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Measurement of localized and systemic resistance of rice plant to *Pyricularia grisea* by foliar spray of chemical inducers

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ABSTRACT

Defense mechanism of plants has been aware of its responses to attempted infection, its responses can be localized or systemic expression. Systemic acquired resistance recovered at 6 hours after spraying oxalic acid (8mM) and $Na_2B_4O_7$ (1mM) under green house condition. Fungitoxicity at concentrations of $Na_2B_4O_7$ of less than 6 mM did not affect the growth of Pyricularia grisea. Systemic acquired resistance expressed at 6, 24, 144 hours after spraying with $Na_2B_4O_7$ inoculation with challenger using fresh inoculum collected from leaves. Spraying natri tetraborac only induced local resistance at time of 6 hours in the treaments inoculated with blast race 006.4 culture. Natri teraborac could enhance stable resistance of rice plant to blast especially to fresh inoculum

Key words: localized and systemic resistance, inducer, enhancer

INTRODUCTION

Defense mechanism of plants has long been known with its responses to attempted infection, its responses can be localized or systemic expression. In systemic acquired resistance (SAR), elicitors enhance level of one or more translocatable signal chemicals which, in turn, results in coordinated induction of genes controlling diverse defense pathways in tissues spatially distant from the initial challenge site (Kuc 1995). The exogenous application of inducer chemicals leads to the induction of systemic resistance in crops (Mitchell & Walterss 1995). In cereal crops like barley and rice, biotic and abiotic elicitors have been used to induce SAR against powdery mildew and blast (Sengupta and Sinha 1987, Manandhar 1996, Manandhar et al. 1998, Kloepper et al. 1992, Lyngs Jorgrnsen et al. 1998, Du et al. 2000). Recently, oxalic acid is reported as chemical elicitor inducing systemic resistance to *Sclerotinia sclerotiorum* in oil seed rape (Toal and Jones 1999).

In Vietnam, rice is major crop which produces anually more than 30 M tons and 50 % of total rice production is from Mekong Delta. However, the intensive system of rice cultivation made rice crop is more conducive to pests and diseases. The effectiveness of using resistant varieties is not well obvious (Noda et al. 1999), because the matching of pathogen virulence has quickly occurred. In 2001 dry season, most of newly realeased varieties became susceptible to blast. It will take much time to replace new resistant genes. In case of good quality rices, it is more difficult to replace it due to farmers' non-preference. Experiences with blast epidemics under field condition with the same susceptible varieties showed that the infection varied in different levels from fields to others. Data of several surveys indicated that different blast severity appeared in two nearby rice fields, it depends on intensification levels by farmers in which rice plants in this field might be more susceptible than other. This means that farmers can continue to grow susceptible varieties with full alert of recommended practices. In Mekong Delta, rice farmers are very progressive, and acceptive for new technologies. Enhancing resistance of rice plant combined with low seeding rate, low nitrogen application can reduce leaf blast severity, and yield loss could also be reduced.

We intended to find out whether rice plant could express its partial resistance as general mechanism by exogenous applying chemical elicitors. Enhancing resistance at

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certain levels might be a useful technique and complemented to conventional method against *Pyricularia grisea* in terms of leaf blast and neck blast .

MATERIALS AND METHODS

Techniques as described by Lyngs et al. (1998), Toal and Jones (1999) to investigate three chemicals oxalic acid ($C_2H_2O_4$), natri tetraborac ($Na_2B_4O_7$) were used to compare di-potassium hydrogen phosphate whether these chemicals could induce resistance to blast in rice. Many experiments have been conducted to check the levels of plant response of localized or systemic resistance and what is time lapsed necessarily for its expression.

Fungal toxicity of Natri tetraborac ($Na_2B_4O_7$) and Oxalic acid ($C_2H_2O_4$),

The experiment was laid out in the laboratory in RCD with three replications, using rice straw agar (RSM) medium. RSM was sterilized at 121^{0}C, in 30', then pour in petri dishes containing $Na_2B_4O_7$ sterilized by filter with 0.8μm diameter to obtain concentration from 0.6 to 30 mM. After ten days of hyphal tip culture of blast, they were then transfererred into those petri dishes and put in incubator at 25^{0}C. After 7 days, colonial diameter were measured. For oxalic acid ($C_2H_2O_4$), concentrations were adjusted from 0.1 to 50 mM under the same procedure.

