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Newly Selected Soybean Genotypes and Their Resistance Against the Two-Spotted Spider mite *Tetranychus urticae* Koch

Hala H. Alakhder
Plant Protec. Res. Inst.. Agric. Res. Center, Dokki, Giza, Egypt.

ABSTRACT

The effect of some factors on the susceptibility of eleven soybean genotypes against *Tetranychus urticae* infestation during two successive seasons 2012 and 2013 was evaluated. The highest population density, in terms of motile and egg stages, was recorded on the genotype H15L27 (4980.3 and 3670) and (2898 and 2248); while the lowest was for the genotype H19L96 (2146 and 1533.3) and (1573.3 and 1309), respectively for the two years. The lowest infested genotypes were characterized by thickness of the upper and lower epidermis (17.67 and 14.28 μ), as well as palisade tissue and the thinness of spongy tissues (90.53 and 51.86 μ), respectively. Mite population density was positively correlated with leaves nitrogen and potassium contents; while negatively correlated with phosphrous. The predatorys, *Phytoseiulus persimilis* and the thrips, *Scolothrips sexmaculatus*, populations were recorded at the lowest density of *T. urricae* and *vice versa*. The susceptibility of soybean genotypes to the spider mite infestation was governed by leaf phytochemical components and histological structure of its surfaces and the presence of natural enemies. These results could be involved in breeding programme cultivated to improve future integrated pest management of soybean in Egypt.

Key words: *Tetranychus urticae*; Soybean genotypes; Suscpitibility; Histological structure; Phytochemical components; Predators.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) and it's have been a part of traditional food for human population. Its plants are attacked by many pests of spider mite, *Tetranychus urticue* (Koch), and can threaten yield quality and quantity (Massoud *et al.*, 2014). It feeds using piercing–sucking process that damages plant cells and tissue leading to the appearance of yellow chlorotic spots on leaves (Martinez-Ferrer *et al.*, 2006). Spider mites are difficult to control with pesticides due to inaccessibility of lower leaf surfaces, short life cycle, high reproductive capacity and ability to develop resistance to miticides (Nahar *et al.*, 2005 and Georghiou, 1990). Assessment of susceptibility/resistance of a crop plant towards pests is among the requirements of integrated pest management (IPM). Soybean variety characteristics can affect susceptibility to pest injury. The relation between population of predatory mites, such as *Phytoseiulus persimilis* Athias Henriot and predatory thrips, six spotted *Scolothrips sexmaculatus* (Pergande) and their host *T.urticae* on susceptible and resistant genotypes of soybean was reported to determine the ability of the predators to regulate their prey populations.

Leaves structure has been shown to be related to mite damage or to symptoms commonly associated with damage. The highest density of phytophagus mites occurred on leaves which, in addition to having higher total nitrogen content, had a thicker palisade mesophyll. Some varieties with thicker cuticle and epidermis were more resistant to spider mite. Besides that, the upper leaf surface was less preferred by mites, because its cuticle and epidermis are thicker than that of the lower surface (Kou *et al.*, 1972; Luczynski *et al.*, 1990 and Jyoti *et al.*, 2001).

This study aims to investigate the susceptibility of some soybeans genotypes to the natural infestation of *T. urticae* under field conditions. Moreover, some factors such as presence of natural enemies, histological structure of leave's surfaces and phytochemical components were investigated.

MATERIALS AND METHODS

Field experiments were carried out at Gemmiza Research Station, Agric. Res. Center, Gharbia, Egypt during 2012 and 2013 seasons to study the performance of eleven soybean genotypes, including eight newly selected strains (H19L96, L117, H32, H4L24, H15L127, H1L1, L127 and H30) in addition to three commercial varieties (Giza11, Giza35 and Crawford) to *T. urticae* infestation level. These genotypes were obtained from the Field Crops Research Institute, Agricultural Research Center, (Giza / Egypt). The experimental design was a randomized complete block (RCBD) with three replications. Each plot consisted of six ridges, 70 cm apart and four m. long. Soybean varieties were sown on the 1st week of June. Regular agricultural practices were conducted and plants were irrigated at the same time and no fertilizers or pesticides were provided. (Modarres, 2012).

Population density of spider mite was determined of different genotypes from germination until harvest, Jul. 2 to Oct. 4, 2012 and Jul. 4 to Oct. 3, 2013. Leaves were selected randomly from top, middle and lower canopy of each pant. Samples were picked up weekly after 25 days post-plantation. Twenty leaves were randomly collected and transferred to laboratory for examination. Motile stages and eggs of *T. urticae,* the predatory mite *Phytoseiulus persimilis* and larvae of the predatory *Scolothrips sexmaculatus* were counted using stereomicroscope.

