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Greenhouse techniques to identify field resistance to the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), in rice cultivars

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ABSTRACT. Two seedbox screening techniques were compared to determine levels of resistance of rice cultivars to three brown planthopper, *Nilaparvata lugens* (Stål), biotypes. The commonly used standard seedbox screening test (SSST) where eight second-instar nymphs were infested on 7-day-old seedlings was compared with a modification (MSST) where four second-instar nymphs were infested on each of 20-day-old plants. In the SSST, the initial infestation killed the susceptible cultivars whereas in the MSST the F_1 progeny killed the susceptible cultivars. The cultivars ASD 11, Wagwag, Utri Rajapan, IR46 and Kencana were susceptible in the SSST but moderately resistant or resistant in the MSST, indicating an increase in the level of resistance with plant age. Feeding rate and population growth of *N. lugens* were the factors involved in the resistance of 30-day-old plants. The MSST provides a rapid method for identifying field-resistant cultivars in the greenhouse and can be used to identify field-resistant lines in a rice-breeding programme for stable resistance to *N. lugens*.

Introduction

Rice cultivars with resistance to the brown planthopper have been developed and released for commercial cultivation in many countries of South-East Asia. IR26, the first cultivar resistant to *N. lugens*, was released in 1973 and was extensively grown in Indonesia, Philippines, and Vietnam. After two or three years of such cultivation, IR26, which has a major resistance gene (*Bph1*) became susceptible because of selection for a virulent population, named biotype 2 (Pathak and Heinrichs, 1982a). In 1976 two further cultivars, IR36 and IR42, with the recessive gene *bph2* for *N. lugens* resistance, were released and these currently occupy much of the rice-growing areas in the Philippines, Indonesia and Vietnam and are planted in several other countries, where IR36 alone occupies about 11 million hectares (IRRI, 1983). *N. lugens* populations that are 'virulent' to both IR36 and IR42 have been reported from Mindanao in the southern Philippines (IRRI, 1983b) and North Sumatra in Indonesia (Sogawa, Kilin Djatnika and Bahagiawati, 1984). The resistance of IR36, however, has been more durable than that of IR42. In attempts to rear these field populations of *N. lugens* continuously from Mindanao on IR36 in the greenhouse, the populations gradually declined on this cultivar, whereas high populations were maintained on IR42 (E. A. Heinrichs, unpublished work).

IR46, which has the *Bph1* gene, is susceptible to biotype 2 of *N. lugens* as a 7-day-old seedling but is resistant to the same biotype as an older plant in the greenhouse and in the field (Panda and Heinrichs, 1983). In international tests co-ordinated by the International Rice Research Institute (IRRI), IR46 has shown field resistance in Asia despite the fact that it is susceptible to the local biotype in the seedling stage in several countries (IRRI, 1982a).

IR36, IR46 and several other cultivars which have been evaluated in the breeding programme for *N. lugens* resistance at IRRI, have either major genes which are not expressed in young seedlings, or both major and minor genes. The minor genes would provide polygenic resistance which may be more effective than major-gene resistance in combating the genetic plasticity of pests (Simons, 1972). According to Takase (1962), major-gene resistance is qualitative, whereas polygenic resistance, which he equated with field resistance, was quantitative. Van der Plank (1968)

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suggested the term 'horizontal resistance' to denote resistance governed by polygenes. Russell (1978) used the terms, 'adult resistance', 'mature-plant resistance' and 'field resistance' for the phenomenon where the cumulative effect of minor genes for resistance is increasingly expressed as plants grow older. In this paper, we are using Russell's definition of field resistance, where the level of resistance increases with plant age regardless of whether measurement of levels of resistance are made under laboratory, or greenhouse or field conditions.

Because of the severity of the *N. lugens* biotype selection problems in rice, the breeding strategy at IRRI is to develop field-resistant varieties which have horizontal resistance to all biotypes and to incorporate field resistance into cultivars having major genes for resistance. In the latter case, the field resistance, although providing a lower level of resistance than the major gene, would continue to provide resistance after selection of a biotype virulent to the major gene has occurred.

