Dichotomous role of jasmonic acid in modulating sorghum defense against aphids

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ABSTRACT

The precursors and derivatives of jasmonic acid (JA) contribute to plant protective immunity to insect attack. However, the role of JA in sorghum (*Sorghum bicolor*) defense against sugarcane aphid (SCA; *Melanaphis sacchari*), which is considered a major threat to sorghum production, remains elusive. Sorghum SC265, previously identified as a SCA resistant genotype among the sorghum nested association mapping founder lines, transiently increased JA at early stages of aphid feeding and deterred aphid settling. Monitoring of aphid feeding behavior using electropenetrography, a technique to unveil feeding process of piercing-sucking insects, revealed that SC265 plants restricted SCA feeding from the phloem sap. However, exogenous application of JA attenuated the resistant phenotype and promoted improved aphid feeding and colonization on SC265 plants. This was further confirmed with sorghum JA-deficient plants, in which JA deficiency promoted aphid settling, however, it also reduced aphid feeding from the phloem sap and curtailed SCA population. Exogenous application of JA caused enhanced feeding and aphid proliferation on JA-deficient plants, suggesting that JA promotes aphid growth and development. SCA feeding on JA-deficient plants altered the sugar metabolism and induced the levels of fructose and trehalose compared to wild-type plants. Furthermore, aphid artificial diet containing fructose and trehalose curtailed aphid growth and reproduction. Our findings underscore a previously unknown dichotomous role of JA, which may have opposing effects by deterring aphid settling during early stage and enhancing aphid's proliferative capacity during later-stages of aphid colonization on sorghum plants.

Keywords: Aphids, fructose, jasmonic acid, plant defense, sorghum, trehalose

INTRODUCTION

Jasmonates are lipid-derived signaling molecules that are involved in modulating plant responses to numerous environmental stresses, including attack by insect pests (Howe and Jander, 2008; Wasternack and Strnad, 2018; Ghorbel et al., 2021). Remarkably, different feeding guilds of insect pests activate distinct plant defense pathways. For example, it is well documented that jasmonic acid (JA) provides enhanced resistance to chewing herbivores (Howe and Jander, 2008; Wang et al., 2019), however, its role in providing defense against piercing-sucking insects needs further characterization. Although salicylic acid (SA) primarily impacts plant defense against piercing-sucking insects (Züst and Agrawal, 2016; Nalam et al., 2019; Zogli et al., 2020), it has been reported that in addition to SA, JA also plays a key role in modulating defense against piercing-sucking insect pests (Zhu-Salzman et al., 2004; Gao et al., 2007; Kuśnierczyk et al., 2011; Wasternack and Strnad, 2018). Moreover, antagonistic interactions between SA and JA fine-tune plant defense responses against various biotic stresses (Pieterse et al., 2012; Thaler et al., 2012; Solano and Gimenez-Ibanez, 2013).

Aphids are important group of piercing-sucking insect pests that cause substantial loss in plant productivity (Nalam et al., 2019). Aphids utilize their needle-like structures present in their mouthparts, known as stylets, to penetrate the plant tissues and to feed unceasingly from the phloem sieve elements, the aphid's primary source of nutrients (Louis et al., 2012; Louis and Shah, 2013; Nalam et al., 2019). While feeding, aphids produce two types of saliva: the 'stylet sheath', a gel tube that forms around the stylet, and 'watery saliva' that aids in ingestion of the phloem sap from the plant as it is released in the plant tissue (Miles, 1999; Will and Vilcinskas, 2015). Both watery and gel saliva of aphids contain proteins, enzymes, and metabolites that potentially interfere with plant defense pathways and/or resistance mechanisms, which in turn benefit the insect and facilitate sustained feeding from the sieve elements (Hogenhout and Bos, 2011; Elzinga and Jander, 2013; Kaloshian and Walling, 2016; van Bel and Will, 2016). For example, effectors present in phloem-feeding insects have been shown to utilize the crosstalk between SA and JA pathways to facilitate their feeding and colonization of host plants (Zarate et al., 2007; Xu et al., 2019). Most aphids, in addition to causing feeding damage, also vector economically important viral diseases (Kennedy et al., 1962; Matthews, 2012), which accentuates yield decline in host plants.

Sorghum (*Sorghum bicolor*), one of the most important monocot crops cultivated worldwide, is known for its versatility as a food, forage, and bioenergy crop. Sugarcane aphid (SCA; *Melanaphis sacchari*) is considered a major threat to sorghum production because it severely damages the plant by sucking sap from leaves, thereby reducing its photosynthetic ability. In addition, SCA vectors plant viruses that result in considerable yield loss (White et al., 2001). Deployment of resistant sorghum plants against SCA is considered as a powerful strategy for sustainable pest management (Limaje et al., 2018; Paudyal et al., 2019). Plants have evolved to utilize a multitude of defense mechanisms to reduce aphid infestation, which include antixenotic factors that deter aphid settling on host plants, and antibiotic factors that adversely impact aphid reproduction, survival, or growth and development (Smith, 2005; Nalam et al., 2019).

Aphid feeding alters the carbohydrate metabolism and signaling in host plants (Dinant et al., 2010; Zhou et al., 2015; Nalam et al., 2019). For example, green peach aphid feeding on

Arabidopsis foliage modulated the expression of genes associated with carbohydrate partitioning and sugar signaling in order to maintain the sucrose concentration in the phloem (Moran and Thompson, 2001; Pegadaraju et al., 2005; Singh et al., 2011). JA also acts as a key regulator of sugar accumulation in insect-infested tissues (Machado et al., 2015). JA depletion in *Nicotiana attenuata* leaves resulted in elevated invertase activity, which converts sucrose into glucose and fructose (Tang et al., 1999; Jin et al., 2009; Bhaskar et al., 2010). For aphids, sucrose acts as a major phloem feeding stimulant, and it also contributes to the high osmolarity of the phloem sap (Douglas et al., 2006). However, how JA-dependent sugar metabolism/signaling influence plant defense against aphids are scantly known.

Previously, exogenous application of methyl jasmonate (MeJA) was shown to deter greenbug aphid (*Schizaphis graminum*) settlement on sorghum plants compared to the untreated control plants, which suggested the JA pathway played a significant role in sorghum defenses against aphids (Zhu-Salzman et al., 2004). We therefore investigated the contribution of JA in the interaction of sorghum with the SCA. Here, we demonstrate that SCA feeding transiently increased JA levels at early stages of aphid feeding in sorghum SC265 genotype, which was previously reported to support fewer number of aphids among the sorghum nested association mapping (NAM) founder lines (Grover et al., 2020a). Concomitantly, aphids preferred to settle on plants that are deficient in JA, which suggests JA is an essential component in aphid deterrence. However, at later-stages, JA promoted aphid feeding and proliferation on sorghum plants. This is reflected by significantly reduced phloem sap consumption by aphids and diminished aphid proliferation on JA-deficient plants, which is independent of JA's antagonistic effect on SA pathway. In addition, our results demonstrate that aphid feeding elevated the levels of fructose and trehalose in JA-deficient plants compared to the wild-type (WT) plants and the direct effect of these compounds potentially contribute to defense against SCA. Thus, we uncover a novel dichotomous role of JA, where JA contributes to initial aphid deterrence, while at later-stages JA promotes aphid feeding and colonization on sorghum plants likely by favoring the desired sugar metabolism to aphids. Taken together, our results highlight the complexity of JA in modulating sorghum defenses against aphids.

RESULTS

Sorghum SC265 plants provide increased resistance to SCA

We have previously shown that, compared to the RTx430 plant, the sorghum genotype SC265 supported fewer number of aphids among the sorghum NAM founder lines (Grover et al., 2020a). Our no-choice bioassay further confirmed that aphid numbers (adult + nymphs) were significantly lower on SC265 plants than the RTx430 plants after 7 days of SCA infestation (Fig. 1A). To determine whether antixenotic factors, which deter aphid settling on host plants, are also involved in SC265's resistance to SCA, host choice by the aphid was studied by introducing 20 aphids at the center of each pot equidistant from RTx430 and SC265 plants. The number of adult aphids that were settled on SC265 plants were significantly less compared to RTx430 plants at 6 and 24 h post aphid release (Fig. 1B). Collectively, our data suggest that SC265 plants provide enhanced resistance to SCA when compared to RTx430plants.

SC265 plants limit SCA feeding from sieve elements

The Electrical Penetration Graph (EPG) technique is a robust tool to monitor the feeding behavior of aphids on host plants (Tjallingii, 1985, 2006; Nalam et al., 2018). Four different waveforms have been delineated from EPG studies: pathway phase, xylem phase, sieve element (phloem) phase, and non-probing phase. In the pathway phase, aphids are involved in various activities prior to sap ingestion, for instance, intra- and inter-cellular probing. The xylem and phloem phases correspond to the ingestion of water and phloem sap, respectively, whereas the non-probing phase refers to no-stylet movement in plant tissues (Tjallingii, 1985, 2006; Nalam et al., 2018). Representative EPG waveforms of SCA feeding for 8 h on different sorghum plants are shown in Supplemental Fig. S1. EPG comparison of SCA feeding behavior on SCA-resistant (SC265) and susceptible (RTx430) plants indicate that the SCA spent significantly less time in the sieve element phase (SEP) and more time in the pathway phase of SC265 plants compared to RTx430 plants (Fig. 1C). There were no significant differences observed for time spent by SCA in xylem and non-probing phases and to reach the first SEP between the RTx430 and SC265 plants (Fig. 1C). These results indicate that SC265 plants can limit aphid feeding from the sieve elements.

SC265 plants exhibit higher basal and SCA feeding-induced levels of SA

Phytohormones play a key role in modulating plant resistance to aphids (Louis and Shah, 2013; Nalam et al., 2019; Zogli et al., 2020). Higher levels of SA have been associated with enhanced resistance to aphids (Mohase and van der Westhuizen, 2002; Coppola et al., 2013; Kloth et al., 2016; Cui et al., 2019). SC265 plants had 10-fold higher basal levels of SA compared to RTx430 plants (Fig. 2A). No significant difference in SA levels were observed at 1 hour post infestation (hpi) between RTx430 and SC265 plants. SCA feeding on SC265 plants significantly enhanced

SA levels at 24 hpi, whereas no significant increase in SA levels was observed on RTx430 plants (Fig. 2A). In addition, SCA feeding-induced SA levels were significantly higher in SC265 plants at 7 days post infestation (dpi) compared to RTx430 plants (Fig. 2B). Taken together, these data suggest that the SC265 plants display higher basal and SCA feeding-induced levels of SA.

SCA feeding triggers transient increase in JA levels in SC265 plants

Although 12-oxo-phytodienoic acid (OPDA), an intermediate in the JA biosynthesis pathway, is known to provide monocot defense against aphids (Varsani et al., 2019; Grover et al., 2020b), both genotypes tested displayed no significant difference in the levels of OPDA before or after SCA infestation at the time points analyzed (Supplemental Fig. S2A and S2B). Basal levels of JA were comparable in RTx430 and SC265 plants, however, SCA feeding significantly induced the JA levels at 1 and 24 hpi in SC265 plants compared to RTx430 plants (Fig. 2C). Like JA, JA-isoleucine (JA-Ile), the biologically active form of JA, albeit transiently, was significantly higher in SC265 plants infested with SCA at 1 hpi (Fig. S2C). Similarly, although not significant, RTx430 plants showed slight increase in JA-Ile at 1 hpi of SCA, and reverted to basal levels at 24 hpi (Fig. S2C). We did not observe any differences in the JA and JA-Ile levels between the RTx430 and SC265 plants after 7 days of SCA feeding (Fig. 2D and Supplemental Fig. S2D). Based on these results, we suggest that SCA feeding activates transient increase in JA levels in SC265 plants at early time points compared to RTx430 plants.