Leaf samples of eleven genotypes were collected during the vegetation period, cleaned, washed and dried in an oven at 70 °C for 48 hours, then grinded into fine powder to determine nitrogen, phosphorous and potassium. Determination of total nitrogen was conducted according to Baker and Thompson (1992). Determination of P and K were measured according to Donohue and Aho (1992).

To clarify the relative susceptibility of soybean genotypes to *T. urticae* infestation in relation to the histological characters of the leaves, leaves from the first fully expanding trifoliate leaves were obtained; cross sections were prepared according to Ruzin (1999). Samples of each variety were placed in the fixed solution FAA, (each 200 ml of FAA composed of 100 ml 95% ethanol, 70 ml dH2O, 20 ml 37% formaldehyde solution and 10 ml glacial acetic acid), four 48 hours then transferred to 70% alcohol. Transverse sections were made according to Jackson (1976). Different measurements (in micron) of leaf thickness of upper epidermis layer, palisade tissues, spongy tissues and lower epidermal layer were determined.

Population of motile stages infesting different varieties and eggs of *T. urticae* were counted and statistically analyzed. Simple correlation between pest and its predators, physical and chemical plant leaf properties were calculated, Duncan (1955).

RESULTS AND DISCUSSION

The accumulated number of *T. urticae*, motile stages (immatures and adults) and eggs on the studied soybean genotypes were estimated during the two successive seasons 2012 and 2013 (Table 1). Statistical analysis indicated significant differences ($P<0.05$) between population densities of *T. urticae* on different tested soybean genotypes. The highest number of motile stages was observed on the genotype H15L27 (4980.3 and 2832) followed by H1L1 (4567 and 2600), whereas the lowest numbers were observed on the genotypes H19L96 (2416 and 1573.33) followed by Giza 111 (2738.7 and 1588) for the two seasons 2012 and 2013, respectively.

It is well documented that, plant genotypes differ greatly in suitability as host for a specific pest (Mohamed and Abdel Hafez 1981; Turhan *et al.*, 1983; Sawires *et al.*, 1990 and Gamieh *et al.*, 2001). The studied genotypes, H15L27, H1L1, L 127 and H 30 were considered as sensitive soybean genotypes; while H19L96, Giza111, L117, H32, H4L24 and Giza35 had the lowest population densities. In a paralleled study, (Alakhdar *et al.*, 2015) revealed significant differences among the yield of soybean genotypes with respect to *T. urticae* infestation. The genotypes, H 19 L 96, H 4 L 24 and H 32 were considered as the best ones regarding to the yield and lowest natural pest infestation, Meanwhile, the H19 L96 genotype surpassed the yield of Giza35 (check). Razmjou *et al.* (2009) indicated significant differences among mite population growth parameters on soybean cultivars, where *T. urticae* displayed lower population growth on Zane cultivar than on other cultivars including Hob 9 Will and L17. El-Sanady *et al.* (2007) revealed high significant differences between soybean varieties in their relative susceptibility to *T. urtica* infestation where Crawford variety was the most susceptible to *T. urticae*; while Giza 35was the highest tolerant to it; results which coincide with our findings.

Relation between histological characters of leaves and *T. urticae* infestation:

Negative significant correlation coefficient values were found between the abundance of *T. urticae* and the thickness of the upper and lower epidermis, and palisade tissues (-0.76,-0.8 and -0.79). On the other hand, a significantly positive correlation with the spongy tissue ($r = 0.89$) was observed (Table 2, Figs. 1 & 2). This proved that, leaf thickness is an important factor influencing mite infestation.

The highest population density of the mite was significantly recorded on H15L27 genotype in comparison with the other soybean genotypes, due to the thinness of upper and lower epidermis, and palisade tissues besides the thickness of spongy tissue of the leaves of this genotype. Similar trends were reported by Hanafy (2004) for cucumber varieties and the abundance of *T. urticae* motile stages. The more thickness of the cuticle epidermis especially that of the lower surface could be considered as a physical tolerance factor against mechanism of spider mite, (El-Sanady *et al.*, 2008). Shakoor *et al.* (2010) reported that leaf thickness is very important factor affecting the reproduction and development of mite populations. Abo-Zaid (2013) indicated significant differences between five tested cucumber cultivars in their relative susceptibility to *T. urticae* infestation in two seasons, and grouped them into highest and lowest infested groups. All the above mentioned data support the results of the present investigation. In

