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Response of the panicles exserted from the caulis and from various effective tillers at four stages of panicle development to neck blast in rice

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Abstract The rice *japonica* variety Nipponbare and the *indica* variety 93-11, the genomic DNA sequences of which are known, were used to analyze the response of the panicles exserted from the caulis and from various effective tillers at four stages of panicle development to neck blast. Disease incidence in the necks (DIN), disease incidence in the rachis nodes (DIRN), lesion length in the necks (LLN), and number of conidia in the necks (NCN) were measured after inoculating the panicles in vitro of two rice varieties with *Magnaporthe oryzae*. Both Nipponbare and 93-11 were susceptible, DIN and DIRN of all panicles being 100% in both the varieties except DIRN in several panicles at stage 1 (the panicle fully exserted) in Nipponbare. Both LLN and NCN of panicles decreased as the panicles continued to develop. However, the patterns of this decrease in the panicles from the caulis and from various effective tillers were substantially different in the two varieties. In Nipponbare, neck blast became progressively severe in the order of emergence of the panicles from the caulis and from six effective tillers: values of LLN and NCN were the lowest in the panicles from the caulis, intermediate in those from first-class tillers, and the highest in those from second-class tillers. In 93-11, however, the source of panicles had no significant influence on LLN and NCN.

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Abbreviations

NCN	number of conidia in the necks
DIN	disease incidence in the necks
DIRN	disease incidence in the rachis nodes
LLN	lesion length in the necks
PP	panicle position
SPD	stage of panicle development

Introduction

Rice blast, caused by *Magnaporthe oryzae* B. Couch, is the most devastating fungal disease of rice (Couch and Kohn 2002). It has been estimated that, each year, the disease kills enough rice to feed 60 million people (Jena 2006). Rice blast occurs in two forms: leaf blast and neck blast. Because it takes longer to study the resistance to neck blast and it is cumbersome to inoculate the rice panicles with *M. oryzae*, most of the research on resistance to blast in rice has focused on the resistance to leaf blast and also that in the seedlings. However, neck blast is the major cause of yield loss, and the research on resistance to neck blast has been receiving increasing attention in recent years, although the difference in resistance shown by the panicles between from the caulis and from various effective tillers (first-class tillers, second-class tillers, etc.) at different stages of panicle development remains one of the neglected aspects (Pattama et al. 2002; Zhuang et al. 2002; Hu et al. 2006; Puri et al. 2009).

The effect of leaf age and nodal position on leaf receptivity to rice blast was studied by inoculating potted plants of 'Rosemont' (*Oryza sativa* L subsp. *japonica*) at different growth stages with *M. oryzae* (Yang et al. 1998). The number of sporulating lesions per square centimetre of inoculated leaves and the average lesion density for a given nodal position gradually decrease as the leaves develop. Lesion density is comparable in leaves less than 1 day old developing from the 4th node to the 7th node but drops sharply in those developing from the 7th node to the 11th node. As to panicles, Tokunaga et al. (1966) have reported that resistance to neck blast increases as the panicles age. Rice spikelets become increasingly resistant with the passage of time and are seldom infected three weeks after heading, whereas the neck and branches of panicles (comprising the rachis, its branches, and pedicels) are susceptible even later compared to spikelets; besides the developmental phase of their proneness is not similar, an observation corroborated by Kobayashi et al. (2009).

Our unpublished work indicates that resistance to neck blast in the panicles exserted from the caulis is positively correlated with that shown by the panicles from tillers in some rice varieties, but in a few varieties these two kinds of panicles differ significantly in the degree of resistance. It is therefore important to ascertain whether such a difference exists while identifying and evaluating the resistance to neck blast in rice varieties or studying the mechanism of resistance to neck blast—as done here with respect to the *japonica* variety Nipponbare and the *indica* variety 93-11 at four stages of panicle development.

Materials and methods

Plant materials and culture

The rice varieties Nipponbare (*Oryza sativa* L subsp. *japonica*) and 93-11 (*Oryza sativa* L subsp. *indica*), the genomic DNA sequences of which are known, were provided by the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. Rice plants were grown under natural light in a greenhouse (20–30°C). The sheath bases of the caulis and all the tillers were tagged with cards of different colours to mark the temporal sequence of appearance of the caulis and tillers right from the seedling stage. The tillers produced by the caulis were designated first-class tillers and those produced by first-class tillers were designated second-class tillers.

