EFFECT OF NITROGEN AND SILICON ON MANAGEMENT OF RICE BLAST (*PYRICULARIA ORYZAE*) IN MWEA IRRIGATION SCHEME OF KENYA

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Abstract
Plant health is an important factor for plant growth and development. Nitrogen is essential and is usually required in large quantities by plants. However, many studies have shown that high nitrogen concentration in plant increases the severity of disease infection by plant pathogen. On the other hand, silicon though regarded as non essential element, has several benefits in crop growth. Its application to the rice plant has been shown to increase resistance to rice blast *Pyriularia oryzae* as well as increased crop yield. This study aimed to establish an effective level of nitrogen and silicon in the management of the rice blast disease. The experiment was carried out at Mwea Irrigation Agricultural Development (MIAD) research station in Kirinyaga District. Seedlings were raised in the nursery before culturing in vertisol filled pots with various treatment combinations of nitrogen (40, 80 and 120 kgN ha^{-1}) and silicon (0,500, 1000 and 1500 kgSi ha^{-1}) in split plots and in completely randomized design (CRD). Plants were inoculated after two weeks with the *Pyricularia oryzae* spore after transplanting and disease assessed in a scale of (0-9) according to IRRI standard. Higher rice blast was realised at 120 KgN and 0kgSi ha^{-1} and in the plots that had neither nitrogen nor silicon. The organic husk ash at $2ton^{-1}$ before burning and $0.7ton^{-1}$ was shown to be good source of silicon and gave results equivalent to those of 120KgN and 1000KgSi.combination.The study established that interaction of nitrogen and silicon at 80 kgN ha^{-1} and 1000 kgSi ha^{-1} was the optimal rate for management of the rice blast disease.

Key words: Silicon, nitrogen, rice blast, rice, hush ash

1.0 Introduction

Rice (*Oryza sativa* L) is a staple food of nearly one-half of world's population contributing high calorie intake to humans. (FAO, 2004) In Kenya it is among the important cereals ranking third after maize and wheat (MOA, 2007). From the varieties grown in Mwea, Basmati 370 is the most preferred by many growers as it fetches higher returns. However, this cultivar is susceptible to rice blast disease which is one of the most important rice diseases that can cause a considerable yield loss. Awoderu, (1990) found over 70 percent yield loss caused by rice blast. In Western Kenya the disease was found to cause a loss of 50% (Anon, 1992). There are various strategies undertaken in management of rice blast that include cultural method, use of resistant varieties, plant nutrition and chemical control. Among these strategies plant nutrition is more appealing as it is considered environmentally friendly. Nitrogen is an essential element in plant growth and development but as reported by Kurschner *et al.,* (1992) high nitrogen concentration in plants increase the severity of infection by obligate parasite. Nitrogen supply influences branching and leaf expansion which together determines the canopy size. A large canopy is conducive to spore transfer and pathogen infection than in sparse canopies. Silicon on the other hand is an important element although it is not classified as an essential element. As reported by Datnoff *et al*., (1990) and Datnoff *et al*., (2001) large amount of silicon has been seen to accumulate in some crops like rice and other grasses. Rice containing inadequate silicon is severely infected by rice blast. In order to manage rice blast disease through plant nutrition there was need to determine effective rates of nitrogen and silicon as important elements curb the disease.

2.0 Materials and Methods

2.1 Rice Culture and Treatment Application

Two experiments were conducted for two seasons (November 08 and May 09). Soil used in the experiment was alluvial soil type that is usually suitable for rice production in Mwea Division. It was obtained from Mwea Irrigation Agricultural Development (MIAD) field and analyzed for nitrogen, phosphorus, potassium and silicon to determine the initial concentration in the soil. Certified seed was sown in the nursery which was preceded by seed treatment through soaking and incubation (pre-germination). Soaking was done for 24hrs while pre-germination took 3 days before sowing. Seedlings were later transplanted into plastic bags after twenty eight days.