Time lapsed for localized or systemic responses to *Pyricularia grisea* by three chemicals $C_2H_2O_4$, $Na_2B_4O_7$ and K_2HPO_4 -

Variety OM2031was used at 18-20 day old seedlings, after treated with the chemicals: oxalic acid (O.A), $Na_2B_4O_7$ and K_2HPO_4, seedlings were inoculated with spores of *Pyricularia grisea* race C 69, at different times as **6, 12, 18 and 24** hours. These inoculated rice seedlings were continuously incubated in cool and dew room (20-23^{0}C) for 24 hours. Then the plants were transferred into room under high humidity condition. Disease observation was taken at 7 days after inoculation for localized and systemic resistance. The experiment was laid out under the green house condition with three replications. Five treatments were [1] Oxalic acid (4mM), [2] Oxalic acid (8mM), [3] $Na_2B_4O_7$ (1 mM), [4] K_2HPO_4 19μM, [5] Control. Collected parameters to access resistance responses were: diseased leaf area (%), total number of lesions (acute, chronic) by Hans Pinnschmidt (1992).

Natri tetraborac ($Na_2B_4O_7$)

Experiment 1a: Induction of **localized and systemic resistance** to *Pyricularia grisea* by Natri tetraborac

The experiment was laid out in RCD with three replications under glass house condition, using susceptible variety OMCS99. Different concentrations of $Na_2B_4O_7$ (form 0.06 mM to 5.6 mM) were sprayed at different time scales**,** 6, 24, and 144 hours before inoculation with challenger. The rice plants were grown in plastic pots, 24cm x 12cm (10 plants per pot) under natural condition. At fourth – fifth leaf stage (17, 20, 21 days after sowing), the first leaf (the youngest leaf) was fixed in a horizontal position on bent plastic plates and covered by nylon before spraying to avoid from contamination by chemical directly affected to systemic response. Chemicals were sprayed until running off. Freshly harvested blast spores were collected and incubated in the dark. After 24 hours, spores were harvested. The plants were inoculated at 150.000 spores/ ml and incubated for 24 hours in darkness at 24 ^{0}C in glass chamber. Then these plants were transferred into greenhouse with RH of 90% at 28^{0}C. Assessment based on lesion numbers was done at 7 days after inoculating (Hans Pinnschmidt 1992) .

Experiment 1b: Induction of **systemic acquired resistance** to *Pyricularia grisea* by Natri tetraborac in rice using race 006.4

This experiment was laid out in RCD with three replications under glass house condition with blast race 006.4 (Noda et al. 1999). Blast fungus culturing was carried out with standard procedure. Rice plants were inoculated at 150.000 spores / ml and kept in cool room at 24^{0}C to maintain high relative humidity for 24 hours in darkness in glass chamber. Then these plants were transferred into greenhouse with RH of 90% at 28^{0}C. Data recording was done as the same way of the experiment 1a.

Experiment 1c: Induction of **local induced resistance** to *Pyricularia grisea* by natri tetraborac in rice using race 006.4

This experiment was laid out as the same way of the experiment 1a. Natri tetraborac was sprayed directly on leaves to induce localized resistance. Plant responses to blast were measured at 7 days after inoculating to access local induced resistance.

Oxalic acid ($C_2H_2O_4$)

Experiment 2a: Induction of **systemic acquired resistance of rice plant** to *Pyricularia grisea* by oxalic acid.

The experiment was laid out in RCD with three replications under greenhouse condition, using susceptible variety OMCS99. Different concentrations of oxalic acid (0.1mM, 0.5mM, 1mM, 5mM) were sprayed with time scales at 6, 24, and 48 hours before inoculation of challenger. The rice plants were grown in plastic pots, 24 x12 cm diameter (10 plants per pot) under the natural condition. At the fourth – fifth leaf stage (19, 20, 21 days after sowing), the first leaf (the youngest leaf) was fixed in a horizontal position on bent plastic plates and covered by nylon before spraying to avoid from contamination by chemical. Chemicals were sprayed until running off. Suspension of spores of race 006.4 was used and inoculation were subsequently done.