A major constraint to the breeding of field-resistant cultivars has been the lack of an efficient technique to evaluate breeding lines for field resistance. We have identified several rice cultivars which are resistant to *N. lugens* both in the vegetative and reproductive stage in the field and as 30-day-old plants in greenhouse studies, but which are susceptible in the seedbox screening of 7–10-day-old seedlings. However, occasionally the field-resistant cultivars are rated as moderately resistant (MR) in the seedbox screening, when plant damage is rated on the day that the susceptible check plants are killed. When ratings are taken a few days later, the MR cultivars are rated as susceptible. The standard seedbox screening test was thus modified to provide for an efficient method of identifying field-resistant cultivars in the greenhouse. This study was conducted to compare the performance of cultivars in the standard and modified seedbox screening tests and to determine the effect of field-resistant cultivars on the feeding activity and population growth of *N. lugens*.

Materials and methods

Screening tests were conducted in the greenhouse at IRRI at an ambient temperature ranging from 20° to 35°C. Three *N. lugens* populations served as test insects. Biotype 1 was reared on cultivar Taichung Native 1 (TN1) from Taiwan, which has no gene for *N. lugens* resistance and is highly susceptible; biotype 2 was reared on Mudgo, an Indian cultivar which has the *Bph1* gene for resistance; and biotype 3 was reared on ASD7, an Indian cultivar with the *bph2* gene for *N. lugens* resistance. The populations were reared on the respective cultivars for about 10 years (100 generations) prior to the tests.

Separate tests were conducted for each biotype in a split-plot design, with the screening method as the main plot, and the cultivars as subplots.

Seedbox screening test 1

Fifteen cultivars were evaluated for resistance to biotype 2 and 3 using the standard seedbox screening test and the modified seedbox screening test. IR26 and ASD7 served as the susceptible checks for biotype 2 and 3, respectively, and IR56 as the resistant check for both biotypes.

Standard seedbox screening test (*SSST*). Seed of each of the cultivars was sown in a 35 cm row in wooden seedboxes measuring 60 × 40 × 10 cm. Seven days after sowing (DS), seedlings were thinned to 20 per row and infested with eight second-instar *N. lugens* nymphs per seedling. Plants were rated for damage 7 days after infestation (DI) when the susceptible check was killed, and again 5 days later, using a 0–9 scale on which 0–3 is classified as resistant, 4–6 as moderately resistant and 7–9 as susceptible.

Modified seedbox screening test (*MSST*). Seed was sown and seedlings thinned as described for the SSST. Twenty DS the plants were infested with four second-instar *N. lugens* per plant and damage ratings were recorded at 25 DI when the susceptible check was killed and again 5 days later. Treatments in both the SSST and MSST in test 1 were replicated five times. In the SSST the initial infestation killed the susceptible plants whereas in the MSST the progeny of the initially infested insects killed the susceptible plants.

Seedbox screening test 2

Six cultivars were evaluated in the SSST and MSST against *N. lugens* biotypes 1, 2, and 3. Cultivars consisted of ASD7, IR46, Kencana and Utri Rajapan. Reactions of the latter three cultivars range from moderately resistant to susceptible in the seedbox screening of 7-day-old seedlings in the SSST but were reported to have high levels of field resistance to biotype 2 (Panda and Heinrichs, 1983). Cv. Sinna Sivappu from Sri Lanka, which has two unnamed genes for *N. lugens* resistance, is highly resistant to the three biotypes and served as the resistant check. TN1 served as the susceptible check.

The technique used in the SSST was the same as that described in test 1 except that ratings were taken at 2-day intervals from 6 to 16 DI. The MSST differed in that 10-day-old seedlings were infested with three nymphs per seedling and plant damage ratings were taken at 2-day intervals from 26 to 36 DI.

Separate experiments were conducted for each biotype. The experimental design consisted of a split-plot with the screening method as the main plot and cultivars as the subplots.

