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Application analysis of monoclonal antibodies in plant

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Abstract. monoclonal antibody is a highly homogeneous antibody produced by the cloning of a single B lymphocyte and directed only against a specific epitope. With the deepening of research on mAb, it can not only be used to treat human diseases, but also be used for the diagnosis of plant bacteria and viruses. In this paper, some applications of monoclonal antibodies in plants were reviewed, and it was found that the recent application direction was mainly focused on plant quarantine and traditional Chinese medicine (TCM) toxicity detection, and it was gradually applied to the research field of TCM, providing a new means for the quality control of TCM. Although McAb has shown good application prospect in many aspects, its development process is complex and there are large differences in the recognition specificity for different strains. Future research can pay more attention to the diagnosis and treatment of plant diseases to provide reference, convenient to speed up the quarantine to reduce the severity of the epidemic; It also provides reference for the research and quality control of toxic TCM containing toxic proteins.

Keywords: Monoclonal antibody, plants, application.

1. Introduction

Antibody is an immunoglobulin produced by the body under the stimulation of an antigen that can specifically bind to the antigen. Conventional antibody preparation is performed by immunizing animals and collecting antisera, so that antisera usually contain antibodies against other unrelated antigens and other protein components in the serum. General antigen molecules mostly contain multiple different antigenic determinants, so the conventional antibody is also a mixture of antibodies against multiple different antigenic determinants. Therefore, the conventional serum antibody is also called polyclonal antibody.

The lymphocyte hybridoma technique was established in 1975 by Kohler and Milstein, who fused mouse spleen cells immunized with a predetermined antigen with myeloma cells that could grow indefinitely in vitro culture to form B-hybridomas. The hybridoma cell has the characteristics of an amphiphilic cell, which can rapidly proliferate indefinitely like myeloma cells in vitro culture and never die, and can synthesize and secrete specific antibodies like splenic lymphocytes. By means of cloning, a monoclonal line derived from a single hybridoma cell, hybridoma cell lines, is obtained, which produces antibodies that are highly homogeneous against the same antigenic determinant, so-called monoclonal antibodies. Compared with polyclonal antibodies, monoclonal antibodies have the advantages of high purity, strong specificity and good repeatability, and can be continuously and indefinitely supplied.

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With global economic integration, the increasing interaction of the international community and international trade, the risk of the introduction of foreign pests into China has become increasingly serious. China has become one of the most serious countries threatened by foreign pests in the world. Bioinvasive species not only pose a serious threat to agroforestry production and ecosystems in the invaded areas but also accelerate species extinction and cause biodiversity loss. 70%–80% of plant diseases are caused by bacteria and viruses, which are the most important cause of yield loss of main crops and cash crops. The plant quarantine bacteria and viruses have great harm. Compared with quarantine pests such as weeds and insects, most of the bacteria are small in form and difficult to identify with human eyes. Therefore, the form identification, detection and identification of quarantine bacteria have high technical difficulty [1]. In addition, that detection time of quarantine bacteria is long, the method adopt the detection technology is complex, and the requirements on manpower, equipment, method and the like are high. Therefore, predecessors found that monoclonal antibody this serological detection method is very suitable for plant quarantine, it can be simple and rapid, specific sensitive, broad-spectrum, efficient detection of an antigen, and no special restrictions on the source of antigen, can greatly improve the efficiency of customs quarantine.

As TCM becoming popular, how to handle the toxic medicinal materials so that they can be used safely is a key step in determining the safety of TCM. At present, the quality control methods of toxic TCM are mainly based on instrument analysis technology. However, these traditional methods are extremely slow and complex in detection, and have high requirements for experimental personnel and laboratory conditions, which are not suitable for the simultaneous detection of a large number of samples. Therefore, immunoassay, a simple, rapid and low-cost technology, has been gradually applied to the research field of TCM in recent years, providing a new detection method for the rapid detection of the quality of TCM and reference for the follow-up research on TCM containing toxic proteins.

