Fruit Research

ARTICLE

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https://doi.org/10.48130/FruRes-2023-0035

Fruit Research **2023**, 3:35

Jujube witches' broom ('Zaofeng') disease: bacteria that drive the plants crazy

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Abstract

Jujube witches' broom (JWB) disease, referred to as 'Zaofeng' disease in Chinese, is associated with the JWB phytoplasma ('*Candidatus* Phytoplasma ziziphi') and causes heavy losses in the jujube industry. JWB disease has been recorded since the 1950s. Diseased trees have symptoms such as shoot proliferation and leaf-like flowers. The JWB phytoplasma is assigned to the 16SrV group, subgroup 16SrV-B, according to 16S rRNA gene sequence. The JWB phytoplasma is transmitted by leafhoppers and can infect a few plants other than jujube. Infection with phytoplasma affects biochemical and physiological process, altering the expression of genes encoding some transcription factors and functional genes, mainly involved in biotic stress response. The genome of JWB phytoplasma 'nky' strain has been sequenced and consists of 750,803 bp within one circular chromosome that encodes 694 protein-coding genes. The pathogenic mechanisms of a few JWB phytoplasma effectors have been investigated. The presence of JWB phytoplasma has been detected through symptoms observation, ELISA, DAPI staining and PCR, but new techniques, such as LAMP and CRISPR/Cas-12 based visual assay, have recently been developed. Some resistant jujube cultivars have been selected by infection screening. Although treatment with tetracycline antibiotics is effective, comprehensive control measures, including orchard management and sanitary measures, are needed for disease control. Further studies are needed in development of JWB phytoplasma culture method, expansion of genomic information, and phytoplasma effectors, resistance-related gene identification and resistant genotype developing.

Citation: Guo S, Gu L, Zhang Y, Wu Y, Tan B, et al. 2023. Jujube witches' broom ('Zaofeng') disease: bacteria that drive the plants crazy. *Fruit Research* 3:35 https://doi.org/10.48130/FruRes-2023-0035

Introduction

Phytoplasmas from the class Mollicutes are Gram-positive bacteria that lack a cell wall and have a small genome[1] . Since the axenic culture of phytoplasmas is difficult, phenotypic characteristics are not known. Therefore, these bacteria are provisional assigned to a '*Candidatus*' genus according to the bacteria taxonomic system[1,2]. Based on 16S rRNA gene sequence, 49 '*Candidatus* Phytoplasma' species were described[2] .

Phytoplasmas are transmitted by phloem-feeding insect vectors, usually leafhoppers (family Cicadellidae), to infect more than 1,000 plant species, including many economically important crops[1]. Infected plants show a wide range of symptoms, such as witches' broom (shoot proliferation), phyllody (leafy flowers), yellowing, stunting, purple top and phloem necrosis, cause serious economic losses worldwide[1].

The fruit Chinese jujube (*Ziziphus jujuba*), also called common jujube, Chinese date, red date, or 'Zao' in Mandarin Chinese, is priced for its delicious and nutritious fruit[3]. The jujube fruit can be consumed both fresh and dried and serves as a traditional herbal medicine in China. Jujube has a long cultivation history of more than 7,000 years and grows in more than 40 countries worldwide[3]. The most destructive disease of jujube is JWB disease, which is related to the presence of JWB phytoplasma ('*Candidatus* Phytoplasma ziziphi')[4,5]. JWB phytoplasma-infected jujube trees show altered fundamental plant development processes, resulting in yield loss, decreased fruit quality and die gradually within a few years, bringing heavy losses to the jujube industry[6,7].

This paper intends to provide an up-to-date review of JWB disease and to describe the prospects.

Historical background

Witches' broom disease in jujube has been present in China for a long time, but it became more serious in some areas in the 1930s[8]. Wang first reported jujube phyllody disease in 1950 in samples that were collected in jujube cultivation areas of Henan province, central China, in 1939 to 1941[9]. Local farmers in northern China called this disease 'Zaofeng', 'Zao' which is jujube, and 'feng' which is uncontrolled or crazy, illustrating the uncontrolled witches' broom symptoms of shoot proliferation and phyllody of the diseased trees[10].