Fungal pathogen and inoculation

A strain of *M. oryzae* (06-257, race ZG_1 (Atkins et al. 1967; The united experimental group of physiologic races of *Pyricularia oryzae* in China 1980; Yamasaki and Kozaka 1980)), isolated and maintained in our laboratory, was grown on oatmeal agar and incubated at 26°C. After 14 days of incubation in the dark, the conidia were harvested by adding sterile water and scraping with a trigonal stick. The conidial suspension was filtered and adjusted to 1×10^5 conidia ml^{-1} as ascertained with a haemocytometer.

Sample collection

On average, the total number of leaves was 13 in Nipponbare and 11 in 93-11, respectively. Nipponbare can produce 7 panicles exserted from the caulis and six effective tillers, 4 first-class and 2 second-class (Fig. 1a); 93-11 can produce 6 panicles from the caulis and 5 effective tillers, 4 first-class and 1 second-class (Fig. 1b). The panicles from the caulis and from effective tillers of the two rice varieties were sampled at each of the four stages of panicle development described as follows, which correspond to the standard evaluation system developed by IRRI for rice panicle exsertion (IRRI 2002): stage 0, the panicle still enclosed in the leaf sheath; stage 1/3, the panicle freshly exserted and shorter than 1/3rd the length of the whole panicle; stage 2/3, the panicle moderately well exserted but not fully so and longer than 2/3rd the length of the whole panicle; and stage 1, the panicle fully exserted with at least one rachis node out of the leaf sheath.

Inoculation

Neck pieces of 6 cm length, containing two or three rachis nodes, were cut from the panicles and placed

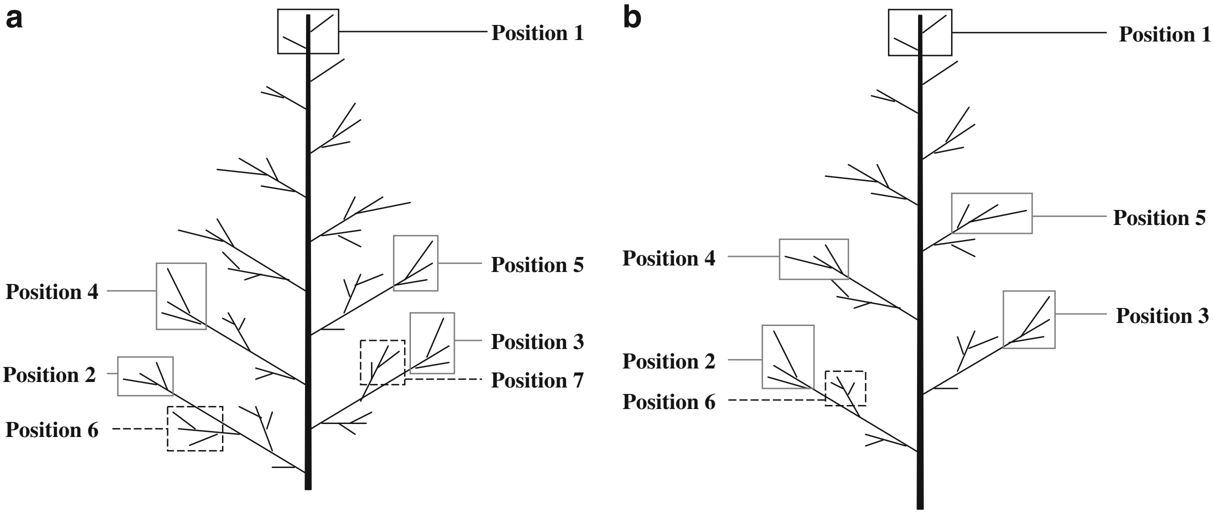

Fig. 1 The caulis and effective tillers in the rice *japonica* variety Nipponbare (**a**) and the *indica* variety 93-11 (**b**). Nipponbare has 6 effective tillers and 93-11 has 5. Position 1 - caulis panicle, position 2 - the first first-class tiller, position 3 - the second first-class tiller, position 4 - the third first-class tiller, position 5 - the fourth first-class tiller, position 6 - a second-class tiller from the first first-class tiller, position 7 - a second-class tiller from the second first-class tiller

onto paper filters that had been pre-soaked in 0.1 mM benzimidazole suspension in glass dishes (10 cm diameter) (Rao et al. 2005; Hao et al. 2009). If a neck piece had two rachis nodes, both the nodes were inoculated; if it had three rachis nodes, the middle one was left out and the other two were inoculated. Each node was inoculated, using a micropipette, with 2 μl conidial suspension containing 2% (*w/v*) carboxymethyl cellulose.