Population growth studies

The cultivars evaluated in Test 2 were further studied to determine whether they affected the population

growth and feeding rate of the three biotypes. Three plant ages were tested—30, 45 and 60 DS. In order that plants of the three ages could be provided simultaneously, seeds were sown at 15-day intervals, in clay pots. At 7 DS, seedlings were transplanted into soil in clay pots (diameter 16 cm) with three seedlings per pot. Each cultivar was replicated six times. When the plants reached the desired ages they were enclosed with a Mylar film cage (90 cm height × 10 cm diameter). The plants in each pot were infested with five pairs (males and females) of 3-day-old *N. lugens* adults. First-generation progeny was counted at 30 DI.

Feeding rate studies

Plants were grown in the same manner and test cultivars, treatments and experimental design were the same as in the population growth test. The honeydew excreted was collected in a feeding chamber, as described in Paguia, Pathak and Heinrichs (1980). The feeding chamber consisted of an inverted plastic cup placed over a bromocresol green-treated filter paper resting on a plastic Petri dish. The plant was inserted through the hole in the centre of the chamber which surrounded the stem portion at the base of the plant. Five 3-day-old adult females which had been starved for 5 hours were placed in the chamber through a hole which was then plugged with cotton to prevent their escape. The insects were allowed to feed overnight, after which the filter papers were examined. Spots produced by the reaction of the honeydew with bromocresol green were traced on filter paper (Pathak and Heinrichs, 1982b). The area of the spots was determined by tracing over the spots with tracing paper and counting the number of squares occupied by the spots over millimetre-squared graph paper. Feeding rate was expressed as the area of the spots produced in a feeding period of 18 h.

Results

Seedbox screening test 1

IR56 and IR60 were resistant to both biotype 2 and biotype 3 in the SSST and MSST (Table 1). ASD 11, Utri Rajapan and Wagwag had ratings of 6 at 7 DI and 9 at 12 DI in the SSST for biotype 2 but had ratings of 1 in the MSST. All other cultivars had ratings of 9 in both screening methods. Results using biotype 3 as the test insect were the same as for biotype 2 except that IR36, which has both seedling and adult-plant resistance to biotype 2 (*bph2* gene), was susceptible to biotype 3 at the seedling stage in the SSST but resistant in the MSST.

Seedbox screening test 2

In comparing the damage ratings of the six cultivars in the SSST and MSST, when infested with *N. lugens* biotype 1, 2, or 3, TN1 was highly susceptible and Sinna Sivappu highly resistant, to the three biotypes with both screening methods (Figure 1). The reactions of the other cultivars varied, depending on the biotypes and the seedbox test employed. In the SSST, with biotype 1, Kencana and Utri Rajapan had ratings of 6·3 and 6·0, respectively, when TN1 was rated 9 at 12 DI, while IR46 and ASD7 had ratings of 1·5 and 2·0, respectively. By 16 DI, Kencana and Utri Rajapan had susceptible ratings of 9 and 7, respectively, while IR46 was moderately resistant. In the MSST Kencana, Utri Rajapan, IR46, and ASD7 were resistant when TN1 was killed at 30 DI. At 36 DI, Kencana and Utri Rajapan were moderately resistant with ratings of 4·4 while IR46 and ASD7 continued to be resistant.

Results using biotype 2 (Figure 1) were generally similar to those with biotype 1. However, biotype 2 was virulent to cultivars with the *Bph1* gene in the

TABLE 1. Resistance of traditional and modern rice cultivars to *N. lugens* biotype 2 and 3 in the standard (SSST) and modified seedbox screening test (MSST). IRRI greenhouse, 1984