In 1956, the incidence in Changli, Hebei province (China) was 30.3%[8].There was a JWB disease outbreak in the 1980s in

Henan province, with an incidence ranging from 20% to 70% in the main jujube cultivation areas such as Xinzheng, Neihuang and Nanyang (China)[11].

Nowadays, JWB disease has spread all over the main jujube cultivation areas in China, from the traditional areas such as Hebei, Henan, Shandong, Shanxi and Shaanxi provinces, to the newly developed production areas such as Xinjiang in the far northwest[12] and Guangdong in the south[13].

In Korea, JWB disease was first observed in the central region in the 1930s and spread outward by the 1950s[14]. Nowadays, JWB-related diseases are distributed in every province throughout Korea[14]. Occurrence of JWB disease has also been reported in Japan[15] and India[16] .

JWB disease

Symptomatology

The symptoms of JWB disease can be observed in all plant organs. Witches' broom and phyllody are the most characteristic symptoms. Diseased trees show precocious development of proliferating secondary shoots, which have an over-abundance of abnormally small and sometimes chlorotic leaves. The sepal and petal become leaf-like, the petiole and pistil elongate, and sometimes the pistil develops as two small leaves[5].

The leaves of diseased trees are smaller, curved and yellow, and do not fall until late winter. The tree roots develop nodules and prolific fibrous roots and suckers. Seriously diseased trees generally do not bear fruit. The fruits on trees with mild disease are smaller and pale in color with a loose pulp and low sugar content[5].

Infected jujube trees show basically the same symptoms, regardless of the cultivar or geographical area. Symptoms are limited to one or a few branches, then the disease spreads progressively throughout the entire canopy. Trees of all ages are susceptible and die within a few years after the symptoms first appear[5].

Agents associated with the JWB disease

Since JWB is a graft-transmissible infectious disease, the pathogen was first considered to be a virus[10]. In 1967, Doi et al. observed wall-less microorganisms resembling mycoplasmas in the phloem of paulownia plants with a witches' broom, and named them mycoplasma-like organisms (MLOs)[17]. Afterwards, MLOs were detected in association with witches' broom in jujube trees through analysis with electron microscopy[5].

In 2004, the MLOs were assigned to the new taxon, '*Candidatus* phytoplasma'[2] and according to 16S rRNA gene sequence analysis, JWB phytoplasma was assigned to '*Candidatus.* Phytoplasma ziziphi' taxon and 16SrV-B subgroup[4].

Although mixed infection of phytoplasmas from the 16SrI and V subgroups has been reported in jujube, '*Ca.* P. ziziphi' is confirmed to the main phytoplasma associated with JWB disease[18].

Phytoplasmas parasitize nutrient-rich tissues, such as phloem sieve tubes in plants and salivary glands in insects[1]. The JWB phytoplasma population in different tree organs fluctuates in different seasons. The phytoplasma densities were low in above-ground organs in the dormant seasons, and higher during the active growing seasons[19]. The distribution and migration of phytoplasma may accompany the phloem stream[19].

The JWB phytoplasma of different areas or different jujube cultivars may be different strains with slight genetic variations. Sequence analysis of the 16S rRNA gene, the 16Sr space region and the *secY* gene of phytoplasma strains collected on 14 witches' broom diseased jujube cultivars from seven provinces in China showed genetic diversity to different extents, although all were in the 16SrV-B subgroup[20]. Virtual RFLP and single-nucleotide polymorphism (SNP) analyses of the 16S rRNA gene sequences of 15 JWB phytoplasma strains from four provinces in North China showed high similarity[21].

