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Research article

Thiamine primed defense provides reliable alternative to systemic fungicide carbendazim against sheath blight disease in rice (*Oryza sativa* L.)

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ABSTRACT

A novel pathogen defense strategy by thiamine priming was evaluated for its efficacy against sheath blight pathogen, *Rhizoctonia solani* AG-1A, of rice and compared with that of systemic fungicide, carbendazim (BCM). Seeds of semidwarf, high yielding, basmati rice variety Vasumati were treated with thiamine (50 mM) and BCM (4 mM). The pot cultured plants were challenge inoculated with *R. solani* after 40 days of sowing and effect of thiamine and BCM on rice growth and yield traits was examined. Higher hydrogen peroxide content, total phenolics accumulation, phenylalanine ammonia lyase (PAL) activity and superoxide dismutase (SOD) activity under thiamine treatment displayed elevated level of systemic resistance, which was further augmented under challenging pathogen infection. High transcript level of phenylalanine ammonia lyase (*PAL*) and manganese superoxide dismutase (*MnSOD*) validated mode of thiamine primed defense. Though minimum disease severity was observed under BCM treatment, thiamine produced comparable results, with 18.12 per cent lower efficacy. Along with fortifying defense components and minor influence on photosynthetic pigments and nitrate reductase (NR) activity, thiamine treatment significantly reduced pathogen-induced loss in photosynthesis, stomatal conductance, chlorophyll fluorescence, NR activity and *NR* transcript level. Physiological traits affected under pathogen infection were found signatory for characterizing plant's response under disease and were detectable at early stage of infection. These findings provide a novel paradigm for developing alternative, environmentally safe strategies to control plant diseases.

1. Introduction

Plants have developed an innate surveillance mechanism that enables them to protect against attempted invasions by pathogens rapidly [1]. This type of acquired tolerance known as systemic acquired resistance (SAR) is now established as an efficient and long-lasting induced resistance against a broad spectrum of pathogens [2]. Disease control with SAR relays without exerting direct pressure on pathogen population unlike traditional pesticides. Beside various categories of SAR inducers, chemical inducers are environmentally safe and well documented for inducing long-lasting resistance. Moreover, chemical defense inducers do not affect the appearance of chemical-tolerant strains or induce the breakdown of resistance [3]. Salicylic acid (SA) [4], methyl jasmonate (Me-JA) [5], probenazole [6], 2,6-dichloroisonicotinic acid (DCINA) [7], benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) [8] and β-aminobutyric acid (BABA) [9] have been successfully characterized for induction of SAR against various pathogens by priming susceptible hosts. In recent years, the importance of vitamins both as nutrients and as disease control agents has been emphasized. Several vitamins such as riboflavin (vitamin B_2), menadione sodium bisulphite and thiamine (vitamin B_1) have been reported to induce SAR [10–13]. Thiamine is a water-soluble B-complex vitamin that is produced in plants and microbes and occurs either as free thiamine or in phosphorylated forms i.e., thiamine monophosphate (TMP), thiamine pyrophosphate (TPP) and thiamine triphosphate [14]. These forms act as coenzymes in several physiological processes, including glycolysis, tricarboxylic acid cycle, pentose phosphate pathway, synthesis of branched

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chain amino acid, isoprenoid and nucleic acids [15,16]. Exogenous application of thiamine is also well known in plants to confer resistance against several environmental stresses i.e. salinity, high temperature [17], fungal, bacterial and viral diseases [18]. In addition, it was reported that thiamine can activate pathogenesis-related gene (PR-1) expression in tobacco [19] and stimulate resistance against tobacco mosaic virus in a SA-dependent manner [20]. However, recent studies further verified that thiamine is systemic, broad-spectrum, long-lasting resistance inducer [12] and dependent on hydrogen peroxide and SA-based signaling [18].

Rice (*Oryza sativa* L.) is one of the three major food crops of the world. Among several biotic factors threatening rice production, fungal diseases account for major losses [21]. Sheath blight caused by *Rhizoctonia solani* anastomosis group1-1A, a common soil-borne fungal pathogen, is one of the most destructive rice diseases worldwide and causes considerable yield loss amounting to 6–10 per cent annually [22,23]. However, under favorable conditions i.e. low plant height, high number of tillers, low spacing and high level of nitrogen fertilizers, sheath blight may cause nearly 50 per cent yield loss [24]. Moreover, high yielding, compact and dwarf to semidwarf rice varieties favor disease spread throughout their growth period [25].