Exogenous JA application promotes SCA growth and feeding on SC265 plants

SCA feeding transiently increased the JA levels at early time points (Fig. 2C). Because JA modulates sorghum defense against a different phloem-feeding aphid, greenbug (*Schizaphis* *graminum*) (Zhu-Salzman et al., 2004), we tested whether JA-Ile application limited SCA proliferation on SC265 plants. Quite to the contrary, compared with the control plants, pretreatment with JA-Ile promoted aphid growth and feeding on SC265 plants compared to JA-Ile untreated control plants (Fig. 3). Aphid no-choice bioassays showed comparable numbers of SCA on RTx430 plants pretreated with JA-Ile and the mock-treated control plants at 1, 3, and 5 dpi. However, RTx430 plants displayed significantly higher numbers of SCA on plants pretreated with JA-Ile compared to control plants at 7 dpi (Fig. 3A). Similarly, SC265 plants exhibited significantly higher number of aphids at 5 and 7 dpi on JA-Ile-pretreated SC265 plants compared to control plants (Fig. 3B). Using EPG technique, we also monitored the feeding behavior of SCA on sorghum plants pretreated with JA-Ile 24 h prior to SCA feeding. Although there was no significant difference in the duration of time spent by SCA in the SEP on RTx430 plants that were pretreated with JA-Ile and control plants, the duration of SEP was significantly enhanced on JA-Ile-pretreated SC265 plants compared to control plants (Fig. 3C), which indicated JA-Ile promoted SCA feeding from sieve elements of SC265 plants. We also observed a diminution in the duration of the pathway phase, during which the aphids were sampling the cells prior to sustained ingestion, on the JA-Ile pretreated SC265 plants compared to control plants (Supplemental Table S1). JA-Ile pretreatment did not impact other feeding behavior patterns of SCA, such as duration of xylem phase, non-probing phase, time to first probe and time to first SEP on both RTx430 and SC265 plants with or without JA-Ile treatment (Supplemental Table S1). Taken together, these results confirmed that JA promoted SCA growth and feeding on sorghum plants.

Sorghum plants impaired in JA synthesis provides enhanced resistance to SCA

To determine whether JA can promote SCA susceptibility and to provide genetic evidence of JA-dependent susceptibility to SCA, we used sorghum *multiseeded* (*msd*) mutant, *msd3* in BTx623 background. *Msd3* encodes for ω-3 fatty acid desaturase 7 (FAD7) (Dampanaboina et al., 2019; Gladman et al., 2019). Basal JA levels are significantly reduced in *msd3* plants, which indicate that Msd3 contributes to the JA synthesis in sorghum (Block et al., 2020). Furthermore, it was shown that Msd3 likely plays an important role in the synthesis of herbivore-induced JA (Block et al., 2020). Aphid no-choice bioassays showed that SCA counts were significantly lower on the *msd3* plants compared to WT plants (Fig. 4A). We further quantified the levels of JA before and after SCA infestation on WT and *msd3* plants after 7 dpi. Phytohormone analysis showed that there was a significant decrease in the JA level in *msd3* plants compared to WT plants, irrespective of the presence or absence of SCA (Fig. 4B). Thus, our results clearly demonstrate that the sorghum plants deficient in JA provided enhanced resistance to SCA.

SCA resistance in JA-deficient plants is independent of SA

Previous studies have suggested that loss of function of certain FADs promote resistance to aphids (Avila et al., 2012; Li et al., 2021). In tomato and Arabidopsis, loss of function of *FAD7*, which encodes the major plastidial 18:2 desaturase, contributed to increased aphid resistance in a salicylate dependent manner (Avila et al., 2012). Sorghum *Msd3* is a homolog of *FAD7* (Dampanaboina et al., 2019). To determine whether the resistance in sorghum *msd3* is dependent on the SA pathway, we analyzed SA levels with and without SCA infestation on WT and *msd3* plants after 7 dpi. Our results suggest that the basal levels of SA were similar in both WT and *msd3* plants (Fig. 4C). In addition, SA levels were comparable in both WT and *msd3* plants after SCA infestation (Fig. 4C). Although antagonistic interactions between SA and JA have been reported (Pieterse et al., 2009; Thaler et al., 2012; Gimenez-Ibanez and Solano, 2013), our results indicate that the resistance in sorghum *msd3* plants is uncoupled with JA's antagonistic effect on SA signaling.

JA deters SCA settling on sorghum plants at early time points

To determine whether JA has a role in aphid settling on sorghum plants, SCAs were given the choice of WT and *msd3* plants to identify if there is a preference. Our results show that the number of adult aphids that were settled on *msd3* plants were significantly higher compared to WT plants at 6 and 24 h after aphid release (Fig. 5A). However, when the *msd3* plants were pretreated with JA-Ile for 24 h, then aphids settled equally on WT and *msd3* plants (Fig. 5B). In addition, aphids preferred to settle on *msd3* control plants compared to *msd3* plants pretreated with JA-Ile (Fig. 5C). To further confirm the role of JA in aphid settling, SC265 plants, which exhibited elevated levels of JA at early time points after SCA attack (Fig. 2C), were pretreated with nordihydroguaiaretic acid (NDGA) that blocks JA synthesis (Louis et al., 2015), and SCA settling was monitored. Our results demonstrate that JA inhibitor treatment on SC265 plants significantly attenuated aphid deterrence effect and aphids settled equally on RTx430 control plants and SC265 plants pretreated with NDGA at 6 and 24 h post aphid release (Supplemental Fig. S3A). This was further supported in choice assays between SC265 control plants and SC265 plants that were pretreated with NDGA, in which aphids preferred to settle on SC265 plants that were pretreated with NDGA (Supplemental Fig. S3A). Collectively, these data confirm JA's involvement in deterring aphid settlement on sorghum plants.

Exogenous JA application attenuates SCA resistance in *msd3* plants

To confirm whether the *msd3* resistance to SCA is dependent on reduced JA levels, we pretreated WT and *msd3* plants with JA-Ile for 24 h prior to SCA infestation. Aphid no-choice bioassays showed that the SCA counts were significantly higher on *msd3* plants that were pretreated with JA-Ile compared to *msd3* control plants, whereas SCA numbers were comparable on WT control plants and JA-Ile pretreated WT plants (Fig. 6A). This result was further supported in EPG analyses, in which the duration of time spent by SCA in the SEP was considerably longer on JA-Ile-pretreated *msd3* plants compared to *msd3* control plants, whereas SCA spent comparable duration of time in the SEP on WT control and JA-Ile-pretreated plants (Fig. 6B). We did not observe significant differences in any other SCA feeding behavior patterns on WT and *msd3* plants with and without JA-Ile treatment (Supplemental Table S2). Thus, our results suggest that JA promotes susceptibility to SCA and facilitates SCA feeding from sieve elements.

SCA feeding alters the free sugars metabolism in *msd3* plants

Previous studies have suggested that jasmonates alter the carbohydrate metabolism that determines the insect performance on host plants (Machado et al., 2015, 2017). To determine whether the JA-mediated susceptibility occurs through changes in sugar concentrations, we analyzed the free sugars in WT and *msd3* plants with and without SCA infestation. We found significant increases in fructose and trehalose levels in *msd3* plants compared to WT plants after SCA infestation for 7 days (Fig. 7A and 7B). Although SCA feeding increased glucose levels in both WT and *msd3* plants, no significant differences were observed in the glucose levels in WT and *msd3* plants before and after SCA infestation (Fig. 7C). The WT plants had significantly higher basal levels of sucrose compared to *msd3* plants, however, sucrose levels were increased upon aphid infestation in both genotypes (Fig. 7D). Raffinose levels declined in WT plants after SCA infestation, but was not altered in the *msd3* plants before or after SCA infestation (Fig. 7E). Xylose and galactose levels were undetectable and unaltered, respectively, after SCA feeding on WT and *msd3* plants for 7 dpi (Supplemental Fig. S4A and S4B). These results suggest that the JA-deficient plants altered sugar metabolism after SCA infestation.

Fructose and trehalose have direct negative impact on SCA reproduction

To determine whether elevated sugar levels in *msd3* plants have direct impact on SCA growth and reproduction, we supplemented SCA artificial diet with two different concentrations of fructose and trehalose, and monitored SCA growth and reproduction. Our feeding trial bioassays showed that both fructose and trehalose have direct negative impact on SCA growth and reproduction after three days (Fig. 8A and 8B). Fructose and trehalose incorporation into aphid diet at both low and high concentrations (50 µM and 200 µM), resulted in a decreased aphid growth and fecundity compared with SCA reared on diet alone. Thus, our results suggest that the elevated levels of fructose and trehalose in JA-deficient plants after SCA infestation may have a direct negative impact on SCA growth and reproduction.

DISCUSSION

Previously, it was shown that JA has a significant role in deterring aphid settling on sorghum plants (Zhu-Salzman et al., 2004). Instead, the results presented here demonstrate that JA, in fact, has a dichotomous role in modulating sorghum defense against SCA. On the one hand, JA deters the aphids on settling, while the aphids initially attempt to establish a feeding site on sorghum plants. On the other hand, once the aphids were able to overcome the early "deterrence" defense, presumably by injecting aphids' salivary effectors into the host tissues during feeding, JA likely acts as a susceptibility factor promoting SCA proliferation on sorghum plants. SC265 plants displayed enhanced JA levels during the initial SCA feeding (Fig. 2C). This result was paralleled by an increased aphid deterrence on SC265 plants at 6 and 24 h (Fig. 1B). The possibility of SA-dependent SC265 resistance to SCA added further complexity to understand the role of JA in sorghum defenses to SCA. Therefore, we used sorghum *msd3* plants that are having deficient levels of JA to provide genetic evidence of JA-dependent sorghum defense responses. Aphids preferred to settle on JA-deficient plants (Fig. 5), which confirmed that JA deters aphid settling on sorghum. Comparable and reduced levels of JA in the SCA-resistant SC265 and *msd3* plants before and after SCA infestation 7 dpi, respectively (Fig. 2D and Fig. 4B), and the ability of exogenous application of JA to promote SCA growth and facilitate sieve element feeding (Fig. 3 and Fig. 6), together confirm the important role of JA in promoting aphid proliferation and feeding on sorghum plants. The higher levels of fructose and trehalose in JA-deficient plants upon SCA infestation suggest the possibility that they interfere with aphid performance, thereby reducing the aphid population.

