potato (*Solanum tuberosum*) genome is largely

14 limited until now. In total, 113 potato LecRLKs (*St*LecRLKs) were first identified, including 85 G-type, 26 L-type

15 and 2 C-type members. Based on phylogenetic analysis, *St*LecRLKs were sub-grouped into seven clades,

16 including C-type, L-type, G-I, G-II, G-III G-IV and G-V. Chromosomal distribution and gene duplication

17 analysis revealed the expansion of *St*LecRLKs occurred majorly through tandem duplication although the whole-

18 genome duplication (WGD)/segmental duplication events were found. *Cis*-elements in the *St*LecRLKs promoter

19 region responded mainly to signals of defense and stress, phytohormone, biotic or abiotic stress. Moreover,

20 expressional investigations indicated that the family members of the clades L-type, G-I, G-IV and G-V were

21 responsive to both bacterial and fungal infection. Based on qRT-PCR analysis, the expressions of

22   PGSC0003DMP400055136 and PGSC0003DMP400067047 were strongly induced in all treatments by both

23   *Fusarium sulphureum* (*Fs*) and *Phytophthora infestans* (*Pi*) inoculation. The present study provides valuable

24   information for LecRLKs gene family in potato genome, and establishes a foundation for further research into

25   the functional analysis.

26   **Keywords** Potato; Lectin receptor-like kinase (LecRLKs); gene duplication; expressional pattern; RNA-seq-based-

27   transcriptomics; fungal disease resistance

1. Introduction

29 Unlike animals, plants lack the basic survival ability to escape from the danger of environmental fluctuations.

30 However, receptor-like kinases (RLKs), as one of the largest receptors, allow the plants to communicate between

31 cells and to interact with the environment [1]. RLKs also have an important role in response to biotic and abiotic

32 stresses [2]. Typical RLKs comprise an extracellular ligand binding domain in N terminus and an intercellular

33 cytoplasmic kinase domain in C terminus, and the two regions are connected via a transmembrane domain [3].

34 Based on sequence analysis of the variable extracellular domain and the kinase domain, a total of 747 RLKs

35 have been categorized into 52 subfamilies [4]. However, Lectin receptor-like kinase (LecRLK) family was first

36 described early in 1996 [5]. Previous study shows that there are a total of 75 and 173 LecRLKs in *Arabidopsis*

37 and rice, respectively [3]. Expression of the LecRLK proteins have been reported in *Lombardy poplar* [6], *Oryza*

38 *sativa* [7], *Nicotiana benthamiana* [8], *Nicotiana tabacum* [9], *Nicotiana attenuata* [10] *Medicago truncatula*

39 [11] and *Pisum sativum* [12], although no LecRLK has been found in yeast and humans [11].

40 Depending on the variability of N terminal lectin domain, LecRLKs has been classified into three types:

41 G-, L- and C-type [13]. G-type LecRLKs possess α-D-mannose specific plant lectins. Many G-type LecRLKs

42 also contain a cysteine-rich epidermal growth factor (EGF) domain and/or a plasminogen/apple/nematode (PAN)

43 domain. However, EGF and PAN domains are only found in the G-type LecRLKs. The L-type LecRLKs contain

44 a characteristic legume-lectin domain, which are similar with soluble lectin proteins found in leguminous plants

45 [14]. The C-type LecRLKs are thought to be homologues of calcium-dependent lectin domain. Previous study

46 has identified 32 G-type and 42 L-type LecRLKs in *Arabidopsis* and 100 G-type and 72 L-type members in rice.

47 However, results showed that both *Arabidopsis* and rice have only one gene encoding C-type LecRLK protein

48 [15].

49 LecRLKs have been reported to play a diverse role in plant multiple development stages, including seed

50 germination, senescence, responses to wounding and salinity [16,17]. Moreover, LecRLKs have been believed

51 to be involved in tolerance to biotic and abiotic stresses, especially in pathogen attack [18-21], which is similar

52 to the role of RLKs reported by Nazarian‑Firouzabadi et al. (2019) [2]. Transcript-level analyses revealed

53 variable gene expression responses to diverse abiotic stresses, such as salt, drought, wounding and extreme

54 temperatures [3,16]. The inhibition of LecRK-b2 can result in salt- and osmotic stress-insensitive root elongation

55 [22]. The overexpression of *Arabidopsis thaliana* L-type lectin-like protein kinase 1 (*AtLPK1*) can enhance seed

56 germination and cotyledon greening under high salinity conditions [23]. The overexpression of *Glycine sojaLec*

57 *RLK* (*GsSRK*) and *Pisum sativum LecRLK* (*PsLecRLK*) can enhance salt tolerance [17,24]. The LecRLKs are

58 also considered to be potential plant immune receptors, which play fundamental role in microbe- and plant-

59 derived molecular patterns associated with pathogen defense as the key pattern-recognition receptors

60 [19,21,25,26]. Several LecRLKs played role in conferring resistance to rice against fungal pathogen [7] and

61 bacterial pathogens [19].

62 Potato (*Solanum tuberosum L.*) is considered as one of the valuable food security crops in the word, with a

63 global production of 375 million tons in 2018 [27]. The cultivated potato ranks fourth food crops planted and

64 consumed worldwideafter rice, wheat and maize [28]. China, India, and Russia are principal potato producers

65 [27]. However, because of the spread of pathogenic microorganisms, crop capacity and quality of potatoes

66 produced by these countries are far behind those in developed countries. Date showed that the total annual potato

67 loss due to bacteria/fungi and viruses worldwide is 14% and 7%, respectively [29]. In Gansu province of China,

68 late blight and dry rot, caused by *Phytophthora infestans* (*Pi*) and *Fusarium sulphureum* (*Fs*), respectively, result

69 in huge losses in yield [30,31].

70   With the development of high-throughput sequencing technology, the transcriptome of potato and some

71   other plants have been published, which have laid a foundation for the identification of large gene families.

72   Besides *Arabidopsis* and rice, LecRLKs gene have been identified in different plants including *Populus*,

73   soybean, shrub moss and *Eucalyptus* [32]. However, limited information is available about LecRLKs in potato.

74   In the current study, we firstly conducted a comprehensive bioinformatics analysis of the LecRLKs in potato

75   genome. The evolutional characteristics were investigated through phylogenetic relationship, chromosomal

76   distribution and gene duplication events. Moreover, based on the published expression data and quantitative real-

77   time PCR (qRT-PCR), the expressional patterns of the family members were analyzed during potato against to

78   biotic stresses, which provided a novel insight for the functional analysis of the LecRLK gene family in potato.

79 **2. Methods**

80 **2.1 Annotation of LecRLKs**

81 The amino acid and nucleotide sequence data of Potato (*Solanum tuberosum*) v3.4, *Arabidopsis thaliana*

82 Araport11, Rice (*Oryza Sativa Japonica* Group) IRGSP-1.0, Apple (*Malus domestica*) GDDH13 v1.1, and

83 Tomato (*Solanum lycopersicum*) SL2.50 were downloaded from the Potato Genome Sequencing Consortium

84 (PGSC) Public Data (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml), The *Arabidopsis*

85 Information Resource (TAIR) (https://www.arabidopsis.org), the Ensembl Genomes

86 (http://ensemblgenomes.org/), the genome database for *Rosaceae* (GDR) (https://www.rosaceae.org/), and the

87 *Solanaceae* Genomics Network (https://solgenomics.net/), respectively. Proteins contained kinase domain

88 (PF00069) or Pkinase_Tyr (PF07714) in above five species were following the method described by Zhu et al.

89 (2018) [33]. After that, sequences contained a N-terminal domain B_lectin (PF01453), Lectin_legB (PF00139)

90 or Lectin_C (PF00059) were considered as LecRLKs. All of the candidate proteins were determined by using

91 online software Pfam (http://pfam.xfam.org/) and SMART (http://smart.embl-heidelberg.de/) (E-value $< 10^{-5}$) to

92 ensure the presence of N-terminal lectin domain, a transmembrane region and C-terminal kinase domain in each

93 putative member.

94 **2.2 Multiple sequence alignment, phylogenetic analysis, physical and chemical properties and subcellular**

95 **localization classification**

96 The sequences of all LecRLKs from six species were aligned using ClustalX 2.0 with default parameters [34]

97 and a phylogenetic tree was constructed using the Neighbor-Joining (NJ) method in MEGA 7 [35]. The

98 parameter partial deletion and P-distance Model were used with 1000 bootstrap replicates. The protein theoretical

99 molecular weight (MV) and theoretical isoelectric point (pI) were predicted using ProtParam

100   (https://web.expasy.org/protparam/)[36]. The subcellular localization of proteins was predicted using the WOLF

101   PSORT (https://www.genscript.com/wolf-psort.html) and CELLO v2.5: subcellular Localization predictor

102   (http://cello.life.nctu.edu.tw/).

