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Research Article

Direct Defense of Common Bean Accessions Against Two-Spotted Spider Mite (*Tetranychus urticae Koch.*) Attack

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- Trichome-based Defense
- Trapping Behavior

Abstract

In order to find direct defense of seedling resistance of common beans, ten cultivars (Naz, Dorsa, Akhtar, Pak, 65-062-107, 65-071-98, 65-071-306, 65-071-410, 65-071-400, and 65-071-405) were evaluated by resistant mechanisms, morphological and physiological attributes against two-spotted spider mite in 2017 at Iran. Among four kinds of resistant mechanisms such as antixenosis, antibiosis, tolerant tests and PRI index, antibiosis mechanism determined to be the most accurate test in evaluating of common bean resistance against TSSM attack. Delaying germination, development and fast growth, early maturity, erected posture, small cotyledon area, the more leaf thickness along with the dark-colored demonstrated as escape mechanism of tolerant genotypes of common beans against TSSM attack. Some epidermal traits as the first defense barrier on common bean like hook-shaped and high density of trichome especially on adaxial epidermis by having trapping behavior showed high relationship with tolerant genotypes. In fact, in tolerant beans, related-traits to trichome on leaves entrapped the body parts of TSSM such as leg tarsi, mouthpart and ovipositor and prevented to move, feed and reproduction of mites and therefore, these features decreased damage leaf area of *Phaseolus vulgaris* as well. The lines of 65-071-98 and 65-062-107 and NAZ, were certainly categorized as the most tolerant cultivars while, Akhtar and 65-071-400 were introduced as the most susceptible accessions numbers of common beans, respectively.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important source of protein, carbohydrates, vitamins, minerals and unsaturated fatty acids throughout the world. Accordingly, cultivated pulse area in Iran was reported around 787 thousand hectares with 670 thousand tons yield. The common bean yield share accounted for 34.3% of the total pulse production, and cultivated commercially in provinces of Markazi, Lorestan, Fars and Zanjan, respectively [1].

The two spotted spider mite (TSSM), *Tetranychus urticae* Koch, is economically one of the most polyphagous herbivores worldwide with around 1,200 species and over 150 host plants of economic value [2-4], whereas is the important pest of variety of agricultural crops special on common bean, which can cause severe damages on broad spectrum [5]. Adults and immature of TSSM feed primarily on leaves producing tiny gray or silvery spots known as stippling damage [6]. At least, plants would be killed quite rapidly by damage effects, because the chloroplasts in leaves are gradually destroyed, while the population of feeding mites increased, photosynthesis declines, stoma close, and transpiration decreases, leading to reduced production [7]. These features, made it as dangerous pest on common bean with and reducing the quantity and quality of agricultural production while, eradicates around 10-20 yield crops in Iran [8].

Not only chemical control, as a common method, is not a sustainable strategy, when considering its capacity of developing resistance to acaricides by high reproductive potential and short life cycle [4,9], but also natural enemies as a fundamental way of biological control cannot reduce the amount of pesticides required to keep pests away from the field (this technique is only limited to greenhouse-based application [9,10]. Hence, use of resistant plants is argued as one of the most appropriate methods for controlling TSSM [11,12].

According to scientific research, seedling stage is one of the most critical and sensitive susceptible levels of common bean to TSSM, while greenhouse makes suitable environment for reproduction and spreading it [13,14]. Plant resistance could be explained by three fundamental mechanisms: non-preference (antixenosis), antibiosis, and tolerance, and have stressed the fact that these mechanisms are most frequently interrelated

although they may also operate independently [16,12]. Antibiotic plant traits negatively impact biology of pest through increases in mortality, reduced growth, longevity, and fecundity [17]. Antibiosis resistance studies on seven lines of Lord Egan Chiti bean to TSSM in field, introduced tolerant in one native cultivar [14]. Survey of population density and distribution effects of *T. urticae* on four Iranian bean varieties showed the highest and lowest population density of TSSM found on Tall ash and Parastoo, respectively [18]. Resistant evaluation of 36 common bean genotypes using standardized tests in greenhouse was carried out in Iran, showed that genotypes KS21163 and KS21235 were the most tolerant genotypes due to antibiosis, antixenosis and tolerance parameters [19].

