ORIGINAL ARTICLE

Evaluation of cucumber (*Cucumis sativus* L.) accessions (cultivars and lines) against the two-spotted spider mite (*Tetranychus urticae* Koch.) and kanzawa spider mite (*T. kanzawai* Kishida, Acari: Tetranychidae)

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Abstract:

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Evaluation of cucumber (*Cucumis sativus* L.) accessions (cultivars and lines) against the two-spotted spider mite (*Tetranychus urticae* Koch.) and kanzawa spider mite (*T. kanzawai* Kishida, Acari: Tetranychidae).
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Forty three cucumber (*Cucumis sativus* L.) accessions (cultivars and lines) were tested against the two-spotted spider mite (*Tetranychus urticae* Koch) and kanzawa spider mite (*Tetranychus kanzawai* Kishida). The highest number of *T. urticae* and *T. kanzawai* eggs /female/ day (5.98, and 6.23 respectively) was recorded on the cucumber genotype Blackish Green while the lowest eggs/female/day (2.95 and 3.31) was recorded on Winter Long Green (WLG) for *T. urticae* and *T. kanzawai* respectively. On a scale of 1 to 5, the highest visual damage rating was recorded on Blackish Green (4.75 for *T. urticae* and 4.78 for *T. kanzawai*) and the lowest damages rating of 1.50 and 1.70 were recorded on WLG for *T. urticae* and *T. kanzawai* respectively. Instead of distinct categories of resistance and/or susceptibility, an array of responses (number of eggs laid/female/ day and damage rating) was recorded on the tested cucumber accessions, suggesting two or more genes with

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additive effects. Based on our findings, Blackish Green was found to be a susceptible cultivar and Winter Long Green proved to be a resistant one. These two cultivars could be used as a differential host in further studies. Among the Korean cucumber lines, K-4, K-6, and K-20 where found susceptible while K-1, K-2, K-13, and K-15 exhibited some type of resistance to the two mite species.

Key words : cucumber, spider mites, damage ratting, resistance

Two-spotted spider mite, *Tetranychus urticae* Koch, is an important pest of many crops (Jeppson *et al.*, 1975) including cucumber (Hussey and Parr, 1963 and Balkema-Boomstra *et al.*, 2003). The Kanzawa spider mite, *Tetranychus kanzawai* Kishida, is primarily a pest of tea but can attack a number of crops (Kim *et al.*, 1996). Hot and dry weather conditions, coupled with suppressed population of natural enemies (due to indiscriminate use of pesticides) could lead to population explosion of the pest, which further demands the use of chemicals (Yang *et al.*, 2002). This chain reaction could ultimately leads to development of resistance in the pest to the pesticides. Management of the *T. urticae* has become difficult, primarily due to miticide resistance (Perring *et al.*, 1981, Bynum *et al.*, 1990).

A number of natural enemies (lady beetles; *Stethorus* spp. and predatory mites) exist and if given protection, can efficiently control the spider mites (Ferguson, 2003). Biological control alone and in combination with the host plant resistance can play a vital role in the management of the pest. Initial work on the resistance in cucumber to two-spotted spider mite was conducted by Hussey and Parr (1963), Dacosta and Jones (1971), Kooista (1971) and De Ponti (1977). Bitter gene (cucurbitacin-C) in the host plant (*C. sativus*) was considered to be responsible for resistance to the two-spotted spider mites. De Ponti (1979), however, denied the absolute role of bitterness against the two-spotted spider mite. The role of cucurbitacin was later confirmed by Bouwmeester *et al.*, (1999) and Balkema-Boomstra *et al.*, (2003).