103   **2.3 Analysis of chromosomal distribution, gene duplication and Gene Ontology (GO) overview**

104   The chromosomal positions of potato LecRLKs (*St*LecRLKs) were obtained from PGSC

105   (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml) and the chromosomal distribution were

106   visualized by using software MapDraw 2.1 [37]. Tandem duplicated genes are defined as those genes that (1)

107   are ≤ l0 genes apart, (2) belong to the same phylogenetic group, and are (3) within 100 kb [38]. The Whole-

108   Genome Duplication (WGD)/segmental duplication was calculated by MicroSyn software [39]. The projected

109   GO annotation for the candidate *St*LecRLK genes was then analyzed for enrichment of GO terms using GOEast with

110   default settings [40]. For *cis*-acting element analysis, genomic DNA sequences in the promoter region (-1500 bp)

111   were downloaded from PGSC and then scanned for in the PlantCARE database

112   (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [41].

113   **2.4 Expression patterns of *St*LecRLKs in response to biotic infection**

114   To investigate the responses of *St*LecRLKs to biotic stress, expression data were obtained from the published

115   studies, including potato infected with pathogenic bacteria *Candidatus Liberibacter solanacearum* (*Cls*) [42],

116   *Pectobacterium carotovorum subsp. brasiliense* (*Pcb*) [43] and *Ralstonia solanacearum* (*Rs*) [44], fungi

117   *Phytophthora infestans* (*Pi*) [45], and *Fusarium sulphureum* (*Fs*) [46]. The expression pattern was extracted by

118   the accession number of *St*LecRLKs. False Discovery Rate (FDR) < 0.05 and |log2 (fold change)| ≥ 1 were used

119   as a threshold to judge the significance of the difference in gene expression. The heatmap was visualized by

120   using software Multi experiment viewer software (MeV 4.9.0) (http://mev.tm4.org/).

121 **2.5 Plant materials, treatment and gene expressional assay**

122 Potato cultivar Helan 15 (Favorita, Qingshenshu 2007001) were cultured in the greenhouse with 24°C. After 5

123 weeks cultivation, leaf tissues were collected, sterilized with 75% ethanol and transferred into a petri dish with

124 a diameter of 9 cm. To maintain humidity, a wet filter paper was placed in each petri dish. *Pi* strain "HB09-16-

125 2 (race 1.2.3.4.5.6.7.9.10.11, collected from Hubei Province, China)" were friendly supplied by Prof. Tian

126 Zhendong, Huazhong Agricultural University. The pathogen was cultured at 19°C for two weeks. The conidia

127 were collected and adjusted to a concentration as 7×10^4 sporangia per milliliter. Inoculation was performed

128 following the method described by He et al. (2018) [46]. For the control, the conidia suspension was replaced

129 with the same volume of sterilized water. Inoculated and control tissues were collected in triplicate at 1 d, 4 d, 6

130 d, and 8 d after inoculation and quick-frozen in liquid nitrogen and stored at -80°C.

131 The method of inoculation of *Fs* was performed as described by Li et al. (2009) [47]. Longshu No.7

132 (Guoshenshu 2009006), without physical injuries or visible infection, was used as the material for the inoculation

133 for 1 d, 2 d, 3 d and 4 d. Before treatment, tubers were surfaced-disinfected with 2% sodium hypochlorite for 3

134 min, and then rinsed with tap water and air-dried.

135 RNA isolation, reverse-transcription and quantitative real-time PCR (qRT-PCR) were performed following

136 the method described by Zuo et al. (2018) [48]. Sequences of primers and actin genes could be found out in File

137 S1. The relative expression levels of each gene were calculated based on the $2^{-\Delta\Delta CT}$ method [49].

138 The above samples at different treatment time were collected from three independent replicates. Each

139 experimental unit consisted of three plants or tubers, three leaves per plant and each leaf or tuber with one

140 inoculation site. The data were statistically analyzed using software Origin 8.0 and the significant differences

141 were analyzed using the t-test (*P<0.05; **P<0.01).

142 **3. Results**

143 **3.1 LecRLKs annotation and classification**

144 Based on domain screening, a total of 113 transcripts encoding LecRLKs were identified in potato, including 85

145 G-type, 26 L-type and 2 C-type members (File S2). For some transcripts, two or more transcripts had the same

146 genome loci and corresponded with the same gene accession, but most of these showed the distinct sequences.

147 Therefore, we determined the number of LecRLKs as the transcript's numbers. With the same criteria, 80, 76,

148 139 and 167 LecRLKs were annotated in Tomato (*Solanum lycopersicum*), *Arabidopsis*, Rice (*Oryza sativa*) and

149 Apple (*Malus domestica*), respectively. From the published study, we also re-identified the LecRLKs in different

150 plant species, including *Populus*, *Eucalyptus*, Shrub, Soybean, Corn and Moss (Table 1; [32]). To ensure the

151 uniformity of the obtained data, domains of all sequences were determined based on the same criterion as above.

152 We excluded several proteins which did not contain typical domains of LecRLKs (File S2). Compared to Moss,

153 the member of G-type and L-type LecRLKs was much larger in other plants, and the number of G-type LecRLKs

154 was exceed that of L-type LecRLKs except for Shrub and *Arabidopsis*. There were only one or two C-type

155 LecRLKs in all the detected plants. Over half of LecRLKs were classified as G-type except in Shrub,

156 *Arabidopsis*, Corn and Moss. In potato, tomato, apple and *Populus*, G-type LecRLKs accounted for more than

157 70% of the LecRLKs, which suggested that the G-type LecRLKs were rapidly expanded in potato, tomato and

158 *Populus* than in the other detected plants.

159   To further investigate the classification and evolution relationships of LecRLKs, phylogenetic tree of three

160 independent types (G-type, L-type and C-type) were constructed separately based on the multiple sequence

161 alignment of the full-length amino acid sequences from five plant species, including potato, tomato, apple,

162 *Arabidopsis* and rice. For G-type LecRLKs, the members could be subdivided into five clades (G-I to G-V)

163 (Figure 1). Clades G-I, G-Ⅱ, G-Ⅳ and G-V contained LecRLKs family members from all the tested plants,

164 while G-Ⅲ only contained members from rice. Potato G-type LecRLKs were discovered from four clades except

165 G-Ⅲ. There were 28, 3, 25 and 29 members in G-I, G-Ⅱ, G-Ⅳ and G-Ⅴ clade which accounted for 25.93%,

166 7.14%, 25% and 26.61% in each subgroup respectively.

167   L-type LecRLKs was subdivided into eight clades (Figure 2). L-V had two *Arabidopsis thaliana* LecRLKs.

168 L-Ⅵ contained members from four investigated plant species except rice. Other subgroups contained members

169 from all five species. Potato LecRLKs located in six clades except L-I and L-V. C-type LecRLKs could be

170 divided into four subgroups (Supplementary Figure 1), in which subgroup Ⅳ contained members from potato

171 and tomato. For the other subgroups, each of these only contained members from one single plant species. Taken

172 together, these results indicated the distinctly evolutional process of LecRLKs in plants. The division of each

173 clade was supported by high bootstrap values.

174 **3.2 *St*LecRLKs classification, domain organization, physicochemical properties and gene ontology (GO)**

175 **analysis**

176 To further confirm the classification and evolutionary relationship of *St*LecRLKs, phylogenetic tree generated

177 from the alignment result of the full-length amino acid sequences (Figure 3 and File S3). *St*LecRLKs could be

178 divided into seven clades, including C-type, L-type, G-I, G-II, G-III, G-IV and G-V, which contained 2, 26, 28,

179 3, 8, 15 and 31 members, respectively.

180   Domain organization was also investigated for each subgroup of *St*LecRLKs (File S2). Unique N-terminal

181 domain was discovered from both L-type (Lectin_legB) and C-type members (Lectin_C). However, complex

182 domain composition was found from G-type LecRLKs. Besides B_lectin domain, multiple additional N-terminal

183 domains were detected, such as S_locus_glycop (PF00954), PAN_2 (Pfam accession number was not detected)

184 and EGF (SM000181). Additionally, two intracellular domains, DUF3660 (PF12398) and DUF3403 (PF11883)

185 were observed from some members. Domain DUF3403 was in C-terminal of specific protein, but its function is

186 still unknow. Above all, G-type *St*LecRLKs, being the largest group, showed a more complex evolutionary

187 relationship and domain organization than the other types.

188 After determining the conserved domains of *St*LecRLKs, the physicochemical properties and subcellular

189 localization of these proteins were also analyzed (File S4). The results showed that 113 *St*LecRLKs had an amino

190 acid size ranging from 419 to 1504 aa, a molecular weight ranging from 47.07 to 169.51 kDa, and pI value

191 ranging from 5.02 to 9.26. The subcellular localization of 113 *St*LecRLKs were predicted by the online software

192 CELLO and PSORT. 87 *St*LecRLKs were located in the plasma membrane, two in the cytoplasm, one was in

193 the extracytoplasmic surface and three in both plasma membrane and cytoplasm. Moreover, there were 20

194 *St*LecRLKs with inconsistent prediction results from the two software. Further, 15 and 20 GO terms were

195 distributed into molecular function and biological process (File S5). Within biological process, a larger number

196 of family members involved in "metabolism process", "cell recognition", and "protein modification process".

