

Defense mechanisms in *Solanum virginianum* against brinjal shoot and fruit borer -a comparative study

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Abstract

A study was taken up to morphologically characterize *Solanum virginianum* accessions to identify suitable lines for resistance breeding. Four accessions of *S. virginianum* were evaluated along with *S. melongena* varieties 'Ponni' and 'Surya'. Plants belonging to *S. virginianum* were prostrate growing and weedy in nature with strongly lobed leaves and small fruits bearing prickles. The plants of 'Ponni' and 'Surya' were erect growing with weakly lobed leaves and large sized fruits and were devoid of prickles. The accessions were screened for brinjal shoot and fruit borer infestation during August-November 2018 and March-July 2019 along with 'Ponni' and 'Surya'. Shoot damage was not observed in the wild accessions during both the seasons. Variety 'Ponni' was highly susceptible as the shoot and fruit damage ranged from 13.33 to 54.16 per cent and 15.87 to 45.56 per cent, respectively. 'Surya' was more prone to damage by borer as the shoot and fruit damage ranged from 15.55 per cent to 54.17 per cent, and 16.58 per cent to 67.25 per cent, respectively. The plants belonging to *S. virginianum* were distinctly characterized by thick walled epidermis, thick cuticle and cortex wall, numerous trichomes, large number of well developed vascular bundles and small pith size. The susceptible varieties 'Ponni' and 'Surya' were characterized by thin walled epidermis, thin cuticle and cortex wall, very few trichomes, small number of poorly developed vascular bundles and large pith size. Hence, anatomical parameters viz., thickness of epidermis and cortex, trichome intensity, number and size of vascular bundles and pith size may serve as morphological markers, and *S. virginianum* could be a promising wild species in pre-breeding programmes as a suitable donor for fruit and shoot borer resistance.

Key words: Brinjal shoot and fruit borer, Floral biology, Morphological characterization, Ponni, *Solanum virginianum*, Surya, Tolerant lines.

Introduction

Brinjal (*Solanum melongena* L.) is an important vegetable crop of the tropics and subtropics. It is preferred by people around the globe, owing to its medicinal and nutritive properties. However, the large scale cultivation of brinjal is limited by the severe infestation of pests and diseases. Brinjal is affected by a large number of pests, of which, brinjal shoot and fruit borer (*Leucinoides orbonalis* Guen.) is the most devastating one. It is regarded as one of

the most destructive pests attacking the crop right from nursery stage to harvesting (Sahu et al., 2018). The yield loss caused by this pest has been estimated to be 70-92 per cent (Chakraborti and Sarkar, 2011). Crop wild relatives share a relatively recent common ancestry with domesticated species and due to that close relationship, serve as reservoirs of genetic traits that can be utilized in crop improvement (Guarino and Lobell, 2011). Brinjal has potential wild relatives that exhibit resistance to a large number of pests and diseases (Behara and Singh,

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2002). Knowledge of the morphological and anatomical traits, flowering behaviour and extent of tolerance is essential for taking up any resistance breeding programme.

Keeping this in view, the present study was taken up to characterize *S. virginianum* accessions, study the flowering biology as well as to elucidate the extent of damage caused by the infestation of brinjal shoot and fruit borer.

Material and methods

Twenty plants each of the accessions JJNS-15/34, JJNS-15/47, JJNS-15/84 and JJNS-15/44 belonging to *Solanum virginianum*, collected from NBPGR Regional Station Vellanikkara, Thrissur and *S. melongena* varieties 'Ponni' and 'Surya' released from KAU formed the material for the study (Table 1). Observations were recorded from twenty plants for vegetative characters, floral and fruit characters

Table 1. Materials used for the study

Genotypes	IC No.	Species	Source
JJNS15/34	618036	<i>S. virginianum</i>	NBPGR, RS, Vellanikkara
JJNS15/84	618054	<i>S. virginianum</i>	NBPGR, RS, Vellanikkara
JJNS15/44	618037	<i>S. virginianum</i>	NBPGR, RS, Vellanikkara
JJNS15/47	618034	<i>S. virginianum</i>	NBPGR, RS, Vellanikkara
Ponni		<i>S. melongena</i>	ARS, Thiruvalla, KAU
Surya		<i>S. melongena</i>	KAU, Vellanikkara

(IPGRI, 1988) and anatomical features. Five leaves, five fruits and 10 flowers from each plant were tagged for observation. Parameters like leaf length and plant height were measured using measuring scale and measuring tape respectively, while plant breadth was measured using a twine. The time of flower opening, anther dehiscence and stigma receptivity were also observed.