Experiment 2b: Induction of **localized resistance of rice plant** to *Pyricularia grisea* by oxalic acid

The experiment was laid out in the same way of the experiment 2a. For checking localized resistance of rice plants, oxalic acid was sprayed directly on leaves.

RESULTS AND DICUSIONS

1. Fungitoxicity at concentrations of $Na_2B_4O_7$ of less than 6 mM did not affect the growth of *Pyricularia grisea* (Figure 1). However, fungal development is gradually reduced from 6 to 27 mM and totally growth of fungus is stopped at 30 mM (Figure 2)

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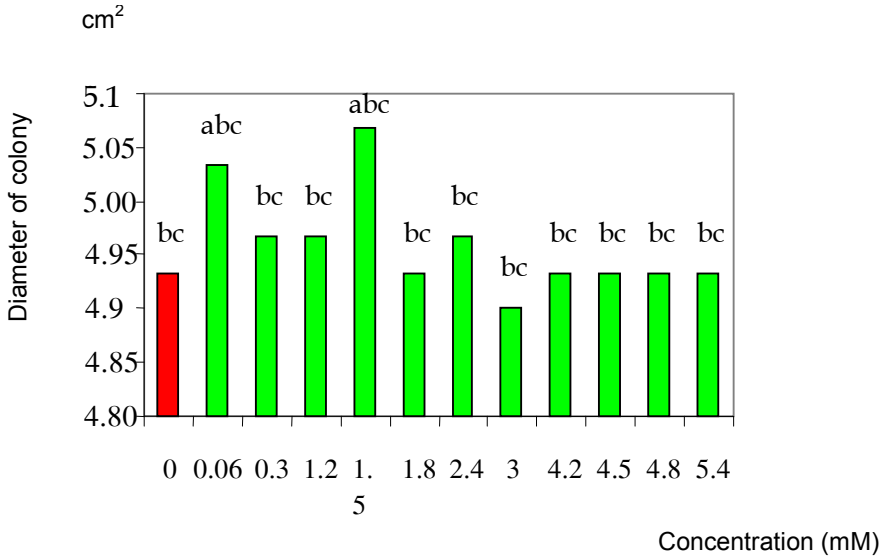

Figure 1: Growth of *Pyricularia grisea* in the medium (Rice straw agar) at concentrations of Natri tetra borac smaller than 6 mM.

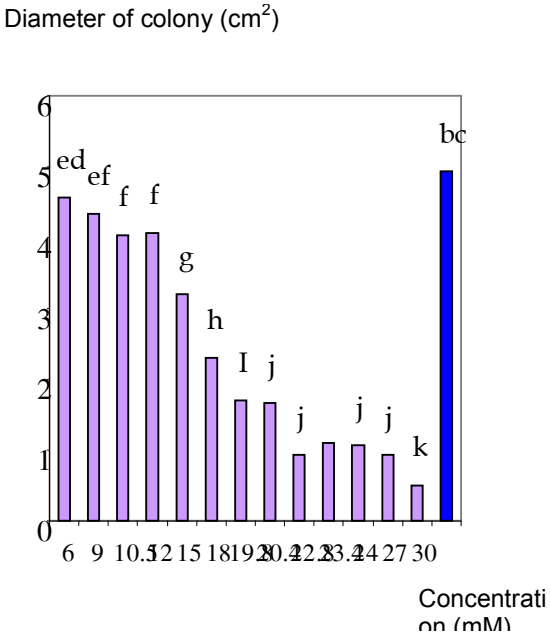

Figure 2: Growth of *Pyricularia grisea* in the RSM incorporated with Natri tetra borate at concentrations from 6 mM to 30 mM

Concentrations of oxalic acid less than 1 mM did not affect on growth of *Pyricularia grisea* (Figure 3). Their colonies at concentration of 2 to 6 mM grew weaker than control (0mM). The fungal growth was gradually stopped at concentrations of 7 to 40mM.