197 For the molecular function categories, genes were mainly associated with "kinase activity", "catalytic activity",

198 "transferase activity" and "binding".

3.3 Chromosomal distribution, gene duplication

200 Tandem duplication mechanism represents a common role in the expansion of RLK family. To identify the gene

201 clusters and to investigate such events, we scanned the gene ID and the corresponded protein ID of *St*LecRLKs based

202 on PGSC database (File S2). For several accessions, unique gene accession was corresponded to two or more protein

203 accessions. For instance, gene PGSC0003DMG400019237 encoded three proteins, including

204 PGSC0003DMP400033418, PGSC0003DMP400033419 and PGSC0003DMP400033420. Finally, 88 of the 113

205   *St*LecRLKs was visualized on chromosome across 12 chromosomes (Figure 4). There were 15, 10, 9, 20, 11 and 7

206   members scattered on Chr 02, 03, 04, 07, 09 and 10, respectively. Additionally, 15 tandem duplicated gene clusters

207   (marked in red vertical lines) and five WGD/segmental duplicated gene pairs (marked in blue dotted lines) were found

208   from *St*LecRLKs. The tandem duplicated gene clusters contained 47 *St*LecRLKsconsisting of 40 G-type (black) and

209   7 L-type (green). On the other hand, WGD/segmental duplicated gene pairs contained seven G-type and two L-type

210   *St*LecRLKs. Above results indicated that tandem duplications played important roles in the amplification of the

211   *St*LecRLKs gene family.

212   **3.4 *Cis*-elements involved in *St*LecRLKs**

213   In order to further explore the functions of the candidate *St*LecRLKs, a 1500 bp promoter region was

214   extracted and PlantCARE was used to analyze the *cis*-elements related to stress challenge. A great number of

215   *cis*-elements responsive to defense and stress, phytohormines (abscisic acid (ABA), methyl jasmonate (MeJA)

216   and salicylic acid (SA)), biotic stressors (elicitor) and abiotic stressors (drought, low temperature and wounding)

217   were identified in the promoter region of *St*LecRLKs (Figure 5 and File S6). Of these, the number of genes

218   which had the *cis*-element involved in ABA-, MeJA- and SA-responsiveness were 57, 57 and 34, respectively.

219   Most of the *St*LecRLKs belonged to the phylogenetic group G-I, G-V and L-type. Additionally, some

220   *St*LecRLKs had elements responsive to abiotic stress, and several members possessed elements responsible for

221   elicitor. The above results indicated that *St*LecRLKs had a potential role in response to signals from

222   phytohormones and multiple stresses (Figure 5).

223   **3.5 Expression patterns of *St*LecRLKs in response to biotic stresses**

224   Based on the published transcriptomic datasets, the expressional patterns of *StLecRLK*s were investigated during

225   potato infected with phytopathogenic bacteria [42-44] and fungi [45,46]. For the bacteria, expression changes of

226   *StLecRLK*s were extracted after potato inoculated with *Cls*, *Pcb* and *Rs* (Figure 6). A total of 55 *St*LecRLKs were

227   differently expressed, including 15 members in G-I, 2 in G-III, 10 in G-IV, 17 in G-V and 11 in L-type, respectively.

228   Furthermore, 28 *St*LecRLKs were detected to be up-regulated under at least one bacterial infection.

229   Additionally, expression patterns of *St*LecRLKs were extracted from published transcriptomic data of potato

230   tuber [50] and leaf [45] inoculated with *Pi* (Figure 7). A total of 26 members, including nine members in G-I (32.14%),

231   two in G-Ⅲ (25.0%), two in G-Ⅳ (13.33%), five in G-Ⅴ (16.13%) and eight in L-type (30.77%) were differentially

232   expressed (Figure 7). PGSC0003DMP400030282 showed the same expressional pattern among resistant tuber and

233   leaves. However, PGSC0003DMP400032146 and PGSC0003DMP400055136 in G-I and PGSC0003DMP400045382

234   in G-IV were detected to be down-regulated under bacteria attack while they were up-regulated under fungi infection.

235   PGSC0003DMP400067047 in G-IV was down-regulated under both bacteria and fungi infection (Figure 6 and Figure

236   7). Combined with the above results, a great number of G-I, G-V and L-type *St*LecRLKs were differentially expressed

237   after potato tissue inoculated with both phytopathogenic fungi and bacteria, which indicated the potential roles of

238   these members in potato disease resistance.

239   **3.6 Expression patterns of *St*LecRLKs during potato infected with *Fs* and *Pi***

240   To further validate the regulating roles of the candidate *St*LecRLKs to fungal disease resistance, the expression

241   analysis of *St*LecRLKs during potato inoculated with *Fs* (Figure 8) and *Pi* (Figure 9) were detected. Two or three

242   members in each phylogenetic group were selected, which were strongly affected under the biotic stress treatment

243   (Figure 6 and Figure 7). Finally, 16 differently expressed members were selected to perform qRT-PCT analysis (File

244   S7). During *Fs* infection, 10 of the 16 detected *StLecRLK*s were differentially expressed at least one time point (Figure

245   8 and File S7), and they were detected to be up-regulated after treatments. The expression of

246   PGSC0003DMP400067047 was gradually upregulated during the experiment, and the expression level was 26.59

247 times that of the control group at 4 d treatment. When the leaves were inoculated with *Pi,* 12 representative genes

248 were differentially expressed (Figure 9 and File S7). PGSC0003DMP400041968, PGSC0003DMP400049549 and

249 PGSC0003DMP400055136 were significantly induced at the later stage (T4), and the expressional levels were 12.33,

250 430.80 and 34.08 times that of the control group. PGSC0003DMP400030282, PGSC0003DMP400001977 and

251 PGSC0003DMP400045383 were significantly induced when the leaves were treated for 6 d (T3).

4. Discussion

LecRLKs are considered as important regulators for external stimuli, such as environmental stresses and pathogen attack [51]. Previous literatures reported the identification, evolution and function of LecRLKs in several plant species [3,32,52-55]. Currently, a comprehensive analysis of the LecRLK family in potato was carried out, and 113 LecRLK genes including 85 G-type, 26 L-type and 2 C-type members were identified and analyzed. Furthermore, investigations for classification, evolution, gene ontology, *cis*-elements and expression patterns revealed several important features of the family members.

4.1 Evolution and expansion of *St*LecRLKs

Besides the differences in genome size, expansion rate is one of the most important contributors to the copy numbers of plant LecRLKs [54]. Compared to Moss, total numbers of LecRLKs were drastically expanded in higher plants (Table 1), which mainly resulted from the expansion of the G-type LecRLKs members, followed by L-type. In potato, tomato, apple and *Populus*, about three-quarter of LecRLKs were identified as G-type members, although L-type LecRLKs were more expanded than G-type members in several species such as Shrub, *Arabidopsis* and Corn (Table 1; [3]). The results showed that different plant species had different numbers of LecRLKs, which was possibly related with the different growth condition and growth behaviors, or maybe with the plant life cycles and the mode of reproduction. Yang et al. (2016) also speculated that the difference in the number of G-type and L-type LecRLKs between *Arabidopsis* and *Populus* may be related with the fact that *Arabidopsis* is a self-fertile plant whereas *Populus* is an obligate outcrossing plant [32]. In our study, the number of LecRLK in *Arabidopsis* and rice is different from the previous researchers, which may be due to the perfection of genome sequence or the difference of identification standards (Table 1).

Phylogenetic analysis revealed the distinct evolutionary process in plant species (Figure 1, 2 and File S1).

273   The rice specific clade was found both in G-type (G-III) and L-type (L-I) LecRLKs. Therefore, we suggested the

274   distinct expanded characteristics between G-type and L-type LecRLKs in plant species. Compare to L-type LecRLKs

275   in potato, more G-type members were found, indicating the crucial roles of G-type LecRLKs in development and

276   environmental response.

277   **4.2 Gene duplications and functional analysis**

278   Duplication can lead to functional divergence of genes, which is a universal phenomenon in plants [56].

279   LecRLKs duplication were mainly resulted from tandem duplication and segmental duplication/WGDs

280   [32,38,57]. In potato, 40 G-type *St*LecRLKs were involved in tandem duplication but only seven members in

281   WGD/segmental duplication (Figure 4). Similarly, there were more tandem duplicated L-type *St*LecRLKs than that in

282   segmental duplication/WGD (Figure 4). Hence, the tandem duplication might be the main reason for expansion of

283   both G-type and L-type *St*LecRLKs. Compared to RLKs involved in development, stress responsive RLKs showed a

284   higher number of tandemly duplicated genes. Based on the above conclusion, we deduced that *St*LecRLKs also took

285   important roles in potato responses to biotic and abiotic stress. Furtherly, we used GO method to analyze the molecular

286   function of these genes. The results showed that the enriched GO terms were kinase activity, catalytic activity,

287   transferase activity and binding (phosphate binding and carbohydrate binding) (Figure 5). Since chitin and β-1,3

288   glucans are components of the cell walls of some higher fungi, some plant lectins sharing chitin-binding domains can

289   recognize the signal then naturally these substrates will be hydrolysed and fungal growth will be disrupted [58]. This

290   type of lectin could inhibit the growth of several phytopathogenic and saprophytic chitin containing fungi in vitro [59].

