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RESEARCH ARTICLE

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Host plant adaptability and proteomic differences of diverse *Rhopalosiphum maidis* (Fitch) lineages

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Abstract

Corn leaf aphid *Rhopalosiphum maidis* (Fitch) can feed on various cereal crops and transmit viruses that may cause serious economic losses. To test the impact of both host plant species and age on *R. maidis*, as well as the proteomic difference of diverse populations, we first investigated the survival and reproduction of six *R. maidis* populations (i.e., LF, HF, GZ, DY, BJ, and MS) via a direct observation method in the laboratory on 10 and 50 cm high maize seedlings, and 10 cm high barley seedlings. Then a proteomic approach was implemented to identify the differentially expressed proteins from both aphids and endosymbionts of BJ and MS populations. Results indicated that the BJ population performed significantly better than the others on both barley and 50 cm high maize seedlings, while no population could survive on 10 cm high maize seedlings. The proteomic results demonstrated that the expression levels of myosin heavy chain (muscle isoform X12) (spot 781) and peroxidase (spot 1383) were upregulated, while ATP-dependent protease Hsp 100 (spot 2137) from *Hamiltonella defensa* and protein SYMBAF (spot 2703) from *Serratia symbiotica* were downregulated in the BJ population when compared to expression levels of the MS population. We hypothesize that the fatalness observed on 10 cm high maize seedlings may be caused by secondary metabolites that are synthesized by the seedlings and the

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MS population of *R. maidis* should be more stress-resistant than the BJ population. Our results also provide insights for understanding the interaction between host plants and aphids.

KEYWORDS
corn leaf aphid, plant age, plant species, population, proteome

1 | INTRODUCTION

Rhopalosiphum maidis (Fitch) (Hemiptera: Aphididae) is a severe sap-sucking pest on maize (*Zea mays* L.) that occurs globally (Foott & Timmins, 1973; Tzin et al., 2015). This insect also attacks barley (*Hordeum vulgare* L.) and some other cereal crops such as sorghum (*Sorghum bicolor* L. Moench), oat (*Avena sativa* L.), and wheat (*Triticum aestivum* L.) (Carena & Glogoza, 2004; Tabikha, 2016). In addition to physical damage on the host plant, *R. maidis* can also transmit viruses, including *Maize dwarf mosaic virus* and *Barley yellow dwarf virus* (Parry et al., 2012; Saksena et al., 1964; Thongmeearkom et al., 1976), which may lead to serious damages in maize production.

Aphids can survive on different cereal crops although some hosts may exhibit a certain degree of resistance. Previous studies on *R. maidis* showed different survival and reproductive rates on different host plants of barley and wheat (Hirano & Ito, 1964; Singh & Painter, 1964). Research on other aphid species also showed that *Toxoptera citricida* (Kirkaldy), for instance, developed at a slower rate on sour orange, Duncan grapefruit, and Mexican lime than on Carrizo and sweet orange (Tang et al., 1999), whereas *Aphis craccivora* (Koch) showed the best potential population growth on *Vicia faba* at 25°C compared to *Trifolium subterraneum* cultivars and *Medicago minima* (Berg, 1984). In this way, resistant host plants or genotypes can be determined to improve biological control efficacy (Narang & Rana, 1999).

Different aphid populations can furthermore have various levels of success on the same host plant (Broeke et al., 2013), as previously reported for *Acyrthosiphon pisum* (Sandström & Pettersson, 1994), *Myzus persicae* and *A. craccivora* (Edwards, 2001) and *Sitobion avenae* (Barrios-SanMartín et al., 2016), demonstrating that long-term population differentiation may endow different populations living in various environments with diverse biological characteristics.

Many aphids harbor secondary symbionts (i.e., facultative symbionts) in nature. Some aphids are single-infected while others are double- or multi-infected with different secondary symbionts (Guo et al., 2019; Weldon et al., 2019). These symbionts are primarily transmitted maternally (i.e., vertically transferred) within populations, or can exhibit horizontal transmission among species (Oliver et al., 2010). According to previous studies, secondary symbionts offer their host aphids benefits such as resistance to natural enemies and increasing the fitness of their host plants to persist and spread (Guo et al., 2017).

With this context in mind, the present study aimed at assessing the performance (i.e., longevity and reproduction) of different populations of an aphid species (*R. maidis*) on host plants that differed in age and species. Six *R. maidis* populations were monitored on barley (10 cm high), small maize seedlings (10 cm high) and high maize seedlings (50 cm high) under laboratory conditions. A proteomic approach, using two-dimensional difference gel electrophoresis (2D-DIGE) analysis and mass spectrometry, was applied to provide insight into the putative molecular mechanisms (including symbiont infestation) underlying the potential variation of aphid performances.

2 | MATERIALS AND METHODS

2.1 | Aphid lineages

Six lineages of *R. maidis*, namely BJ, DY, GZ, HF, LF, and MS populations, were collected from maize field in Beijing, Dongyang, Guangzhou, Hefei, Langfang and Mangshi cities of China respectively in 2014 (Figure 1). All clones were reproduced initially from one female and then reared separately at 23 ± 1°C, L16: D8 photoperiod and 60%–80% relative humidity (RH) in laboratory on barley seedlings for more than ten generations before test.

2.2 | Host plants

Three treatments of host plants, barley seedlings (10 cm high), small maize seedlings (10 cm high) and high maize seedlings (50 cm high), were used for this study. The height of the host plants was measured with a ruler. To ensure germination rate and uniformity, the maize seeds were treated with accelerating germination before sowing into the soil. Rearing conditions for plants were 20 ± 1°C, 60%–80% RH and a photoperiod of L16: D8 before inoculation of aphid. All plants were moved to aphid rearing conditions as described above after inoculation.