2.2 Treatments Rates of Nitrogen and Silicon

The experiment was arranged in Split plot design laid in complete randomized design (CRD) replicated four times. There were seventeen treatments that included four levels of silicon (0, 500, 1000 and 1500Si kg ha^{-1}), four nitrogen levels (0, 40, 80 and 120NKg ha^{-1}) and 2tons of ash husk. Calcium silicates was the source of silicon at 0, 2500, 5000 and 7500kg ha^{-1} This translated to 0, 0.06,0.12,0.18gCasio3/ 200 g soil per pot respectively. Sulphate of ammonia (SA) was the source of nitrogen at (0, 0.46,0.91 and 1.37 gm/pot). The potassium and phosphorus were applied as basal fertilizer at recommended rates of 30 Kg K_2O ha^{-1} and 58 kgP_2O_5 ha^{-1}. Due to the calcium contained in the calcium silicate as the source of silicon the calcium element was added to ensure the effect of silicon was not influenced by calcium. Individual treatments were thoroughly mixed with 200 gms of soil, filled in plastic pots (12x8cm). Each of the units/treatment contained six plants replicated four times. The mixture was well-irrigated four days before the seedlings were transplanted. The seedlings that were of the same height and thickness were selected for uniformity purposes.

2.3 Preparation of Media

The media was prepared by dissolving 35.5 gms of malt extract agar (MEA) in 1 litre of distilled water and mixed on hot plate/stirrer, mixed homogeneously before autoclaving /sterilizing for 15 min at 121^0C. The media was supplemented with 0.05g Chlorotetracycline, 0.1g penicillin G and 0.2 g Streptomycin-sulphate per litre to prevent bacterial contamination. The media was dispensed into culture plates after attaining a temperature of 30-40^oC. It was then allowed to cool, gel and preserved for culturing.

2.4 Preparation of Inoculums

Infected plant tissues were collected from MIAD and the surrounding infected fields as the source of inoculums. The conidia (asexual spores) were harvested from different plant parts (stems, panicles, sheaths), washed thoroughly with tap water. Under aseptic conditions, the infected plant tissue were cut in small section 5-10mm square from the margin of the infected lesion such that it contained both diseased and healthy looking tissue. The tissues were surface sterilized for two minutes in 90% Ethanol, washed with three changes of sterile water and blotted dry on clean sterile paper by use of forceps and finally plated in 90mm Petri dishes containing malt extract agar (MEA). The cultured media was incubated under continuous light at 25°C for 24 hours after which the light was put off and incubation continued for seven days allowing the growth of mycelia. Under sterile condition a drop of sterile water was put on the slide and a small piece of mycelia placed on it, covered with a cover slip and placed under a light microscope for observation. After the identification of the fungus, the pathogen was sub-cultured in order to isolate rice blast fungus and accelerate the sporulation. The Petri dishes were re-incubated in the laboratory for seven days during which

growing fungi were viewed under a light microscope (x40 magnification). Further sub-culturing was done to obtain pure cultures.

2.5 Pathogenesity Tests

Once the pathogen was identified and isolated, pathogenesity test of the fungi was necessarily for verification that the fungus was the real cause of the rice blast disease. This was done through inoculation of grown plants in the screen house. The method used was as described by Matsuo and Hoshikama, (1993). The soil was collected from the field, heat sterilized (121^{o}C, 102 kPa, and 60 min) and allowed to cool before potting in the plastic containers (200gms volume). The plants were planted in two different pots of which one pot was inoculated while the other was left with healthy plants. Distilled water was used to flood the soil as required by rice plant. The inoculums were foliar sprayed by use of a hand sprayer on the plants in one pot and then covered with a plastic bag to maintain the water-saturated atmosphere. The plastic bag was removed after 24hours after which the plants were left at room temperature. The other pot was left with clean plant without any inoculation. The infection from the fungus was observed four days after inoculation. The symptoms of the lesions that appeared on the leaves of the inoculated plants were similar to the symptoms described on the naturally infected plants from the field. The plant parts of the other plants that were not inoculated were surface sterilized with 95% ethanol for 1 to 2 min. Similarly the infected plants parts too were surface sterilized the same way and placed on the malt extract agar media and incubated for 24 hours of light and observed on the seventh day. The fungus was observed in the infected plants while it was absent in the healthy parts.

