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Exogenous Methyl Jasmonate Alters Trichome Density on Leaf Surfaces of Rhodes Grass (*Chloris gayana* Kunth)

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Abstract Jasmonates, including jasmonic acid and its derivatives such as methyl jasmonate (MeJA), are plant growth substances that control various responses. Jasmonates regulate leaf trichome density in dicotyledonous plants, but their effects on the trichome density of monocotyledonous plants, such as those in the Poaceae, remain unclear. In the present study we examined the effects of exogenous MeJA on the trichome density of Rhodes grass, which has three kinds of trichomes: macrohairs, salt glands, and prickles. Exogenous MeJA significantly increased the densities of macrohairs and salt glands on the adaxial and abaxial leaf surfaces and those of prickles on the adaxial leaf surface. Because exogenous MeJA significantly reduced the leaf area, we calculated the number of trichomes per 1000 epidermal cells to eliminate the effects of reduced leaf area. Exogenous MeJA significantly increased the number of macrohairs per 1000 epidermal cells on both adaxial and abaxial leaf surfaces, but it significantly decreased the number of salt glands per 1000 epidermal cells on both surfaces. Exogenous MeJA had no significant effects on the number of prickles per 1000 epidermal cells on either of the leaf surfaces. These results indicate that exogenous MeJA alters the trichome density by affecting leaf area and trichome initiation, and the effects of exogenous MeJA on trichome initiation differ among the various trichome types.

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Introduction

Plants have epidermal appendages, known as trichomes, on their surfaces. Trichomes are defined as unicellular or multicellular protuberances extending from epidermal cells (Levin 1973). They develop outward on the surface of plant organs and vary in size, shape, and location among plant species. Trichomes play diverse roles such as reflecting radiation, reducing leaf wetness, secreting substances, and protecting the plant from herbivores and pathogens (Levin 1973; Wagner and others 2004).

Jasmonates, including jasmonic acid and its derivatives such as methyl jasmonate, are plant growth substances that control various responses. For example, jasmonates promote the formation of storage organs such as tubers, induce anthocyanin accumulation, and enhance tolerance against abiotic and/or biotic stresses (Rohwer and Erwin 2008). Recent studies have indicated that exogenous jasmonates increase the trichome density in tomato and *Arabidopsis* (Traw and Bergelson 2003; Boughton and others 2005), and that endogenous jasmonates regulate trichome development in these species (Li and others 2004; Yoshida and others 2009). However, to our knowledge, the contribution of jasmonates to trichome formation in monocotyledonous plants such as those in the Poaceae has not been examined.

Plant species in the Poaceae have several different types of trichomes, such as macrohairs, prickles, and microhairs (McWhorter and others 1993; Snow 1996). Macrohairs are generally unicellular structures that are visible to the naked eye. Prickles are structures with swollen bases and short, sharp apices. Microhairs are bicellular structures and are

found throughout the Poaceae, except for the subfamily Pooideae. From a functional point of view, trichomes are divided into two types: glandular and nonglandular (Levin 1973; Wagner and others 2004). Microhairs secrete substances such as polysaccharides, proteins, and minerals (Amarasinghe 1990; Ramadan and Flowers 2004) and, therefore, are classified as glandular trichomes. In some grasses of the subfamily Chloridoideae, microhairs act as "salt glands" that secrete excess salts transported into the shoots (Ramadan and Flowers 2004; Kobayashi and others 2007). For example, salt glands of Rhodes grass (*Chloris gayana*) secrete approximately 47% of the sodium ions that are transported into the shoots (Kobayashi and others 2007). Other trichomes such as macrohairs and prickles are classified as nonglandular trichomes. These structures physically deter insect pests and spray droplets (Levin 1973; Kumar 1992; McWhorter and others 1993). For example, the stem borer (*Chilo partellus*) exhibits a strong ovipositional preference for maize cultivars without macrohairs on the abaxial surface over those bearing macrohairs (Kumar 1992). Thus, Poaceae plants have diverse types of trichomes with different roles. The diversity of trichomes in the Poaceae contrasts with the single type of trichome in *Arabidopsis*.