Determination of anther dehiscence

Ten mature flower buds of each type were examined with hand lens on a day prior to anthesis, at one hour intervals starting from 5 am till the dehiscence of pollen grains to determine the time of anther dehiscence (Prasad and Krishnaprasad, 1994).

Determination of stigma receptivity

The stigmatic surface of selected buds was observed at hourly intervals starting from 5 am on the previous day of flower opening for any change in colour or appearance to find out the onset of stigma receptivity. Duration of stigma receptivity was determined as described by Radford et al. (1984). Moist conditions of stigmatic surface and/or change in colour of stigmatic surface were considered as indications of onset of receptivity. Loss of receptivity was indicated by fading of the colour or drying up of stigmatic surface.

Anatomical characterization of the resistant and susceptible lines

Mature shoots from 100 day old plants were collected. Thin sections were made using microtomy. Sections were stained using 2 per cent Safranin solution. Slides were observed under a phase contact microscope with image analyser (Johansen, 1941).

Screening for resistance against brinjal shoot and fruit borer

Thirty five days old seedlings were planted in two replications at a spacing of 90×75 cm. Twenty plants each of four accessions were screened in the field for resistance to brinjal shoot and fruit borer along with 'Ponni' and 'Surya' varieties of *S. melongena*. Recommended cultural operations were carried out (KAU, 2011). Observations were recorded in 10 randomly selected plants per replication. The first season crop was raised from August to November and the second season crop from March to July.

The following observations were recorded from randomly selected plants at vegetative and reproductive stages.

Shoot damage: The total number of terminal shoots drying/drooping from ten randomly selected plants was counted. After each observation, the damaged shoots were removed and the per cent shoot infestation was calculated.

Fruit damage: The number of infested and uninfested fruits from each cultivar was counted from ten randomly selected plants and per cent fruit infestation was calculated. Based on the mean infestation throughout growing season, the brinjal cultivars were categorized as per the grade index of Subbaratnam and Butani (1981) (Table 2).

Table 2. Scoring index for the incidence of brinjal shoot and fruit borer infestation

Grade	Fruit infestation	Shoot damage
Tolerant	< 15	< 2
Moderately tolerant	16-25	2.1-3.0
Susceptible	26-40	3.1-5.0
Highly susceptible	> 40	> 5

Results and Discussion

Morphological characterization

Observations were recorded based on twenty one vegetative characters (Table 3). Anthocyanin content was absent in all the accessions of *S. virginianum* during the seedling stage. However, anthocyanin colouration was observed in the stem and hypocotyl of the varieties 'Ponni' and 'Surya'. The intensity of colouration was weak in 'Ponni' and strong in 'Surya'. Presence of anthocyanin is found to have some significance in the defense mechanism of the plant. Scott (1999) reported that plant tissues containing anthocyanin are often resistant to drought stress.

Moderate pubescence was observed on the stems of all the accessions of *S. virginianum*. However, stem pubescence was absent in the varieties 'Ponni' and 'Surya'. Trichomes or pubescence are hair like epidermal outgrowths that occur on leaves, shoots, and roots. Their impact on insect pests depends on density, erectness, length, and shape of trichomes. Some insects experience difficulty in feeding on and ingesting plants with these small hairs. Trichomes might also release sticky substances that could trap or inhibit movement of small insects (Zehnder, 2010). Norris et al. (1980) reported that plant hairiness could partially suppress a few pests *viz.*, bean aphid and potato leaf hopper on beans, two spotted spider mites on strawberry, and whitefly on

tomatoes, peppers, and potatoes. Ali et al. (2012) reported that hair density and length of hair on the lamina, midrib, and veins showed a highly significant negative correlation with the jassid population. In cotton, the hairy or pilose condition imparted resistance to jassids (Knight, 1952). In the present study, accessions belonging to *S. virginianum* with moderate to large number of hairs on stem and leaves showed lesser incidence of brinjal shoot borer. Plants of 'Ponni' and 'Surya', which were devoid of hairs were found to be highly prone to shoot damage by borer.

According to Freeman and Beattie (2008), prickles were outgrowths of the epidermis that served as a modified appendage that aided in mechanical protection from herbivores, pathogens and pests. Prickles were present in all the accessions of *S. virginianum*. Prickliness could be considered as an important attribute that contributed to pest resistance in crop plants. Shoot damage due to the incidence of brinjal shoot and fruit borer was absent in the wild genotypes bearing large number of prickles, while the varieties 'Ponni' and 'Surya' (without prickles) were highly infested by the insect.