Epidemiology of the JWB disease

Phytoplasmas are transmitted by insect vectors that feed on phloem sap, such as leafhoppers, plant hoppers, and psyllids[1]. Transmission experiments confirmed that *Hishimonus sellatus*[22], *Hishimonides chinensis*[23], *Hishimonoides aurifascialis*[24], *Typhlocyba sp.*[24] and *Hishimonus lamellatus*[24] are insect vectors of JWB phytoplasma. PCR analysis indicated that *Cicadella viridis* and *Kolla paulula* are putative insect vector of JWB phytoplasma[25].

JWB phytoplasma can be spread by grafting with infected materials. Grafting of improved or selected cultivars onto sour jujube (*Ziziphus spinosa*) rootstocks or jujube root suckers are the most popular jujube propagation methods. As a phloem-colonizing pathogen, JWB phytoplasma may spread via grafted saplings, especially in areas being developed for jujube cultivation where the pathogen is not yet present[5].

Inoculation with diseased phloem sap does not result in infection, and JWB phytoplasma was not transmitted by pollen, seeds, root contact, or soil[24].

Other than *Ziziphus jujuba*, the plant hosts of '*Ca.* P. ziziphi' include a few other plants in the *Ziziphus* genus, several other common fruit and garden trees, and some herbaceous and crop plants (Table 1). The JWB phytoplasma could be experimentally transferred to *Arabidopsis* and periwinkle (*Catharanthus roseus*), which may be convenient for pathogenesis studies[6].

Table 1. Plant hosts of '*Ca.* P. ziziphi'.

Category	Common name	Scientific name	Reference
Genus *Ziziphus*	Chinese jujube	*Ziziphus jujuba*	[4]
	sour/wild jujube	*Ziziphus spinosa*	[14]
	Indian ber	*Ziziphus mauritiana*	[16]
		Ziziphus nummularia	[16]
		Ziziphus oenoplia	[26]
Other fruit trees	Apple	*Malus pumila*	[27]
	Peach	*Prunus persica*	[28]
	Sweet cherry	*Prunus avium*	[29]
	Persimmon	*Diospyros kaki*	[30]
	Plum	*Prunus salicina*	[31]
	Date palm cherry	*Phoenix dactylifera*	[32] [33]
Garden trees	Locust tree	*Sophora japonica*	[34]
	Willow	*Salix babylonica*	[35]
	Meadowsweet	*Spiraea salicifolia*	[36]
Herbaceous plants and crops		*Amaranthus retroflexus*	[37]
		Orychophragmus violaceus	[38]
	Eggplant	*Solanum melongena*	[39]
	Sweet potato	*Ipomoea batatas*	[40]

Pathogenesis of the JWB disease

Biochemical and physiological effects

Photosynthesis

Leaf yellowing is one of the typical symptoms of phytoplasma disease, and reduced photosynthesis is a major physiological impairment[1]. When the resistant jujube cultivar Xingguang and the susceptible cultivar Pozao were grafted onto JWB-diseased rootstocks, the susceptible cultivar had significantly decreased total chlorophyll content, carotenoid levels, and photochemical parameters (Fv/Fm, FPSII and qP) at later stages of infection. Meanwhile, in the resistant cultivar, the total chlorophyll and carotenoid levels increased, while the main photochemical parameters decreased at earlier disease stages[41].

Mineral elements

The mineral element content in the leaves of healthy, JWB-diseased and tetracycline-treated, JWB-diseased jujube trees show significant variation. The Ca, Mg and Mn contents in the diseased leaves are lower than in the healthy leaves, while the diseased leaves have higher K content. The Cu and Zn content in both healthy and diseased leaves are similar. The mineral elements contents were similar in the healthy trees and the diseased trees treated with tetracycline[42].

Anatomic structure

In additional to the external morphological modifications, phytoplasma infection may also change anatomical structures of jujube trees. Longitudinal dissection of the stems of healthy and JWB-diseased jujube trees showed that the vessels in the xylem of diseased branches are shorter and smaller, with decreased lumen diameters, while the vessel frequency and wall-to-lumen ratio is increased. These responses of the vessels to JWB phytoplasma may be due to disrupted developmental processes and may be one cause of the decline and death of JWB-diseased trees[43].

