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Identification of sheath blight tolerant lines through detached leaf method in samba mahsuri (BPT-5204) rice variety

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Abstract

Rice is an important staple food crop and primary diet source for majority of the world's population. However, biotic stress such as Sheath blight (ShB) is one of major disease which effects 50-60% yield loss. ShB disease is mainly caused by *Rhizoctonia solani*, however, no rice cultivar has been found to be completely tolerance. We developed the BPT5204 mutant lines through EMS method. The mutant lines (BPT5204) were screened through detached leaf method (Dath 1987) under standard glass house conditions and the protocol was standardized in ICAR-IIRR. After 72hrs of infection, the lesion length of each cut leaf was measured and according to tolerance we scored 0-9 scale. We observed that 13 out of 40 were showed tolerance against sheath blight. ShB-1, ShB-5, ShB-11, ShB-12, ShB-13 (score-0) lines were showed highly tolerance, ShB-2 and ShB-8 (score 1-8) were moderately tolerant, wild type (BPT5204) was complete susceptible (scores up to 9). Therefore, this standardized detached leaf assay can be used to assess against sheath blight disease.

Keywords: sheath blight tolerant, rice variety, food crop and primary diet source

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. One of the most devastating diseases of rice worldwide is sheath blight caused by the soil-borne necrotrophic fungal pathogen, *Rhizoctonia solani* Kuhn (*R. solani*). ShB is a serious problem in all rice-growing countries and one of the most destructive rice diseases in the world (Srinivasachary and Serge, 2011) [2]. Over the years, many researchers have worked to develop sheath blight-resistant cultivars through conventional breeding and achieved only marginal success due to the lack of resistant germplasm, limited efficiency and effectiveness of the available screening methods (Willoquet *et al.*, 2012) [3]. Consequently, there has been growing interest in using chemical mutagenesis in functional genomics research (Liu *et al.*, 1999; Nadeau and Frankel, 2000) [4, 5]. To achieve this goal, diverse genetic resources including germplasm, near isogenic lines, mapping and mutant populations held and developed. Identification of genetic variation and genetic resources, mutant stocks with discrete genetic lesions are essential to determining gene function and dissecting biochemical and metabolic pathways useful for trait improvement. In rice, there are several advantages of using chemical mutagenesis to produce mutant populations suitable for both forward and reverse genetics. First advantage is mutant populations can developed in any genotype. Second, because of the high density of mutations; genome-wide saturation mutagenesis can be achieved using a relatively small mutant population (Koornneef *et al.*, 1982; Henikoff and Comai, 2003) [6, 7]. Third, it provides a large allelic series as a complement to the knockout mutants produced by insertional mutagenesis or transformation methods (over- and under-expression). The developed mutants can screen for various biotic and abiotic stresses and identified the tolerant lines. Among biotic stress screening, detached plant part screening assays are useful because they enable assessments to be made under highly controlled conditions, serve as rapid screening techniques that can be adopted by breeding programs and can assist in the understanding of host pathogen interactions (Kim and Y. K., Lee, 2000; MacKill, D. J and Bonman, 1992) [8, 9]. A detached leaf assay conducted in vitro is only of benefit to the plant breeder if it correlates well to field responses. In the present study, 40 EMS generated mutant lines along with wild type BPT5204 were screened against the sheath blight disease. 13 lines out of 40 showed various levels of tolerance when compared with wild type BPT5204.

2. Materials and Methods

A detached cut leaf inoculation technique was reported by Dath (1987) [1] for assessing reaction of large number of varieties in laboratory to sheath blight. This technique involves placing the leaf blades (6-8 cm long) were placed over thin layer of 5% of benzimidazole solution/water/moist filter paper in petridish and then inoculated cut leaf blades by placing 10 days old sclerotia or agar block with mycelium (Wgl-12-1*R.solani* ICAR-IIRR strain) over them. The Petri plates are incubated at 26-28°C. The water soaked lesions appeared within in 24hr of incubation and clear cut lesions are formed between 48hr-72hr. Since the spread of infection very fast, the leaf pieces could be floated on tap water, after 72 hr the lesion length of each were measured and each leaf visually rated using 0-9 scale, Scored 0 for no lesions, 9 for 90-100% of lesions on leaf surface. Visual score of 1-8 represented 10-80% of infection on leaf surface. The laboratory screening compares well with field screening.

3. Results & Discussion

In current study we screened EMS mutant lines from Samba Mahsuri (BPT – 5204) for sheath blight tolerance. BPT 5204 was popular rice cultivar in India especially in East and south regions. The 40 promising EMS mutant lines were screened against sheath blight through detached leaf method.

13 out of 40 mutant lines showed highly tolerance against sheath blight. In ShB-1, ShB-5, ShB-11, ShB-12, ShB-13 (score-0) lines not observed any lesions on leaf surface and considered as highly tolerant when compare with WT. ShB-2 and ShB-8 (score 1-8) were moderately tolerant, wild type (BPT5204) was fully infected and considered as susceptible (scores up to 9). Generally, the results obtained from the experiment showed that detached leaf assay can be applied efficiently for tolerance screening to sheath blight in rice.

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Fig 1: Comparison of Sheath Blight infection severity between susceptible cultivar Samba Mahsuri (BPT5204 - Wild Type) and Tolerant mutants (ShB-1, ShB-11 and ShB-13) using Detached Leaf Assay

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