

1 Short communication

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3 **Defensive forwards: stress-responsive proteins in cell walls of crop plants**

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12 **ABSTRACT**

13 As the vital component of plant cell wall, proteins play important roles in stress response
14 through modifying wall structure and involving in wall integrity signaling. However, the
15 potential of cell wall proteins (CWPs) in improvement of crop stress tolerance has probably
16 been underestimated. Recently, we have critically reviewed the predictors, databases and
17 cross-referencing of subcellular locations of possible CWPs in plants (*Briefings in*
18 *Bioinformatics* 2018;19:1130-1140). In this study, taking maize (*Zea mays*) as an example,
19 we retrieved 1873 entries of probable maize CWPs recorded in UniProtKB. As a result, 863
20 maize CWPs are curated and classified as 59 kinds of protein families. By referring to GO
21 annotation and gene differential expression in Expression Atlas, we highlight the potential of
22 CWPs as defensive forwards in abiotic and biotic stress responses. In particular, several
23 CWPs are found to play key roles in adaptation to many stresses. String analysis also reveals
24 possibly strong interactions among many CWPs, especially those stress-responsive enzymes.
25 The results allow us to narrow down the list of CWPs to a few specific proteins that could be
26 candidates to enhance maize resistance.

27 **Keywords:** Cell wall proteins (CWPs); stress-responsive proteins; abiotic and biotic stresses;
28 stress-resistant crops; *Zea mays*

29 **1. Introduction**

30 Crop plants, especially cereals such as wheat, rice and maize, are the main source for food
31 and feed worldwide. In nature, crops are often suffering from abiotic and biotic stresses,
32 which adversely affect plant growth, development and eventually production. Therefore,
33 developing stress-resistant crops is crucial for global food security [1].

34 The major abiotic stresses that limit maize production includes drought, heat, cold and
35 flooding [2,3]. Maize yield decreases sharply when the plants are exposed to high
36 temperature (>29°C), particularly in the USA and in northern China [4]. Maize is sensitive to
37 flooding at seedling stage; about 25~30% annual yield loss is caused by flooding in India
38 alone [5]. It is often plagued by insect pests (e.g., corn borer and nematode) and fungal
39 invasion [6,7]. Alone the fungus *Colletotrichum graminicola*, which induces maize
40 anthracnose, is responsible for annual loss of up to one billion dollars in the USA [8].

41 In response, plants have developed the sophisticated mechanisms to adapt to both abiotic
42 and biotic stresses, such as forming structural barriers and recruiting chemical compounds.
43 As the outermost layer facing the environments, the cell wall provides stability and
44 protection to the plants and is involved in stress perception [9,10]. The role of plant cell wall
45 in stress response is increasingly emphasized, especially the surveillance of its structure is
46 closely associated with innate immunity in plants [11]. Notably, cell wall proteins (CWPs)
47 have been implied in various stresses through modifying wall structure and involving in wall
48 integrity signaling [12,13]. However, the potential of CWPs in crop improvement of stress
49 tolerance has probably been underestimated.

50 The composition of cell walls varies with plant species, cell type and developmental stage.
51 Pectin, cellulose and hemicellulose are the main components (>90%) of the primary cell wall,
52 whereas cellulose, hemicellulose and lignin are dominant in the secondary wall [14]. Despite
53 present in minor amounts (5–10% of the primary cell wall mass), CWPs are actively

54 involved in cell wall integrity signaling and innate immunity during plant development and
55 adaptation to environmental cues [12,13]. Most CWPs are basic proteins with a signal
56 peptide and are post-translationally modified, especially by hydroxylation, N-glycosylation
57 and O-glycosylation [14]. After synthesis in the cytosol, CWPs are targeted to the cell wall
58 and/or the extracellular space via the secretory pathways from endoplasmic reticulum (ER)
59 and Golgi apparatus to the cell wall [15].

