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Research Article

Blast disease influence on agronomic and quality traits of rice varieties under Mediterranean conditions

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Abstract: The effects of *Pyricularia oryzae*, the causal agent of blast disease in rice, on plant growth, grain yield, and grain quality under Mediterranean conditions were studied in 2002 and 2003. Four rice varieties were grown under high and low disease pressure, achieved by early inoculation with the blast fungus and by natural infection, respectively. Results showed that inoculation affected the overall plant growth and yield and resulted in a reduction in plant height, number of productive tillers per plant, grain weight, and grain yield. The magnitude of the reduction was dependent on the varietals' susceptibility to the disease. Both types of the disease (leaf blast and neck blast) were negatively correlated with grain yield. It was estimated that each unit increase in leaf blast rating and neck blast resulted in 5.97 and 0.23 g plant^{-1} yield reduction, respectively. Grain quality traits were less affected by the disease treatments compared to the agronomic traits. The most pronounced effect of the disease was on total milling yield, which in some cases was reduced by inoculation up to 11%.

Key words: Grain quality, grain yield, leaf blast, neck blast, *Pyricularia oryzae*

Introduction

Blast disease [*Pyricularia oryzae* Cavara (synonym *P. grisea* Sacc (teleomorph *Magnaporthe grisea* (Hebert) Barr)], is one of the most destructive diseases of rice (*Oryza sativa* L.) worldwide (Ou 1985). The pathogen is most common on leaves, causing leaf blast during the vegetative stage of growth, or on neck nodes and panicle branches during the reproductive stage, causing neck blast (Bonman 1992). Leaf blast lesions reduce the net photosynthetic rate of individual leaves to an extent far beyond the visible diseased leaf fraction (Bastiaans 1991). Neck blast is considered the most destructive phase of the disease and can occur without being preceded by severe leaf blast (Zhu et al. 2005).

Climatic conditions affect greatly the disease establishment, development, and severity, resulting in large genotype-by-environment interactions. Most of the field research on rice blast disease has been conducted in tropical and subtropical environments. Several studies have shown that panicle and neck blast incidence was linearly related to yield loss (Tsai 1988;

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Torres and Teng 1993). In Europe, rice was planted in 594,000 ha in 2004 (International Rice Research Institute database, http://www.irri.org), the majority of which is in the Mediterranean region, where the climatic conditions favour high grain yield and quality. Serious losses of productivity due to blast infection have been reported in all European rice growing countries (Chataigner 1996). However, detailed information on the effect of blast disease on grain yield and its importance as a pre-harvest factor affecting grain quality of European rice is limited.

Therefore, the objective of this study was to investigate the effect of blast disease on rice growth, grain yield, and quality under Mediterranean conditions, and furthermore to identify associations between the disease and plant traits.

Materials and methods

Field experiments

The experiments were carried out at the Experimental Station of the Cereal Institute in Kalochori, Thessaloniki, Greece, in 2002 and 2003. The soil was a silty loam (Aquic Xerofluvents) with a pH of 7.5 and 1.6% organic matter. Four rice varieties of varying origin were used, namely Maratelli and Selenio from Italy, Senia from Spain, and Roxani from Greece. Apart from Maratelli, the other 3 varieties are currently cultivated in Europe. These varieties were chosen after a preliminary study in controlled environments, in order to represent a wide degree of response to blast disease and variability in agronomic and quality traits (Ntanos et al. 2002). Maratelli and Roxani are susceptible, while Selenio and Senia have moderate resistance to blast.

Plants were grown under 2 blast disease levels achieved by inoculation with conidia of the *P. oryzae* (high disease pressure) and by natural fungus infection (low disease pressure). The inoculated plants were grown 300 m away from the non-inoculated plants to avoid any cross infection. Seeds were sown in pots on 9 May 2002 and 6 May 2003. The seedlings were transplanted by hand into the field at the 5^{th} to 6^{th} leaf stage and arranged in a randomised complete block design with 3 replications. Plots were 1 m long and consisted of 3 rows 0.25 m apart with a 0.10 m on row spacing.

