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1 Transcriptional profiling of potato (*Solanum tuberosum* L.) during a compatible interaction with the
2 root-knot nematode, *Meloidogyne javanica*
3

4 **Running title: “” Response of potato (*Solanum tuberosum* L.) to root-knot nematode,**
5 ***Meloidogyne javanica***

6

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14 **Abstract**

15 Root-knot nematode (RKN, *Meloidogyne javanica*) presents a great challenge to *Solanaceae*
16 crops, including the potato. In this report, we conducted an investigation to understand the
17 transcriptional regulation of molecular responses in potato roots during a compatible
18 interaction following RKN infection. In this study, analysis of gene expression profiles using
19 RNA-seq of *Solanum tuberosum* cv Mondial with RKN interaction at 0, 3- and 7-days post-
20 inoculation (dpi). In total, 4,948 and 4,484 genes were respectively detected as differentially
21 expressed genes (DEGs) at 3 and 7 dpi. Functional annotation revealed that genes associated
22 with metabolic process were enriched at the transcriptional level suggesting they have an
23 important role in RKN disease development. Nematode infection caused down-regulation of
24 282 genes associated with pathogen perception hence interfering with activation plant immune
25 system. Further, late activation of pathogenesis-related genes, down-regulation disease
26 resistance genes and activation of host antioxidant system contributed to a susceptible response.
27 Activation of Jasmonic acid (JA) pathway and protease inhibitors was due to wounding during
28 nematode migration and feeding. Nematode infection suppressed ethylene (ET) and salicylic
29 acid (SA) signalling pathway hindering SA/ET responsive genes involved with defense.
30 Induction of auxin biosynthesis genes, regulation of cytokinin levels and up-regulation of
31 transporter genes facilitated of nematode feeding sites (NFSs) initiation. The regulation of
32 several families of transcription factors (TFs) in the plant, such as WRKY, GRAS, ERF BHLH
33 and MYB, was affected by RKN infection disrupting plant defense signalling pathways. This
34 clearly suggest that TFs played an indispensable role in physiological adaptation for successful
35 RKN disease development. This genome-wide analysis revealed the molecular regulatory
36 networks in potato roots which are successfully manipulated by RKN. Being the first study
37 analysing transcriptome profiling of RKN diseased potato, it will provide unparalleled insight
38 into the mechanism underlying disease development.

39 **Key words:** Root-knot nematode, *Solanum tuberosum,* nematode feeding sites, differentially
40 expressed genes

41 **Introduction**

42 Potato, *Solanum tuberosum* (L) belongs to the *Solanaceae* family, which comprises several
43 economically important crops such as tomato, pepper, aubergine, and tobacco. Plant parasitic
44 nematodes particularly root-knot nematodes, are among the most destructive and economically
45 important pests of potatoes worldwide (Scurrah et al., 2005, Jones et al., 2017). In this context,

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46 *Meloidogyne* spp are obligate and highly polyphagous pests that form an intricate relationship
47 with their host causing drastic morphological and physiological changes in plant cell
48 architecture (Gheysen & Fenoll, 2002). A typical life cycle of RKNs spans between 4-6 weeks
49 depending on the nematode species and environmental conditions. Following the embryonic
50 phase, the infective second-stage juveniles (J2s) hatch from the egg. At 3dpi the nematodes
51 have already penetrated the host root tips and migrating towards the elongation zone
52 (Castaneda et al., 2017). At this stage the J2s select target cells to initiate reprogramming of
53 host cells to giant cells (GCs). The nematodes are completely dependent on the induced GCs
54 for supply of nutrients. During the induction stage the parasitic J2 abandons its migratory
55 lifestyle becomes sedentary to concentrate on feeding, development and reproduction (Bartlem
56 et al., 2013). As the GCs enlarge, surrounding cells undergo rapid division causing swelling of
57 roots and discontinuity of the vascular tissue. The sedentary nematode further moults into J3,
58 J4 stages and finally into the adult stage when the nutrient acquisition stage commence from
59 7dpi. The developing, nematode, GCs and surrounding tissue contribute to the formation of
60 RKN symptom (Bartlem et al., 2013). Analogous to other plant pathogens, nematode secretions
61 play a crucial role in manipulation of the host cellular function. Secreted molecules suppress
62 host defense to initiate a successful infection process including establishment and maintenance
63 of NFSs (Hewezi & Baum, 2013). In the genus *Meloidogyne*, several effectors have been
64 reported such as: MiLSE5, which interferes with host metabolic and signalling pathways; or
65 MjTTL5, Misp12 and MgGPP, which suppress the host immune responses facilitating
66 successful nematode parasitism (Vieira & Gleason, 2019).

67 Due to their capability to infect plant species from diverse families, RKN species pose a great
68 challenge to crop production globally (Sasser & Freckman, 1987). In 2014, 22 species of RKN
69 were reported in Africa causing damage to various vegetable and field crops (Onkendi et al.,
70 2014). Both tropical and temperate RKN species are present in potato growing regions of
71 South Africa with *M. javanica* and *M .incognita* being the prevalent species impairing the
72 potato production sector (Onkendi & Moleleki, 2013). For decades the use of nematicides has
73 been effective in managing RKN populations. However, their usage is coupled with adverse
74 effects to the ecosystem. This has led to withdrawal of the most effective nematicides from the
75 agro-markets, further aggravating crop losses due to RKN (Onkendi et al., 2014). Plant host
76 resistance through the use of resistant cultivars is an effective and environmentally safe
77 alternative method of controlling RKN species (Onkendi et al., 2014). Nevertheless, the current
78 cultivated potato cultivar lack resistance against RKN (Dinh et al., 2015). Thus, studies

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79 involving plant-nematode interactions will deepen our understanding of the molecular
80 regulatory networks associated with resistance or susceptibility. The insights drawn from such
81 studies will be useful in breeding programs to develop novel target-specific control strategies
82 against nematodes.

83 RNA-Seq has become a powerful instrument for gene expression profiling and detection of
84 novel genes (Wang et al., 2009, Ozsolak & Milos, 2011) which has been used widely to study
85 the expression profiles of RKN diseased Solanaceae plants (Xing et al., 2017, Li et al., 2018,
86 Shukla et al., 2018). RNA-seq profiling has been used to decipher potato responses to various
87 abiotic (Zuluaga et al., 2015, Gálvez et al., 2016) and biotic stresses (Kwenda et al., 2016, Yang
88 et al., 2018) where large sets of genes and pathways associated with either biotic and abiotic
89 stress were revealed. To date most research has focused on potato gene expression in response
90 to potato cyst nematodes (Jolivet et al., 2007, Walter et al., 2018, Kooliyottil et al., 2019) while
91 potato responses to RKN infection remain poorly understood. Here, we set out to evaluate the
92 responses of potato cultivars to root-knot nematode infection. Our results revealed that seven
93 commercially tested South African potato cultivars were susceptible to *M. javanica.* Further,
94 in order to investigate the molecular basis of this compatible interaction, we employed RNA-
95 Seq to analyse differential gene expression patterns in *Solanum tuberosum* cv. Mondial
96 subjected to *M. javanica* infection at two early stages (3 and 7dpi).

## 97 Results and Discussion

### 98 Susceptibility of potato cultivars to *Meloidogyne javanica*

99 In this study, we evaluated the susceptibility to RKN in seven commercially available potato
100 cultivars in South Africa. The number of galls induced, and reproductive potential of the
101 nematodes was used to assess the host status of the potato cultivars. Our results show that all
102 the seven potato cultivars were efficient hosts to *M. javanica* as indicated by the high
103 reproductive factor (Rf >1) **(Fig 1A).** This further supports the findings by Pofu and Mashela
104 (2017) which concluded that South African potato cultivars are efficient hosts to *Meloidogyne*
105 species. Based on the gall numbers, cultivars were classified as highly susceptible (BP1,
106 Mondial and Lanorma), susceptible (Up-to-date, Sifra, and Valor), and moderately resistant
107 (Innovator) **(Fig 1B)** according to the ranking scale coined by Taylor and Sasser (1978)
108 *Meloidogyne Javanica* infection generally reduces plant growth and yield of potato (Vovlas et
109 al., 2005). Similarly, RKN infection caused reduction in root length and shoot length in various
110 potato cultivars compared to their corresponding controls (**Fig 1 C** and **D**). The reduced growth

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111 is attributed to root injury caused during nematode penetration and feeding, which impairs the
112 plant root systems, hence reducing the efficiency of roots to absorb water and nutrients. As a
113 result, the top growth of the plant is reduced, and this explains the reduced shoot lengths.
114 Interestingly, increased root length was recorded in four diseased cultivars in comparison to
115 untreated controls. The production of secondary roots may be a characteristic of nematode
116 infected plant's efforts to recompense for root injury (Mcdonald & Nicol, 2005). In addition,
117 mature galls exhibited either a single or more egg masses, which indicates that *M. javanica*
118 was infective on potato cultivars (**Fig 1E and F**). Therefore, nematodes were able to penetrate
119 the host system, subdue the host defense responses during the entire infection process, complete
120 their life cycle and reproduce.

121 **Transcriptome data analysis and functional annotation of differentially expressed genes**

122 Aiming to understand the molecular basis of this compatible interaction between RKN and
123 potato, RNA sequencing was performed on the highly susceptible cultivar *Solunum tuberosum*
124 cv Mondial. Two infection stages were selected for the analysis: 3 and 7 dpi. These time points
125 correspond to nematode stages of induction of feeding sites at 3 dpi and nutrient acquisition
126 stage that starts from 7 dpi to 8 weeks after infection (Bartlem et al., 2013). Approximately 1.3
127 billion paired-end reads were generated yielding an average of 23 million high quality reads
128 for individual samples. Successfully mapped reads onto the *S. tuberosum* reference genome
129 (v4.03) (Consortium, 2011) accounted for 78-86% of the total of reads generated per sample
130 **(Table S1).** $Log_2$ fold change $\geq \pm 1$ and adjusted p-value (FDR) $< 0.05$ were used as cut off
131 values to obtain DEGs through pairwise comparison between the mock-inoculated samples and
132 infected samples at 3 and 7 dpi. Overall, 4948 potato genes were differentially expressed at
133 3dpi. Of these, 2867 were down-regulated and 2081 up-regulated. At 7dpi, 2871 and 1613
134 genes were detected to be down and up-regulated respectively **(Fig 2A)**. Collectively, 3108
135 genes were regulated at both 3 and 7 dpi: 2069 down- and 1022 up-regulated. As biotrophic
136 organisms, RKNs need to actively suppress the host defense during the infection process. This
137 might explain the current observation where 57.75% of the DEGs (3,652 out of 6,324) were
138 suppressed (**Fig 2Aand B**).

