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MODIFIED KAPPA CARRAGEENAN AS INDUCER OF RESISTANCE IN RICE AGAINST BACTERIAL LEAF BLIGHT

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ABSTRACT

Modified Kappa Carrageenan (MKC) was evaluated in the greenhouse as possible inducer or elicitor of resistance in rice against bacterial leaf blight (BLB) using two hybrid varieties: NSIC Rc202H (Mestizo 19) and NSIC Rc204H (Mestizo 20). Effects of MKC concentration, induction time, frequency of application and spray intervals on blight severity were evaluated using *Xanthomonas oryzae* pv. *oryzae* Race 3 (Xoo R3) from July 2014 to April 2015 at the National Crop Protection Center, University of the Philippines Los Baños. Effect of root soaking in MKC solution as a means of resistance induction was also evaluated. The study showed that Mestizo 19 (M19) and Mestizo 20 (M20) hybrid rice varieties have induced resistance trait. M19 gained resistance against BLB 6 hours to 12 days after spraying with 50 to 100 ppm solution of MKC as shown by the significantly shorter blight lesions compared with the untreated plants. M20 gained resistance after 6 hours up to 12 days after induction with 100 ppm solution of MKC. Spraying plants three times with 100 ppm solution of MKC at 7 days interval or spraying two times with 200 ppm solution of MKC at 10 days interval starting at 30 days after planting resulted in shorter blight lesions than the untreated plants. Soaking roots of Mestizo 20 hybrid in 100 ppm solution of MKC for five minutes resulted in shorter blight lesions compared with the untreated plants, an indication of induced resistance.

Key words: hybrid rice, induced resistance, *Oryza sativa*, *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food in Asia. The demand for rice is increasing steadily due to the rapidly increasing human population. To meet this demand rice production has to increase by more than 40% in 2030 (Khush 2005). One way to increase rice production to meet this demand is to develop high yielding varieties with tolerance to biotic and abiotic stresses. The Department of Agriculture in the Philippines is encouraging farmers to plant hybrid rice varieties due to their 15% yield advantage over the inbred varieties (Virmani 2001). However, bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) and other diseases are among the biotic stresses limiting hybrid rice production (Ou 1985). Xoo is widespread in occurrence and 10 races or pathotypes have already been identified which can attack different varieties depending on the resistance gene that they carry (Adhikari et al. 1999). The use of pesticides for control is costly and environmentally undesirable. There is a need to develop strategies to produce durable resistance in rice.

Rice plants in the field are exposed continuously to possible attack by various plant pathogens throughout their lifetime. To protect themselves from these threats, they have developed several layers of constitutive and inducible defense system. Inducible plant defense mechanisms are mainly regulated by three types of signaling molecules or hormones: salicylate (SA), jasmonate (JA) and ethylene (ET) (Loake and Grant 2007; Pieterse et al. 2009). Their signaling pathways influenced each other through a complex network of synergistic and antagonistic interactions (Koornneef and Pieterse 2008) allowing the plant to tailor its defense reaction depending on the type of attacker encountered.

Induced resistance is an example of a defense mechanism by plants with potential to provide long-lasting resistance against pathogens and insect pests and promising for sustainable rice production in the future (Song and Goodman 2001). Induced resistance expression is dependent on plant genotype, nutrition, stage of the crop, the kind of inducer or effector, timing and method of application. Studies are being carried out in rice-growing countries in Asia to investigate the growth promotion and plant protection effects of modified kappa carrageenan (MKC) under different environmental conditions. MKC as obtained from Philippine Nuclear Research Institute (PNRI) is a radiation modified kappa carrageenan with an average molecular weight (Mw) of less than 5kDa.

Studies on induced resistance in rice against bacterial blight are very limited. The role of MKC as an inducer or effector of resistance against bacterial blight needs to be elucidated. The successful adoption of induced resistance in disease management depends on the use of appropriate elicitors at the right concentration on the right time with the right frequency applied to a responsive crop or cultivar. Whether induced resistance can be used in practice against bacterial blight of hybrid rice varieties using MKC was the focus of this study because the Philippines is a major producer of processed carrageenan.