The field was flooded 1 day before transplanting and the water maintained between 5 and 10 cm deep until the grains reached at physiological maturity. Plots were fertilised with 55 kg N ha^{-1} as ammonium sulphate (21% N), 33 kg P ha^{-1} as super phosphate, and 62 kg K ha^{-1} as potassium sulphate (42% K and 17% S), all applied by hand broadcasting before transplanting. A further 145 kg N ha^{-1} was applied when rice was at the tillering stage, 50 kg N ha^{-1} at the stem elongation, and finally 50 kg N ha^{-1} at booting. The experimental area was kept free of weeds by hand weeding.

Inoculum preparation and disease assessment

Stock cultures were created from infected plant seeds of the Greek variety Roxani. The inoculum was prepared as described by Katsantonis et al. (2007). The fungus from the stock cultures was grown on rice flour agar medium (15 g L^{-1} rice flour, 20 g L^{-1} agar, 2.5 g L^{-1} yeast extract and 40 mg L^{-1} streptomycin). After 10 days a conidial suspension at a concentration of 5×10^4 per mL was prepared from the overgrown cultures. When the plants reached the 6^{th}-7^{th} leaf stage, 3 mL of conidial suspension was sprayed on each individual rice plant. After inoculation free water was sprinkled on the plants to facilitate the disease development. Blast assessment was performed on an individual plant basis. Leaf blast was recorded 20, 40, and 60 days after inoculation (DAI), using the lesion type rating scale from 1 to 6, where 1 = complete resistant plants, with no symptoms, 2 = resistant plants, with small brown spots of pin-point size or larger brown spots without sporulating centre, 3 = partially resistant plants, with small roundish to slightly elongated necrotic sporulating spots, 1-2 mm diameter with a brown margin or yellow border, 4 = susceptible plants, with grey narrow or elliptical lesions, 1-2 mm in breadth and 3 mm long surrounded by brown border, 5 = highly susceptible plants, with wide spindle-shaped grey lesions with brown or chlorotic border, and 6 = highly susceptible plants, with coalescent greyish to whitish lesions (dead leaves) (Notteghem 1981). Neck blast assessment was performed 55 days after the time of 50% heading as a percentage of the infected panicles against the total number of them. The area under the disease progress curve (AUDPC) was calculated for each variety according to Shaner and Finney (1977).

Data collection and plant sampling

Plant height, number of productive tillers per plant, and grain weight were determined at maturity. Grain yield was determined by harvesting the middle row from each plot. Grain quality traits, i.e. total milling yield, grain vitreosity, grain length, and grain length to width ratio, were determined according to Ntanos and Roupakias (2001). Weather data for each growing season were collected from a meteorological station located in the experimental site (Table 1). The weather was characterised by high relative humidity, an important prerequisite favouring disease development.

Results

In 2002, the rain events that occurred during the summer maintained the temperature at moderate levels and the relative humidity at higher levels compared to those in 2003 (Table 1). The mean relative humidity during the experimentation period was 92.9% in 2002 and 68.2% in 2003. Blast disease symptoms were observed on leaves and necks of plants in both disease treatments (Table 2). The disease level was dependent on variety and year of the experimentation. Leaf blast rating in the inoculated plants ranged from 2.67 (Senia) to 5.67 (Maratelli) in 2002 and from 3.17 (Senia) to 4.17 (Maratelli) in 2003. The corresponding values for neck blast varied from 30% (Senia) to 100% (Maratelli) in 2002 and from 30% (Senia) to 81% (Maratelli) in 2003. The infection of the non-inoculated plants was on average significantly ($P < 0.05$) lower compared to that of the inoculated plants in both years. Additionally, the AUDPC was significantly ($P < 0.05$) higher in the inoculated plants compared to non-inoculated plants in both years (Table 2), making possible the evaluation of agronomic and quality traits vs. disease. Leaf blast rating at 60 DAI was significantly correlated with neck blast ($r = 0.891$, $P < 0.01$).