Phytohormones

Several reports have shown how JWB disease affects phytohormone content. In grafting experiments, phytoplasma infection results in reduced auxin content and increased jasmonic acid (JA) content at the early stages of JWB disease[7]. In a tetracycline treatment experiment, phytoplasma elimination resulted in a decrease in the JA content[44]. When the resistant cultivar T13 and the susceptible cultivar Pozao are grafted onto JWB-diseased rootstocks, JA significantly accumulated in the susceptible Pozao diseased plants, while salicylic acid (SA) decreased significantly in the resistant T13 compared to Pozao[45].

Molecular mechanisms underlying JWB disease

Genes and pathways responding to phytoplasma infection

The infection and colonization with phytoplasma modulate gene expression patterns of the host plants. To reveal the key genes and pathways that respond to phytoplasma infection, suppressive subtraction hybridization (SSH), qRT-PCR, transcriptomics, proteomics, and metabolomics analyses have been performed between healthy and diseased jujube plants. Some transcription factors and functional genes involved in the plant-phytoplasma interaction were identified. Most of these genes are involved in biotic stress responses, phytohormone biosynthesis, or metabolism pathways (Table 2).

Genome of the JWB phytoplasma

The genomic DNA of phytoplasma was difficult to purify and sequence due to the inability to cultivate pure strains in axenic culture. However, as prokaryotes, phytoplasma genomes have relatively lower G + C content than the genomic DNA of the eukaryote hosts. Thus, the phytoplasma genome can be enriched from total DNA of infected host plants or insect vectors *via* pulsed-field gel electrophoresis (PFGE) or density gradient centrifugation (DGC). Both PFGE and DGC enrichment were carried out to study the JWB phytoplasma genome[46].

A genome of a strain of jujube witches' broom phytoplasma, 'nky,' was sequenced in 2018 from JWB-infected jujube samples[46]. The jwb-nky genome was small, at 750,803 bp, and consisted of one circular chromosome with a G + C content of 23.3% that was predicted to encode 694 protein-coding genes, two operons for rRNA genes, and 31 tRNA genes. Interestingly, there was no heterogeneity observed in the 16S rRNA gene sequence of this strain, as both copies were identical (16SrV-B F = 1.00). Additionally, four potential mobile units (PMUs) containing clusters of DNA repeats were also identified[46].

KEGG pathway analysis revealed that the JWB phytoplasma had reduced metabolic capabilities, indicating that the phytoplasma is an obligate parasite. The metabolic pathways for oxidative phosphorylation, amino acid and fatty acid biosynthesis, the pentose phosphate pathway, and the tricarboxylic acid cycle are not complete in the JWB phytoplasma genome. None of the ATP-synthase subunits were identified, while genes involved in glycolysis were detected. The phosphoenolpyruvate-dependent sugar-phosphotransferase systems responsible for sugar importation and phosphorylation were not identified, whereas genes encoding ABC-type maltose transporters were found in JWB phytoplasma[46].

Effectors

Phytoplasmas encode a functional Sec-dependent secretion pathway and secrete effectors into host cells, modulating host defense and morphogenesis[1]. These effector proteins migrate systemically in plants and interact with target proteins in the plant cell cytoplasm or nucleus. The aster yellows phytoplasma ('*Ca*. P. asteris') genome encodes 56 secreted proteins (SAPs). SAP11 and its homologs interact with and destabilize TCP (TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR) transcription factors, induce stem proliferation and alter leaf development through affecting the jasmonate pathway[47]. SAP54 and homologs, named phyllogens, degrade MADS-box transcription factors and induce the development of leaf-like flowers[48]. Phyllogen induces the degradation of MADS-box transcription factors by mediating interactions between MADS and the 26S proteasome shuttle protein RADIATION SENSITIVE 23C (RAD23C)[48]. SAP05 hijacks the plant ubiquitin receptor PRN10 within the 26S proteasome to mediate the concurrent degradation of SPL and GATA transcription factors through an ubiquitination-independent process, inducing witches' broom and sterile shoot[49].TENGU, from onion yellows phytoplasma, induce witches' broom, dwarfism and flower sterility[50]. The wheat blue dwarf phytoplasma ('*Ca*. P. tritici') effector SWP12 degrades the WRKY transcription factor TaWRKY74 and suppresses wheat resistance.[51]

