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# Screening of Common Bean Accessions to Two-Spotted Spider Mite (*Tetranychus urticae Koch*) under Growth Chamber Condition

**Alireza Taleei[1]*, Azar Mohammadi[1], Reza Maali-Amiri[1], Mohammad Khanjani[2]**

[1]Department of Agronomy and Plant Breeding, Tehran University, Tehran, Iran

[2]Department of Plant Protection, Bu–Ali Sina University, Hamedan, Iran

Research Article

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***For Correspondence:** Alireza Taleei, Department of Agronomy and Plant Breeding, Tehran University, Tehran, Iran

**E-mail: mohamadi.azar@ut.ac.ir**

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## ABSTRACT

The two-spotted spider mite *Tetranychus urticae Koch* is one of the economically most important pests in a wide range of protected crops worldwide. Due to its short life cycle, abundant progeny and arrhenotokous reproduction, it is able to develop resistance to these compounds very rapidly. Also, *Tetranychus urticae* is the most important pest of common bean (*Phaseolus vulgaris*) in Iran, and seedling stage is susceptible to the mite. Therefore, screening and using resistant cultivar of common bean is the most effective strategy to decrease mite damage on crops. In order to finding seedling resistance ten cultivars of common beans (Naz, Dorsa, Akhtar, Pak, 65-062-107, 65-071-98, 65-071-306, 65-071-410, 65-071-400, and 65-071-405) were selected to evaluate resistant mechanisms, morphological and physiological attributes to two-spotted spider mite under growth chamber condition included 25 ± 3°C temperature, RH=55% ± 5% humidity, 14:8 (D: L) photoperiod, and 13000 Lux light intensity in 2017 at Iran. For evaluating antixenosis resistance, 100 adult female mites were released in the center of platform with 100 cm diameter and 40 cm height in which selected leaves isolated above and the pots located in the below of platform, as long as the mites were counted after 72 hours.

Antibiosis and tolerant test were studied by counting adult female mites after two weeks while, leaf damage scale was evaluated by 1 to 6 scoring. According to ANOVA analysis there was significant difference among resistance mechanisms. It was determined antibiosis mechanism was the most accurate test for evaluating of common bean resistance, and there were high correlation between antibiosis resistance, leaf thickness and abaxial trichome type.

Finally, cluster analysis by Word method showed both of Naz and 65-062-107 were grouped as the most tolerant genotypes.