Cultivar	Origin		Plant damage rating*			
			Biotype 2		Biotype 3	
			SSST	MSST	SSST	MSST
ASD 11	India	T†	9·0 a	1·0 b	9·0 a	1·0 b
Binato	Philippines	T	9·0 a	9·0 a	9·0 a	9·0 a
CO29	India	T	9·0 a	9·0 a	9·0 a	9·0 a
CO32	India	T	9·0 a	9·0 a	9·0 a	9·0 a
GEB24	India	T	9·0 a	9·0 a	9·0 a	9·0 a
Intan	Philippines	T	9·0 a	9·0 a	9·0 a	9·0 a
Peta	Indonesia	T	9·0 a	9·0 a	9·0 a	9·0 a
Raminad	Philippines	T	9·0 a	9·0 a	9·0 a	9·0 a
Wagwag	Philippines	T	9·0 a	1·0 a	9·0 a	1·0 a
Utri Rajapan	Indonesia	T	9·0 a	1·0 b	9·0 a	1·0 a
Vazhaipoo Samba	India	T	9·0 a	9·0 a	9·0 a	9·0 a
IR36	Philippines	M‡	1·8 b	1·0 b	9·0 a	1·0 b
IR60	Philippines	M	1·6 b	1·0 b	1·4 b	1·0 b
IR56 (R check for biotype 2 and 3)		M	1·4 b	1·0 b	1·4 b	1·0 b
IR26 (S check for biotype 2)		M	9·0 a	9·0 a	—	—
ASD7 (S check for biotype 3)		T	—	—	9·0 a	9·0 a

* 1–3 = resistant; 9 = susceptible. In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.
† Traditional cultivar. ‡ Modern high-yielding cultivar.

