

Induced systemic resistance against rice grassy stunt virus - a promising field for ecological rice production

Kích kháng lưu dẫn đối với bệnh vàng lùn trên lúa – triển vọng trong việc sản xuất lúa theo hướng sinh thái

Research article

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Most rice protection methods have currently used toxic chemicals to control pathogens and pests, which leads to environmental pollution. Systemic acquired resistance (SAR) taking advantage of natural defence reaction of plants could be proposed as an alternative, ecologically friendly approach for plant protection. Its application into rice production could minimize the chemicals quantity used and could contribute to the decrease of environmental pollution and the development of sustainable agriculture. The research was conducted to select the most effective chemical and suitable method to improve the health of rice plants infected by grassy stunt disease in net-house of Can Tho University. SAR chemicals were used at very low concentrations (in mM). Results showed that the height of rice plants treated with SAR chemicals was higher than that of plants untreated. Besides, the number of diseased plants was reduced and the ratio of firm grain and yield increased when plants were applied by SAR. Among the used substances, oxalic acid provided the best systemic acquired resistance. With oxalic acid, seed soaking was better than seed coating in systemic acquired resistance against rice grassy stunt disease.

Hầu hết các phương pháp sản xuất lúa hiện nay đều sử dụng các hóa chất độc hại trong việc phòng trừ bệnh và côn trùng gây hại, nên dẫn đến ô nhiễm môi trường. Kích thích tính kháng lưu dẫn giúp kích hoạt cơ chế tự nhiên kháng bệnh của cây có thể là giải pháp bảo vệ thực vật thay thế an toàn với môi trường. Việc ứng dụng tiến bộ này vào trong sản xuất lúa có thể làm giảm lượng hóa chất sử dụng, đóng góp vào việc giảm thiểu ô nhiễm môi trường và sự phát triển của một nền nông nghiệp bền vững. Nghiên cứu đã được thực hiện tại nhà lưới trường Đại học Cần Thơ để tuyển chọn hóa chất và phương pháp sử dụng hóa chất để tăng cường sức khỏe giúp cây lúa vượt qua bệnh vàng lùn. Hóa chất kích kháng được sử dụng ở một nồng độ rất thấp (đơn vị là mM). Kết quả cho thấy chiều cao cây lúa khi xử lý chất kích kháng tốt hơn so đối chứng không xử lý. Bên cạnh đó, số cây lúa nhiễm bệnh giảm, tỉ lệ hạt chắc và năng suất tăng khi cây lúa được xử lý với chất kích kháng. Trong số các chất kích kháng đã sử dụng, acid oxalic cho hiệu quả vượt trội. Với chất acid oxalic, phương pháp ngâm hạt cho hiệu quả kích kháng tốt hơn phương pháp áo hạt.

Keywords: ecological rice production; systemic acquired resistance; rice grassy stunt disease

1. Introduction

Rice production in Vietnam is largely based on the use of fungicides, bactericides and insecticides – chemical compounds toxic to disease agents or pests. However, the harmful effect of these chemicals on the environment and human health demands the researches for new harmless means of disease control. Induced plant resistance against pathogen infection has been described for more than 100 years (early reviewed by Chester, 1933; Mahy and Van

Regenmortel, 2010). Exploiting the plant potential to resist pathogens, the induced resistance may diminish the use of toxic chemicals to disease and pest control, and thus could be proposed as an alternative, non-toxic and ecologically friendly approach for plant protection and hence for sustainable agriculture (Heil, 2001; Edreva, 2004).

Most of the work on the induced resistance, however, was with dicotyledonous plants. Considering the economic

importance of monocotyledonous crop plants, the number of studies on this Order is comparatively small (Steiner and Schonbeck, 1995).

