AGRIVITA VOLUME 34 No. 1 FEBRUARY-2012 ISSN : 0126-0537

EXPRESSION OF RESISTANCE OF SOYBEAN TO THE POD SUCKING BUG *RIPTORTUS LINEARIS* F. (HEMIPTERA: COREIDAE)

Suharsono[1*] and Liliek Sulistyowati[2]

[1] Indonesian Legumes and Tuber Crops Research Institute (ILETRI)
Jl. Raya Kendalpayak Km 8 PO Box 66 Malang East Java Indonesia
[2] Dept. of Plant Protection, University of Brawijaya Jl. Veteran Malang 65145 East Java Indonesia
[*] Corresponding author Phone: +62-341-801468 E-mail: suharsono_kabi@yahoo.com

Received: March 6, 2011/ Accepted: May 12, 2011

ABSTRACT

Factors involved in the mechanism of soybean resistance to pod sucking bug *R. linearis* were identified using resistant soybean genotypes, IAC-100, and IAC-80-596-2 and the susceptible variety, Wilis as a check. The role of trichomes in resistance was assayed removing trichomes from the pod shell, and seed coat and the resistance was determined based on the number of stylet punctures made by the bug. Seed of IAC-100 and IAC-596-2 that had longer, denser trichomes, higher crude fiber content and suffered fewer stylet punctures than Wilis. This suggested that denser and longer trichomes interfered with stylet piercing of the pod shell. When the trichomes of IAC-100 and IAC-596-2 were removed these genotypes were more susceptible to insect feeding. In further studies, replacement of IAC-100 and IAC-596-2 seed with seed of Wilis in the pods of resistant genotypes resulted less stylet punctures on the Wilis seed. It was concluded that denser and longer trichomes on pods along with harder pod shells acts as a physical barrier in antixenosis resistance of soybean to the pod sucking bug. Therefore, IAC-100, and IAC-596-2 genotypes have good potential for used as resistant parents in a soybean breeding program.

Keywords: resistance, pod characters, trichomes, antixenosis, pod stink bug

INTRODUCTION

The first plant cells that interact with an insect pest of areal tissues are trichomes on the plant surface. The more glabrous (less trichome dense) genotype of the perennial herbaceous plant *Arabidopsis lyrata* was more damaged than the more pubescent type with denser trichomes (Løe *et al.,* 2007).

Trichomes are single celled or multi-cellular outgrowths of the epidermis on plant stems, leaves and other organs that may be glandular or non-glandular (Kitayama *et al.,* 2010) and collectively constitute the pubescence (hairiness) of the plant surface. They may be in host acceptance of insect pest (Werker, 2000), and function as a defense against herbivores (Traw and Dawson, 2002) and pathogens (Sheprad *et al.,* 2005; Shepard and Wagner,2007). There is much evidences that damage of plants by herbivorous insects is reduced by dense trichomes (Hare and Elle, 2002).

The epidermal hairs in many plant species are specialized for plant defense against attack by insect pests. The defense by trichomes may be determined by whether they are nonsecretory or glandular trichomes as well as by their density, length, shape, and degree of erectness. High densities of nonsecretory trichomes create a physical barrier to insects feeding on the underlying surface or internal tissues (Tingey, 2001).

Single-cell trichomes occur on most of the surface of soybean pods with varying from glabrous to pubescent types, with shorter or longer trichomes, and with erect and irregular types. Damage from pod feeding insects by stylet punctures of pod suckers and direct feeding by pod feeders cause considerable yield loss of soybean.

In Indonesia three species of pod sucker insects (*Riptortus linearis* F., *Nezara viridula* L. and *Piezodorus hybneri* Gmel.) (Talekar, 1997; Suharsono, 2001), and five species of pod borers (*Etiella zinckenella* Treit., *E. hobsoni* Butler, *E. chrysoporella* Meyrick, *E. grisea drococsia* Meyrick and *E. behrii* Zeller) are

Accredited SK No.: 81/DIKTI/Kep/2011
http://dx.doi.org/10.17503/Agrivita-2012-34-1-p055-059

common pests of soybean (Hirano *et al.,* 1992). These insects are widely distributed and have wide range of alternative host plants throughout the soybean production centers in sub-tropical and tropical countries, feeding on pulse legumes such as soybean, mung bean, cowpea, string bean, and pigeon pea.