Although there are no prior reports on dichotomous role of JA in modulating aphid performance on host plants, few studies have identified that some oxylipins, including JA, have shown to promote susceptibility to various pathogens and aphids (Louis et al., 2010; Makandar et al., 2010; Nalam et al., 2012; Avila et al., 2012; Gorman et al., 2020; Li et al., 2021). Moreover, antagonistic interactions between oxylipins/JA and SA likely influence the outcome of plant-pathogen/pest interactions (Pieterse et al., 2009; Thaler et al., 2012; Solano and Gimenez-Ibanez, 2013). For example, it was shown that elevated levels of SA due to loss of FAD7 activity in tomato and Arabidopsis resulted in enhanced resistance to aphids (Avila et al., 2012). However, loss of function of *Msd3*, a homolog of *FAD7*, -mediated resistance to SCA in sorghum was independent of the SA pathway. SA accumulation was comparable between the WT and *msd3* plants before and after SCA infestation (Fig. 4C), which suggests JA does not influence SA accumulation in response to SCA feeding on sorghum plants. SC265 plants also have elevated levels of SA after 7 days of SCA infestation (Fig. 2B). On the basis of our results, SA is highly likely to contribute to SC265's resistance to SCA, which does not exclude the possibility of other hormones/metabolites synergistically interacting with SA in modifying sorghum defenses.

In Arabidopsis, *LIPOXYGENASE 5* (*LOX5*), which encodes a 9-lipoxygenase, was required for green peach aphid (*Myzus persicae*) colonization and facilitated aphid feeding from the sieve elements and water consumption from xylem tissues (Nalam et al., 2012). The aphids' inability to tap into the xylem of the *lox5* mutants correlated with reduced water content in the aphid body, and apparently negatively impacted aphid performance. Intermittent ingestion of xylem contents is critical for aphids, which allows them to avoid dehydration while feeding on a sugar-rich phloem sap (Powell and Hardie, 2002; Douglas et al., 2006). However, although SCA spent reduced time in the SEP of SC265 and *msd3* plants, no discernible differences were observed in the duration of xylem phases between SC265 and *msd3* plants compared to their respective controls (Fig. 3C and Fig. 6B; Supplemental Tables S1 and S2), which suggests the resistant phenotype of both SC265 and *msd3* plants are not associated with the inability of aphids to tap into the xylem tissues and/or reduced water content in aphids.

Oxylipins have been identified in the phloem sap (Madey et al., 2002; Harmel et al., 2007; Benning et al., 2012). In fact, phloem sap-derived oxylipins were detected in green peach aphids (Harmel et al., 2007), suggesting that oxylipin is highly likely to be consumed by aphids. Oxylipin consumption by aphids may impact physiology of the insect. However, we have previously shown that OPDA does not have a direct impact on aphid growth and fecundity (Varsani et al., 2019). Rather, OPDA modifies maize resistance to corn leaf aphids (*Rhopalosiphum maidis*), independent of the JA pathway, through ethylene signaling and interaction with the Maize insect resistance1-Cysteine Protease (Mir1-CP) defensive protein (Varsani et al., 2019). However, we did not observe any temporal changes in OPDA levels before or after SCA infestation (Supplemental Fig. S1A and S1B). Furthermore, loss of function mutation in the Arabidopsis *SUPPRESSOR OF SALICYLIC ACID INSENSITIVITY2* (*SSI2*) gene, which encodes a desaturase involved in lipid metabolism, resulted in enhanced resistance to green peach aphids, but did not deter aphid settling and feeding from sieve elements (Louis et al., 2010). Like Arabidopsis *ssi2* plants, sorghum *msd3* plants also have lower basal levels of oleic acid (18:1) compared to WT plants (Block et al., 2020; Li et al., 2021). However, the fact that oleic acid and stearic acid (18:0), the precursor of oleic acid, does not have a direct impact on aphid feeding and settling (Louis et al., 2010), it is highly unlikely that depletion of oleic acid levels in *msd3* plants contribute to SCA resistance. Loss of function of *SSI2* in Arabidopsis also resulted in elevated levels of nitic oxide (NO) (Mandal et al., 2012), which is a well-known defense regulator involved in plant biotic stress (Wilson et al., 2008; del Río, 2015). In fact, NO is involved in providing plant resistance to aphids (Moloi et al., 2014; Woźniak et al., 2017). Because multiple defense mechanisms are being utilized by plants to limit aphid colonization

(Nalam et al., 2019), further work is required to understand how precisely NO and JA are related in modulating sorghum resistance to SCA.

Sugars are required for primary metabolism in plants, however, sugars can also act as metabolic signals to further augment the plant defense responses (Rolland et al., 2006; Bolouri Moghaddam and Van den Ende, 2012; Formela-Luboińska et al., 2020). Several reports have shown the plant's ability to alter sugar metabolism in response to insect attack (Singh et al., 2011; Machado et al., 2015, 2017). For instance, jasmonate-dependent sugar depletion rendered *Nicotiana attenuata* plants more susceptible to *Manduca sexta* caterpillar (Machado et al., 2015, 2017). Similarly, sucrose was shown to induce defense response against pathogen attack (Morkunas et al., 2005; Tauzin and Giardina, 2014) and trehalose has been proposed as a signaling molecule (Singh et al., 2011; Govind et al., 2016). Furthermore, trehalose has been shown to provide direct toxicity against green peach aphids (Singh et al., 2011). Besides providing direct effects, trehalose was also shown to enhance the expression of *PHYTOALEXIN DEFICIENT4* (*PAD4*) gene, an important modulator of resistance to aphids, in Arabidopsis that curtails insect infestation (Singh et al., 2011; Louis and Shah, 2015). The higher trehalose and glucose levels in pea aphid's (*Acyrthosiphon pisum*) body has been shown to affect the feeding behavior of aphid, resulting in lesser phloem feeding on fava beans (Wang et al., 2021). Furthermore, elevated fructose content in tomato has been associated with heightened defense against necrotrophic fungus, *Botrytis cinerea* (Lecompte et al., 2017). Our results showed that JA-deficient plants induced higher levels of fructose and trehalose content upon SCA infestation. It is highly likely that JA-dependent altered sugar levels in sorghum affect the SCA performance directly through altered aphid feeding behavior and growth, and possibly indirectly through triggering the plant defenses as signaling molecules, which ultimately lead to a reduced aphid population.

Insect secretions contain phytohormones (Tooker and De Moraes, 2006; Dafoe et al., 2013; Schwartzberg and Tumlinson, 2014; Acevedo et al., 2019). JA has been identified in the saliva of fall armyworm (*Spodoptera frugiperda*) and is involved in regulating plant defense responses (Acevedo et al., 2019). Furthermore, the amount of JA in the insect saliva was highly influenced by the host plant that supported the insect. Although not much is known about the salivary components of SCA, it is possible that JA could be present in the aphid saliva. Alternatively, during the initial brief sampling of cells and/or phloem sap ingestion, aphids may uptake JA or JA-derived factor(s) from host plants and sequester these factors for reintroduction into the host during sieve element feeding. Toxins produced by pathogens, for example, coronatine (COR) produced by plant pathogenic strains of *Pseudomonas syringae* that acts as a molecular mimic of JA–Ile, hijacks plant's defense machinery to promote pathogen virulence (Bender et al., 1999; Zhao et al., 2003; Katsir et al., 2008; Jiang et al., 2013). Whether SCA salivary effectors manipulate host JA signaling to ensure successful colonization, in a manner similar to COR phytotoxin or through other mechanism(s), remains unknown.

Our findings demonstrate an unanticipated and evidently dichotomous role of JA in modulating sorghum defense against SCA. At early stages, JA contribute to feeding deterrence to aphids. However, at later-stages, JA alters the sugar metabolism, which further enhance the aphid growth and reproduction (Fig. 9). Efforts to improve sorghum resistance to aphids focusing on JA-regulated defenses should consider the complexity of JA exerting an initial "deterrent" effect on aphids and subsequently promoting aphid colonization. Uncovering the underlying sorghum defense mechanisms against aphids could significantly accelerate the development of sorghum plants through modern breeding techniques and/or through transgenic approaches.

MATERIALS AND METHODS

Plants and growth conditions

The sorghum RTx430 and SC265 lines, which are among the NAM founder lines (Bouchet et al., 2017), used in this study were obtained from USDA-GRIN global germplasm (Grover et al., 2019, 2020a). The BTx623 and *msd3* mutants have been described previously (Xin et al., 2008; Jiao et al., 2016). For all experiments, two-week-old sorghum plants at the three-leaf stage were used (Vanderlip and Reeves, 1972). All plants were grown in Cone-Tainers (Ray Leach SC10; Stuewe & Sons, Inc., Tangent, OR) that were filled with soil mixed with vermiculite and perlite (PRO-MIX BX BIOFUNGICIDE + MYCORRHIZAE, Premier Tech Horticulture Ltd., Canada) at the University of Nebraska-Lincoln (UNL) greenhouse. The greenhouse conditions were set at 25 °C, 16-h-light/8-h-dark photoperiod, and 50–60% relative humidity. Plants were watered regularly and fertigated once per week.

Insect colony

A SCA colony was propagated as described previously (Tetreault et al., 2019) on SCA susceptible BCK60 sorghum genotype in a growth chamber with 16-h-light/8-h-dark photoperiod, 140 μE m^{-2} s^{-1} light quality, 25 °C, and 50–60% relative humidity. Plants for aphid propagation were grown in the greenhouse until it reached the 7-leaf stage. Adult aphids were used for all the experiments.

Aphid bioassays

Two-week-old sorghum plants were used for both no-choice and choice bioassays. For no-choice assay, each plant was infested with five adult apterous aphids and covered with tubular clear plastic cages to avoid aphid escape. The cages were ventilated with organdy fabric on the sides and top of cage for proper aeration. All plants were randomly arranged and infested with aphids. After 7 days of infestation, the cages were removed and aphids including both nymphs and adults were counted. For choice assays, plants already grown in cone-tainers were placed in each pot (10 inches diameter by 9 inches height) while maintaining equal distance from each other (~ 5.0 cm) and supported with soil. Twenty adult aphids of similar age and condition were introduced at the center of pot on a filter paper placed on soil. The settled adult aphids on each plant were counted after 6 and 24 hours of aphid release.

Chemical treatment on plants

For all chemical treatments, two-week-old plants were dipped in a JA-Ile (Cayman Chemical Company, Ann Arbor, MI) solution containing a surfactant (CapSil). Plants were treated with 50 μM JA-Ile in 0.1% CapSil and the control plants were dipped in 0.1% CapSil. JA-Ile treated plants and control plants were maintained in different greenhouse chambers to avoid the spread of JA volatiles. To block the JA biosynthesis pathway, plants were sprayed with 1 mM nordihydroguaiaretic acid (NDGA) dissolved in 0.1% (v/v) methanol (MeOH) diluted in water (Louis et al., 2015). Plants that were sprayed with 0.1% (v/v) methanol (MeOH) in water were used as the controls. After 24 hours of treatment, plants were used for choice or no-choice aphid bioassays.

Monitoring aphid feeding behavior

The EPG technique (Walker et al., 2000; Nalam et al., 2019) was used to assess the SCA feeding behavior on sorghum plants as previously described (Tetreault et al., 2019; Grover et al., 2019). Eight channels were used of EPG recordings simultaneously over an 8 h period of SCA feeding, and at least 7 replicates of individual aphids (one aphid per plant) were obtained for each sorghum plant. The waveform recordings obtained were analyzed using the EPG analysis software *Stylet*$^{+}$ (EPG Systems, Wageningen, The Netherlands).

