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ORIGINAL ARTICLE

Interaction between *Phaseolus* plants and two strains of Kanzawa spider mites

Rika Ozawa[a]*, Ryo Matsushima[b], Massimo Maffei[c] and Junji Takabayashi[a]

[a]*Center for Ecological Research, Kyoto University, Otsu, Japan;* [b]*Institute of Plant Science and Resources, Okayama University, Okayama, Japan;* [c]*Department of Plant Biology and Centre of Excellence CEBIOVEM, University of Turin, Turin, Italy*

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Kanzawa spider mites, *Tetranychus kanzawai*, are polyphagous herbivores that feed on various plant families including legumes. Lima bean leaves infested by *T. kanzawai* emit a specific blend of volatiles. We found that two strains of spider mites were able to induce different blends of volatile to lima bean plants infested by either strain. Here we describe the genetic properties of the two strains and the responses of lima bean plants to the mite damage in terms of expression of defensive genes, emission of herbivore-induced plant volatiles, and phenotypic response of lima bean and other legume leaves (leaf color).

Keywords: color of the scar; herbivore-induced plant volatile (HIPV); lima bean; plant defense response; *Tetranychus kanzawai*

Introduction

The Kanzawa spider mite, *Tetranychus kanzawai*, feeds on many plant families, including Fabaceae. When they feed on lima bean plants (*Phaseolus lanatus*) or common bean plants (*Phaseolus vulgaris*), scars with different colors are observed (Yano et al. 2003; Matsushima et al. 2006). The mites can be divided genetically into two types according to the color of the scar. Since the color of scars is considered to be one of the responses of damaged plants, gene(s) associated with this phenomenon might be involved in the interaction of these mites with plants. In this report, we focus on the differences in interactions between the *Phaseolus* plants and each strain of these mites, and discuss how this phenomenon is involved in the survival of the mites.

Genetic properties of two strains of Kanzawa spider mites

When lima bean plants and common bean plants are infested by Kanzawa spider mites, scars of two different colors – white and red – are observed on the leaves. The mites were divided into two strains by artificial selection based on the colors of scars on the *Phaseolus* leaves (Yano et al. 2003; Matsushima et al. 2006). The spider mites causing red and white scars were named Red and White, respectively. Reciprocal crosses between the two strains revealed that the Red is fully dominant over the White (Yano et al. 2003; Matsushima et al. 2006). Backcrossing also revealed that this phenotype is dominated by a single locus of the mite by using common bean leaves (Yano et al. 2003).

The two different strains show different behavior on common bean plants. When the mites were placed on one of two leaf discs which were connected by a vinyl bridge, the Red strain moved to another leaf disc faster than the White strain did (Yano et al. 2003). Furthermore, the Red strain showed higher performance than the White strain on lima bean leaves: randomly selected Red females (mixture of young and old adults) laid a larger number of eggs than White females after 3 days and also after 7 days of infestation, although the number of eggs laid by newly emerging females of the Red and the White strains was not significantly different at 3 days of infestation (Matsushima et al. 2006). These results suggest that the Red strain suppresses the direct defense of lima bean plants more efficiently than the White strain. When common bean plants were infested by the same number of each strain, the plants infested by the Red blasted earlier than those infested by the White (Yano et al. 2003). This phenomenon is considered to be caused by the larger number of eggs laid by the Red females. Thus, the defense response of the *Phaseolus* plants against each strain could be different. To test this possibility, we studied the physiological response of lima bean plants to damage by each strain.