291   Moreover, the *cis*-element prediction furtherly demonstrated that *St*LecRLK*s* have the potential role against multiple

292   stresses (Figure 5 and File S6). Previous study confirmed LecRLKs participated in the regulation of abiotic stress

293   [17,19,60] and biotic stress [7,61].

4.3 Complex domain architecture and organization of G-type *St*LecRLKs

Protein domains are some complex regions of a protein's structure, which often perform some specific function [62]. Architecture and composition of domain of G-type *St*LecRLKs were observed (File S2). In addition to B_lectin domain, most G-type members contained an S_locus_glycoprotein, EGF and PAN_2 or PAN-AP domain, in which PAN domains are involved in interactions of protein-protein and protein-carbohydrate [63]. Furthermore, some G-type *St*LecRLKs also contained intracellular domain DUF3660 or DUF3403. Our result was consistent with the findings in *Populus* although the function of the two domains was largely limited until now [32]. Nevertheless, the complex domain architecture suggested the diverse functions of G-type *St*LecRLKs.

4.4 *St*LecRLKs in response to biotic stresses

Increased investigations reported the crucial role of both G-type and L-type LecRLKs in plant immunity [55, 61,64]. Over-expression of LecRK-V (L-type) significantly enhanced the resistance of Yangmai158 to powdery mildew [65]. Similar studies have been done in rice and tomatoes. *Pid2* (G-type *LecRLK* in rice) and *NbLecRK* (L-type *LecRLK* in tomato) were shown to increase the resistance to fungal pathogen and *Phytophthora*, respectively [7,21]. In this study, we found a large number of *StLecRLK*s were differentially expressed during potato infected both bacterial and fungal pathogens (Figure 6 and 7). Most of these were distributed in clades G-I, G-V and L-type. Genes which contributed to plant resistance to a wide of pathogens could be classified as broad-spectrum resistant genes [66,67]. In *Populus*, the root-expressed *Pt*LecRLKs maybe have the potential function for perceiving signals from soil microbes [32]. Intriguingly, the expression of PGSC0003DMP400030282 was induced under signals from both phytopathogenic bacteria and fungi, which suggested that the crucial roles in broad-spectrum disease resistance of potato, while PGSC0003DMP400055136 and PGSC0003DMP400067047 showed distinct expression, indicating a different role in response to different

315   pathogen signal (Figure 7, 8 and Figure 9).

316   **5. Conclusions**

317   In conclusion, a systematic study was carried out to identify and characterize the LecRLKs family genes in

318   potato. The phylogenetic analysis showed the distinctly evolutionary processes of the family members in

319   different plants. G-type *St*LecRLKs exhibited a rapid enlargement of family members, followed by L-type

320   members. The expansion of both G-type and L-type *St*LecRLKs was mainly resulted from the tandem

321   duplication. In addition, a large number *St*LecRLKs were differentially expressed in response to bacteria and

322   fungi. Among these, PGSC0003DMP400055136 and PGSC0003DMP400067047 were strongly induced by both

323   *Fs* and *Pi*, which could be used as candidate genes for functional analysis of resistance. Our study provides

324   insight into further research on the functions and mechanisms of this important RLK subfamily.

325   **Author Contributions:** ZWN and QSH conceived the study idea, executed its design and coordination. ZWN

326   performed the experiments and wrote the manuscript. ZWN and CZJ collected, analyzed, and deposited the data.

327   FYL, KYC, YXY, SMF, YK and LYH proofread the final draft. All authors have read and approved the

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339   **Availability of data and materials**

340   The data supporting the results of this article are provided as additional files. All the data pertaining to the present

341   study has been included in the tables/figures of the manuscript and the raw data of RNA sequencing has been

342   deposited at the Sequence Read Archive (SRA) database of NCBI (SRS4823473).

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Figure 1

Phylogenetic analysis of G-type LecRLKs from five different plants.

The phylogenetic tree was generated from the alignment result of the full-length amino acid sequences by the neighbor-joining (NJ) method. All *St*LecRLKs members, together with homologues of *Arabidopsis thaliana*, apple (*Malus domestica*), rice (*Oryza sativa*) and tomato (*Solanum lycopersicum*), were classified into five distinct clades. LecRLK subgroups were shown in different colors.

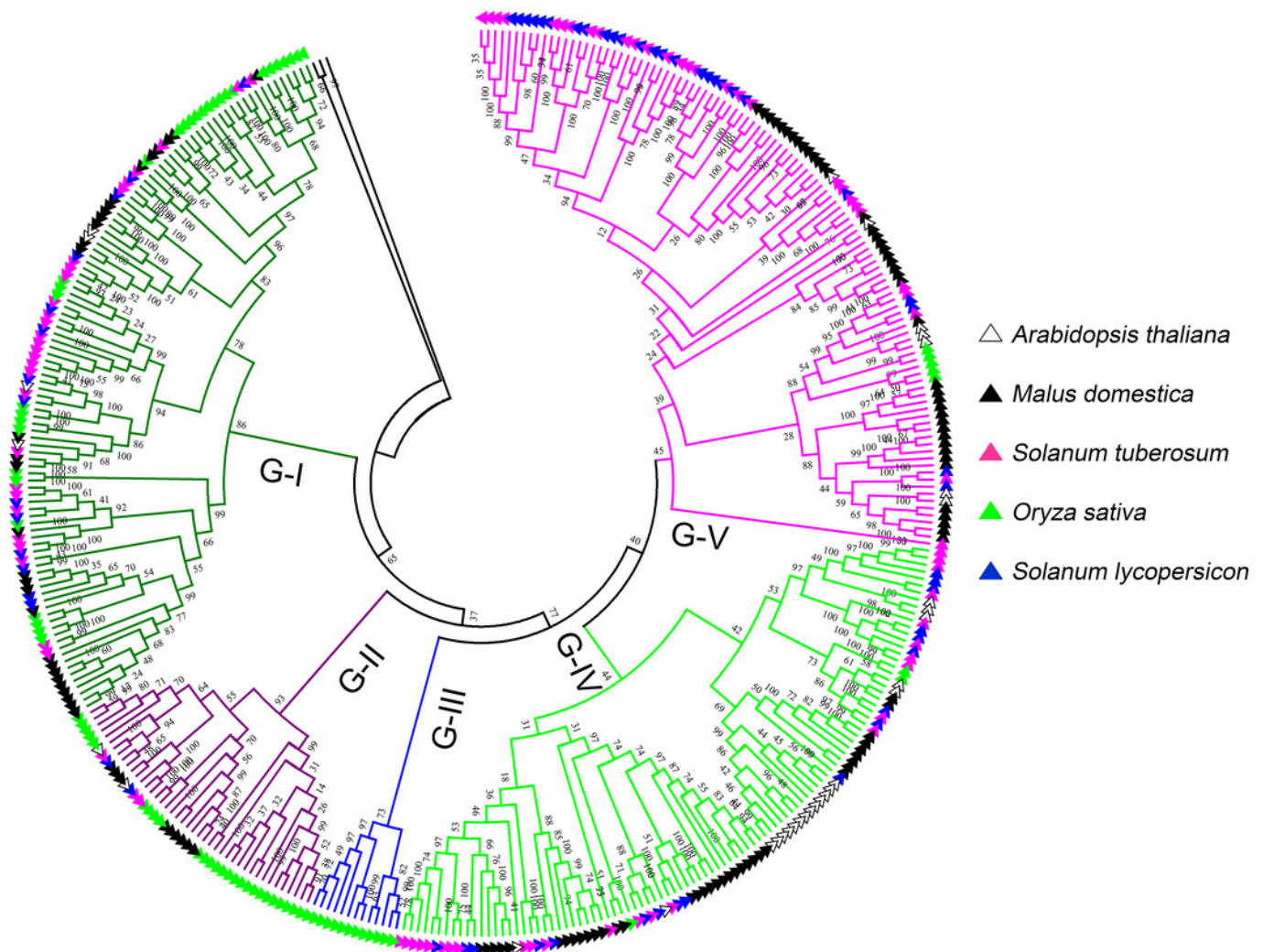



Figure 2

Phylogenetic analysis of L-type LecRLKs from five different plants.