60 Identification and cloning of resistance genes is an important prerequisite for targeted
61 breeding of stress-resistance crops, especially through genome editing technologies. To date,
62 only a small number of resistance genes have been identified and modulated in transgenic
63 maize to enhance resistance and improve production [16-21]. All these known resistance
64 genes encode intracellular proteins [22], whereas CWPs have not yet been targeted for crop
65 improvement of stress tolerance. However, many experimental data suggest the role of
66 CWPs in stress response. For example, proteomic analysis in rice and chickpea revealed that
67 many different abundance proteins in extracellular space may be involved in various cellular
68 processes, e.g. cell wall modification, metabolism, signal transduction, cell defense and
69 rescue [23,24].

70 Over the past 10 years, genome sequencing [25,26] and high-throughput profiling
71 analyses [27-29] in maize have generated huge amounts of CWPs data that have been stored
72 in public databases, especially in the UniProt Knowledgebase (UniProtKB). In this study, we
73 have performed the bioinformatic analysis of CWPs in maize. Our results highlight the
74 potential of CWPs as defensive forwards in stress adaptation. It may provide candidates for
75 targeted improvement of stress resistance in maize and other crops.

76 **2. Methods**

77 *2.1 Retrieving possible maize CWPs entries*

78 UniProtKB is the central hub for the collection of functional information on proteins, with

79 accurate, consistent and rich annotation [30]. Protein entries in UniProtKB have either been
80 confirmed with experimental evidence at protein level or are entirely predicted. Search of
81 UniProtKB with the keyword ‘*Zea mays*+ cell wall or apoplast or secreted protein’
82 (December 1, 2019) retrieved 1873 entries of possible maize CWPs, with only 50 curated
83 entries. Notably, many sequences were over-represented in UniProtKB, because the fact that
84 different maize lines have been sequenced and submitted separately. Therefore, we have
85 merged redundant sequences and only kept the entries with complete sequences. This applied
86 equally to protein isoforms produced from a single gene.

87 *2.2 Curating possible maize CWPs entries*

88 We curated the subcellular locations of all maize CWPs entries retrieved from UniProtKB.
89 Only a small number of the entries are annotated with definite localizations in cell wall
90 and/or extracellular spaces, whereas the majority of the records are computationally analyzed
91 and have no localization annotations. For the latter entries, we predicted their locations with
92 the software HybridGO-Loc [31], as we previously recommended [15]. Only those with
93 definite locations of cell wall or extracellular space were retained for further analysis. For
94 uncharacterized proteins with definite locations of cell wall or extracellular space, BLAST
95 was run against UniProtKB to find their homologues for functional assignment. The gene
96 differential expression of maize CWPs was referred to Expression Atlas
97 (<https://www.ebi.ac.uk/gxa/>).

98 *2.3 Protein-protein interaction analysis*

99 Protein-protein interaction networks were analyzed using the publicly available program
100 STRING (<http://string-db.org/>). STRING is a database of known and predicted
101 protein-protein interactions.

102 **3. Results and Discussion**

103 The cell wall proteome consists mainly of *strict sensu* CWPs present only in the wall and

104 secreted proteins present in the extracellular space (**Fig. 1A**). Inside a plant, the space
105 outside the plasma membrane can be defined as the apoplast where material can diffuse
106 freely. Gene Ontology (GO) definitions of CWPs include cell wall (GO:0005618) protein,
107 apoplast (GO:0048046) protein or secreted protein. Thus, apoplast proteins represent the
108 generalized CWPs. To an extent, cell wall, apoplast and extracellular space are partially
109 overlapping in scope and include related proteins.

110 After thorough curation, the maize CWPs dataset included 863 entries (Supplementary
111 Table S1), belonging to 56 kinds of protein families (Supplementary Table S2). We
112 functionally classified maize CWPs (**Fig. 1B**) by referring to functional classes of
113 Arabidopsis CWPs [14,32]. According to the number of entries, the top 10 families are
114 expansin (109), pectinesterase (108), xyloglucan endotransglucosylase/hydrolase (XTH) (80),
115 peroxidase (82), polygalacturonase (69), pectin acetyltransferase (66), α -L-arabinofuranosidase
116 (64), pectin lyase (51), germin-like proteins (42), and galactosidase (27). Some members of
117 different families are highly similar, such as chitinase (D0EM57) and endochitinase (P29022)
118 (96.44% identity), non-classical arabinogalactan protein (A0A3L6FGF2) and pistil-specific
119 extensin-like protein (B6UHE3) (99.58% identity).