## INTRODUCTION

Common bean is one of the most critical crops in the world and makes up stable natural protein for human beings. Two-Spotted Spider Mite (TSSM) is one of the important pests of variety of agricultural crops, which can cause severe damages on broad spectrum [1]. It has now become one of the most important and widely grown crops in the world [2]. Based on statistics by the Iranian Ministry of Agriculture in 2017, pulse area cultivated in Iran was reported around 787 thousand hectares about 670 thousand tons yield. From these, the common bean yield share accounted for 34.3% of the total pulse production, and commercially it was cultivated in Markazi, Lorestan, Fars and Zanjan provinces of Iran, respectively [3]. The spider mite genus *Tetranychus* includes 149 species, some of which are of cosmopolitan agronomical pests, such as *Tetranychus urticae*. *T. urticae* is one of the most polyphagous pod herbivores special on common beans [4]. Adults and immature of TSSM feed primarily on leaves producing tiny gray or silvery spots known as stippling damage. Damage to the leaves inhibits photosynthesis, and severe infestations can result in premature leaf fall, shoot dieback and, decreased plant vigor [5]. Finally plants would be killed quite rapidly by damage effects, because the chloroplasts in leaves are gradually destroyed, while the population of feeding mites increase, photosynthesis declines, stoma close, and transpiration decreases, leading to reduced production [6]. The mites spin webbing, which can cover all the surfaces of the plant. TSSM feeds on more than 180 host plants [7]. It supposed to an adult TSSM measuring 0.5 mm in length passes through egg, larva, protonymph and deutonymph stages before becoming a consumer [8]. By the other hand, one generation of TSSM is completed in 19 days, at 21-23°C but, at 30°C the development is completed in 12 days [9]. These features, has made it as dangerous pest and reducing the quantity and quality of agricultural production. So it is an economical important pest while, eradicates around 10-20 yield crops. Furthermore, TSSM is most important pest on common beans [10]. Although chemical control is a common method, it cannot decrease mite population even by frequent chemical sprays to achieve effective control, and this is not a sustainable measure against that when considering its capacity of developing resistance to acaricides by high reproductive potential and short life cycle [11]. Therefore, the main target of plant breeding program are both of genetic resistance identification and resistance evaluation cultivars on all crops and then, crop damage assessment of TSSM on them [12]. In fact achieving to TSSM resistance of bean cultivar is early preparation to breed of common bean in order to produce high yield [13]. According to scientific researches, 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 [14]. A study in the United States showed that selected bean lines for resistance to TSSM had high correlation to some traits such as early maturity, growth habits, stand tall or rising, red-brown seed coat and white and black leave [15]. Accordingly, plants employ multiple strategies to defend against, tolerate or avoid insect herbivore which can be categorized into three categories: antibiosis, antixenosis or non-preference, tolerance or some combinations of these mechanisms [16]. 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 Lordegan Chiti bean to TSSM in field, introduced tolerant in one native cultivar [18]. Survey of population density and distribution effects of *T. urticae* on four Iranian bean varieties showed that the highest and lowest population density of TSSM found on Tallash and Parastoo, respectively [19]. Resistant evaluation of 36 common bean genotypes using standardized tests in greenhouse was carried out in Iran, which genotypes KS21163 and KS21235 introduced as the most tolerant genotypes according to antibiosis, antixenosis and, tolerance parameters [20]. Plant structural attributes might benefit plant health by contributing to plant resistance to mites. These plant architectural attributes be used by plant breeders in order to develop new cultivars resistant or tolerant to insects and diseases. The outermost defensive barrier of plant leaves against pathogens and the environment were the epicuticular wax crystalloids and the cuticle. When pathogens interact with the surface of a leaf, they might be deprived of water preventing in this way their germination. In addition, the cuticle was a considerable physical barrier to the penetration of pathogens. Therefore, a thicker cuticle may prevent mite penetration [21]. This was the first report describing the presence of diversity among epidermal characters in *P. vulgaris* that might contribute to plant resistance to pathogens [22]. On the other hand, epidermal micro characters such as deposition of wax crystalloids, cuticle thickness, trichome types, size and density, stomata types on the surface of leaves of *P. vulgaris* showed that the stomatal density and the number of trichomes on the abaxial and adaxial leaf epidermis were different between resistant bean accessions [23]. In this research, resistance of common bean cultivars/genotypes to TSSM was surveyed under seedling stage in growth chamber condition at plant protection department of Bu–Ali Sina University, Iran in 2017 summer. The main aim of this experiment basically was to investigate difference types of resistant mechanisms on common bean under TSSM attack in order to determine the most effective and efficiency of TSSM resistance mechanism in common bean cultivars. Moreover, to detect the most tolerant accessions, assessing of damage score of mite activity on leaves in seedling stage was the next aim. Finally, main morphological and physiological attributes were compered on both of resistance and sensitive cultivars/genotypes.

## MATERIALS AND METHODS

### Plant material, experiment design and field

Ten different common bean cultivars/genotypes which, were selected by lecture reviews, were prepared from central gene bank of agricultural and natural resources campus of Tehran University. The experimental design was done by Completely Randomized Design (CRD) with 10 replications at a growth chamber in Bu–Ali Sina University in summer 2017.

### Growth condition of common bean

This study carried out under seedling stage of plant (seedling phase in which the plant had two developed trifoliate leaves. In order to make similar condition for germination, the seeds were disinfected by Rovral-TS fungicide and, were placed on a water-saturated filter paper in petri dishes. Thereafter, they have been transferred into the plastic pots (20 cm diameter × 25 cm depth) in which had filled with fertilized and sterilized loam field soil so that one seed planted per pot individually. Irrigation regularly applied every two days, which have been kept in a growth chamber condition that has been set up at 25 ± 5 °C, RH=55 ± 5%, photoperiod of 16:8 (D: L) and 13000 Lux light intensity with the Osraml Fluora 36W77 lamps [24].