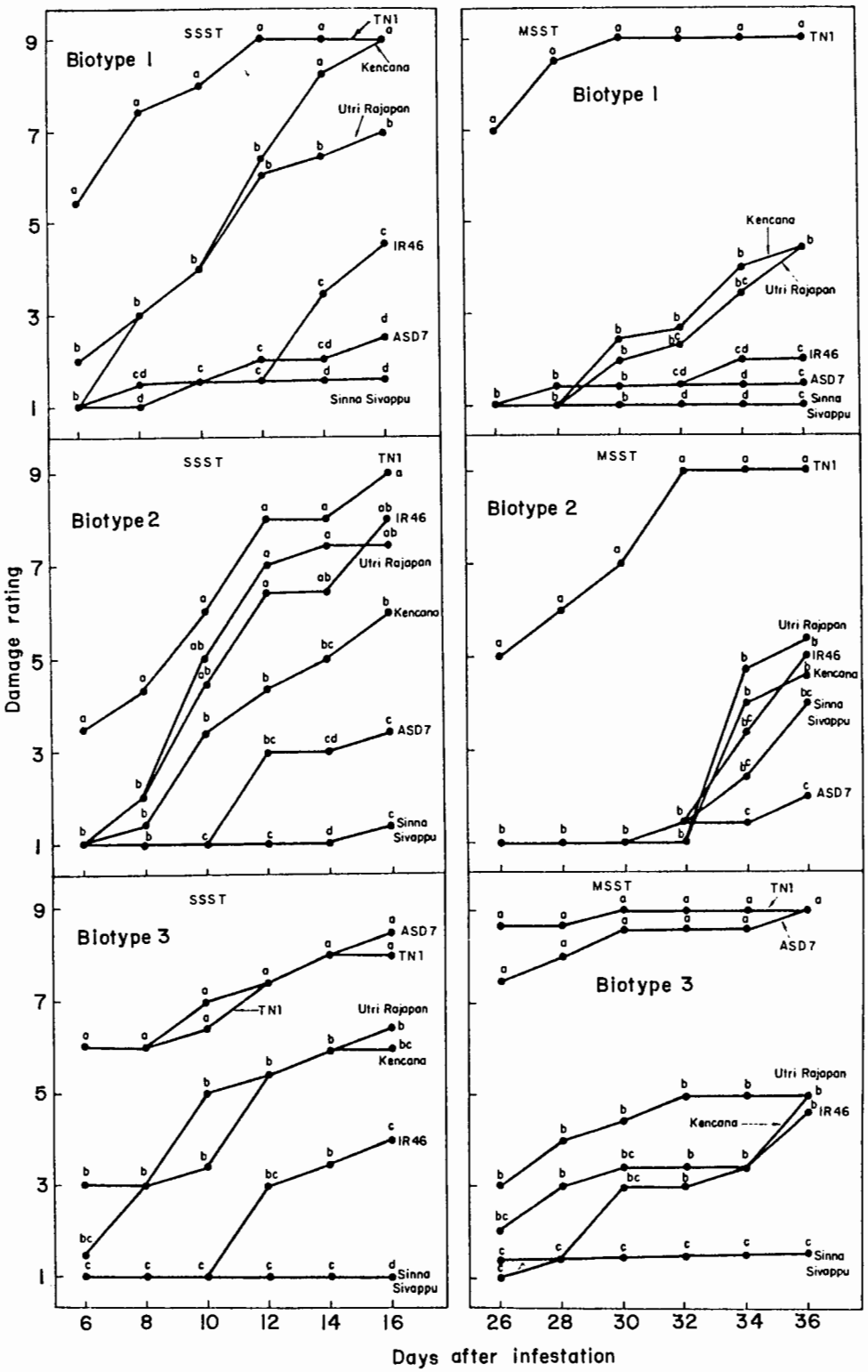

FIGURE 1. Damage ratings of selected cultivars to *N. lugens* biotypes 1, 2, and 3 in the standard seedbox screening test (SSST) (left) and modified seedbox screening test (MSST) (right) at given days after infestation (DI). For each biotype, screening method and DI points with a common letter are not significantly different at the 5% level by Duncan's multiple range test.

SSST and thus IR46 had a rating of 8 at 16 DI. In the MSST there was a distinct separation in damage ratings between TN1 and the other cultivars. IR46, Utri Rajapan, and Kencana were moderately resistant.

ASD7 was susceptible in both the SSST and MSST in the biotype 3 test. Utri Rajapan, Kencana, and IR46 had ratings of 6·3, 6·0 and 4·0, respectively in the SSST whereas they had ratings of about 5 in the MSST.

Population growth studies

Because *N. lugens* populations generally differed little on plants aged 30, 45 or 60 DS, only the 30 DS data are

TABLE 2. Population growth and area of honeydew excreted by *N. lugens* biotypes infesting 30-day-old rice plants.* IRRI greenhouse, 1984

Cultivar	Population growth (no.)			Area of honeydew (mm^2)		
	Biotype 1	Biotype 2	Biotype 3	Biotype 1	Biotype 2	Biotype 3
Sinna Sivappu	1 d	4 d	7 d	71 b	29 c	34 c
ASD7	95 c	137 c	1222 a	174 b	545 b	891 a
IR46	120 bc	382 b	69 c	51 b	273 b	48 c
Kencana	274 bc	122 c	370 b	172 b	205 c	95 c
Utri Rajapan	262 bc	470 ab	359 b	208 b	512 b	302 b
TN1	1189 a	1204 a	1233 a	882 a	1265 a	802 a

* Mean of six replications. Means within a column, followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

presented (Table 2). Plants in the MSST were at a similar age to those in the population growth test (30 DS) and results of the two tests are comparable. Sinna Sivappu was highly resistant to all three biotypes, with numbers of progeny ranging from one to seven, whereas TN1 was highly susceptible with about 1200 progeny of all three biotypes. Progeny on ASD7 was highest (1222) with biotype 3, being 10 times that of biotype 1 and 2, and not differing significantly from that on TN1 (1233). Progeny number on IR46 was highest with biotype 2 (382) but was still only one-quarter that on TN1. The number of *N. lugens* produced on Kencana was significantly less than that on TN1 with the three biotypes while biotype 1 and biotype 3 progeny were less on Utri Rajapan than on TN1.

Feeding rate studies

Results of the feeding test were similar to that of the population growth test. The lowest honeydew excretion was on the resistant check, Sinna Sivappu, with the highest on the susceptible check, TN1 (Table 2). Biotype 3 fed the most on ASD7, the feeding being equal to that on TN1. Biotype 2 fed most on IR46 but only one-quarter as much as that on TN1. Feeding was significantly less on Kencana and Utri Rajapan than that on TN1 with all three biotypes.