139 GO enrichment analysis was performed using the AgriGo tool (Tian et al., 2017) to reveal the
140 main regulatory trends in root tissues on the course of RKN infection. The GO terms were
141 grouped into three main functional categories at adjusted *p-value < 0.05* and categorized using
142 WEGO software (Ye et al., 2018). Within the biological process class, the highest percentage
143 of the DEGs was down-regulated and fell under metabolic process category. Within this

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144 category, we found the following sub-categories: Primary metabolic process, cellular metabolic
145 process, biosynthetic process, oxidation-reduction process and regulation of metabolic process.
146 Accordingly, past studies revealed that nematode infection modulates the expression of genes
147 involved in metabolic activities, particularly the primary metabolic process due to the high
148 demand for nutrients and energy (Hofmann et al., 2010). Other significant GO terms in this
149 category include response to stimulus, cellular process, localization and signalling processes
150 and regulation of biological processes **(Fig 3 and S2 Table).**

151 **Plant signal transduction, pathogen perception, and defense-related genes are modulated**
152 **by *M. javanica* infection**

153 Plants have developed the innate immune system to inhibit pathogen invasion and
154 multiplication. Pattern triggered immunity (PTI) (plants first line of defence) relies on
155 perception of pathogen/damage-associated molecular patterns (PAMPs/DAMPs) by pattern
156 recognition receptors (PRRs) (Zipfel, 2014, Macho & Zipfel, 2014). Plant PRRs are either
157 surface localized receptor-like proteins (RLPs) or receptor-like kinases (RLKs) that perceive
158 and transmit danger signals to activate defense response (Zipfel, 2014). In this study, genes
159 encoding for RLKs and RLPs (e.g. serine-threonine protein kinase, leucine-rich repeat (LRR)
160 receptor-like protein kinase) and wall-associated receptor kinases (WAKs) were detected
161 among the DEGs. The majority of the PRRs (68.28%) were repressed by nematode infection
162 at 3 and/or 7 dpi following nematode infection **(Fig 4A and S3 Table)**. Previous reports show
163 that phytonematodes are able to induce plant basal defense responses through recognition by
164 large arsenals of plant receptors (Peng & Kaloshian, 2014, Teixeira et al., 2016, Mendy et al.,
165 2017). Mendy et al. (2017) reported the first surface localized LRR receptor-like kinase
166 (NILR1) that was up-regulated in response to nematode attack in *Arabidopsis.* Arabidopsis
167 *nilr1* mutants were found to be hyper susceptible to a wide range of phytonematodes (Mendy
168 et al., 2017). Similarly, our results revealed that six out of seven WAK-encoding genes were
169 down-regulated by nematode infection **(Fig 4A and S3 Table).** The WAK proteins perceive
170 danger signals to activate PTI responses (Ferrari et al., 2013). Past research shows that WAK
171 proteins are important components of potato disease resistance against various microbes
172 (Kwenda et al., 2016, Yang et al., 2018). For instance, WAK genes were induced in a tolerant
173 cultivar (BP1) which correlated with enhanced perception of *Pectobacterium brasiliense*
174 (formerly *Pectobacterium carotovorum* subsp *brasiliense*) (Kwenda et al., 2016). Similarly,
175 WAK genes were up-regulated conferring resistance to *Phytophthora infestans* in potato
176 genotype SD20 (Yang et al., 2018) Additionally, plants defective of PRRs or PTI signaling

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177 components are typically susceptible to both adapted and non-adapted microbes (Macho &
178 Zipfel, 2014). This notion is confirmed by *M. javanica* ability to interfere with the functioning
179 of RLKs, RLPs and WAK genes lead to successful disease development.

180 Transmission of perceived signals from the PRRs is mediated through the MAPK cascade and
181 calcium ($Ca^{2+}$) signaling pathway which transfers downstream components of plant immunity.
182 Here, we found the expression of MAPKs genes was largely repressed (20 out of 22 genes) by
183 nematode infection **(Fig 4B and S3 Table)**. The MAPK cascade basically entails three-tiered
184 kinases (a) a MAP kinase kinase kinase (MAPKKK) (b) a MAP kinase kinase (MAPKK), and
185 (c) the MAP kinase (MAPK) which mediates transmission of extracellular signals to activate
186 an appropriate defense output (Jagodzik et al., 2018). The role of MAPK in plant defense
187 against nematodes has been demonstrated previously. It was reported by Zhang et al. (2017)
188 and Postnikova et al. (2015) that the induction of MAPK genes leads to resistance against cyst
189 nematode and RKN, respectively in soybean plants. Further, *M. javanica* repressed 91.6% of
190 the genes involved in $Ca^{2+}$ signaling pathway (33 out of 36) **(Fig 4C and S3Table)**. This
191 includes calmodulin (CaM), calcineurin B-like proteins (CBL), $Ca^{2+}$ dependent kinases (CPKs)
192 and $Ca^{2+}$ receptors that transmit $Ca^{2+}$ signatures into a specific cellular and physiological
193 response after a pathogen challenge (Zhang et al., 2014). Takabatake et al. (2007) demonstrated
194 that repression of CaM/CML members' expression or loss of function in mutated plants
195 strongly affects immunity. Furthermore, in plant-nematode interaction, calcium burst was
196 associated with the release of ROS causing cell death and inhibiting establishment of (GCs) in
197 potato (Davies et al., 2015). In connection to this, our results indicate that *M. javanica* ability
198 to repress MAPK and $Ca^{2+}$ pathways interfered with the transmission of signals responsible for
199 activation of precise and prompt defense response, hence a susceptible response.

200 NBS-LRR disease resistance proteins have been implicated in mediating resistance against
201 various phytonematodes (Williamson & Kumar, 2006). In our study, 114 disease resistance
202 genes (out of the 641 in the potato genome) (Sharma et al., 2013) were differentially expressed
203 following root knot nematode infection. The highest proportion of these proteins (54.35%)
204 including NBS-LRR disease resistance proteins, was repressed at 3 and/or 7 dpi **(Fig 4D and**
205 **S3 Table).** Similarly, repression of 12 NBS-LRR genes by cereal cyst nematode in wheat led
206 to a susceptible response (Qiao et al., 2019). Altogether, down-regulation of resistance genes
207 indicates repression of plant resistance by *M. javanica* infection.

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208 Apart from the activation of MAPK and $Ca^{2+}$ signaling, PTI activation is associated with
209 expression of pathogenesis related (PR) proteins. In this study, we found the expression of
210 several PR proteins under the regulation of *M. javanica* including 11 chitinase encoding genes
211 (PR-3 and PR-4). The PR-3 and PR-4 are markers for JA-mediated defense response with 8
212 genes specifically up-regulated at 7dpi **(Fig 5A and S3 Table).** In a similar fashion, it has been
213 previously reported that increased chitinase activity do not correlate with resistance in potato
214 to potato cyst nematode (Wright et al., 1998). Therefore, chitinase might be functioning as a
215 signalling molecule to stimulate other PR proteins, or alternatively, its induction may be due
216 to wounding response (Wright et al., 1998). Regarding SA-responsive genes (PR-1 and PR-5),
217 14 were up-regulated and 11 were down-regulated by nematode infection specifically at 7dpi
218 **(Fig 5A and S3 Table).** According to previous studies, the PR-5 transcripts were induced
219 following RKN and CN infection in *S. lycopersicum* and *Brassica nigra*, respectively (Sanz-
220 Alférez et al., 2008, van Dam et al., 2018). These observations are corroborated in the current
221 study where the majority of thaumatin-like and osmotin genes (55.82%) were induced by
222 nematode infection. Moreover, the stimulation of PR-5 protein has been associated with
223 osmotic stress induced during nematode invasion (Sanz-Alférez et al., 2008). Generally, there
224 was delayed activation of the PR genes as the majority of the PR encoding genes were induced
225 at 7 dpi. This could reflect the strategy adopted by the RKN to suppress PR encoding genes in
226 early stages of colonization to ensure successful nematode infection.

227 Rapid generation of reactive oxygen species (ROS) is one of the early PTI cellular events that
228 trigger a number of defense responses such as activation of several defense genes and cell wall
229 reinforcement (Goverse & Smant, 2014) In this study, NADPH oxidase, respiratory burst
230 homologue (RBOHs) and peroxidases, which are important players in production of ROS in
231 plants, were differentially regulated both at 3 and 7dpi **(Fig 5B and S3 Table)**. Genes encoding
232 for peroxidases (30 genes) were repressed to a larger extent at 7 dpi than at 3 dpi **(Fig 5B and**
233 **S3 Table)**. This implies that *M. javanica* suppresses ROS-mediated defense signaling during
234 induction and acquisition of nutrients in the GCs. In addition, two genes coding for 2-oxoacid-
235 dependent dioxygenase were down-regulated at 3 and/or 7 dpi. 2-oxoacid-dependent
236 dioxygenase enzyme mediates a variety of oxidative reactions and synthesis of secondary
237 metabolites (Prescott & Lloyd, 2000) and has toxic effects on a wide range of pathogens
238 including phytonematodes (Hansen et al., 2008). Genes encoding for 2OG-Fe (II) oxygenase
239 superfamily were up-regulated (23 genes out of 34) following nematode infection in this study
240 **(Fig 5B and S3 Table)**. Patel et al. (2010) reported that the interaction between host 2OG-Fe

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241 (II) oxygenase and a nematode effector HS4F01 increased the plant susceptibility to cyst
242 nematode. This illustrates that *M. javanica* effectors might interact with the host proteins
243 responsible for oxidative responses hence interfering with ROS mediated defense signaling.

244 Genes encoding for glutathione, glutaredoxin, thioredoxin, peroxiredoxins, ascorbate and
245 peroxidases comprise plant's antioxidant network, which is responsible for controlling ROS
246 levels (Laporte et al., 2012). These genes were differentially regulated by nematode infection
247 in this study **(Fig 5B and S3 Table)**. Emerging evidence shows that RKN can utilize the host
248 ROS scavenging system to reduce the damaging effects of oxygen species (Lin et al., 2016,
249 Guan et al., 2017). Here, we detected one gene encoding for peroxiredoxin
250 (PGSC0003DMG401002721) , the main detoxifying antioxidant enzyme in the plant-nematode
251 interface (Goverse & Smant, 2014), being up-regulated at both timepoints **(Fig 5B and**
252 **S3Table)**. In addition, 16 out 23 genes encoding for glutathione S transferase (GST) and UDP-
253 Glycosyltransferase (6 out of 9 genes) **(S3 Table)** were up-regulated following *M. javanica*
254 infection **(Fig 5B and S3Table)**. Qiao et al. (2019) reported that CN nematode can utilize the
255 GST and UDP-Glycosyltransferase antioxidant enzymes to ameliorate the ROS effects as well
256 control plant defense. In this context, it is likely that in the current interaction, the nematode
257 activated host antioxidant mechanism to interfere with defense response and to avoid the
258 harmful effect of ROS molecules.