Evaluation of disease severity

All the observations were recorded 10 days after inoculation and comprised the following: disease incidence in the necks (DIN), disease incidence in the rachis nodes (DIRN), lesion length in the necks (LLN), and number of conidia in the necks (NCN) of panicles. LLN was the average of two lesions produced on each neck piece. To measure NCN, each inoculated neck piece was put into a test tube with 1 ml sterile water, and the conidia were rinsed off by votexing for 1 min and then counted using a haemacytometer (Rao et al. 2005).

Statistical analysis

Each value was the mean of three independent experiments with 10–12 inoculated neck pieces in each experiment. Two-way ANOVA was used to detect the differences due to the two main effects, namely stage of panicle development and panicle position, and the interaction effect of these two factors; the response-surface methodology was used for regression analysis (Hohnson and Berger 1982; Momen et al. 2001). Regression equations and the corresponding graphs were obtained with Statistica 6.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results

Nipponbare

DIN of the panicles exserted from the caulis and from six effective tillers was 100% at every stage of panicle development and so was DIRN of all panicles except in the panicles from several panicle positions at stage 1 (data not shown).

LLN of the panicles for a given position became shorter as the panicles continued to develop (Fig. 2a). Furthermore, the difference in LLN between two designated stages of panicle development in the panicles from the caulis and from six effective tillers also decreased gradually ($P<0.01$) in the order of appearance of the caulis and tillers. For example, LLN of the panicles from the caulis at stage 0 was

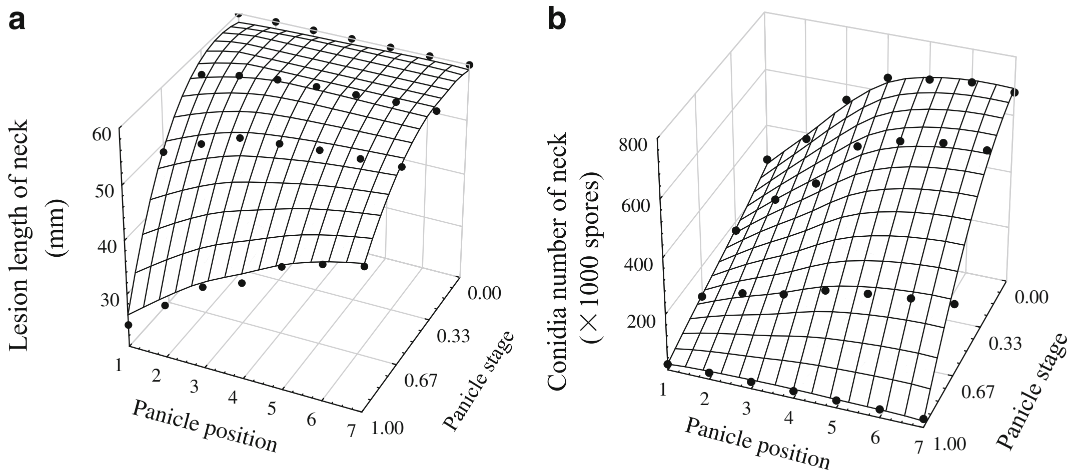

Fig. 2 Lesion length (**a**) and number of conidia (**b**) in the necks of the panicles exserted from the caulis and from six effective tillers at four stages of panicle development in the rice *japonica* variety Nipponbare. X axis: stage 0 - the panicle still enclosed in the leaf sheath, stage 1/3 (0.33) - the panicle freshly exserted and shorter than 1/3rd the length of the whole panicle, stage 2/3 (0.67) - the panicle moderately well exserted but not fully so and longer than 2/3rd the length of the whole panicle, stage 1 - the panicle fully exserted with at least one rachis node out of the leaf sheath. Y axis: position 1 - caulis panicle, position 2 - the first first-class tiller, position 3 - the second first-class tiller, position 4 - the third first-class tiller, position 5 - the fourth first-class tiller, position 6 - a second-class tiller from the first first-class tiller, position 7 - a second-class tiller from the second first-class tiller. Black dots indicate lesion length or number of conidia in the necks of the panicles from the caulis and from effective tillers at four stages of panicle development. Each value was the mean of three independent experiments with 10–12 samples in each experiment