The objectives of the present study were to determine whether jasmonates affect trichome formation in the Poaceae, and whether they have similar effects on diverse trichomes with different roles. To address these aims, we examined the effects of exogenous methyl jasmonate (MeJA) on the trichome density of Rhodes grass. Rhodes grass is a popular and widespread fodder crop as pasture and hay in tropic and subtropic regions and has three kinds of trichomes: macrohairs, prickles, and salt glands (Fig. 1).

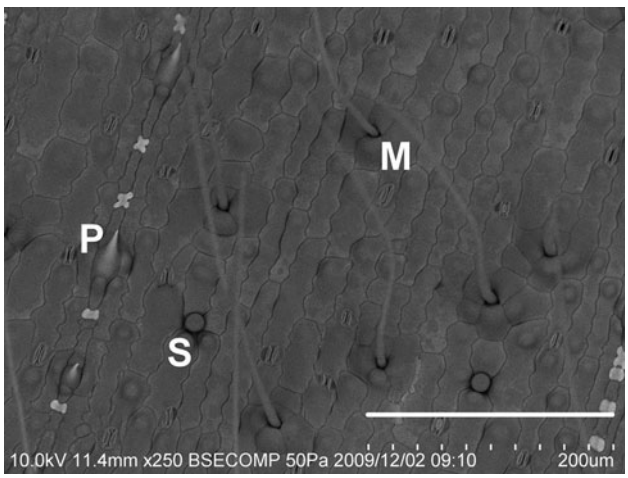

Fig. 1 Trichomes on leaf surface of Rhodes grass. Image shows abaxial surface of leaf blade without methyl jasmonate treatment. *M* macrohair, *P* prickle, *S* salt gland. Scale bar = 200 μm

Materials and Methods

Plant Material and Methyl Jasmonate Treatment

Rhodes grass (*Chloris gayana* Kunth cv. Asatsuyu) was cultivated as described previously (Kobayashi and others 2007). MeJA (Sigma Aldrich, St. Louis, MO, USA) was applied at the early four-leaf stage (leaf blades of the 5th leaves were elongating and leaf blades of the 6th leaves were not yet visible). The base of the shoot was wrapped in cotton (18 × 75 mm, 0.6 g) wetted with 4.5 ml of MeJA solution, and further wrapped in plastic wrap to prevent water evaporation. The concentrations of MeJA in the treatment solution were 0, 0.2, and 0.4 mM, and each solution contained 2% ethanol. The treatment was conducted for 48 h, and the cotton remained wet during the treatment. The plants were further cultivated until the 6th leaves were completely expanded.

Analysis of Leaf Structure

Leaf structure was analyzed by examining leaf blades of the 6th leaves. Leaf area was measured using an automatic area meter (AAM-9, Hayashi Denko co., Tokyo, Japan). Leaf surface structure was examined with a scanning electron microscope equipped with a cool stage (S-3400N, Hitachi High-Technologies, Tokyo, Japan). Fresh leaves were observed in the low-vacuum-pressure mode (50 Pa) at −25°C and 10 kV accelerating voltage. We selected leaf samples from four randomly selected positions on each blade: two for the observation of the adaxial surface and two for the abaxial surface. For each leaf sample, the numbers of macrohairs, salt glands, and prickles were counted under 100-fold magnification (area = 1.197 mm^2). The number of epidermal cells in an area of 0.04 mm^2 was counted under 250-fold magnification.

Statistical Analyses

The experiment was repeated twice and the results showed similar trends. Data presented in this report are from one experiment. Data were statistically analyzed using JMP software (4.0.5 J, SAS Institute Japan Inc., Tokyo, Japan). All data presented are the mean values ± standard error of nine leaves. Means were compared by Tukey's test and significance was set at $p \leq 0.05$.