Plant structural attributes might benefit its health by contributing to plant resistance to mites. The outermost physical defensive barrier is epicuticular wax crystalloid and the cuticle as direct defense. So, a thicker cuticle often prevented the mite penetration feeding and ovipositor [20]. The presence of diversity among epidermal micro characters such as cuticle thickness, trichome types, size and density, stomata types on the leaf surface of *Phaseolus vulgaris* showed that the number and structure of trichomes on the abaxial and adaxial epidermis were different between resistant plants to pathogens [13], whereas, there were two types of the non-glandular trichomes on *Phaseolus vulgaris* consisted straight or hooked with variable densities, and distributions on the plant [21]. By the way, trichomes played important role for the passive resistance of plants to pathogens, parasites and others stresses [22]. So that, [20] investigated non-glandular and four types of glandular trichomes on both sides of the leaf, and observed the growth regulators influenced qualitative and quantitative profiles of the volatile organic compounds and the number and distribution of hairs on the leaf surface to develop plant resistance. Furthermore, increased production of VOC had correlation with biotic stress, because trichomes were directly involved with the storage and secretion of phytochemical compounds [23].

On the other hand, the ventral surface of the leaf was more effective in trapping flies than other parts of the plant, Even though insects were frequently entrapped and killed by trichome traps. In a study capture-events monitoring showed the mouthparts, legs, and ovipositor of *Liriomyza trifolii* adults were the body parts involved in entrapment by surface hooked trichomes on *Phaseolus vulgaris* plants, and subsequently, deterred their ability to feed, walk, and oviposit [24].

In this research, resistance mechanisms (antixenosis, antibiosis, tolerance and PRI index) of common bean accessions against TSSM was studied in order to find both the most efficiency mechanism and the most tolerant accession number. Also, related attributes to direct defense on resistant plants, i.e. trichome-ased defense and trapping behavior were investigated.

MATERIALS AND METHODS

Plant material, experiment design

Ten divers common bean accessions were selected based on previous researches and provided by Gene bank of the department of agronomy and plant breeding, college of agricultural and natural resources, University of Tehran, Iran. Completely randomized design (CRD) with 10 replications was carried out at a growth chamber in Bu–Ali Sina University in summer of 2017.

Growth conditions of common bean

Sampling was carried out under seedling stage (after the plant had two developed trifoliate leaves) [15], in three different infection intervals i.e. 1, 3 and 5 days after infections [25].In order to make similar germination, the seeds were disinfected by Rovral-TS fungicide and were placed on a water-saturated filter paper in petri dishes, thereafter, one seedling has been transferred into the plastic pots (20cm diameter× 25cm depth) in which had filled with fertilized and sterilized loam field soil individually. Irrigation regularly applied every two days, which have been kept in a growth chamber conditions that has been set up at 25±2°C, RH=65±5%, photoperiod of 16:8 (D: L) and 13000 Lux light intensity with the Osraml Fluora 36W77 lamps [5].

Scrutinizing of morphological and physiological attributes

In order to look for correlation among attributes and resistant genotypes, some related traits were measured during common bean growth as seed color, plant posture, day to germination, day to seedling, cotyledon area, leaf thickness, density, size and type of trichome [13,26]. Since the leaf surface features such as epidermal cells play an important role in the variability of optical properties, the trichome length (in hooked trichomes and straight), trichome density (number per mm^2) and size (µ) were counted on a cutting width of the leaflet for both the adaxial and abaxial epidermis on three replicates, respectively [20,13]. Measurements carried out using stereo microscope Olympus were equipped with an ocular micrometer.

