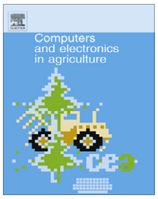
Computers and Electronics in Agriculture 121 (2016) 404–411

Contents lists available at ScienceDirect

Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag



Original papers

Detecting Bakanae disease in rice seedlings by machine vision

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ARTICLE INFO

Article history:
Received 25 April 2015
Received in revised form 2 January 2016
Accepted 6 January 2016
Available online 2 February 2016

Keywords:
Foolish seedling
Fusarium fujikuroi
Disease screening
Early detection
Image processing
Machine learning

ABSTRACT

Bakanae disease, or "foolish seedling", is a seed-borne disease of rice (*Oryza sativa* L.). Infected plants can yield empty panicles or perish, resulting in a loss of grain yield. The disease occurs most frequently when contaminated seeds are used. Once the seeds are contaminated, the pathogen *Fusarium fujikuroi* spreads in the field. Therefore, infected plants must be screened at early developmental stages. This work proposes an approach to nondestructively distinguish infected and healthy seedlings at the age of 3 weeks using machine vision. Seeds of the rice cultivars Tainan 11 and Toyonishiki were inoculated with a conidial suspension of *F. fujikuroi*. The seedling were cultivated in an incubator for 3 weeks. The images of infected and control seedlings were acquired using flatbed scanners to quantify their morphological and color traits. Support vector machine (SVM) classifiers were developed for distinguishing the infected and healthy seedlings. A genetic algorithm was used for selecting essential traits and optimal model parameters for the SVM classifiers. The proposed approach distinguished infected and healthy seedlings with an accuracy of 87.9% and a positive predictive value of 91.8%.

1. Introduction

Rice (*Oryza sativa* L.) is an essential staple food consumed worldwide. Rice production is threatened by Bakanae disease, which is primarily caused by the fungus *Fusarium fujikuroi*. Bakanae disease induces sterility, resulting in a considerable loss of grain yield (Ou, 1985; Zainudin et al., 2008). The pathogen infects rice grains in fields and during storage, colonizes seedlings grown from contaminated grains, and spreads from seedbeds to fields through transplanting. For disease control, rapidly and nondestructively identifying infected rice plants at early stages is helpful for determining contamination rate. In this study, we developed an approach for screening rice seedlings infected with Bakanae disease by machine vision.

Biological approaches have been proposed for identifying plants infected with Bakanae disease. Conventional methods of diagnosing Bakanae disease involve isolating and culturing *F. fujikuroi* from infected plants, followed by microscopically examining the morphological characteristics of the mycelia, sporodochia, microconidia, and macroconidia (Leslie et al., 2006; Zainudin et al., 2008). In addition, *F. fujikuroi* can be identified by sequencing the genes encoding translation elongation factor (TEF), histone H3, and β-tubulin (Jeon et al., 2013; Wulff et al., 2010). Amatulli et al. (2010) designed a TEF-based primer pair and used it to detect *F. fujikuroi* in rice seedlings and seeds by conventional and real-time polymerase chain reaction (PCR). With a peptide *N*-glycanase-based primer pair, Hwang et al. (2013) used real-time PCR for quantifying the growth of *F. fujikuroi* in the root and crown of rice seedlings. Although these biological methods are accurate, they are often too time-consuming or costly to be suitable for field application.

F. fujikuroi-infected plants demonstrate morphological and color abnormalities (Fig. 1). These abnormalities include elongation, stunting, a large angle between leaf and stem, and yellowish-green leaves (Amatulli et al., 2010; Jeon et al., 2013; Wulff et al., 2010; Yamanaka and Honkura, 1978; Ou, 1985). The types and manifestation of Bakanae symptoms can be affected by rice resistance, *F. fujikuroi*-produced secondary metabolites, and/or environmental factors. *F. fujikuroi* at different levels of soil moisture produced varying amounts of gibberellin and fusaric acid, which have been found associated with elongation or stunting of diseased rice plants, respectively (Nyvall, 1989; Ou, 1985; Singh and Sunder, 2012). Because the symptoms of Bakanae disease are complex, occasionally contradictory (e.g., elongation and stunting),

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http://dx.doi.org/10.1016/j.compag.2016.01.008
0168-1699/

Fig. 1. Symptoms of Bakanae disease of rice.

and can vary depending on the cultivar, accurately identifying infected plants through visual inspection is challenging.

Machine vision approaches have been used for disease detection in plants (Barbedo, 2013). Machine vision combines image analysis and machine learning techniques to provide automated inspection. Because machine vision techniques are rapid, nondestructive, and objective, various techniques have been implemented for grading the level of leaf spot diseases (Shen et al., 2008); distinguishing blast, brown spot, and narrow brown spot diseases (Kurniawati et al., 2009); screening bacterial leaf blight, sheath blight, and blast diseases (Yao et al., 2009); identifying plant-hopper infestation (Zhou et al., 2013); and recognizing the infection of brown spot and blast (Sanyal et al., 2007) in rice.