120 Notably, 36 kinds of the CWPs represent various enzymes, implied in various
121 physiological or biological processes, especially cell wall organization (including
122 polysaccharide biogenesis, degradation and modification), protein proteolysis, cell redox
123 homeostasis, and abiotic and biotic stress responses. Moreover, STRING analysis implied that
124 maize CWPs participate in MAPK and Wnt signaling pathways. It is suggested that MAPK
125 and Wnt signaling pathways may play pivotal roles in linking perception of external stimuli
126 with changes in cellular organization or gene expression [33].

127 The importance of the CWPs that function in cell wall organization is obvious because
128 polysaccharides constitute the largest components of plant cell walls and are constantly

129 subjected to remodeling during plant development or in response to environmental cues.
130 Likewise, glycoside hydrolase family proteins are very important due to their activities to
131 hydrolyze chitin (a primary component of fungus cell walls) to confer resistance to fungus.
132 In addition, the importance of several oxidoreductases (e.g. L-ascorbate oxidase, malate
133 dehydrogenase, peroxidase, and polyamine oxidase) was expected in maintaining cell redox
134 homeostasis that may subjected to change under various stresses [34].

135 Regarding subcellular localizations, 32 kinds of the CWPs exist only in the cell wall, 11
136 only in the extracellular space, and 13 both in the cell wall and the extracellular space. The
137 CWPs present in the cell wall, such as structural proteins, may interact with other wall
138 components by non-covalent linkages to form insoluble networks [35]. The CWPs present in
139 the extracellular space, especially between the cell plasma membrane and the cuticle in aerial
140 organs or the suberin layer in roots, may confer to the plant surface waterproof qualities and
141 protection against biotic and abiotic stresses [36,37]. In addition, many CWPs, such as
142 peroxidase (A5H8G4), malate dehydrogenase (B4FRJ1), peroxiredoxin (B6T2Y1), purple
143 acid phosphatase (B4FR72), NADH-cytochrome b5 reductase (B6TCK3),
144 carbohydrate-binding-like fold (A0A1D6FHT0) and peroxiredoxin (B6T2Y1), also have
145 intracellular locations.

146 It should be noted that the 863 entries of maize CWPs collected here are sufficiently
147 representative. By comparison, the WallProtDB, a specialized collection of cell wall
148 proteomic data [38], records only 2,170 protein sequences from 11 different plant species
149 (without maize). In particular, the 270 entries of rice CWPs in the WallProtDB, belonging to
150 46 kinds of protein families, are homologous with the corresponding maize CWPs.
151 Undoubtedly, the list of maize CWPs entries remains incomplete. For example,
152 pectinesterase, endoglucanase and endoxylanase inhibitors are pathogenesis-related proteins
153 found in cereals and dicots [39], but only maize pectinesterase/pectinesterase inhibitor

154 (A0A1D6KNZ1) are retrieved from UniProtKB. This is possibly due to the difficulty in
155 proteomic analysis of the CWPs and the lack of complete annotation of all sequenced genes.
156 With the technical advance in cell wall isolation, proteomic analysis of maize cell walls will
157 identify more the ‘missing’ CWPs.