Table (1): Total number of motile stages and eggs of *T. urticae* during the two seasons 2012 and 2013

Genotypes	2012		2013	
	Motile stages	Eggs	Motile stages	Eggs
H19L96	2416±5.7^{f}	1533.3±1.2^{g}	1573.33±15.5^{f}	1309±1.15^{f}
Giza 111	2738.7±5.2^{ef}	1709±4.6^{fg}	1588±3.46^{ef}	1490±4.6^{e}
L117	2853.6±1.2^{def}	1795± 3.5^{f}	1618±5.48^{ef}	1541±3.46^{e}
H32	3197.7±1.7^{cdef}	2163.3±6.4^{e}	1672.3±17.47^{ef}	1591.3±6.35^{de}
H4L24	3197.3±5.19^{cdef}	2209d±5.2^{e}	1750±5.77^{e}	1656±5.19^{cd}
Giza 35	3785.7±3.4^{bcde}	2359±5.8^{cde}	1947.67±10.9^{d}	1666. 7±5.77^{cd}
Crawford	3836±1.2^{bcde}	2429±1.2^{cd}	2194.6±8. 6^{c}	1752±1.15^{c}
H30	3879±1.2^{abcd}	2565.3±4.6^{c}	2202.6±4.6^{c}	1867±4.6^{b}
L127	4184.3±3.5^{abc}	2963±1.7^{b}	2310.67±16.8^{c}	1873±1.7^{b}
H1L1	4567± 4.6^{ab}	3462.6±3.5^{a}	2600.67±8.1^{b}	2193±3.5^{a}
H15L27	4980.3± 3.5^{a}	3670±1.7^{a}	2832±7.2^{a}	2248±1.7^{a}

Each value is a mean ± SD; n=20, Means followed by the same superscript (s) are not significantly differed by the least significant difference ($p<0.05$) (Duncan, 1955).

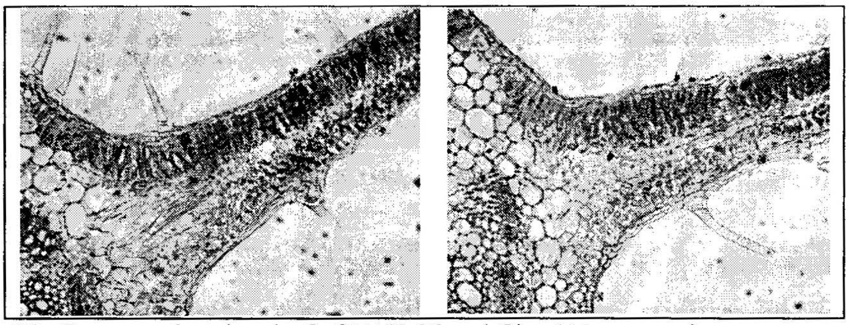

Fig. (1): Cross sections in a leaf of H19L96 and Giza 111 as a resistance genotypes.

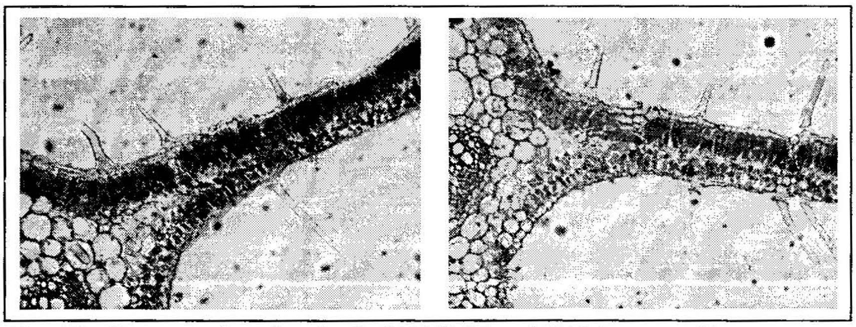

Fig. (2): Cross sections in a leaf of H15L27 and H1L1 as sensitive genotypes.

Table (2): Histological characters of leaves and their correlation coefficient with the population density of motile stages and eggs of *T.urticae* during the two seasons 2012 and 2013.