The study sought to determine the effective concentration of MKC, frequency of application and spray intervals that will induce resistance of hybrid rice against BLB; and determine if root soaking in MKC solution can induce resistance of hybrid rice against BLB.

METHODOLOGY

The modified kappa carrageenan (MKC) was evaluated as possible inducer or elicitor of resistance in rice against bacterial leaf blight (BLB). The MKC was provided by the Philippine Nuclear Research Institute (PNRI). The kappa carrageenan was modified by irradiation to break the large molecular weight into smaller fragments (oligomers) to facilitate absorption into the plant tissue. MKC concentration, induction time, frequency of application and spray intervals were evaluated.

Two susceptible hybrid rice varieties namely: NSIC RC 202H (Mestizo 19) and NSIC RC 204H (Mestizo 20) were evaluated for induced resistance trait against the BLB pathogen, *Xanthomonas oryzae* pv. *oryzae* Race 3 (*Xoo* R_3) using the MKC in the greenhouse of the National Crop Protection Center (NCPC), University of the Philippines Los Baños from July 2014 to April 2015.

The rice seedlings were grown in plastic pots (21 cm diameter and 22 cm depth) with two hills per pot and three seedlings per hill, transplanted when the seedlings were 20 days old. The seedlings were fertilized with 200 kg of complete fertilizer (14-14-14) and 200 kg of urea (46-0-0) per hectare. The complete fertilizer was applied basally while urea was split; 100 kg was applied at 20 days after transplanting (DAT) and the other 100 kg at 45 DAT. The amount of fertilizer applied per pot was computed based on a planting distance of 20 cm x 20 cm or a population of 250,000 hills per hectare.

In the initial experiment, two concentrations (50 and 100 ppm) of MKC were evaluated with induction time (time from spraying to inoculation) of 6 hours (0 day), 4, 8, 12 and 16 days. The treatments were arranged in a split-plot design with concentration as the main plot and induction time as the sub-plot. Each treatment was replicated three times. Application of the treatments started at 40 DAT. MKC concentrations were sprayed to leaves and stems in the morning using a hand sprayer.

Xoo was cultured in potato dextrose peptone agar (PDPA) for two days. The bacterial suspension was prepared, homogenized in a beaker at approximately $1x10^8$ cells per ml and inoculated to leaves by clipping about 2 cm of the leaf tip with a pair of scissors previously dipped into the bacterial suspension. Untreated seedlings that were inoculated with the pathogen served as control. Blight lesion length was measured fourteen days after inoculation. A mean lesion length of six leaves per replicate was calculated. The data were analyzed statistically using SAS Version 9.1.

Effects of MKC concentration, frequency and spray interval on blight severity

The effects of MKC concentration and frequency of spraying on blight severity were evaluated using the Mestizo 20 (M20) hybrid. The following treatments were evaluated:

1. 100 ppm sprayed 4 times at 5 days interval
2. 100 ppm sprayed 3 times at 7 days interval
3. 200 ppm sprayed 3 times at 7 days interval
4. 200 ppm sprayed 2 times at 10 days interval
5. 300 ppm sprayed 3 times at 7 days interval
6. 300 ppm sprayed 2 times at 10 days interval
7. Untreated plants (water only)

The treatments were arranged in a randomized complete block design (RCBD) with four replications. First treatment application was at 30 DAT. All treatments were inoculated with 48-hour old culture of *Xoo* five days after the last application of MKC following the same procedure described previously. Lesion length was measured at 14 days after inoculation. A mean lesion length of six leaves per replicate was calculated.