Results and Discussion

Exogenous methyl jasmonate (MeJA) significantly reduced the area of leaf blades in Rhodes grass (Fig. 2a). To investigate the reduction of leaf size in detail, we examined

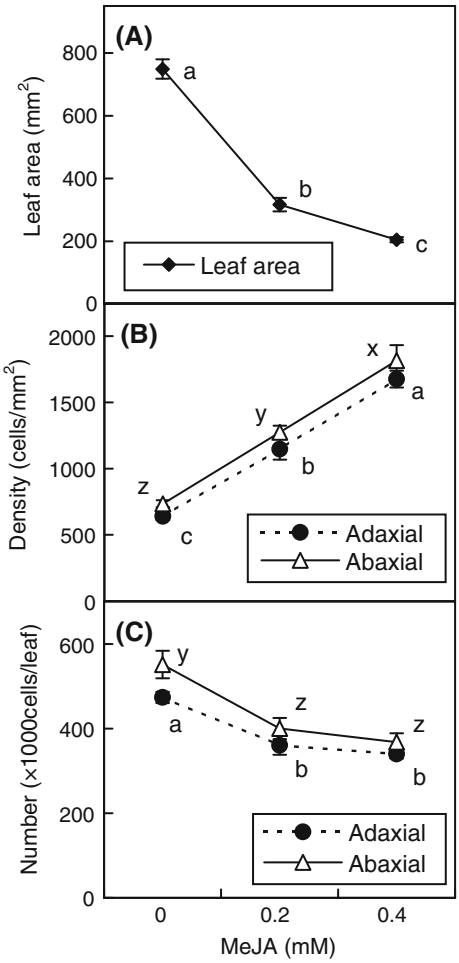

Fig. 2 Effect of exogenous MeJA on **a** area of leaf blades, **b** epidermal cell density, and **c** epidermal cell number per leaf. Data are mean ± standard error (SE) ($n = 9$). Data followed by the same letter are not significantly different (Tukey's test, $p < 0.05$)

the effects of exogenous MeJA on epidermal cells. Exogenous MeJA significantly increased the density of epidermal cells on both adaxial and abaxial leaf surfaces (Fig. 2b), which indicated that exogenous MeJA reduced the size of epidermal cells. In addition, exogenous MeJA significantly decreased the number of epidermal cells per leaf on both leaf surfaces (Fig. 2c), which was calculated from leaf area (Fig. 2a) and the density of epidermal cells (Fig. 2b). These results indicate that the reduction of leaf area by exogenous MeJA resulted from both a reduction in the epidermal cell size and a decrease in the number of epidermal cells per leaf.

Exogenous MeJA significantly increased the densities of macrohairs and salt glands on the adaxial and abaxial leaf surfaces, and those of prickles on the adaxial leaf surface (Figs. 3, 4a, c, e). Roy and others (1999) reported that leaf hair density is a complex character comprising two separable traits: leaf area and the number of hairs initiated per leaf. Because exogenous MeJA significantly reduced the leaf area (Fig. 2), we calculated the number of trichomes per 1000 epidermal cells to eliminate the effects of leaf area reduction and to evaluate the effects of exogenous MeJA on the number of trichomes initiated.

Exogenous MeJA significantly increased the number of macrohairs per 1000 epidermal cells on both adaxial and abaxial surfaces (Fig. 4b). These results are consistent with those reported previously for nonglandular trichomes of *Arabidopsis* and glandular trichomes of tomato (Traw and Bergelson 2003; Boughton and others 2005). Moose and others (2004) reported a number of similarities between the initiation of macrohairs in maize and that of trichomes in *Arabidopsis*. They proposed that initiation of macrohairs in maize is regulated by a complex of interacting proteins, similar to the complex that regulates trichome initiation in *Arabidopsis*. The complex in *Arabidopsis* is composed of the MYB domain (GLABROUS1), basic helix–loop–helix (GLABRA3), and WD-40 repeat (TRANSPARENT TESTA GLABRA1) families. Jasmonates are thought to regulate trichome density by acting upstream of this MYB-bHLH-WD-40 complex (Yoshida and others 2009). Therefore, jasmonates may regulate macrohair initiation in Poaceae plants via a complex similar to that found in *Arabidopsis*.

