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Advances in Integrated Control Techniques of Rice Blast

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Abstract Rice blast is the most devastating disease in rice, and it seriously threatens the safety of rice production. Improving the ability of integrated prevention and control of rice blast has always been an important part of ensuring food production safety. In the past decade, scientists have made great progress in the prevention and control techniques of rice blast, which paves the way for the green controlling of rice diseases. In this article, the advances in the intelligentization of rice blast field monitoring techniques, the optimization of prediction and forecast modeling system, the research and development of low-toxic and high-efficiency chemical pesticides, biogenic pesticides and inducers and the regulation of multiple ecological factors including variety and cultivation are reviewed, and the new strategies for green controlling of rice blast based on these techniques are summarized. Further, the problems such as high pesticide prices and pesticide residue faced by rice blast prevention and control and the challenge of slow research and development of low-toxic and economical biogenic pesticides are discussed. Finally, the development direction of green controlling of rice blast based on molecular targets, small interfering RNA (siRNA) and CRISPR/Cas9 technologies is predicted, with a view to guaranteeing the safety of rice production.

Key words Rice, Rice blast, Integrated prevention and control

1 Introduction

Rice blast, caused by *Magnaporthe oryzae*, is the most devastating disease in rice. The average annual reduction in rice production caused by rice blast is about 10% – 30%, and harvest failure even occurs in severe cases. Rice blast still can not be completely controlled so far[1]. In recent years, with the continuous optimization of prevention and control techniques by scientific research institutes, the enhancement of early warning and guidance capability of plant protection departments, and the strengthening of prevention and control awareness of farmers, the losses caused by rice blast to rice production has decreased significantly, and the average annual output loss has dropped to about 450 000 t. However, in years when the rainy area covers most of the rice planting area, the probability of severe occurrence of rice blast still increases significantly under the conditions of double cropping and high water and fertilizer cultivation for high quality late season rice varieties with low resistance level. Therefore, controlling rice blast is still of great significance to ensure food production safety. Carrying out resistance breeding by utilizing variety resistance is considered to be the most economical, effective and environmentally friendly control method, but practice has proved that most of the disease-resistant varieties come to be susceptible after 2 – 3 years of popularization and application in a large area. In summary, improving the capacity and enhancing the technical research of rice blast integrated prevention and control are important guarantees for food production safety.

In recent years, around the green development concept of innovation, opening and sharing, green production has become the future development trend of China's agriculture. Based on the domestic and foreign literature in the past decade, this article reviews the research progress of prediction and forecast technology, pesticide control, ecological control, and resistance monitoring of rice blast, and analyzes the future directions of research on rice blast prevention and control combining green integrated prevention and control technology (e.g., utilization and distribution of resistant varieties), so as to provide reference for researchers and technical extension staff.

2 Prediction and forecast and field monitoring techniques of rice blast

M. oryzae directly damages the ears of rice, and has the most serious damage to the yield and quality of rice. It is difficult to find before the symptoms appear. Therefore, early monitoring and forecasting is essential for the prevention and control of rice blast. As the forerunner to guide prevention and control, the accuracy and operability of prediction and forecast are of great significance for the disease controlling. Rice blast is a multi-cycle disease spread by air current, and its prevalence is particularly affected by meteorological factors. Therefore, establishing prediction and forecast model with high accuracy and easy operation has a multiplier effect on the early control, and it is one of the hot spots of rice

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blast research in recent years.

A prediction and forecast model was established using the three-layer BP neural network (back propagation neural network) method for the incidence of rice blast. It can well reflect the functional relationship between the occurrence of leaf and neck blast and the meteorological factors, and the prediction accuracy for rice blast is significantly better than that of the stepwise regression model[2]. The ant colony gray RBF neural network combined forecast model based on the complex non-linearity of the rice blast gray system affected by a variety of climatic factors has a prediction accuracy of 96.77%[3]. The optimal correlation and spatial topology techniques were used to analyze the influencing factors of the disease index of rice blast in Jiangsu rice area, the most significant, stable and independent large-scale predictors were selected, and a long-term prediction model for the meteorological grade of rice blast based on atmospheric circulation and sea temperature factors was established. And the model was proved to be ideal by history fitting and trial test[4]. In addition, based on the monitoring of disaster-causing climatic factor and amount of spores floating in the air in the field, a rice blast epidemic trend prediction model was constructed, and it can predict the occurrence trend of panicle blast[5].