35.9 mm longer than that at stage 1, but the corresponding difference in LLN between these two stages of panicle development in the panicles from a second-class tiller from the second first-class tiller was only 13.3 mm. LLN of the panicles from all positions was 60 mm at stage 0, in other words, the lesion had covered the entire length of the 6 cm long pieces, and the black layer of conidia was clearly visible to the naked eye. The pattern of change in LLN in all panicles at stage 1/3, stage 2/3, and stage 1 was similar: LLN of the panicles from the caulis was the shortest, that of the panicles from first-class tillers was intermediate, and that of the panicles from second-class tillers was the longest. In Nipponbare, LLN of the panicles from the caulis and from six effective tillers was intuitively ranked as follows: caulis panicle (position 1) < the first first-class tiller (position 2) ≤ the second first-class tiller (position 3) ≤ the third first-class tiller (position 4) ≤ the fourth a first-class tiller (position 5) ≤ a second-class tiller from the first first-class tiller (position 6) = a second-class tiller from the second first-class tiller (position 7) ($P<0.01$). The differences in LLN in the panicles from between two designated positions were the largest at stage 1. For instance, LLN of a panicle from the caulis was 24.1 mm whereas that of a panicle from position 7 was 46.7 mm at stage 1, a difference of 22.5 mm. However, the differences were not as wide at the later stages, the differences in LLN between these two panicle positions being 3.3 mm at stage 1/3 and 7.8 mm at stage 2/3, respectively. The relationship among stage of panicle development (SPD), panicle position (PP), and LLN was $Y_{LLN}=-3.741X_{SPD}-32.159X_{SPD}^2+1.189X_{PP}-0.204X_{PP}^2+3.604X_{SPD}\cdot X_{PP}+58.749$ ($R^2=0.9435$, $P<0.01$).

NCN of the panicles for a given position decreased as the panicles continued to develop (Fig. 2b), especially at stage 1 ($P<0.01$). The differences in NCN between two designated stages of panicle development in the panicles from the caulis and from six effective tillers gradually increased ($P<0.01$) in the order of appearance of the caulis and tillers. There were no significant differences in NCN between any two panicle positions at stage 1, and the pattern of change in NCN in the panicles at stage 0, stage 1/3, and stage 2/3 was similar to that in LLN in the panicles at stage 1/3, stage 2/3, and stage 1. In Nipponbare, NCN of panicles was intuitively ranked as follows: position 1 ≤ position 2 ≤ position 3 < position 4 ≤ position 5 ≤ position

6 = position 7 (P<0.01). The relationship among SPD, PP, and NCN was $Y_{NCN}=-69.602X_{SPD}-181.698X_{SPD}^2+154.596X_{PP}-8.946X_{PP}^2-76.8X_{SPD}\cdot X_{PP}+130.364$ (R^2=0.9482, P<0.01).

93-11

DIN of the panicles exserted from the caulis and from six effective tillers was all 100% at every stage of panicle development and so was DIRN of all panicles.

LLN of the panicles for a given position became shorter as the panicles continued to develop (Fig. 3a). However, the differences in LLN between two designated stages of panicle development in the panicles from the caulis and from five effective tillers hardly changed. The pattern of change in LLN in all panicles at all the four stages was similar: LLN of the panicles from the caulis was the longest, LLN of the panicles from the four first-class tillers gradually increased in the order of appearance of the tillers, and LLN of the panicles from second-class tillers from the first first-class tillers began to decrease. However, LLN did not differ significantly in the panicles between from the caulis and from five effective tillers (P<0.01). The relationship among SPD, PP, and LLN was $Y_{LLN}=6.392X_{SPD}-19.701X_{SPD}^2-0.304X_{PP}+0.044X_{PP}^2+0.114X_{SPD}\cdot X_{PP}+59.607$ (R^2=0.9719, P<0.01).

NCN of the panicles for a given position decreased as the panicles continued to develop (Fig. 3b), especially at stage 1 (P<0.01), a pattern similar to that observed in Nipponbare. The differences in LLN between any two designated stages of panicle development in the panicles from the caulis and from five effective tillers were minimal. The pattern of change in NCN of all panicles at all the four stages was similar to that in LLN, there were no significant differences in LLN between any two panicle positions at a given stage of panicle development (P<0.01). The relationship among SPD, PP, and NCN was $Y_{NCN}=-245.406X_{SPD}-147.824X_{SPD}^2+0.634X_{PP}-0.638X_{PP}^2+6.098X_{SPD}\cdot X_{PP}+610.574$ (R^2=0.9397, P<0.01).