2.3 | Treatment settings

Both barley seeds and germinated maize seeds were sown in 10 × 10 cm square plastic pots. We kept 12 ± 1 barley seedlings and one maize seedling in each pot. Five adult apterous aphids were inoculated to the seedlings of required height in each pot, then the pots were separated by a plastic tube (10 cm in diameter) covered by gauze element. Each treatment was replicated 10 times and the total survival number of aphids was observed at the same time every day for 1 week. We recorded the survival number of inoculated adults and number of nymphs for 4 days since it was difficult to distinguish the inoculated adults from the fifth day.

FIGURE 1 Collecting locations of six *Rhopalosiphum maidis* populations in China. 1, MS; 2, LF; 3, BJ; 4, HF; 5, DY; 6, GZ

2.4 | 2D-DIGE and protein identifications

BJ and MS populations were selected to do proteomic analysis since BJ population performed the best while MS population was more abundant in endosymbionts according to our previous study (Guo et al., 2019). Total protein from around 50 mg fresh *R. maidis* of BJ and MS populations were extracted, purified and quantified as described by Francis et al. (2010). Three Cy dyes (GE Healthcare) were used for labelling and protein samples of BJ and MS aphid clones were labelled either with Cy3 or Cy5 and mixed with an internal reference standard protein mixture (pooled from equal aliquots from all experimental samples) labelled with Cy2. Two replicates from each treatment with one dye (Cy3 or Cy5) and a third replicate with the other of the two Cy dyes were established for a conventional dye swap of DIGE. The first and second-dimensional electrophoresis, the excision of protein spots and the process of protein identification were performed following the description in Francis et al. (2010).

2.5 | Statistical analysis

Generalised linear mixed effect models (package "lme4," function "glmer"; Bates et al., 2014)) with Poisson error distribution (log-link function) were fitted to test whether the abundance of aphids (i.e., the nymphs, the survived inoculated adults, and the total abundance of aphids) (i) differed between populations on each crop separately (i.e., populations analyzed as fixed factor), (ii) differed between host plants for each population separately (i.e., host plants as fixed factor). The observed plants (i.e., 10 repetitions per crop) were included as random effects as measurements were repeated each time on the same plant. For each model, the effect of the fix factor was tested using a likelihood-ratio test ($p < 0.05$) and means (i.e., between the different populations or between the different host plants) were compared using a post hoc test of Tukey ($p < 0.05$, package "multcomp," function "glht," Hothorn et al., 2008). The statistical analyses regarding the survival and reproduction of *R. maidis* were performed using R program (R Core Team, 2013). For the proteomic analysis, the fold-change ratios were used to compare the expression levels of proteins between two populations. Only fold-change ratios with $p < 0.05$ were statistically significant.

3 | RESULTS

3.1 | Number of nymphs reproduced per female and survival of inoculated adults on different hosts plants

Host plants had a significant effect on the abundance of aphid nymphs produced for all *R. maidis* populations (BJ: $\chi^2 = 88.6$, $df = 2$, $p < 0.001$; DY: $\chi^2 = 38.8$, $df = 2$, $p < 0.001$; GZ: $\chi^2 = 40.2$, $df = 2$, $p < 0.001$; HF: $\chi^2 = 79.7$, $df = 2$, $p < 0.001$; LF: $\chi^2 = 43.1$, $df = 2$, $p < 0.001$; MS: $\chi^2 = 58.2$, $df = 2$, $p < 0.001$). Aside from the HF population, the number of nymphs for the other five populations was significantly higher on barley than on high maize ($p < 0.05$), and significantly higher (for all six populations) on high maize than on small maize ($p < 0.05$) (Figure 2). It was also found that the number of nymphs produced by each female per day on high maize reached maximum on the second or third day (for all populations), while the number was still increasing on the fourth day for five populations (except LF) when tested on barley. On small maize, less than or only one nymph was produced by each female per day for all six populations (Figure 2).

Host plants also had a significant effect on the survival of inoculated adults for BJ ($\chi^2 = 18.2$, $df = 2$, $p < 0.001$), GZ ($\chi^2 = 18.0$, $df = 2$, $p < 0.001$), HF ($\chi^2 = 33.1$, $df = 2$, $p < 0.001$), and MS ($\chi^2 = 31.1$, $df = 2$, $p < 0.001$) populations but not for DY ($\chi^2 = 1.9$, $df = 2$, $p = 0.395$) or LF ($\chi^2 = 3.2$, $df = 2$, $p = 0.201$) populations. The survival number of adults was significantly higher on barley than on high maize and small maize ($p < 0.05$) with the exception of LF and DY