Cucumber has bitter foliage and non-bitter fruits, but fruits may become bitter under stress. The gene *Bi* confers bitterness to the entire plant that could be determined in the cotyledons (Balkema-Boomstra, 2003). In a preliminary study with dihaploid (completely homozygous) progenies from the F1 generation of a cross between the non-bitter, susceptible inbred line G6 and the resistant bitter accession 9140, Balkema-Boomstra *et al.*, (2003) noticed an absolute link between spider mite resistance and bitterness (judged by testing the young cotyledons) in cucumber. All non-bitter-tasting dihaploid lines were highly susceptible, and the bitter-tasting dihaploid lines were shown to be resistant. Pierce and Wehner (1990) reported that the *Bi* gene confers bitterness to the entire plant while the plants with bi (the recessive allele) are bitter-free.

The objective of this study was to evaluate the bitter-taste cucumber cultivars and lines for any possible variation in term of resistance among them, and hypothesized that resistant in cucumber to the two-spotted spider mite and the Kanzawa spider mite was dependent on the level of cucurbitacin.

Materials and Methods

Out of forty three Cucumber (*Cucumis sativus* L) accessions, twenty lines (K1 - K20) were obtained from RDA (Rural Development Administration), Suwan, South Korea. Five Korean cultivars (Winter Long, Winter Long Green, Big Bong Dado-Gi, Dol-Pung Dado-Gi and Jan- Lok - Huk Jinju) were purchased in the open market in Suwan, Korea. Three modern cultivars (Encore 10, Sharp 1, and Suisei Fushinari) and ten cucumber lines (LCJ series) were received from Japan and five western cultivars (Market more 76, Poinset 76, Bet Alpha, Blackish Green and Five Rings) were purchased in Peshawar, Pakistan in open market. Two to three seeds of each genotype were sown in a plastic cup (7.5 cm diameter by 7.5 cm deep) in potting materials (Borokur) containing sterilized soil with vermiculite (minimum size) in a 3:2 ratio. After planting, the cups were watered and placed in an incubator at 25ºC with 16:8 photoperiods. Plants were watered twice a week with no fertilizers or chemicals added throughout the experiment.

Colonies of *T. urticae* were established from stock, maintained at the Ecology Laboratory, Seoul National University, since 1990, while field collected *T. kanzawai* (from *Prunus* spp.) were reared on kidney bean (*Phaseolus vulgaris* L) plants, at 2-5 leaf stage for about five generations before using in the experiment. All the experiments were conducted at 27±1ºC, 65% R.H. and 16:8 (L:D) photoperiod.

At two-leaf stage one set of all the test cucumber plants were inoculated with spider mites and individually caged. These plants provided us mature females for egg production on the required leaf disc of the test cultivars. A second set of test plants was maintained in isolated incubator at 25ºC with 16:8 (L:D) photoperiod for the regular supply of leaves for leaf discs.

Circular leaf discs of 2 cm diameter were cut with the help of a punch from leaves of kidney bean and from each cucumber accession and placed upside down on wet cotton (8 cm diameter) in a petri dish (8.5 cm). Leaf discs were arranged in a circular fashion. The position of each disc was marked for proper identification. Additional water was added to each petri dish to arrest the movement of the female mites from one disc to another. Each petri dish was covered with a lid (9 cm diameter) having 50-60 tiny holes to allow the escape of excessive moisture. A single young female of *T. urticae* was placed with a help of a fine camel hairbrush. The experiment was replicated 25 to 30 times depending on the availability of young females. For easy handling, a set of ten replicates was handled at a time. The same procedure was adopted for *T. kanzawai*.

At three day intervals, the numbers of eggs were counted and the females were transferred to a fresh leaf disc. The numbers of eggs were counted for 9 days and number of eggs laid/female per day was calculated for each accession.

In a separate experiment, two cucumber plants per accession were grown in plastic pots (15 cm diameter and 15 cm deep) with the same material as stated above. The kidney bean was also tested for comparison for damage rating. Plants were raised on string as suggested by De Ponti (1977). The experiment was run at 25±1ºC, 65% R.H. and 16:8 (L:D) photoperiod. At four-leaf stage, each plant was inoculated with 20 adult female mites. After six weeks of infestation, plant damage was scored using1-5 damage rating scheme of De Ponti (1977) where, 1 = slight damage (1-20%) and 5 = heavy damage (80-100%). The experiments for the two mite species were carried out separately to avoid cross infestation and each experiment was replicated three times. Data were analyzed using SAS Packages. Means were separated using DMR.