Discussion

The levels of resistance expressed by the cultivars tested were dependent on plant age. The MSST provided a better separation of levels of resistance than the SSST, where young plants were attacked by the hoppers. However, when damage ratings in the SSST were taken at the time when TN1 was first killed (at 7 DI), cvs ASD 11, Wagwag and Utri Rajapan were rated as moderately resistant, with ratings of 5·8, but were rated as 9 at 12 DI in the biotype 2 test. Thus, if daily gradings for plant damage are taken, some of the field-resistant cultivars may be detected in the SSST but they will often be rated as susceptible.

The mechanism involved in the field resistance of IR46, Utri Rajapan and Kencana was a low feeding rate of *N. lugens* and a low reproductive rate. However, tolerance, although not measured, may have also been involved, as was reported by Panda and Heinrichs (1983) for biotype 2 on Utri Rajapan and Kencana.

The MSST is a rapid method of identifying field-resistant cultivars in the greenhouse. The cultivars identified as having field resistance in the MSST—IR46, Kencana, and Utri Rajapan—were previously shown to have field resistance to biotype 2 under field testing (Panda and Heinrichs, 1983). These cultivars were field resistant to all three biotypes in this study and have been reported to have field resistance in Korea, Philippines, Solomon Islands, Thailand and India (IRRI, 1982a,b); this indicates that the type of resistance which they express is probably general or horizontal.

Three of the traditional cultivars, which formerly were widely grown by farmers, were identified as having high levels of field resistance in the MSST. Wagwag, a Philippine cultivar, is still grown on a small scale because the Filipinos prefer its grain quality to that of the modern high-yielding cultivars. Utri Rajapan and Kencana are traditional cultivars from Indonesia, where Kencana is still grown on a small scale. Kencana has been reported to be resistant under field conditions, as an older plant in Indonesia, although it is susceptible at the 7–10-day-old seedling stage in the greenhouse (Mochida, Wahyu and Suryani, 1979). Utri Rajapan, in addition to having field resistance to biotype 2, as reported by Panda and Heinrichs (1983), also has field resistance to biotypes 1 and 2 as shown in this study and is resistant to rice tungro and grassy stunt virus diseases (Panda, Heinrichs and Hibino, 1984).

Rice breeders are interested in utilizing field-resistant cultivars as donors in the breeding programme. Cultivars such as Utri Rajapan, which have field resistance to *N. lugens* and the whitebacked planthopper, *Sogatella furcifera* (N. Panda and E. A. Heinrichs, unpublished work) and resistance to rice viruses, are of interest in the development of high-yielding cultivars with stable resistance to insects and diseases. The lack of an efficient means of evaluating large numbers of breeding lines has hampered the use of such cultivars as donors. However, with the use of the MSST, such breeding lines can be evaluated and the MSST is now being used for this purpose at IRRI where field-resistant cultivars IR46, Utri Rajapan and Triveni have been used as parents. The difference between susceptible and resistant plants is sufficiently distinct to allow the use of the MSST for genetic analyses.

One strategy in the breeding for cultivars resistant to *N. lugens* at IRRI is to incorporate vertical resistance controlled by major genes with field resistance controlled by either major or minor genes. When vertical resistance 'breaks down', through the selection of a virulent biotype, field resistance can be expected to continue to provide stable yields, especially if tolerance is also involved. IR46 is an example of such a cultivar. The major gene *Bph1* provides vertical resistance to biotypes 1 and 3, whereas other major or minor genes provide field resistance to biotype 2, which is virulent to young seedlings of cv. IR46. Field resistance would be expected to act against all biotypes. Although field resistance generally gives a less effective control of *N. lugens* than that of seedling resistance, combinations of field resistance and the action of biocontrol agents in an integrated control programme have provided excellent control and minimized yield losses in field tests (E. A. Heinrichs, unpublished work).

The two screening methods have been tested in China, where cv. Utri Rajapan was rated as susceptible in the SSST, but resistant in both the MSST and in field tests (Wu, Liangyou and Ziguang, 1984). The MSST provides a method of identifying field resistance under greenhouse conditions and is expected to be a useful tool in the breeding of high-yielding rice cultivars which have durable resistance to *N. lugens*.

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