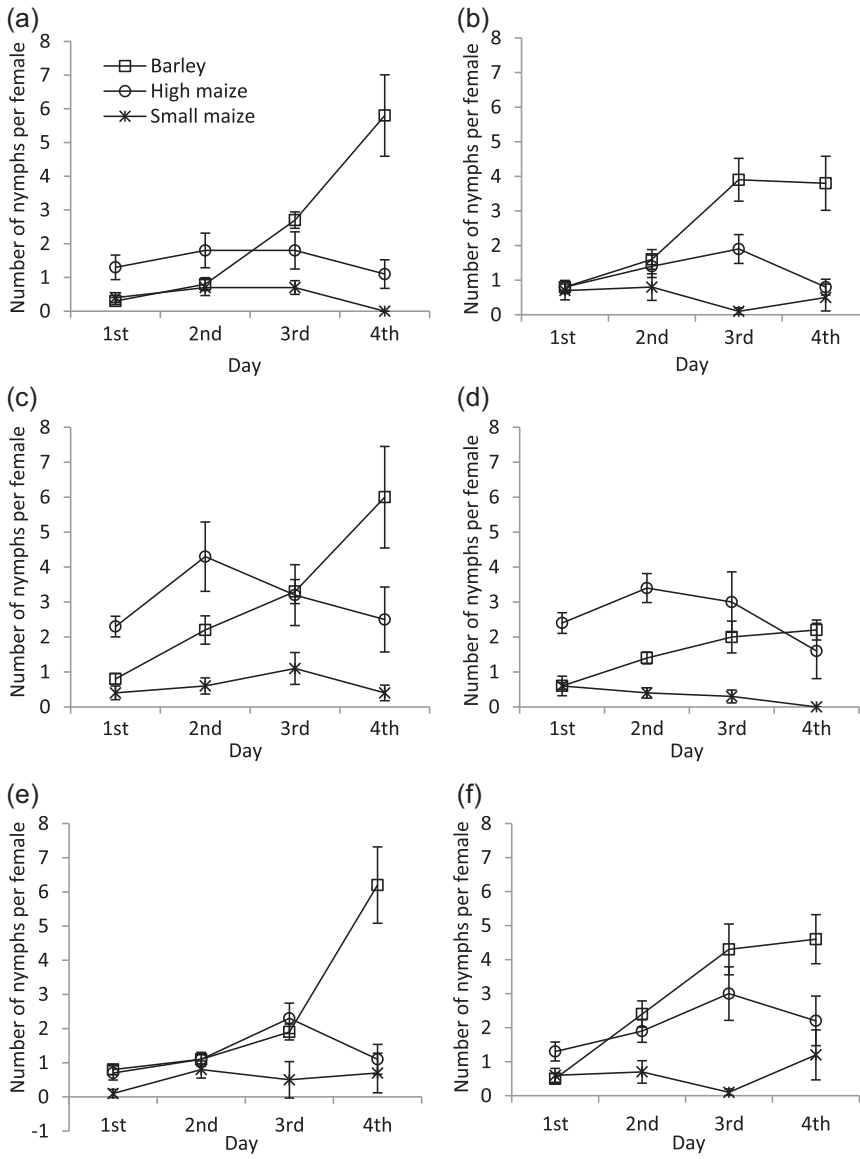

FIGURE 2 Number of nymphs produced by each female per day on different host plants of six *R. maidis* populations. (a), MS; (b), LF; (c), BJ; (d), HF; (e), DY; (f), GZ

populations. Aside from the MS population, for which the survival number of adults was significantly higher on small maize than on high maize ($p < 0.05$), there were no significant differences with regards to the adult survival for the other five populations (Figure S1).

3.2 | Total aphid survival and reproduction on different plant hosts

Host plants had a significant effect on the survival and reproduction of aphids for all populations (BJ: $\chi^2 = 3575.2$, $df = 2$, $p < 0.001$; DY: $\chi^2 = 1005.1$, $df = 2$, $p < 0.001$; GZ: $\chi^2 = 1907.4$, $df = 2$, $p < 0.001$; HF: $\chi^2 = 1922.5$, $df = 2$,

$p < 0.001$; LF: $\chi^2 = 1439.5$, $df = 2$, $p < 0.001$; MS: $\chi^2 = 2288.7$, $df = 2$, $p < 0.001$). The survival and reproduction of *R. maidis* populations on barley were significantly higher than on high maize ($p < 0.001$) (Figure S2) and the total survival numbers on high maize were significantly higher than that of small maize ($p < 0.001$) for all six lineages (Figure S3). Figure 3a shows that no clones were able to survive on small maize. Although several progeny nymphs were deposited in the first and second day, the total number of aphids decreased rapidly from the third to the seventh day. We observed that the nymphs were unable to grow and most of them died at the first or second instar.

Contrastingly, *R. maidis* populations could survive on high maize seedlings, although the aphid numbers increased significantly slower than on barley (especially from the fourth day onward) (Figure S2). By the seventh day

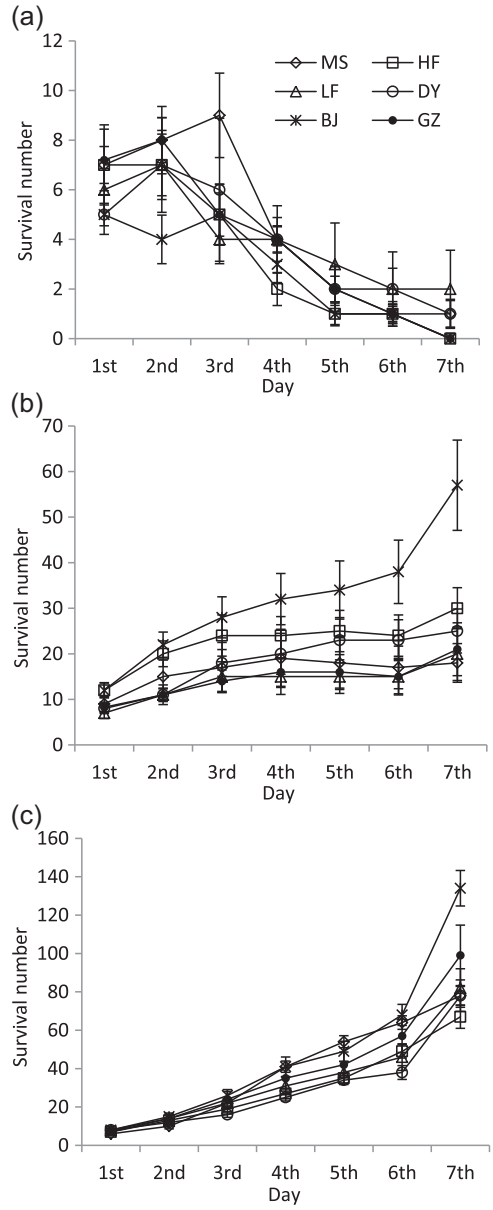

FIGURE 3 Survival number of six *R. maidis* populations on different host plants. (a), small maize; (b), high maize; (c), barley

on high maize, the highest aphid number ($n = 57$) of the BJ population was about three times that of the lowest number ($n = 18$) of the MS population (Figure 3b).