Phytohormone quantification

Two-week-old sorghum plants were infested with 10 adult apterous SCA and aphids were removed from plants at different time points with a soft paint brush. Control plants were uninfested with aphids. Leaf tissues were collected and fresh weight were recorded (~50 mg), and flash frozen in liquid nitrogen. These leaf tissue samples were ground using a 2010 Geno/Grinder (SPEX SamplePrep) for 40 s at 1,400 strokes min^{-1} in the presence of liquid nitrogen. Further, liquid chromatography-mass spectrometry (LC-MS) assay and quantification of OPDA, JA, JA-Ile, and SA were performed at the Proteomics and Metabolomics Facility at the Center for Biotechnology/University of Nebraska-Lincoln using deuterium-labeled internal standards as previously described (Chapman et al., 2018; Varsani et al., 2019; Grover et al., 2020a).

GC-MS Single Ion Monitoring (SIM) analysis of sugars

Two-week-old sorghum plants were infested with 10 adult apterous SCA and SCA were removed from plants 7 dpi and leaf tissues were harvested. Control plants were uninfested with aphids. Leaves were weighed (50-100 mg) and immediately flash-frozen in liquid nitrogen. An aliquot of samples was extracted using cold methanol:acetonitrile (50:50, v/v) and the tissue samples were disrupted and homogenized by adding two stainless steel beads (SSB 32) using the TissueLyser II (Qiagen) at 10 Hz for 15 mins. After centrifugation at 16,000 g, the supernatants were collected, and the extraction of the pellet was repeated. The supernatants were pooled and vacuum dried down using a speed-vac. The extracted sample was further used for the GC-MS analysis of sugars. The samples were resuspended in 20 mg/mL methoxyamine hydrochloride reagent prepared in pure pyridine and incubated for 2 h at 37°C. Samples are then derivatized using MSTFA + 1% TMCS (ThermoFisher Scientific) incubated for 30 min at 37°C followed by a centrifugation for 10 min at 16,000 g. Sugars were separated on a HP-5MS 30 m, 0.25 mm, 0.25 μm capillary column (Agilent Technologies), at constant flow 1.5 ml min^{-1} of helium as a carrier gas. The temperature of the column was initially set to 80 °C and increased at a rate of 15°C min^{-1} to 175 °C, followed by an increase at 5 °C min^{-1} to 220°C, and a final ramping to 320 °C at 25 °C min^{-1}. A SIM scan method using selected ions was used to analyze the sugars (xylose, fructose, glucose, galactose, sucrose, trehalose and raffinose). The generated data was analyzed with Agilent Mass Hunter Quantitative Analysis. For quantification, an external standard curve was prepared using a series of standard samples containing different concentrations of sugars and fixed concentration of pinitol (Thermo Scientific Chemicals) as the internal standard (Lopez-Guerrero et al., 2022).

Artificial diet feeding trial bioassays

Aphid feeding trial bioassays were carried out in a growth chamber with 16-h-light/8-h-dark photoperiod, 140 μE m^{-2} s^{-1} light quality, 25 °C, and 50–60% relative humidity. An artificial aphid diet for SCA that contains 20% sucrose solution (Sigma-Aldrich) (Toledo-Hernández et al., 2018) was used for the SCA feeding trial bioassays. Aphid feeding chambers and experimental set up was prepared as described previously (Louis et al., 2010). Over each aphid feeding chamber, 700 μL of aphid diet was sandwiched between two stretched parafilm layers with or without supplementation of test sugars, fructose and trehalose (Acros Organics). Five adult apterous SCA were added to each feeding chamber and total number of aphids (both adults and nymphs) were counted in each feeding chamber for three days.

Statistical analyses

The aphid no-choice and artificial diet bioassays data were analyzed using mixed model and replications were considered as random effects (PROC GLIMMIX, SAS 9.3, SAS Institute). Pairwise comparisons were computed using Tukey's adjustment with an experiment-wise error rate of $\alpha = 0.05$. For choice assays, proportions were taken for total aphids settled on each plant based on the total aphids that made choice in each replication. The aphid proportion data were analyzed following square root transformation to correct for heterogeneous variances. We used generalized linear models (GLM) with a likelihood ratio and Chi-square test to assess the treatment effects on aphid settling behavior. For EPG data, non-parametric Kruskal–Wallis test was used to compare the duration of different feeding parameters/phases between different sorghum plants using PROC NPAR1WAY procedure, considering the non-normally distributed data. Data related to phytohormones and sugars were analyzed using one-way ANOVA. For phytohormones and sugars, means were separated using Fisher protected least significant difference (LSD) procedure when appropriate ($P < 0.05$).

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AUTHOR CONTRIBUTIONS

S.G. and J.L. conceived and designed the research; S.G. and H.P. performed the research; Z.X. contributed sorghum jasmonic acid deficient plants; S.E.S. contributed reagents, methods development and provided guidance on experiments; S.G. and J.L. wrote the paper. All authors reviewed and edited the manuscript.

SUPPLEMENTAL DATA

The following materials are available in the online version of this article.

Supplemental Figure S1. Representative Electrical Penetration Graph (EPG) waveform patterns over an 8 h period of SCA feeding on RTx430 and SC265 sorghum plants.

Supplemental Figure S2. Time course of changes in 12-oxo-phytodienoic acid (OPDA) and jasmonic acid-iso leucine (JA-Ile) levels before and after sugarcane aphid (SCA) infestation on RTx430 and SC265 plants.

Supplemental Figure S3. Choice assay comparison of aphid preference for RTx430 vs SC265 plants pretreated with NDGA, and SC265 control mock-treated plants vs SC265 plants pretreated with NDGA.

Supplemental Figure S4. Levels of xylose and galactose after sugarcane aphid (SCA) feeding on BTx623 and *msd3* plants for 7 dpi.

Supplemental Table S1. Sugarcane aphid (SCA) feeding activities on the sorghum RTx430 and SC265 plants with and without JA-Ile treatment.

Supplemental Table S2. Sugarcane aphid (SCA) feeding activities on the sorghum BTx623 and *msd3* plants with and without JA-Ile treatment.

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FIGURE LEGENDS

Figure 1. Sorghum SC265 plants provide enhanced resistance to sugarcane aphids (SCA). (A) Total number of SCA adults and nymphs recovered 7 days post infestation of two-week-old sorghum RTx430 and SC265 plants with 5 adult apterous aphids/plant (n = 14–15). Error bars represent mean ± SE. Different letters indicate significant differences between sorghum plants ($P < 0.05$; Tukey's test). (B) Choice assay comparison of aphid preference for RTx430 vs SC265 plants by releasing 20 adult SCA at the center of a pot containing one plant of each indicated sorghum line. Proportion of adult SCA that had settled on RTx430 and SC265 plants were monitored after 6 and 24 h post aphid release (n = 10). An asterisk (*) indicate values that are significantly different from each other ($P < 0.05$; χ^2 test). Aphid no-choice and choice bioassays were conducted three times with similar results. (C) Electrical Penetration Graph (EPG) monitoring of mean time spent by SCA for various feeding behavior activities over an 8 h feeding by SCA on RTx430 and SC265 sorghum plants (PP, pathway phase; XP, xylem phase; SEP, the total duration of sieve element phase; NPP, non-probing phase; f-SEP, the time to reach first SEP) on RTx430 and SC265 sorghum plants. Each value is the mean ± SE of 15–16 replications. An asterisk (*) represents significant difference ($P < 0.05$; Kruskal-Wallis test) in the time spent by SCA for the indicated activity on the RTx430 and SC265 sorghum plants.

Figure 2. Sugarcane aphid (SCA) feeding on sorghum plants alters salicylic acid (SA) and jasmonic acid (JA) levels. Time course of changes in SA (A and B) and JA (C and D) levels before (0 h or –SCA) and after (1 h, 24 h, and 7 dpi) sugarcane aphid (SCA) infestation on RTx430 and SC265 plants. (n = 3–5). FW, fresh weight; dpi, days post infestation. Different

letters indicate significant differences between sorghum plants (*P* < 0.05; Tukey's test). Error bars represent mean ± SE.

Figure 3. Jasmonic acid-isoleucine (JA-Ile) pretreatment promotes aphid growth and feeding from phloem sieve elements. Total number of sugarcane aphid (SCA) adults and nymphs recovered 1, 3, 5, and 7 days after infestation of RTx430 (A) and SC265 (B) sorghum plants that were pretreated with JA-Ile (50 µM JA-Ile in 0.1% CapSil) or mock-treated with 0.1% CapSil (solvent control) for 24 h. Plants were infested with five adult apterous aphids per plant after 24 h of chemical/solvent treatment. Error bars represent mean ± SE (*n* = 15–19). Asterisks above the bars indicate values that are significantly different from each other (*P* < 0.05; Tukey's test), while "ns" indicates no significant differences between treatments. These experiments were conducted twice with similar results. dpi, days post infestation. (C) Mean time spent by SCA in the sieve element phase (SEP) of RTx430 and SC265 plants with and without JA-Ile pretreatment for 24 h. Each value is the mean ± SE of 9–10 replications. An asterisk (*) represents significant difference (*P* < 0.05; Kruskal-Wallis test).

Figure 4. Jasmonic acid (JA)-deficient sorghum plants provide heightened resistance to sugarcane aphids (SCA). (A) Total number of SCA adults and nymphs recovered 7 days post infestation (dpi) of two-week-old sorghum wild-type (WT) and *msd3* plants with 5 adult apterous aphids/plant (*n* = 13–14). This experiment was conducted twice with similar results. Levels of (B) jasmonic acid (JA) and (C) salicylic acid (SA) before (–SCA) and after SCA infestation for 7 days on WT and *msd3* plants. (*n* = 3–5). FW, fresh weight; dpi, days post infestation. For A-C,

different letters indicate significant differences between sorghum plants ($P < 0.05$; Tukey's test). Error bars represent mean ± SE.

Figure 5. Jasmonic acid (JA) is required for sugarcane aphid (SCA) deterrence on sorghum plants. Choice assay comparison of aphid preference for (A) wild-type (WT) vs *msd3* plants, (B) WT vs *msd3* plants pretreated with JA-Ile, and (C) *msd3* control mock-treated plants vs *msd3* plants pretreated with JA-Ile. Twenty adult SCA were released at the center of a pot containing one plant of each indicated sorghum line. Proportion of adult SCA that had settled on each plant combination were monitored after 6 and 24 h post aphid release ($n = 9$–14). An asterisk (*) indicate values that are significantly different from each other ($P < 0.05$; χ^2 test), while "ns" indicates no significant differences between sorghum plants on aphid settling. Aphid choice bioassays were conducted twice with similar results.

Figure 6. Exogenous jasmonic acid-isoleucine (JA-Ile) application attenuates *msd3* resistance to sugarcane aphids (SCA). (A) Total number of SCA adults and nymphs recovered 7 days post infestation (dpi) of two-week-old sorghum wild-type (WT) and *msd3* plants that were pretreated with JA-Ile (50 μM JA-Ile in 0.1% CapSil) or mock-treated with 0.1% CapSil (solvent control) for 24 h. Plants were infested with five adult apterous aphids per plant after 24 h of chemical/solvent treatment. Error bars represent mean ± SE ($n = 15$–19). Different letters indicate significant differences between sorghum plants ($P < 0.05$; Tukey's test). This experiment was conducted twice with similar results. (B) Mean time spent by SCA in the sieve element phase (SEP) of WT and *msd3* plants with and without JA-Ile pretreatment for 24 h. Each

value is the mean ± SE of 7–9 replications. An asterisk (*) represents significant difference ($P < 0.05$; Kruskal-Wallis test).