Two spotted Spider mite colony

Colony of TSSM was collected on common bean field in agricultural greenhouses of Bu-Ali Sina University, Hamedan, Iran, and reared on the potted Akhtar cultivar (susceptible control genotype) in greenhouse conditions. Furthermore, in order to growth and feed of mites, the plants were substituted with fresh potted plants once every two weeks [25,27].

Making the same age of the mite population

To make the same age of mites, cutting completed leaves placed in the petri dishes, while their trifoliate leaf trails were covered into water-saturated cotton vials. Thereafter, several adult female mites have been transferred on the leaves by brush carefully, after 24 hours from oviposition the mites removed from the field. At least everything left were indeed larva in the same age which applied in experiments [6].

Antixenosis test

To antixenosis evaluation (non-preference test), ten cultured

genotypes with their pots were randomly arranged in a circle around the center of platform (100cm diameter× 40cm height) carefully, while selected leaves isolated above of the platform and the pots located in the below. This situation repeated for three replications. At the next step 100 adult female mites of the same age released into the center of platform [6,27] and the platforms surrounded by cellophane for 72 hours in climatic chamber. Finally, number of live female mites on leaves counted by the stereoscope [28]. The mite density on leaves stated the amount of desire and preference of TSSM for every genotype.

Antibiosis test

For mite infestation, 30 adult female mites were basically released on target compound leaves of potted common beans by smooth brusher e.g. 10 mites for every trifoliate leaves, then whole of potted plants covered and isolated completely by mesh cloth which was consisted less than 250 micron pores during the test in climatic chambers for two weeks (this size was less than TSSM body width size). At the end of two weeks, all the adult female mites on leaves were counted [29,27]. In antibiosis test of genotypes evaluation criteria were mite reproduction on leaves.

Tolerant test

Tolerance carried out in the same way of antibiosis test. Moreover, the only difference was to assess damage levels on leaves ratio to evidence plant by damage score as described below table. The mite damage on each leaf of cultivars/genotypes was scored on a 1 to 6 scales [6,19,28].

Plant Resistance Index (PRI)

There were four steps to calculate plant resistance index [6].

a) Measuring the average of cultivars/genotypes with each resistant attributes (antibiosis, antixenosis, and tolerance)

b) Making data normalization of each attribute with normality test by Anderson-Darling test.

c) Calculating data standardization in which all above data were divided on the biggest data, independently.

d) Determining plant resistance index (PRI) that was calculated by Webster et al. 1993 formula.

PRI index = [1/(Antibiosis.Antixenosis.Tolerance)]

Data Analysis

Statistical analysis on data carried out by SAS software version 19 [30]. Before analysis of variance, normalization test was used and, for abnormal distribution data was done using square root transformation $(\sqrt{x+0.5})$. Mean comparison was done by Tukey's multiple range tests. Clustering of common bean genotypes based on damage score and number of mite on leaf in infested conditions was drawn using cluster analysis with Ward method and Pearson distance.

RESULTS AND DISCUSSION

Results

Analysis of variance: Results of analysis of variance using completely randomized design with 10 replications showed high significant difference between genotypes and all four resistance indices (α=0.01), indicating high genetic diversity among selected accessions to TSSM resistance. Other researches were confirmed existence of genetic diversity in treats, similarity [6,19]. Furthermore, low amount of replication effect stated accurate statistical design selection, while coefficient of determination (R^2) in CRD design confirmed experiment design, subsequently [Table 1].

Mean comparisons: Results of mean comparisons showed that kind of trifoliate which had the lowest amount of adult mites or eggs, possessed the highest antixenosis and antibiosis levels. According to Table 2, by evaluating the antixenosis test (non-preference), Akhtar and 65-071-400 with the highest mites and egg stayed in the same group as susceptible genotypes, while Naz as the most resistant genotypes were classified in contrast group. Using antibiosis test, 65-062-107, 65-071-98 and Naz categorized in same group as the most resistant cultivars, in comparison 65-071-400 and, Akhtar were as the most sensitive cultivars. Also tolerance test showed that the 65-071-98 genotype was grouped as the most tolerant cultivar in front of 65-071-400 and Akhtar as the sensitive with the most leaf damage. At least, using plant resistant index, 65-062-107 was grouped as tolerant variety, while both of 65-071-400 and Akhtar was in a same position as the most sensitive cultivars. Some of these results had similarity to [19,6,25] results. Without the doubt, results of cluster analysis obviously confirmed that antibiosis test, with classifying the

Table 1: Results of analysis of variance for indices of resistance mechanisms.