The phylogenetic tree was generated from the alignment result of the full-length amino acid sequences by the neighbor-joining (NJ) method. All *St*LecRLKs members, together with homologues of *Arabidopsis thaliana*, apple (*Malus domestica*), rice (*Oryza sativa*) and tomato (*Solanum lycopersicum*), were classified into eight distinct clades. LecRLK subgroups were shown in different colors.

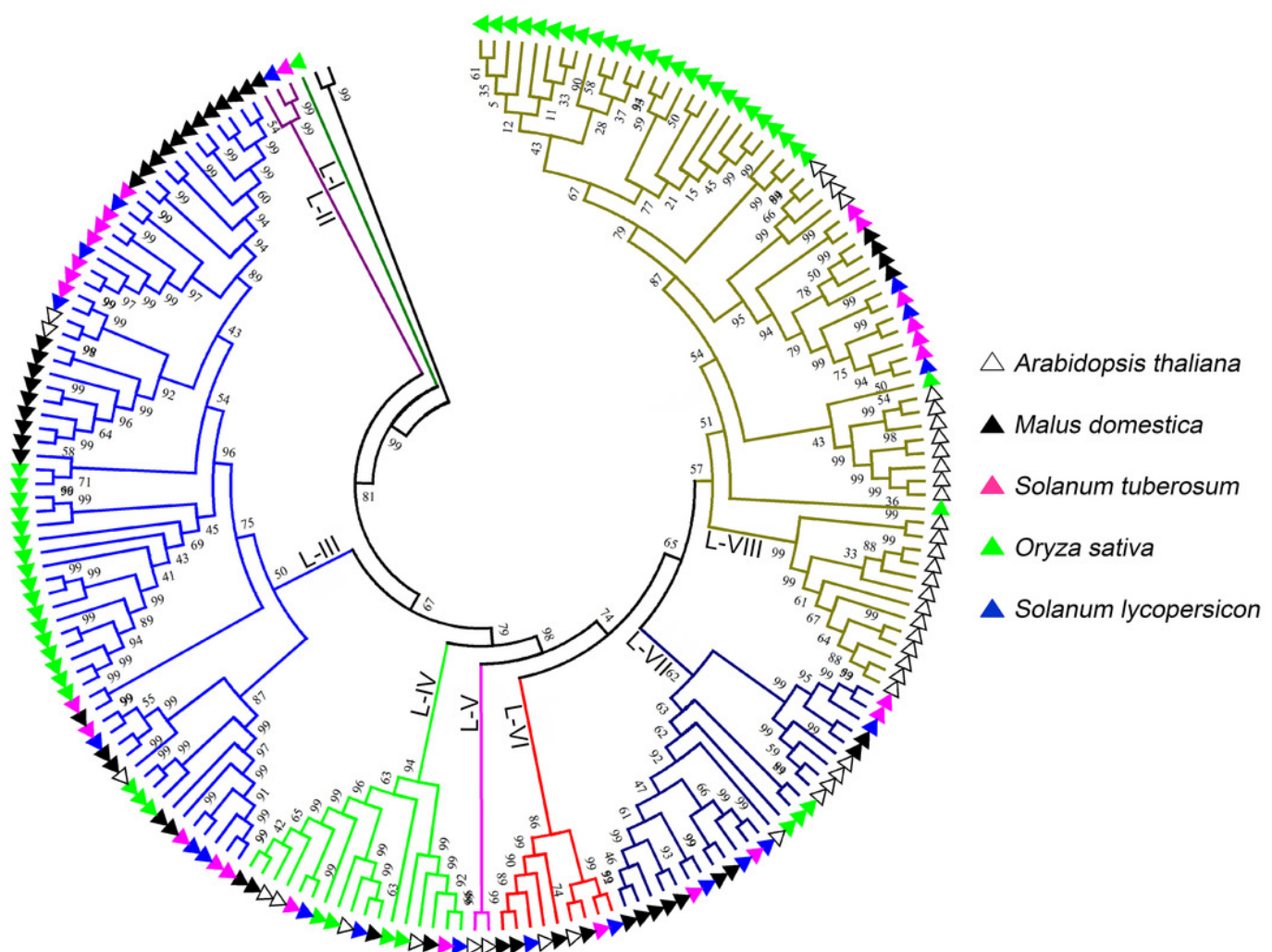



Figure 3

Phylogenetic analysis of LecRLKs in potato (*Solanum tuberosum*).

The phylogenetic trees were constructed using MEGA 7 software. The numbers were bootstrap values based on 1000 iterations. Note that three different types of *St*LecRLKs were categorized clearly in seven clades. Different color was used to distinguish different clades.

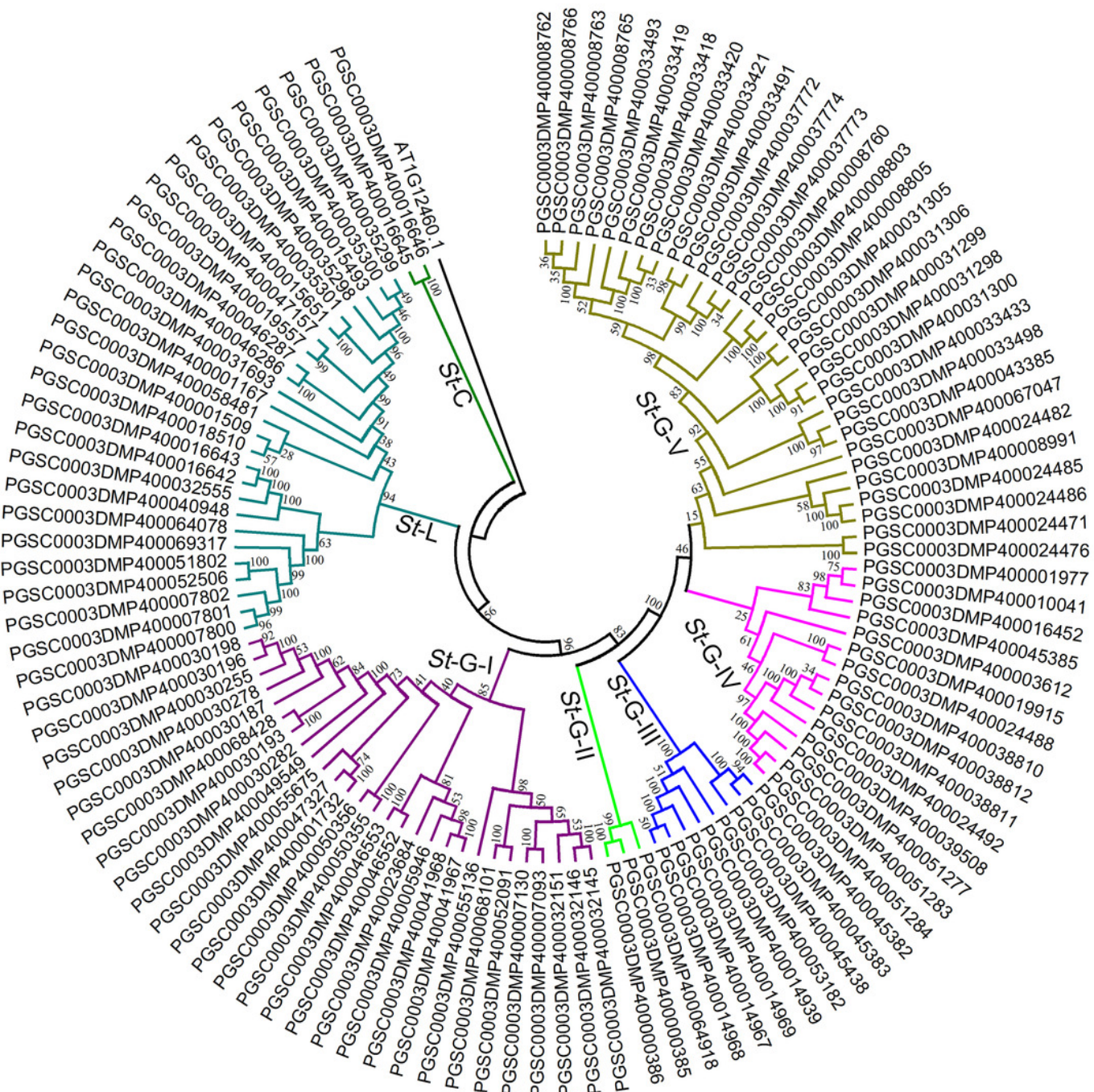



Figure 4

Distribution of LecRLKs genes on potato (*Solanum tuberosum*) chromosomes.

Graphical representation of physical locations for each LecRLK on chromosomes. Tandem duplicated genes on the particular chromosome were depicted by red vertical lines. Chromosomal distances were given in Mb.