2.6 Inoculation

Inoculation was done at the time of emergence on the seventh leaf from the main tiller as described by Matsuo and Hoshikama (1993). A conidial suspension of *Pyricularia oryzae* (4×10^4 conidia/ ml) was used. This was achieved by placing a cover slip over the hemocytometer counting chambers. By use of a Pasteur pipette a drop of conidia suspension was placed at the edge of the V-shaped allowing the suspension to be drawn into the chamber by capillary action. One of the nine 1mm square represented a volume of 10^{-4}mls. Using the 10X objective the conidia of 1mm square area was counted using tally counter. The conidia amounted to more than100 conidia per 1mmsquare area. Other four squares were counted and calculated using a formula: c=n/v where c=conidia concentration in conidia/ml, n=average number of conidia/square mm, v=volume counted=$10^{-4.}$ Thus: c=nx10^4. This suspension was applied as a fine mist to the upper leaf blades of six plants per pot until runoff with a hand sprayer. Gelatin (1%, wt/vol) was added to the sterile water to aid conidial adhesion to the leaf blades. Immediately after inoculation, plants were covered with a plastic bag to increase humidity for 24 hrs. The pots that contained the plants were retained with water to maintain high relative humidity of approximately 85% and above throughout the experiments.

2.7 Disease Assessment

The data regarding the occurrence of the rice blast disease was collected one week after the inoculation by using the disease rating scale 0-9 developed by International Rice Research Institute (IRRI, 2002). Four plants were randomly selected out of the six plants planted in a pot. The fourth leaf was tagged in each of the selected plant and the largest lesion measured in both width and length. Area under the disease progress curves (AUDPC) was used for disease determination where the lesion size was measured using the formula.

Lesions area= width/15 [(6*length) +8{square root (width2+length2)/4}] as described by Pinnschmidt *et al.* (1993)

Table 1: Visual score rating for disease and plant response

Code	*Type of lesions*	*Host Behavior*
0	No lesions observed	Highly resistant
1	Small brown specks of pinpoint size or larger brown specks without sporulating center	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin	Moderately resistant
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves	Moderately resistant
4	Typical susceptible blast lesions 3 mm or longer, infecting less than 4% of the leaf area	Moderately susceptible
5	Typical blast lesions infecting 4-10% of the leaf area	Moderately susceptible
6	Typical blast lesions infection 11-25% of the leaf area	Susceptible
7	Typical blast lesions infection 26-50% of the leaf area	Susceptible
8	Typical blast lesions infection 51-75% of the leaf area and many leaves are dead	Highly susceptible
9	More than 75% leaf area affected	Highly susceptible

Sources: International Rice Research Institute (2002). *Standard evaluation systems for rice*

2.8 Data Analysis

Data on rice infection of plant by rice blast was analyzed using statistical package (SAS,2001). The analysis conducted were (ANOVA) and means separations using LSD.

3.0 Results and Discussion

The results in figure 1 indicate that infection rate in the first sampling date did not show any significant ($P > 0.05$) difference, implying that all the plants acquired the same score with a mean of 1. However, the subsequent weekly sampling that followed indicated significant ($P< 0.05$) difference among different levels of nitrogen all through to the last sampling. Nitrogen applied at 120Kg ha^{-1} (N3) gave a higher level of rice blast infection while a lower infection was realized in those plots that were treated with husks ash at 2tons ha^{-1}.

