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Novel PCR-Based Multiplex Assays for Detecting Major Quality and Biotic Stress in Commercial and Weedy Rice

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Simple Summary: Rice, the staple food for more than half of humanity, is grown predominantly in Asia, the world's most populous continent with the fastest-growing economy. The present-day rice industry must not only meet increasing demand but also changing consumer demands, with a strong emphasis placed on producing high-quality rice. While the rapid development of advanced genotyping methods can be useful for modern rice breeding programs, some methods (such as capillary electrophoresis or sequencing) can be costly to apply in laboratories with limited resources. To address this issue, we developed six novel multiplex polymerase chain reaction (PCR) assays that employ a standard agarose-based gel electrophoresis system to simultaneously detect at least two major grain quality (amylose content and fragrance) and biotic stress (blast, sheath blight, and bacterial leaf blight) genes in rice. One of these assays, which can detect all three targeted biotic stresses, was found to be useful in screening Malaysian weedy rice that may contain novel sources of disease resistance. The universal protocol described in this study can be used in routine molecular laboratories to aid rice breeding initiatives in Malaysia and other resource-constrained countries.

Abstract: While previous research has demonstrated that multiplex polymerase chain reaction (PCR) can be a cost-effective approach to detect various genes in crops, the availability of multiplex assays to simultaneously screen both grain quality and biotic stress resistance traits in rice (*Oryza sativa*) is limited. In this work, we report six novel multiplex assays that use a universal protocol to detect major rice grain quality (amylose content and fragrance) and biotic stress (blast, sheath blight, and bacterial leaf blight) traits with amplified products consisting of up to four primer pairs that can be analyzed using a standard agarose-based gel electrophoresis system. Recent studies have suggested that weedy rice has novel sources of disease resistance. However, an intensive screening of weedy biotypes has not been reported in Malaysia. Accordingly, we employed one of the developed multiplex assays to screen reported genes or quantitative trait loci (QTLs) associated with blast, sheath blight, and bacterial leaf blight diseases in 100 weedy rice biotypes collected from five local fields, with phenotyping performed to validate the genotyping results. In conclusion, our universal multiplex protocol is effective for the large-scale genotyping of rice genetic resources, and it can be employed in routine molecular laboratories with limited resources.

Keywords: agarose gel electrophoresis; amylose content; bacterial leaf blight; blast; fragrance; multiplex polymerase chain reaction; rice; sheath blight; weedy rice

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1. Introduction

Most rice (*Oryza sativa* L.) is grown in Asia, the world's most populous continent with the fastest-growing economy [1]. The present-day rice industry must not only meet increasing demand but also changing consumer demands, with a strong emphasis placed