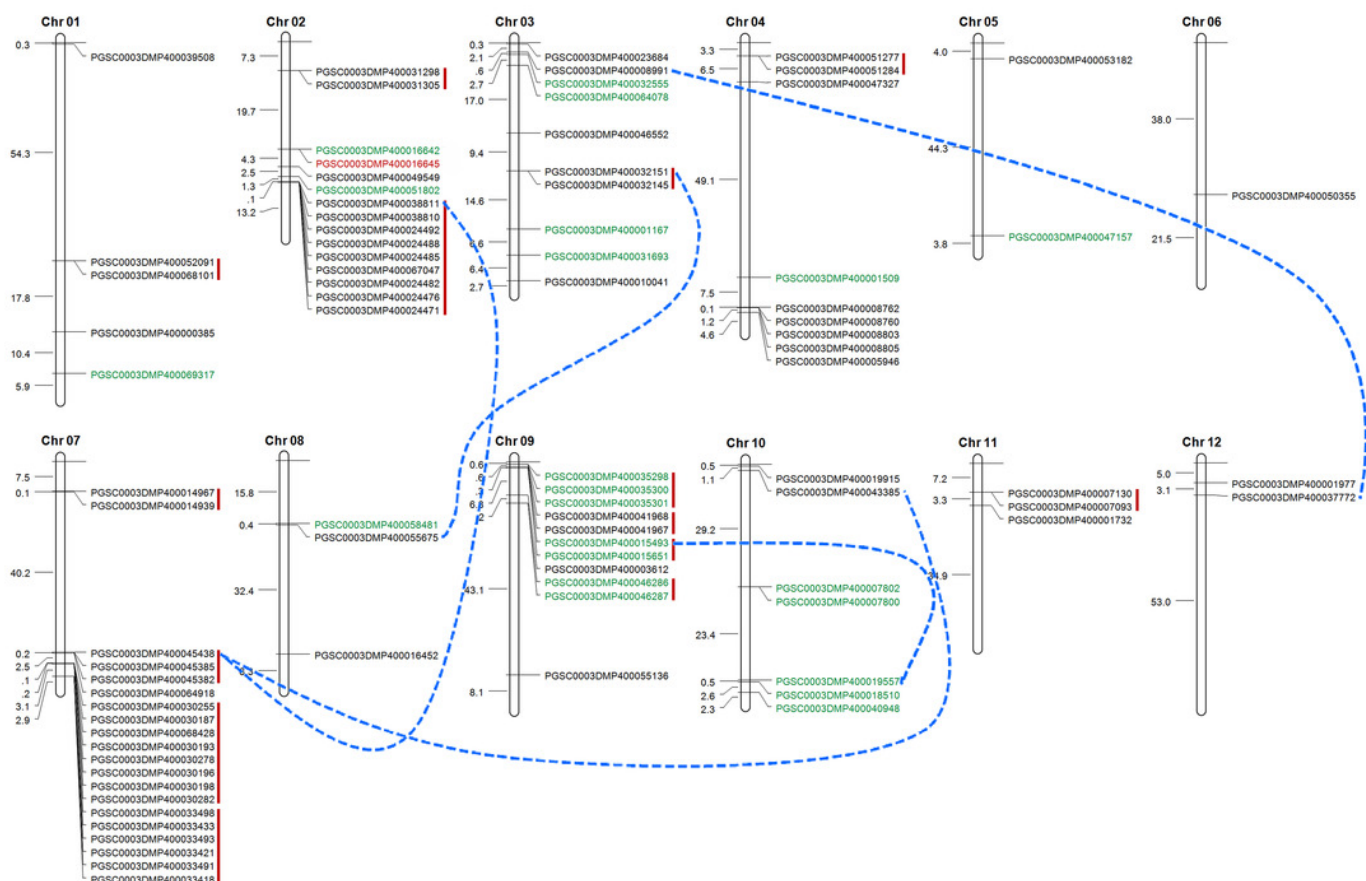



Figure 5

Cis-element detection in the promoter region of *St*LecRLK.

Signals of *cis*-element were responsive for stress challenge, including deference and stress, elicitor, low-temperature, drought, wounding and hormone (SA, ABA and MeJA). Different color was used to distinguish different clades.

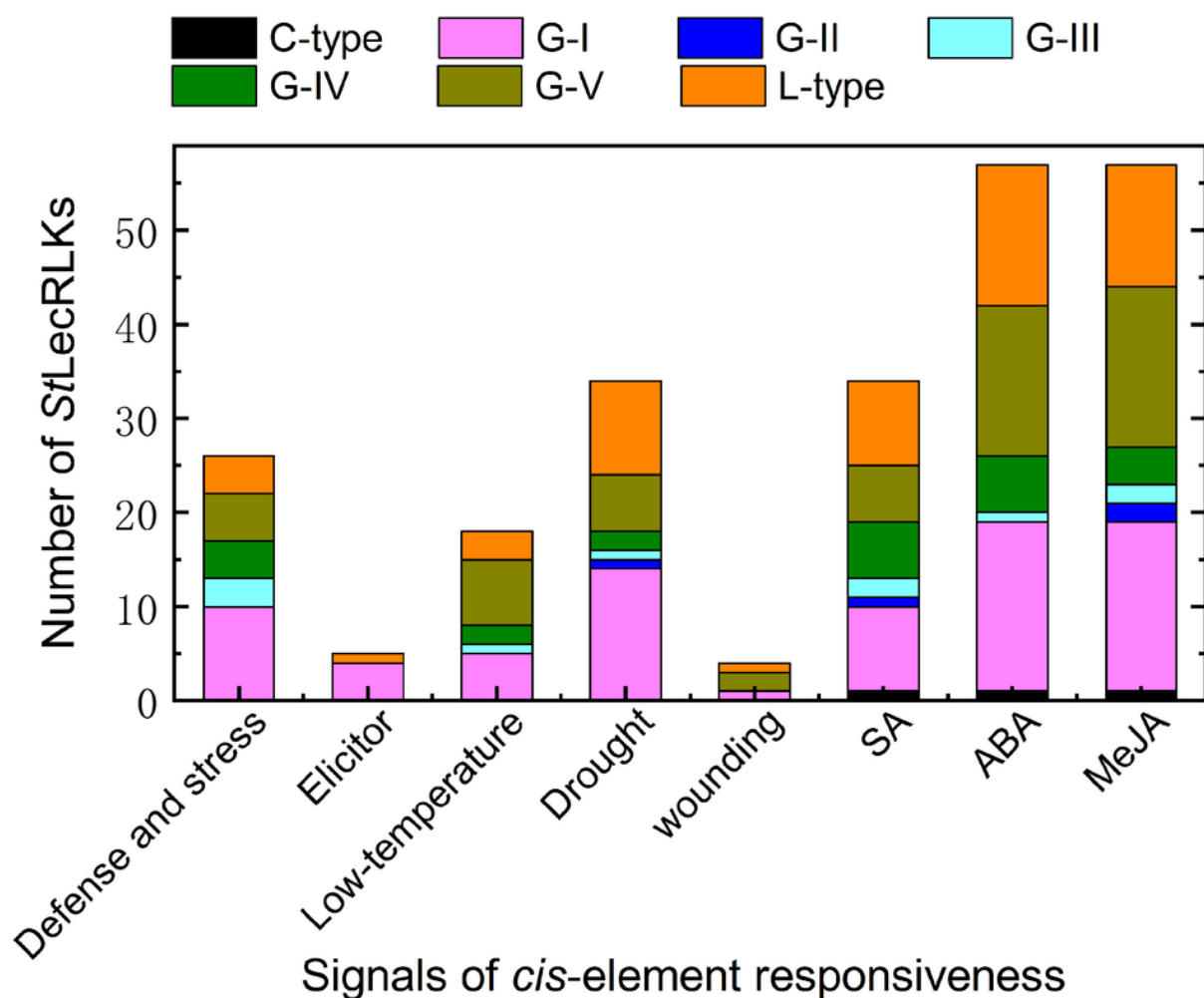



Figure 6

Expression patterns of *St*LecRLKs in response to phytopathogenic bacteria.

The heatmap showed the differentially expressed genes of Waneta potato infected with *Candidatus* Liberibacter solanacearum (*Cls*), *Solanum tuberosum* cv. Valor compared with *S. tuberosum* cv. BP1 at each time-point inoculated with *Pectobacterium carotovorum* ssp. *brasiliense* (*Pcb*), and differentially-expressed genes in inoculated potato roots with *Ralstonia solanacearum* (*Rs*). The classification of genes in phylogenetic trees were listed next to the gene name. Yellow indicated genes that were up-regulated, blue indicated genes that were down-regulated, and white indicated genes that were not changed significantly.