### Scrutinizing of morphological and physiological attributes

In order to look for correlation among attributes and resistant cultivars/genotypes, some related attributes were measured during common bean growth which were included seed color, plant posture, day to germination, day to seedling, cotyledon area, leaf thickness, density, size and type of trichome [25]. 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 on both the adaxial and abaxial epidermis on three replicates, respectively. Measurements were taken using an LM Nikon equipped with an ocular micrometer [26].

### Two spotted spider mite colony

Colony of TSSM was collected on common bean field in Hamedan province and, reared on the potted Akhtar cultivar (susceptible control genotype) in greenhouse condition. Furthermore, in order to growth and feed of mites, the plants were substituted with fresh potted plants once every two weeks. The TSSMs were transferred by a 4 zero smooch brush from the leaves at all stages [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 a water-saturated cotton vials. Thereafter, several adult female mites have been transferred on the leaves by brush carefully while, 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 [28].

### 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 carefully, while selected leaves isolated above of the platform, and the pots located in the below. This situation repeated three replications. At the next step 100 adult female mites in the same age released into the center of platform and, the platforms surrounded by cellophane for 72 hours in climatic chamber. Finally, number of alive female mites on leaves counted by the stereoscope [29]. The mite density on leaves stated the amount of desire and preference of TSSM on every genotype.

### Antibiosis test

In order to 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. At the end of two weeks, all the adult female mites on leaves were counted. In antibiosis test genotypes evaluation criteria were mite reproduction on leaves [30].

### 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 are shown in Table 1.

**Table 1.** The definition of damage levels caused by TSSM on common bean leaf under growth camber condition.

| Infested level | Detail |
|---|---|
| 1 | No damage |
| 2 | Chlorosis of leaf back area less than 5% |
| 3 | Chlorosis of leaf back area between 5%-25% |
| 4 | Chlorosis of leaf back area between 25%-45% |
| 5 | Chlorosis of leaf back area between 45%-65% |
| 6 | Necrosis of leaf back area more than 65% |

### Plant resistance index

There were four steps to calculate Plant Resistance Index (PRI), which include below stages [31].

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 statistic test.

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

### Data analysis

Statistical analysis on data carried out by SAS software version 19 [32]. Before analysis of variance, normalization test was used and, for abnormal distribution data was done using square root transformation (√(x+0.5)). Mean comparison was done by Duncan Multiple Range Test (DMRT). Clustering of common bean genotypes based on damage score and number of mite on leaf in infested condition was drawn using cluster analysis with Ward method and Pearson distance.

## RESULTS AND DISCUSSION

### ANOVA analysis

Results of analysis of variance using completely randomized design with 10 replications showed high significant difference between genotypes and all four resistance indices ($\alpha$=0.01), which expressed high genetic diversity among selected accessions to TSSM resistance. At other researches were confirmed existence of genetic diversity in treats, similarity [32]. Furthermore, low amount of replication effect stated accurate statistical design selection, while, coefficient of determination ($R^2$) in CRD design confirmed experiment design, subsequently are shown in Tables 2 and 3.

**Table 2.** Results of analysis of variance on indices of resistance mechanisms.

| | | Mean of square | | | |
|---|---|---|---|---|---|
| **S.O.V** | **df** | **Antixenosis** | **Antibiosis** | **Tolerance** | **PRI** |
| Genotype | 9 | 0.31 | 0.187 | 0.165 | 0.271 |
| Error | 90 | 0.003 | 0.004 | 0.07 | 0.007 |
| Total | 99 | - | - | - | - |
| CV | - | 9.222 | 15.96 | 38.63 | 28.9 |
| $R^2$ | - | 0.98 | 0.924 | 0.512 | 0.94 |

**Table 3.** Results of comparisons of means on genotypes by multiple range test (Duncan's test) at 0.05 level. **Note:** a,b,c,d,e,f,g: The geotypes at different salinity levels.