Worldwide, many scientists have successfully conducted their research by using systemic acquired resistance (SAR) to control plant viral diseases. Ghoshroy et al. (1998), Naylor et al. (1998), Chivasa and Carr (1998), Anfoka (2000), Dong and Beer (2000), Chirkov et al. (2001), Ahn et al. (2005), Mayers et al. (2005), Faheed and Mahmoud (2006), Man-dal et al. (2007) and Sudhakar et al. (2007) had successfully used chemicals such as cadmium, salicylic acid, 2,6-dichloroisonicotinic acid, antimycin A, riboflavin, vitamin B1, chitosan, benzo-(1,2,3)-thiadizole-7-carbothioic-S-methyl ester, kinetin, acibenzolar-S-methyl and ozone to induce plants against Tobacco mosaic virus, Potato virus X, Cucumber mosaic virus, Tobacco necrotic virus, Tomato spotted wilted virus. Raupach et al. (1996), Murphy et al. (2000), Zehnder et al. (2000), Elbadry et al. (2006) and Al-Ani et al. (2011) succeeded in using plant growth-promoting rhizobacteria (PGPR), Pseudomonas fluorescence and Azospirillum irakense to induce resistance in plants against Cucumber mosaic virus, Tomato mottle virus, Bean yellow dwarf virus. In rice, induction of resistance by applying biotic and abiotic inducers have been demonstrated by Iwano (1987), Smith and Metraux (1991), Steiner and Schonbeck (1995), Du et al. (2001), Nandakumar et al. (2001), Thuy et al. (2004), Kagale (2004), Saikia et al. (2006), Taheri (2010), Pal et al. (2011), Chithrashree et al. (2011) and Govindappa et al. (2011). Following these SAR achievements with viral diseases in tobacco, papaya, chilli, potato, cucumber etc; SAR method could be applied to rice grassy stunt disease.

The area of rice production in Vietnam is about 7.5 millions of hectares. The epidemics of rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV) have begun to cause serious damage in Vietnam since 2006. According to Vietnam Plant Protection Department (2006), rice area seriously infected by RGSV and RRSV was 43,887 ha. Crop loss in the Mekong Delta was approximately 428,000 tons due to the damage caused by brown plant hopper (BPH), RGSV and RRSV. The farmers used a lot of chemicals to kill BPH and keep rice plants' vigour. Only 20-30 % of chemicals (by quantity) reached the goal or was kept by plants; the remaining was useless and accumulated in the soil. The unused chemicals will pollute the soil, water, and even groundwater. This epidemic has continued to do damage to Vietnam's rice production, affect national food security and rice export. Many methods for reducing rice grassy stunt disease (RGSD) and rice ragged stunt disease (RRSD) have been applied in many places. The method of sowing at a time in accordance with lowest population of migratory BPH helped the farmers in restricting damage of viral diseases. In addition, protecting young rice plants also had a part in reducing the damage. However, there is no rice variety that can be tolerant of RGSV and RRSV, the rice price is at high level, the farmers grow overlapping crops and thus RGSD and RRSD will always occur in the field, at different ratios. In this situation, to keep farmers "living" together with RGSD, RRSD as well as to avoid another outbreak of epidemics in the future, systemic acquired resistance is considered to be a feasible and cheap method, as well as safe to the environment. If successful, the method can be introduced to farmers to decrease damage when they cannot avoid BPH at the beginning of crop. To reach this aim, the research was conducted to find the most effective chemicals in SAR to RGSD for the rice plants infected early (at 7 days) after sowing. Further on, the paper focuses on finding the suitable way to use the effective chemicals.

2. Materials and methods

The experiment was conducted in the net-house of Plant Protection Department, College of Agriculture and Applied Biology at Can Tho University. The rice variety was a BPH susceptible variety that was widely cultivated in the Mekong Delta.

RGSD source was collected in Can Tho city and purified to separate from RRSD, then used for the experiment. Clean BPHs used for transmitting RGSD were collected in the field and their eggs were laid in pickerelweed's sheaths (*Momochoria vaginalis*). The eggs hatched, the BPH larvae did not held RGSV and RRSV (rechecking by Enzyme-linked Immunosorbent Assay - ELISA). BPH larvae 1 to 2 took RGSV in grassy stunt rice for 2 days and then they were fed on healthy rice to be latent. After being latent RGSV, the BPH larvae 4 to 5 (infected with RGSV) were used for the experiment.