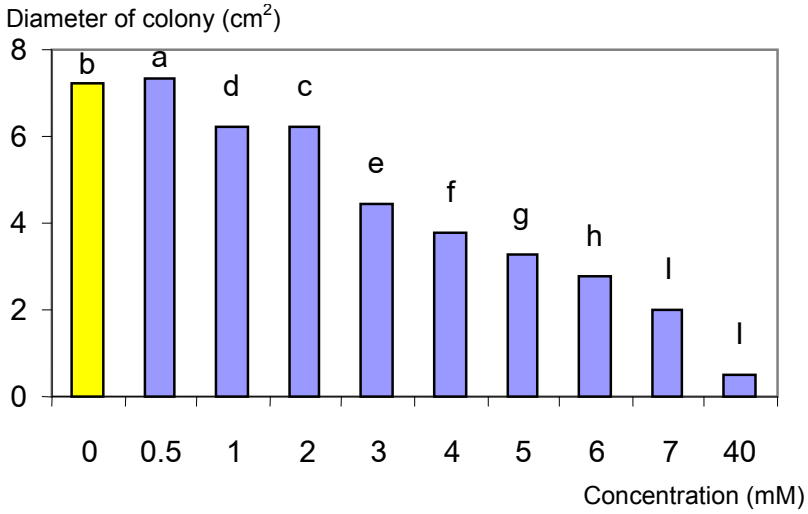

Figure 3: Growth of *Pyricularia grisea* in the medium (Rice straw agar) at concentrations of Oxalic acid from 0 to 40 mM.

2. Rice variety OM2031 treated with oxalic acid at 8mM and $Na_2B_4O_7$ 1mM expressed local resistance as well as systemic one to challenger at 6 hours after spraying chemical. The observation was based on diseased leaf area covered (%), it was significantly different to control treatment (Figure 4). However, the introduction of challenger from 12, 24 and 48 hours, rice plant could not express resistance any more (Figure 5,6,7).

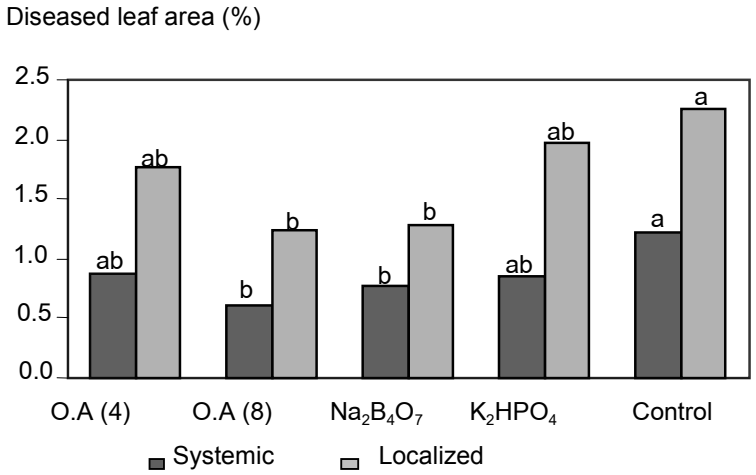

Figure 4: Localized and systemic resistance of variety OM2031 expressed to *PPyricularia grisea* at 6 hours after treated with chemical inducers, Oxalic acid (O.A = 4 mM and 8 mM), $Na_2 B_4 O_7$ (2mM), and K_2HPO_4 (19 mM).

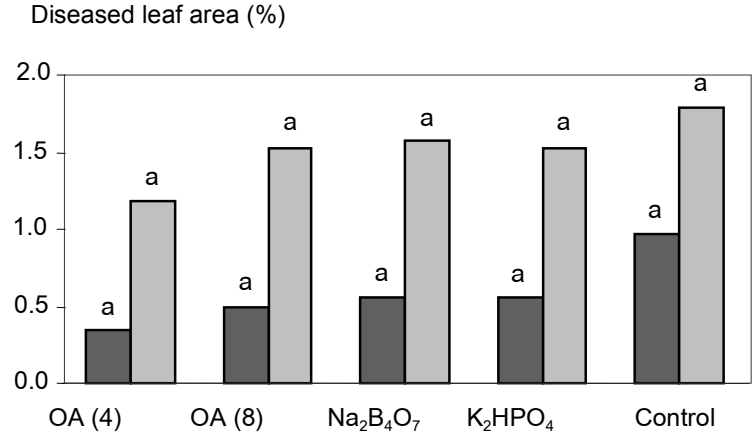

Figure 5: Localized and systemic resistance of variety OM2031 expressed to *Pyricularia grisea* at 12 hours after treated with chemicals inducers, Oxalic acid (O.A = 4 mM and 8 mM), $Na_2 B_4 O_7$ (2mM), and K_2HPO_4 (19 mM)