Generally the inoculated plants showed lower plant height compared to the non-inoculated plants (Table 2). The reduction in plant height due to inoculation was significant ($P < 0.05$) for Maratelli and Selenio in both years and for Senia only in 2002. In Roxani there were no significant differences in plant height between inoculated and non-inoculated plants in both years.

Inoculation reduced the grain yield compared to the non-inoculated plants in both years and the magnitude of the reduction was dependent on the variety and year of the experimentation. Grain yield reduction due to inoculation in both years was consistently higher for the susceptible varieties Roxani, Selenio, and Maratelli and lower for Senia, a variety with moderate resistance to blast disease. Averaged across susceptible varieties, yield reduction due to inoculation was 22% in 2002 and 26% in 2003. The corresponding yield reduction for Senia was 8% in 2002 and 15.4% in 2003. Both leaf and neck blast levels were negatively correlated with grain yield (Figure 1). A significant negative correlation was also found between the AUDPC and grain yield ($r = -0.73$, $P < 0.01$).

Table 1. Monthly temperature, precipitation, and relative humidity at the farm of the Cereal Institute of Thessaloniki, Greece, during the growing season in 2002 and 2003

Month	Average temperature (°C)						Total precipitation (mm)		Average relative humidity (%)	
	Minimum		Maximum		Mean					
	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003
May	11.8	14.0	23.2	26.1	17.6	17.8	17	85	91.7	83.2
June	16.5	17.7	29.7	31.5	23.3	24.6	24	5	77.3	69.2
July	18.9	18.8	31.2	30.9	25.3	25.1	32	9	96.3	64.2
August	18.8	18.7	30.1	31.8	24.3	25.3	11	34	98.8	70.5
September	14.1	13.5	24.3	25.8	18.8	19.7	134	14	99.2	69.0
October	10.5	10.2	20.7	20.5	14.9	15.1	53	95	99.5	80.2

Table 2. Leaf blast rating, neck blast severity, area under the disease progress curve (AUDPC), and agronomic and grain quality traits of 4 rice varieties inoculated and non-inoculated with *P. oryzae* in field experiments conducted in 2002 and 2003