Resistance induction by root soaking

The effect of soaking roots of rice to various concentrations of MKC as a means of resistance induction against bacterial leaf blight was evaluated using Mestizo 19 (M19) and Mestizo 20 (M20) hybrid rice varieties. Roots of 24-day old rice seedlings were soaked for 5 minutes in 50, 100, 150 and 200 ppm solution of MKC and transplanted to plastic pots containing paddy soil. Fifty percent of the root-soaked plants were sprayed with the same concentration of MKC at 20 DAT. Untreated plants served as control. The following treatments were compared:

1. Roots soaked only in 50 ppm solution of MKC
2. Roots soaked in 50 ppm MKC + spraying with 50 ppm MKC at 20 DAT
3. Roots soaked only in 100 ppm MKC
4. Roots soaked in 100 ppm MKC + spraying with 100 ppm MKC at 20 DAT
5. Roots soaked only in 150 ppm MKC
6. Roots soaked in 150 ppm MKC + spraying with 150 ppm MKC at 20 DAT
7. Roots soaked only in 200 ppm MKC
8. Roots soaked in 200 ppm MKC + spraying with 200 ppm MKC at 20 DAT
9. Untreated control

The treatments were arranged in RCBD with 4 replications. The plants were inoculated with *Xoo* ten days after spraying with MKC solution using the clipping method. Blight lesions were measured 14 days after inoculation. A mean lesion length of six leaves per replicate was calculated.

RESULTS AND DISCUSSION

Effects of MKC concentration and induction time on blight lesion length of Mestizo 19 and Mestizo 20 hybrid rice varieties

The results of the greenhouse trial showed that the M19 and M20 hybrid rice varieties have induced resistance trait. In fact, M19 had gained resistance to BLB infection in just 6 hours after spraying with 100 ppm MKC solution as shown by the shorter mean blight lesion of 145.3 mm compared to the mean blight lesion of 176.80 mm in the untreated plants (Table 1). The best induction time was at four days but the plants remained resistant to BLB infection up to 12 days after spraying with the MKC solution. The plants sprayed with 50 and 100 ppm concentrations of MKC had mean lesion length of 156.32 and 138.44 mm, respectively across induction time that were significantly shorter than the mean lesion length of the untreated plants.

Table 1. Mean lesion length in Mestizo 19 hybrid 14 days after inoculation with *X. oryzae* pv. *oryzae* due to MKC concentration and induction time.

Concentration	Mean Lesion Length (mm)[1] Induction Time (Days)[2]					Mean concentration[3]
	0(6 hrs)	4	8	12	16	
0	176.80[a]	166.10[a]	183.90[a]	203.60[a]	171.70[a]	180.41[a]
50 ppm	151.70[b]	142.80[a]	147.00[b]	163.20[b]	176.90[a]	156.32[b]
100 ppm	145.30[b]	124.10[b]	132.40[b]	137.70[c]	152.70[a]	138.44[b]
Mean Induction Time	157.93[ab]	144.34[b]	154.43[ab]	168.16[a]	167.09[a]	

[1] Each figure is the mean of 3 replications with 6 leaves per replicate.
[2] Refers to the number of days after spraying of the MKC to inoculation of the bacterial blight pathogen
[3] In a column, means with the same letter are not significantly different using Least Significant Difference (LSD) test.

The M20 hybrid rice variety gained resistance to BLB six hours after spraying with 100 ppm solution of MKC as shown by the significantly shorter blight lesions with a mean of 153.0 mm compared to the mean lesion length of 212.37 mm in the uninduced or untreated plants. The induced plants remained resistant for twelve days as shown by the significantly shorter blight lesions with a mean of 158.73 mm compared to the mean lesion length of 190.13 mm in the uninduced plants (Table 2). The results show that M19 and M20 can respond quickly to MKC induction against possible invasion by the BLB pathogen. The study shows that MKC can be used as elicitor or inducer of resistance against BLB in these two hybrid rice varieties.

Despite the significant reduction in blight lesion length, the MKC-induced plants showed a susceptible reaction (greater than 10 cm lesion length), based on the standard varietal evaluation for BLB of the International Rice Research Institute (IRRI). This is due to the fact that M19 and M20 are highly susceptible to the isolate used (*Xoo* Race 3) that some of the blight lesions extended up to the leaf sheath. There are hybrid rice varieties in the Philippines that are resistant to *Xoo* Race 3 such as NSIC 114, NSIC 116, Bigante, SL8, MRH-005 and MRH-007 and have intermediate mean disease reaction to ten *Xoo* races (Balidion et al. 2012). These hybrid varieties can be used in future studies for their reaction to the MKC treatment in controlled environment and field conditions.