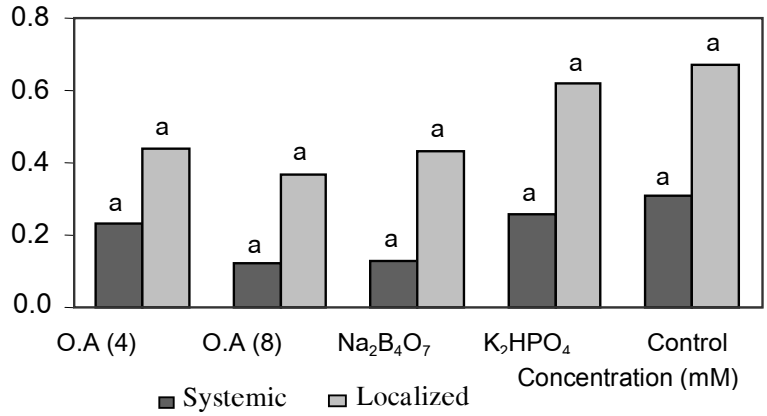

Figure 6: Localized and systemic resistance of variety OM2031 expressed to *Pyricularia grisea* at 24 hours after treated with chemical inducers, (O.A = 4 mM and 8 mM), $Na_2 B_4 O_7$ (2mM), and K_2HPO_4 (19 mM)

Interestingly, when challenger could be collected freshly from rice leaves infected, rice plant would response very clearly. The very low concentrations of $Na_2B_4O_7$ are able to induce systemic acquired resistance to rice blast disease (concentration less than 6 mM) at different time scales as 6, 24, 144 hours (Figure 8). However, resistance was not expressed obviously at some concentrations (0.3mM, 1.8mM, 3.6mM) (Data were not shown)

However, experiments conducted with cultured fungal race 006.4 indicated that local induced resistance at 6 hours before inoculation clearly expressed with spraying natri tetraborac and systemic reaction at 6, 24, and 144 hours did not very clearly express. This may be due to a contradiction of systemic acquired resistance when we inoculated with fresh inoculum of challenger (Figure 9).

In case of **oxalic acid**, diseased leaf areas which addressed the localized and systemic resistance by spraying O.A (0.5mM) at 6 and 24 hours before introduction of challenger were not obvious. However, enhancing resistance was clearly recovered at 48 hours before inoculation (Figure 10,11)

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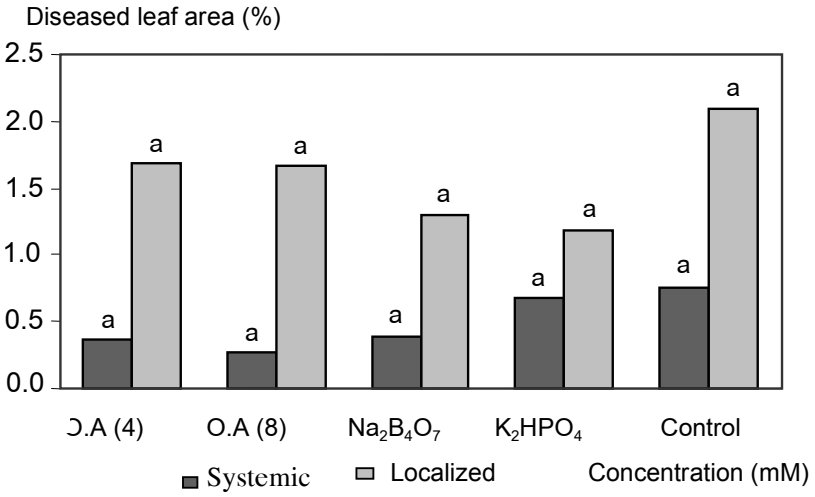