Year	Disease treatment	Variety	Leaf blast rating at 60 DAI (1-6)	Neck blast (%)	AUDPC	Plant height (cm)	Grain yield (g plant^{-1})	Productive tillers per plant (no.)	100-grain weight (g)	Total milling yield (%)	Grain length (mm)	Grain length to width ratio	Grain vitreosity (%)
2002	Inoculated	Maratelli	5.67	100	180	101	13.8	10	2.4	63.7	6.0	1.86	0.0
		Roxani	4.33	51	110	100	20.1	11	2.7	55.0	7.1	2.28	0.0
		Senia	2.67	30	110	91	29.8	13	2.6	66.4	6.2	1.98	0.0
		Selenio	5.00	91	137	87	18.7	11	2.3	66.7	5.2	1.72	50.0
		Mean	4.42	68	134	95	20.6	11	2.5	62.9	6.1	1.96	12.5
	Non-inoculated	Maratelli	4.67	97	77	107	15.4	11	2.8	63.9	6.1	1.88	7.8
		Roxani	2.33	41	53	101	30.2	14	3.1	61.1	7.2	2.34	0.0
		Senia	2.00	22	50	98	32.4	15	3.0	66.5	6.3	2.02	0.0
		Selenio	4.00	87	70	94	23.6	14	2.3	66.2	5.2	1.72	64.5
		Mean	3.25	62	62	100	25.4	14	2.8	64.4	6.2	1.99	18.1
	LSD 0.05[a]		0.39	5.1	9.3	1.9	1.65	1.2	0.09	1.43	0.10	0.060	4.20
	LSD 0.05[b]		0.78	10.2	18.5	3.9	3.29	2.4	0.19	2.86	0.21	0.120	8.41
2003	Inoculated	Maratelli	4.17	81	130	107	21.3	8	2.6	65.7	5.9	1.77	0.0
		Roxani	3.83	52	105	103	24.9	9	2.7	61.2	6.9	2.16	0.0
		Senia	3.17	30	98	93	31.8	10	3.1	68.9	6.1	1.92	0.0
		Selenio	3.67	55	97	90	22.9	9	2.3	67.5	5.3	1.73	65.5
		Mean	3.71	55	108	98	25.2	9	2.7	65.8	6.1	1.90	16.4
	Non-inoculated	Maratelli	3.67	64	67	121	29.5	10	2.9	66.3	5.8	1.82	0.0
		Roxani	2.67	47	57	108	31.9	11	3.1	61.6	6.7	2.25	0.0
		Senia	2.00	28	50	98	37.6	13	3.2	68.9	6.1	1.97	0.0
		Selenio	3.17	48	62	102	31.9	10	2.5	70.2	5.3	1.74	66.0
		Mean	2.88	47	59	107	32.7	11	2.9	66.8	6.0	1.95	16.5
	LSD 0.05[a]		0.41	12.7	7.2	3.9	1.69	1.2	0.09	0.96	0.06	0.026	0.75
	LSD 0.05[b]		0.83	25.5	14.4	7.8	3.38	2.3	0.19	1.92	0.14	0.053	1.50

[a]Least significant difference for comparing disease treatment means in each year.
[b]Least significant difference for comparing varieties in each year.

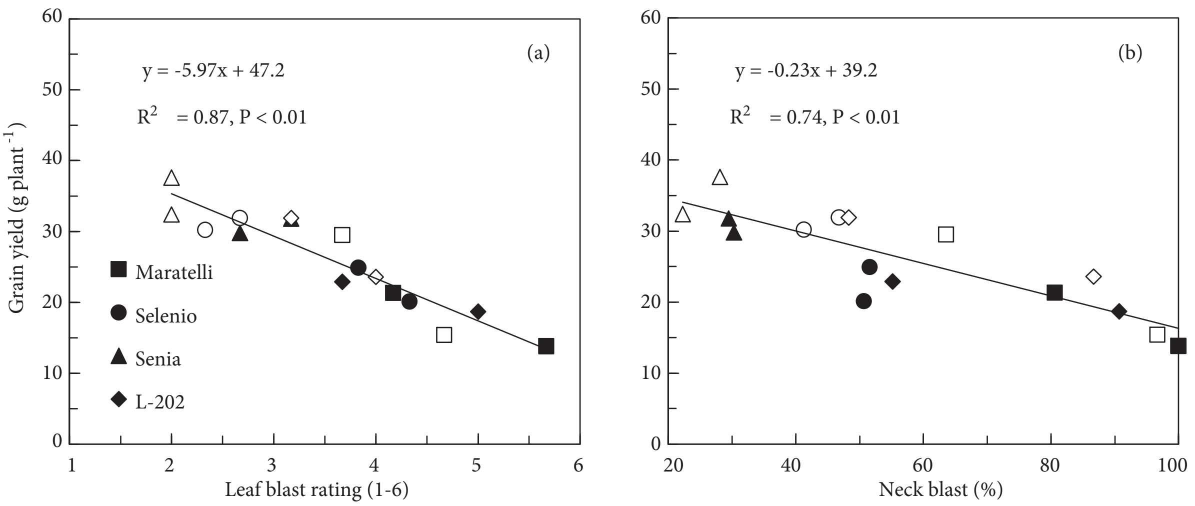

Figure 1. Relationships between (a) leaf blast rating at 60 days after inoculation and grain yield, and (b) between neck blast and grain yield in rice. Each relationship was based on means derived from 4 varieties, 2 disease treatments (inoculated, filled symbols; non-inoculated, empty symbols), and 2 years of experimentation (n = 16)

