Integrated management of Bacterial Leaf Blight disease of rice using nitrogen fertilizer and growth regulators

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Abstract

A factorial experiment was conducted in 2010 and 2011 in Bende, Abia State, South Eastern zone of Nigeria, to determine if these growth regulators (chlormequat and ethephon) could be used in the control of Bacterial leaf blight (BLB) disease in a place where it is highly prevalent, using a susceptible rice variety (Faro 44) under high nitrogen fertilization. Three rates of nitrogen fertilizer: 0, 30, 60 kg N/ha, three inoculation rates: no inoculation (control), inoculation at tillering and seed soaking overnight in 600 mg/1 chlormequat and ethephon. These were tested in a split-plot design to establish if BLB could be controlled with the concentration (600 mg/1) of these chemicals using seed soaking technique. Application of nitrogen fertilizer to rice crop increased the incidence of BLB disease. The two growth regulators, especially chlormequat, reduced incidence of BLB disease and increased grain yield. This occurred even at high rates of nitrogen fertilization when the incidence of BLB was usually increased. Both chlormequat and ethphon also reduce plant height, root length, leaf water potential and stomatal resistance. However, chlormequat was better than ethephon in reducing the susceptibility of rice (Faro 44) to BLB and suppressing the incidence of the disease. It is therefore desirable to apply both chlormequat and supplemental nitrogen fertilizer where BLB susceptible rice varieties are planted.

Keywords: Chlormequat, ethephon, Bacterial leaf blight, susceptibility, inoculation and rice.

Introduction

Rice in Nigeria is mostly grown under rain-fed or irrigated lowland and a high proportion of farmers use high yielding Faro varieties like Faro 44, Faro 51, Faro 52 and Faro 57 along with other traditional varieties like MAS and EDU. All these are susceptible to BLB. The disease has been reported to destroy completely varieties like PUSA Basmati No. 1 and MAS (Habarurema et al., 2012; Maji and Shaibu, 2012). In many places the disease is often wrongly diagnosed as deficiency syndrome or lack of proper irrigation or symptoms caused by some fungal diseases and in an attempt to cure the symptoms urea has been applied wrongly (Maji and Imolehin, 2010). Urea, in fact aggravates BLB and the crop is destroyed within a week depending on the dosage applied (Maji and Imolehin, 2003).

The bacterial leaf blight (BLB) of rice caused by Xanthomonas oryzae pv. Oryzae is an important disease of rice (Oryza sativa L.) in tropical and subtropical countries. Yield losses of 20-30% are common, occasionally up to 50% (Mew, 1992; Verdier et al., 2011). In India yield losses have been higher, ranging from 74-81.3% depending on the cultivar and the growing season (Ahmed and Singh, 1975 and Singh et al., 1977). In farmers field around Bende in Abia State Nigeria, the region where the disease prevalence is high, the yield of Faro 44 was only 2.5-3.0 t/ha in that year

2007 when there was severe epidemic of BLB as against 4.0-5.0 t/ha in years with moderate rainfall and low BLB prevalence (Kurata and Yamazaki; Maji and Shaibu 2012). The disease is prevalent in most of the rice growing field of Nigeria, frequently occurring in the Northern States, Edozhigi and Wishishi (Maji and Imolehin, 2003). Appropriate use of fertilizer improves the photosynthetic activities in the plants. The resultant photosynthate will be used for the metabolic activities, hence increased tolerance to pathogenic attack. The growth regulator, on the other hand, help to increase the resistance of the propagule penetration through the stomata by reducing the size of their pores, by this, the degree of infestation will be minimized (Maji and Imolehin, 2003). It was noted that, inappropriate use of N-fertilizer, aggravates the virulence of BLB, so use of growth regulator in an integrated manner was seen to have increased yield in rice (Khan et al. 2000). The area under study is in rain forest zone, the environment has frequent and prolonged rain periods with cool temperature in the day time and under rainfed lowland so favourable to BLB (IRRI, 2010). Also, lack of flooding during planting season predisposes plants to the pathogen (Singh et al. 2004).

This trial was carried out to determine if integrated use of nitrogen fertilizer and growth regulators could be used in management of bacterial leaf blight to avoid any field out-break.

Materials and method

Preparation of Inoculum

Four hundred seeds were collected from the harvest of the rice field infested with BLB in Bende, south eastern zone, Nigeria.These were grouped into eight replicates of 50 seeds each in randomized complete block design. Seeds were equally spaced between two paper towels 45 x 28cm size (ordinary blotting paper) which have previously been soaked in tap water. The towels were carefully rolled and closed at the ends with rubber bands placed in polythene bags and finally incubated in an upright position at temperature 30^0C, twelve hours of light and dark, after 9 days, they were unrolled and the seedlings, examined carefully; seedling having water soaked areas, brown discolored coleoptiles or yellow discoloration in leaves were suspected to be infected with the bacteria leaf blight (BLB) organism (Veena et al. 2000). To confirm that the observed symptoms were caused by the (BLB) pathogens, small sections of the affected tissues were cut and placed on a glass slide in drop of water. The drop was covered by cover slips. The cut edges of the leaves were examined for bacterial ooze under compound microscope, where bacterial ooze was observed, pathogenic ity test was carried out. All seedlings showing infections were cut into small pieces (except roots) completely immersed in sterile water in a beaker. They were allowed for 15 minutes for the bacteria to ooze out of the tissue. These bacterial inoculums suspension were used to inoculate 30 – 40 leaves of 6 weeks – old rice plants of faro 44 earlier identified to be susceptible. This was done by dipping a pair of scissors in the bacterial suspension and then used to cut leaf tips ($3/4^{th}$ of an inch below the tip). The inoculated plants were covered for 24hrs with a polythene bag and incubated at 30^0C and exposed to sunlight for 12 hours. Within 48 – 72hrs they were checked for water soaked areas in the inoculated leaves (Veena, 1997).