The screening of potential chemicals was conducted with 10 types of chemicals, three concentrations for each chemical (data not shown). The SAR chemicals chosen were CuCl₂ (0.05mM), oxalic acid (0.5mM), K₂HPO₄ (20mM). The experiment of chemicals' selection was carried out with randomized complete design, 10 replications, 6 rice plants/replication, and 6 treatments namely (1) CuCl₂; (2) oxalic acid; (3) K₂HPO₄; (4) mixing of CuCl₂ and K₂HPO₄; (5) a control with diseased rice plants and (6) a control with healthy rice plants. The SAR chemicals were CuCl₂ (concentration is 0.05mM), oxalic acid (0.5mM), K₂HPO₄ (20mM). The seed was soaked for 24 hours and the leaf was sprayed at 10, 20, 30 and 40 days after sowing (DAS).

The experiment of oxalic acid's usage was performed with randomized complete design, 5 replications, 6 rice plants/replication, and 9 treatments namely (1) seed coating; (2) seed coating and leaf spraying at 10, 20, 30 and 40 DAS; (3) seed soaking; (4) seed soaking and leaf spraying at 10 DAS; (5) seed soaking and leaf spraying at 20 DAS; (6) seed soaking and leaf spraying at 10, 20 and 30 DAS; (7) seed soaking and leaf spraying at 10, 20, 30 and 40 DAS; (8) a control with infected rice plants and (9) a control with healthy rice plants. The SAR chemical was oxalic acid (0.5 mM).

For all experiments, RGSD was transmitted to rice plants at 7 DAS with 3 BPH larvae, 4 to 5 for each tiller. The BPH had transmitted for 2 days and then was killed. After transmitting, the rice plants were put in the net-house. The data collected include the ratio of infected plants and the length of rice plant. Yield/pot and elements of yield were recorded after harvesting. The elements of yield included the effective tillers per pot (effective tiller: tiller has floccules) and weight of firm grain per pot. The data was analysed by using MSTATC software.

3. Results and discussion

3.1 The experiment of chemicals' selection to rice grassy stunt disease

The data presented in Table 1 shows that the control with infected rice plants had a high ratio of diseased plants (68.3%). This result proved that experiment's inoculation was good. Meanwhile, ratios of diseased plant in systems treated by chemicals were lower and significantly different to the control with healthy rice plants. This result indicates that the chemicals had an important effect in reducing the RGSD compared to the control with infected rice plants (without treating chemicals). Among the treated samples, the chemicals had the same ability in decreasing ratio of diseased plants. For the average latent time, there are no significant differences between the infected treatments. This implies that the chemicals did not affect the average latent time. Recording the beginning time for diseased plants' death, the control with diseased rice plants was 21 days after inoculation (DAI). Treatment treated with K₂HPO₄ was 43 DAI, the treatment treated with CuCl₂ and K₂HPO₄ was 42 DAI and treatment treated with oxalic acid was 60 DAI. For this parameter, the treatments oxalic acid, K_2HPO_4 and mixing of CuCl₂ and K_2HPO_4 were effective SAR chemicals; among them, oxalic acid was the most valuable chemical that can prolong rice's life. There is no data of this parameter on the control with healthy rice plants.

At 35 DAI, the treatments treated by chemicals (CuCl₂ or K_2 HPO₄ or their mixing or oxalic acid) had the increasing rate of height in significant difference in comparison to the control with diseased rice plants. Therefore, induced chemicals can maintain the height of rice plant.

The data presented in Table 1 indicates a significant difference of effective tillers per pot between diseased and non-diseased treatments. The number of effective tillers per pot in treatments infected virus was 21.5 to 34.9, while the number of effective tillers per pot in the control with healthy rice plants was 52.3. The weight of firm grain per pot of chemical's treatments was equivalent to the control with healthy rice plants, but significantly different in comparison to the control with diseased rice plants. The weight of firm grain per pot of these treatments was 25.40 g (CuCl₂), 27.63 g (K₂HPO₄), 28.01 g (CuCl₂ and K₂HPO₄), 33.27 g (oxalic acid), 12.11 g (the control with diseased rice plants) and 31.13 g (the control with healthy rice plants).