The *S. virginianum* accessions differed from the varieties 'Ponni' and 'Surya' with respect to all phyto-graphic characters.

Observations were recorded on thirteen leaf features (Table 3). Lobing of leaf blade was strong in all the accessions of *S. virginianum*. Leaves were medium in size with green colour, long green petiole, very strong lobing, and acute leaf tip with large number of prickles. Accessions were similar to each other with respect to most of the leaf characters except intensity of leaf blade colour, vein colour and spininess. Kavitha (2004) reported that leaves of *S. virginianum* were ovate or elliptic, sinuate or sub pinnatifid, obtuse or subacute, stellately hairy on both sides, armed on the midrib and often on the nerves with long yellow sharp prickles. However, in the present study, prickles were green in colour. The leaves of 'Ponni' and 'Surya' were medium

sized, with dentate margin and without prickles. Leaves of 'Ponni' were light green while they were dark green in 'Surya'. Presence of prickles could be considered as the most distinguishing factor between the wild and the cultivated types.

Corolla colour at anthesis was bluish violet in all the accessions of *S. virginianum* (Table 3). Flower buds were greenish white in colour at the time of initiation of flowering and colour gradually changed to pale violet in two to four days after initiation of flowering. The flowers of 'Ponni' were bright white and colour changed to light violet on the day prior to opening. Intensity of corolla colour changed from light violet to dark violet after anthesis. Naujeer (2009) reported that flower (corolla) colour intensity increased from pale violet to light violet in *Solanum* genotypes, and this was true with variety 'Ponni' but not observed in accessions of *S. virginianum*. The corolla colour was bluish violet in 'Surya'.

Large number of prickles was observed in the calyx of flowers in all the accessions of *S. virginianum*. This was in accordance with the earlier report by Gunjeet et al. (2008). The flowers of *S. virginianum* appeared in clusters. Number of flowers in each cluster was more in the accessions JJNS 15/84 and JJNS 15/44 compared to JJNS-15/34 and JJNS-15/47 which ranged from three to six. However, in the varieties 'Ponni' and 'Surya' only a single flower was present per inflorescence. This conformed with the study by S kara and Bieniasz (2008) where they reported that the number of flowers per inflorescence was more in wild species.

Observations on the time of flower opening, anther dehiscence, and time and duration of stigma receptivity are presented in Table 3. Seasonal influence on the time of anthesis was not prominent in the flowers of *S. virginianum* as well as 'Ponni' and 'Surya'. The time of flower opening remained constant during both the seasons.

Determination of anther dehiscence

The time of anther dehiscence varied with the

accessions. Dehiscence once started, continued for three days in all the accessions. The pollen grains were dehisced through the apical pore of anthers in all the accessions evaluated.

Determination of stigma receptivity

In all the accessions of *S. virginianum* as well as the varieties 'Ponni' and 'Surya', the stigmatic surface at the time of peak stigmatic receptivity was observed to be moist plumpy and glossy green. The colour was found to fade with the decline in stigma receptivity. This was in accordance with the study of Neeraja (2017) where a similar pattern was observed in *S. melongena* variety Haritha.

Morphological characterization based on fruit features (IPGRI 1988)

Morphological characterization of the fruits showed that the fruits of *S. virginianum* were small, globular in shape, pendent in position, green with white patches at commercial maturity and deep yellow at physiological maturity (Table 3). All the four accessions were similar with respect to most of the fruit characters except fruit colour distribution, colour at commercial ripeness and fruiting pattern. Fruits of JJNS-15/34 and JJNS-15/47 were light green with white stripes at commercial maturity and they exhibited striped distribution as well as mixed fruiting pattern. Both clustered and solitary bearing was observed in the same plant. Fruits belonging to JJNS-15/84 and JJNS-15/44 were light green with white mottles at commercial maturity. Colour distribution was mottled and fruiting pattern was clustered. Large number of prickles were present on the fruit calyx of the accessions of *S. virginianum*. This was a limiting factor in harvesting and post harvest handling.