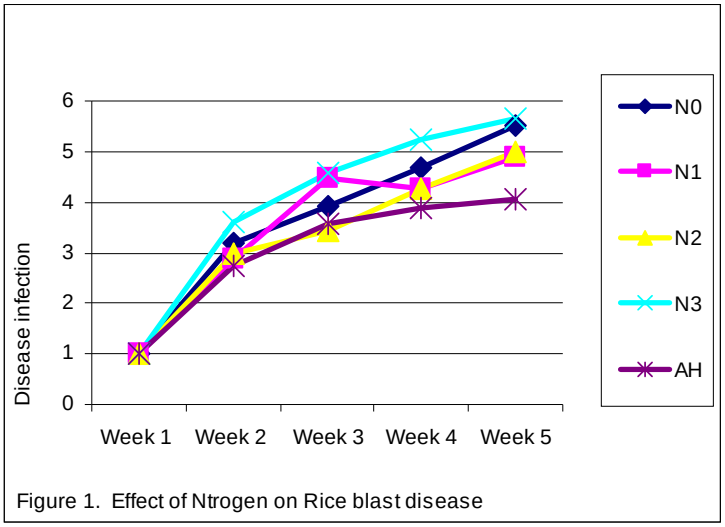

Figure 1. Effect of Ntrogen on Rice blast disease

As was the case of nitrogen, the results shown in figure 2 indicated that silicon levels did not show any significant ($P > 0.05$) difference in the first sampling period. The second and the subsequent sampling showed significant ($P < 0.05$) difference in the level of infection. Infection was higher where there was no silicon (S0) and on a lower rate 500 Kg Si ha^{-1} (S1) of application while the least infection of rice blast occurred where silicon was applied at 1500 kg Si ha^{-1} (S3) and 1000Kg Si ha^{-1} (S2). Disease infection decreased with increase in silicon rate. Husk ash applied at 2tons ha^{-1} (0.7 tons ha^{-1}) managed the rice blast at a higher level than 500 Kg Si ha^{-1} (S1) Figure 2. Contrary to nitrogen, high silicon level decreased the rice blast infection.