Treatments	Ratio of in- fected plants (%)	Average latent time (DAI)	Beginning time for in- fected plants' death (DAS)	Increasing rate of height at 35 DAI (%)	Effective tillers/pot (tiller)	Weight of firm grain /pot (g)
CuCl ₂ 0.05mM	35.0 b	13.72 b	20	23.6 b	30.1 bc	25.40 a
K ₂ HPO ₄ 20mM	26.7 b	13.75 b	43	23.2 b	21.5 c	27.63 a
$CuCl_2\ 0.05mM\ +$	36.7 b	12.55 b	42	19.4 b	26.2 bc	28.01 a
K_2 HPO ₄ 20mM						
Oxalic acid 0.5mM	26.7 b	13.75 b	60	29.8 b	34.9 b	33.27 a
Control with dis- eased rice plants	68.3 c	12.78 b	21	0.0 c	27.0 bc	12.11 b
Control with healthy rice plants	0.0 a	0.00 a	-	58.0 a	52.3 a	31.13 a
Significant level	*	*	-	*	*	*
CV (%)	28.4	36.5	-	35.1	13.2	16.6

Numbers followed by the same letter do not differ significantly according to Duncan's multiple range test at P=0.05

*: differ significantly according to Duncan's multiple range test at P=0.05

-: do not record the parameter on healthy rice plant

The chemical's treatments such as CuCl₂, K₂HPO₄, mixing of CuCl₂ and K₂HPO₄, and oxalic acid, at used concentrations in this experiments, had the ability in maintaining the rice height better than the control with diseased rice plants. In addition, these chemicals also helped the rice plant in decreasing the ratio of diseased plants, prolonging the survival time of diseased plants, having the high weight of firm grain per pot comparable to the control with diseased rice plants. Moreover, there were some tolerant tillers on a diseased rice plant after treating these SAR chemicals (Figure 1). This result demonstrated that CuCl₂, K₂HPO₄, mixing of CuCl₂ and K₂HPO₄, and oxalic acid were the effective chemicals in SAR to RGSD; among them, oxalic acid was more dominant than in lengthening the rice plant's live. This was a special phenomenon that rarely occurs in virus-infected-plants. As a result, oxalic acid was selected as one of the most effective chemicals.

Our conclusion was in accordance with the results of Tri et al. (2011). The authors showed that $CuCl_2$ and oxalic acid helped the rice plants in decreasing the ratio of diseased plants, maintaining the weight of firm grain and increasing the activities of ribonuclease and protease inside the rice plants infected RGSD.

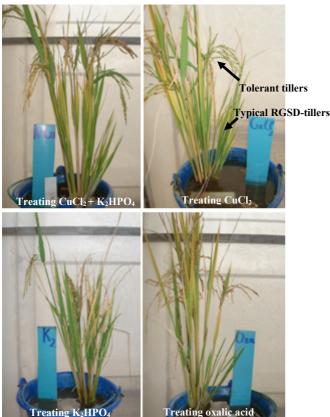


Figure 1. Rice plants at chemicals treatments

3.2 The experiment of oxalic acid' usage on rice grassy stunt disease

The data presented in Table 2 shows that the seed-soaking group had the ratio of infected plants at around 46.7% - 63.3%, significantly different in comparison with the control with diseased rice plants (96.7%). The ratios of seed coating group had not been different to the diseased control. Therefore, soaking the seed into SAR chemicals

before sowing can help the rice plant take RGSD fewer although all plants of this experiment were transmitted RGSD equally. The following leaf sprayings did not decrease the ratio of infected plants.

For average latent time, seed coating group expressed no difference in comparison with the diseased control. Howrs ever, seed soaking had the ability to prolong the latent time, longer than the diseased control's time. This was an effect in inducing plants to resist viral disease.

