大 豆 科 学
SOYBEAN SCIENCE

The Status of Soybean Rust Research in China*

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Abstract Soybean rust caused by *Phakopsora pachyrhizi* Syd was first reported in 1899 in Jilin, a province in northeast China. It became the most destructive disease for soybean production in tropical or sub-tropical areas since the 1960s. Systematic research in China started since 1970s. The disease distribution, yield loss, pathogen life cycle, host range, interaction of pathogen and host, disease epidemiology, host resistance identification and screening, genetics of rust resistance and breeding, integrated management were addressed in this paper.

Key words Soybean rust; Epidemics; Resistance mechanism; Resistance resource; Physiological race

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Soybean rust was first reported in 1899 in Jilin, a province in northeast China[11]. Then it was found in other 23 provinces in China by the end of 1980s. As early as in 1914, soybean rust had already become the most serious disease in Taiwan and became a destructive disease in tropical regions after 1960s. The risk of soybean rust attracted more attention in mainland of China when it first broke out in 1975 in Henan, a province in center part of China, where soybean rust had never been reported[34]. As a member of the International Working Group on Soybean Rust (IWGSR), more detail research in soybean rust was conducted in China since that time.

1 Pathogen distribution and host range

It is clear that the pathogens causing soybean rust are *Phakopsora pachyrhizi* and *Phakopsora meibomiae*. The pathogens, once regarded as synonymous by the early investigators, were separated by Ono et al[18]. The teliospores of *Phakopsora pachyrhizi* were thin-walled, and pale-colored, irregular arranged in several layers in the sorus while those from *Phakoposora meibomiae* were relatively thick-walled, moderately pigmented and arranged in vertical rows of a few spore depth in the sorus. It was concluded that *Phakopsora pachyrhizi* existed both in Asia and America while *Phakopsora meibomiae* only existed in America[47]. *Phakopsora pachyrhizi* is the only pathogen causing soybean rust in Asian countries.

Phakopsora pachyrhizi has been found in 24 provinces in China, including Taiwan, Hebei, Sichuan, Tibet, Jilin, Shananxi, Jiangxi, Fujian, Hunan, Heilongjiang, Liaoning, Hubei, Guangxi, Guangdong, Guizhou, Yunnan, Jiangsu, Zhejiang, Anhui, Shandong, Gansu, Hainan, Henan and Shanxi[3, 9~11, 13, 14, 25, 34, 42, 43, 46]. Tan et al. confirmed that the pathogen in 19 provinces were

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Phakopsora pachyrhizi while the pathogens in other 4 provinces (Hebei, Liaoning, Jilin and Heilongjiang) had not been found again. The annual area reached about 1,333,333 hm^2[35].

It has been reported that the pathogen can infect 139 species belonging to 49 genera of leguminous plants. The natural hosts included at least 30 species belonging to 17 genera, among thoes 21 species belonging to 12 genera distributed in China[25,40,47]. Ono et al[18] and other investigators reported that more than 100 species were infected by *Phakopsora pachyrhizi* through artificial inoculation. *Glycine max* is a preferred host in the legumes family. Other common legume hosts are *Pachyrhizi*, *Dolichos*, *Vigna*, *Crotalaria*, and *Lupinus*. It was not clear if there was any alternative host species outside legumes.

2 Biological characteristics and physiological race differentiation

Phakopsora pachyrhizi had its own asexual and sexual stage. Uredospores and teliospores were the main spore forms at these two stages respectively. Uredospores were obovoid or broadly elliptical, minutely and densely echinulate and colorless to pale browning[40], wall thick 1 ~ 1.5 μm, pycnium was not obvious. Uredopsores from China were 29.36 × 17.88 μm in size in average[27]. The size of the spores varied with regions[25]. The life span of uredospore usually is 61 days at 13 ~ 24 ℃. The vigor can be kept under −140 ℃ for 3 months. The spores start germinating under 8 ~ 36 ℃ with 100% relative humidity (RH)[34]. Uredospore germ tube growth was anti-phototropic[27]. The pH required for spore germination was at pH 2.2 ~ 10 with the best germination ratio at pH 5 ~ 6[27].