Control of rice sheath blight is difficult with conventional breeding due to the low inherent level of resistance against sheath blight, wide host range of *R. solani*, its ability to survive in soil and high genetic variability [24]. Although use of fungicides for disease suppression gives promising results, it is environmentally undesirable and has serious health concerns with its indiscriminate use [26,27]. Thus, pest management approaches with emphasis on use of chemicals having no fungicidal properties but are able to prime the plant defense for broad range of pathogens assuming special significance. Although, previous studies have established thiamine as a potential SAR inducer, its effectiveness in comparison to conventional fungicide is needed before using as alternative against sheath blight disease in rice. Also the effects of thiamine priming on growth physiology and yield are perquisite for its environmentally safe application especially for quality rice production in future.

In view of above, the present study was carried out to evaluate the efficacy of thiamine priming on high yielding (3.3–4.3 t ha^{-1}) basmati rice variety, Vasumati [28] in comparison to systemic fungicide, carbendazim (BCM). With its semidwarf stature (110 cm) and good tillering capacity, Vasumati was taken as susceptible host for sheath blight pathogen. Moreover, no study hitherto, examined the effect of sheath blight infection on different physiological traits related to growth and yield. Hence, a systemic approach was made to investigate physiological traits that can be used as phenotypic markers to characterize varietal response under pathogen infection at early growth stages.

2. Results

2.1. Growth, yield and disease severity

R. solani infection showed significant decline in all growth parameters studied. It was observed that pathogen infection caused decrease in total biomass (Fig. 1a), number of tillers per hill (Fig. 1b), number of panicles per hill (Fig. 1c), number of grains per panicle (Fig. 1d) and grain weight per plant (Fig. 1e). However, reduction in growth and yield due to pathogen infection was significantly minimized in plants pre-treated with thiamine and BCM. Thiamine treatment alone did not affect yield traits significantly except for increase in grain weight per plant (Fig. 1e). Similarly, BCM-treated plants showed no influence on yield traits, though produced less number of tillers (Fig. 1b) in comparison to healthy control plants.

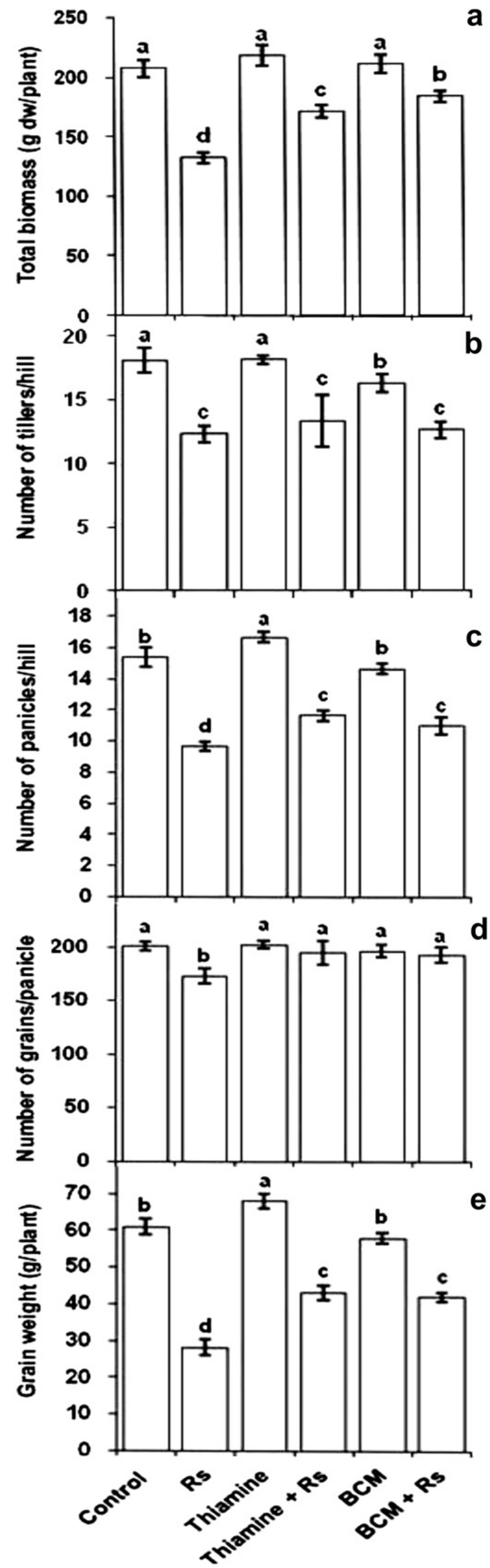

Fig. 1. Effect of thiamine (50 mM) and BCM (4 mM) treatments without and with challenge inoculation of sheath blight pathogen, *Rhizoctonia solani* AG-1A, on total biomass (g)/plant (a), number of tillers/hill (b), number of panicles/hill (c), number of grains/panicle (d) and grain weight (g)/plant (e). Sampling was done at the maturity. Bar represents the means ± SE of four replicates. Treatment means that do not have a common letter are significantly different by Duncan's multiple range test. Rs: *Rhizoctonia solani* AG-1A; BCM: carbendazim.