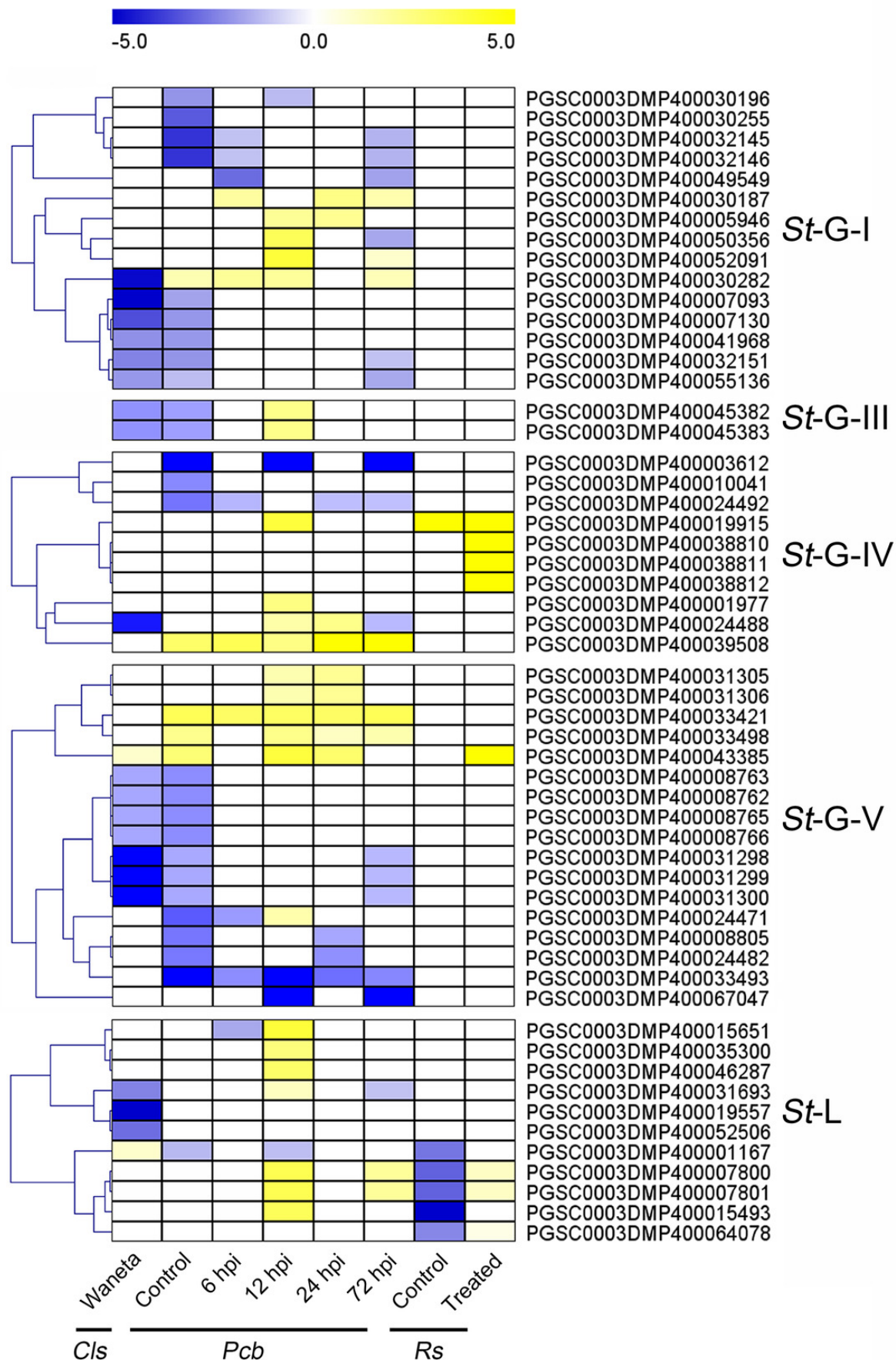



Figure 7

Expression patterns of *St*LecRLKs in response to *Phytophthora infestans* (*Pi*).

The heatmap of differentially expressed genes between F06025 (derived from AWN86514-2 × N06993-13 cross) and F06037 (derived from frontier russet × N1503-19 cross) genotypes, RB (tubers of transgenic 'Russet Burbank' line SP2211 potato) and WT (tubers of nontransformed 'Russet Burbank' (WT) potato) inoculated with *Pi*. The classification of genes in phylogenetic trees were listed next to the gene name. Bins in yellow were significantly up-regulated; bins in blue were significantly down-regulated and bins in white did not change significantly.

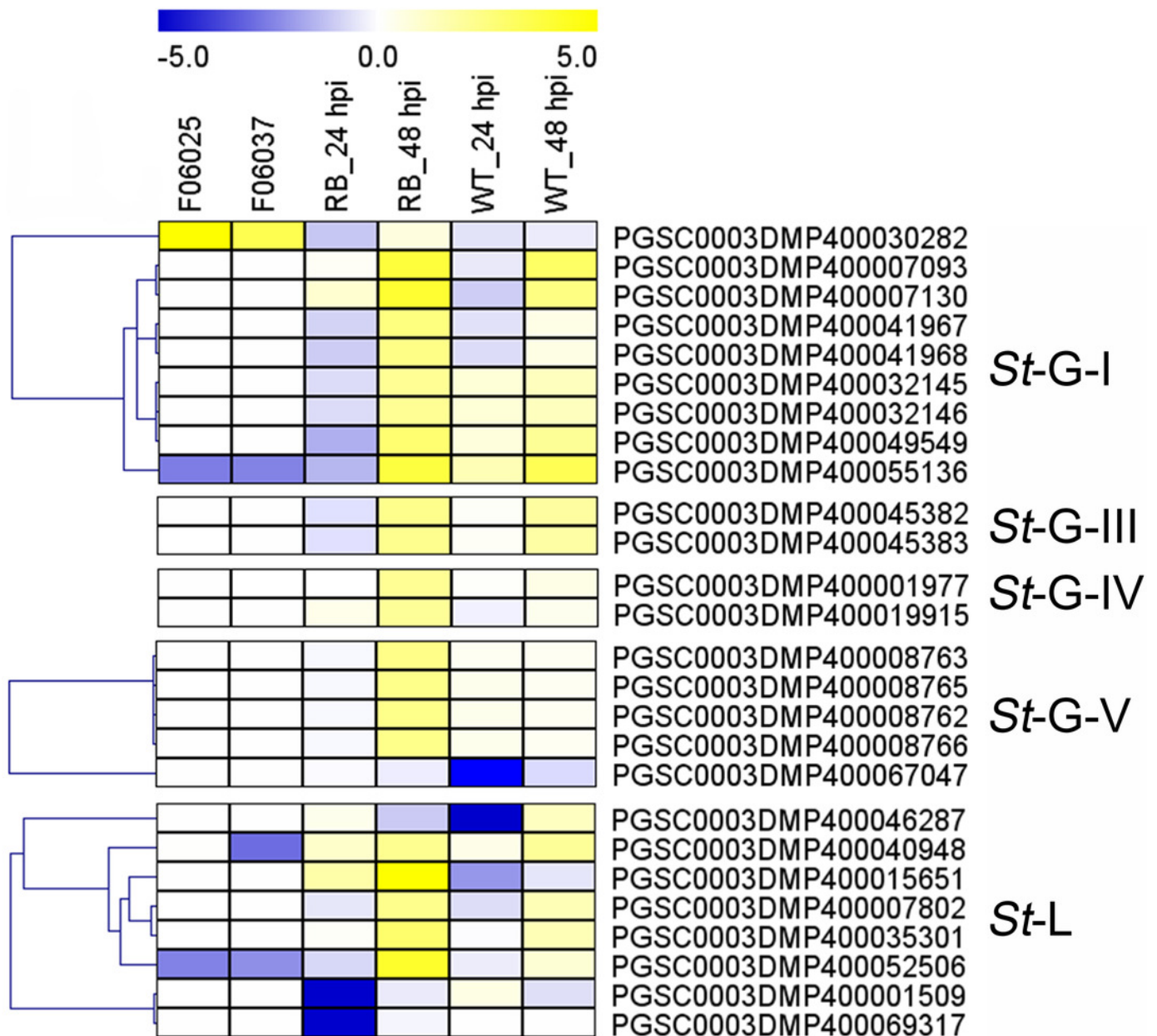



Figure 8

Expressions of 10 *StLecRLK*s during potato inoculated with *Fusarium sulphureum* (*Fs*).

(A-H) represent eight G-type LecRLK genes, in which A-G belonged to G-I clade and H was in G-III clade; (I-J) two L-type LecRLK genes. Samples at 1-, 2-, 3- and 4-days post *Fs* inoculation were collected. The qRT-PCR data presented here were from three independent biological replicates and error bars indicated the standard deviation (SD). The asterisks indicated a significant difference as compared with the group inoculated with sterilized water at each time point (* $p < 0.05$ and ** $p < 0.01$).