259 **Nematode responsive transcription factors**

260 Several transcription factors (TFs) were detected as DEGs in response to *M. javanica* infection.
261 This includes ERF (77), MYB and MYB-related (62), bHLH (49), bZIP (23), WRKY (33) and
262 GRAS (32). In total, these differentially expressed TFs represent 75% of the TFs found in *S.*
263 *tuberosum*. Most of the differentially expressed TFs in the current data set were down-regulated
264 (298/532) after nematode infection **(Fig 6A and S4 Table)**. Classification and identification of
265 the differentially expressed TFs was attained from the Plant Transcription Factor Database
266 (http://planttfdb.cbi.pku.edu.cn/ v .4.0) (Jin et al., 2016).Transcription factors (TFs) are key
267 regulators of plant response to various biotic stress in potato. For instance, in response to *P.*
268 *brasiliense* infection in potato, 4 families of TFs (WRKY, bHLH, MYB, and AP2/ERF) were
269 regulated (Kwenda et al., 2016). Similarly, in potato, several TFs were found to be important
270 regulators of resistance response against *P. infestans* (Yang et al., 2018).

271 The ERF TFs are associated with hormone signal transduction of sacylic acid (SA), jasmonic
272 acid (JA) ethylene (ET), and PR via binding to the GCC box of target genes that positively or
273 negatively regulate transcription of various stress responses (Li et al., 2017). In this study, most

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274 of the genes encoding for AP2/ERF TF family were down-regulated (70.37%) at 3 and/or 7
275 dpi. In addition, 15 genes were suppressed to a greater extent at 7 than 3 dpi **(Fig 5C and S4
276 Table)**. This could be ascribed to the secretion of nematode effectors and subsequent
277 suppression of defense response associated with the activation of AP2/ERF TFs. Among the
278 down-regulated ERF TFs we found 7 genes encoding DREB (out of 9 DREB genes), which
279 are regarded as main regulators of abiotic stress responses (Zhou et al., 2010). **(Fig 6C and S4
280 Table)**. Qiao et al. (2019) recently reported that two genes encoding for DREB were strongly
281 repressed in wheat following a compatible interaction with CN. This indicates that DREB
282 genes may regulate signaling pathways associated with defense response to nematode
283 infection. This can be subjected to further analysis to identify their specific role in plant-
284 nematode interactions. Further, we found three ERF6 TFs activated in response to *M. javanica*
285 infection **(Fig 6C and S4 Table)**. In this context, ERF6 has been described to positively
286 regulate JA/ET and resistance against *Botrytis cinerea* in *A. thaliana* (Moffat et al., 2012). In
287 addition, Warmerdam et al. (2019) showed that ERF6 regulates *M. incognita* disease
288 development in *A. thaliana.* ERF6-mutated plants recorded a higher number of RKN egg
289 masses indicating a role of ERF6 in enhancing host susceptibility to *M. incognita* as a result of
290 deteriorated plant defenses (Warmerdam et al., 2019). In this case, ERF6 TFs have a role in
291 mediating potato susceptibility to RKN. Our findings indicate that down-regulation of
292 AP2/ERF TFs following nematode infection could have debilitated plant defense through
293 targeting the defense signaling pathways regulated by the AP2/ERF family of TFs.
294 It is generally accepted that pathogen-directed modulation of WRKY genes in plants is an
295 important aspect that enhances success rates of pathogen infection. Cyst nematode's successful
296 infection process in *A. thaliana* roots was attributed to the nematode’s control over the
297 expression of WRKY genes (Ali et al., 2014). In agreement with that notion, we found 23 genes
298 down-regulated WRKY-encoding genes, including *WRKY40, WRKY23,* and *WRKY29* at both
299 infection stages **(Fig 6B and S4 Table)**. In cotton plants, *GhWRKY40* has been reported to
300 regulate wounding and resistance response against *Ralstonia solanacearum* (Wang et al.,
301 2014). Furthermore, the up-regulation of *WRKY23* influenced an early resistance response to
302 *M. incognita* infection in cucumber plants (Ling et al., 2017). It has also been reported that in
303 *Arabidopsis, AtWRKY29* is an important constituent of MAPK mediated defense pathway
304 against microbes (Asai et al., 2002). Here we detected 20 genes encoding for MAPK
305 suppressed by nematode infection. The suppression of *WRKY29* might have influenced the
306 expression of *MAPK* genes interfering with transmission of signals that elicit a defense
307 response. Moreover, we found WRKY75 to be up-regulated by nematode infection at 7dpi. In

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308 tomato, *SlyWRKY75* regulates the JA-signal transduction system (López-Galiano et al., 2018)
309 indicating activation of the JA pathway by *M. javanica* infection. Our data reveals that RKN
310 interferes with important defense signaling components such as MAPK and JA pathways
311 eliciting a susceptible response through down regulation of WRKY TFs.

312 Among the 34 down-regulated MYB TFs **(S4 Table)**, we found three genes encoding for
313 MYB108 at 3 and 7 dpi. *Arabidopsis* AtMYB108 has been characterized as an important
314 regulator of both biotic and abiotic stresses (Mengiste et al., 2003) It is also known that the
315 expression of *GhMYB108* in cotton, responds to application of defense-related phytohormones
316 such as SA, JA and ET (Cheng et al., 2016). The absence of *GhMYB108* led to increased
317 susceptibility of cotton plants to *Verticillium dahliae* infection while its ectopic overexpression
318 enhanced tolerance to the fungal pathogen (Cheng et al., 2016). This would, therefore, indicate
319 that down-regulation of MYB108 coding genes interfered with the defense signaling pathway
320 leading to a compatible response. MYC2, MYC3, and MYC4 from the bHLH family are a part
321 of the JA signal transduction system (Pireyre & Burow, 2015). bHLH activates various sets of
322 plant genes in response to environmental factors such as phytohormone signaling, and
323 development (Pireyre & Burow, 2015). Here, we found 49 genes encoding for bHLH TFs with
324 a total of 23 up-regulated and 24 down-regulated in response to RKN infection **(S4 Table)**.
325 Our results indicate that nematode infection interferes with these important regulators of JA
326 mediated defenses by blocking the expression of some of the bHLH TFs responsible for
327 mounting sufficient defense responses against *M. javanica.*

328 Out of 71 GRAS representatives in the potato genome, 32 were differentially expressed
329 following nematode infection in the present data set, out of which 65.63% were up-regulated
330 **(Fig 6D and S4 Table)**. This includes 17 scarecrow-like (SCL) encoding genes important for
331 root physiology (Hirsch & Oldroyd, 2009). A nematode effector conserved in *Meloidogyne*
332 spp. acts as a signalling molecule that specifically targets the plant SCL transcription regulators
333 to induce root proliferation (Huang et al., 2006).Therefore, our results support the notion that
334 the induction of SCL led to increased cell proliferation in the roots, which is essential for GCs
335 induction and expansion. Further, we detected three nodulation-signaling pathway (NSP) genes
336 1 and 2 under positive regulation **(Fig 6D and S4 Table)**. In addition, we found that genes
337 encoding nodulin-like proteins were either induced (7 genes) or repressed (5 genes) by
338 nematode infection **(S5 Table)**. It has been reported previously that RKN can invoke similar
339 host signals involved during the formation of nodules necessary for nitrogen fixation (Favery
340 et al., 2002). In various phytopathosystems, nodulin-like genes are involved in solute supply

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341 during these interactions (Denancé et al., 2014). In the same way, nematode infection might
342 have induced nodulin-like genes and their transcriptional regulators NSP1 and NSP2 in the
343 GRAS family to aid in solute transportation in the GCs, a crucial process for a successful
344 nematode infection. overall, our results show that potato susceptibility to RKN is controlled at
345 the transcriptional level by a complex gene regulatory network.

346 **Nematode responsive phytohormones**

347 Plant hormone signal transduction pathways are typically targeted by pathogens to either
348 disrupt or avoid plant defense responses. Pathogen invasion results in changes in various plant
349 hormone levels (Bari & Jones, 2009). In this context, our study shows that nematode infection
350 influenced the expression of genes associated with the synthesis of JA, SA, ET, auxin,
351 gibberellic acid (GA) and cytokinin (CK) signaling pathways **(Fig 7 and S5 Table).** Salicylic
352 acid signaling pathway positively regulates immunity to biotrophic parasites while JA and ET
353 hormones usually function synergistically to regulate defense against necrotrophic microbes
354 and herbivorous insects (Bari & Jones, 2009). Differential expression of genes involved in the
355 phenylpropanoid metabolic pathway was detected including three key enzymes, one gene
356 encoding phenylalanine ammonia lyase (HAL), and one encoding trans-cinnamate (C4H, 3
357 genes) were repressed at 3 and 7dpi. Further, two genes encoding 4-coumarate-CoA ligase
358 (4CL1) were repressed at 7 dpi and one gene slightly activated at 3dpi during RKN disease
359 development **(S3 Table)**, which can affect other downstream activities such as SA, lignin and
360 flavonoids biosynthesis (Vogt, 2010). Here, we detected regulation of genes in the flavonoid
361 biosynthetic pathway, including chalcone synthase (induced, one gene), chalcone-flavone
362 isomerase and flavanol sulfotransferase-like (repressed, one gene) at 3 and 7dpi **(S5 Table)**.
363 Moreover, enzymes that participate in lignin biosynthesis were detected including cinnamyl
364 alcohols dehydrogenase (CAD, one gene) and lignin-forming anionic peroxidase (five genes)
365 were repressed in response to RKN. O-methyltransferase encoding genes in the lignin pathway
366 were either slightly up-regulated or down-regulated at 3 and/or 7dpi **(S5 Table).** This
367 demonstrates the involvement of phenylpropanoid metabolic pathway in RKN disease
368 development.