A few effectors from JWB phytoplasma have been identified. Two secreted JWB phytoplasma proteins, SJP1 and SJP2, were determined to target the TCP transcription factor ZjBRC1, to induce witches' broom with increased lateral branches, and to promote the accumulation of endogenous auxin (indole-3-acetic acid) in jujube callus[52]. SJP3 disrupts the expression of MADS-box transcription factors associated with floral organ identity and flowering time, inducing phyllody in both jujube and *Arabidopsis*[53]. A JWB phytoplasma effector was also identified and, named Zaofeng6, it interacts with ZjTCP7, a homolog of the *Arabidopsis* BRC1 through its first two α-helix domains in the cell nucleus, where it then down-regulates expression of genes in the strigolactone signaling pathway, which induces shoot proliferation[6].

Detection of JWB phytoplasma infections

The symptoms of JWB disease, especially witches' broom and phyllody, are quite typical and recognizable, making it possible to distinguish it from other jujube disorders[5]. Earlier, researchers were able to detect JWB phytoplasma, or other mycoplasma-like organisms (MLOs) with an electron microscope[5]. Later, observation of witches' broom-associated MLOs with a fluorescence microscope after staining with 4'-6-diamidino-2-phenylindole·2HCl (DAPI) were developed[5]. Antiserum to JWB MLOs (phytoplasmas) were also developed, which made ELISA detection possible[70].

The rRNA genes of phytoplasmas are highly conserved, with slight variation. Thus, 16S rRNA gene sequences could be used for phylogenetic analysis as well as molecular detection. JWB phytoplasma could be detected by 16S rRNA gene PCR with either phytoplasma universal primers or JWB phytoplasma specific primers[4].

Based on PCR detection and symptom observation, a JWB disease grading system has been established on the tissue, branch, tree, orchard and regional levels. This standardized assessment provides a reference for both research and management[5].

In more recent years, new techniques such as loop-mediated isothermal amplification of DNA (LAMP) and CRISPR/Cas12-based visual assays have been developed for pathogen detection[71]. An all-in-one Dual CRISPR assay technique for phytoplasma detection has been developed, which could also be used for JWB phytoplasma detection. This technique is sensitive, accurate, and efficient, can be assessed visually, and does not rely on expensive equipment, meaning it can be used in the field[72].

Table 2. Genes and pathways involved in the interaction between JWB phytoplasma and jujube.