158 To summarise the potential functions of maize CWPs, we checked gene differential
159 expression of maize CWPs in Expression Atlas (<https://www.ebi.ac.uk/gxa/>). Analysis of the
160 inducible genes suggested that 36 kinds of maize CWPs are implied with a definite role in
161 various biotic and abiotic stresses (**Table 1**, Supplementary Table S3). In particular, at least
162 ten CWPs are simultaneously involved in 5-7 kinds of stresses, including germin-like protein
163 (B4FAV5), UDP-arabinopyranose mutase (P80607), β -fructofuranosidase (P49174),
164 chitinase (D0EM57), peptidase A1 domain-containing protein (B4G1Q7), peroxidase 1
165 (A5H8G4), β -D-xylosidase (B4F8R5), eukaryotic aspartyl protease (A0A1D6DSN9),
166 NADH-cytochrome b5 reductase (B6TCK3), O-glycosyl hydrolase superfamily protein
167 (K7V329), and subtilisin-like protease SBT2.6 (C0P6H8). Our analysis also revealed that
168 maize may recruit a different set of CWPs (**Table 1**) to respond to abiotic and biotic stresses,
169 although both stress responses may share common CWPs.

170 The roles of some CWPs in abiotic stresses have been proved in Arabidopsis and other
171 plant species. Here we just referred some examples, because we did not aim to
172 comprehensively review previous studies. In Arabidopsis, pectinesterase 1 acts as negative
173 regulators of genes involved in salt stress response [40]; pectin methylesterase is required for
174 guard cell function in response to heat [41]; purple acid phosphatase 17 is reducible by
175 ABA(abscisic acid), H₂O₂, senescence, phosphate starvation and salt stress [42]. In rice,
176 β -galactosidase gene responds to ABA and water-stress [43] and germin-like proteins are
177 associated with salt stress [44]. In other plants, β -galactosidase was found to be related to
178 abiotic stress, especially heavy metals [45]; glycine-rich proteins [46,47] and cell wall

179 invertase (copper stress, [48]) are stress-induced. Stress upregulates the expression of
180 expansins and xyloglucan-modifying enzymes that can remodel the wall under abiotic stress
181 [49]. In *Medicago truncatula*, XTH respond to heavy metal mercury, salinity and drought
182 stresses [50], possibly through incorporating newly deposited xyloglucan to strengthen cell
183 walls. However, the role of similar CWPs in maize under abiotic stresses needs to be
184 characterized.

185 Many maize CWPs are implied to play a role in response of plants to biotic stress. For
186 example, aspartyl protease AED3 (B4FMW6) may be involved in systemic acquired
187 resistance against fungal invasion. Transcription profiling revealed that its transcript
188 (*Zm00001d027965*) was increased by a Log₂-fold change of 4.3 in maize infected with
189 *Ustilago maydis*. The role of some CWPs in biotic stress have been studied in different plant
190 species. For example, chitinase and endochitinase A have antifungal activity against
191 chitin-containing fungal pathogens [51,52]. Overexpressing extensin enhanced Arabidopsis
192 resistance to *Pseudomonas syringae* by promoting cell wall stiffness [53]. Pectin-degrading
193 enzymes (polygalacturonases, pectatylases, and pectinmethyl esterases) and
194 xylan-degrading enzymes (endoxylanases) are key virulence factors for pathogens. As a
195 counterattack, plants respond these attacks with a wide range of protein inhibitors of
196 polysaccharide-degrading enzymes [54], e.g. maize pectinesterase/pectinesterase inhibitor
197 (A0A1D6KNZ1) and XIP (xylanase inhibitor protein) [27]. In fruits, polygalacturonases and
198 pectatylases contribute substantially to the softening of fruit. Suppression of these enzymes
199 delays fruit softening and simultaneously confers enhanced resistance to pathogens like
200 Botrytis [55,56].

201 As demonstrated in **Table 1**, many maize CWPs may have a role in both abiotic and biotic
202 stress, such as aspartyl protease AED3, HRGPs, β -D-xylosidase, germin-like protein,
203 peroxidase, β -fructofuranosidase etc. It is recognized that HRGPs play major roles in plant

204 defense against abiotic and pathogen attacks [57]. Arabidopsis β -fructofuranosidase (Q43866)
205 is involved in defense to fungus, karrikins and wounding.

206 However, many maize CWPs did not imply to have significant roles in stress response,
207 such as dirigent protein, expansin, heparanase-like protein, proline rich cell wall protein,
208 exopolygalacturonase, heparanase-like protein 3 etc. However, the roles of dirigent protein
209 and expansins in stress response have been suggested in Arabidopsis [58]. Heparanase
210 activity is strongly implicated in structural remodeling of the extracellular matrix of animals,
211 a process which can lead to invasion by tumor cells [59].