Table 2. Mean lesion length in Mestizo 20 hybrid 14 days after inoculation with *X. oryzae* pv. *oryzae* as affected by MKC concentration and induction time.

Concentration	Induction Time (days) 0 (6 hrs)	4	8	12	16	Mean Concentration[2]
	Mean Lesion Length (mm)[1]					
0	212.37[a]	189.57[a]	190.37[a]	190.13[a]	193.77[a]	195.24[a]
50 ppm	198.57[a]	162.40[ab]	171.00[a]	162.97[ab]	171.00[a]	173.19[b]
100 ppm	153.03[b]	158.73[b]	147.90[b]	158.73[b]	170.67[a]	157.81[c]
Mean Induction Time	187.99[a]	170.23[b]	169.76[b]	170.61[ab]	178.48[ab]	

[1] Each figure is the mean of 3 replications with 6 leaves per replicate.
[2] In a column, means with the same letter are not significantly different using Least Significant Difference (LSD) test.

Effects of MKC concentration, frequency of application and spray interval on blight severity

In terms of practical disease control, the frequency of application and spray intervals in relation to crop growth stages are crucial considerations in induced resistance. Results of greenhouse trial using Mestizo 20 hybrid showed that spraying plants four times with 100 ppm MKC every five days starting at 30 DAT gave significantly shorter blight lesions with a mean lesion length of 84.47 mm (intermediate reaction) than the untreated plants with a mean length of 135.25 mm (susceptible reaction), a 35% blight reduction. Plants sprayed three times with 100 ppm MKC every 7 days had mean lesion length of 95.10 mm (intermediate reaction), a 30% blight reduction (Table 3). When 200 ppm MKC was sprayed twice every 10 days, the plants had a mean lesion length of 108.35 mm (susceptible reaction) or blight severity reduction of 20%.

Mean treatment comparison showed that there were no significant differences among treatments (3 concentrations of MKC, frequency of spraying and spray intervals). All the treatments had the same effectiveness in reducing lesion length due to blight. Since the differences in lesion length of 100 ppm sprayed 4 times every 5 days, 100 ppm sprayed 3 times every 7 days and 200 ppm MKC sprayed 2 times every 10 days are not statistically significant, it is practical and economical to use 100 ppm MKC sprayed 3 times at 7 days interval or 200 ppm MKC sprayed 2 times at 10 days interval. Increasing the MKC concentration to 300 ppm did not improve the blight control.

The 20 to 35% blight reduction due to two to four sprays of MKC may not be enough to increase rice yield. For instance, wheat plants sprayed with benzothiadiazole (BTH) to control Septoria leaf blotch caused by *Septoria triticii* had 46% reduction in disease severity but there was no increase in yield (Vallad and Goodman 2004). Huang et al. (2012) found that weekly application of acibenzolar-S-methyl (ASM) as inducer provided considerably better control of bacterial spot of tomato than applications every two weeks.

Table 3. Lesion length in Mestizo 20 hybrid as influenced by MKC concentration, frequency of application and spray intervals.

Treatment	Lesion Length (mm)				Mean[2]	% Blight Reduction
	Replication					
	1	2	3	4		
Control (no spray)	133.0[1]	117.0	138.2	152.8	135.25[a]	
100 ppm, 4 sprays every 5 days	93.5	89.7	79.0	87.7	87.47[b]	35
100 ppm, 3 sprays every 7 days	113.7	91.8	82.7	92.2	95.10[b]	30
200 ppm, 3 sprays every 7 days	115.5	101.5	108.0	95.2	105.05[b]	22
200 ppm, 2 sprays every 10 days	124.2	105.7	108.5	95.0	108.35[b]	20
300 ppm, 3 sprays every 7 days	114.5	80.0	97.7	112.2	101.17[b]	25
300 ppm, 2 sprays every 10 days	105.2	84.8	117.2	102.5	102.42[b]	24

[1] Each figure is the mean of four replications with six leaves per replicate.
[2] In a column, means with the same letter are not significantly different using Tukey's Honest Significant Difference (HSD) test.