Figure 7: Localized and systemic resistance of variety OM2031 expressed to *PPyricularia grisea* at 48 hours after treated with chemical inducers, (O.A = 4 mM aand 8 mM), $Na_2 B_4 O_7$ (2mM), and K_2HPO_4 (19 mM)

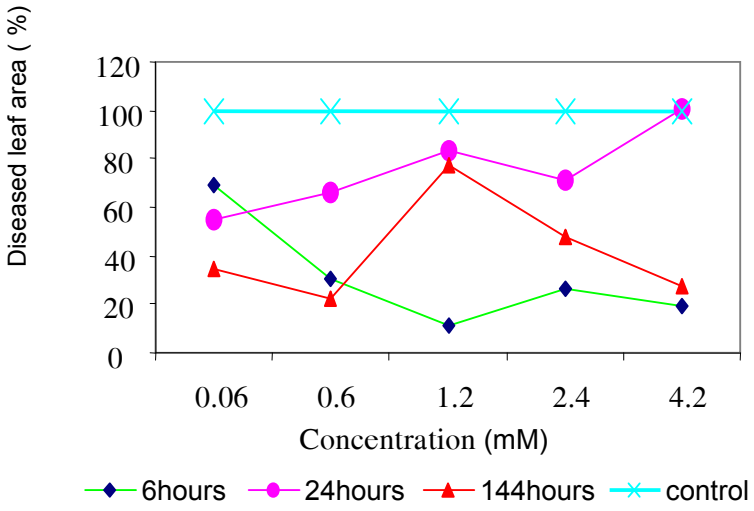


Figure 8: Inducing systemic acquired resistance to Rice blast disease by Natri tetra borate at different time in 6, 24, 144 hours before inoculation with freshly harvested spore.

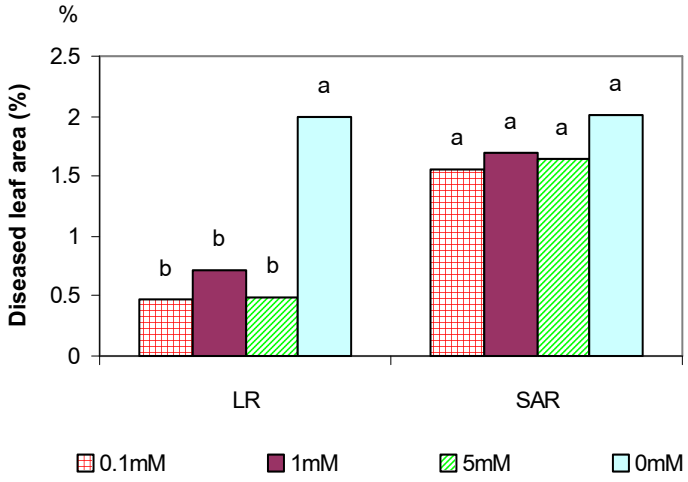
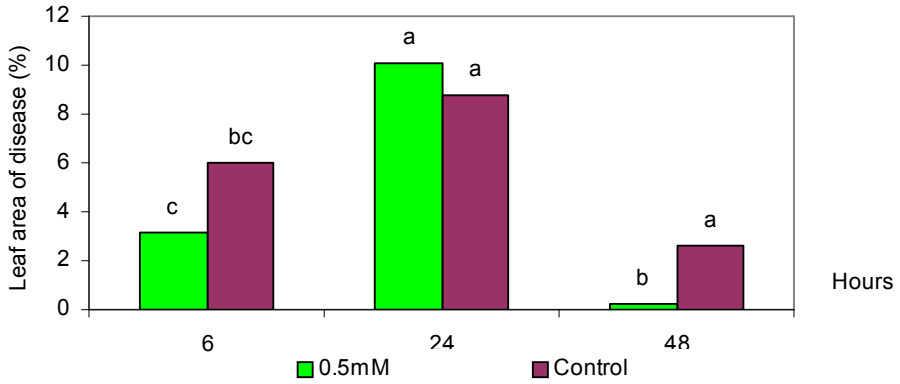


Figure 9: Induction of localized resistance to blast in rice by spraying Natri tetra borate at 6 hours before inoculation with blast race 006.4.