2. Basic principles

Cell fusion is a random physical process. In a mixed cell suspension of mouse spleen cell and mouse myeloma cells, that fuse cells will appear in various forms, such as fused spleen cell and tumor cells, fused spleen cells and spleen cells, fused tumor cells and tumor cells, unfused spleen cells, unfused tumor cells, multimeric forms of cells, and the like. Normal spleen cells survive in culture medium for only 5 to 7 days without special screening and the multimeric form of the cells easily dies. Unfused tumor cells require special screening removal.

After cell fusion, hybridoma cells should be screened out from the above five cells generally by HAT culture, which contains hypoxanthine, aminopterine and thymine.

There are two ways of DNA synthesis in cells, namely, endogenous pathway (main pathway) and exogenous pathway (bypass pathway). The endogenous pathway is DNA synthesis catalyzed by glutamine or monophosphate uridylic acid in dihydrofolate reductase, while the exogenous pathway is DNA synthesis catalyzed by hypoxanthine or thymine in hypoxanthine-guanine phosphoribosyl transferase or thymidine kinase. Aminopterine in HAT medium is an inhibitor of dihydrofolate reductase and can effectively block the endogenous pathway of DNA synthesis.

B lymphocytes have two enzymes, HGPRT and TK, so they can still use hypoxanthine and thymine in HAT medium to synthesize DNA after the endogenous pathway is blocked. They can survive in HAT medium, but B lymphocytes are normal cells, so they cannot survive for a long time. The SP2/0-Ag14 myeloma cell used in that hybridoma technique are HGPRTTTK- deficient and lack HGPR enzyme and TK enzymes that are incapable of exogenous synthesis of DNA after the endogenous pathway is blocked and therefore cannot survive in HAT medium.

Only hybridoma cells can synthesize HGPR enzyme and TK enzyme by inheriting the dual characteristics of B lymphocyte and myeloma cell, and thus can survive for a long time in HAT medium. Therefore, after the fused mixed cells were cultured in HAT medium for two weeks, only the hybridoma cells could survive and become the cell source for producing monoclonal antibodies (Figure 1).

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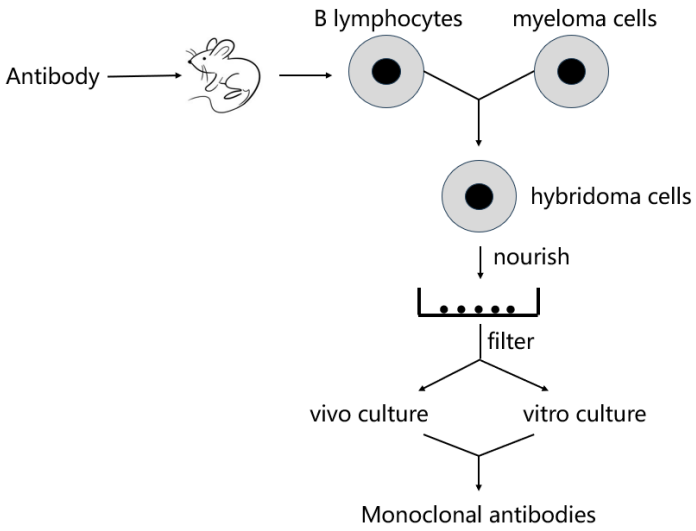

Figure 1. Preparation of monoclonal antibody.

3. Application of monoclonal antibodies in plants

3.1. Bacteria

Pseudomonas syringae pv. tomato is a quarantine pest of imported plants in China, which is a Gram-negative bacterium. It mainly infects tomatoes, but also infects plants such as chili, broccoli, and arabidopsis thaliana. The artificial inoculation hosts are eggplant and potato. Nowadays, Pst has become a global disease, and the pathogen can overwinter on seeds, diseased bodies, soil and weeds. The disease may occur during the seedling stage when the seed with bacteria is planted. If 10% of the plants in the field develop the disease, it can be transmitted to the whole plot when the environmental conditions are suitable [2].