Inducing resistance by root soaking

In most induced resistance studies, elicitors or inducers are usually applied as either foliar or root drench. A more convenient means of applying a treatment is by root soaking (dipping) or seed treatment which can result to primed plants for enhanced defense against pathogens (Worrall et al. 2012). If the priming effect is long-lasting, the need for further treatment with elicitors would be reduced.

Our greenhouse study showed that MKC can be used as elicitor to induce the resistance of some high breed rice varieties against the BLB pathogen through root soaking. Soaking roots of M19 for five minutes in 50-200 ppm solution of MKC produced plants with relatively shorter blight lesions than the untreated plants. However, the magnitude of blight reduction in each treatment, whether root soaking alone or root soaking + spraying with MKC solution after 20 days, was not statistically significant compared to the untreated plants. All the MKC-treated plants had a susceptible reaction to BLB (lesion length greater than 10 cm). Spraying with the various MKC concentration 20 days after root soaking did not improve the resistance of M19 hybrid to BLB infection (Table 4).

In the case of M20 hybrid, the MKC-treated plants had intermediate reaction to BLB, except the plants whose roots were soaked only in 50 ppm MKC solution that had susceptible reaction to BLB. The untreated plants had susceptible reaction to BLB (Table 4). Only the treatments involving root soaking + spraying with 50 ppm MKC solution, root soaking alone in 100 ppm MKC solution and root soaking + spraying with 200 ppm MKC solution produced significantly shorter blight lesions compared to the untreated plants.

This result suggests that hybrid rice varieties differ in their response to root soaking as a means of inducing resistance to BLB and that M20 is more responsive to the treatment than M19. Responsive plants can remain resistant to BLB infection thirty days after induction by root soaking. This method of inducing resistance is more convenient and can be used in the management of early occurring diseases in the field. Whether application of inducer to growing plants early in the growing season is necessary will depend upon the likelihood of pathogen attack. This earlier application of the inducer is warranted when there is a threat of early infection in the field due to high inoculum pressure of the BLB pathogen and frequent heavy rain.

Table 4. Blight lesions in Mestizo 19 and Mestizo 20 hybrid varieties as influenced by root-soaking in MKC solution.

Treatment	**Mean Blight Lesion Length (mm)[1]**	
	Mestizo 19	**Mestizo 20[2]**
1. Untreated	139.50	110.12[a]
2. Root-soaking alone (50 ppm)	118.98	108.15[a]
3. Root-soaking + spraying (50 ppm)	125.45	77.03[b]
4. Root-soaking alone (100 ppm)	125.20	78.08[b]
5. Root-soaking + spraying (100 ppm)	121.40	91.30[ab]
6. Root-soaking alone (150 ppm)	121.92	84.08[ab]
7. Root-soaking + spraying (150 ppm)	120.97	90.08[ab]
8. Root-soaking alone (200 ppm)	119.42	87.25[ab]
9. Root-soaking + spraying (200 ppm)	112.85	80.08[b]

[1] Each figure is the mean of four replications with six leaves per replicate.
[2] In a column, means with the same letter are not significantly different using Tukey's Honest Significant Difference (HSD) test.

The influence of host genotype on the expression of induced resistance in several cultivars of spring barley (*Hordeum vulgare*) to the foliar pathogen *Rhynchosporium secalis* using the elicitors acibenzolar-S-methyl (ASM), β-aminobutyric acid (BABA) and *cis*-jasmone (CJ) varied greatly across a range of spring barley varieties (Walters et al. 2011). BTH applied as seed treatment at the rate of 2 gram/kg of corn seeds reduced downy mildew incidence by 37% (Morris et al. 1998). Since induced resistance is a host response, its expression under field conditions can be influenced by crop genotype or variety, environment (temperature, moisture stress, etc.), crop nutrition, concentration of effector or inducer, timing and frequency of application in relation to crop growth and vulnerability to infection (Walters et al. 2013).