Exogenous MeJA significantly decreased the number of salt glands per 1000 epidermal cells on both surfaces (Fig. 4d). This contrasts with its effects on macrohairs in Rhodes grass (Fig. 4b) and trichomes of dicotyledonous plants (Traw and Bergelson 2003; Boughton and others 2005). These contrasting results suggest that regulation of salt gland initiation differs from that of other trichomes. This idea is partly supported by previous reports that exogenous gibberellic acid has little effect on the number of salt glands per 1000 epidermal cells in Rhodes grass (Liphschitz and others 1974), whereas it induces trichome initiation in *Arabidopsis* (Traw and Bergelson 2003). Although it remains unclear how jasmonates reduce salt gland initiation, there are two possible mechanisms: one is that jasmonates directly inhibit salt gland initiation, and the other is that they have antagonistic effects on the actions of other phytohormones. Previous reports indicate that exogenous benzyl adenine, a cytokinin, increases the number of salt glands and microhairs per 1000 epidermal cells in Rhodes grass and maize (Liphschitz and others 1974; Ramadan and Flowers 2004). Therefore, antagonistic regulation between jasmonates and cytokinins may occur in the initiation of salt glands.

At the concentrations used in this study, exogenous MeJA did not affect the number of prickles per 1000 epidermal cells (Fig. 4f). However, prickles are also reportedly under hormonal regulation. For example, exogenous benzyl adenine increased the number of prickles per 1000 epidermal cells in maize (Ramadan and Flowers 2004).