Figure 7. Sugarcane aphid (SCA) feeding alters the free sugars metabolism in jasmonic acid (JA)-deficient plants. Free sugar concentrations of (A) fructose, (B) trehalose, (C) glucose, (D) sucrose, and (E) raffinose in BTx623 (WT) and *msd3* plants before (–SCA) and after SCA infestation (7 dpi). Aphid uninfested plants were used as controls ($n = 3–4$). FW, fresh weight; dpi, days post infestation. Different letters indicate significant differences between sorghum plants ($P < 0.05$; Tukey's test). Error bars represent mean ± SE.

Figure 8. Fructose and trehalose have direct negative impact on sugarcane aphid (SCA) reproduction. Comparison of SCA numbers on artificial diet supplemented with two different concentrations of (A) fructose and (B) trehalose are shown. For feeding trial bioassays, five adult apterous SCA were introduced into each feeding chamber and allowed to feed on the diet. The total numbers of aphids (adults and nymphs) in each chamber were counted after three days ($n = 10–15$). This experiment was conducted twice with similar results. Different letters indicate significant differences between treatments on each day ($P < 0.05$; Tukey's test). Error bars represent mean ± SE.

Figure 9. Model depicting the dichotomous role of jasmonic acid (JA) in modulating sorghum defense against aphids. At early stages of aphid feeding, JA deters the aphid settling on the plants. However, at later-stages, JA alters the sugar metabolism that increases the aphid

reproduction on sorghum plants. Black lines ending in arrows show positive effects and black lines ending with perpendicular bar indicate oppressive effects.

Figure 1
Grover et al.

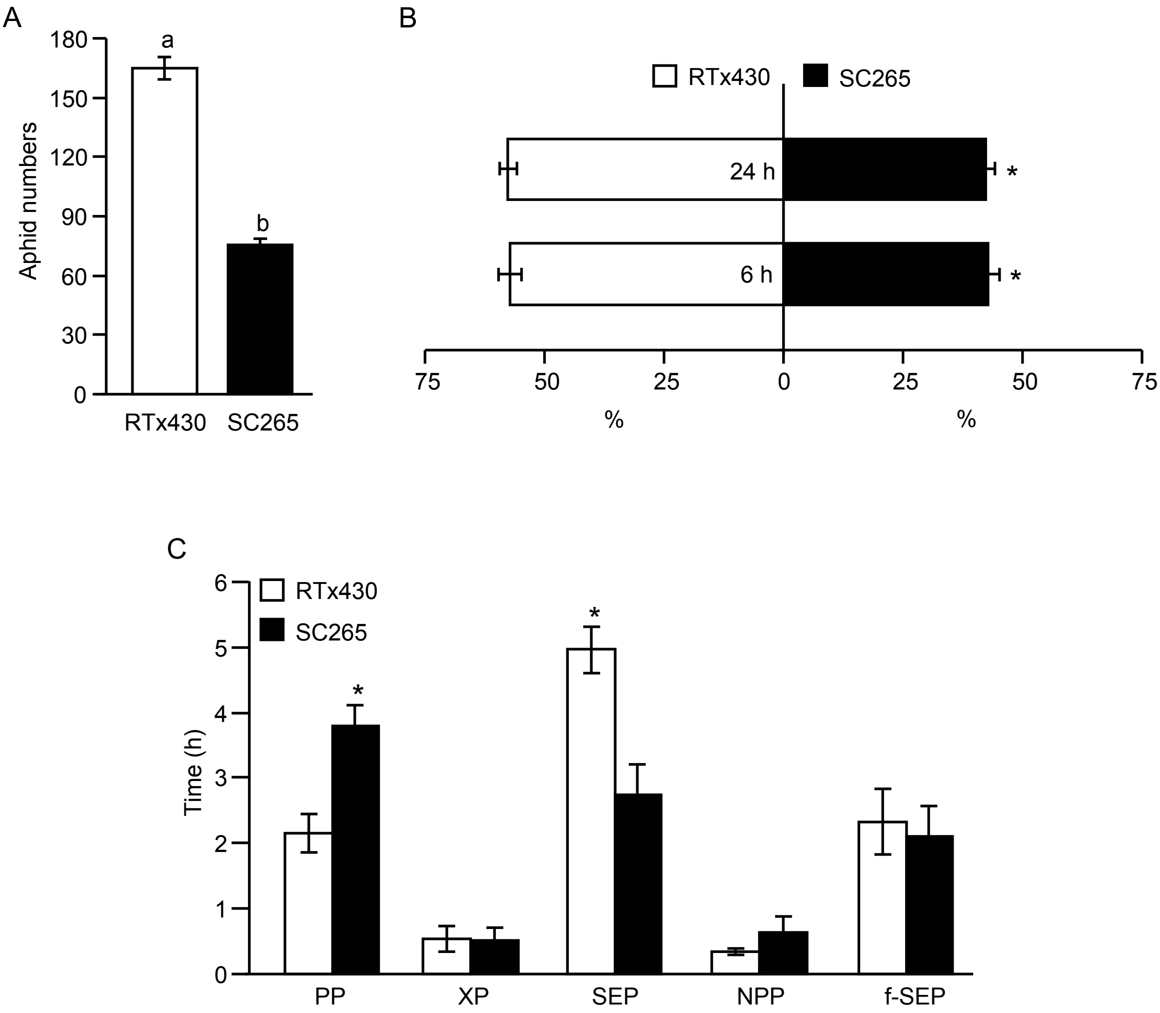

Figure 1. Sorghum SC265 plants provide enhanced resistance to sugarcane aphids (SCA). (A) Total number of SCA adults and nymphs recovered 7 days post infestation of two-week-old sorghum RTx430 and SC265 plants with 5 adult apterous aphids/plant (*n* = 14–15). Error bars represent mean ± SE. Different letters indicate significant differences between sorghum plants ($P < 0.05$; Tukey's test). (B) Choice assay comparison of aphid preference for RTx430 vs SC265 plants by releasing 20 adult SCA at the center of a pot containing one plant of each indicated sorghum line. Proportion of adult SCA that had settled on RTx430 and SC265 plants were monitored after 6 and 24 h post aphid release (*n* = 10). An asterisk (*) indicate values that are significantly different from each other ($P < 0.05$; χ^2 test). Aphid no-choice and choice bioassays were conducted three times with similar results. (C) Electrical Penetration Graph (EPG) monitoring of mean time spent by SCA for various feeding behavior activities over an 8 h feeding by SCA on RTx430 and SC265 sorghum plants (PP, pathway phase; XP, xylem phase; SEP, the total duration of sieve element phase; NPP, non-probing phase; f-SEP, the time to reach first SEP) on RTx430 and SC265 sorghum plants. Each value is the mean ± SE of 15–16 replications. An asterisk (*) represents significant difference ($P < 0.05$; Kruskal-Wallis test) in the time spent by SCA for the indicated activity on the RTx430 and SC265 sorghum plants.

Figure 2
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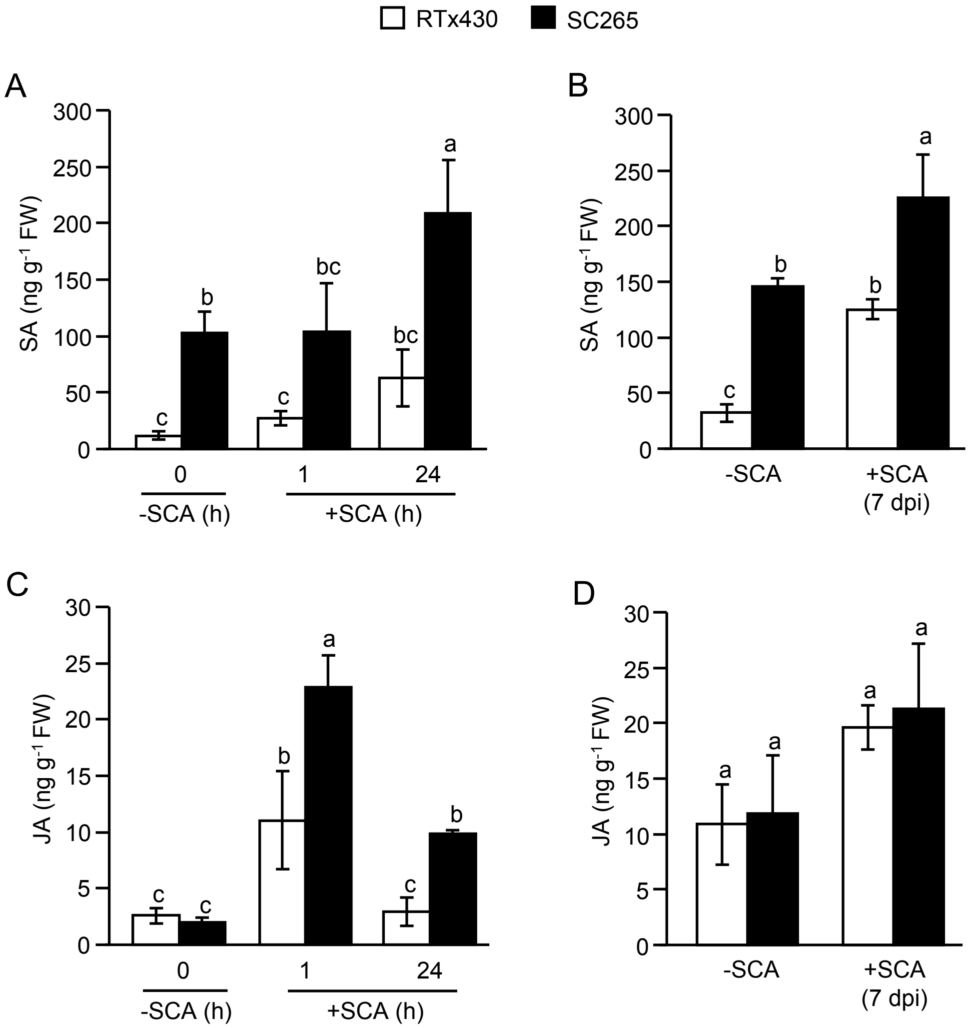