CONCLUSION AND RECOMMENDATIONS

MKC is a potential inducer or elicitor of resistance in hybrid rice against BLB. Soaking roots of rice in a solution of MKC is a possible and convenient way of inducing resistance against BLB. Since induced resistance is a host response, its expression under field conditions can be influenced by crop genotype or variety, environment, crop nutrition and vulnerability of the rice plant to infection by different races of the BLB pathogen. The carrageenan technology for resistance induction should be further tested or verified in several locations with different soil types and races of the BLB pathogen using different rice varieties.

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REFERENCES CITED

Adhikari, T.B., R.C. Basnyat and T.W. Mew. 1999. Virulence of *Xanthomonas oryzae* pv. *oryzae* on rice lines containing single resistance genes and gene combinations. Plant Dis. 83: 46-50.

Balidion, J.F., G.B. Amar, F.A. dela Peña, M.P. Natural and C.M. Vera Cruz. Pathotypic and genetic diversity of *Xanthomonas oryzae* pv. *oryzae* (ex Ishiyama) Swings, et al. from selected hybrid rice-growing areas in the Philippines. J. Tropical Plant Pathology. 48: 31-54.

Huang, C.H., G.E. Vallad, S. Zhang, A. Wen, B. Balogh, J.F.L. Figueiredo, F. Belau, J.B. Jones, M.T. Momol, and S.M. Olsen. 2012. Effect of application frequency and reduced rates of acibenzolar-s-methyl on the field efficacy of induced resistance against bacterial spot on tomato. Plant Disease. 96: 221-227.

Khush, G.S. 2005. What it will take to feed 50 billion rice consumers in 2030? Pl. Mol. Biol. 59: 1-6.

Koornneef, A. and C.M.J. Pieterse. 2008. Crosstalk in defense signaling. Plant Physiol 146: 839-844.

Loake, G. and M. Grant. 2007. Salicylic acid in plant defense: the players and protagonists. Curr Opin Plant Biol. 10: 466-472.

Morris, S.W., B. Vernooji, S. Titatarn, M. Starrett, S. Thomas, C.C. Wiltse, R.A. Frederiksen, A. Bhandhufalck, S. Hulbert and S. Uknes. 1998. Induced resistance responses in maize. Molecular Plant-Microbe Interactions. 11: 643-658.

Ou, S.H. 1985. Rice diseases, 2nd ed. Kew Survey, U.K.: Commonwealth Mycological Institute.

Pieterse, C.M.J., A. Leon-Reyes, S. Van der Ent and S.C.M. Van Wees. 2009. Networking by small molecule hormones in plant immunity. Nat Chem Biol, 5: 308-316.

Song, F. and R.M. Goodman. 2001. Molecular biology of disease resistance in rice. Physiological and Molecular Plant Pathology. 59: 1-11.

Vallad, G. E. and R.M. Goodman. 2004. Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop Sci. 44:1920-1934.

Virmani, S.S. 2001. Opportunities and challenges of developing and using hybrid rice technology in the tropics. In: Peng SB, Hardy B, editors. Rice Research for Food Security and Poverty Alleviation. Proc. International Rice Conference. Philippines, 407p.

Walters, D.R., N.D. Havis, L. Paterson, J. Taylor and D.J. Walsh. 2011. Cultivar effects on the expression of induced resistance in spring barley. Plant Disease. 95: 595-600.

Walters, D.R., J. Ratsep and N.D. Havis. 2013. Controlling crop diseases using induced resistance: Challenges for the future. J Experimental Botany 18 pages.

Worrall, D., G.H. Holroyd, J.P. Moore, M. Glowacs, P. Croft, J.E. Taylor, N.D. Paul and M.R. Roberts. 2012. Treating seeds with activators of plant defense generates long-lasting priming of resistance to pests and pathogens. New Phytologists 193: 770-778.