S.O.V.	df	Mean of Square			
		Antixenosis	Antibiosis	Tolerance	PRI
Genotype	9	0.31 **	0.187 **	0.165 **	0.271 **
Error	90	0.003	0.004	0.070	0.007
Total	99				
CV		9.222	15.96	38.63	28.90
R^2		0.98	0.924	0.512	0.94

**: Significant at 1% level.

Table 2: Results of comparisons of means of genotypes using Turkey's multiple range tests (α< 0.01)

Accession Number	Antixenosis test	Antibiosis test	Tolerance test	PRI
Dorsa	0.471 e	0.353 bc	0.573 e	10.497 d
(65-071-410)	0.658 d	0.494 b	0.630 d	4.882 c
(65-062-107)	0.113 g	0.206 c	0.515 f	83.415 h
(65-071-98)	0.346 f	0.240 c	0.332 g	36.272 e
(65-071-400)	0.948 a	0.843 a	0.962 a	1.301 a
Naz	0.101 h	0.202 c	0.733 cd	66.876 f
(65-071-306)	0.768 c	0.560 b	0.888 c	2.618 b
Akhtar	0.969 a	0.832 a	0.945 a	1.312 a
KS41128	0.519 e	0.447 bc	0.389 bc	11.091 d
(65-071-405)	0.869 b	0.617 b	0.905 b	2.071 b

genotypes to the four separated groups, was the most accurate test for evaluating of common bean as well (Figure 1).

Cluster analysis: Obtained results of cluster analysis presented by Ward method and Pearson distance (Figure 1), clearly showed that all cultivars were divided to four groups consisted as tolerance, semi-tolerance, semi-susceptible and susceptible accessions. Furthermore, Akhtar and 65-071-400 genotypes with the most similarity located to one group as susceptible cultivars against TSSM attack, while 65-071-98, 65-062-107, and Naz were grouped as tolerant cultivars. As well as, there were two middle groups: semi-tolerance (Dorsa and KS41128) and semi-susceptible cultivars (65-071-410, 65-071-306 and 65-071-405) [27,19]. found the similarity in results, too.

Simple correlation coefficients: Results of simple correlation coefficients between different mechanisms of resistance (Table 3) showed there were moderate correlations among resistant mechanisms. Although antibiosis and antixenosis tests are not easily separated from each other [31], there were high significant, positive correlation between antibiosis and antixenosis mechanisms (r=+0.956). Therefore, non-preference of TSSM to feed on common bean was directly related to the antibiosis. In other word, the mites preferred to reproduce, feed and survey the life cycle on common bean with high antixenosis. Also there were positive and significant correlation among the tolerance index, the antibiosis, and the antixenosis mechanisms. Since the tolerant mechanism was determined by low scale and scoring of leaves damage by TSSM attack, in conclusion those kinds of genotypes which had the low number of alive mite and egg on leaves, had high level of antibiosis and antixenosis resistances [27].

In addition, there was negative, significant correlation between all three mechanisms ratio the PRI index [6]. Despite this fact which determined the importance and contribution of all these mechanisms to the identification of the plant resistance index, can be said that the most effective mechanisms for estimating the resistance were the antixenosis and tolerant mechanisms at 1% significant levels (r= -0.783). It should be noted that negative sign in the correlation coefficients among all parameters belonged to the nature of the computational PRI formula [32]. Thus, the more amount of PRI index expressed high level of the resistant mechanism. These conclusions were certainly conformed to results of [19].

Physiological and morphological traits: In order to look for relationships among traits and direct defense mechanisms on accession, some morphological and physiological traits were measured during plant growth (Table 4).