All *R. maidis* populations performed best on the barley seedlings (Figure 3c). Notably, the BJ population showed the fastest reproduction cycle with the number of aphids reaching 134 on the seventh day—nearly two times that of the number of MS and DY populations ($n = 78$). The HF population produced the lowest number of surviving aphids ($n = 67$) on barley by the seventh day.

The mean survival numbers per day were significantly different among populations for small maize ($\chi^2 = 43.48$, $df = 5$, $p < 0.001$), high maize ($\chi^2 = 860.53$, $df = 5$, $p < 0.001$) as well as barley ($\chi^2 = 439.73$, $df = 5$, $p < 0.001$) (Figure S4). The mean numbers per day on small maize were no more than five (Figure S4a), while the mean number of the BJ population on high maize was significantly higher ($p < 0.001$) than that of the other five populations (Figure S4b). No difference was found ($p > 0.1$) between LF and GZ populations (exhibiting the lowest mean survival numbers on high maize). With barley as the host plant, a significantly higher mean survival number of the BJ population was observed in comparison to the other five lineages ($p < 0.001$) (Figure S4c). There was no difference between the GZ and MS populations ($p > 0.1$), nor was there a significant difference between the HF and DY populations ($p > 0.1$) which were significantly lower in survival number than the other four populations ($p < 0.05$).

3.3 | Identification of differentially expressed proteins

In total, 466 proteins were identified from the 2D gel among which 77 protein spots varied significantly ($p < 0.05$) (Figure S5). The complete properties of over- and under-expressed proteins in the BJ population on MS population of *R. maidis* were listed in Tables 1 and 2 according to their relations to metabolic pathways in aphids and bacterial endosymbionts. We found that 57 proteins were related to aphids (Table 1) and 20 proteins were related to endosymbionts (Table 2). The fold change ratios of these proteins between the two *R. maidis* populations ranged from −1.9 to 1.8 (Tables 1 and 2). These differentially expressed proteins showed that 16/57 (Table 1) in the aphid group and 11/20 (Table 2) in the endosymbiont group were overexpressed in the BJ population versus the MS population. In particular, the amount of myosin heavy chain (muscle isoform X12) (spot number: 781) and peroxidase (spot number: 1383) from the aphid group in the BJ population was 1.7 and 1.6 times of that in the MS population respectively. However, the amount of bifunctional glutamate synthase subunit beta (spot number: 1664) in *Rickettsia rickettsii* from the endosymbiont group in the BJ population was −1.9 times that of the MS population. Also, the expression levels of ATP-dependent protease, Hsp 100 (spot number: 2137) from *Hamiltonella defensa*, and protein SYMBAF (spot number: 2703) from *Serratia symbiotica* were −1.4 and −1.5 times in the BJ versus MS population.

With regards to proteins in the aphid group, 86% were detected in *A. pisum* while fewer were detected in *Aphis citricidus* (5%), *Aphis glycines* (2%), *Aphis laciniariae* (2%), *Rhopalosiphum padi* (3%) and *Schizaphis graminum* (2%), respectively (Figure 4a). With regards to the endosymbiont group, various proteins were detected in *Buchnera aphidicola* (10%), *H. defensa* (5%), *Regiella insecticola* (10%), *Rickettsia* (40%), *S. symbiotica* (5%) and *Spiroplasma* (30%) (Figure 4b).

The distribution of proteins with different expression levels in BJ and MS populations, as they relate to metabolic pathways, is shown in Figure 5. We found that proteins in the aphid group accounted for various roles in metabolic pathways such as cell function and structure (18%), energy metabolism (12%), folding, sorting and degradation (11%), protein–protein interactions (11%), and stress tolerance (11%). On the other hand, proteins involved in metabolic pathways of carbohydrate metabolism (15%), energy metabolism (10%), signaling molecules (10%), and interaction, replication and repair (35%) were prominent in the endosymbiont group.

In the aphid group, 7/10 proteins that take part in metabolic pathways of cell function and structure were upregulated, while 6/7 proteins that participate in energy metabolism, 5/6 proteins that participate in folding, sorting and degradation, 5/6 proteins that participate in stress tolerance and all six proteins that participate in

TABLE 1 A list of identified proteins related to metabolic pathways in aphids that differ in abundance between two populations[a]

Spot number	Fold change	Protein identification	Organism	Mascot score	MS coverage	Peptide number	Protein MW	pI	Metabolic pathways
1784	−1.20	Glutamate dehydrogenase	*Acyrthosiphon pisum*	92	44	27	59,929	8,6	Amino acid metabolism
1878	−1.30	Serine hydroxymethyltransferase 1	*Acyrthosiphon pisum*	45	17	11	52,156	6,9	Amino acid metabolism
1648	−1.40	Transketolase-like protein 2	*Acyrthosiphon pisum*	60	17	13	67,859	7,1	Carbohydrate metabolism
1406	1.20	NADH-ubiquinone oxidoreductase	*Acyrthosiphon pisum*	79	14	9	80,850	6.0	Carbohydrate metabolism
1780	−1.40	Glucose-6-phosphate 1-dehydrogenase	*Acyrthosiphon pisum*	137	38	24	61,370	7,2	Carbohydrate metabolism
2792	1.40	Diacetyl/L-xylulose reductase	*Acyrthosiphon pisum*	69	47	14	26,317	7,7	Carbohydrate metabolism
523	−1.20	Laminin subunit gamma-1 isoform X1	*Acyrthosiphon pisum*	71	16	23	184,715	4,8	Cell function and structure
789	1.30	Myosin heavy chain	*Acyrthosiphon pisum*	106	15	29	223,739	5,7	Cell function and structure
781	1.70	Myosin heavy chain, muscle isoform X12	*Acyrthosiphon pisum*	207	26	57	225,732	5,7	Cell function and structure
825	1.30	Myosin heavy chain	*Acyrthosiphon pisum*	189	25	51	223,739	5,7	Cell function and structure
818	1.20	Myosin heavy chain, muscle isoform X7	*Acyrthosiphon pisum*	225	24	55	225,440	5,7	Cell function and structure
1239	−1.50	Filamin-A isoform X4	*Acyrthosiphon pisum*	230	43	33	91,385	6,3	Cell function and structure
2051	−1.20	LIM domains protein 2 isoform X3	*Acyrthosiphon pisum*	80	40	12	41,861	8,9	Cell function and structure
2188	1.30	Muscle actin	*Acyrthosiphon pisum*	168	50	18	42,158	5,2	Cell function and structure
3114	1.40	Actin-1	*Acyrthosiphon pisum*	111	68	8	12,794	5,3	Cell function and structure
1786	−1.30	G2/mitotic-specific cyclin-B3	*Acyrthosiphon pisum*	74	32	16	50,521	9,1	Cell growth and death
1394	−1.20	2-Oxoglutarate dehydrogenase	*Acyrthosiphon pisum*	143	26	30	117,106	6,9	Energy metabolism
2058	1.30	Isocitrate dehydrogenase [NADP]	*Acyrthosiphon pisum*	73	37	17	46,850	6,2	Energy metabolism
1982	−1.30	Hydroxyacid-oxoacid transhydrogenase	*Acyrthosiphon pisum*	69	34	17	50,998	6,4	Energy metabolism
1905	−1.30	Aldehyde dehydrogenase	*Acyrthosiphon pisum*	75	27	15	48,197	6,5	Energy metabolism
1622	−1.30	ATP synthase alpha	*Acyrthosiphon pisum*	52	21	20	59,987	9,6	Energy metabolism