Results and Discussion

The number of eggs laid per female and the damage ratings of various cucumber accessions were significantly different in the mite species, *T. urticae* and *T. kanzawai* (Table 1 and 2). In case of the *T. urticae*, the highest number of 10.2 eggs/female/day was recorded on the Kidney bean followed by 5.98 eggs/female /day on the cucumber cultivar Blackish Green. On the other hand 2.95 eggs/female/day were recorded on Winter Long Green. Our results are some what supported by the finding of De Ponti (1985) who reported 21 eggs/female/3 days of *T. urticae* on a susceptible, bitter free slicing cucumber line which is equivalent to 7 eggs/female/day.

In case of *T. kanzawai*, the highest number of eggs laid/female per day was also recorded on Kidney bean (10.97 eggs/female/day). On Blackish green the numbers of eggs laid were 6.23/female/day and the lowest number of eggs laid/female/day was recorded on Winter Long Green (3.31). It could be concluded from these findings that Blackish Green is susceptible and Winter Long Green is somewhat resistant. These two genotypes could be used as differential hosts in further studies.

The Korean germ plasms showed a wide range of response. In case of *T. urticae* 5.60 eggs laid/female/day were recorded on K-4 (IT # 110903) while 3.43, 3.17 and 3.17 eggs were recorded on K-15 (# 110921), K-1 (#110871), and K-13 (# 110916) respectively. In the case of *T. kanzawai*, the highest numbers of 5.85, 5.83, and 5.82 eggs laid/female/day were recorded on K-6, K-4 and K-20, respectively. Lowest numbers of 3.7, 4.14, 4.41, and 4.72 eggs/female/day were recorded on K-13, K-15, K-2, and K-1 respectively. Somewhat similar response was exhibited by the Japanese cucumber genotypes. There seems some discrepancy in the numbers of eggs laid/female/day between the two mite species but this is quite natural. The number of eggs laid by a female on a particular cucumber accession could be an index of acceptance of the host for oviposition and the amount of nutrients or secondary metabolites provided by the host.

The damage rating of the various cucumber genotypes was also significantly different in response to the feeding by *T. urticae* and *T. kanzawai* (Table 1 & 2). Higher damages were recorded in cucumber accessions where greater numbers of eggs were produced and vice versa. There seems a positive correlation between the number of eggs laid/female/day and the extent of damages.

The variation among the cucumber accessions in terms of number of eggs laid/female/day and extent of damages may be due to the variable levels of the secondary plant metabolites (cucurbitacin-C). Balkema-Boomstra *et al*. (2003) reported variable levels of cucurbitacin-C in bitter dihaploid cucumber lines. The response (% survival rate) was negatively correlated to the level of cucurbitancin-C. The survival rate of the *T. urticae* was 26.3% on a cucumber line with 314.4 ug/g fwt, while 46.5% survival was recorded on cucumber line with 49.5 ug/g fwt cucurbitacin-C, which means the higher the level of cucurbitacin-C, the greater the mortality percentage in the two spotted spider mites.

Agrawal *et al*. (1999) reported that cucurbitacin formation in cucumber (*Cucumis sativus* L.) is induced in response to the spider mite feeding and this induction of cucurbitacin decreases the spider mites population by about 40%.

On the resistant cultivars, the mites develop two strategies; they either avoid feeding, remaining slim but weak, or they keep on feeding but cannot digest the food material, get swell up, becoming more or less bloated and turning black. This phenomenon was more prominent in *T. urticae* as compared with *T. kanzawai*. These changes become evident within a few days of feeding on the resistant cultivars. This might be one of the reasons for variability in response of the two mite species to the same cucumber genotype. Another reason could be the variability in the level of cucurbitacin among the various tested cucumber genotypes. This hypothesis is supported by the finding of Wehner *et al*. (1998) who reported a second recessive gene (bi-2) which segregates independently of the first gene (bi). The role of the dominant gene, Bi-2 has yet to be investigated (Balkema-Boomstra *et al*., 2003).