Based on the hyperspectral imaging technology and the classification standards established by the International Rice Research Institute for rice blast, the prediction and forecast accuracy of 166 samples reached 96.39%, which can provide research basis for the detection of field disease degree of rice leaf blast[6]. Using chlorophyll fluorescence spectroscopy technology and principal component analysis (PCA) method, three rice blast prediction models, PCA-DA, PCA-MLRA, and PCA-MLP were established, respectively, further improving the remote sensing and detection technology of rice blast[7].

With the rapid development of artificial intelligence and network technology, the construction of field monitoring stations is becoming more and more dense. The field meteorological factors and field disease monitoring spectral data can be collected and analyzed in real time, providing strong support for the early warning, prediction and real-time monitoring of rice blast. The development of rice blast forecasting technology tends to be more accurate and practical.

3 Research progress in pesticide prevention and control

3.1 Research & development and rational utilization of high-efficiency chemical pesticides

3.1.1 Research & development of high-efficiency chemical pesticides. Chemical control has the advantages of high efficiency, convenience, high speed, *etc.*, and it is the first choice for emergency prevention and control of rice blast.

The main chemical agents commonly used for the prevention and control of rice blast are tricyclazole (preventative) and isoprothiolane (preventive and therapeutic effect). In recent years, people have developed new ingredients pyraclostrobin, azoxystrobin and AzTop with both preventive and therapeutic effects and epoxiconazole with preventive effect on rice blast. Among them, some of high-efficiency dosage forms have been commercialized, such as the suspension of 9.5% pyraclostrobin microcapsule, 30% azoxystrobin and 12.5% epoxiconazole. The 25% pyraclostrobin suspension and 9.5% pyraclostrobin microcapsule suspension perform very well to leaf blast and neck blast in the field[8-9]. Indoor and field experiments have proved that 30% azoxystrobin suspension has a good control effect on rice blast, and it can be used as a substitute product for carbendazim and kitazin[10]. AzTop has an ideal control effect on rice blast with long lasting effect[11].

Long-term use of a single agent is easy to cause drug resistance, therefore, it is important to develop some safe and efficient compound formulations and combination medicine technology.

The currently reported compound formulations for controlling rice blast generally consist of single agents with preventive and therapeutic effects or two chemical agents with therapeutic effect. For example, 75% trifloxystrobin · tebuconazole WG, 75% azoxystrobin · tebuconazole WG, 30% blastamide · tebuconazole SC, 30% Fumile (27% isoprothiolane +3% hexaconazole) EC, 23% kresoxim-methyl · epoxiconazole SC, 32.5% benzyl · azoxystrobin SC, *etc.* have good control effect on leaf blast and neck blast, and have yield-improving and health care effects on rice[12-14]. A study[15] shows that 30% azoxystrobin · prochloraz SC is a safe compound for rice and river crabs and a new pesticide for preventing and controlling rice blast in ecological fields. With the rise of rice-duck, rice-shrimp, and rice-fish co-breeding and rice field tourism, the research and development of new pesticides suitable for ecological planting-breeding has broad prospects.