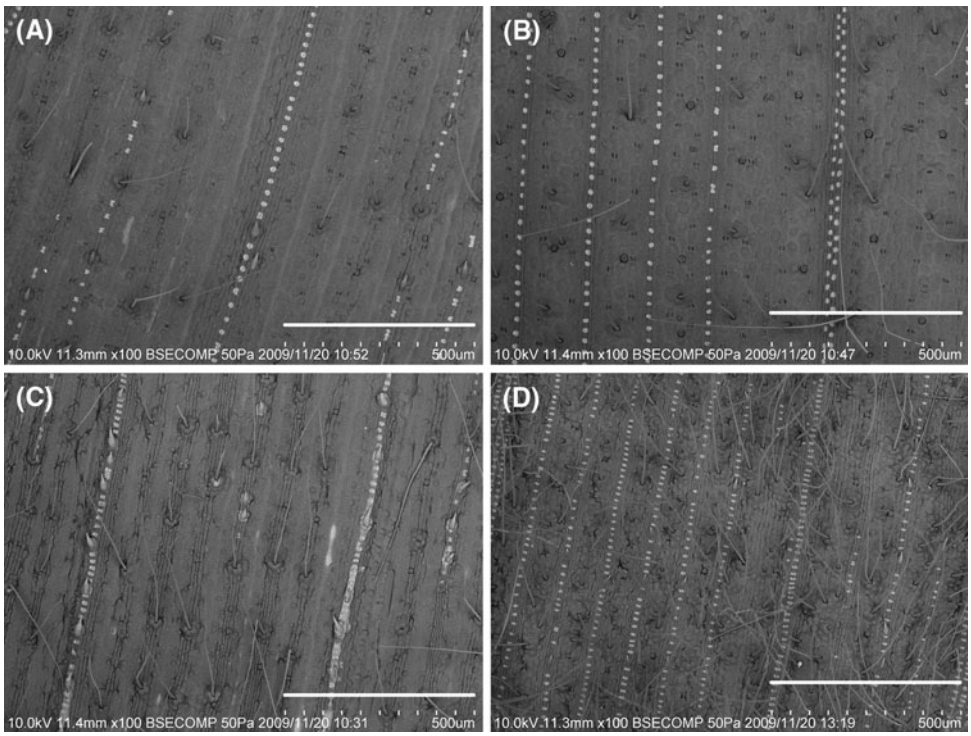

Fig. 3 SEM micrographs of leaf blade of Rhodes grass. **a** Adaxial surface of control plant. **b** Abaxial surface of control plant. **c** Adaxial surface of plant treated with 0.4 mM MeJA. **d** Abaxial surface of plant treated with 0.4 mM MeJA. Scale bars = 500 μm

Application of higher concentrations of MeJA may be required to clarify the effects of exogenous MeJA on initiation of prickles. However, the present results suggest that the response of prickle initiation to exogenous MeJA is not as sensitive as that of the other trichomes in Rhodes grass.

The contrasting responses of macrohairs and salt glands to exogenous MeJA (Fig. 4b, d) indicate that there may be an interaction between these two types of trichomes. A similar phenomenon in which the number of glandular trichomes decreased while that of nonglandular trichomes increased was reported in birch (*Betula pubescens*) after a defoliation treatment. This phenomenon was explained by the trade-off between organs (Rautio and others 2002). A "trade-off" means that allocation of resources to one type of defense occurs at the expense of other defenses. Trade-offs among defenses are also observed after application of plant growth substances, including jasmonates (Rojo and others 2003). In tomato, for example, exogenous jasmonic acid induces expression of the antiherbivore defense-related enzyme polyphenol oxidase, but it reduces expression of the pathogenesis-related protein P4 (Thaler and others 1999). The present results indicate that the trade-off theory may be applied to the relationship between macrohairs, which contribute to herbivore resistance, and salt glands, which contribute to salinity tolerance. In addition, the present results suggest that the trade-off between the two kinds of trichomes is mediated by jasmonates, which have roles in signaling a herbivore attack in Poaceae plants and in dicotyledonous plants (Schmelz and others 2009). However, it remains unclear whether endogenous jasmonates induce the trade-off between the types of trichomes. Therefore, further studies on the role of endogenous jasmonates in the formation of trichomes are required.

Exogenous MeJA significantly reduced the leaf area of Rhodes grass (Fig. 2), and this reduction affected the density of trichomes. The 0.4 mM MeJA treatment increased macrohair density (number of macrohairs per square millimeter) to 3.5 and 3.6 times that of the control on adaxial and abaxial leaf surfaces, respectively (Fig. 4a), whereas it increased the number of macrohairs per 1000 epidermal cells to only 1.3 and 1.5 times that of the control on adaxial and abaxial surfaces, respectively (Fig. 4b). Furthermore, exogenous MeJA significantly increased salt gland density on both leaf surfaces (Fig. 4c), whereas it significantly decreased the number of salt glands per 1000 epidermal cells (Fig. 4d). These results indicate that the reduction of leaf area by exogenous MeJA greatly contributes to the increase in trichome density in Rhodes grass.

In summary, the results of the present study show that exogenous methyl jasmonate alters the densities of macrohairs and salt glands in Rhodes grass by reducing leaf area and affecting trichome initiation. Furthermore, the effects of exogenous MeJA on trichome initiation differ among the various trichome types: exogenous MeJA increases the initiation of macrohairs but decreases that of salt glands. These different responses may be attributed to the trade-off between trichomes that play different roles in tolerance to abiotic and biotic stresses.