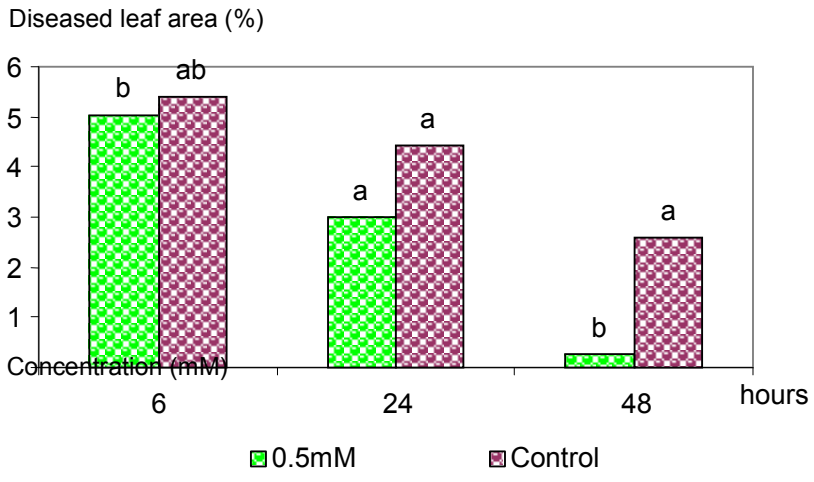


Figure 11: Localized resistance expressed against rice blast by Oxalic acid at concentration 0.5mM

SUMMARY

Systemic acquired resistance recovered at 6 hours after spraying oxalic acid (8mM) and $Na_2B_4O_7$ (1mM) under green house condition. Foliar spray of DHP in this case showed less effective than the two above treatments. In other experiment, spraying oxalic acid (0.5 mM) at 48 hours before inoculation induced local resistance as well as systemic one to blast.

Systemic acquired resistance expressed at 6, 24, 144 hours after spraying with $Na_2B_4O_7$ (less than 6 mM concentration) inoculation with challenger using fresh inoculum collected from leaves. Rice plant responsed more clearly with fresh inoculum. Otherwise, spraying natri tetraborac only induced local resistance at time of 6 hours in the treaments inoculated with blast race 006.4 culture

It seems that localized and systemic acquired resistance of rice plant by these chemicals to

blast were still depending on time between introduction of each chemical and challenger, concentration of each chemical, and strains (virulence) of pathogens. Further research is needed how to apply efficiently of each chemical, foliar spray and seed treatment techniques.

Natri teraborac could enhance stable resistance of rice plant to blast especially to fresh inoculum, more applications of chemical are recommended under field condition.

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SUMMARY IN VIETNAMESE

Kết qủa kích kháng bệnh đạo ôn trên lúa bằng hóa chất có tính chất kích kháng (inducer) phun trên lá

Sử dụng hóa chất kích thích tính kháng bệnh đạo ôn trên nguyên lý khai thác tiềm năng tự bảo vệ của cây lúa trong điều kiện bị nấm bệnh tấn công, đã được nghiên cứu. Phun oxalic acid nồng độ 8mM, và $Na_2B_4O_7$ nồng độ 1mM sẽ kích thích tính kháng bệnh đạo ôn sau 6 giờ xử ly, và tính kháng này ở dạng lưu dẫn. Phun oxalic acid nồng độ 0,5mM sẽ kích thích tính kháng bệnh đạo ôn sau 48 giờ, tính kháng thể hiện cả hai dạng "tại chổ" và "lưu dẫn". Phun $Na_2B_4O_7$ nồng độ thấp hơn 6mM sẽ kích thích tính kháng dạng lưu dẫn, tính kháng này thể hiện sau 6, 24, 144 giờ. Cây lúa thể hiện rõ nhất tính kháng khi chúng ta chủng bệnh với những bào tử mới, còn tươi. Phun natri tetraborac sẽ kích thích tính kháng tại chổ sau 6 giờ. Thí nghiệm được thực hiện với sự lây nhiễm bệnh bằng nòi nấm gây bệnh đạo ôn có số mẫu 006.4