TABLE 1 (Continued)

Spot number	Fold change	Protein identification	Organism	Mascot score	MS coverage	Peptide number	Protein MW	pI	Metabolic pathways
2321	−1.40	Glyceraldehyde-3-phosphate dehydrogenase	*Rhopalosiphum padi*	90	49	13	35,579	8,7	Energy metabolism
2330	−1.60	Glyceraldehyde-3-phosphate dehydrogenase	*Rhopalosiphum padi*	111	50	13	35,579	8,7	Energy metabolism
1367	1.40	Nucleoprotein TPR isoform X2	*Acyrthosiphon pisum*	73	22	32	130,929	4,8	Folding, sorting and degradation
1182	−1.30	Transitional endoplasmic reticulum ATPase	*Acyrthosiphon pisum*	376	67	64	89,914	5.0	Folding, sorting and degradation
1178	−1.20	Transitional endoplasmic reticulum ATPase	*Acyrthosiphon pisum*	210	37	33	89,914	5.0	Folding, sorting and degradation
2385	−1.20	Proteasome subunit alpha type-1	*Acyrthosiphon pisum*	115	52	18	30,934	6,8	Folding, sorting and degradation
2623	−1.20	Proteasome subunit alpha	*Acyrthosiphon pisum*	82	50	16	28,366	7,6	Folding, sorting and degradation
3554	−1.30	Ubiquitin, partial	*Schizaphis graminum*	89	69	8	8552	7,6	Folding, sorting and degradation
2795	1.20	ATP synthase oligomycin sensitivity conferral protein	*Acyrthosiphon pisum*	98	63	18	110,012	6,1	Ion transport
1173	−1.50	Translation elongation factor 2	*Acyrthosiphon pisum*	176	46	49	95,558	6.0	Protein processing
2454	−1.20	Elongation factor 1-alpha, partial	*Aphis laciniariae*	103	46	16	30,157	6,7	Protein processing
1584	−1.20	Heat shock 70 kDa protein cognate 4	*Acyrthosiphon pisum*	140	36	24	71,626	5,2	Protein–protein interactions
1203	−1.30	Heat shock 70 kDa protein 4	*Acyrthosiphon pisum*	102	22	18	89,426	5,3	Protein–protein interactions

(Continues)

TABLE 1 (Continued)

Spot number	Fold change	Protein identification	Organism	Mascot score	MS coverage	Peptide number	Protein MW	pI	Metabolic pathways
1295	−1.30	Heat shock protein 83	*Acyrthosiphon pisum*	147	39	30	83,707	4,8	Protein–protein interactions
1532	−1.20	Heat shock protein cognate 3 precursor	*Acyrthosiphon pisum*	116	35	26	72,993	5,1	Protein–protein interactions
1736	−1.20	Chaperonin containing TCP1, subunit 5	*Acyrthosiphon pisum*	160	48	28	59,844	5,5	Protein–protein interactions
1928	−1.20	RAB GDP dissociation inhibitor alpha	*Acyrthosiphon pisum*	201	57	28	50,201	6,2	Signal transduction
2326	−1.30	Protein kinase C receptor	*Aphis citricidus*	135	62	21	36,522	6,7	Signal transduction
2641	−1.30	GTP-binding nuclear protein Ran	*Acyrthosiphon pisum*	139	69	20	24,861	6,6	Signaling molecules and interaction
1812	−1.20	Leucine-rich repeat protein	*Acyrthosiphon pisum*	80	14	8	62,913	6,7	Signaling molecules and interaction
1598	−1.40	Stress-induced-phosphoprotein 1	*Acyrthosiphon pisum*	70	25	21	61,879	6,4	Stress tolerance
890	−1.20	Hypoxia upregulated protein 1	*Acyrthosiphon pisum*	108	22	21	100,244	5,2	Stress tolerance
1673	−1.40	T-complex protein 1 subunit alpha	*Acyrthosiphon pisum*	188	54	33	60,431	5,2	Stress tolerance
1955	1.30	Peroxiredoxin 1	*Acyrthosiphon pisum*	38	22	3	21,781	5,3	Stress tolerance
1709	−1.20	T-complex protein 1 subunit zeta	*Acyrthosiphon pisum*	125	48	31	58,617	6,2	Stress tolerance
2718	−1.50	Peroxiredoxin-6	*Acyrthosiphon pisum*	184	77	25	25,245	4,6	Stress tolerance
1980	−1.20	TAR DNA-binding protein 43	*Acyrthosiphon pisum*	109	41	21	45,677	6,6	Transcription
2003	−1.20	Eukaryotic initiation factor 4A	*Acyrthosiphon pisum*	167	61	33	46,989	5,3	Transcription
1676	−1.20	Zinc finger MYM-type protein 1	*Acyrthosiphon pisum*	60	19	7	71,306	7,2	Transcription
2042	−1.30	Guanine nucleotide-binding protein subunit beta-like protein	*Acyrthosiphon pisum*	122	55	19	36,582	6,7	Translation