Soybean Genotypes	Mean diameters of leaf layers(µ)			
	Upper epidermis	Palisade tissue	Spongy tissue	Lower epidermis
H19L96	17.67±1.2^{a}	90.53±2.72^{a}	51.86±1.84^{ef}	14.28±0.96^{a}
Giza 111	16.21±0.43^{ab}	84.75±2.44^{ab}	64.45±1.7^{fg}	13.87±0.96^{a}
L117	16.1±0.46^{ab}	81.95±1.38^{b}	67±2.96^{fg}	13.38±0.5^{ab}
H32	15.9±1.37^{abc}	73.34±1.78c	73.4±1^{ef}	12.97±0.8^{abc}
H4L24	14.19±0.59^{bcd}	70.75±1.53^{cd}	76.7±5.45^{de}	11.82±0.46^{bcd}
Giza 35	13.93±1.38^{bcd}	66.35±2.28^{de}	79.77±2.17^{de}	11.24±0.69^{cd}
Crawford	13.49±0.73^{cd}	64.3±3.51^{de}	83.34±3.17^{cd}	11.1±0.36^{d}
H30	12.93±0.84^{d}	61.8±1.38^{ef}	89±0.86^{b}	10.89±0.6^{e}
L127	9.95±0.55^{e}	60.87±2.31^{ef}	91.45±2.16^{b}	8.59±0.91^{e}
H1L1	9.46±0.44^{e}	56.43±2.51^{f}	104.7±2.47^{a}	7.9±0.76^{e}
H15L27	9.08±0.63^{e}	55.56±2.48^{f}	107.9±0.9^{a}	7.68±0.52^{e}
	Correlation Coefficient values(r)			
Motile stages	-0.766***	-0.79***	0.89***	-0.8***
Eggs	-0.3089*	-0.29*	0.4***	-0.3024*

Each value is a mean ± SD; n=20, Means followed by the same superscript (s) are not significantly differed by the least significant Difference ($p<0.05$) (Duncan, 1955).

general, genotypes characterized by thicker layers of the upper and lower epidermis, and palisade tissues were less susceptible to spider mite infestation and subsequently, infested by fewer population and *vice versa*.

Relation between phytochemical constituents of leaves from different soybean genotypes and *T.urticae* infestation:

Data indicated a positive significant correlation between mite infestation levels and leaves nitrogen and potassium contents (0.7788 and 0.7513) and (0.556 and 0.2586) for motile stages and eggs population of *T.urticae* respectively, while negative correlation was found with phosphorous (-0.69 and -0.295), for the two seasons 2012 and 2013, respectively (Table 3).

The lowest population density of total life stages of *T. urticae* was obtained for H19L96 genotype as shown in Table 3. The decrease of phosphorus and nitrogen and the increase of potassium contents may be an important reason for unsuitability and decrease of the population density of the mite. Several studies (e.g., Yokama, 1978; Taha *et al.*, 1993; Taha and Raies, 1996; Gamieh and El-Basuony, 2001 and El-Sanady *et al.*, 2007) found significant negative correlation between leafe contents of potassium and positive correlation between phosphorus and nitrogen with respect to population densities of *T.urticae*.

Relation between population of each of *Phytoseiulus persimilis* and *Scolothrips sexmaculatus* and that of *T. urticae* on soybean genotypes:

Total population of the predatory mite *P. persimilis* and the thrips *S. sexmaculatus* are shown

Table (3): Phytochemical characters of leaves and their correlation coefficient with the population density of different stages and eggs of *T.urticae* during the two seasons 2012 and 2013

Soybean Genotypes	Nitrogen%	Phosphorous%	Potassium%
H19L96	0.28±0.02^{h}	0.72±0.01^{a}	0.95±0.9^{e}
Giza 111	0.32±0.28^{g}	0.29±0.01^{b}	0.98±0.01^{de}
L117	0.35±0.1^{f}	0.25±0.02^{bc}	1.01±0.04^{de}
H32	0.38±0.04^{ef}	0.22±0.01^{cd}	1.06±0.1^{cde}
H4L24	0.4±0.3^{e}	0.22±0.02^{cd}	1.12±0.07^{bcde}
Giza 35	0.41±0.3^{c}	0.18±0.01^{de}	1.47±0.3^{abcde}
Crawford	0.46±0.1^{d}	0.16±0.02^{ef}	1.25±0.02^{abcd}
H30	0.47±0.08^{d}	0.14±0.01^{ef}	1.33±0.02^{abc}
L127	0.53±0.02^{c}	0.12±0.01^{f}	1.37±0.02^{ab}
H1L1	0.57±0.04^{b}	0.06±0.02^{g}	1.47±0.02^{a}
H15L27	0.63±0.08^{a}	0.05±0.02^{g}	1.52±0.01^{a}
	Correlation Coefficient values(r)		
Motile stages	0.7788***	-0.6903***	0.7513***
Eggs	0.5572***	-0.2953	0.2586