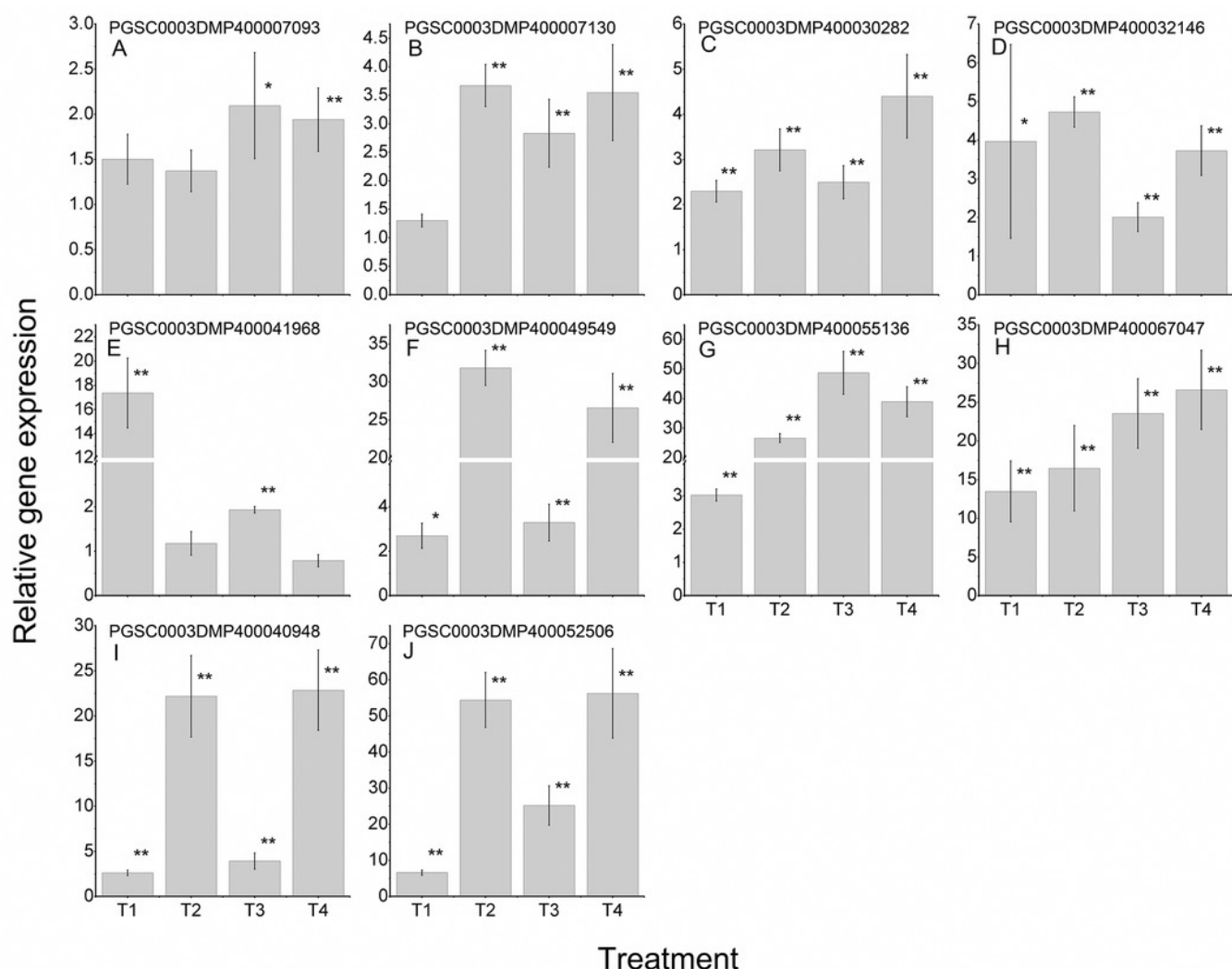
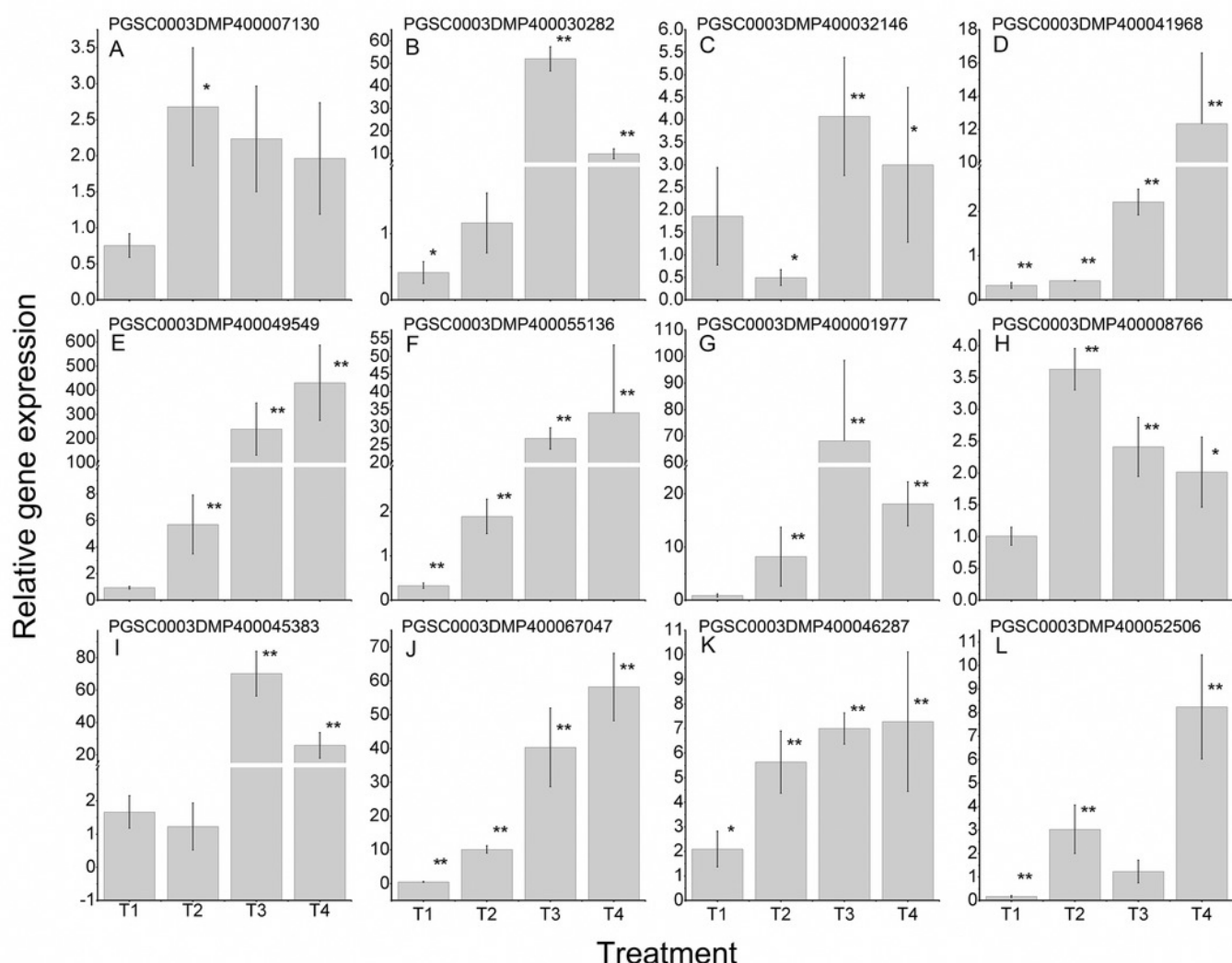



Figure 9

Expressions of 12 *StLecRLK*s during potato inoculated with *Phytophthora infestans* (*Pi*).

(A-J) represent 10 G-type LecRLK genes, in which A-F belonged to G-I clade, G was in G-II clade and H-J were in G-III clade; (K-L) two L-type LecRLK genes. Samples at 1-, 2-, 3- and 4-days post *Pi* inoculation were collected. The qRT-PCR data presented here were from three independent biological replicates and error bars indicated the standard deviation (SD).The asterisks indicated a significant difference as compared with the group inoculated with sterilized water at each time point (*p< 0.05 and **p< 0.01).

The number of G-type, L-type and C-type LecRLKs in 11 plant species.

Plant species	Sub-group			Total
	G-type	L-type	C-type	
Solanum tuberosum	85 (75.22%)	26 (23.01%)	2 (1.77%)	113
Solanum lycopersicon	60 (75%)	19 (23.75%)	1 (1.25%)	80
Malus domestica	118 (71.08%)	46 (27.71%)	2 (1.2%)	166
Populus	180 (77.92%)	50 (21.65%)	1 (0.43%)	231
Eucalyptus	118 (59.6%)	79 (39.9%)	1 (0.51%)	198
Shrub	25 (44.64%)	30 (53.57%)	1 (1.79%)	56
Soybean	123 (65.08%)	64 (33.86%)	2 (1.06%)	189
Arabidopsis thaliana	34 (44.74%)	41 (53.94%)	1 (1.32%)	76
Oryza sativa	80 (57.55%)	57 (41.01%)	2 (1.44%)	139
Corn	46 (48.42)	48 (50.53%)	1 (1.05%)	95
Moss	2 (40%)	1 (20%)	2 (40%)	5