3.1.2 Research and development of new compounds and discovery of pesticide targets. Following with the long-term use of isoprothiolane, tricyclazole, fenaminstrobin and other agents, the rice regions of both southern and northern China are facing the risk of pesticide resistance[16-18]. Thus, there is an urgent need to develop and reserve a batch of high-efficiency and safe new pesticides (synergists), medication technologies and prevention and control strategies. Through the research on the pathogenic mechanism of pathogenic bacteria and the targets of rice blast pesticides, the research and development of new compounds with novel mechanisms of action has been expanded. Studies[19-20] have shown that new methoxy acrylate pesticides fluoxastrobin and syringostrobin, carbamate pesticide Tolprocarb (test code MTF-0301) and fluoroquinoline amide compounds obviously inhibit the hyphal growth of *M. oryzae*, and have a very good control effect on rice blast. The new quinoline pesticide ZJ5337 (benzyl carbonate-2,3,8-trimethyl-6-heptafluoroisopropyl-4-quinolinate) can significantly inhibit the formation of attachment spores of *M. oryzae*, and its field control effect against rice blast can reach 66% - 91%, and it is safe for rice[21]. The methylenetetrahydrofolate reductase encoding gene *MoMet*13 identified from *M. oryzae* can affect the growth and asexual reproduction of *M. oryzae* by participating in the biosynthesis of methionine. It plays an important role in regulating the invasion of appressoria, infecting hyphae growth and pathogenicity of *M. oryzae*. This provides important reference value for the development of new pesticides targeting key genes in the methionine synthesis pathway[22]. *MoPEX*1 is a gene encoding peroxisomes, necessary for infection-related morphogenesis and pathogenicity of *M. oryzae*. In deletion mutant △*Mopex*1, the hyphal growth slows down, the spore production is significantly reduced, the appressorium formation rate is reduced, and the appressoria are abnormal in morphology and enable to penetrate the host epidermis, leading to the loss of pathogenicity[23]. In *M. oryzae*, the gene *MoSOK*1 encoding Ste20 protein kinase, a member of the GCK (germinal center kinase) family, is up-regulated during the critical period of appressorium differentiation, and it participates in the development and pathogenic process of *M. oryzae*[24]. *MoAp*1 and several related genes of its regulatory network play a key role in the growth, sporulation, infection and pathogenic process of *M. oryzae*. Feeding asiRNA targeting *MoAP*1 (*i. e.* asiR1245, asiR1362 and asiR1115) causes silencing of *MoAP*1 gene, leading to inhibition of growth, abnormal spores, and reduction of pathogenicity. Among them, asiR1115 has the strongest inhibitory effect on *M. oryzae*[25].

3.2 Discovery of botanical pesticides Botanical pesticides are derived from plant metabolites with antibacterial activity. Generally, they are economical and safe, and have low risk of drug resistance, with good application prospects. Starting with the crude extracts of plants that are naturally resistant to pests and diseases, botanical resources that can inhibit *M. oryzae* are discovered. For example, the alcohol extracts of *Schima superba* leaves, *Sapindus mukorossi* peel, *Magnolia grandiflora* leaves, *Camellia oleifera* leaves, *Castanopsis sclerophylla* leaves and *Cedrus* leaves have a strong inhibitory activity on *M. oryzae*[26]; the volatiles and extracts of the arils of spice crops such as *Allium sativum*, *Alliumcepa*, *Allium fistulosum* and *Torreya grandis* have obvious antibacterial activity against various pathogens such as *M. oryzae*[27-28]; the n-hexane extracts of *Garcinia kola* nuts, *Piper guineense* seeds and Eugenia aromatica, which are rich in aromatic lipids, can inhibit the growth and development of *M. oryzae* by 97% or more, and when they are used to treat rice seeds, the occurrence of leaf blast is alleviated[29]; *in vivo* and *in vitro* experiments prove that the hydrated extracts of *Chrysanthemum coccineum*, *Aloe vera* (25%) and *Coffee arabica* (25%) can inhibit *M. oryzae* by 78.83%, 79.45% and 89.40%, respectively, and they are not phytotoxic to seed germination, plant height, root length, dry weight, seedling growth and seedling vigor index of rice, and can be used safely[30]; the plant-derived pesticide 0.1% isopsoralen chalcone EC has better control effect on rice blast than 40% isoprothiolane EC; the by-product tea saponin of *C. oleifera* has activity against *M. oryzae*, significantly better than that of tricyclazole; and two alcohol extracts of *S. superba* and *S. superba* saponin monomers all have strong activity against *M. oryzae*[31-33].

In terms of research on the formulation of botanical pesticides, the control effect of the mixture of alcohol extracts of *S. mukorossi*, *M. grandiflora*, *C. oleifera* and *S. superba* (1:4.6:1:2.4) on rice blast is higher than that of tricyclazole, and it is moderately toxic to bees, carp, *etc.*, is safe to use[26, 34]. The mixture of chamaejasmenin B (root extract of *Stellera chamaejasma* L.) and tricyclazole (2:1) have a significant synergistic effect on the indoor virulence of *M. oryzae*[35].

However, the research on the production technology and formulation of plant-derived pesticide products is not yet mature and has not been commercialized. Therefore, the research and development of plant-derived pesticides is still in its infancy.