A) Posture, seed germination, seedling stage and cotyledon area

There were basically relationships among early germination and posture in common bean genotypes to TSSM attack. Overall, resistant genotypes structurally prefer to escape from insect

Table 3: Results of simple correlation coefficients of resistance mechanisms.

Resistance Mechanism	Antixenosis	Antibiosis	Tolerance	PRI
Antixenosis	1			
Antibiosis	0.956 **	1		
Tolerance	0.992 **	0.951 **	1	
PRI	-0.781 **	-0.750 *	-0.783 **	1

* And **: Significant at 5% and 1% levels, respectively.

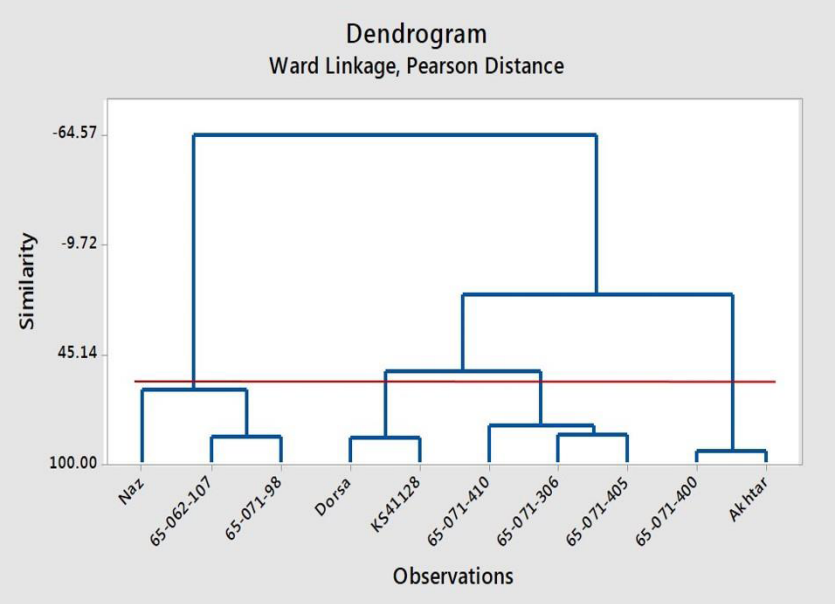

Figure 1 Dendrogram of accessions numbers clustering under TSSM resistant.

onslaught by delaying in germination like 65-071-98. So, resistant genotypes of beans had delay germination and development in mite stress situation. Moreover, whatever common bean could be tolerant to TSSM, preferably had erected posture. That was why Akhtar and 65-071-400 as susceptible cultivars had prostrate type style and afterwards, semi-resistant genotype such as Dorsa had erected-prostrate. It seems that scrollable plant style had more maintenance moisture ability in their canopy surface which is ideal for establishment to mite living and survey [26]. As well as, susceptible cultivars had more cotyledon area and leaf surface according to results of this research.

B) Epidermal traits: leaf thickness, Trichome density, size and type and damage score

Microscopic cut of leaves showed that there were three shapes of trichomes on the both of leaves surface of common beans (abaxial and adaxial), which consisted long straight, short straight and hooked shape with different density and size in the microscopic model (Figure 2). Morphologically, that kind of genotypes with hooked and high density of trichome shape in epidermis had resistant characteristic ratio that straight one with low density on the leaf surface. As well, leaves surface with short straight trichome showed more resistance to TSSM in front of long straight type. The results can be inferred that existence of hook-shaped and short epidermal characters directly prevented mite movement as barrier defense on common bean [13]. Apparently, anatomical structure of mite leg in term of being hooked engaged by leaf curly trichome and that was why movement and plant selecting for mite was difficult, so the mite prefer to live and reproduced on leaf of long straight trichome as well (Figure 3).