The fruits of 'Ponni' were pale green colour, with a slight purplish tinge on the side, uniform colour distribution, cylindrical fruit shape, elliptic cross section, dense flesh, strong glossiness at harvesting, white flesh, solitary bearing and without spines. However, the fruits of Surya were dark purple in colour, with uniform colour distribution, obovate

shape and dense flesh. This was in accordance with Vorontsova et al. (2014) who reported that the fruits of *S. melongena* types were globular to ovoid, ellipsoid, or oblong to curved berry, with one fruit per inflorescence and the fruiting pedicels were woody and pendulous, without prickles.

From this study it was evident that cultivated brinjal *S. melongena* bore large and showy fruits that were devoid of prickles, while the fruits belonging to the wild type *S. virginianum* were small with large number of prickles. Fruit characters as well as the presence or absence of prickles were the most distinguishing features between the wild and the cultivated types. Plants with large sized fruits that were free from prickles are preferred in breeder’s perspective.

Anatomical characterization of the resistant and susceptible lines

Anatomical characterization revealed that all the accessions had single layered epidermis and numerous vascular bundles. However, the resistant lines JJNS 15/34, JJNS 15/47 (Fig.1a), JJNS 15/84 and JJNS 15/44 were distinctly characterized by thick walled epidermis, thick, dense and compact cortex wall and small pith size. Contrary to this, the varieties ‘Ponni’ and ‘Surya’ (Fig.1b) were characterized by thin walled epidermis, thin, loose and spongy cortex and large pith size. From this study it was evident that thick walled epidermis, thick cuticle and numerous trichomes in *S. virginianum* aided in imparting resistance to brinjal shoot and fruit borer. Among the biophysical characters, the higher density of trichomes on shoot

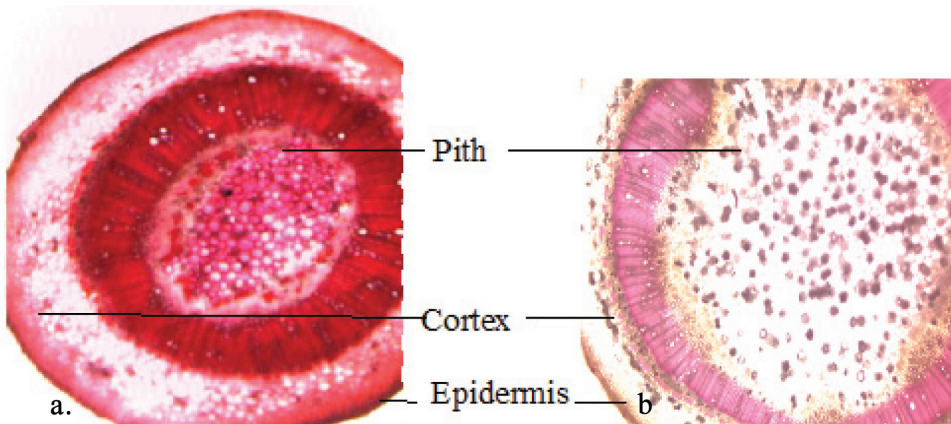


Figure 1. a. Thick epidermis, thick, dense, compact cortex and small pith in the resistant line JJNS 15/47 b. Thin epidermis, thin, loose and spongy cortex and large pith in the susceptible line Surya

Table 4. Anatomical parameters contributing to resistance in *Solanum* spp

	JJNS /34	JJNS15/47	JJNS15/84	JJNS 15/44	Ponni	Surya
1. Epidermis						
1a. Layer	Single	Single	Single	Single	Single	Single
1b.Size	Small and large thick walled	Small and large thick walled	Small and large thick walled	Small and large thick walled	Small and large thick walled	Uniform, thin walled
1c.Cuticle	Thick	Thick	Thick	Thick	Thin	Thin
1d.Trichome	Numerous	Numerous	Numerous	Numerous	Very few	Very few
2. Cortex						
2a. Wall	Thick wall	Thick wall	Thick wall	Thick wall	Thin wall	Thin wall
3. Vascular bundle						
3a Development	Well developed	Well developed	Well developed	Well developed	Poorly developed	Poorly developed
3b Number	Many	Many	Many	Many	Many	Many
3c Size	Large	Large	Large	Large	Small	Small
4. Pith size	Small	Small	Small	Small	Large	Large

apex, lower midrib, lower pith area, compact vascular bundles in shoot apex and tight or semi tight calyx in fruit were found to impart resistance to borer infestation (Nair, 1983). The morphological and biophysical characteristics of shoot and fruits were associated with attraction, feeding and oviposition of the pest. Plants defended themselves against herbivores using their biophysical and structural features (Nirmala and Irene, 2016).