In one of the cucumber genotypes, (Dol pung Dado Gi), sticky materials were produced in response to the mites feeding (data not given). This material glued the mouthparts of the mites then the mite would die away of starvation. There is a need for further investigation on the nature of such material, which will serve as additional source of resistance in the cucumbers.

We got an array of response (number of eggs laid/ female and plant damages ratings) rather than distinct categories (resistant or susceptible). It could be speculated that two or more genes are involved in the resistance of cucumber to the mites. De Ponti (1979) hypothesized polygenic resistance in cucumber cultivars to the two-spotted spider mites. These genes for resistance in the cucumber segregate independently (Balkema-Boomstra *et al*., 2003), and their effects are cumulative in nature. The resistance mechanism is the same for both mite species. Further studies are needed to investigate the level of cucurbitacin in these cucumber genotypes and their impacts on the life table parameters of the mite species.

Table 1. Mean number of eggs laid per female/day* by *Tetranychus urticae* and damage rating response of various cucumber accessions (cultivars and lines) in comparison with kidney beans.

Cultivars/Lines	Eggs/female	Damage rating (mean±SD[1])
Kidney Bean	10.21	4.90±0.06a[2]
Blackish Green	5.98	4.75±0.11a
K-4 (11091)	5.60	4.75±0.83ab
Big Bong Dado-Gi	5.14	4.40±0.40ab
Five Ring	5.11	4.75±0.11ab
K-6 (110906)	5.10	3.90±0.23ab
Sharp-1	5.08	4.70±0.15ab
K-17 (110926)	5.04	4.67±0.13abc
Ao Naga (LCJ 780250)	5.02	4.50±0.15abcd
K-8 (110909)	4.98	4.40±0.12abcde
K-18 (110927)	4.94	4.50±0.14abcd
Shin dome (LCJ870153)	4.90	4.20±0.13abcdefg
Bieth Alpha	4.90	4.10±0.23abcdefgh
K-3 (110902)	4.89	3.60±0.16defghij
K-16 (110924)	4.83	3.70±0.21defghi
Daisen kema (LCJ 770046)	4.80	4.50±0.17abcd
Poinsett 76	4.73	4.70±0.13ab
K-14 (110918)	4.72	4.40±0.22abcde
Winter Long	4.71	3.90±0.23bcdefg
Jan- Lok -Huk Jinju	4.68	4.00±0.21abcdefgh
K-2 (110901)	4.63	2.55±0.17kl
Sue Je (LCJ 860239)	4.59	3.50±0.16efghij
K-12 (110914)	4.47	2.75±0.19jkl
K-10 (110912)	4.46	2.35±0.18l
Shogan (LCJ 770057	4.32	4.55±0.14abcd
Shagami Hanjiro (LCJ 770160)	4.23	4.20±0.20abcdefg
K-11(110913)	4.12	4.30±00.17abcdef
K-9 (110911)	4.09	3.40±0.16ghijk
Sushi Fushinari	3.99	4.05±0.13abcdefgh
Market More 76	3.90	3.80±0.15bcdefgh
San Tau (LCJ 900085)	3.86	3.85±0.18bcdefgh
Ao Fushinari (LCJ 910006)	3.79	3.30±0.13ghijkl
K-7 (110908)	3.76	2.70±0.17jkl
Dol-Pung Dado-Gi	3.68	2.55±0.13kl
K-5 (110904)	3.68	2.65±0.15kl
Natsu Fushinari (LCJ 810001)	3.61	2.80±0.13aijkl
K-20 (142335)	3.58	2.55±0.14kl
Sakata(LCJ 870024)	3.58	3.15±0.15hijkl
Encore-10	3.54	2.80±0.13ijkl
K-15 (110921)	3.43	2.80±0.15ijkl
K-1 (110871)	3.17	2.35±0.11l
K-13 (110916)	3.17	1.72±1.52 lm
Winter Long Green	2.95	1.50±0.05m