The objective of this study was to identify rice plants infected with *F. fujikuroi* at the seedling stage by machine vision technologies. Specifically, the objectives were (1) to define and quantify the morphological and color traits of rice seedlings at the age of 3 weeks, (2) to identify traits that differ between the healthy and infected seedlings, and (3) to develop machine learning classifiers to distinguish healthy and infected seedlings.

2. Materials and methods

2.1. Sample preparation

Two rice cultivars, Tainan 11 and Toyonishiki, were used. Preliminary inoculation tests on approximately 700 rice accessions in the germplasm showed that the two cultivars had different levels of susceptibility to Bakanae disease (data not shown). Toyonishiki was among the most susceptible accessions; Tainan 11, a principle cultivar comprising approximately 50% of the cultivation area in Taiwan, was moderately resistant. These two cultivars were included in this study to develop a screening system that could identify infected seedlings of different levels of susceptibility.

The seeds of the two cultivars were inoculated with *F. fujikuroi* spores. *F. fujikuroi* was cultured on 1/2 potato dextrose agar (Difco, BD Diagnostics; Franklin Lakes, NJ, USA) for 10–14 days at 24 °C under continuous lighting. The spores were dislodged using a plastic Pasteur pipette and sterile distilled water and were then filtered using Kimwipes paper to remove the mycelium. The amount of spores was counted using a hemocytometer, a microscope chamber slide with a grid etched to the surface (Celis, 2005). The inoculum was prepared by adjusting the concentrations of the spore suspension to 10^3 and 10^5 spores/mL (Hsu et al., 2013). Before inoculation, rice seeds were surface-disinfected in distilled water at 60 °C for 10 min and were then immersed in running water for 2 days. The pregerminated seeds were soaked in the spore suspensions or sterile water and were shaken for 1 h. The seeds treated with sterile water were used as healthy controls. By contrast, the seeds treated with spore suspensions of 10^3 and 10^5 spores/mL were considered as having been exposed to low and high levels of inoculum, respectively. The treated seeds were grown in pots ($L \times W \times H = 3.5 \times 4.5 \times 5.5$ cm) filled with Akadama soil, with 10 seeds per pot. The seeds were directly placed on the soil surface and were then cultivated in a walk-in incubator at 28 °C and 90% relative humidity in a 12 h photoperiod. For each cultivar, 40 seedlings for each treatment were collected as specimens on the 21st day post inoculation.

2.2. Anatomical description of rice seedlings

A typical 3-week-old rice seedling grows three completely elongated leaves (Yoshida, 1981). In this study, the traits of an entire seedling and some seedling parts were defined as shown in Fig. 2. A leaf is composed of leaf sheath and leaf blade. Only the leaf blade areas were considered as the leaf traits. The first and second nodes were defined as the joints of the leaf sheath and leaf blade for the first and second leaves, respectively. The second internode was the region between the defined first and second nodes. The third node sometimes did not appear at the age of 3 weeks. In this case, the second node was considered the joint of the leaf sheath and leaf blade for the third leaf.

2.3. Image acquisition

Two flatbed color scanners (Perfection V37, Epson; Long Beach, CA, USA) were used for acquiring images of the seedlings. The

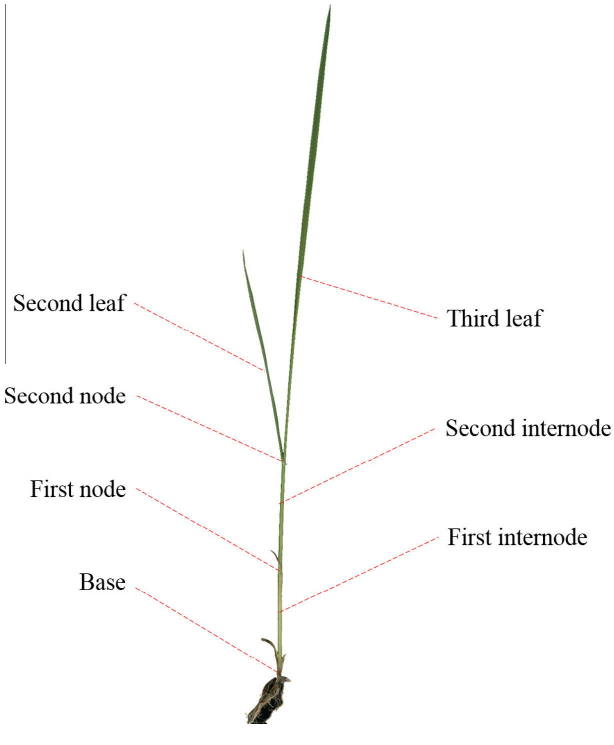

Fig. 2. Parts of a rice seedling at the age of 3 weeks.