Incidence of brinjal shoot and fruit borer

As brinjal shoot and fruit borer is a devastating pest of brinjal the selected accessions of *S. virginianum* were screened for borer infestation in two different seasons along with highly susceptible high yielding varieties of brinjal, ‘Ponni’ and ‘Surya’. The results are presented in Table 5. The accessions were screened for natural infestation of borer during August-November 2018 (Table 5).

Shoot infestation was not observed in any of the *S. virginianum* accessions and they were observed to be moderately tolerant, with scores which ranged from 0.14 to 0.67 per cent. However, shoot infestation to an extent of 13.33 per cent was observed in the variety ‘Ponni’ which was graded as highly susceptible. The extent of shoot damage in ‘Surya’ ranged from 15.55 per cent to 54.17 per cent:

Nandi et al. (2017) reported that peak infestation of borer was observed during summer season as the pest population was less in the first season (August-November). Confirmatory screening was done during March- July. The incidence of pest was

higher compared to the previous season. The variety ‘Ponni’ was observed to be highly susceptible with 54.16 per cent shoot and 45.57 per cent fruit damage. However, shoot damage was not observed in the *S. virginianum* accessions. Fruit damage to an extent of 0.14 to 0.94 per cent was observed. Kumar and Singh (2013) observed that seasonal incidence of shoot and fruit borer (on shoot) was more prevalent during vegetative phase of the crop. On initiation of fruiting stage there was a continuous decline in the infestation on shoots and it disappeared during fruiting stage of the crop.

In the present study, shoot damage in ‘Ponni and ‘Surya’ was higher during the second season (March- July) wherein pest population was high. The mean value for shoot and fruit damage for two seasons in ‘Ponni’ was 33.745 and 30.72 per cent respectively. However fruit damage was higher in ‘Surya’ compared to ‘Ponni’, where it ranged from 16.33 per cent to 67.25 per cent.

Morphological characterization revealed that variety ‘Ponni’ and ‘Surya’ were desirable with respect to fruit characters, but they were susceptible to borer. However, the accessions of *S. virginianum* were tolerant to borer, but the fruit characters were undesirable. *S. virginianum* being tolerant to borer with respect to both shoot and fruit damage, could be selected as a donor parent to transfer the genes for resistance to the susceptible line ‘Ponni’.

Fruit traits (shape, size and colour) could be considered as important marker traits that distinguished between *S. melongena* accessions,

Table 5. Scoring of *Solanum* accessions based on percentage of infestation of shoot and fruit borer

Accessions	Shoot damage(%)				Fruit damage(%)			
	Season1	Score	Season 2	Score	Season1	Score	Season 2	Score
Ponni	13.33	1	54.16	5	15.86	5	45.57	5
Surya	15.55	1	54.17	5	16.58	5	67.25	5
JJNS 15/34	0	1	0	1	0.67	0	0.94	1
JJNS 15/47	0	1	0	1	0.37	0	0.81	1
JJNS 15/84	0	1	0	1	0.31	0	0.14	1
JJNS 15/44	0	1	0	1	0.14	0	0.22	1
SE	2.52		9.57		2.58		6.82	
CD (0.05)	4.40		16.75		1.75		11.93	

0-Tolerant, 1-Moderately tolerant, 5- Highly susceptible

their related species and wild types. Other useful marker traits were prickles on leaf and fruit calyx, leaf blade lobing and corolla colour that could differentiate the wild types from *S. melongena* accessions and their related species. *S. virginianum* bore large number of small sized fruits with numerous prickles on stem, leaves, calyx and pedicel. Various morphological adaptations helped the plants to remain unaffected by the common pests and diseases of brinjal. The variety ‘Ponni’ was characterized by tall plants bearing large sized fruits. Prickles were absent in leaves, shoot, calyx and pedicel. The variety Surya was characterized by medium sized plants bearing large sized fruits. All the accessions of *S. virginianum* were tolerant to the infestation by brinjal shoot and fruit borer. However, the varieties ‘Ponni’ and ‘Surya’ were prone to fruit and shoot damage by borer. Considering this, *S. virginianum* accessions could be selected as parents in hybridization programmes. Anatomical parameters, viz., thickness of epidermis and cortex, trichome intensity, number and size of vascular bundles and pith size might serve as markers for resistance. Further studies may be conducted to evaluate the sensory and culinary properties of *S. virginianum*. Cross compatibility might be elucidated to utilize these accessions as donor sources in pre-breeding programmes.

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