TABLE 1 (Continued)

Spot number	Fold change	Protein identification	Organism	Mascot score	MS coverage	Peptide number	Protein MW	pI	Metabolic pathways
2829	−1.30	Aspartyl-tRNA synthetase	*Acyrthosiphon pisum*	60	48	13	31,884	5,2	Translation
1383	1.60	Peroxidase	*Acyrthosiphon pisum*	189	43	28	79,341	5,7	Xenobiotics biodegradation and metabolism
2476	1.20	Fructose 1,6-bisphosphate aldolase	*Aphis citricidus*	117	53	16	40,275	6,8	Carbohydrate metabolism
2640	1.30	Muscle actin	*Aphis citricidus*	79	28	10	42,158	5,2	Cell function and structure
1569	−1.20	Heat shock protein 70	*Aphis glycines*	146	45	27	71,399	5,3	Protein–protein interactions
3146	1.60	MPA13 allergen-like isoform X1	*Acyrthosiphon pisum*	89	58	12	15,458	6,2	Other

Note: Fold change, fold change ratios of differentially expressed proteins of BJ population versus MS population; Organism, related original organism for the protein identification; Mascot score, Mowse score according to Mascot search; MS coverage, percentage of the protein sequence identified; Peptide number, number of peptide hits for each protein; Protein MW, molecular weight; pI, isoelectric point.

[a]Proteins that significantly varied ($p < 0.05$) between two populations are listed by spot number according to the gel analysis.

TABLE 2 A list of identified proteins related to metabolic pathways in bacterial endosymbionts that differed in abundance between two populations[b]

Spot number	Fold change	Protein identification	Organism	Mascot score	MS coverage	Peptide number	Protein MW	pI	Metabolic pathways
2137	−1.40	ATP-dependent protease, Hsp 100	*Hamiltonella defensa*	48	27	21	96,395	5,9	Energy metabolism
2323	−1.20	ATP-dependent DNA helicase	*Spiroplasma apis*	64	25	20	83,617	9,1	Energy metabolism
2980	1.80	chaperone protein DnaK, partial	*Rickettsia bellii*	48	10	4	29,247	4,7	Protein–protein interactions
2627	1.30	Glucose-6-phosphate isomerase	*Regiella insecticola*	60	26	14	63,058	6,4	Carbohydrate metabolism
3150	−1.40	Dihydrolipoamide succinyltransferase	*Regiella insecticola*	70	19	9	45,076	6,8	Carbohydrate metabolism
2196	1.20	Isocitrate dehydrogenase [NAD]	*Spiroplasma chrysopicola*	122	47	21	39,697	6,9	Carbohydrate metabolism
2638	1.40	Succinyl-CoA:3-ketoacid-coenzyme A transferase	*Rickettsia bellii*	60	31	11	23,072	4,8	Lipid metabolism
1656	−1.20	NACHT family NTPase	*Rickettsia bellii*	69	24	13	56,674	6,1	Transcription
2541	1.50	GTP-binding protein YsxC	*Rickettsia prowazeki*	42	15	4	24,580	10,3	Signaling molecules and interaction
1914	1.30	GTP-binding protein	*Rickettsia rickettsii*	50	27	20	40,030	4,7	Signaling molecules and interaction
1994	−1.50	NIFR3-like protein	*Rickettsia prowazeki*	41	9	4	36,108	9,8	Stress tolerance
1664	−1.90	Bifunctional glutamate synthase subunit beta/2-poly	*Rickettsia rickettsii*	51	23	19	119,326	6,2	Amino acid metabolism
826	1.40	DNA helicase II	*Spiroplasma apis*	64	26	18	82,464	6,3	Replication and repair
2702	1.20	Transposase	*Rickettsia rickettsii*	44	15	3	21,620	5,6	Replication and repair
3036	1.50	Hypoxanthine-guanine phosphoribosyltransferase	*Spiroplasma chrysopicola*	51	53	10	22,031	4,8	Replication and repair
1932	−1.30	DNA primase	*Spiroplasma syrphidicola*	47	31	9	39,664	6,3	Replication and repair
1338	1.20	Superfamily I DNA/RNA helicase	*Spiroplasma taiwanense*	56	18	26	150,160	8,1	Replication and repair

TABLE 2 (Continued)

Spot number	Fold change	Protein identification	Organism	Mascot score	MS coverage	Peptide number	Protein MW	pI	Metabolic pathways
1619	−1.40	DNA polymerase III beta subunit	*Buchnera aphidicola*	43	7	4	42,291	9,5	Replication and repair
1045	1.40	DNA topoisomerase I	*Buchnera aphidicola*	85	22	22	100,640	10,1	Replication and repair
2703	−1.50	Protein SYMBAF	*Serratia symbiotica*	63	31	8	23,031	8,8	Other

Note: Fold change, fold change ratios of differentially expressed proteins of BJ population versus MS population; Organism, related original organism for the protein identification; Mascot score, Mowse score according to Mascot search; MS coverage, percentage of the protein sequence identified; Peptide number, number of peptide hits for each protein; Protein MW, molecular weight; pI, isoelectric point.