212 Many protein/protein interactions are expected in cell walls and between CWPs with those
213 spanned in plasma membrane, not to mention leucine-rich repeat (LRR) family protein and
214 pectinesterase/pectinesterase inhibitor enzymes that exist in maize cell walls. Recently, a
215 spanning the plasma membrane protein ZmWAK that confers quantitative resistance to
216 maize head smut, possibly acting as a receptor-like kinase to perceive and transduce
217 extracellular signals [20]. String analysis revealed that many stress-responsive CWPs,
218 especially chitinase, β -hexosaminidase, glycoside hydrolase, α -galactosidase, pectinesterase,
219 and β -fructofuranosidase may form strong-interaction networks in maize (**Fig. 2**). These
220 stress-responsive CWPs can act as a frontline defense or involved in cell signaling process
221 under abiotic and biotic stresses.

222 As the interface with the environment, the existing CWPs faces with intense selection
223 pressure to evolve new functions or recruit new proteins to the apoplast through gene
224 duplication and retargeting [60]. Genetic and transgenic evidence in Arabidopsis and other
225 species supported that cell wall gene families associated with cell wall remodeling during
226 abiotic stress and pathogen attack [9]. Therefore, the approach of modifying CWPs provides
227 a novel and rational means of enhancing crop resistance.

228 At present, many CWPs are suggested as stress-responsive only based on the RNA-Seq

229 data. It was not clear if the protein abundance increased accordingly. So, their accumulation
230 at protein level need to be validated under normal and stress conditions. Another possibility
231 is that not all stress-responsive CWPs play a key role in stress tolerance. This highlights a
232 need to extend genome editing technologies toward specific CWPs. Further molecular and
233 genetic characterization of maize CWPs, especially those with intracellular localizations,
234 will clarify their functions in mediating the plant response to various stresses. Importantly,
235 specific protein abundance in cell walls may not be enough under high stress severity or
236 where crop is exposed to multiple stresses like flooding, drought and diseases, thus
237 enhancing stress-responsive CWPs in crop plants with gene transfer and genome editing is a
238 straightforward approach to enhance crop resistance.

239 In conclusion, we highlight the potential role of stress-responsive CWPs as defensive
240 forward in maize defense response to various stresses. After clarification of the functions of
241 stress-responsive CWPs during growth, development and stress adaptation, specific CWPs
242 can be candidates for application in genetic modification of stress tolerance in maize and
243 other crops. This may have an important impact on global food security.

244

245 **FIGURE LEGENDS**

246 **Fig. 1.** Subcellular localizations highlighting cell wall and extracellular space (**A**) and major
247 molecular functions (**B**) of the examples of maize CWPs. 1, α -L-arabinofuranosidase 1; 2,
248 α -L-fucosidase 2; 3, ankyrin repeat family protein; 4, aspartyl protease AED3; 5, auxin-induced
249 β -glucosidase; 6, basic endochitinase; 7, β -D-xylosidase; 8, β -fructofuranosidase; 9, β -glucosidase; 10,
250 β -hexosaminidase; 11, carbohydrate-binding-like fold; 12, cell wall invertase; 13, chitinase; 14,
251 chitin-binding type-1 domain-containing protein; 15, dirigent protein; 16, DUF1005 family protein; 17,
252 endochitinase; 18, eukaryotic aspartyl protease; 19, exopolysaccharidase; 20, expansin; 21,
253 α -galactosidase; 22, β -galactosidase; 23, germin-like protein; 24, glycine-rich cell wall structural protein;
254 25, glyco_hydro_19_cat domain-containing protein; 26, glycoside hydrolase; 27, group 3 pollen allergen;