Discussion

Both Nipponbare and 93-11 were susceptible to *M. oryzae*, DIN and DIRN of all panicles being 100% in both the varieties except DIRN in several panicles at stage 1 in Nipponbare. These results suggest that DIN and DIRN for measuring the severity of neck blast between panicles in different positions or at different stages of development are not compatible measures in Nipponbare and 93-11.

The patterns of changes in LLN and NCN in the panicles exserted from the caulis and from various effective tillers at the same stage of panicle development in Nipponbare were totally different from those in 93-11. The neck blast became progressively severe in the order of appearance of the panicles from the caulis and from

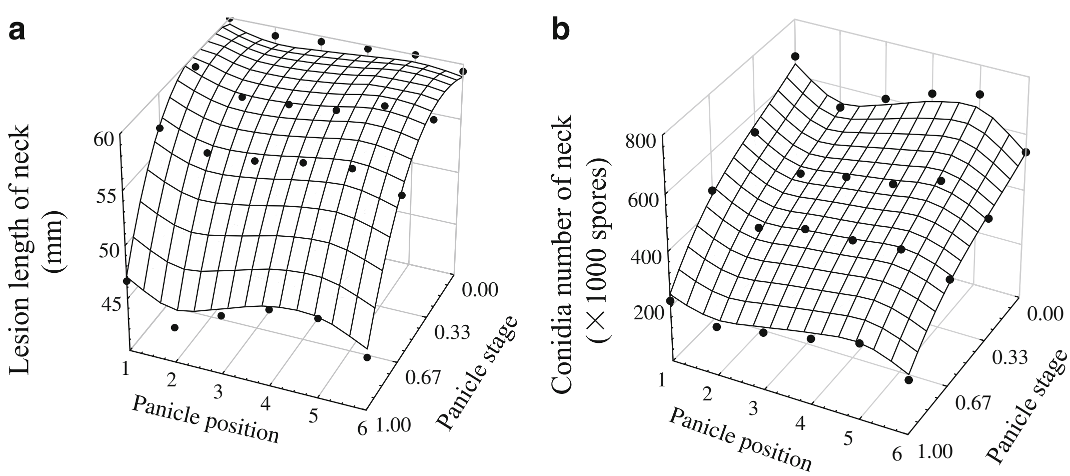

Fig. 3 Lesion length (**a**) and number of conidia (**b**) in the necks of the panicles exserted from the caulis and from five effective tillers at four stages of panicle development in the rice *indica* variety 93-11. The note is the same as that for Fig 2

six effective tillers in Nipponbare, whereas there were no significant differences in LLN and NCN in the panicles between from the caulis and from five effective tillers in 93-11. Therefore, we should choose the panicles in different positions in studies on the resistance to neck blast or on the mechanism of resistance to neck blast in Nipponbare; in 93-11, the choice is not critical—any of the panicles from the caulis and from five effective tillers can be chosen. These results suggest that the differences in resistance to neck blast between the panicles from different positions (from the caulis and from effective tillers) vary with the varieties and should be ascertained before choosing panicles as experimental material.

Our earlier research showed that, according to the standard evaluation system developed by IRRI (IRRI 2002), Nipponbare is susceptible (resistance level 7) and 93-11 is moderately susceptible (resistance level 5) to leaf blast caused by *M. oryzae* (strain 06-257, race ZG_1). However, the small lesions of *M. oryzae* in the necks of panicles were visible to the naked eye 3 days after inoculation in 93-11 but 5 days after inoculation in Nipponbare, which suggests that the incubation period of *M. oryzae* in the necks of panicles is shorter in 93-11 than in Nipponbare. Furthermore, LLN in the panicles in many positions at stage 1/3, stage 2/3, and stage 1 was longer in 93-11 than in Nipponbare, especially at stage 2/3 and stage 1 (LLN of the panicles from the caulis and from effective tillers was nearly 60 mm at stage 0 in Nipponbare and 93-11). NCN of the panicles at stage 2/3 and stage 1, and of the panicles from the caulis, the first first-class tiller, and the second first-class tiller at stage 1/3 and stage 0 was significantly higher in 93-11 than in Nipponbare. These results show that resistance to neck blast was possibly poorer in 93-11 than in Nipponbare ($P<0.01$, *t*-test), a pattern differs from that to leaf blast in 93-11 and Nipponbare. In 93-11, the resistance is therefore stage-specific: resistance to leaf blast (*M. oryzae* strain 06-257) at the seedling stage is not positively correlated with resistance to neck blast at the panicle stage, and similar differences also exist in a few other rice varieties (Koh et al. 1987; Bonman 1992; Zhuang et al. 2002; Hao et al. 2009)

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