3.3 Biocontrol bacteria-derived pesticides Using antagonistic bacteria (biocontrol bacteria) to control rice blast is also a safe and environmentally friendly disease prevention measure. It is reported that the application of antagonistic bacteria to control rice blast is increasing. Among 486 strains of bacteria isolated and purified from rice rhizosphere soil, it was found that 12 strains have inhibitory effects on *M. oryzae*. Among them, 7 *Bacillus subtilis* strains and 3 *Bacillus pumilus* strains inhibited the mycelial growth and spore germination of *M. oryzae* by 100% and 80%, respectively[36]. The fermentation supernatant of rice endophytic antagonistic bacteria B10 has a strong inhibitory effect on *M. oryzae*, and the inhibition rates of its 100-fold dilution on the mycelial growth and spore germination of *M. oryzae* reached 79.37% and 63.42%, respectively, and the field disease prevention effect reached more than 70.2%[37]. The control effect of *B. subtilis* wettable powder (100 billion live spores/g) on leaf blast and neck blast was above 90%[38-39]. The high-frequency endophytic fungi *Absidia* and *Acremonium* isolated from Sri Lanka's traditional rice species Suwandel and Kaluheenati can effectively inhibit the expansion of rice blast by 100%[40]. Application of wild rice endophytic fungus *Falciphora oryzae* can effectively prevent rice blast when mixed with seeds or sprinkled once after sowing, and its control effect on leaf blast and panicle blast reached more than 70%, therefore, it can be used for prevention and control of rice blast in directly sown rice[41]. The crude extract of the endogenous actinomycete *Streptomyces rochei* YL-2 isolated from the leaves of *Azadirachta indica* had an inhibitory rate of 82.65% against rice blast, significantly higher than that of 25 mg/L kasugamycin WP, by up to 85.41%. The alcohol extract of *Inonotus obliquus* can inhibit the germination of *M. oryzae* spores by up to 100%, and it can cause hyphae malformation and thickened cell wall in *M. oryzae*[42-43].

Five components have been isolated from *B. subtilis* B-332, with the effect of resisting rice blast. Among them, 3 components and B-332 original bacteria have teratogenic effect on appressoria of *M. oryzae*. It is speculated that these components are small molecule cycloaliphatic peptide compounds of the antifungal bacillomycin D class. Nongkang 702, a metabolite isolated from *Streptomyces* JXAU4324, has a strong inhibitory effect on conidium germination and hyphae growth of *M. oryzae*, and 0.3% Nongkang 702 aqueous solution can achieve the effect of the commercially available pesticides jinggangmycin and kasugamycin[44-45]. *In vitro* and *in vivo* experiments proved that trichothecin (TCN), griseofulvin (griseofulvin) and the antimicrobial peptide MSI-99 can effectively inhibit the expansion of *M. oryzae* and have a good control effect on *M. oryzae*[46-48]. Field trials have shown that the control effects of the biological-sourced fungicides 3.5% polyoxin aqueous solution, 4% - 6% kasugamycin aqueous solution and Yidiling aqueous solution (biological fertilizer with actinomycete fermentation liquid as the main component) on rice blast were equivalent to that of conventional medicine, and they can be popularized and applied in a large area. The 2% jinggangmycin · 800 million spores/g *Bacillus cereus* suspension has significantly higher control effects on rice leaf blast and panicle blast and significantly higher yield-preserving effect than 20% tricyclazole wettable powder[49-51]. In addition, the optimized formula and biological control method (dry spraying) have simplified the application method of *B. subtilis* T429[52].

Similarly, although biocontrol bacteria are safe and environment-friendly control measures, the use of them is restricted by strict cultivation conditions, high separation costs, strict storage and transportation conditions, limited storage time, and complex release methods. Existing biocontrol bacteria-derived pesticide products are still difficult to promote and apply on a large scale.

3.4 Research and utilization of inducers Inducing plant to produce disease resistance is a new technology and new approach for plant protection and disaster prevention. People are working on applying inducers as pesticide substitutes for disease control. The plant-derived inducers reported in recent years that can induce rice blast resistance mainly include nano-SiO_2, allylbenzothiazole, β-aminobutyric acid, salicylic acid, and so on. Research shows nano-SiO_2 can enhance the resistance of rice to blast, and the application of nano-SiO_2 can significantly increase the chlorophyll content of leaves, increase the growth of new roots, reduce the inclination of rice leaves, and reduce the attachment of fungi, conducive to the normal growth of rice[53]. Application of 8% allyl thiazole granules to the roots significantly improves the activity of the defense enzymes PAL, POD and PPO and the maintenance time of their high activity in rice, and stimulates the immune response of the plant. It has excellent control effect on leaf blast and neck blast in the field, and it is a new type of protection and treatment pesticide[54-55].