Responses of lima bean plants to the mite damage – expression of defensive genes

When plants undergo various kinds of stress, various kinds of response are induced. A typical example is the expression of defensive genes such as pathogenesis-related (PR) genes and genes involved in the phyto-oxylipin pathway. To investigate the differences of responses of plants that were infested by the Red or the White, we analyzed the expression of PR genes in infested leaves. Higher expression levels of

*Corresponding author. Email: ozawar@ecology.kyoto-u.ac.jp

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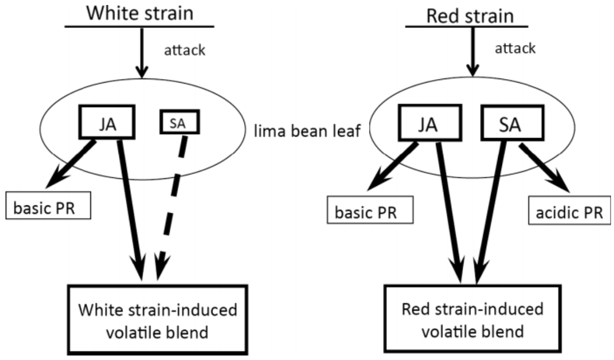

Figure 1. A model of induced responses and signal transductions in lima bean leaves infested by either the Red strain or the White strain. JA: jasmonic acid; PR: pathogenesis-related protein; SA: salicylic acid.

the acidic PR gene (gene for acidic chitinase) were detected in Red-infested leaves, while expression of the basic PR gene (gene for basic chitinase) were similarly induced in the leaves infested by either the Red or the White (Matsushima et al. 2006). Since the acidic PR gene is activated by salicylic acid (SA), we analyzed the SA levels in the infested leaves. Levels of SA were increased in Red-infested leaves but not in White-infested leaves. The results of gene expression and SA level analyses thus suggest that both the jasmonate-related signaling pathway and salicylate-related signaling pathway are activated in Red-infested leaves, while mainly the jasmonate-related signaling pathway is activated in White-infested leaves (Figure 1). Exogenously added jasmonic acid (0.1 mM) reduced the number of eggs laid by two spotted spider mites in lima bean plants (Choh et al. 2004). Crosstalk between the jasmonate-related signaling pathway and salicylate-related signaling pathway is expected to be involved in direct defense against Kanzawa spider mites. Reciprocal crosses between the two strains revealed that the Red strain is also dominant for the expression of the acidic PR gene over the White strain (Matsushima et al. 2006). Thus, the genetic trait of induction of the red color in the scars appears to be linked to activation of salicylate-related signaling pathway.

Upon infestion by caterpillars, early events such as influx of calcium ions and generation of hydrogen peroxide are induced in the infested leaves (Maffei et al. 2006). Staining for hydrogen peroxide by Amplex Red and subsequent observation by confocal laser scanning microscopy were performed in the scars on lima bean leaves infested by Kanzawa spider mites. Green fluorescence of hydrogen peroxide was observed on the red scars but not on the uninfested parts, and it was mainly localized at the plasma membrane, where most of it was produced (Figure 2). These results suggest that generation of hydrogen peroxide is induced by at least the Red infestation. Differential early events in the response to damage by either strain must be confirmed in future studies.

Responses of lima bean plants to the mite damage – emission of herbivore-induced plant volatiles (HIPVs)

In response to herbivory, plants emit a specific blend of volatiles that attracts natural enemies of herbivores. The emission of the blend of volatiles (so-called herbivore-induced plant volatiles (HIPVs)) is considered to be one of the induced indirect defenses. Common bean plants infested by Kanzawa spider mites emit HIPVs. The HIPVs attract predatory mites, *Neoseiulus womersleyi*, that prey on Kanzawa spider mites (Teramoto, personal observation). We analyzed headspace volatiles emitted from lima bean leaves infested by each strain. Among the compounds we tested, the amounts of (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) induced by the Red strain were higher than those induced by the White strain in lima bean plants infested by 10 mites par a plant for 3 days (Matsushima et al. 2006). It has been reported that emission of DMNT was controlled under both jasmonate- and salicylate-related signaling pathways and emission of TMTT was controlled under salicylate-related signaling pathway in lima