Table (4): Density of Predators and their correlation coefficient with the population density of motile stages and eggs of *T. urticae* during the two seasons 2012 and 2013

Genotypes	2012		2013	
	P. persimilis	*S. sexmaculatus*	*P. persimilis*	*S. sexmaculatus*
H19L96	82.7±5.2^{g}	51.3±5.2^{g}	209±5.19^{h}	48±1.7^{d}
Giza 111	104±9.2^{fg}	70±4^{f}	316±9.2^{g}	48±4.1^{d}
L117	112±4^{fg}	83±1.7^{e}	402.7±4.1^{e}	44±5.2^{d}
H32	143 ±6.9^{ef}	107±1.7^{d}	403±1.7^{e}	77±2.3^{c}
H4L24	157.3±4.6^{e}	145±2.3^{c}	372±6.9^{f}	55±4.1^{d}
Giza 35	184.7±3.5^{de}	142±4^{c}	428±4.6^{d}	53±2.3^{d}
Crawford	180±1.7^{de}	139±2.3^{c}	533±1.7^{c}	75±2.8^{c}
H30	202.66±1.7^{cd}	155±3.7^{c}	526±3.4^{c}	70±2.8^{c}
L127	234±8.7^{bc}	145±2.8^{c}	535±8.6^{c}	81±3.4^{c}
H1L1	247±4.6^{b}	184±2.3^{b}	688.3±4.6^{b}	94±2.3^{b}
H15L27	330±15.2^{a}	270±4^{a}	765±15.3^{a}	147±4^{a}
	Correlation Coefficient values(r)			
P. persimilis	-0.535**	-0.807***	-0.9131***	-0.3884*
S. sexmaculatus	-0.5805***	-0.865***	-0.7455***	-0.7455 ***

Each value is a mean ± SD; n=20, Means followed by the same superscript (s) do not significantly differ by the least significant difference ($p<0.05$) (Duncan, 1955).

in Table 4. *P. persimilis* populations on the most susceptible genotype (H15L27) was 330 and 270 significantly greater than the most resistant genotype (H19L96) 82.7 and 51.3 at the two successive seasons, respectively, while those of the predatory thrips *S. sexmaculatus* populations were765 and 147 on (H15L27) and 209 and 44 on (H19L96).

Total predator populations were significantly greater on sensitive leaflets as shown in Table 4. The reduction in its populations on resistant genotypes was due to the absence of sufficient prey on resistant leaflets resulting in emigration and cannibalism among the predators; this agreed with findings of Sabelis (1981). According to Wheatleyl and Boethel (1992), predator populations could achieve control of spider mites faster on resistant than on susceptible ones, or both. It's interestingly to mention that, the number of preys consumed daily by the first and second instars larvae of *Scolothrips longicornis* was estimated to be 6.8 and 4.6 of motile stages of *T. urticae*, respectively, Pakyari, *et al.* (2009). In comparison with other spider mite predators, the daily consumption of *T. urticae* eggs by *S. longicornis* female (24 eggs per day at 26°C) was higher than reported for the phytoseiid females *Phytoseiulus persimilis* Athias-Henriot (14.9 eggs per day at 25°C) Friese and Gilstrap (1985) and *Amblyseius californicus* (McGregor) (13.4 eggs per day at 25°C) Gotoh *et al.* (2004a). (Pakyari and Enkegaard, 2011). These differences can be attributed to variations in their genetic makeup. It can also be of significant importance in a variety introduction programme as a source of resistance for further improvement of soybean genotypes.

Through the methods of hybridization and genetic recombination, pest resistant traits from resistant sources can be transferred to the agronomic acceptable genotypes. The obtained data can be utilized as a baseline for IPM programme for achieving better control against *T. urticae* on soybean.

Thus, in Integrated Pest Management (IPM) programs, choice of resistance genotypes is one of the major components in this strategy; by other words, the use of resistant genotypes in association with the use of natural enemies could promote better control of the target pests. This research demonstrated that the tested soybean genotypes had significant effects on the population density of *T. urticae*. The highest population density of the pest was seen on H15L27 and the lowest was on H19L96 genotype; while Crawford had an intermediate values. In this respect, our assessment was based on efficiency of natural enemies, histological and phytochemical characters of leaves of the different soybean genotypes. Hence, the use of resistant genotypes in association with the use of natural enemies or pesticides could promote better control of pest.

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