Chemical's treatments indicated that the beginning time for diseased plants' death was over 60 days, but for the diseased control was only 36 days. Although this data could not be analysed statistically, this gap was worthy to be noticed. This result contributed to the oxalic acid's effect to induce resistance on rice. The beginning time for the diseased plants' death was from 61 to 68 days. The longest time was recorded for the seed soaking and leaf spraying at 10, 20, 30 and 40 DAS. As a result, the late spraying times could help the rice plant in prolonging the survival time.

At 35 DAI, the increasing rate of height of chemical treatments was equivalent and significantly different in comparison to the control with diseased rice plants. The chemical's treatments had effective tillers/pot higher than the infected control, but lower than the healthy control. There was no difference noted among these chemical treatments. The weight of firm grain /pot of oxalic acid's treatments (except seed coating) was equivalent to one of the healthy control, and higher than one of infected control. This parameter was symbolised for rice plant's yield. Therefore, oxalic acid induced rice plants, helped them maintain the yield. Among these different ways used, seed coating had the lowest effect while the others had a high effect.

Treatments	Ratio of infected plants (%)	Average latent time (DAI)	Beginning time for in- fected plants' death (DAS)	Increasing rate of height at 35 DAI (%)	Effective tillers/pot (tiller)	Weight of firm grain /pot (g)
Seed coating	80.0 cd	10.50 ab	61	45.9 bc	5.1 b	9.4 bc
Seed coating and leaf spraying at 10, 20, 30 and 40 DAS	76.7 bcd	10.48 ab	63	23.8 c	8.3 b	12.6 b
Seed soaking	53.3 bc	10.94 b	63	35.8 bc	6.4 b	15.8 b
Seed soaking and leaf spraying at 10 DAS	46.7 b	11.79 b	64	46.0 bc	11.5 b	19.4 b
Seed soaking and leaf spraying at 20 DAS	53.3 bc	10.19 a	63	40.6 bc	10.7 b	18.8 b
Seed soaking and leaf spraying at 10, 20 and 30 DAS	63.3 bc	11.53 b	65	36.2 bc	10.9 b	15.9 b
Seed soaking and leaf spraying at 10, 20, 30 and 40 DAS	50.0 bc	11.07 b	68	49.9 b	10.8 b	22.8 b
Control with diseased rice plants	96.7 d	9.66 a	36	0.0 d	1.6 c	2.3 c
Control with healthy rice plants	00.0 a	-	-	87.5 a	37.7 a	56.8 a
Significant level	*	*	-	*	*	*
CV (%)	30.4	36.5	-	7.9	34.0	36.1

Notes: Numbers followed by the same letter do not differ significantly according to Duncan's multiple range test at P=0.05

* differ significantly according to Duncan's multiple range test at P=0.05

- criteria on healthy rice plant no recorded

In general, the different usage of oxalic acid could help rice plants in maintaining the height, lengthening the survival time, increasing effective tillers/pot, especially maintaining the weight of firm grain /pot, in comparison to the diseased control. Seed soaking had better ability than seed coating in keeping rice plant's health.

However, at the field-scale applications, migratory BPHs can come into the rice fields many times. They not only arrive at the field early (at 7 DAS) but also come to later. Farmers need to protect their field at a late stage, so leaf spraying could contribute to prolong the SAR efficiency. Tuzun et al. (1986) showed that immunized plants has reduced lesion number and size compared to the nontreated controls; and, even in the absence of disease, immunized plants grew more vigorously and yield were increased up to 20%, compared to the controls. Besides, Kuc (1995) reported that induced systemic resistance was not a final solution to all plant disease problems. It did deserve to be extensively and vigorously explored for its potential in providing an effective, inexpensive, natural, consumer-friendly and environmentally-friendly technology for plant disease control.

4. Conclusion

In conclusion, under our experimental conditions, oxalic acid seems to be a promising inducer for systemic resistance on rice to a challenge RGSV infection under nethouse conditions. The treatment - soaking the seeds into oxalic acid for 24 hours before sowing – showed better ability in SAR to RGSD than other ways of seed treatment. This result could be important in practice since it may offer a simple, environmentally safe and economically accepted mean to protect rice plants from RGSV infection and decrease the pollution by agricultural chemicals. However, additional studies are needed to confirm the results under field condition.

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