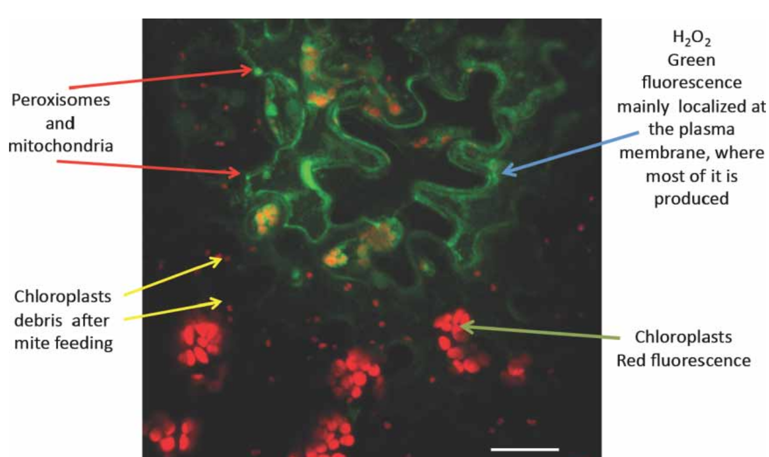

Figure 2. Generation of hydrogen peroxide. Amplex Red was used to stain lima bean leaves infested by the Red strain. A scale bar represents 50 µm.

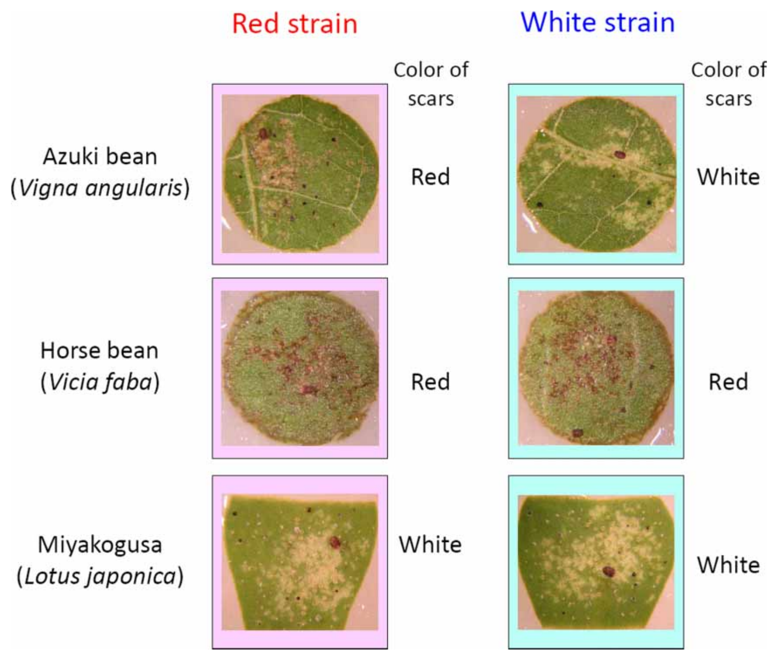

Figure 3. Color variation of scars on various legume species. Adult females of either the red strain or white strain were allowed to feed on leaf discs of each species for 2–4 days.

bean plants (Hopke et al. 1994; Koch et al. 1999; Ozawa et al. 2000). The elevated emission levels of these compounds in Red-infested plants were partly explained by induction of salicylate-related signaling pathway.

A predatory mite, *Phytoseiulus persimilis*, also preys on Kanzawa spider mites. DMNT attracts the predatory mites and the predatory mites use TMTT in discrimination between plants infested with prey or nonprey (De Boer et al. 2004; Kappers et al. 2005). Thus, damage made by Red is predicted to more robustly induce the indirect defense response.