In order to detect Pst, many previous studies have been conducted, such as PCR gel electrophoresis of diseased leaves to see if DNA of the pathogen can be extracted. Although this method has certain effects, it takes a long time and the result is not necessarily accurate. Therefore, somebody [3] designed and screened out the specific amplification primers based on the hrpZPst gene sequence of Pst and established the RPA detection method for tomato bacterial leaf spot disease. The method has high efficiency and low requirement on equipment and is suitable for rapid detection in a simple laboratory at the grass-roots level. But obviously still need to wait for amplification time, and cannot be directly measured in the field, still has certain limitations.

Meisheng Wei et al. [4] obtained the results from the American type Culture Collection, the National Collection of Plant Pathogenic Bacteria, and the Institute of Plant Inspection and Quarantine of the China Academy of Inspection and Quarantine Sciences, and successfully obtained a hybridoma cell strain that stably secreted Pst monoclonal antibodies. The secreted monoclonal antibodies were of type IgG1 and belonged to high-affinity antibodies, and the antibodies were firmly bound to the antigens. The established DAS-ELISA method could detect three Pst strains, but the test results were negative with those of eight other bacteria, indicating that the monoclonal antibody had strong detection specificity and could be used for routine immunological detection.

Although the results of this study had a good prospect for the detection of Pst, only the test strains cultured in the above three laboratories were detected, and the field test was not conducted actually, and the resistance detection was not conducted with other Pst strains in China and abroad. Therefore, the field effect of this monoclonal antibody and the test with other isolates need to be further improved. However, since the monoclonal antibodies required for the previous production of plant pathogen reagents were basically developed by foreign companies, the Pst monoclonal antibodies obtained by this

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research institute can provide support for the independent development of other types of immunological diagnostic reagents against this pathogen in China in the future.

3.2. Virus

3.2.1. Nightingale Telosma mosaic virus, East Asian passiflora virus, Passion fruit severe mottle associated virus. The potato y virus's cordate Telosma mosaic virus, TelMV, EAPV and PFSmAV are very susceptible to co-infection and have seriously endangered the Passiflora edulis industry.

At present, the detection of the virus mainly relies on PCR/RT-PCR, qPCR/RTqPCR, deep sequencing of small RNA, RNA-seq, gene hybridization and gene chip [5–7]. Although these molecular biological detection methods have high sensitivity and good specificity, the detection steps are tedious and require professional personnel and equipment for determination, so they are only suitable for laboratory small sample detection and cannot be applied to the rapid detection of large samples in grass-roots and fields. Studies have shown that the isothermal amplification detection technology of TelMV can quickly detect TEL mV in passion flower plants [8], but this method is difficult to apply to field testing, and the false positive rate is high during the operation.

Therefore, Xue Li et al. [9] obtained monoclonal antibodies capable of specifically and broad-spectrum detecting the three viruses through hybridoma technology by purifying virus particles from diseased leaves and using the mixed virus particles as immunogens of mice using field-collected Passiflora edulis infected by the combination of TelMV, EAPV and PFSMaV. Western blot analysis showed that the four monoclonal antibodies could simultaneously recognize the capsid proteins of TelMV, EAPV and PFSMaV. However, the four selected mAbs were all broad-spectrum mAbs against TelMV, EAPV and PFSMaV, and no specific mAb that could distinguish the three viruses was selected. Hence, it is still necessary to further prepare the mAb with the diseased leaves infected with a single virus.

3.2.2. Tomato black ring virus. Tomato black ring virus was first found on tomatoes in the United Kingdom in 1946. TBRV has now become a global important crop virus. The virus has a wide host range and can infect a variety of monocotyledonous and dicotyledonous herbs and woody plants, causing serious harm. It has been listed as a quarantine pest at China Port. The tomato infected by TBRV could produce black ringspot, causing ringspot on kidney beans, beet and lettuce, and also causing yellow vein of celery, bouquet and coral shape of potato, dwarf of new peach branch, etc. Infection with TBRV can also lead to smaller and ulcerated berries with reduced fruiting rates.