Teliospore, a key indicator of *Phakopsora* taxology, was the main spore type in the sexual stage of *Phakopsora pahcyrhizi*. Telia were 2 ~ 7 spores layered, often mixed with uredinia. The size varied with the isolates origin. The shape of teliospore was oblong to ellipsoid, light yellowish, single or cluster-celled scattered or cluster arrangement in the leaf tissue[27].

Teliospores and telia could be formed on many host plants such as *Glycine species*, *Cajanus cajan*, *Pachyrhizi*, *Phaseolus lunatus*, *Phaseolus vulgaris*, *Rhychos minina*, *Sesbania exaltata*, *Sesbania vesicaria* and *Vigna univulata*[40,41]. Teliospores stage has not been observed in tropical regions under natural conditions. Usually a low-temperature duration was required for teliopsore formation[40,41]. The controlled condition for telliospores formation was 13 ~ 25 ℃ with 200 lux light, and the teliospore formation reached to the peak when daily average temperature was around 16 ℃[29,31]. Teliospores usually had 3 ~ 6 months dormancy[12,19]. Teliospores germinated and developed into basidia and basidiospores by using dry-wet alternative treatment[12], no further development was observed. However, no teliospore germinating was observed when the experiment was repeated again in China (unpublished data).

The knowledge about teliospores is quite limited yet. It is not clear where and how teliospores germinate and develop into uredospores under natural conditions, and if there are any alternative hosts beside legumes. If there exist, how to find them. Tan[31] suggested that teliospores might not germinate under nature conditions; they may not be the primary inoculums in north China. The primary inoculums were probably airborne from south China. However, if this was true, how to explain the rust outbroke in some temperate places, where there was no wind and rain records within 2 ~ 3 weeks, no spores caught, then rust disease seemed spread to the whole field in one night, where the pathogen came from (unpublished data)? And why rust is more seriously in autumn than in spring in the same area as the weather is favorable for pathogen multiplication in both seasons? Uncovering of mystery of teliospore will give us a more clear understanding of soybean rust.

It was noticed that isolates virulence varied from different geographic regions or legume hosts.

Lin divided 9 isolates from 3 places into 6 physiological races by using a tentative differential set consisting of six soybean varieties and 5 legumes species, however no marked differences in virulence among the isolates was observed on the soybean varieties. Ding[4] classified 12 isolates into 5 races by using 11 soybean varieties. Yeh[38] classified isolated from 5 areas into 3 races (1, 2, 3) by using 5 soybean genotypes. Tan[26] inoculated isolates from Hubei on 8 soybean genotypes, identified 4 races: A, B, C and D. Among them, A and B were same as "1, 2" in Taiwan identified by Yeh. A was the most virulent isolate. C was different from "3", so the C and D were regarded as new races. Mo et al[16] classified 4 races from the isolates in Guangxi by using 10 differentials; only one was the same as B race identified by Tan, indicating the complexity of pathogen population structure in different geographic regions. So far there has been no a set of standard differential for pathogen isolates virulence identification. The research on the virulence of pathogen isolates was restricted to the limited scales or regions.

3 Rust epidemic

Rust epidemic was caused by its multi-cyclic foci and copious production of uredopsores[1]. Uredia formed 10 ~ 14 days after uredospores infected host plants, and then the released new uredospores infected the surrounding tissues again. In the regions of 19 ~ 27°N, uredopsores from natural hosts such as *Glycine max*, *Glycine soja*[47], *Glycine tomentosa* Benth[10], *Pachyrhizus erosus* Urban[47], Pueraria lobata (Willd) Ohwi[47], Pueraria tonkinensis Gagnep[47] could become the primary inoculums. However, the above hosts could not be grown in north China (north from 27°N) during the winter. Whether or not some perennial legumes such as *Apio carnea*, *Aphicarpaea trisperma* Bake, *Desmodium elegans* DC and *Desmodium williamsii*[46] can become the over-season hosts in north China still needs further investigation. Seed or soil transmission has been proved to be impossible for the pathogen spreading[39].