Plant material	Analysis methods	Corresponding pathways	Corresponding genes	Reference
cv. Xingguang, grafted in healthy and diseased trees	SSH, rRT-PCR	Disease/defense	*TLP, PR10, HSP70, ERF, kinase-related protein*	[54]
cv. Junzao, grafted in healthy and diseased trees	rRT-PCR, western blotting		*GSTU1*	[55]
Healthy and diseased trees	HiSeq, RT-PCR, 5' RLM RACE		*miRNA156a, 156b, 156c, 156d, 156e, 156h, 159a, 159e, 172, 2111, 2950, 319a, 395a, 395b,399, 477, 858b, n2, n8, n16, n23, n24*	[56]
cv. Xingguang (resistant) and Zanhuang-dazao (susceptible) grafted in healthy and diseased trees			*ZjeEF-1a*	[57]
cv. Langzao, symptomatic and healthy trees	RNA-seq	Amino acid metabolism, carotenoid synthesis		[58]
cv. Dongzao, *in vitro* healthy and diseased plantlets	qRT-PCR		*ZjMPK1, 2, 3, 4,5, 9, 10, ZjMKK1, 2, 3*	[59]
Healthy and diseased trees	qRT-PCR		*ZjSPL6, 7, 9, 12, 13, 16, 17, 18*	[60]
Healthy and diseased trees	RT-PCR, qRT-PCR		B, C/D, E-type MADS-box genes	[61]
cv. Huizao, healthy and diseased trees	RNA-seq, iTRAQ proteomic, mass spectrometry	Phenylpropanoid biosynthesis, flavonoid biosynthesis	*LOX2*	[44]
cv. Huizao, healthy and diseased trees	RNA-seq, qRT-PCR	ABA, CTK, JA, SA, BR, ET, IAA	*MYB, WRKY, PAL, 4CL, DELLA, LOX2*	[62]
cv. Huizao, healthy and diseased trees	qRT-PCR		*ZjTCP6, 16*	[63]
Healthy and diseased trees	RT-PCR, qRT-PCR		*ZjbHLH12,18, 23, 24, 34, 49, 53, 62, 63, 79, 83, 88*	[64]
cv. Goutouzao, *in vitro* healthy, diseased, and recovered* plantlets; cv. Dongzao, healthy and diseased trees, cv. Xingguang, cv. Junzao	qRT-PCR		*ZjMPK2, ZjMKK2, 4*	[65]
cv. Huizao, *in vitro* diseased and tetracycline-treated plantlets	RNA-seq, iTRAQ proteomic, mass spectrometry	JA biosynthesis	*PLA1, LOX, AOC, OPRs, JIP*	[44]
cv. Dongzao, healthy and diseased trees, healthy and diseased *in vitro* plantlets	qRT-PCR		*ZjMPKKK3, 4, 7, 10, 17, 18, 25, 26, 30, 34, 35, 37, 40, 41, 43, 44, 45, 46, 50, 52, 53*	[66]
cv. Fanchangchangzao, healthy and diseased trees	qRT-PCR		*ZjARF1, 2, 3, 4, 8*	[67]
cv. Dongzao, healthy and diseased trees	RT-PCR, qRT-PCR		*ZjbZIP3, 11, 12, 15, 17, 18, 19, 20, 26*	[68]
Healthy and diseased trees	RNA-seq, iTRAQ proteomic, qRT-PCR		*ZjLOX2, 5, 6, 8*	[69]
cv. Pozao, cv T13, healthy and diseased trees	RNA-seq, qRT-PCR	JA, SA		[45]

* The recovered plantlets are the JWB diseased plantlets cultured in medium with tetracycline.

Control of phytoplasma in jujube

Breeding for resistance

As one of the longest-cultivated fruit tree species in the world, jujube has abundant germplasm resources after undergoing long periods of evolution and selection[10]. According to the 'China Fruit Tree Records on Jujube', there are more than 700 jujube cultivars based on synonym and homonym identification[3].

Different jujube cultivars and different trees of the same cultivar can show different resistance to JWB disease. Among the six most common jujube cultivars in Henan province, Lingbaozao and Jiuyueqing are resistant, Huizao and Bianhesuan are susceptible, while Jixinzao and Guangyangzao show mild resistance[11]. In Hebei province, Hupingzao and Hamazao are the most resistant cultivars[73]. Some trees of the cultivar Pozao and Junzao also showed high resistance to JWB disease[73].

Screening 42 jujube cultivars by grafting onto JWB-diseased stocks resulted in identification of two cultivars with the highest resistance, four with high resistance, and 15 with general resistance. By grafting diseased scion to healthy rootstock and healthy scions onto diseased stock, four jujube strains were selected from the 29 different combinations of germplasms[74]. Among the tested accessions, a resistant individual of cultivar Junzao that also produced big fruit with a high dry-fruit weight, was selected and named as a new cultivar, Xingguang[75]. In another study, seedlings were propagated from 147 ancient individual jujube trees growing in Beijing and infected by grafting with a diseased bud. Among these accessions, seven resistant strains were found after two years of observation[76].