255 28, heparanase-like protein 3; 29, hydroxyproline-rich glycoprotein (HRGP); 30, leucine-rich repeat (LRR)
256 family protein; 31, malate dehydrogenase; 32, NADH-cytochrome b5 reductase; 33, non-classical
257 arabinogalactan protein 31; 34, nudix hydrolase domain-containing protein; 35, O-glycosyl hydrolase
258 superfamily protein; 36, pectin acetyesterase; 37, pectin lyase; 38, pectin methylesterase 1; 39,
259 pectinesterase; 40; pectinesterase/pectinesterase inhibitor; 41; pepsin A; 42, peptidase A1
260 domain-containing protein; 43, peroxidase 1; 44, peroxiredoxin; 45, L-ascorbate oxidase; 46, polyamine
261 oxidase 1; 47, polygalacturonase; 48, proline and lysine rich protein; 49, protein EXORDIUM-like 3; 50,
262 purple acid phosphatase (PAP); 51, pyrroline-5-carboxylate reductase; 52, subtilisin-like protease SBT2.6;
263 53, UDP-arabinopyranose mutase; 54, wall structural protein; 55, vegetative cell wall protein gp1; 56,
264 xyloglucan endotransglucosylase/hydrolase.

265 **Fig. 2.** String analysis of possible protein-protein interaction among maize CWPs.

266

267 SUPPLEMENTARY MATERIAL

268 **Supplementary Table S1** Cell wall proteins of maize retrieved in UniProtKB

269 **Supplementary Table S2** Summary of GO annotation of maize CWPs and their gene
270 differential expression

271 **Supplementary Table S3** Summary of GO annotation of representative maize CWPs and
272 their gene differential expression

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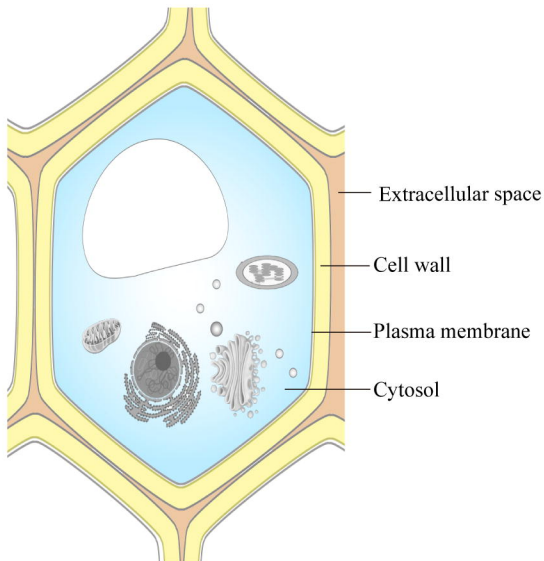
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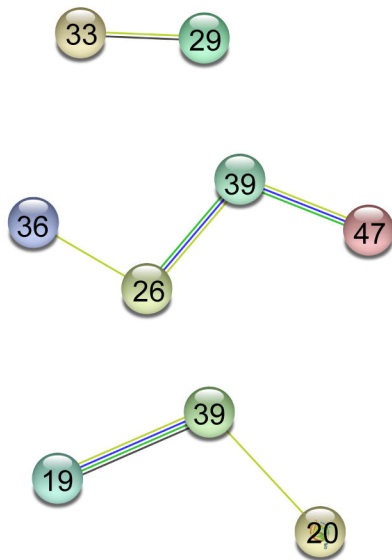
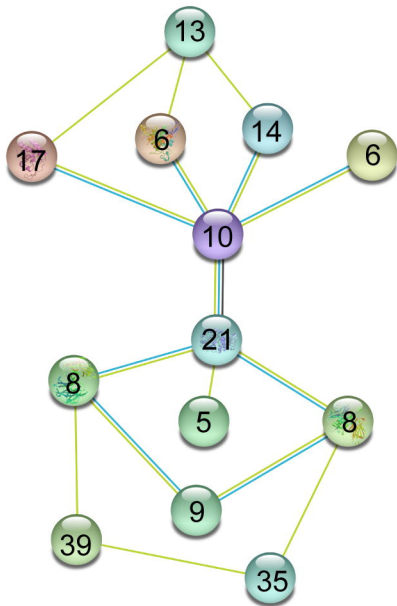
Table 1 Subcellular localizations of stress-responsive CWPs