| Accessio n number | Antixenosis test | Antibiosis test | Tolerance test | PRI |
|---|---|---|---|---|
| Dorsa | 0.471 e | 0.353 de | 0.573 abc | 10.52 c |
| (65-071-410) | 0.658 d | 0.414 cd | 0.630 abc | 5.82 ab |
| (65-062-107) | 0.113 g | 0.306 de | 0.515 abc | 43.48 d |

| | | | | |
|---|---|---|---|---|
| (65-071-98) | 0.346 f | 0.240 e | 0.332 c | 40.00 d |
| (65-071-400) | 0.948 a | 0.643 b | 0.962 a | 1.71 a |
| Naz | 0.101 g | 0.202 e | 0.733 abc | 71.43 e |
| (65-071-306) | 0.768 c | 0.800 a | 0.888 ab | 1.84 a |
| Akhtar | 0.969 a | 0.832 a | 0.945 a | 1.83 a |
| KS41128 | 0.519 e | 0.547 bc | 0.389 bc | 8.01 c |
| (65-071-405) | 0.869 b | 0.847 a | 0.905 a | 1.51 a |

### Coefficient of correlation

Results of simple correlation coefficient between different mechanisms of resistance that there were moderately some correlations among resistant mechanisms. Although, antibiosis and antixenosis agents are not easily separated from each other. In this research, there were high significant, positive correlation between antibiosis and antixenosis mechanisms. 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. Because the tolerant mechanism was determined by low scale and scoring of leaves damage by TSSM attack, in conclusion those kind of genotypes which had the low number of alive mite and egg on leaves, had high level of antibiosis and antixenosis resistance are shown in Table 4.

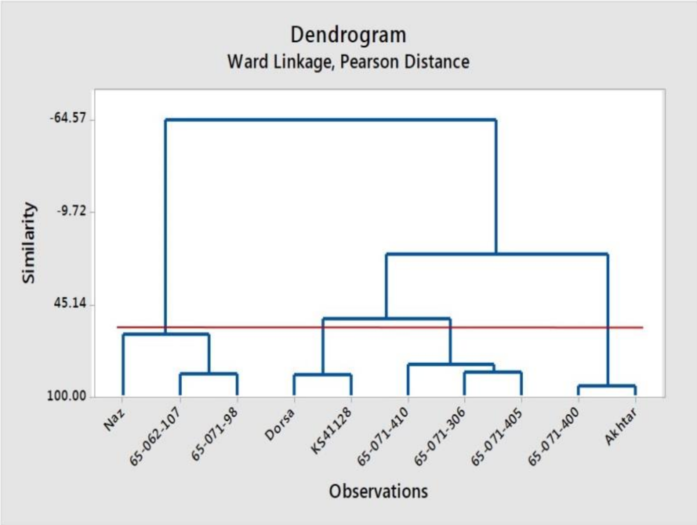
**Table 4.** Results of simple correlation coefficient of resistance mechanisms.

| 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 |

### Cluster analysis

Obtained results of cluster analysis presented by Ward method and Pearson distance in Figure 1. According to observations, all cultivars were divided to four groups which were consisted tolerance, semi-tolerance, semi-susceptible and susceptible accessions. Furthermore, Akhtar and 65-071-400 common bean genotypes with the most similarity located to a group as susceptible cultivars against TSSM attack, while 65-071-98, 65-062-107, and Naz were grouped as tolerant cultivars in experiment. Also, there were two middle groups as semi-tolerance and semi-susceptible genotypes, respectively: Dorsa and KS41128 in front of 65-071-410, 65-071-306 and 65-071-405. These conclusion were conformed to results [33].

**Figure 1.** Dendrogram of accessions numbers clustering under TSSM resistant.




## Physiological and morphological attributes

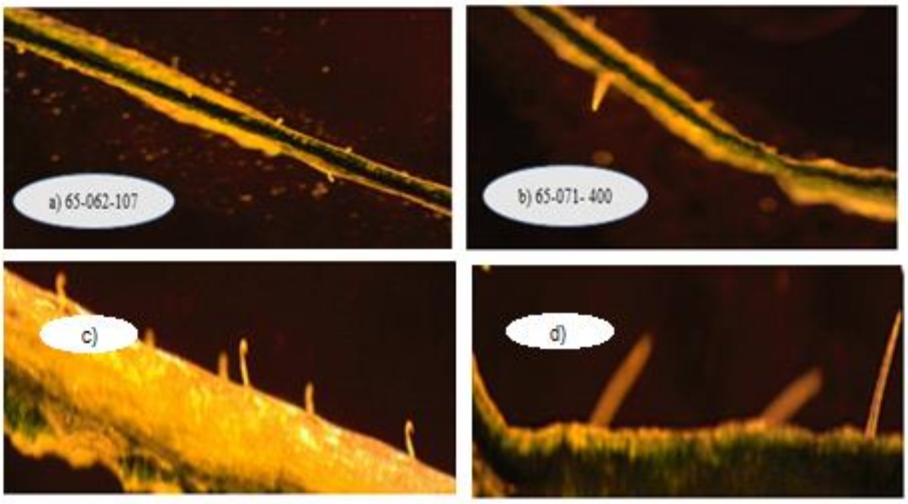
In order to look for relationship among attributes and direct defense mechanisms on accession, some morphological and physiological attributes were measured during plant growth.