Thee most dominant species of pod sucking pests in Indonesia are the brown stink bug (*R. linears*), southern green stink bug (*N.viridula*) and smaller green stink bug (*P. hybneri*). Both young (nymphs) and adults insects feed on soybean seed by piercing the pod shell and sucking out the seed content. The symptoms of damage, and yield loss of the first two species are less similar (Talekar, 1997). Tengkano *et al.,* (1988a) reported that there were three species of *Riptortus* with different morphologies and distributions in Indonesia, i.e. *R. linearis, R. pedestris* and *R. annulicornis.*

Brown stink bug (*R. linearis*) is the most common species of pod sucking bug distributed in Indonesia. Feeding on pods and developing seed stage causes empty pods, seed crinkle, pod abortion, and reduces seed vigor by 46-67%, and prolongs harvesting time when the insects attack on pod maturity (Tengkano, *et al.,*1988b). The brown stink bug causes 75% yield reduction when occurs 45-55 days after planting (DAP) (Winoto, 1986). The severity of damage depends on the susceptibility of the soybean and environmental conditions, especially thr degree of water stress..

A previous study indicated that that soybean genotypes IAC-100 and IAC-80-596-2 were consistently less damaged by *R. linearis* and it was suggested that genotypes possesses some resistance to the stink bug (Suharsono, 2001). The present study investigated factors involved in the mechanisms of resistance.

MATERIALS AND METHODS

The two soybean genotypes referred to above: (IAC-100 and IAC-80-596-2) were selected as being field resistant to *R. linearis* and the variety Wilis variety was used as a susceptible check. All soybeans were grown in potting mix in 10 kg polybags in screen house, and were harvested for use in experiments at 55-65 days after planting (DAP).

Determination of the site of resistance.

Experiment 1

Matured pods of each of IAC-100, IAC-80-596-2 and Wilis R5-R6 (55-65 DAP) were harvested, and their seeds were removed, and then inserted into empty soybean pods. The seeds of ten pods of each of IAC-100, and IAC-80-596-2 (resistant genotypes) were replaced by seeds of the susceptible variety Wilis, and seeds of IAC-100 and IAC-80-596-2 were each inserted into ten Wilis pod shells. All filled pods were infested by 5 nymphs at 3^{rd} - 4^{th} instars and the batches of ten pods were maintained separately in 10 x 10 x 15 cm plastic boxes for five days under normal conditions in the laboratory. The experiment was completely randomized design with five replicates. The number of stylet punctures on pod shells and seed was observed at 4 days after infestation (DAI) by examination under a stereomicroscope (40x) following staining with 1% Fuchsine acid. Pod shells and seed of each soybean genotypes were separately deeping in 250 ml beaker glass contained 100 ml of 1% Fuchsin acid solution for five minutes. Subsequently these pod shells and seeds were taken out, and rinsed with tap water to clean the remaining fuchsin acid. All pod shells and seeds were kept dry under room temperature. Detection of pod shells and seeds damage under stereomicroscope based on number of red spots both on pod shells and seeds as the symtomps of stylet punctures of stink bug.

Experiment 2

In a further experiment, pods of soybean at R5-R6 (55-65 DAP) were harvested and then used in a feeding test. Ten pods with trichomes (normal pods), pods with trichomes removed and seeds of resistant genotype: IAC-100 and IAC-80-596-2, susceptible variety Wilis were each exposed to 10 3^{rd} instar nymphs of *R. linearis* in 10 x 10 x 15 cm plastic boxes for five days under normal laboratory conditions. The trichomes were removed from pod shells surface by shaven off using scalpell. The experiment was laid in a completely randomized design with five replicates. Number of stylets puntures on pod shell and seed was observed at 4 DAF under stereomicroscope 40x stained in 1% Fuchsin acid.

RESULTS AND DISCUSSIONS

More stylet punctures occured on pods of the variety Wilis when they were filled by seed of the resistant genotypes IAC-596-2 and IAC-100 than on pods of the resistant genotypes when they were filled by seed of Wilis (Table 1). More punctures occured on sedds of resistant genotypes when they were enclosed in Wilis pod than on seeds of Wilis when they were enclosed on pods of the resistant genotyps. It is evident that pod characters rather than seed characters are involved in defense against stink bug feeding. Plant defenses against insect feeding may involve both physical and chemical characters (Smith, 1989; Suharsono, 2000). Pod characteristics (wall thickeness, hairiness, and hardeness) may directly affect stink bug *R.linearis* feedof ing (Suharsono, 2001). It was found that thrichomes length and density and fiber content of pods were greater on pods of IAC-80-596-2 and IAC-100 than Wilis (Table 2) and these differences were correlated with resistance of the pods to by brown stink bug feeding. Its mean that pod characteristics determine the resistance of IAC-80-596-2 and IAC-100 to pod stik bug.