A novel fungal-derived stress protein isolated from *Alternaria alternate* can induce resistance to rice blast and bacterial blight by stimulating the metabolism of active oxygen in rice[56]. chitin Fermentation filtrate of *Trichodema aureoviride* and alcohol extract WS of wild reed root endophytic fungus Fusarium solani can improve the activity of PPO, PAL, SOD, chitinase and other defense enzymes in rice, inducing rice to resist the invasion and expansion of rice blast[57-58]. In the early stage of rice blast, spraying the mixture of kasugamycin (500 μg/mL), phytochemical inducer β-aminobutyric acid BABA (250 μg/mL) and sodium silicate (250 μg/mL) has a good control effect on rice blast[59].

4 Ecological control techniques with multiple ecological factors

Multi-ecological factor control technology is to control the occurrence and damage of rice blast in multiple ways, starting from early warning and prevention, cultivation and drug monitoring, and combining the rational application of various drugs. The use of rice variety diversity and high-efficiency cultivation techniques to improve rice resistance is currently the most widely used ecological control measure for rice blast control in the field. Finding out the resistance of existing rice varieties and making a reasonable layout is the most green and ecological prevention and control measure, which has gradually attracted widespread attention from rice science and technology workers. Taking 24 rice blast-resistant monogenic lines and 22 *M. oryzae* identification strains as reference, 120 *M. oryzae* monospore strains collected and isolated from various districts and counties in Harbin as selection pressure, and 12 rice varieties as targets, Ma Juntao *et al.* [60] carried out research on resistance distribution of the rice varieties based on resistance gene analysis, clarified the local utilization value of rice blast resistance genes, analyzed the types of rice blast resistance genes in various varieties, and preliminarily formulated layout plans for single varieties and multiple varieties[60]. Cultivation measures such as dry nursery and thin planting, shallow-wet inter-irrigation, scientific fertilization, and application of silicon fertilizer help to balance the content of chlorophyll, total phenols, flavonoids and crude fiber with yield and silicon cell structure, *etc.*, improving resistance to rice blast[61-62]. Intercropping of rice varieties or of hybrid and glutinous rice reduces selection pressure of *M. oryzae* due to a single host genetic background and also control rice blast to a certain extent and increase yield[63-65].

5 Conclusions, existing problems and prospects

The prevention and control of rice blast is currently still dominated by chemical control, which has the disadvantages of high price, lack of therapeutic effect, unscientific spraying, being prone to residue, *etc.*, and so, developing and utilizing safe, environmentally friendly and cost-effective biogenic pesticides and taking ecological control, biological control, physical control and other environmentally friendly measures to control the behavior of harmful organisms are important development directions for integrated prevention and control of rice blast. Although biogenic pesticides are being researched and developed actively these years, the key components and mechanisms of action of biogenic drugs are still unclear. China has a vast territory, with extremely rich biological and environmental resources and extensive and profound research background on Chinese herbal medicine, and it still has great potential to be tapped in resource utilization. However, the research and development of plant- and bacteria-derived pesticide products in China is still in its infancy, and they have neither shown obvious advantages. The prospects for accelerating the development and utilization of these resources to make them better serve the prevention and control of rice blast are promising. In addition, developing ecological control technology based on multiple ecological factors can more comprehensively control the occurrence and spread of rice blast, which is of great significance to the standardized production of rice.

With the rapid development of molecular biotechnology, molecular targets, small interfering RNA (siRNA) and host-induced gene silencing (HIGS) technologies have been proven to be new strategies for controlling fungal diseases[23-25], and successful case of using CRISPR/Cas9 technology to directionally improve the resistance of rice to rice blast has also appeared[66]. In-depth research on these target genes and new technologies lays a foundation for the development of green and precise strategies for rice blast prevention and control and also opens up a new path for the green and safe production of rice.

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