Field experiment

Field experiments were conducted for two years (2010 and 2011) to determine the effects of applying nitrogen fertilizer and growth regulators on rice bacterial leaf blight incidence. Trials were conducted in inoculated plots of BLB-susceptible rice (variety Faro 44) in Bende experimental field of National Cereals Research Institute (NCRI) Amakama The experimental field is about sixteen hectares with its source of water from the dam constructed by the Ministry of Agriculture, Abia state. The site is in Bende Local Government Area, located at North eastern part of Umuahia, capital of Abia state, Nigeria. This is an irrigated lowland ecology, the water supply comes from the dam constructed by Abia State Ministry of Agriculture.

The experiment was arranged as 3x3x2 factorial in a randomized split-plot design with four replicates. The main plots were inoculation treatments – no treatment and soaking of seeds overnight in 600mg/1 (as the recommended dose for seed soaking) of both chlormequat ethephon. Sub-sub plots were nitrogen application rates – no nitrogen, 30 and 60kgN/ha. Each sub-plot was 3.8 m long, with 11 rows spaced 10 cm apart. Each main plot was separated by a 9m border plot of rice and the replicates were separated by 3m bare walk way. One week after seeding, half of the required doses (30 and 45 kgN/ha) was applied and the remaining half at panicle initiation stage. At tillering the plants were inoculated with bacterial suspension of BLB by dipping a pair of scissors in the bacterial suspension and then used to cut leaf tips ($3/4^{th}$ of an inch below the tip). The inoculated plants were covered for 24hrs with a polythene bag and incubated at 30^{0}C and exposed to sunlight for 12 hours. Within 48 – 72hrs they were checked for water soaked areas in the inoculated leaves. Un-inoculated plots served as the control. Plant height was measured in centimeter with a meter rule from the base to apex of the mother tiller in five randomly selected hills and the mean was calculated. Measurement of leaf stomatal resistance was by using a diffusive resistance meter (Lambda Instrument Corporation, Nebraska U.S.A Model) (LI-60) between 12.00 noon and 1.00 pm when the cloud was clear. Flag leaf of the mother tiller from five randomly selected hills was inserted in a chamber to read the rate of water diffusion from the guard cell to the surrounding cells. Root length was measured on 10^{th} day after seed soaking treatment and 9^{th} day after foliar application of the growth regulators. This was by digging a pit around a plant from five hills selected at random and directing a jet of water on the root system to dissociate the rootlets from the adhering soil particles. The length of the longest root was measured with a meter ruler in centimeter. Leaf water potential was determined in a pressure chamber [soil moisture equipment corporation, Califonia, U.S.A]. This was again carried out [12.00 – 13.00 hour] when the intensity of the sunlight was at maximum, 90 days after sowing. Grain yield was obtained by harvesting grains from 1 meter square per plot, winnowed, dried to 14% moisture content and weighed on a weighing balance and recorded in tons per hectare. The incidence of the disease was assessed using the Standard Evaluation System [SES] scale (0-9) developed by the International Rice Research Institute, Philippines [1988]. Statistical analysis was carried out by subjecting the data to analysis of variance procedures to determine the significance of the treatment effects and their interactions. A protected Duncan Multiple Range Test [DMRT] at p=0.05 level was carried out to compare treatment means.

Results

Means of the data collected for 2010 and 2011 experiments were used. Chlormequat and ethephon caused a significant [p=0.05] increase in the root length of rice compared to the control [Table 1). However, bacterial suspension inoculation and N fertilization rates did not show any significant effect on the root length. Plant height was significantly [p=0.05] reduced by both plant regulators (Table 1). There was no significant difference between the two chemicals in their effect on plant height. Application of nitrogen significantly increased plant height in the plots not treated with growth regulators. A significant reduction (p=0.05) in plant height was observed only in plots inoculated with *X. oryzae* under nitrogen application.Leaf water potential was significantly (p=0.05) increased by chlormequat and ethephon (Table 2). However, only chlormequat caused a significant (p=0.05) increase in stomatal resistance (Table 2).

Table 1. Effect of chlormequat and ethephon on root length and plant height of rice inoculated with *Xanthomonas oryzae* under different rates of nitrogen fertilization.