The number of productive tillers per plant was significantly reduced by inoculation in Roxani and Selenio in 2002 and in Senia in 2003. In the other varieties, the inoculated plants had lower number of productive tillers per plant compared to the non-inoculated plants but the differences were not significant. Leaf blast rating and AUDPC were significantly correlated with the number of productive tillers per plant ($r = -0.51$, $P < 0.05$ and $r = -0.52$, $P < 0.05$, respectively).

Inoculation resulted in lower grain weight compared to the non-inoculated plants. Averaged across varieties, grain weight reduction due to inoculation was 11% in 2002 and 7% in 2003. Varieties responded differently to inoculation concerning the grain weight. Maratelli and Roxani in both years, Senia in 2002, and Selenio in 2003 had significantly lower grain weight under inoculation compared to the non-inoculated plants. Both leaf and neck blast levels were significantly correlated with grain weight ($r = -0.68$, $P < 0.01$ and $r = -0.62$, $P < 0.05$, respectively). Moreover, a negative correlation between the AUDPC and grain weight was found ($r = -0.59$, $P < 0.05$).

The mean over varieties total milling yield of inoculated plants was significantly lower compared to that of the non-inoculated plants in both years (Table 2). However, the response of the most varieties to inoculation was marginal except for Roxani in 2002 and Selenio in 2003 in which the total milling yield was significantly reduced by inoculation. Grain length and grain length-to-width ratio were similar in both disease treatments. Grain vitreosity was reduced by inoculation in Maratelli and Selenio in 2002, while it was not affected in the other varieties.

Discussion

The yearly differences that occurred in blast infection levels were most likely due to the corresponding differences in the weather parameters recorded during the growing season. Both leaf and neck blast infections were higher in 2002 probably due to the development conditions being more favourable for the disease (high relative humidity and moderate temperature) compared to 2003. It is well documented that the environmental conditions, especially relative humidity, are one of the most important factors affecting sporulation, release, and germination of blast conidia (Ou 1985). Inoculated plants were found to have higher disease levels than the non-inoculated plants and more constant disease development across years, indicating that inoculation was essential for achieving a high and uniform disease level in the field.

The differences in leaf and neck blast levels detected among the varieties followed the corresponding differences in varietal susceptibility to disease. The values of disease levels for both types of symptoms were found to be consistently higher in the variety Maratelli and lower in Senia. Previous studies have shown that Maratelli is susceptible to blast disease, while Senia has moderate resistance (Ntanos et al. 2002). The positive correlation detected between leaf blast and neck blast probably suggests that a high level of leaf blast achieved by early inoculation resulted in severe neck blast during the later stages of plant development.

The soil properties were similar in inoculated and non-inoculated plots and therefore the differences found in agronomic and quality traits between the disease treatments could be attributed to the effect of the disease. The greater disease level of the inoculated plants was found to have a shortening effect, causing a mean plant height reduction of 5.5% in 2002 and 8% in 2003 compared to the non-inoculated plants. These results are in agreement with those given by Torres and Teng (1993), who reported a negative effect of blast disease on plant height proportional to disease level.