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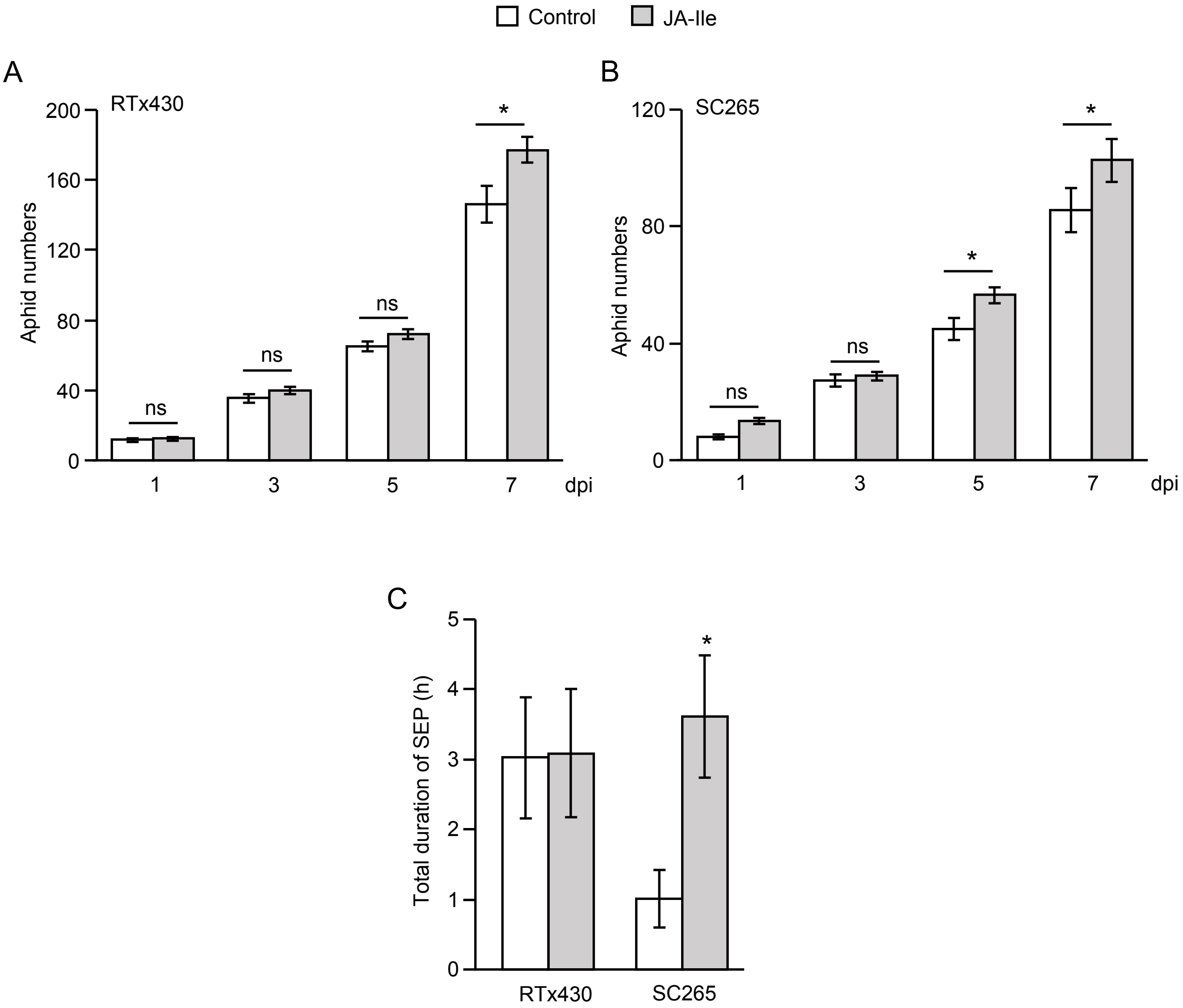
Figure 3
Grover et al.



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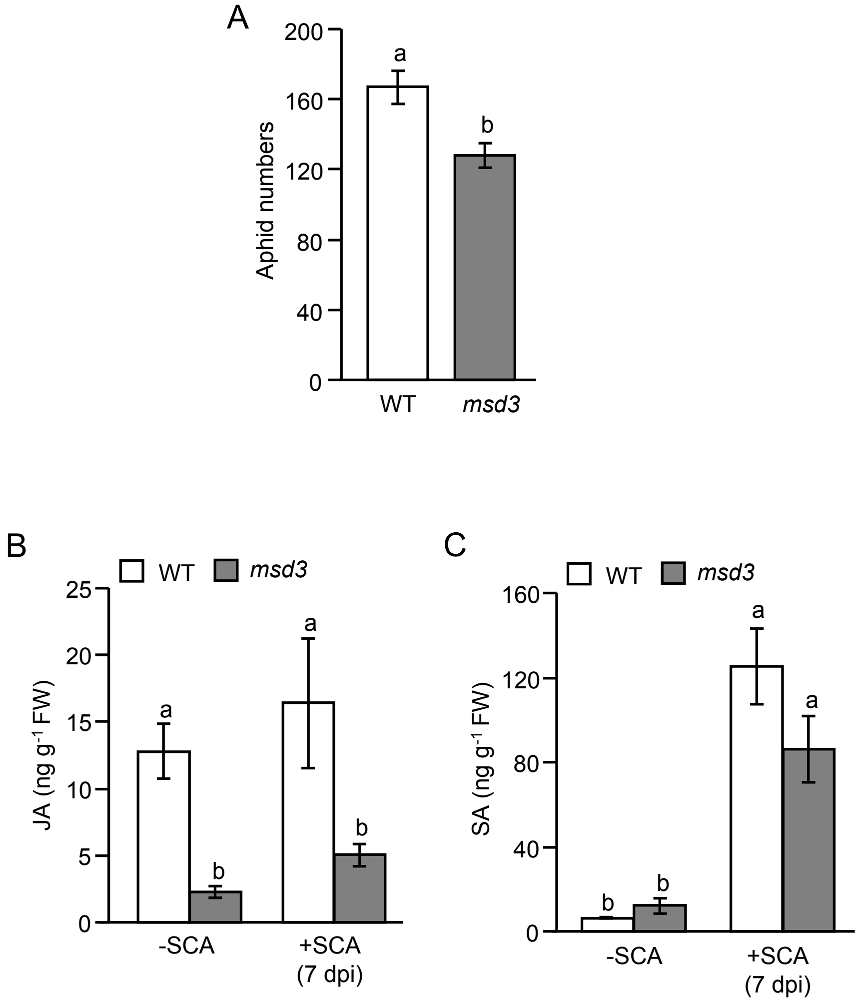

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Figure 5
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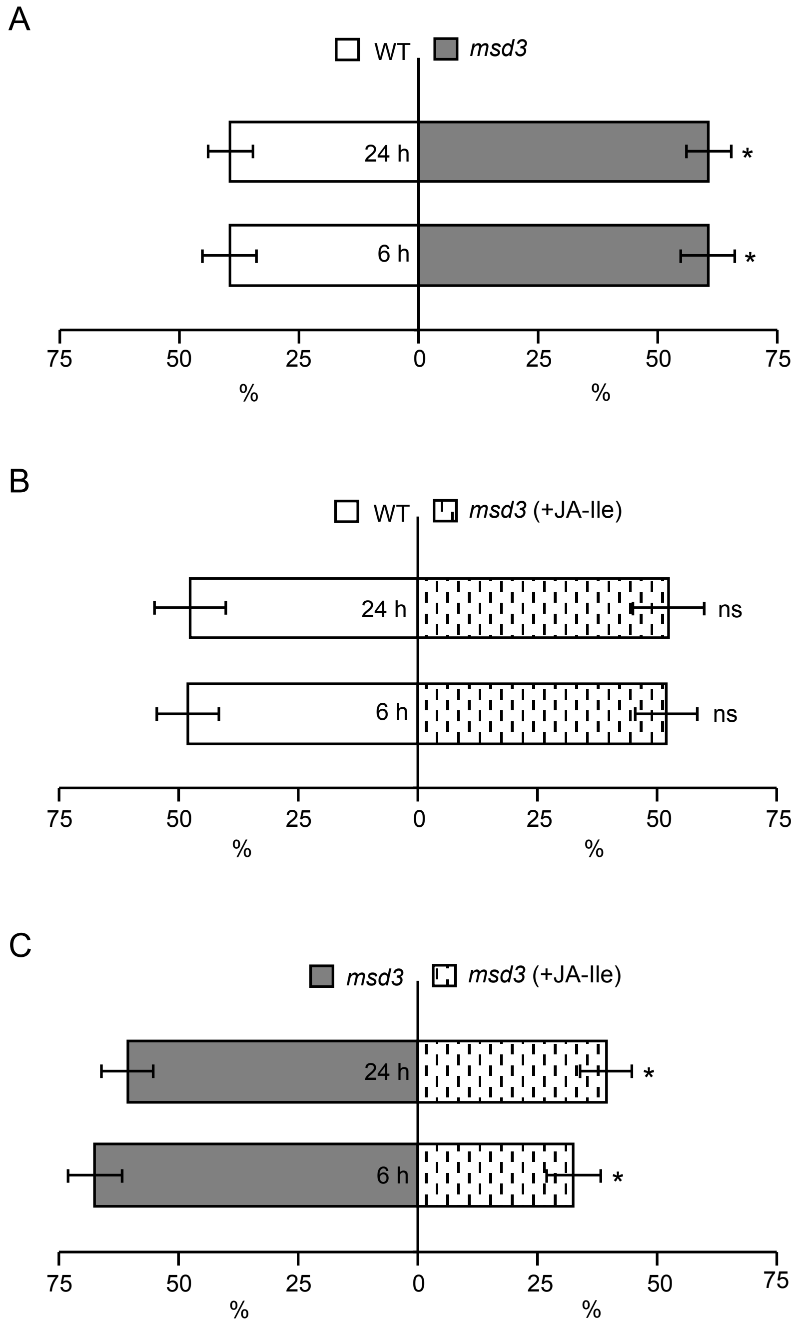

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Figure 6
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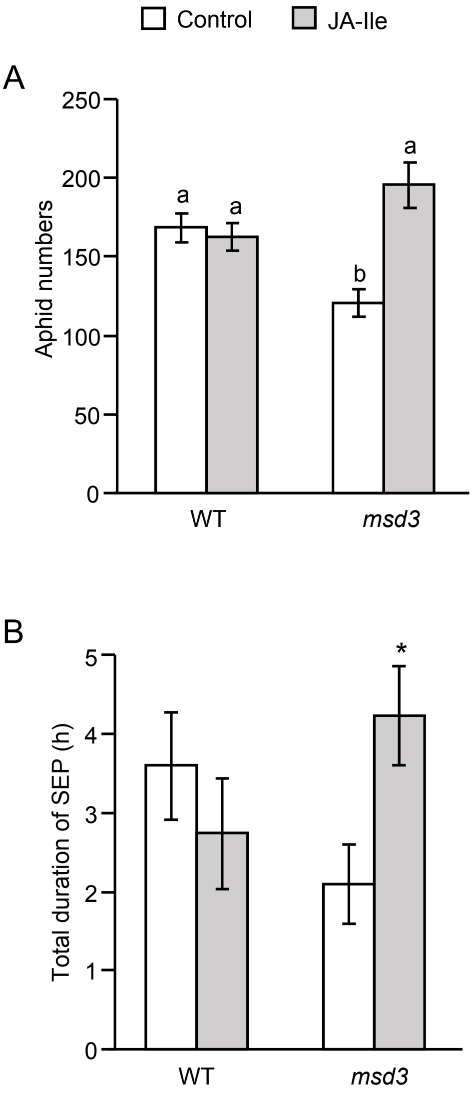