369 For SA signalling, chorismate mutase (one gene) and SA-carboxyl methyltransferase (one
370 gene) encoding genes implicated in SA synthesis (D'Maris Amick Dempsey et al., 2011) were
371 down-regulated following nematode infection in addition to differential regulation of a gene
372 encoding for key enzymes of PAL pathway **(S5 Table)**. Further, a subset of the WRKY family
373 specifically involved in SA signalling pathway (i.e. WRKY70, WRKY40, WRKY17, and

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374 WRKY8) was suppressed according to our data set **(Fig 6B and S4 Table)**. These genes are
375 important regulators of SA-dependent responses and have been implicated in the antagonistic
376 crosstalk between SA-JA pathways (Pieterse et al., 2012) indicating repression of SA pathway
377 by RKN infection.

378 Genes encoding for enzymes involved in the JA-signaling pathway were largely down-
379 regulated. These include genes encoding allene oxide synthase (AOS 2 genes), allene oxide
380 cyclase (AOC, one gene), lipoxygenase (LOX, 3 genes) and 12-oxophytodienoate (12-OPR, 5
381 genes) **(Fig 7A and S5 Table)**. The LOX pathway mediates resistance against pathogens,
382 insects, and nematodes (Gao et al., 2008). Gleason et al. (2016) demonstrated that the 12-OPR
383 enzyme, a JA-precursor, is a vital defense-signaling molecule that mediates plant immunity
384 against nematodes. Moreover, plants incapable of producing JA or 12-oxo-phytodieonoic acid
385 (OPDA) are more susceptible to phytonematodes (Gleason et al., 2016). In this perspective,
386 the down-regulation of LOX and 12-OPR enzymes in the current study might have played a
387 role in initiating a susceptible interaction through interfering with JA-mediated defense
388 pathway. In addition, jasmonate O-methyltransferase, an additional regulatory point for the
389 accumulation of jasmonate derivatives in the cytoplasm and production of signal transmitters
390 other than JA (Seo et al., 2001) was up-regulated according to our dataset **(Fig 7A and**
391 **S5Table)**. Interestingly, 5 genes of the TIFY protein family, which includes jasmonate-Zim-
392 domain protein 10 (JAZ10), that represses JA signaling pathway, were down-regulated in this
393 study **(Fig 7A and S5 Table)**. This indicates the activation of this pathway although not
394 sufficient to mount the defense against RKN. Apart from pathogen and herbivory attack, the
395 JA pathway can be activated as result of wounding. It also enhances accumulation of protease
396 inhibitors which hinder exogenous proteases from insects to halt their development and
397 reproduction (Koo & Howe, 2009). It is likely that JA-mediated defenses are effective against
398 phytonematodes. However, the strong induction of several classes of protease inhibitors in our
399 study **(S7 Table)** did not correlate with RKN resistance. Thus, the activation of the JA signaling
400 pathway in this study might be due to wounding caused by nematode migration and feeding
401 rather than by defense response.

402 Activation of the ET pathway upon pathogen attack leads to accumulation of defense-related
403 through a cascade of events leading to activation of ERF TFs (van Loon et al., 2006). In the
404 current data set, genes encoding for key enzymes involved in ethylene biosynthesis including
405 1-aminocyclopropane-1-carboxylate (ACC, 4 genes) synthase and 1-aminocyclopropane-1-
406 carboxylate (ACO, 5 genes out of 8 genes) were down-regulated. We also detected three up-
407 regulated membrane receptors (which perceive ET) at 3 and/or 7dpi **(Fig 7B and S5 Table)**.

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408 This includes ETHYLENE RESPONSE1 and 2 (ETR1 and ETR2) and ETHYLENE
409 RESPONSE SENSOR2 (ERS2) negative regulators of ET responses (Ju & Chang, 2015)
410 suggesting ET suppression. Further, ETHYLENE INSENSITIVE3 BINDING F-BOX (EBF)
411 proteins (2 genes) that degrade EIN3/ETHYLENE INSENSITIVE3-LIKE1 (EIL) key positive
412 regulators of ET responses (Ju & Chang, 2015) were activated in our data set **(Fig 7B and S5
413 Table)**. Our results show that apart from suppressing ET synthesis genes, RKN induced both
414 negative regulators of ET responses and EBF responsible for proteasomal degradation of
415 EIN3/EIL TFs that positively regulate ET responsive genes. This hindered the activation of
416 defense-related genes associated with ERF branch of TFs.

417 Auxin stimulates several changes such as cell wall ingrowths, cell cycle activation and cell
418 expansion occurring in nematode feeding sites (Gheysen & Mitchum, 2018). Here, we found
419 51 auxin signaling genes differentially expressed, including tryptophan aminotransferase-
420 related protein 4 (auxin biosynthesis), GH3.3 and SAUR family (auxin-responsive genes),
421 auxin repressors (e.g. IAA13, IAA19) and auxin transporters (e.g. TIR, LAX1) **(Fig 7C and
422 S5 Table)**. It has been recently reported that nematode invasion results in induced auxin
423 biosynthesis and responsive genes while genes encoding for repressors are switched off
424 (Gheysen & Mitchum, 2018). Similar to this scenario, in our dataset, auxin repressors were
425 repressed further highlighting the importance of auxin manipulation in nematode parasitism.
426 Overall, *M. javanica* modulates auxin signaling pathway to facilitate successful formation of
427 GCs.

428 Cytokinin and auxin hormones have been implicated in the induction and development of NFS
429 (Gheysen & Mitchum, 2018). In the present study, genes involved in CK signaling pathway
430 were differentially expressed. These include cytokinin dehydrogenase and cis-zeatin O-
431 glucosyltransferase involved in CK homeostasis, of which 5 genes out of 7 were induced at 3
432 and/or 7 dpi **(Fig 7D and S5 Table).** Cytokinin dehydrogenase is involved in the degradation
433 of CK. Transgenic plants overexpressing this enzyme had decreased gall formation and
434 consequently reduced susceptibility to nematode infection (Lohar et al., 2004, Siddique et al.,
435 2015). Due to their involvement in nutrient mobilization and cell division, CKs are believed to
436 play a role in formation and maintenance of NFS infection (Lohar et al., 2004, Siddique et al.,
437 2015). Our study shows that RKN regulates CK levels by regulating genes associated with the
438 homeostasis of CK further underlining the significance of CK in GCs formation.

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439 Next, our RNA-seq data revealed differential expression of the genes encoding for enzymes
440 involved in GA biosynthesis including 3 up-regulated GA2OX1 encoding genes, and 5 down-
441 regulated at both infection stages. Two genes encoding for DELLA proteins, which are
442 negative regulators of GA response, were up-regulated while GA receptors were either up (2
443 genes) or down-regulated (one gene) by nematode infection at 3 and 7dpi **(Fig 7E, S5, and S4
444 Table)**. GA2OX1 enzymes reduce endogenous GA content in *Arabidopsis* plants that
445 stimulates plant elongation process (Lee et al., 2014, Hu et al., 2017). A similar observation
446 was made on tomato and rice plants, where GA2OX and GA receptors were strongly activated
447 following RKN attack (Bar-Or et al., 2005, Kyndt et al., 2012). Further supporting this notion,
448 GA foliar application on tomato plants enhanced resistance to *M. javanica* (Moosavi, 2017).
449 Collectively, these results show that *M. javanica* modulates GA signaling process by activating
450 GA20X1 enzymes and GA repressors, which reduce the active GA and stimulate root
451 elongation that might be essential during GCs induction (Fuchs et al., 2013).

452 Several components of ABA stress-responsive hormone signaling, including ABA receptors,
453 protein phosphatase 2C (PP2C) and SNF1-related protein kinases (SNRK) were differentially
454 regulated in the present data set **(S5 Table)**. Genes encoding for PP2Cs were repressed (26 out
455 of 38) by nematode infection according to our data set **(S3 Table)**. PP2Cs encoding genes are
456 major players in stress signalling (Fuchs et al., 2013) that transmit ABA signaling directly from
457 receptors to their downstream regulators. The SNRK regulators then activate an
458 ABF/AREB/AB15 clade of bZIP-domain TFs through protein phosphorylation process finally
459 to induce physiological ABA response(Sun et al., 2011). Apart from regulating stress responses
460 and plant development, members of bZIP TF family are also implicated in plant defense
461 response (Singh et al., 2002). In this study 23 bZIP genes were differentially expressed with
462 14 genes activated and 9 genes repressed **(Table S4)**. Therefore, we can hypothesize that
463 nematode infection modulates the main stress-signaling pathway through repression of ABA
464 receptors, which blocks the expression of some of the bZIP TFs responsible for defense
465 response initiation.

466 **Genes associated with metabolic activities and transport activity are regulated by *M.***
467 ***javanica* infection**

468 As obligate biotrophs, RKN fully depends on host-derived nutrients and solute transport to
469 establish feeding sites. The differentiation of giant cells is coupled with massive changes in
470 structure and metabolism of the host cells (Siddique & Grundler, 2015). GO enrichment
471 analyses showed that genes involved in primary metabolism and cellular metabolism were

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472 overrepresented among the down-regulated genes **(S2 Fig and S1 Table).** Repression of these
473 genes might be a strategy adopted by the host to save energy which is diverted for defense
474 responses (Rojas et al., 2014). For instance, in this study, genes associated with lipid
475 metabolism such as GDSL esterases/ lipases were repressed (14 genes) by nematode infection
476 at both infection stages **(Fig 8A and S6 Table)**. Lipid and their metabolites have a role in
477 mediating plant resistance (Gao et al., 2017). This indicates that *M. javanica* interfered with
478 the host lipid-based defenses when initiating a compatible interaction. Nematode infection is
479 associated with drastic reorganization of infected plant cells as well reprogramming of plant
480 primary metabolism (Hofmann et al., 2010). It is also believed that nematodes may trigger
481 biosynthesis of essential nutrients for their development, hence new metabolic pathways maybe
482 induced in the host plants (Hofmann et al., 2010). Other genes in plant primary metabolism
483 category under differential regulation of RKN parasitism include glycolytic process, trehalose
484 metabolism, fatty acid biosynthetic process, sucrose and protein metabolism **(S6 Table)**.
485 Similar to other studies, our transcriptomic data revealed that 165 genes encoding for cell wall
486 modifying/degrading enzymes (CWM/DEs) annotated under the carbohydrate metabolic
487 processes were differentially expressed. This includes genes encoding for glucan endo-1-3
488 beta-glucosidase, xyloglucan endo-transglycosylase, expansins, and extensins which were
489 differentially expressed at 3 and/or 7dpi by RKN infection **(Fig 8B and S6 Table)** This shows
490 that the regulation of these CWD/MEs is important during cell wall modification in the NFS.
491 Glucan endo-1-3beta-glucosidase (members of PR-2 protein family) were differentially
492 expressed with 14 genes down-regulated and 6 genes up-regulated at 3 and/7 dpi in this study
493 **(Fig 8B and S6 Table)**. These cell wall modifying enzymes and also linked to plant defense
494 against pathogens (van Loon et al., 2006). Callose deposition of a 1,3-ß glucan polymer is one
495 of the first events occurring during pathogen invasion to slow down its progression (Voigt,
496 2014). Therefore, suppression of Glucan endo-1-3beta-glucosidase genes by nematode
497 infection interferes with callose formation as well as plant defense. Among the 14 repressed
498 genes encoding for xyloglucan endo-transglycosylase, two genes (PGSC0003DMG400004670
499 and PGSC0003DMG400021877) were induced by nematode infection at 7dpi (**Fig 8B and S6**
500 **Table**) suggesting a tight regulation of CWMEs during GCs formation. Similarly, 3 expansin
501 encoding genes were specifically up-regulated at 7 dpi. **(Fig 8B and S6 Table)**. Shukla et al.
502 (2018) also demonstrated the up-regulation of genes encoding for expansins between 5 and 7
503 dpi in a susceptible tomato response to RKN. Expansins are cell wall loosening proteins that
504 might play key role during the expansion of GCs (Gheysen & Mitchum, 2008). In addition,
505 genes encoding for hydrolytic enzymes involved in pectin degradation such as