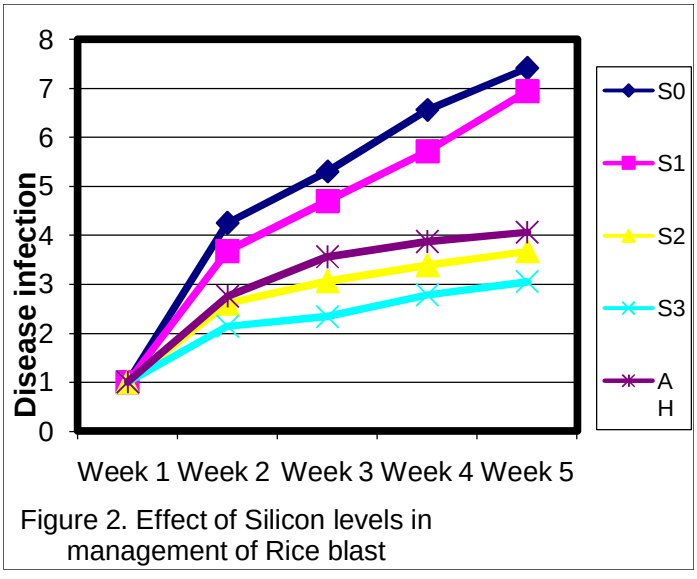

Figure 2. Effect of Silicon levels in management of Rice blast

Figure 2: Effect of silicon levels in management of rice blast

The results (Table 2) for interaction of nitrogen and silicon in the first season indicated that the plots that had no silicon (0KgSi ha^{-1}) or nitrogen (0 KgN ha^{-1}) N0S0 experienced a higher rice blast infection. Similarly 80 KgN and 0 KgSi ha^{-1} (N2S0), 0 KgN and 500 KgSi ha^{-1} (N0S1), 40 KgN and 500 KgSi ha^{-1} (N1S1), 40 KgN and 0 KgSi ha^{-1} (N1S0), 80 KgN and 500 KgSi ha^{-1} (N2S1) gave a disease mean score above 7 indicating the plants were susceptible to the pathogen. These results revealed low nitrogen and low silicon might have contributed to increase of rice blast infection of rice plants. However, husk ash at 2tons ha^{-1} and 0KgN and 1000SiKg ha^{-1} /ha (N0S2) were moderately susceptible. Plots treated with 80 KgN and 500 SiKg/ha (N2S1), 80KgN and 1500 SiKg ha^{-1} a (N2S3), 120 KgN and 1000SiKg ha^{-1} (N3S2), 120 KgN and 1500 SiKg/ha (N3S3) showed the plants were moderately resistant to the pathogen. That disease was well managed at 80 KgN, 1000 SiKgha^{-1} (N2S2) and 80 KgN and 1500 SiKg ha^{-1} (N2S3) which were not significantly differently with a mean score of 2-3 (moderately resistant). The trend was similar in the second season although the means in the second season were higher (Table 3). The plots that weretreated with the husk ash were not significantly different from those treated with 120 KgN and 1000 SiKgha^{-1} (N3S2).

Table 2: Interaction of nitrogen silicon and husks ash in management of rice blast season one

Nitrogen	*Silicon*	*Sampling period*			
		Week 1	*Week 2*	*Week 3*	*Week 5*
AH	AH	2.75**d**	3.56**e**	3.88e	4.06**g**
N0	S0	4.06**b**	5.00**bc**	6.31**b**	7.31abc
N0	S1	3.75**b**	4.88**c**	5.81**c**	7.19c
N0	S2	2.81**d**	3.56**e**	4.00**e**	4.63**f**
N0	S3	2.13**e**	2.19**g**	2.63g	2.94lk
N1	S0	3.81**b**	4.69**c**	6.25**b**	7.25bc
N1	S1	3.19c**d**	4.19**d**	5.13**d**	6.25**e**
N1	S2	2.56**d**	2.94**f**	3.25**f**	3.38**ij**
N1	S3	2.00**e**	2.06**g**	2.44**g**	2.69**l**
N2	S0	4.63**a**	5.38**b**	6.69**ab**	7.38abc
N2	S1	3.31**c**	4.00**d**	5.13**d**	6.69**d**
N2	S2	2.06**e**	2.25**g**	2.63**g**	2.88**l**
N2	S3	2.00**e**	2.19**g**	2.69**g**	3.00**lk**
N3	S0	4.50**a**	6.13**a**	7.00**a**	7.60**ab**
N3	S1	4.50**a**	5.75**ab**	6.81**a**	7.63**a**
N3	S2	3.00**cd**	3.50**e**	3.69**eg**	3.81**gh**
N3	S3	2.44**de**	2.94**f**	3.38**f**	3.67hi

Means of the same letter along the column are not significantly different

Table 3: Interaction of nitrogen silicon and ash husks in management of rice blast season 2

Nitrogen	*Silicon*	*Sampling period*			
		Week 1	*Week 2*	*Week 3*	*Week 5*
AH	AH	2.63**d**	3.63**d**	3.94**d**	4.13**f**
N0	S0	4.06**b**	5.31**bc**	6.50**b**	7.19**c**
N0	S1	3.75**b**	4.94**c**	6.13**b**	7.06**c**
N0	S2	2.81**d**	3.56**d**	4.19**d**	4.69**e**
N0	S3	2.19**e**	2.50**f**	2.81**f**	3.00**ih**
N1	S0	3.75**b**	5.06**c**	6.25**b**	7.25**bc**
N1	S1	3.18**cd**	4.50**c**	5.31**c**	6.05**d**
N1	S2	2.60**d**	3.00**e**	3.44**e**	3.75**g**
N1	S3	2.00**e**	2.19**f**	2.63**f**	2.75**i**
N2	S0	4.56**a**	5.56**b**	6.81**ab**	7.55**ab**
N2	S1	3.31**c**	4.00**d**	5.19**c**	6.56**d**
N2	S2	2.00**e**	2.25**f**	2.69**f**	2.94**ih**
N2	S3	1.87**e**	2.19**f**	2.75**f**	3.00**ih**
N3	S0	4.63**a**	6.25**a**	7.06**a**	7.75**a**
N3	S1	4.50**a**	5.81**ab**	7.00**a**	7.63**a**
N3	S2	3.00**cd**	3.63**d**	3.94**d**	4.25**ih**
N3	S3	2.50**d**	2.88**ef**	3.25**e**	3.56**h**