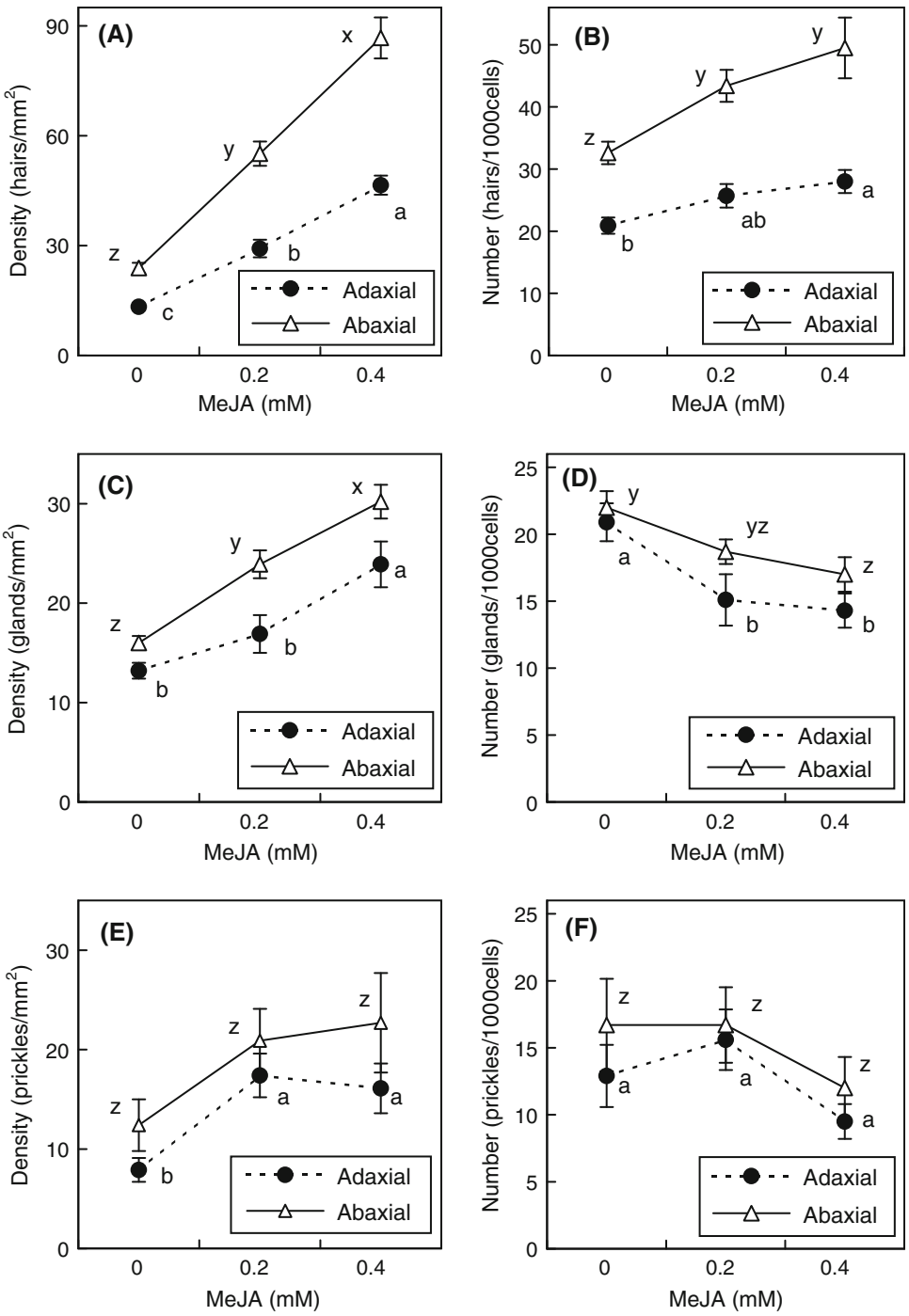

Fig. 4 Effect of exogenous MeJA on density of **a** macrohairs, **c** salt glands, **e** prickles, and number of **b** macrohairs, **d** salt glands, and **f** prickles per 1000 epidermal cells. Data are mean ± SE ($n = 9$). Data followed by the same letter are not significantly different (Tukey's test, $p < 0.05$)

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