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506 polygalacturonase (PG), pectate lyases (PL) and pectin esterase (PE) were differentially
507 expressed at 3 and 7 dpi by nematode infection **(Fig 8B and S6 Table).** Pectin degradation
508 leads to plant tissue maceration that is essential in disease development (Lionetti et al.,
509 2012).Therefore, the reduction of pectin content may increase nutrient accessibility to
510 nematodes (Jammes et al., 2005). Furthermore, four genes encoding pectin methyl esterase
511 inhibitor (PMEI or PEI) were down-regulated upon nematode challenge at 3 and 7 dpi **(Fig 8B
512 and S6 Table)**. Plants produce PMEI in an effort to counteract the increasing pectin methyl
513 esterase (PME) upon pathogen attack (Lionetti et al., 2012). Repression of PMEI by nematode
514 attack shows that activity of PME was activated leading to the breakdown of pectin bonds,
515 which increases the vulnerability of the cell wall to microbial pectic enzymes and other
516 degrading enzymes and culminates in a susceptible response. Generally, the differential
517 regulation of genes associated with cell wall architecture suggests that *M. javanica* was able to
518 break down plant cell wall to facilitate migration and formation of GCs. Furthermore, RKN
519 interferes with defense role associated with the plant cell wall architecture leading to
520 compatible interaction.
521 With the increased demand for nutrients in nematode feeding cells, nematodes deploy
522 specialized membrane transporters to control the flow of nutrients in and out of the NFS
523 (Rodiuc et al., 2014, Siddique & Grundler, 2015). In agreement with previous studies (Hammes
524 et al., 2005, Shukla et al., 2018), we found that several families of transporter genes were
525 differentially regulated upon RKN infestation in our analyses. These include peptide
526 transporters, aquaporins, amino acid transporters, ion transporters, sugar transporters and
527 glutathione S transferase **(Fig 8C and S6 Table)**. Overall, we found that 54.8% of transporter
528 encoding genes in the DEGs were up-regulated following nematode infection. The activation
529 of genes encoding amino acid transporters (8 genes) and sugar transporters (9 genes) indicates
530 activation of amino acid and carbohydrate metabolism, respectively. For instance, according
531 to Zhao et al. (2018), the induction of sugar transporters increases soluble sugar contents in
532 RKN infected tomato plants, which is crucial for nematode development. Furthermore,
533 multidrug transporter-encoding genes were differentially expressed in our samples as well **(Fig
534 8C and S6 Table),** which encompasses ATP- binding cassette (ABC, 16 genes) and multidrug
535 and toxin extrusion proteins (MATE, 17 genes). These are secondary active transporters
536 involved in plant immunity and transporting and trafficking of xenobiotic, small organic
537 molecules, and secondary metabolites (Peng et al., 2011). In a similar study involving tomato
538 and *M. incognita,* 15 MATE efflux proteins exhibited differential expression (Shukla et al.,
539 2018), nevertheless, their role in plant-nematode interaction is yet to be defined. Hence, it is

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540 likely that nematodes recruit some of these transporters to flush out toxic secondary metabolites
541 or to disperse nematode effectors produced following nematode invasion.
542 Nematode effectors that target plant ubiquitin-proteasome system (UPS) have been reported
543 previously (Rehman et al., 2009, Chronis et al., 2013). In the present study, we found that
544 several genes involved in protein ubiquitination and proteolysis including the U-box domain
545 (27 genes) RING finger protein (3 genes) zinc finger domain (8) and the F-box (30 genes) were
546 down-regulated **(Fig 8D and S7 Table)**. This could indicate an immense turnover of proteins
547 due to constant nematode feeding leading to a compatible interaction. Wang et al. (2015)
548 reported an enzyme E3 ligase, U-box/ARM repeat protein (OsPUB15) which interacts with a
549 receptor-like kinase to regulate programmed cell death as well as disease resistance. Similarly,
550 E3 ubiquitin ligase protein is known to control plant immunity to a broad range of microbes in
551 rice through orchestrating plant immunity homeostasis and coordinating the trade-off between
552 defense and growth in plants (You et al., 2016). Indeed, the findings further support that the
553 UPS system might be a primary target to *M. javanica* effectors, which modulate the various
554 facets of plant defense linked with the UPS system.
555 Collectively, this study uncovers the molecular networks regulated during compatible
556 interaction between potato and RKN. This provides further insights on plant-nematode
557 interactions and will enhance further studies in this area including development of target-
558 specific control strategies against *Meloidogyne* species.
559

560 **Materials and methods**

561 **Plant material and RKN inoculations**

562 Certified seed (tubers) of seven potato cultivars were grown under greenhouse conditions to
563 evaluate resistance to *M. javanica* under greenhouse conditions. The seed tubers were pre-
564 germinated in the dark 20 ± 3ºC for seven days to allow sprouting. Stocks of *M. javanica* were
565 originally obtained from Dr. Pofu (ARC Roodeplaat, Pretoria, South Africa) and maintained
566 on susceptible tomato cultivar, *S. lycorpersicum* Cv Floradade in glasshouse environment with
567 a temperature of 24 ºC-30 ºC for eight weeks. *Meloidogyne javanica* eggs were extracted from
568 infected roots as described (Hussey, 1973). Egg suspension was poured onto an extraction tray
569 for collection of second juveniles' (J2s) nematodes. Five- week old potato seedlings were
570 inoculated with 1000 freshly hatched J2s per plant and control plant mock-inoculated with
571 water. The number of galls using Taylor and Sasser (1978) ranking scale to determine
572 susceptibility and reproduction factor (RF) using Sasser et al. (1984) RF formula was used to

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573 assess the host status of potato cultivars to RKN infection 8 weeks after infection For RNA
574 experiment, whole root tissues of a compatible potato cultivar were collected at 0, 3 and 7 days
575 post-inoculation (dpi) with two biological replicates per time point. Samples were washed and
576 immediately frozen in liquid nitrogen to prevent RNA degradation and later stored at -80 $^{o}$C
577 until RNA extraction.

578 **RNA extraction, library preparation, and sequencing**

579 RNA extraction, library preparation, and sequencing were carried out at Novogene (HK)
580 Company Limited. Total RNA for individual time course and replicates was extracted using
581 TiaGen extraction kit (Biotech Beijing Co., Ltd) and treated with sigma DNase1 (D5025). RNA
582 degradation and contamination was measured on 1% agarose gel while RNA purity was
583 assessed using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA
584 concentration and integrity were assessed using Qubit ® RNA Assay kit in Qubit ®2.0
585 Fluorometer (Life Technologies, CA, USA) and RNA Nano 6000 Assay Kit of the Bioanalyzer
586 2100 system (Agilent Technologies, CA, USA), respectively. Three micrograms of RNA
587 samples were used as input for library construction. Libraries were constructed using
588 NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) according to the
589 manufacturer's instructions and index codes were added to attribute sequences to each sample.
590 Finally, PCR products were purified using AMPure XP system and quality of the library
591 assessed using the Agilent Bioanalyzer 2100 system. A cBot Cluster Generation System using
592 HiSeq PE Cluster Kit cBot-HS (Illumina) was used to cluster the index-coded samples. After
593 cluster generation, the library preparations were sequenced on an Illumina Hiseq platform 2500
594 generating 150 bp paired-end reads.

595 **Transcriptomic data analysis**

596 Quality analysis of sequenced reads were initially analyzed using FASTQC package (https://
597 www.bioinformatics.babraham.ac.uk/projects/fastqc). Clean reads were obtained by removing
598 reads containing adapter reads with poly-N and low-quality reads from raw data. Trimming of
599 low-quality regions was performed using Trimmomatic v 0.36 (Bolger et al., 2014). All the
600 subsequent downstream analyses were based on high- quality data. *Solanum tuberosum*
601 genome v4.03 (Consortium, 2011) was used for reference-guided mapping of RNA-seq reads.
602 Paired-end clean reads were aligned to the potato genome using hisat2 v 2.1.0 software (Kim
603 et al., 2015). Unmapped reads were progressively trimmed at the 3'end and re-mapped to the
604 genome. Next, featureCounts package (Liao et al., 2014) was used to perform raw-reads counts
605 in R environment (https://www.r-project.org/). The read counts were then used for differential

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606 expression analysis using edgeR package (Robinson et al., 2010). Further, to investigate the
607 responses at different time points (3 dpi and 7 dpi), the expression profiles were compared to
608 mock-inoculated (0dpi) data sets. The transcripts were then classified as differentially
609 expressed genes (DEGs) based on both (a) false discovery rate (FDR) (Benjamini & Yekutieli,
610 2005) cut-off of 0.05 and (b) $\log_2$ fold change $\geq 1$ or $\leq -1$ for induced and repressed genes,
611 respectively.