[b]Proteins that significantly varied ($p < 0.05$) between two populations are listed by spot number according to the gel analysis.

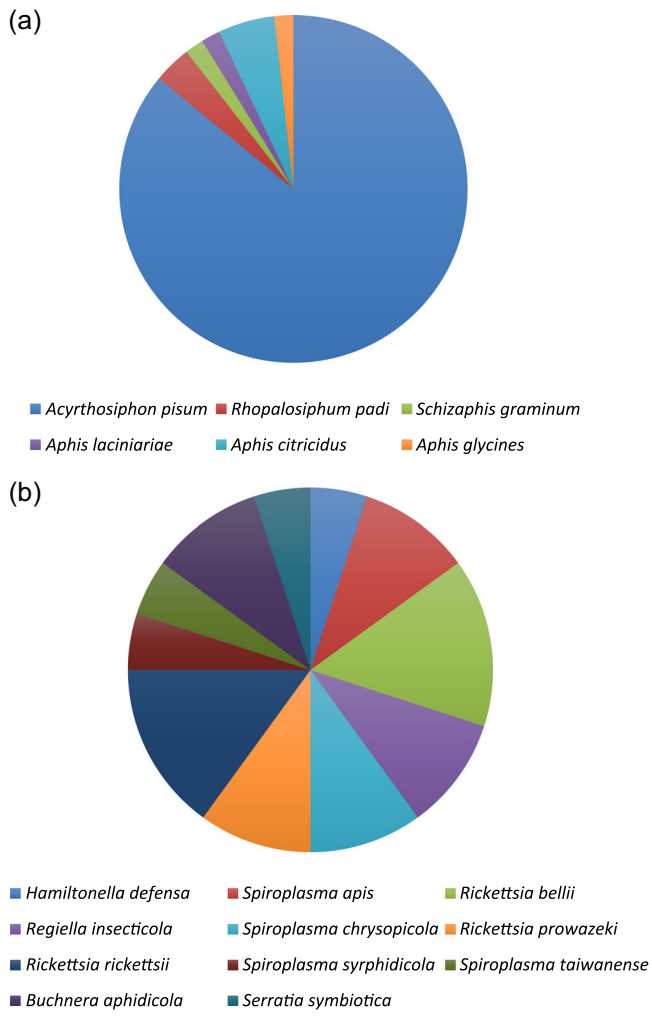

FIGURE 4 Distribution of organisms for proteins with different expression levels in *R. maidis* of BJ and MS populations. (a) proteins related to aphids, (b) proteins related to endosymbionts

protein–protein interactions were downregulated in the BJ population compared to that of the MS population (Table 1). Similarly, all proteins involved in energy metabolism and stress tolerance in the endosymbiont group were downregulated in the BJ population (Table 2), whereas 5/7 proteins that participate in replication and repair were upregulated (Table 2).

4 | DISCUSSION

Rhopalosiphum maidis is known to be one of the major pests of maize in the field. Our laboratory data indicated that although all six aphid populations best performed on barley seedlings, they showed a suboptimal reproductive potential on 50 cm high maize seedlings. As previously reported (Hirano & Ito, 1964), the survival rates of *R. maidis* were different between barley and wheat hosts, and performed differently between different wheat ages.

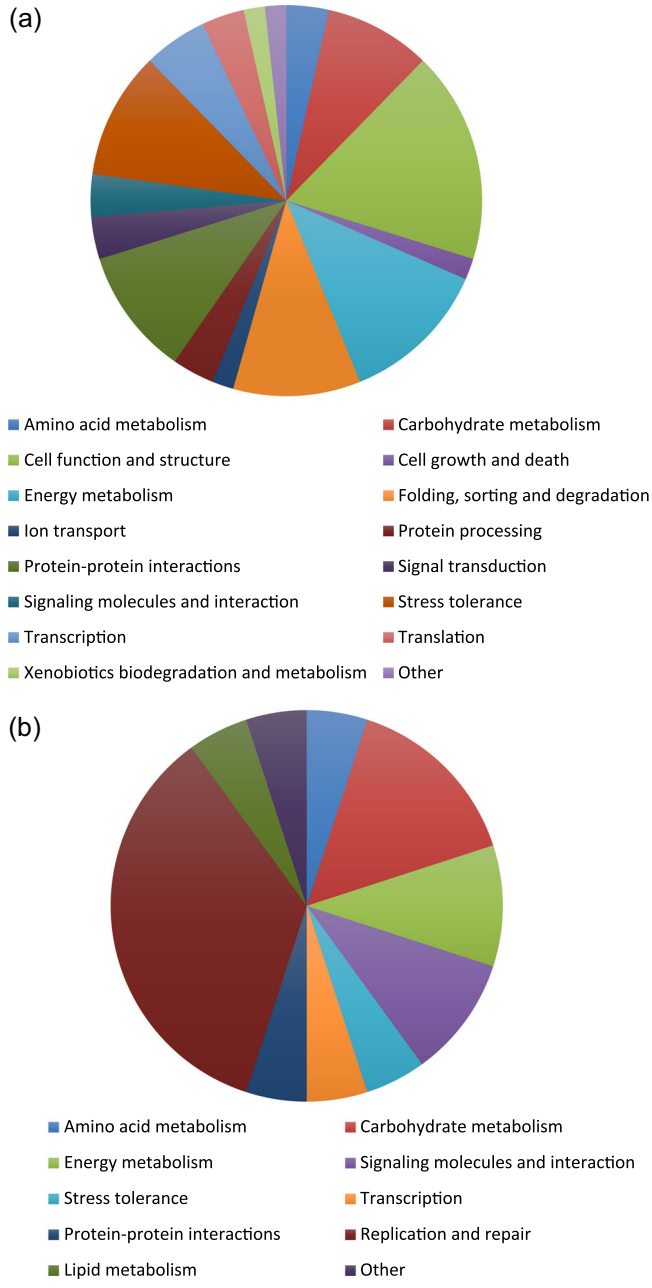

FIGURE 5 Distribution of related metabolic pathways for proteins with different expression levels in *R. maidis* of BJ and MS populations. (a) proteins related to metabolic pathways in aphids, (b) proteins related to metabolic pathways in bacterial endosymbionts