At present, the inspection and quarantine methods for TBRV in China and abroad are mainly molecular biological methods such as reverse transcription, RT-PCR, qRT-PCR, immune capture RT-PCR and reverse transcription loop-mediated isothermal amplification. Although the PCR-based virus nucleic acid amplification method is specific and sensitive, the operation steps are tedious, time-consuming and labor-consuming, and is not suitable for the rapid and large sample inspection and quarantine at ports.

The four monoclonal antibodies created by Li Liu et al. [10] all specifically recognize the capsid protein subunit of TBRV without any immunoreactivity with healthy plant tissues. The created monoclonal antibody is used as a detection antibody to establish a Dot ELISA and a Tissueprint-ELISA serological method capable of specifically and sensitively detecting the TBR of the diseased plant and carrying out detection, and plants infected with other viruses such as TRSV and TSWV or healthy plants are all negative; The sensitivity for detect diseased leave infected with TBRV is extremely high. Therefore, the serological method established with the four prepared monoclonal antibodies has high specificity and sensitivity in the detection of TBRV, and can be accurately and effectively used for the inspection and quarantine of port TBRV and the detection and diagnosis of field plant TBRV.

3.2.3. Summary. Compared with bacteria, viruses are more difficult to diagnose from the appearance of diseased plants. Moreover, the size of the viruses is much smaller than that of bacteria. Bacteria can be

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observed under the microscope, but the viruses can be seen through the submicroscopic structure, which further aggravates the diagnosis difficulty of plant virus diseases. Bacterial diseases can be preliminarily judged through symptom observation or bacterial spraying phenomenon, while viral diseases are diagnosed through serology and molecular biology technology, so the quarantine method with strong specificity, high homogeneity, single biological activity and stable source of monoclonal antibodies is adopted for diagnosing the viral diseases, the detection efficiency is greatly improved, and the method is of great significance for disease observation on a large scale in the field and speeding up plant quarantine by the customs.

3.3. Endogenous toxic protein substances

3.3.1. Tripterygium wilfordii hook. f. Tripterygium wilfordii hook. f. is an important medicinal plant resource in China. currently, it is mainly obtained by cultivation, wild cultivation and tissue culture. the part used for medicine is the xylem of root, and the sheath has great toxicity, so it is usually scraped off when used for medicine. However, due to differences in planting, harvesting and processing methods and other factors, the quality of Radix Tripterygii Wilfordii is uneven, and many poisoning incidents have occurred in recent years. As Tripterygium Glycosides is more and more widely used in clinical practices, there are also many fake products in the market. The traditional methods can not completely identify the authenticity and quality evaluation. Therefore, it was necessary to establish an accurate, simple and rapid quality evaluation method for Tripterygium Glycosides, which was of great significance for achieving rapid quality field screening and ensuring the safety and effectiveness of clinical medication.

At present, the quality control methods of toxic TCM are mainly instrument analysis techniques, such as high performance liquid chromatography (HPLC) and thin layer scanning method (TLC). These traditional methods have the disadvantages of large amount of test samples, complex sample pretreatment and slow detection speed. In addition, they have certain requirements for laboratory personnel and laboratory conditions, which are not suitable for the simultaneous detection of a large number of samples and cannot meet the requirements of rapid detection on the spot. Therefore, immunoassay, a simple, rapid, low-cost, and Qualcomm-based technology, has been gradually applied to the research field of TCM in recent years, and provides a new detection method for the on-site rapid detection of the quality of TCM.

Yaping Duan [11] prepared the monoclonal antibody against Tripterygium Glycosides. The icELISA detection method established based on the monoclonal antibody had the advantages of rapid detection, high sensitivity, low cost, strong specificity, and was suitable for the rapid detection of Tripterygium Glycosides in Tripterygium Glycosides medicinal materials and preparations. The triptolide colloidal gold immune detection test strip prepared by the method is low in cost and easy to carry, can be directly detected in the field and can quickly and directly obtain a result, fills the blank of the immune rapid detection technology in the detection of the traditional TCM tripterygium wilfordii, lays a foundation for batch on-site quality rapid screening of tripterygium wilfordii, and provides feasible technical ideas for quality detection of other TCM.