612 **Gene ontology (GO) and enrichment analysis**

613 The GO and enrichment analysis were performed using agriGO v.2.0 (Tian et al., 2017) and
614 categorized by WEGO v 2.0 tool (Ye et al., 2018). Parametric gene set enrichment analysis
615 based on differential expression levels ($\log_2$ fold change) was performed and FDR correction
616 was performed using the default parameters to adjust the *p*-value. Functional annotations and
617 pathway analyses were obtained through sequence search performed on eggNOG database
618 utilizing *eggmapper* (Huerta-Cepas et al., 2017). Annotations from eggNOG were then
619 integrated with Kyoto Encyclopedia of Genes and Genomes (KEGG) database in order to reach
620 pathway annotation level.

621 **Validation for DEGs by qRT-PCR**

622 For qRT-PCR, first-strand cDNA was done from total RNA using Superscript IV First-Strand
623 cDNA Synthesis SuperMix Kit (Invitrogen, USA) following manufacturer's protocol.
624 Quantitative real-time PCR was performed using SYBR Green Master Mix in the QuantStudio
625 12k Flex Real-Time PCR system (Life Technologies, Carlsbad, CA, USA) to validate DEGs.
626 Two micrograms of the sample was added to 5 µl of Applied Biosystems SYBR Green Master
627 Mix and primers at a concentration of 0.4 µM. The implication cycle consisted of following:
628 initial denaturation at 50 °C for 5 min and 95 °C for 2 min followed by 45 cycles of 95 °C for
629 15 s and 60 °C for 1 min. Each sample was run in triplicates. Specific qRT-PCR primers for
630 six target genes were designed using an online tool Prime-Blast
631 (http://www.ncbi.nlm.nih.gov/tools/primer-blast) **(S8 Table).** Each sample was run in
632 triplicates. The 18S rRNA and elongation factor 1-α (PGSC0003DMG400020772,ef1α),
633 (Nicot et al., 2005) were used as the reference genes for normalization and the mock-treated
634 samples used as calibrators. The comparative $2^{-\Delta\Delta Ct}$ method was used to determine the relative
635 fold change according to Schmittgen and Livak (2008). Despite, the two techniques (RNA-seq
636 and qRT-PCR) being different, the expression patterns of selected genes upon nematode
637 infection was consistent between the two procedures **(Fig S9)**.
638

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639 **Data access**

640 Both raw and processed sequencing data have been deposited to the Gene Expression Omnibus
641 (GEO) repository at the National Center for Biotechnology Information (NCBI) with accession
642 no. GSE134790.

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649 **Author's Contributions**

650 L.N.M conceived, designed this study and funding acquisition. T.N.M set-up the experiment
651 for nematode inoculations, analyzed and visualized data and wrote the original draft. D.R.B
652 carried out the bioinformatics work. L.N.M and D.R.B revised the manuscript. All authors
653 reviewed and made changes to the initial draft and approved the final version.

654 **Abbreviations:** Cytokinin-CK; Differentially expressed genes-DEGs; Ethylene-ET; Gene
655 ontology-GO; Giant cells-GCs; Gibberellic acid-GA; Jasmonic acid-JA; Nematode feeding
656 sites-NFSs; Pathogenesis-related protein-PR; Pattern triggered immunity-PTI; Reactive
657 oxygen species-ROS; Root-knot nematode-RKN; Salicylic acid-SA; Transcription Factors-
658 TFs.

659 **Competing Interests**: The authors have no competing interests declare.

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955 **Figures**

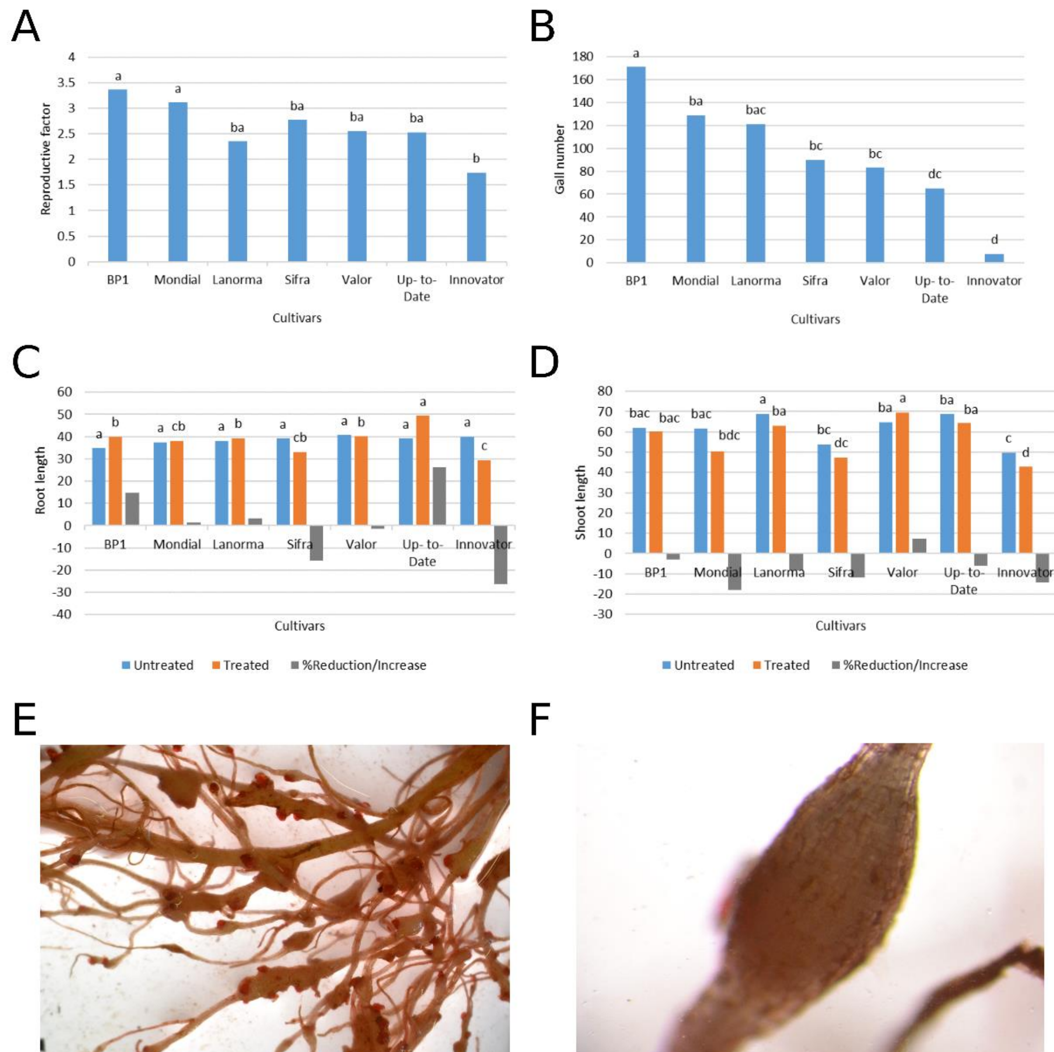


**Figure 1**: Responses of seven potato cultivars to *M. javanica* infection. **(A)** and **(B)** Reproductive factor and the number of galls, respectively induced by RKN. **(C)** and **(D)** Show the effect of nematode infection on root length and shoot length of potato cultivars. % increase or reduction= $\frac{Untreated - Treated}{Untreated}$*x100*. Values are means of five replicates. Statistical significance between the cultivars was determined by one-way ANOVA analysis with Fisher's least significant difference test at $P < 0.05$. (E) and (F) show nematode damage on potato roots, the egg masses stained pink and a mature gall, respectively.

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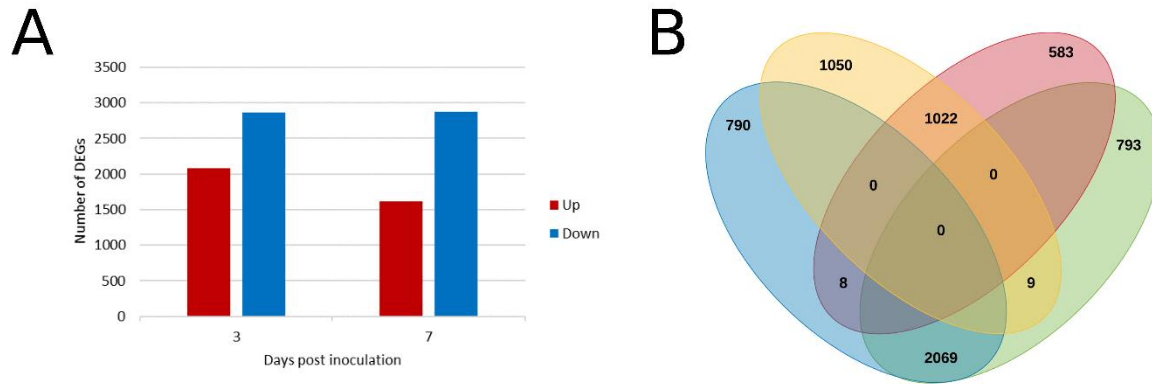


**Figure 2:** Schematic representation of DEGs in potato roots following *M. javanica* infection **(A)** Number of differentially expressed genes detected at 3 and 7 dpi compared to the mock-inoculated samples. 'Down' designates down-regulated genes. 'Up' designates up-regulated genes. **(B)** Venn diagram of the distribution of DEGs between 3 and 7 dpi. Yellow and blue ovals represent up-regulated and down-regulated DEGs at 3dpi, respectively. Red and green ovals indicate the genes upregulated and downregulated at 7 dpi, respectively.