On the other hand, there were a relationship between resistant cultivar (damage score) and leaf thickness. This means that 65-062-107 as a resistant cultivar with the low damage score (less than 5% of leaf back chlorosis) had the most leaf thickness (1125µ) in addition of high density (62.5 per 5mm^2 area) and hook-shaped trichome on epidermis. In return, the lowest leaf thickness (350µ) with low density (3.45 per 5mm^2 area) and straight shape of trichome belonged to 65-071-400 as a susceptible genotype (necrosis of leaf back area more than 65%). These results were similar to [20,33] results.

Discussion

In conclusion of this research demonstrated that screening of common bean based on resistant mechanisms consisting antibiosis and tolerant test and PRI index were the most effective and efficiency strategy to select tolerant cultivars on common beans against TSSM attack. Considering that each stress including

Table 4. Morphological and physiological characteristics of common bean accession numbers.

Accession Number	Origin	Seed Color	Plant Posture	Day to Germination	[1]Day to Seedling
Akhtar	Iran (Breeding Variety)	Bright Red	Prostrate	5	12.5
65-071-400	Shahrood, Iran	Chiti	Prostrate	4.5	13.4
65-071-405	Bam, Iran	Dark Red	Erected-prostrate	5	12.5
65-071-306	Dareh gaz, Iran	Red	Prostrate	5.5	14
65-071-410	Rafsanjan, Iran	White	Prostrate	7	11.4
KS41128	Iran (Line)	White	Erected-prostrate	7.2	12.5
Dorsa	Iran (Breeding Variety)	White	Erected-prostrate	6.5	13.5
65-071-98	Fars, Iran	White	Erected	9	18.2
65-062-107	Guatmala	Black	Erected	8.5	16.7
Naz	Iran (Breeding Variety)	Dark Red	Erected	9.5	17.5

[1]Appearing two completed composited leaves

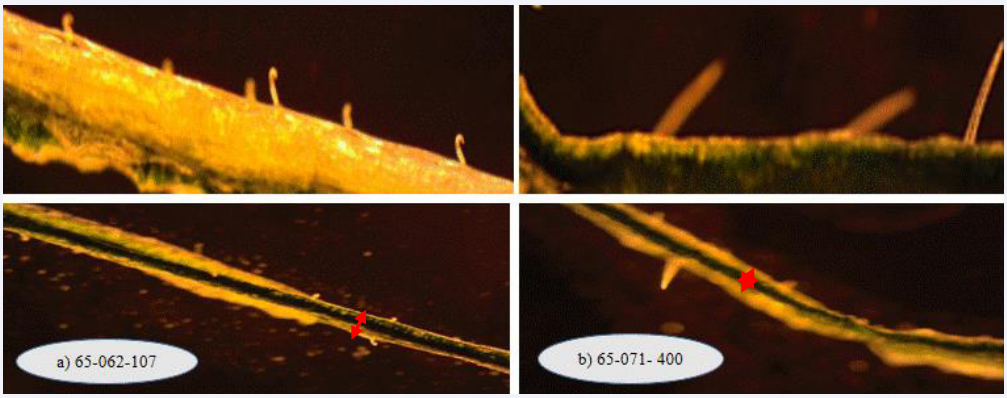

Figure 2 Trichome types, density and leaf thickness: a) resistance genotypes with hooked and high density of trichome, b) susceptible genotypes with long straight and low density of trichome, c) leaf thickness in resistant genotype and d) leaf thickness in susceptible genotype.