The dispersal of fungal spores by the wind was thought to be the primary inoculums in the regions in north from 27°N in China[31]. The evidence was obvious in hilly land. The pathogen infested center firstly formed in southern slope, then the infested area expanded to north part in '<' type with the wind. However, it was difficult to explain why rust occurred only in autumn or occured seriously on autumn-sown soybean or winter-sown soybean in south China.

Climate played an important role in rust spreading. The daily average temperature at 15 ~ 26 ℃ was favor for uredospores germination. Rainfall and rainy days mainly influenced the development of rust in inland and flatlands[27]. Rust occurrence was delayed during the less rainfall season whereas the disease outbreak was shifted to an earlier time and spread rapidly when the weather was continuous cloudy and drizzly. In coastland areas and mountain areas, fog or dew occurred frequently through year. A long duration of dew or fog weather played an important role in rust development in Fujian, Hainan and Yunnan where the disease was prevalent[34].

Elevation also influenced the disease development. Disease severity was reported to be positive related with elevation below 600m, whereas such relation disappeared when the elevation was above 600m because rust was mostly seriously[17].

Soybean could be infected in whole growing stage, but the flowering stage was more sensitive to the pathogen[28]. Infested rate was positively related with plant growth stage[34].

The disease severity in field also varied with landform, drainage condition, irrigation patterns, and plant density. The conditions that increased the humidity in the field such as lowlands, high plant density[34], blocked drainage, and flood irrigation would enhance the disease severity.

Combined the meteorological information with rust investigation, Tan et al.[35] divided soybean production area in China into three regions: heavy rust regions, frequent rust regions and occasional rust regions. Most heavy rust areas covered the regions of 19～27°N. Rust occurred every year and resulted in yield loss except drought years, the yield loss in normal year reached 30%～50%, serious yield loss reached 50%～70% or even 100% when the weather was favor for rust development. The frequent rust areas were distributed in the regions of 27～34°N. When the rainy days during flower stage were more than 15 days, and monthly rainfall was above 150mm, rust can result in 50%～70% yield loss. In regions north from 34°N, rainfall was less, so rust just occurred occasionally.

4 Identification and screening for rust resistance

The common evaluation method in field is the 3-digital rating system that was established by IWGSR[36]. It is easy to be manipulated for large-scale screening.

Rust resistance rate was determined according to the spots type (RB, O and Tan type), amount of uredospores multiplication in host leaves[1] in field or in lab. However, this method could identify typical resistance genotypes. Some RB type spots also produced a lot of spores when the inoculated leaves were cultured for long time especially in field. Thus, Tan et al.[33] divided 5 response types: immune (IM), high resistant (HR), moderate resistant (MS), moderate susceptible (MS) and high susceptible (HS). Their standard has been used for germplasm resistance rating in field or lab.

Combined primary screening in field plants and re-identification in lab, about 11000 germplasm lines had been screened for rust resistance[22,30]. No immune or highly resistance lines was identified. There were 83 resistant accessions (MR) obtained, most were autumn-sown soybean. There were only 9 from summer-sown or spring-sown soybean. It was reported that the wild germplasm materials possessed a lot of resistance genes, so far no related research have been investigated in China.

5 Rust resistance genetics, breeding and mechanism

It had been reported that there were at least 4 locus linked to soybean rust resistance[7,8]. The resistant genotype may carry one or two dominant genes. By means of diallel analysis of PI459025 (R), AGS181(MS), AGS129(MS) and Houzimao (HS), Tan et al[32] confirmed that the resistance in PI459025 was controlled by one dominant gene, while the tolerance to rust in AGS181 and AGS129 was controlled by multiple genes, and the super dominance was observed in the tolerant lines.