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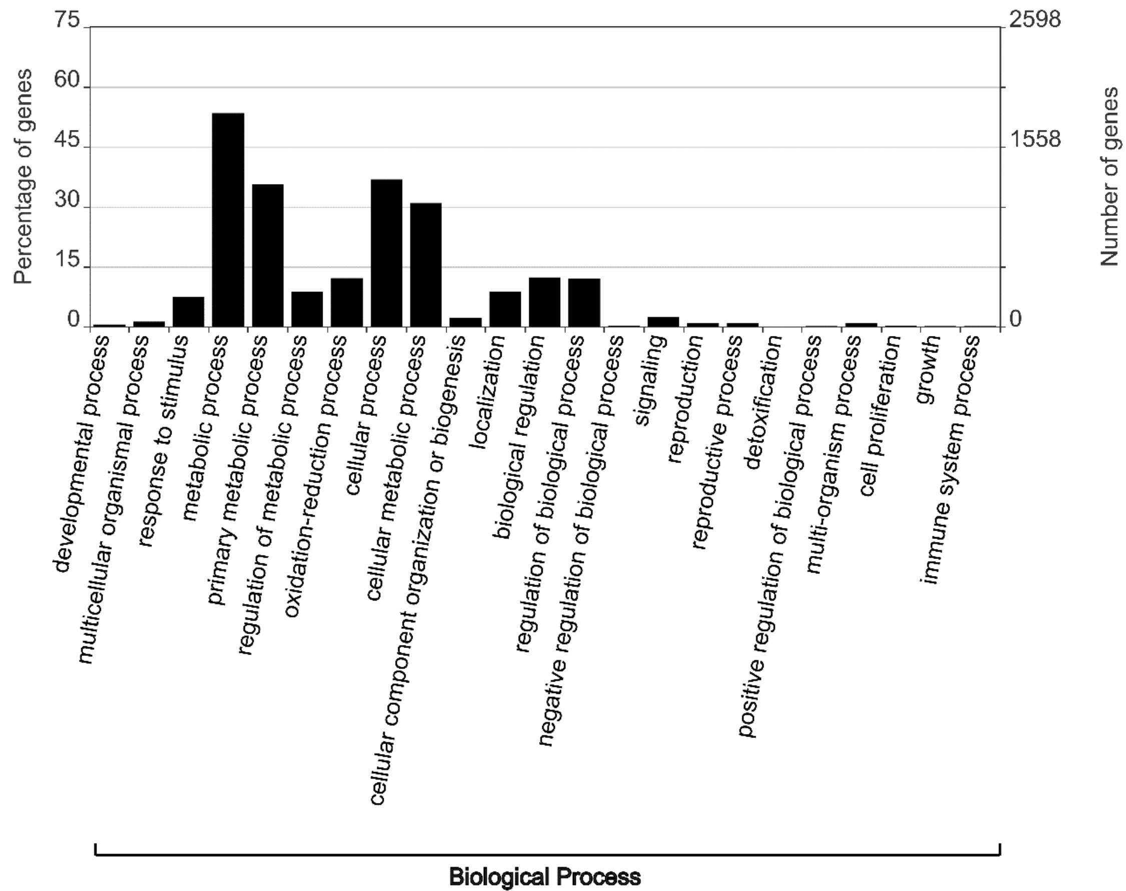


**Figure 3:** A representation of GO analysis demonstrates the percentage of DEGs enriched within the Biological Process category.

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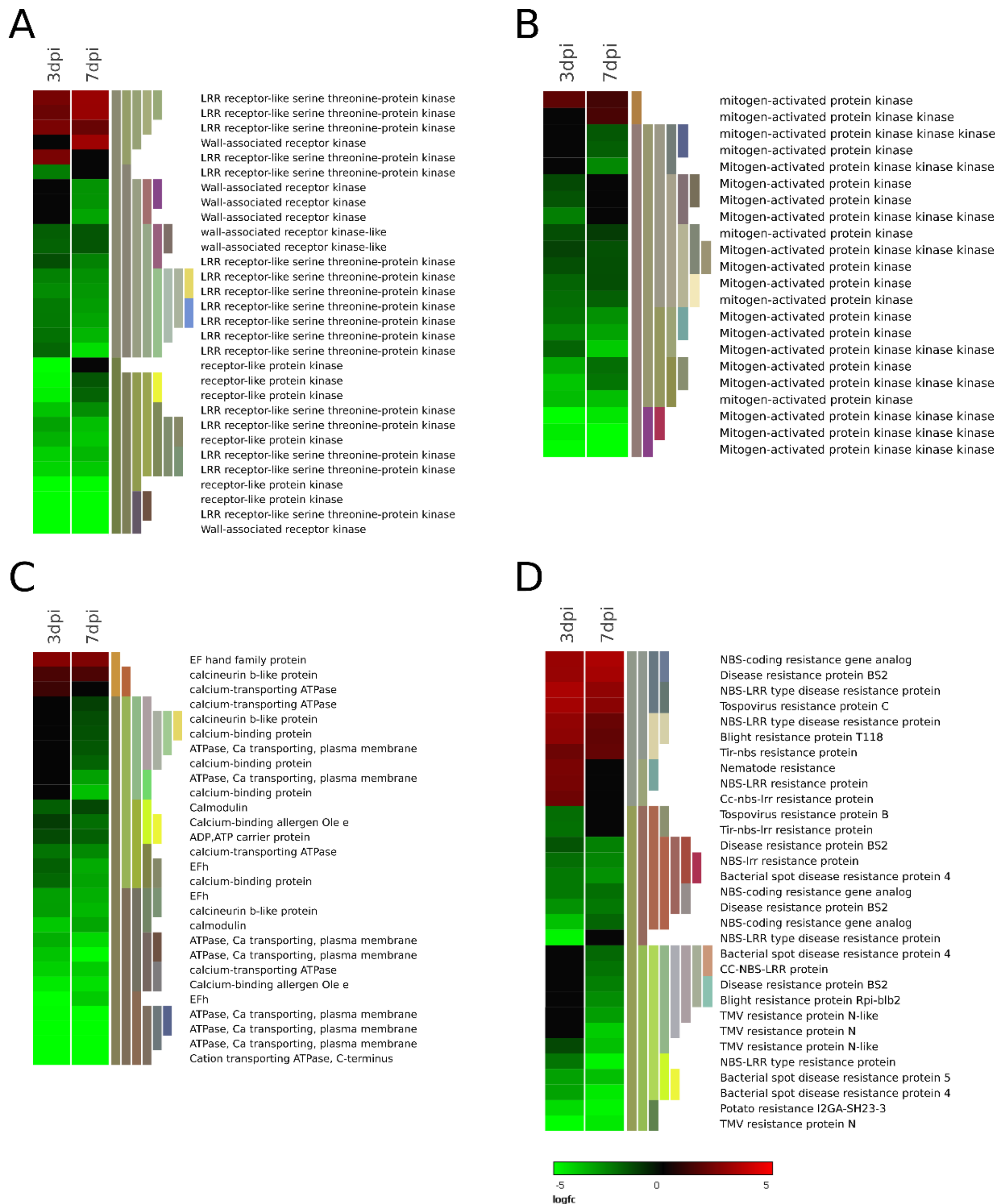


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**Figure 4:** Heat map representation of selected DEGs associated with pathogen perception **(A)** PRRs-RLKs, RLPs and WAKs **(B)** MAPK signaling pathways **(C)** Ca2+ signaling pathways, and Disease resistance proteins. **(D)** (The heat map illustrates a subset of genes from each group. Refer supporting information for all DEGs in each group).

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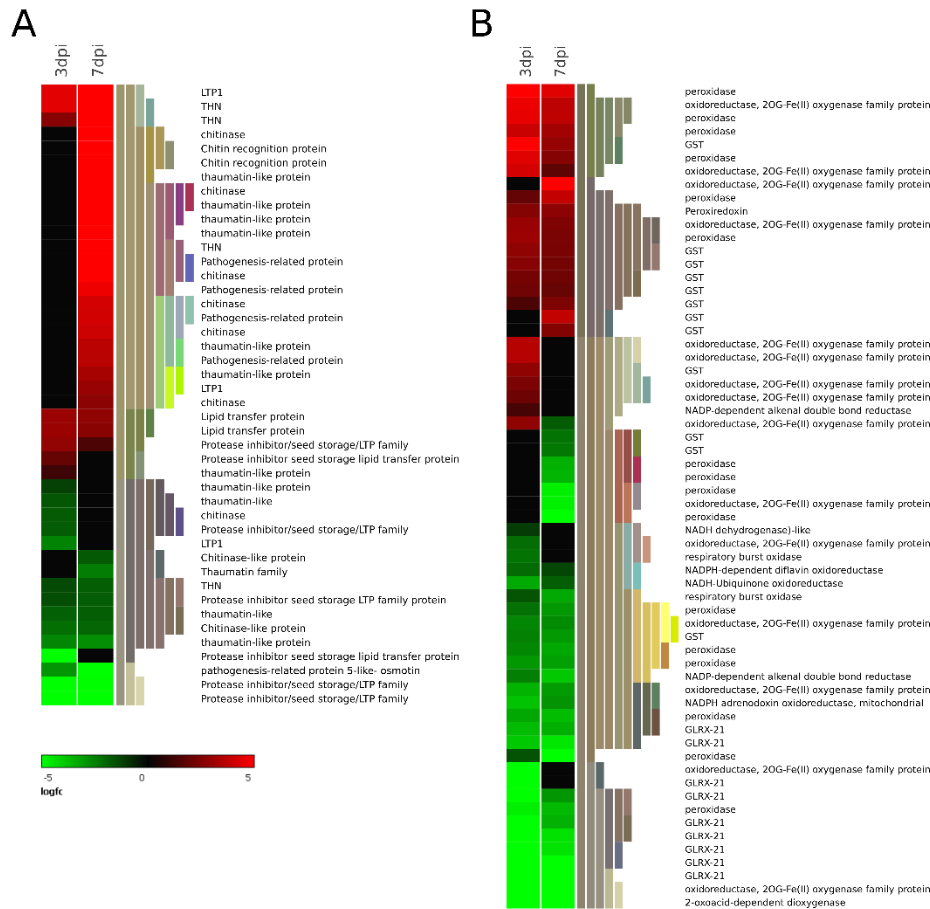


**Figure 5:** Heat map representation of selected DEGs associated with plant defense **(A)** Pathogenesis-related protein. **(B)** Oxidative stress-related gene (The heat map illustrates a subset of genes from each group. Refer to supporting information for all DEGs in each group).