In the present study, 10 cm high maize seedlings proved fatal to all *R. maidis* populations. We hypothesize that it was due to potential detrimental chemicals in young maize seedlings (10 cm high) such as DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one). As a secondary metabolite (and hydroxamic acid) in many cereals, DIMBOA plays an important role in insect resistance (Klun et al., 1967; Pérez & Niemeyer, 1989; Yan et al., 1995). Moreover, the concentration of DIMBOA varies according to plant organ and age (Cambier

et al., 2000), research has shown that DIMBOA is synthesized concurrently with germination in maize and that the concentration reaches its highest level 24–36 h after germination (Ebisui et al., 2000). It has furthermore been reported that the concentration of DIMBOA decreases during subsequent plant growth and, in the aerial parts of maize, can drop to almost zero by the 20th day after germination (Cambier et al., 2000). In our study, *R. maidis* populations were introduced 4–5 days after host plant germination for the 10 cm high maize seedling treatment, with the expectation that biosynthesis of DIMBOA should still be ongoing (Cambier et al., 2000). Contrastingly, the inoculation of *R. maidis* populations was performed 19–20 days after germination for maize seedlings that were 50 cm in height (expecting that there would be almost no DIMBOA left in aerial parts at this time; Cambier et al., 2000). In addition, the barley seeds used in this study were from a cultivar that is unable to biosynthesize DIMBOA due to the elimination of the necessary benzoxazinone genes (Nomura et al., 2003). Future research is however required to confirm the resistance effect of DIMBOA in small maize seedlings.

Previous research found that the performance of different populations of *A. craccivora* was significantly different on faba bean, field pea as well as narrow-leafed lupin (Edwards, 2001). Also, when highly resistant wheat seedlings were used as host plants, the population growth rate of one *S. avenae* superclone was significantly higher than that of nonsuperclones (Barrios-SanMartín et al., 2016). Moreover, different genotypes of *S. avenae* identified through microsatellite analysis performed diversely on Poaceae (Figueroa et al., 2004). In this study, the total survival numbers of all *R. maidis* populations on high maize were significantly higher than on small maize (Figure S3). However, no significant difference in adult survival was detected between high and small maize for five of the populations (Figure S1), demonstrating that the adults were more adaptive to the detrimental chemicals in small maize than the nymphs. There was, however, also a significant decline in adult fecundity (Figure 2). The BJ population showed the best performance on both high maize and barley, and its mean survival number per day was also significantly higher than that of the other populations, suggesting that the BJ population was more adaptable and fertile. The aphid number of other populations slowly increased on 50 cm high maize seedlings (when compared with the BJ population), especially after the fourth day. The total number of the MS population even declined upon comparison of the seventh day to the fourth day. Nikolakakis et al. (2003) found a significant effect of "region/host plant origin" based on the different performances of various *M. persicae* clones. The six populations of *R. maidis* in the present study that collected from different regions of the same plant (maize) were tested, however, the effect of "host plant origin" should be tested in future research.

According to protein identification results, both myosin heavy chain, muscle isoform X12 (spot number: 781), and peroxidase (spot number: 1383) were overexpressed in the BJ population. Myosin heavy chain, muscle isoform X12 is involved in cell structure and function (Table 1) and it has been reported that myosin heavy chains could activate ATPase and be responsible for mechanochemical energy transduction (Kiehart et al., 1989). Additionally, peroxidase takes part in the pathway of xenobiotics biodegradation and metabolism (Table 1), and it has previously been shown that suppression of a peroxidase gene could reduce the survival rate in *S. avenae* (Fei et al., 2016). Based on this, these two proteins might thus be beneficial to the BJ population—allowing for stronger vitality and faster reproduction.

Conversely, both ATP-dependent protease, Hsp 100 (spot number: 2137) and protein SYMBAF (spot number: 2703) were overexpressed in the MS population. We infer that these proteins may be involved in heat shock resistance since both *H. defensa* (the organism of ATP-dependent protease, Hsp 100) and *S. symbiotica* (the organism of protein SYMBAF) have been reported to enhance the heat shock resistance for their host aphid (Russell & Moran, 2006). Furthermore, the MS population was collected from Mangshi City (24°26′ N, 98°35′ E), Yunnan Province where the average temperature is higher than that of Beijing City (40°2′ N, 116°16′ E) which served as the collection site of the BJ population.

Secondary symbionts have been suggested to enact various effects on their host aphids such as host plant fitness, heat shock resistance, parasitoids resistance, longevity, and fecundity (Oliver et al., 2005; Russell & Moran, 2006; Tsuchida et al., 2011; Vorburger et al., 2013). Both BJ and MS populations were infected with various secondary symbionts while *Rickettsia* and *Spiroplasma* exhibited a relatively high frequency of infection in these two

populations of *R. maidis*, supporting previous findings (Guo et al., 2019). Nevertheless, the fold change ratios of all differentially expressed proteins were no more than twofold since both BJ and MS populations were originally collected from maize and reared under the same conditions.

In conclusion, the BJ population of *R. maidis* performed the best, both on high maize and barley seedlings, while maize seedlings in 10 cm height were fatal to all populations. Secondary metabolites may be responsible for the unfitness of small maize seedlings, but this hypothesis requires further verification. Proteomic analyzes showed that proteins involved in stress tolerance and energy metabolism were mostly downregulated in the BJ population (in both aphid and endosymbiont groups), indicating that the MS population might be more stress-resistant (especially to heat-shock) than the BJ population—even though the BJ population of *R. maidis* showed a faster reproduction rate.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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