Inoculation was found to reduce the grain yield up to 33% compared to the non-inoculated plants. However, the magnitude of the reduction was dependent on the variety and year of the experimentation, reflecting the variation in genotype response to blast disease and the differences in the level of disease between inoculated and non-inoculated plants in each year. Yield losses due to rice blast reported in the literature exceed 50% over large areas in some parts of the world (Ou 1985), and they have been ascribed to several different reasons. Bastiaans and Kropff (1993) reported that the disease leads to a reduction in canopy photosynthesis mainly due to an adverse effect of lesions on leaf photosynthetic rate and also to shading by dead leaf area resulting from disease induced senescence. Koutroubas et al. (2009) found that blast disease reduced the accumulation and remobilisation of the pre-anthesis assimilates to the grains of rice. In the current study, both types of the disease (leaf blast and neck blast) were found to be negatively correlated with grain yield. Based on the linear equations relating these parameters, it could be estimated that each unit increase of leaf blast rating corresponded to a decreased grain yield of 5.97 g $plant^{-1}$. The corresponding reduction in grain yield for each unit increase of neck blast was 0.23 g $plant^{-1}$. The associations between blast severity and grain yield reported in the literature are rather variable, since they are dependent on the environment and the level of disease infection. Torres and Teng (1993) reported that neck blast infection was directly related to yield loss, while it was less reduced by collar infections. The associations between grain yield and disease severity found in the present study concerned the environment in which the experiments were carried out. Controlled experiments are needed to extract more generalised conclusions.

Rice grain yield is the final product of a combination of different yield components, the relative importance of which varies with the location, season, crop duration, and cultural system (Yoshida 1983; Koutroubas and Ntanos 2003). Both yield components evaluated in our study (productive tillers per plant and grain weight) contributed to the differences in grain yield obtained between the disease treatments. Inoculation caused a reduction in grain weight compared to the non-inoculated plants. The negative effect of the disease on grain weight was also confirmed by the negative correlations detected between this trait and leaf and neck blast. These results could be explained taking into account the specific nature of blast disease in rice. Infection of plants during the generative growth stages mainly results in panicle or neck infections that may cause necrosis of the plant neck and incomplete grain filling. Candole et al. (2000) reported that rough rice from blast-infected panicles was drier and thinner than on blast-free panicles. Inoculation was also found to reduce the number of productive tillers per plant in both years. The reduction was proportional to the disease level as suggested by the negative correlation found between leaf blast and number of productive tillers per plant.

Rice quality is of great importance for all people involved in producing, processing, and consuming, since it affects the nutritional and commercial value of grains. In this study, grain quality traits were less affected by the disease treatments compared to the agronomic traits. No direct effect of blast disease on grain quality seemed to exist, as suggested by the lack of significant correlations between leaf blast rating or neck blast and quality traits (data not shown). However, the effect of the disease on grain quality could be indirect though the influence of various physiological parameters during the grain filling process. Total milling yield, a parameter involved in the determination of milling quality of rice, was reduced by inoculation only for Roxani in 2002 and for Selenio in 2003. The magnitude of the reduction was 11% and 4%, respectively, revealing an important negative economic influence for farmers and rice mills. The values of grain length and grain length to width ratio were found to be similar in inoculated and non-inoculated plants. One explanation for this could be that grain size and shape, as it is characterised by the grain length and grain length to width ratio, are traits that are little affected by the environment (Chandraratna 1964). It has been reported that grain shape is simultaneously controlled by genes expressed in the triploid endosperm, cytoplasm, embryo, and maternal tissues (Shi and Zhu 1996; Shi et al. 2000). A remarkable reduction in grain vitreosity due to inoculation was found only for Maratelli (100%) and Selenio (22%) in 2002. The lack of response obtained in the other 2 varieties was due to their very low values of grain vitreosity (Koutroubas et al. 2004).

Conclusions

Our results emphasise the important effects of blast disease on rice growth and productivity under Mediterranean conditions. Plants grown under high disease levels (inoculated) showed reduced plant height and grain yield compared to those grown under low disease levels (naturally infected). The magnitude of the reduction was dependent on the differences in the disease pressure between the treatments and the varietal susceptibility to the disease. Both grain weight and productive tillers per plant were reduced by inoculation, contributing to the differences in grain yield obtained between the disease treatments. The effect of the disease on grain quality traits was in most cases marginal, but in some varieties blast caused a remarkable reduction in total milling yield and grain vitreosity.

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