Rust resistant landraces, such as Zhangziwu, Yushanqingpi and Jiuyuehuang, could be directly used in production but most resistant germplasm lines could not be used directly due to their poor agronomic traits, such as long maturity, low yield, logging and dark coat[21]. Conventional rust resistance breeding started in Taiwan in 1960s. Tainung 3, Tainung 4 and Kaoshung 3 were derived from hybrid progenies of PI200492, PI200490 and PI200451, respectively[2]. PI203970 and PI 230971 were used for the breeding of AGS181, AGS182, AGS183, AGS229, and AGS233[24], Tan et al. used PI459025 as a parent crossed with AGS181, and some resistance lines such as R-34 and R-50 were selected. In addition, it was observed some progenies such as You 84-87, Zhongdou 19 from non-resistant crossing combination behaved moderate resistance, this may be due to the improvement of horizontal resistance through hybridization. Resistant lines also could be obtained through mutation. Zaochun No. 1 was obtained from the mutation progenies of Aijiaozao, a landrace in Hubei province, treated with ^{60}Co, and 9854 from Zhongdou-24 by γ-ray irradiation[23]. Unfortunately, there were many examples that resistant germplasm or breeding varieties lost their resistance gradually due to the emergence of new races[18, 21].

Histological study showed that the leave trichome of resistant genotypes were short and thick in regular arrangement while those on susceptible genotypes were soft and thin in irregular arrangement[5]. The mastoid protuberance was formed in resistant genotype 3 days after inoculation on leaf surface while no mastoid protuberance formation occurred in susceptible ones. Lignification occurred much rapidly than that in susceptible ones[6]. Isoenzymes changed obviously in amount and types after inoculation. Such responses may efficiently prevent spores dispersing on leaf surface[5]. The acidic amino acids in resistant genotypes increased significantly 24 hour after inoculation while the neutral or alkaline amino acids in susceptible genotypes changed greatly[20], indicating that rust resistance might possess some special features.

6 Soybean rust management

Chemical application during the potent infected stage can effectively prevent the disease. Bayleton, Plantvax and Dithane M45 were found to be effective in controlling rust. Bayleton was the best one for 90% control efficiency and over 70 days-long effect duration. Daconil and Mebenil were efficient to reduce the yield loss in autumn-sown soybean area[45]. Plantvax, Benomyl, Mancozeb, Saprol (Triforine) and Triazone were also effective to control rust[44].

Integrated protect management (IPM) includes growing resistant varieties, changing cultivation patterns such as spring-sown soybean instead of autumn-sown, applying potassium, decreasing the field humidity by reducing planting population, and assisted with chemical applying at early stage. The IPM strategies exert an efficient effect in soybean production in rust infected area.

7 The prospect of rust research

As has been reported in 39 countries distributed in Asia, America, Oceania, Europe and Africa, soybean rust has become a worldwide disease in soybean production. However, soybean rust possess much potential risk in production due to the complexity of the pathogens and lacking of immune or high-level resistance gene source compared to other pests. Being an obligate fungus, *Phakopsora pachyrhizi* cannot be cultured under artificial conditions. In most cases, the mixtures of races were used to identify resistance accessions, the lacking of an overview about *Phakopsora pachyrhizi* differentiation made the releasing of resistance cultivars not synchronize with the evolution of the pathogen, thus reduced life span of resistance cultivars. The establishment of pathogen culture method will provide us an exact resistant spectrum for each resistant variety, avoid 'escape' of resistant lines due to improper evaluation way, facilitate our understanding about pathogen population evolution and interaction between host and pathogen. The knowledge about teliospore will help us to understand the gametic copulation, story in meiosis, new physiological race emergence, the whole life cycle of *Phakopsora pachyrhizi* and the primary inoculums in temperate regions. There has been no genotype immune to rust all over the world, screening new resistant germplasm lines in wild soybean perhaps will provide us some exciting result for soybean resistance breeding. The advances in plant functional genomic and comparative genomic will also provide some clue for rust resistance research.

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中国大豆锈病研究

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摘要 1899 年在中国吉林首次报道了由豆薯层锈菌(*Phakopsora pachyrhizi* Syd)引起的大豆锈病。20 世纪 60 年代大豆锈病成为热带、亚热带地区大豆生产中最严重的病害。中国于 20 世纪 70 年代开始了较为系统的研究, 本篇综述介绍了中国在大豆锈病的病原菌分布及其寄主、产量损失, 病害流行、病原生活周期、病原与寄主的互作、抗锈资源鉴定、抗锈遗传、抗锈育种和综合防治等方面的研究进展。

关键词 大豆锈病; 流行; 抗性机制; 抗锈资源; 生理小种