3.3.2. Pinellia tuber. Pinellia tuber is a commonly used TCM in clinic. Its raw products have a strong excitant toxicity, and its processed products are often used as medicine in clinic. The irritant toxicity of unprocessed Rhizoma Pinelliae is a combination of mechanical stimulation caused by the sharp end of needle crystal penetrating into body and chemical stimulation caused by the entry of pinellia ternate lectin (PTL) into the body.

Yuwei Xie et al. [12] reviewed the data and found that the toxicity of Rhizoma Pinelliae was decreased after processing, and the decrease of PTL content was consistent with the decrease trend of irritant toxicity, indicating that PTL could be used as the toxicity control index of processed Rhizoma Pinelliae. And the double-antibody sandwich ELISA method can be used for detecting the antigen in the sample to be detected, and the capture antibody is specifically combined with the specific antigen, so

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that the sample to be detected does not need to be purified in advance, and the sample processing step is greatly simplified; and at the same time, the ELISA method can be used for carrying out quantitative detection on the sample in batch, quickly and simply. The content determination results of PTL in Rhizoma Pinelliae and its processed products showed that the content of PTL in raw Rhizoma Pinelliae was high, and it was significantly reduced after processing. Moreover, the content of PTL in different processed products varied significantly. This result was consistent with the result of different processing methods for Rhizoma Pinelliae on PTL content discovered in the previous study.

Many Chinese medicinal materials have certain toxicity before treatment. How to judge whether the medicinal materials meet the level of drug use after treatment is a crucial step in determining the safety of drugs. In the above study, the hybridoma fusion technology was used to prepare the PTL specific monoclonal antibody and the double-antibody sandwich ELISA antibody was established for quantitative detection, which provided a new idea for judging the toxicity of Chinese medicinal materials. This experiment improved the content control method of toxic substances in processed pinellia tuber, and provided a scientific basis for improving the quality standard of decoction pieces, and also provided a reference for the research of toxic TCM containing toxic proteins.

4. Conclusion

With the increasing popularity of international cooperation, the epidemic situation of many crops has gradually spread to the world. For example, the sudden oak death pathogen Physiothothora Ramorum Werres, De Cock & Man in 'Tveld, which is popular in California, USA, has caused economic losses of up to tens of billions of US dollars within 10 years [13]. Verticillium dahliae Kleb. was introduced into China along with American Gossypium hirsutum in 1935, causing an extensive outbreak of verticillium dahliae in China and annual economic loss exceeding 1.2 billion yuan [14]. TCM is also growing, with more and more medicinal materials with international recognition. However, due to the inherent toxicity of many TCM, the differences due to planting, harvesting and processing methods and other factors, as well as the existence of more fake products in the market, the quality of many Chinese herbal medicines (CHMs) is uneven.

Therefore, monoclonal antibody, with its superior sensitivity and specificity, autonomy and Qualcomm content detection method, has attracted more and more scientists' attention and application, and has developed many drugs for plant field measurement, port quarantine and CHM quality detection. However, they also found that although monoclonal antibodies have a good prospect in these fields, the preparatory work for the early development of each monoclonal antibody specific to a specific effect is relatively complicated, and the difference in recognition specificity between different strains of virus and bacteria is large, so that some strains may not be recognized; In addition, the active components in CHMs need to be accurately identified, the specific toxic protein substances can be clearly studied and extracted so that the monoclonal antibody can be prepared. Nevertheless, there is no denying the great significance of the monoclonal antibody in these fields. With the continuous improvement of technology, it is believed that more phytosanitary and TCM toxicity diagnosis methods will be developed in the future to provide more protection for human safety.

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