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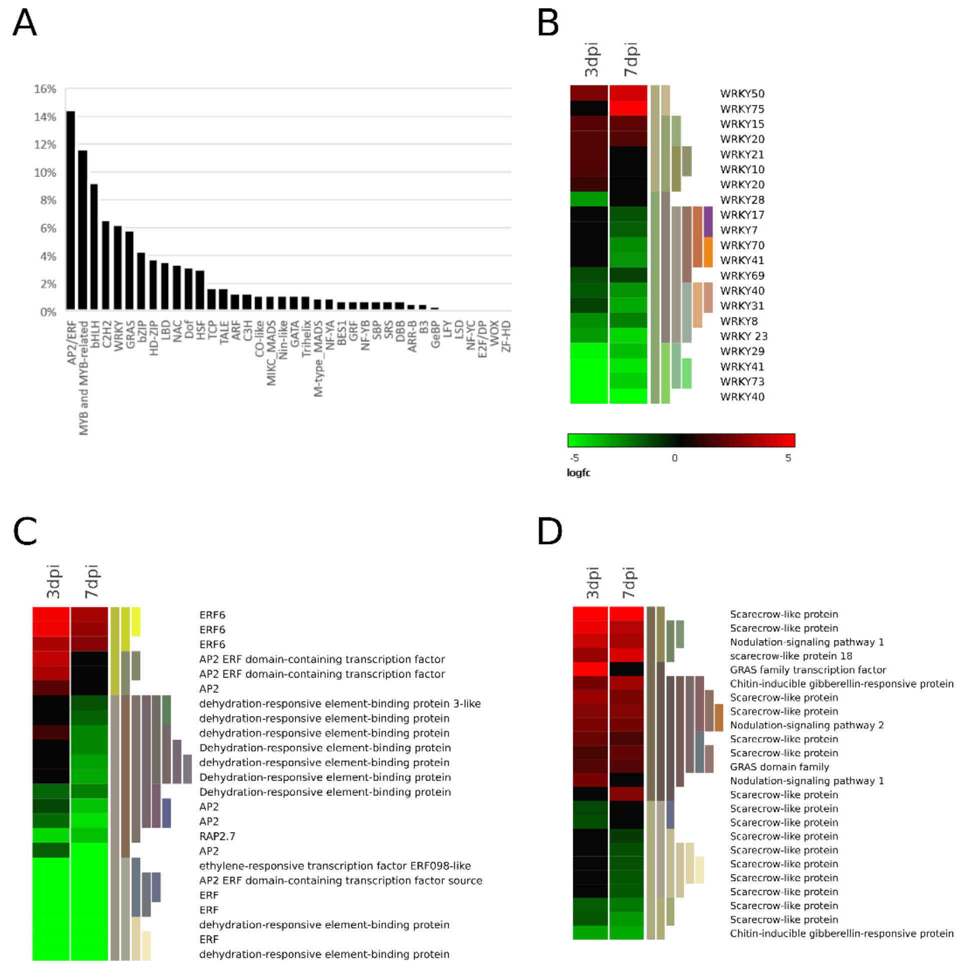


**Figure 6:** Heat map representation of differential regulation of TFs. **(A)** Represents various families of TFs under the regulation of RKN. **(B)** WRKY family. **(C)** AP2/ERF family. **(D)** GRAS family. (The heat map represents a subset of the differentially expressed family of TFs. Refer to supporting information for all TFs family displaying differential expression).

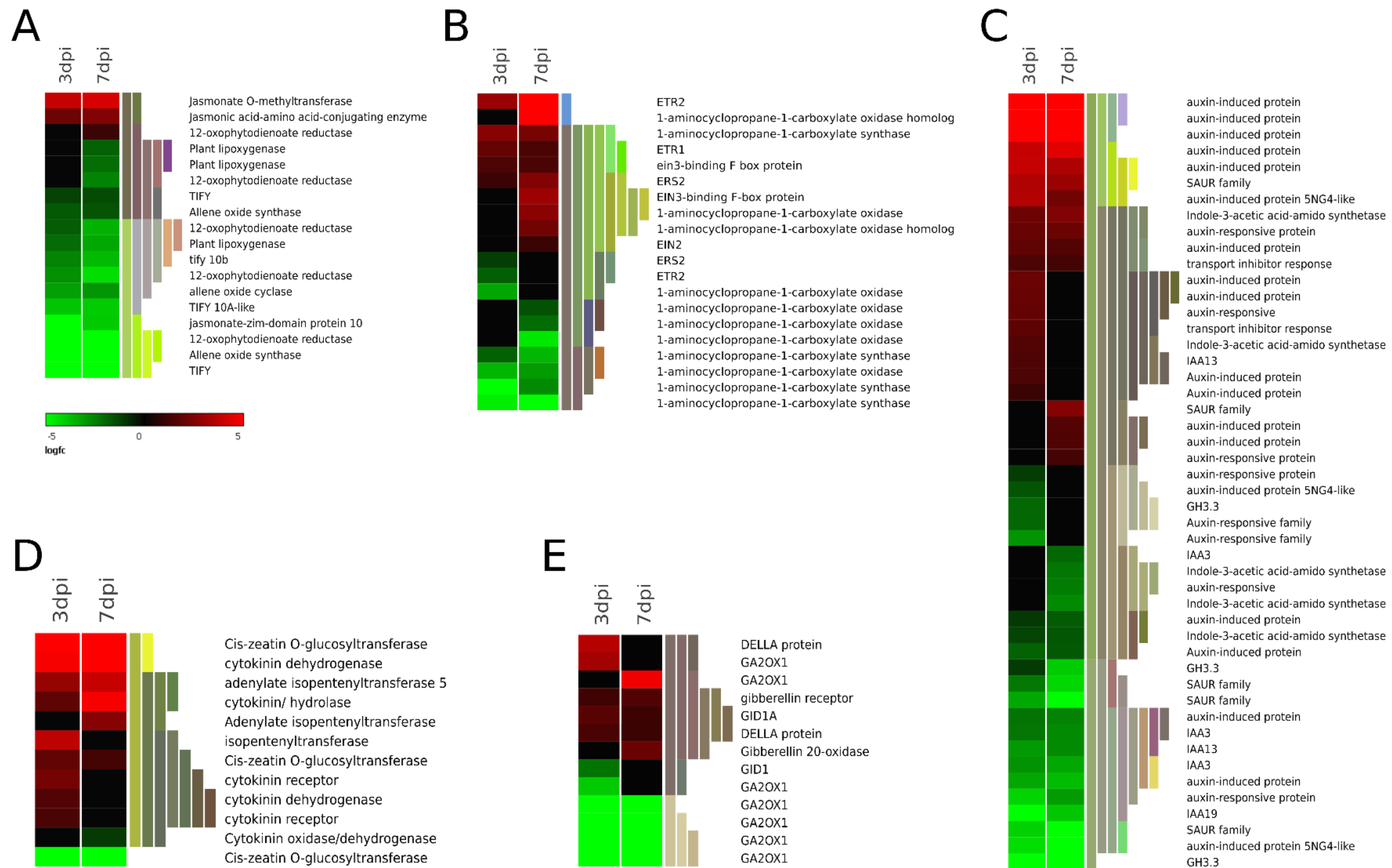


**Figure 7:** Heat map illustration of DEGs involved in hormone signal transduction. **(A)** JA signaling pathway. **(B)** ET signaling pathway. **(C)** Auxin signaling pathway. **(D)** Cytokinin signaling pathway. **(E)** GA signaling pathway. (The heat map illustrates a subset of genes from each group. Refer supporting information for all DEGs in each group).

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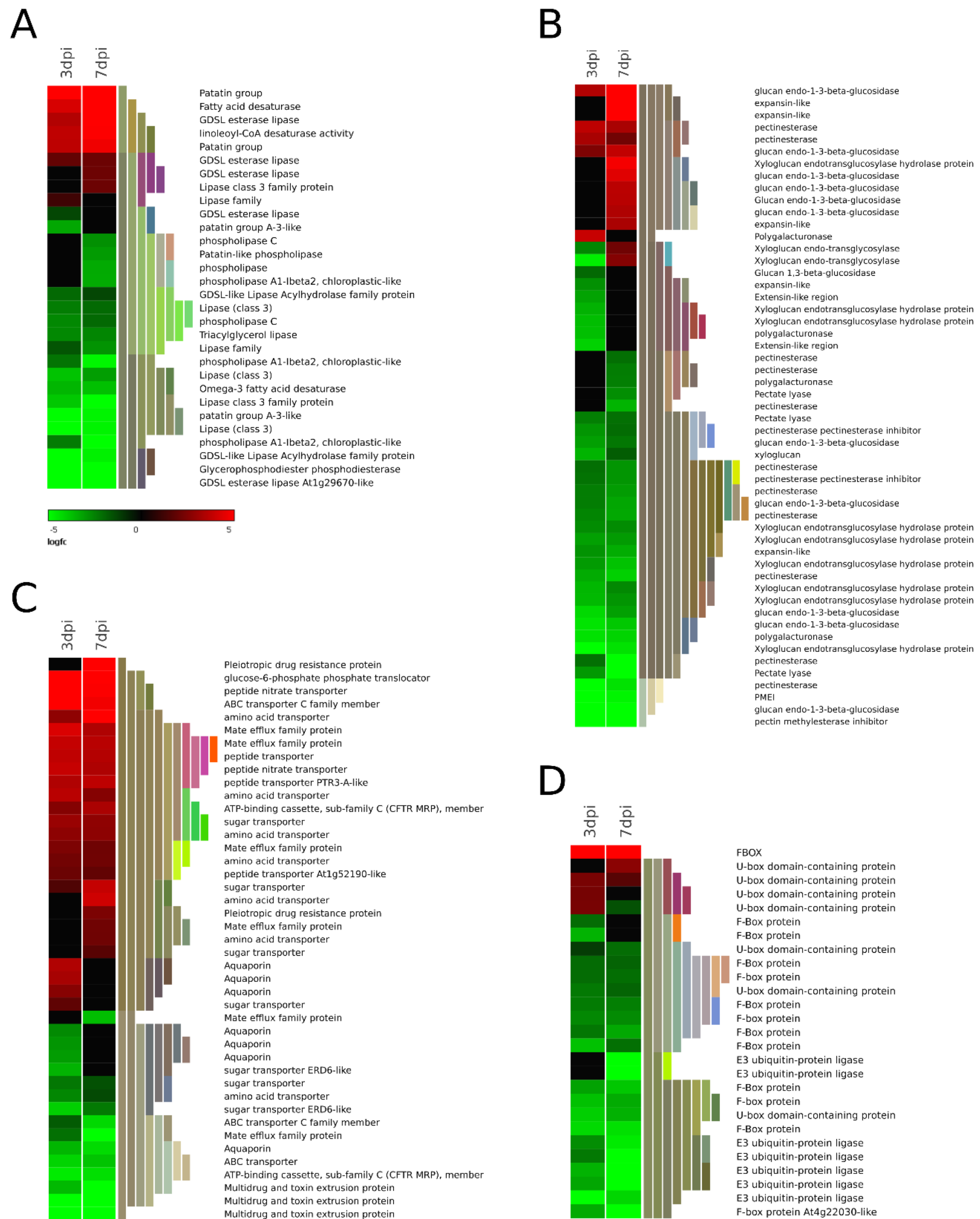


**Figure 8:** Heat map representation of gene expression patterns of genes associated with metabolism and transport activity **(A)** Lipid metabolism. **(B)** Cell wall architecture. **(C)** Transporters **(D)** Proteolysis and ubiquitination processes. (The heat map illustrates a subset of genes from each group. Refer supporting information for all DEGs in each group).