**Posture, seed germination, seedling stage and cotyledon area**: There were basically relationship among early germination and posture in common bean genotypes to TSSM attack. Overall, resistant genotype structurally prefer to escape from insect onslaught by delay 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 seem that scrollable plant style had more maintenance moisture ability in their canopy surface which is ideal for establishment to mite living and survey. As well as, susceptible cultivars had more cotyledon area and leaf surface according to results of this research.

**Epidermal attributes:** Cross section of leaves showed that there were three shapes of trichome 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 are shown in Figure 2.

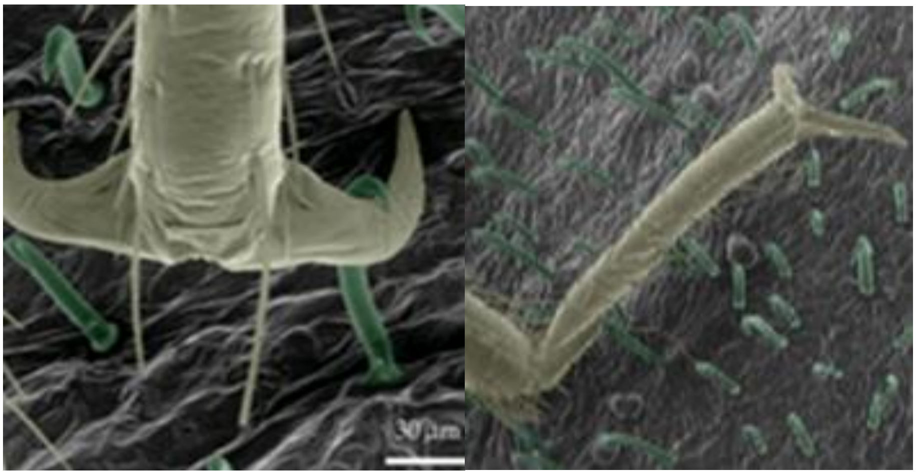
Morphologically, that kind of genotypes with hooked and high density of trichome shape in epidermis had resistant characteristic ratio that straight one with low density on the leaf surface. As well, leaves surface with short straight trichome showed more resistance to TSSM in front of long straight type of the results can be inferred that existence of hook-shaped and short epidermal characters directly prevented mite movement as barrier defense on common bean.

**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 d) leaf thickness in susceptible genotype.




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 as shown in Figure 3.

**Figure 3.** Entrapment of bed bugs by leaf trichomes inspires microfabrication of biomimetic surfaces.



Final results of this research demonstrated that screening of common bean based on some morphological attributes and resistant mechanisms like antibiosis and tolerant test, and PRI index were the most effective strategy to select tolerant cultivars on common beans against TSSM attack. Of these, It detected antibiosis mechanism was the most accurate test for evaluating of common bean resistance.

## CONCLUSION

Overall, Epidermal attributes such as hook-shaped and high density of trichome specially on the back of leaf surface (adaxial form) and leaf thickness had high relationship with tolerant genotypes by barely mite movement and behind it decreasing damage area as a the first defense barrier on common bean. By the way, some physiological traits like early germination, delay maturing traits and erected posture to be identified as common bean escape trick which belonged to tolerant cultivars, as well. Moreover, susceptible cultivars had more cotyledon area and leaf surface according to results of this research.

Total conclusion of this research showed 65-071-98 and 65-062-107 lines, and Naz breeding variety were categorized as tolerant cultivars altogether while, Akhtar and 65-071-400 were introduced as susceptible accessions numbers by the highest damage level, respectively.

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