Antibiotics application

Phytoplasmas are sensitive to tetracycline group of antibiotics. Hence, tetracycline antibiotic treatment has the ability to suppress phytoplasma symptoms. Trunk injection on diseased trees is feasible for practical control of jujube witches' broom disease in the jujube orchard[5]. Before grafting, soaking the scion with tetracycline is efficient to obtain phytoplasma-free grafted jujube saplings[5]. Tetracycline treatment is also efficient in phytoplasma elimination on *in vitro*-grown jujube shoots[44].

Management

Comprehensive control of JWB disease includes implementing measures during seedling cultivation, grading of symptom, and managing the orchard, especially for sanitation[5]. Phytoplasma-free plants can be obtained through phytoplasma-free tissue culture[77], tetracycline treatment[5], heat treatment[78] or cryopreservation[79].

Farmers must carefully manage the irrigation, fertilizer, and vegetation to keep the trees strong and healthy. An integrated pest management strategy must include the regular application of pesticides to control insects, especially phloem-feeding insects, that can carry and transmit JWB phytoplasma. The removal of the diseased branch on lightly diseased trees can stop an infection, while severely diseased trees must be dug out. The use of comprehensive control measures may not eliminate phytoplasmas completely, but control the phytoplasma density at a relatively economical level[5,11].

Prospects

Great efforts have been made on understanding the mechanism of JWB disease and on ways to control it. Yet, some basic properties of JWB phytoplasma are still poorly understood. The development of an axenic culture protocol may be helpful for the isolation of JWB phytoplasma, which can support whole genome sequencing and the study of the pathogen life cycle. Finding the specific culture media and growing conditions for JWB phytoplasma is the key to *in vitro* culture.

The genome of the JWB phytoplasma 'nky' strain has been sequenced. Sequencing more JWB phytoplasma strains would allow more thorough study of the diversity, evolution and molecular properties of these bacteria.

Pathogenic mechanisms of a few JWB phytoplasma effectors were revealed[6,52,53]. We still need to identify more JWB phytoplasma effectors and their target proteins to better understand JWB disease progression. The mechanisms by which JWB phytoplasma effectors direct the degradation of host proteins still needs further study.

Modification of host targets may produce plants that are resistant to phytoplasma pathogen. A two amino substitution within plant RPN10 generate a functional variant that is resistant to phytoplasma effector SAP05 activities[49]. Based on deep understanding of the pathogen-host interaction, further study should try to identify more plant targets responding to phytoplasma effectors and generate more resistant variants by modifying target protein.

Traditional resistant breeding by selection needs a relatively longer period of time. Screening genes and pathways involved in the pathogen-host interaction, analysis of the function of these genes and identification of the key genes which play roles in plant resistance, will be helpful to understand the jujube resistance mechanism. Sequence variations of these genes among the jujube populations could be developed as DNA markers, which will accelerate the screening of resistant jujube varieties.

Natural recovery from phytoplasma disease has been reported on grapevines, apple and apricot, with recovered plants showing a lower susceptibility to re-infection compared to those that were never infected, suggesting the presence of a potential resistance mechanism[80]. Tetracycline, cold or heat treatment could induce phytoplasma free jujube seedlings[44,78,79]. The advent of molecular technologies and their refinement for the study and detection of JWB phytoplasmas has created an opportunity for molecular biologists, plant pathologists, and plant breeders to gain an upper hand on this devastating disease. Armed with the experience of farmers and the scientific knowledge, it makes it possible to limit the devastating effects of phytoplasmas on the production of jujube worldwide.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Feng J.; data collection: Zhang Y, Tan B, Wu Y, Wang W, Qiao Z; Chen P; analysis and interpretation of results: Zheng X, Ye X, Cheng J, Bi S, Huang Y; draft manuscript preparation: Li J, Gu L, Guo S. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (32071803), and Tree Germplasm Reservation Project of Henan Province (2022003). We thank Anita K. Snyder for help in correcting the English of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 14 May 2023; Accepted 19 September 2023; Published online 5 December 2023

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