Stress	Subcellular localizations		
	Cell wall	Cell wall & extracellular	Extracellular
Abiotic stress			
Drought	③ ⑦ ⑫ ⑮ ⑲ ⑳ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	④ ⑧ ⑬ ⑯ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	㉞
Salinity	㉞ ㉟		
Heat	⑦ ⑨ ⑬ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	④ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	⑬
Cold	③ ⑤ ⑨ ⑲ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	④ ⑧ ⑬ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	
Flooding	⑤ ⑦ ⑮ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	④ ⑧ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	⑬
Acidic soil	① ⑩ ⑮ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	④ ⑧ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	⑬
Biotic stress			
Fungus	① ⑤ ⑦ ⑨ ⑩ ⑫ ⑮ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	④ ⑧ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	⑥ ⑬ ⑰ ㉞
Bacterium	㉞		
ECB larvae	① ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	⑧ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	⑬
Corn leaf aphids	⑤	㉑	
Nematode	⑦ ⑮ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	⑧ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘ ㉙ ㉚ ㉛ ㉜ ㉝ ㉞ ㉟ ㊱ ㊲ ㊳ ㊴ ㊵ ㊶ ㊷ ㊸ ㊹ ㊺ ㊻ ㊼ ㊽ ㊾ ㊿	

Note: the serial numbers represent the CWPs numbered in Figure 1 legend.

A**B**

Molecular function	CWPs	Protein family
Enzyme (α -L-arabinofuranosidase, α -L-fucosidase, β -glucosidase, xylan 1,4- β -xylosidase, β -fructofuranosidase) activity in carbohydrate (arabinan, xylan, pectin, etc.) metabolic process	① ② ⑤ ⑦ ⑧ ⑨ ⑩ ⑫ ⑬ ⑭ ⑮ ⑯ ⑰ ⑱ ⑲ ⑳ ㉑ ㉒ ㉓ ㉔ ㉕	Glycoside hydrolase superfamily
Carbohydrate binding and modification	⑳ ㉑ ㉒	Pectinesterase family
Structural constituent of cell wall	㉑	Expansin family
Structural constituent of cell wall	㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘	Glycine-, hydroxyproline-, or proline and lysine-rich protein
Chitin catabolic process in defense response to <i>fungus</i>	⑥ ⑬ ⑭ ⑰ ㉑	Glycoside hydrolase superfamily
Protein metabolic process (binding, proteolysis)	③	Ankyrin repeat family protein
Oxidoreductase activity in cell redox homeostasis	④ ⑧ ⑩ ⑫ ㉑ ⑪ ⑬ ⑭ ⑮ ⑯ ㉑ ㉑	Peptidase
Oxidoreductase activity in cell redox homeostasis	⑪ ⑬ ⑭ ⑮ ⑯ ㉑ ㉑	Dehydrogenase oxidase, reductase, peroxidase, etc.
Signaling process	㉑	Leucine-rich repeat (LRR) family protein
Miscellaneous functions	⑪ ⑬ ⑰ ⑲ ㉑ ㉒ ㉓ ㉔ ㉕ ㉖ ㉗ ㉘	Various proteins



- ⑤ Auxin-induced beta-glucosidase
- ⑥ Basic endochitinase
- ⑧ Beta-fructofuranosidase
- ⑨ Beta-glucosidase
- ⑩ Beta-hexosaminidase
- ⑬ Chitinase
- ⑭ Chitin-binding type-1 domain-containing protein
- ⑰ Endochitinase
- ⑱ Exopolygalacturonase
- ⑳ Expansin-B11
- ㉑ Alpha-galactosidase
- ㉒ Glycoside hydrolase
- ㉓ Hydroxyproline-rich glycoprotein
- ㉔ Non-classical arabinogalactan protein 31
- ㉕ O-Glycosyl hydrolase
- ㉖ Pectin acetylesterase
- ㉗ Pectinesterase
- ㉘ Polygalacturonase