Color of the scars on leaves of bean plants and distribution of each strain in the field

Two strains (Red and White) which were artificially selected by the color of the scars they induce on *Phaseolus* leaves induced different defensive responses of the *Phaseolus* plants. Although the trait of White is seemingly disadvantageous, resulting in phenotypes such as a low number of eggs on lima bean leaves, it might be an advantage when these mites feed on plants other than *Phaseolus* leaves. Color variation of the scars induced by the Red and the White is not always observed in bean plants other than *Phaseolus* (Figure 3). Among the other plants we tested, the color variation caused by Kanzawa spider mites was observed on leaves of azuki bean (*Vigna angularis*) alone. On the leaves of horse bean (*Vicia faba*), kudzu vine (*Pueraria lobat*) and suckling clover (*Trifolium dubium*), the scars caused by both strains were seen as red, while on narrowleaved vetch (*Vicia angustifolia*), *Lotus japonica*, white clover (*Trifolium repens*), and black locust (*Robinia pseudoacacia*), which is a woody plant, the scars caused by both strains were seen as white (Matsushima and Ozawa, unpublished data). Thus, the color of scars caused by these two strains might be the same in many genuses of Fabaceae. In the cases where both strains cause scars of the same color, it has been unclear whether the defense responses of the plant are the same or not. If the defense responses of a certain plant are not different in scars of the same color, the genetic variation of the mites may not be necessary on such a plant.

The number of eggs laid by both strains differed depending on the plants on which they fed (Yano et al. 2003). The distribution of each strain in the field was investigated by observing the color of the scars on common bean leaves infested by the mites collected from various kinds of plants (Yano et al. 2003). The Red strain was predominant (70–80%) on all grass plants that those authors tested (*Boehmeria nivea*, *Cayratia japonica*, and *P. lobata*). In contrast, on woody plants, around 50% of the mites on *Nerium indicum* and 50–100% of the mites on *Orixa japonica* were the White strain. Thus, the predominant strains differ according to the host plant species in the field. Experiments examining the interaction between each strain and the plants on which the White strain is predominant could be performed to clarify the effects of the traits of Red and White on the survival of Kanzawa spider mites.

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References

Choh Y, Ozawa R, Takabayashi J. 2004. Effects of exogenous Jasmonic acid and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analogue of salicylic acid, on the egg production of a herbivorous mite *Tetranychus urticae* (Acari: Tetranychidae). Appl Entomol Zool. 39:311–314.

De Boer JG, Posthmus MA, Dicke M. 2004. Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. J Chem Ecol. 30:2215–2235.

Hopke J, Donath J, Blechert S, Boland W. 1994. Herbivore-induced volatiles: the emission of acylic homoterpenes from leaves of *Phaseolus lunatus* and *Zea mays* can be triggered by a β-glucosidase and jasmonic acid. FEBS Lett. 352:146–150.

Kappers IF, Aharoni A, van Herpen TWJM, Luckerhoff LLP, Dicke M, Bouwmeester HJ. 2005. Genetic engineering of terpenoid metabolism attracts bodyguards to Arabidopsis. Science. 309:2070–2072.

Koch T, Krumm T, Jung V, Engelberth J, Boland W. 1999. Differential induction of plant volatile biosynthesis in the lima bean by early and late intermediates of the octadecanoid-signaling pathway. Plant Physiol. 121:153–162.

Maffei ME, Mithoefer A, Arimura G, Uchtenhagen H, Bossi S, Bertea CM, Cucuzza LS, Novero M, Volpe V, Quadro S, et al. 2006. Effects of feeding *Spodoptera littoralis* on lima bean leaves. III. Membrane depolarization and involvement of hydrogen peroxide. Plant Physiol. 140:1022–1035.

Matsushima R, Ozawa R, Uefune M, Gotoh T, Takabayashi J. 2006. Intraspecies variation in the Kanzawa spider mite. J Chem Ecol. 32:2501–2512.

Ozawa R, Arimura G, Takabayashi J, Shimoda T, Nishioka T. 2000. Involvement of jasmonate- and salicylate-related signaling pathways for the production of specific herbivore-induced volatiles in plants. Plant Cell Physiol. 41:391–398.

Yano S, Kanaya M, Takafuji A. 2003. Genetic basis of color variation in leaf scars induced by the Kanzawa spider mite. Entomol Exp Appl. 106:37–44.