Means of the letter along the column are not significantly different

The mean score of the first observation was the same in all the treatments which may have indicated that all the plants were similarly infected once the inoculums was introduced. It was likely that the disease was still latent hence no physical symptoms were detected. The trend indicated that as the level of nitrogen increased the infection of rice blast also increased with highest infection occurring at 120 KgN ha^{-1}. This might have been brought about due to creation of canopy providing a suitable environment for the rice blast to thrive. Although the application was done in two split the infection at this rate was high. These results disagreed with what was found by Helms, (1990); Kurschner *et al.,* (1992) who found that splits application of nitrogen reduced the rice blast severity. This implied that as long as the nitrogen is excessive, the disease tend to increase. The soil in Mwea being vertisols could have had little leaching and probably the first split had not been exhausted by the time the second split was applied. Since the rice plants were grown in the pot there was no likelihood of leaching of nitrogen hence retaining high amount of nitrogen in the soil readily available to the plants. The growth contributed by nitrogen may have provided the pathogen with readily available substrate. As reported by Marchner (1995) that an increase in low molecular weight organic nitrogen compound as a substrate contributes to the high pathogen infection.

Plots applied with husk ash showed the least infection (Figure 1) indicating that the silicon present in the husk may have contributed to the low rice blast infection. High infection of rice blast was also realized in the plots that had no nitrogen 0 KgN ha^{-1} (N0) and in 40 KgN (N1) which was a low application rate. Much as high nitrogen level increased rice blast infection, nitrogen deficiency is also detrimental and there is an optimal level required by the rice plant to enables it resist *Pyricularia oryzae* pathogen. These observations indicate that rice blast infection would occur if nitrogen is limiting or if applied in excess (Figure 1).

Plots with higher silicon rates (1000 and1500 KgSi ha^{-1}) showed that plants were moderately resistant to the pathogen indicating that silicon played a role in disease management probably through its deposition in the cell wall creating mechanical barrier to pathogen penetration. It was also likely that the erectness caused by the silicon allowed more light to the leaves preventing the formation of large canopy hence un-conducive environment for the disease to thrive. Although it is not easy to establish the hypothesis with the prevailing data, there is a possibility that silicon triggers the rice plant cell defense mechanism such that by the time the appressoria penetrate the plant it hasphysiologically prepared its defenses. Since all plants produces phytoalexins against infection it is unclear on how silicon contributes to plant disease resistance. Further research is necessary.

Conclusion

The findings of this research indicate that rice blast disease can be managed through proper nutrition management. Nitrogen application at 120 Kg ha^{-1} led to high rice blast infection as well as in low (40 kgN ha^{-1}) application and where no (0 kgN ha^{-1}) nitrogen was applied. This brings to a conclusion that when nitrogen was deficient or applied below 80 kg/ha the plant resistance to rice blast was low. Since silicon managed the disease at 1000 and 1500 kgSi/ha and the two levels were not significantly different it would be reasonable to adopt a lower level. The interaction of silicon and nitrogen at 80KgN/ha, 1000 kgSi/ha (N2S2) may be adopted to manage the rice blast in Mwea irrigation scheme. The organic husk ash at 2 tons/ha may be useful in management of the disease since it played positive role in suppression of the disease. The husk ash performance was found to be as effective as those plots that had 120 kgN and 1000 KgSi/ha. It may therefore be used as an alternative source of silicate since it produced similar results as those produced by the commercial silicate. These husk have no other alternative use and hence farmers could make good savings inform of purchase of silicate fertilizer through the use of husks. Since the trial was conducted under screened house condition further work is recommended verifying these results under field conditions.

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