Fertilizer rates (Kg N/ha)	Inoculation treatment	Root Length (cm)			Plant height (cm)		
		Control	Chlormequat	Ethephon	Control	Chlormequat	Ethephon
0	no sclerotia	13a	18b	17b	76a	72b	71b
	Scleratia at tillering	10a	17b	16b	75a	70b	69b
30	no sclerotia	14a	19b	18b	82a	78b	79b
	Scleratia at tillering	12a	20e	12b	79a	69e	72e
60	no sclerotia	15a	18b	17b	83a	76b	77b
	Scleratia at tillering	13a	20e	18b	80a	68c	69c

Figures carrying a similar letter in each row and column are not significantly different at p=0.05 using Duncan Multiple Range Test [DMRT]. Figures are means of two years (2010 and 2011).

Table 2. Effect of chlormequat and ethephon on leave water potential and stomatal resistance of rice inoculated with Xanthomonas oryzae under different rates of nitrogen fertilization.

Fertilizer rates (Kg N/ha)	Inoculation treatment	Leaf water potential (-bar)			Leaf stomatal resistance (sec./cm)		
		Control	Chlormequat	Ethephon	Control	Chlormequat	Ethephon
0	no sclerotia	18a	13c	16c	8.3a	13.4b	9.6a
	Scleratia at tillering	16a	13c	15a	6.3a	15.2b	8.1a
30	no sclerotia	17a	12c	13c	7.3a	15.1b	10.5a
	Scleratia at tillering	18a	13c	14c	8.5a	17.3b	9.9a
60	no sclerotia	16a	11c	12c	8.2a	17.8b	12.2a
	Scleratia at tillering	17a	12c	11c	8.9a	17.6b	11.9a

Figures carrying a similar letter in each row and column are not significantly different at p=0.05 using Duncan Multiple Range Test (DMRT). Figures are means of two years (2010 and 2011) data

Also, neither increase in N-fertilization application rate nor inoculation with BLB pathogen showed any significant effect on these parameters.The differences in yield between inoculation and non-inoculated plants were not statistically significant (Table 3), chlormequat treated plots gave significantly (p=0.05) higher yield than the ethephon – treated and control plots. Nitrogen fertilization at different rates (0, 30 and 60 kg N/ha showed no significant effect on yield in the presence of both growth regulators (Table 3). The growth regulators significantly [p=0.05] reduced BLB incidence (Table 3). The variation in BLB incidence between the inoculated plots was not significant. No significant (P=0.05) differences in BLB incidence were recorded among inoculating treatments associated with either chlormequat or ethephon growth regulators across fertilizer application rates.

Discussion

The growth regulators tremendously reduced the incidence of BLB disease even where high doses of N-application would have increased it, thereby increasing yield. The growth regulators, especially chlormequat effectively reduced disease incidence in both inoculated and un-inoculated plants. This observation is different from the report by Veena et al., (2004) where growth hormones showed no influence on the disease severity of *Fusarium graminearum* on wheat and bacterial leaf blight of rice.

The reduction in plant height observed in plants treated with chlormequat and ethephon even where high rates of nitrogen were applied, was in line with earlier findings by Marthur, (1997). This is important because it will enable the plants to remain sturdy and dwarf even under maximum fertilization thereby reducing lodging. Improvements in the root length, leaf water potential and stomatal resistance which are indices for quantifying water status in plants, contributed to the suppressive effects of these growth regulators on BLB disease severity.

Table 3. Effect of chlormequat and ethephon on grain yield and bacterial leaf blight incidence after inoculation with Xanthomonas oryzae under different rates of nitrogen fertilizer.

Fertilizer rates (Kg N/ha)	**Inoculation treatment**	**Grain yield [t/ha]**			**BLB incidence (SES scale, 0-9)**		
		Control	**Chlormequat**	**Ethephon**	**Control**	**Chlormequat**	**Ethephon**
0	no sclerotia	0.7a	1.3b	1.0a	3a	0c	1c
	Scleratia at tillering	0.5a	1.2b	0.8b	5a	1c	3c
30	no sclerotia	1.0a	1.8c	1.5b	5a	0c	2c
	Scleratia at tillering	0.7a	1.6b	0.9b	8b	1c	3c
60	no sclerotia	1.0a	2.0c	1.5b	7b	1c	2c
	Scleratia at tillering	0.6a	1.7c	1.0b	9b	2c	3c

Figures carrying a similar letter in each row and column are not significantly different at p=0.05 using Duncan Multiple Range Test (DMRT). Figures are means of two years (2010 and 2011) data

Under a drought situation, this chemical would encourage the plants to extend their roots to the deeper soil layers to absorb water which is then distributed within the plants. In this manner, the casual organism for BLB disease was kept under check because a balanced water status was maintained in the plant making the plant to more resistant. (Maji and Rahaman,1995).

Our earlier report (Maji and Rahman, 1995) indicated the efficacy of foliar application of growth regulators, especially chlormequat, at a concentration of 1000 mg/1 in controlling blast disease. However, soaking seeds overnight prior to sowing in a 600 mg/1 concentration of the growth regulators is preferable.

The latter is less cumbersome, since it requires a lower quantity of chemical and could be adopted by the farmers. It is also useful where BLB-susceptible rice varieties are planted with a desirable supplemental application of nitrogen fertilizer.

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