*** During 9 day period of reproduction**
[1] damage rating is based on 1-5 scale (1 being no or low damage and 5 highly damaged)
[2] Means in a column followed by the same letter are not significantly different (P>0.05)

Table 2. Mean number of eggs laid per female/day* by *Tetranychus kanzawai* and damage rating response of various cucumber accessions (cultivars and lines) in comparison with kidney beans.

Cultivars/Lines	Eggs/female	Damage rating (mean±SD[1])
Kidney Bean	10.97	4.80±0.13a[2]
Blackish Breen	6.23	4.78±0.09a
K-6 (110906)	5.85	4.51±0.08ab
K-4 (11091)	5.83	4.31±0.15abcd
K-20 (110921)	5.82	4.10±0.06abcde
Market More 75	5.73	4.25±0.18abcd
Natsu Fushinari (LCJ 810001)	5.68	4.14±0.26abcde
Five Ring	5.65	4.01±0.11abcdef
K-12 (110914)	5.62	4.00±0.12abcdef
K-17 (110926)	5.58	4.20±0.13abcd
Shogan (LCJ 770057)	5.57	4.35±0.17abc
K-10 (110912)	5.54	4.52±0.08ab
Encore-10	5.54	3.95±0.23abcdef
Jan- Lok -Huk Jinju	5.47	4.00±0.21abcdef
K-14 (110918)	5.45	3.97±0.19 abcdef
Sushi Fushinari	5.41	3.85±0.07abcdef
K-11 (110913)	5.38	3.82±0.18abcdef
K-18 (110927)	5.38	3.80±0.15abcdef
Winter long	5.34	3.75±0.21abcdef
Beth Alpha	5.33	3.73±0.30abcdef
K-8 (110909)	5.30	3.86±0.21abcdef
Poinsett 76	5.24	3.70±0.12abcdef
K-3 (110902)	5.14	3.80±0.32abcdef
Sagami Hanjiro (LCJ 770160)	5.13	3.63±0.22abcdef
K-16 (110906)	5.05	3.60±0.08abcdef
K-9 (110911)	4.97	3.50±0.14abcdefg
K-7 (110908)	4.96	3.55±0.32abcdefg
K-19 (110929)	4.96	3.53±0.90abcdefg
Ao Fushinari (LCJ 910006)	4.84	3.40±0.89 abcdefg
K-5 (110904)	4.80	3.39±0.31abcdefg
K-1 (110871)	4.72	2.55±0.11 efgh
Dol-Pung Dado-Gi	4.67	2.70±0.21defgh
Sakata (LCJ 870024)	4.43	3.00±0.11bcdefgh
K-15 (110921)	4.41	2.40±0.13fgh
K-2 (110901)	4.14	2.80±0.22cdefgh
K-13 (110916)	3.27	1.95±0.08gh
Winter Long Green	3.31	1.70±0.61h
Sharp-1	—[3]	4.35±0.90abc
Big Bong Dado-Gi	—	4.30±0.09abcd
Shindom (LCJ 870153)	—	4.15±0.0.29abcde
Ao Naga (LCJ 780250)	—	4.00±0.20abcdef
Daisen Kema (LCJ 770046)	—	3.80±0.21abcdef
Sue Je (LCJ 860239)	—	3.70±0.31abcdefj
San Tau (LCJ 900085)	—	3.25±0.12abcdefgh

***During 9 day period of reproduction**
[1] damage rating is based on 1-5 scale (1 being no or low damage and 5 highly damaged)
[2] Means in a column followed by the same letter are not significantly different (P>0.05)
[3] Eggs were not collected

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