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Figure 7
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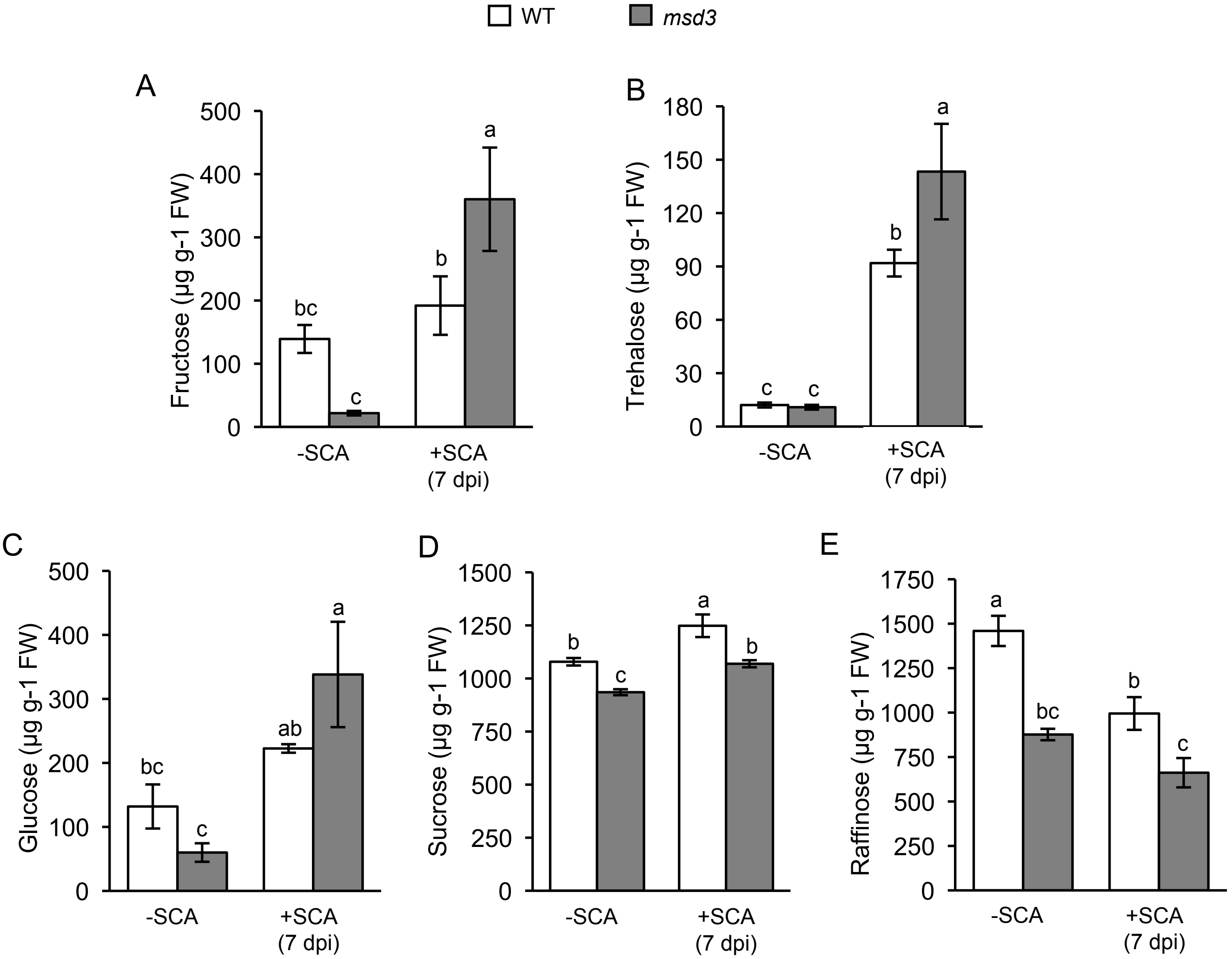

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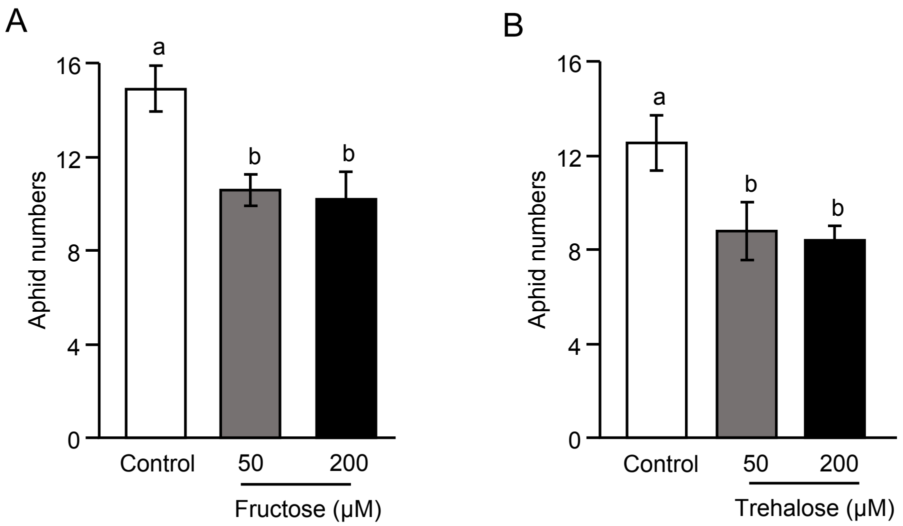

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Figure 9
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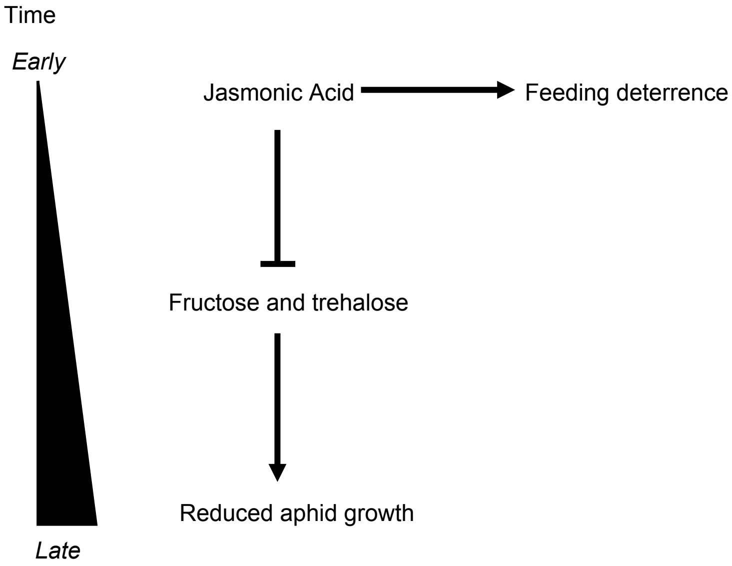

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SUPPLEMENTAL MATERIALS

Dichotomous role of jasmonic acid in modulating sorghum defense against aphids

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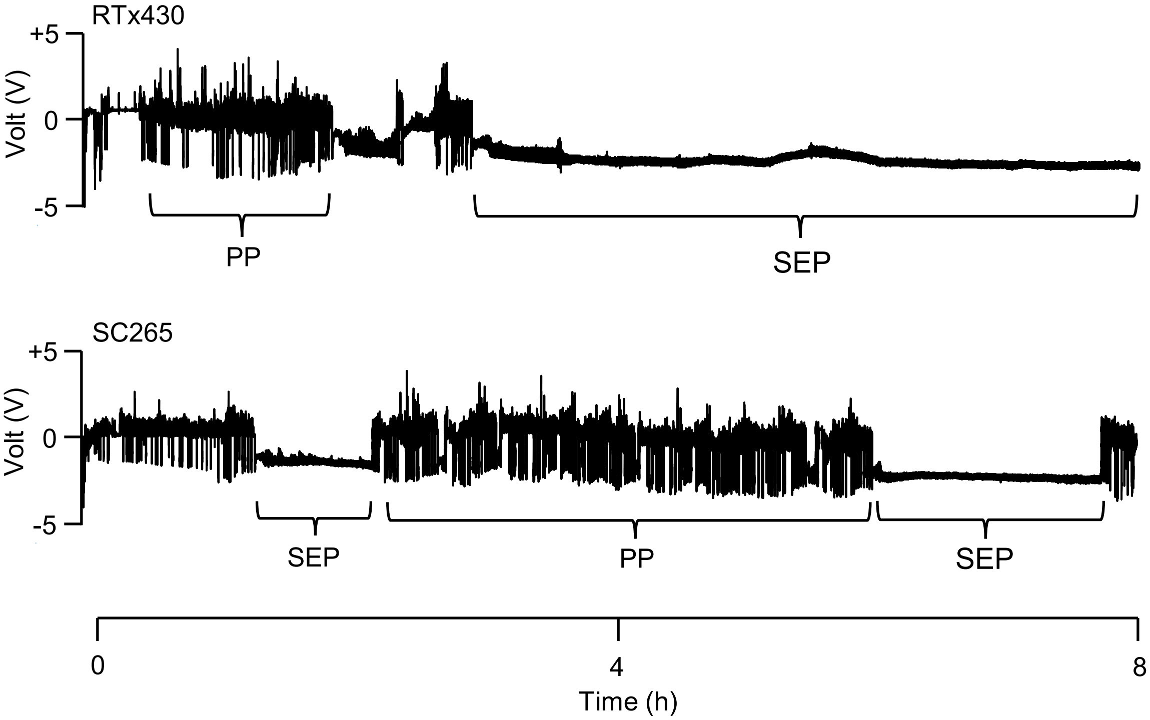
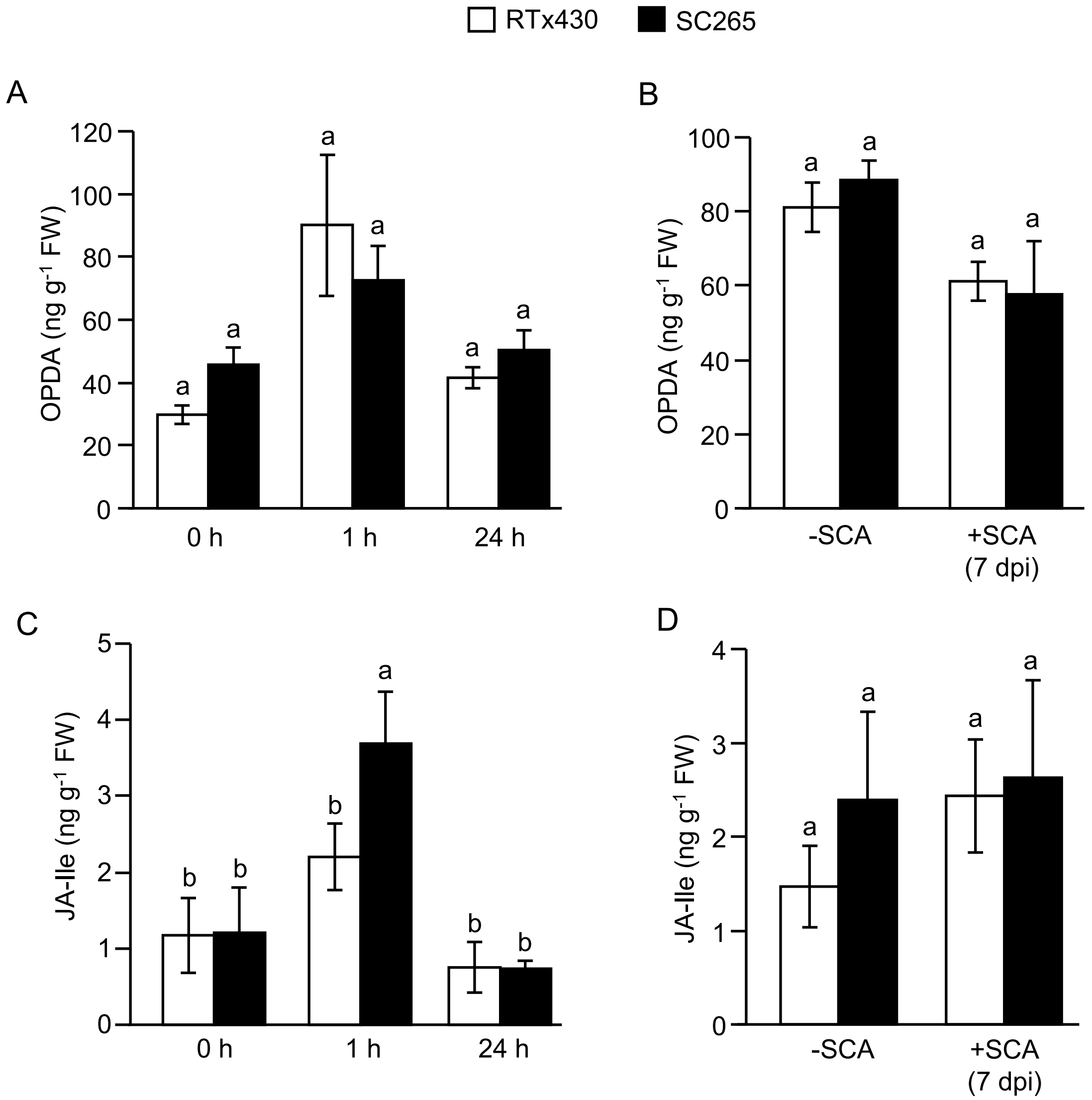
Supplemental Materials include:

Figures S1 - S4

Tables S1 and S2

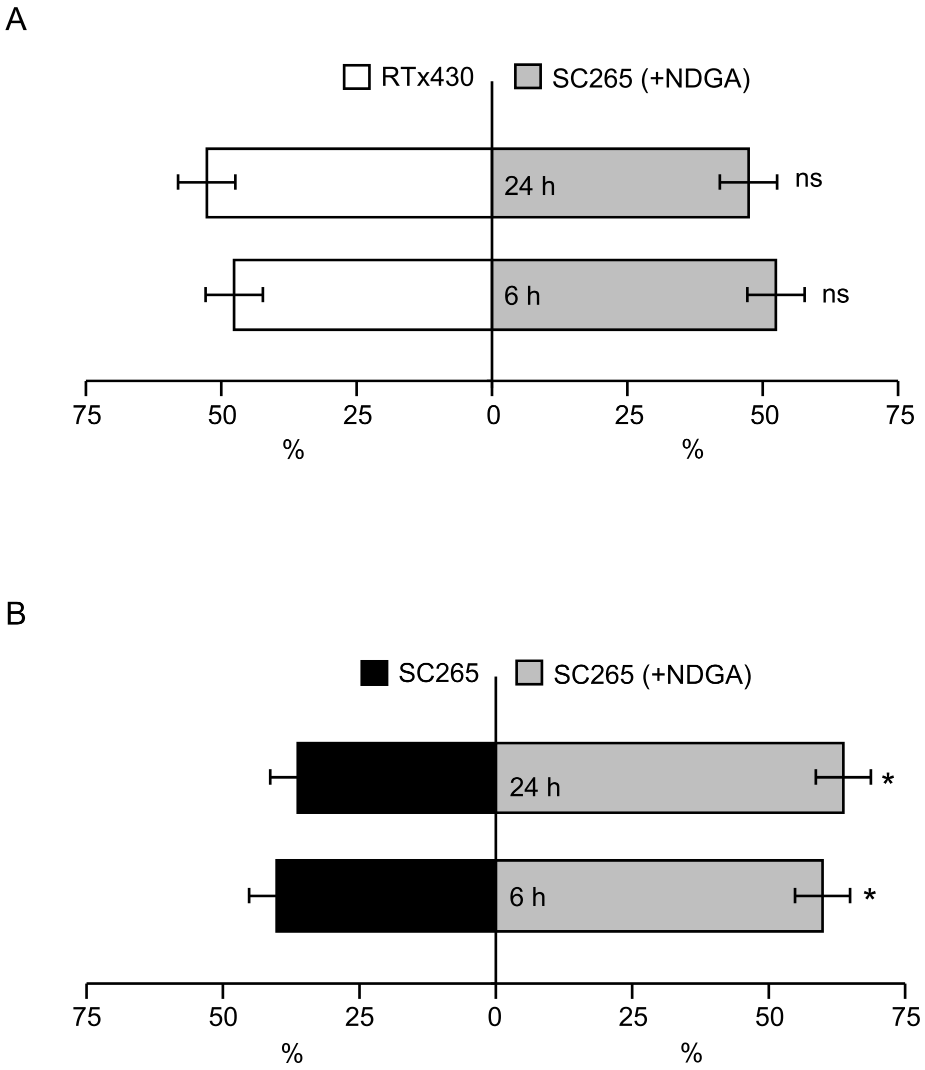


Supplemental Figure S1. Representative Electrical Penetration Graph (EPG) waveform patterns over an 8 h period of SCA feeding on RTx430 and SC265 sorghum plants (PP, pathway phase; XP, xylem phase; SEP, the total duration of sieve element phase) on RTx430 and SC265 sorghum plants.



Supplemental Figure S2. Time course of changes in 12-oxo-phytodienoic acid (OPDA) (A and B) and jasmonic acid-iso leucine (JA-Ile) (C and D) levels before (0 h or –SCA) and after (1 h, 24 h, and 7 dpi) sugarcane aphid (SCA) infestation on RTx430 and SC265 plants. ($n = 3–5$). FW, fresh weight; dpi, days post infestation. Different letters indicate significant differences between sorghum plants ($P < 0.05$; Tukey's test). Error bars represent mean ± SE.

Figure S3
Grover et al.



Supplemental Figure S3. Choice assay comparison of aphid preference for (A) RTx430 vs SC265 plants pretreated with NDGA, and (B) SC265 control mock-treated plants vs SC265 plants pretreated with NDGA. Twenty adult SCA were released at the center of a pot containing one plant of each indicated sorghum line. Proportion of adult SCA that had settled on each plant combination were monitored after 6 and 24 h post aphid release (n = 13–14). An asterisk (*) indicate values that are significantly different from each other ($P < 0.05$; χ^2 test), while “ns” indicates no significant differences between sorghum plants on aphid settling. Aphid choice bioassays were conducted twice with similar results.

Figure S4
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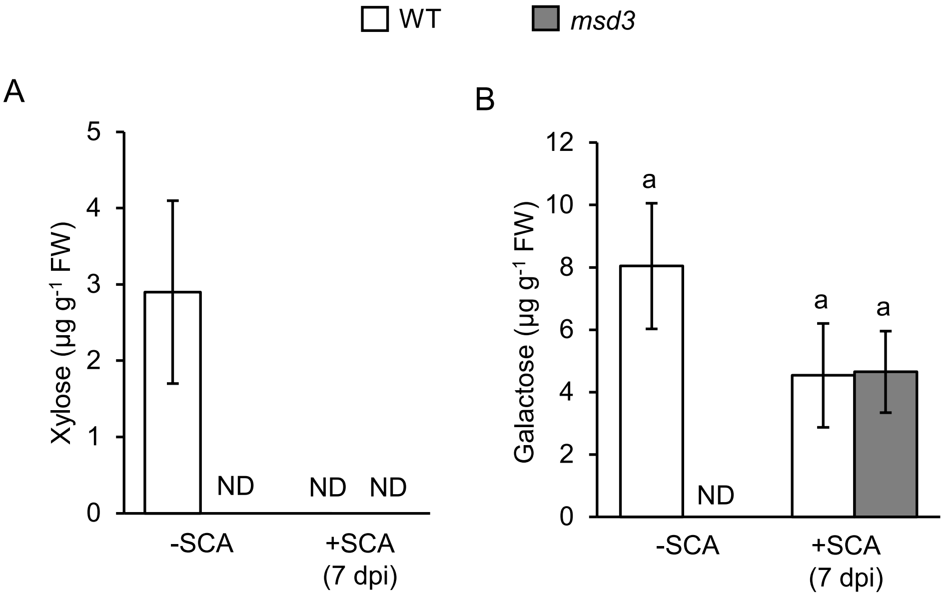

Supplemental Figure S4. Levels of xylose (A) and galactose (B) after sugarcane aphid (SCA) feeding on BTx623 (WT) and *msd3* plants before (–SCA) and after SCA infestation (7 dpi). Aphid uninfested plants were used as controls (n = 3–4). FW, fresh weight; dpi, days post infestation; ND, not detected. Different letters indicate significant differences between sorghum plants ($P < 0.05$; Tukey's test). Error bars represent mean ± SE.

Table S1

Sugarcane aphid (SCA) feeding activities on the sorghum RTx430 and SC265 plants with and without JA-Ile treatment.

SCA feeding activity	RTx430		SC265	
	Control	JA-Ile	Control	JA-Ile
Total duration of pathway phase (PP)	2.83 ± 0.73	4.5 ± 0.43	4.6 ± 0.62	2.53 ± 0.52*
Total duration of xylem phase (XP)	2.64 ± 1.75	0.5 ± 0.38	1.08 ± 0.45	0.78 ± 0.51
Total duration of non-probing phase (NPP)	1.1 ± 0.49	0.61 ± 0.21	1.26 ± 0.64	1.04 ± 0.48
Time to first probe	0.9 ± 0.58	0.12 ± 0.08	0.76 ± 0.72	0.5 ± 0.38
Time to first sieve element phase (f-SEP)	1.98 ± 0.59	2.98 ± 0.72	2.43 ± 0.75	2.05 ± 0.59

Values represent mean time (h) ± SE spent by SCA on various activities in each 8 h of recording (*n* = 9-10). An asterisk represents a significant difference between control and JA-Ile treatment across each genotype ($P < 0.05$, Kruskal-Wallis test) in the time spent by SCA for the indicated activity on the RTx430 and SC265 plants.

Table S2

Sugarcane aphid (SCA) feeding activities on the sorghum BTx623 and *msd3* plants with and without JA-Ile treatment.

SCA feeding activity	BTx623		*msd3*	
	Control	JA-Ile	Control	JA-Ile
Total duration of pathway phase (PP)	3.50 ± 0.67	3.45 ± 0.63	3.41 ± 0.57	2.87 ± 0.66
Total duration of xylem phase (XP)	0.48 ± 0.27	0.39 ± 0.28	0.7 ± 0.35	0.13 ± 0.07
Total duration of non-probing phase (NPP)	0.72 ± 0.55	1.47 ± 0.99	0.84 ± 0.31	0.31 ± 0.1
Time to first probe	0.68 ± 0.67	0.25 ± 0.17	0.19 ± 0.19	0.14 ± 0.09
Time to first sieve element phase (f-SEP)	1.31 ± 0.51	2.64 ± 0.96	3.31 ± 0.62	2.55 ± 0.61

Values represent mean time (h) ± SE spent by SCA on various activities in each 8 h of recording (*n* = 7-9). Statistically significant differences were not observed for any of the parameters between control and JA-Ile treatment across each genotype ($P < 0.05$, Kruskal-Wallis test) on the BTx623 and *msd3* plants.