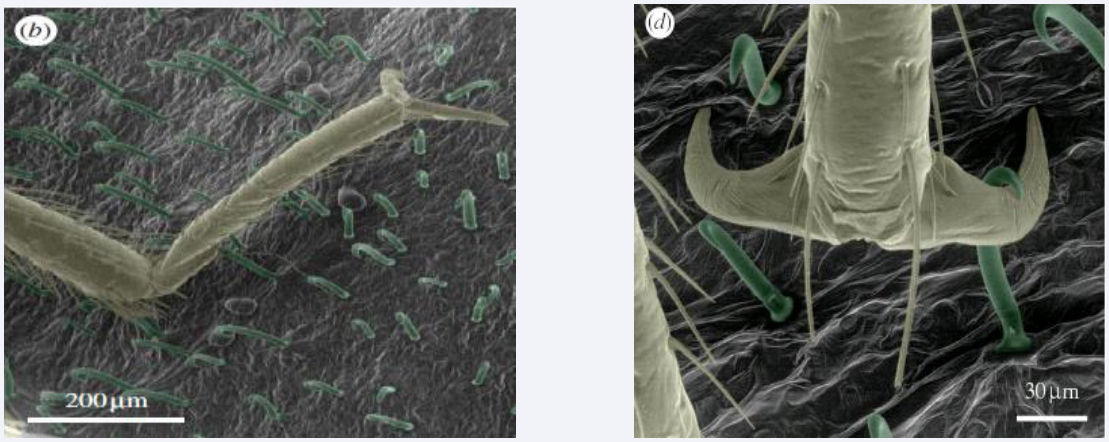

Figure 3 Adapted from lecture: "Entrapment of bed bugs by leaf trichomes inspires micro fabrication of biomimetic surfaces". Writhed by Szyndler et al. 2013. Hook- shaped trichomes of bean leaf surface by surrounding the leg tarsi prevented insects from moving.

Table 5: continued. Morphological and physiological characteristics of common bean accession numbers.

Accession Number	Cotyledon Area (cm^2)	Leaf Thickness (µ)	Trichome Density ($5mm^2$)	Trichome Size (µ)	Trichome Type (in frequency)	Damage Score
AKHTAR	27.5	430	5.25	1120.5	Long straight	6
65-071-400	31.12	350	3.45	1170	Long straight	6
65-071-405	20.5	650	15.5	720.5	Long straight	5
65-071-306	21.4	540	20.7	370.5	Short straight	4
65-071-410	20.12	425	9.7	265.5	Short straight	4
KS41128	22.4	845	17	450.5	Short straight	3
Dorsa	26.5	870	25.5	125	Hook- shaped	3
65-071-98	17.04	820	49.5	76	Hook- shaped	2
65-062-107	12.12	1125	62.5	62.5	Hook- shaped	2
Naz	18.25	950	48	87.5	Hook- shaped	2

biotic and abiotic can easily activate the cycle of the signaling cascade in the plant, we applied natural and uncut leaves (not leaf disk method), in order to prevent of the secretion of biochemical compound metabolites derived from direct defense mechanism during investigating of resistant mechanisms. Therefore, we found that the antibiosis mechanism was the most accurate test for evaluating of common bean resistance indeed.

Evidently, delaying germination was the characteristic of the tolerant genotypes of common beans, so that is the reason why they could tolerance TSSM attack in seedling stage. Subsequently, development in growth, early maturity, stand tall or erected rising posture, small cotyledon area, more leaf thickness with the dark-colored leaves, as escape mechanism, were correlated with this kind of genotypes against TSSM attack.

Behind it, some epidermal traits as the first defense barrier on common bean like leaf thickness, hook-shaped and high density of trichome especially on adaxial epidermis by having trapping behavior showed high relationship with tolerant genotypes against TSSM attack. In fact, related-traits to trichome by entrapping the body parts of TSSM such as leg tarsi, mouthpart and ovipositor on leaves prevented to move, feed and reproduction of mites. Hence, by decreasing damage area, all of the above features existed in tolerant cultivars of *Phaseolus vulgaris* as well.

In contrast, the susceptible cultivars had obviously low level of antibiosis, antixenosis resistances, and high significant level of damage score and PRI index. Moreover, more cotyledon area, thinner and brighter leaf, high long straight-shaped trichome along with low density on epidermis belonged to susceptible ones.

Above all, accession numbers of 65-071-98 and 65-062-107, along with breeding variety of NAZ, were certainly categorized as the most tolerant cultivars while, Akhtar and 65-071-400 line were introduced as the most susceptible accessions numbers of common beans by the highest damage levels of TSSM attack, respectively.

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