Physical characteristics of soybean pods (i.e longer and denser trichomes of IAC-100 and IAC-80-596-2 breeding lines) was reported to play a role in resistance mechanisms against pod sucking insect *R.linearis* (Suharsono, 2001). Denser and longer trichomes and high fiber content in IAC-80-596-2 and IAC-100 genotypes (Table 2) acting as mechanical barrier for stink bug feeding on pod and seed as indicated when trichomes was removed the stylet punctures increased (Table 3 and 4).

Removal of pod trichome resulted in an increased man number 0f stylet punctures on pod on resistant genotypes but not on susceptible Wilis. However, it resulted in an increased number of punctures on the sedds of all three genotypes (Tabel 3). Trichome production is an important component of resistance against herbivorous insects (Traw and Dowson, 2002) and damage from many insect pests is negatively related to trichome density (Handley *et al.,* 2005). The glabrous (non hairy) tyApes of the perenial herb *Arabidopsis lyrata* is more damaged by insect herbivores than the pubesence type (Løe *et al.,* 2007).

Table 1. Number of stylet punctures on pods and seeds of susceptible Wilis and resistant genotype as affected by seed insertion

Treatments	Mean no. of stylet punctures	
Pod shell genotype /seed genotype	Pod shells	Seeds
Wilis/IAC-80-596-2	20.25 a	10.20 a
Wilis/IAC-100	18.55 ac	13.60 a
IAC-80-596-2/Wilis	11.40 b	5.60 b
IAC-100/Wilis	14.65 bc	4.20 b
LSD 0.05	5.25	4.13

Remarks: Means within a collumn followed by the same letter are not significantly different at p = 0.05

Table 2. The characterstics of trichomes on pods and fiber content of pods of resistant genotypes IAC-80-596-2 and IC-100 and susceptible variety Wilis

Soybean gen./var.	Trichome length (mm)	No. of trichome/mm^2	Fiber content (mg/g)
Wilis	1.36 ± 0.67 b	4.98 ± 0.98 a	9.85 ± a 0.52
IAC-80-596-2	1.74 ± 0.44 a	11.49 ± 1.46 b	21.95 ± b 0.46
IAC-100	1.90 ± 0.56 a	13.05 ± 1.89 b	20.99 ± b 0.35
LSD 0.05	0.28	5.45	6.25

Remarks: Means within a column followed by same letter are not significantly different at p = 0,05

Table 3. Effects of trichome removal on mean number of stylet punctures on pod shells of resistant soybean genotypes IAC-80596-2 and IAC-100 and susceptible variety Wilis..

Soybean gen./var.	No. Stylet punctures on pod		Mean
	Normal pod	Pod no trichome	
IAC-80-596-2	8.60 Ab	12.80 Bb	10.70 b
IAC-100	8.00 Ab	13.85 Bab	10.92 b
Wilis	16.25 Aa	17.07 Aa	16.66 a
Mean	10.95 ns	14.57 ns	-
LSD	7.50	4.56	

Remarks: Means within a column followed by same lower case letter and within a row followed by same capital letter are not significantly different at p = 0.05

Table 4. Number of stylet puncture on seeds of resistant and susceptible Wilis either normal pod or without trichome

Soybean gen./var.	No. Stylet punctures on seed		Mean
	Normal pod	Pod no trichome	
1. IAC-80-596-2	2.20 Ab	5.40 Bb	3.80 b
2. IAC-100	3.25 Ab	4.58 Bb	4.27 b
3. Wilis	14.48 Aa	17.07 Ba	15.77 a
Mean	6.64 ns	9.02 ns	-
LSD	4.55	6.50	

Remarks: Means within a column followed by same lower case letter and within a row followed by same capital letter are not significantly different at p = 0.05

This facts indicate that pod characters along with trichomes act as mechanical barier interfering the stink bug stylet piercing on pod surface. Thus, pod trichomes on soybean involved in a mechanism of resistance to pod sucking insect.

CONCLUSSIONS

It was shown that trichomes on soybean pods constitute mechanical barrier to stink bug stylet piercing of the pod surface and seeds, and probably involved in the resistance of soybean to pod stink bug. Thus removal of trichome from the pod surface allowed more insect punctures and damage. From this it could be predicted that soybean genotypes bearing fewer or shorter thricomes will be more susceptible to pod stink bug.

The soybean genotypes IAC-100 and IAC-80-596-2 wto ere shown possesses some resistance to brown stink bug *Riptortus linearis* and probably to other stink bug species. Therefore, a further study the resistance of these genotypes to other species of stink bug is needed.

ACKNOWLEDGMENTS

We like to Agrivita Peer Reviewer for the valuable comments and review.

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