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EFFECTS OF APHID DISOPERATION ON PHOTOSYNTHETIC PERFORMANCE AND AGRONOMIC TRAITS OF DIFFERENT SORGHUM VARIETIES

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Abstract

The current study used photosynthetic indexes to investigate the mechanism of aphid resistance in two sorghum varieties with different aphid tolerance. (Pn, Tr, Gs, Ci), chlorophyll fluorescence parameters (Fo, Fm, Fv/Fm, Y (II), qP, NPQ) as well as some agronomic traits (chlorophyll content, plant height, fresh weight, dry weight, root length, root number) that treated at different aphid densities (0, 10, 20 and 40 aphids/plant) and at different infestation times (4d, 8d, 12d and 16d). The results showed that, as aphid density and infestation time increased, the agronomic traits, the most photosynthetic parameters and the most chlorophyll fluorescence parameters showed a larger decrease in sensitive sorghum variety, whereas a minor decrease was observed correspondingly in the resistant variety. Comprehensive analysis indicated that five indicators, including Pn, Tr, Gs, Y(II), qP and chlorophyll content, were easily disturbed under large density of aphid and long infestation time. Among which three indicators, including chlorophyll content, Pn and Y (II), played a crucial role in promoting crop yield and thus could be considered as the key indicators in studying the aphid resistance in sorghum.

Key words: Sorghum, Sugarcane aphid, Photosynthesis, Chlorophyll fluorescence parameters, Agronomic Traits.

Introduction

Sugarcane aphid is a major kind of pest that causes damage to sorghum, sugarcane, sudangrass and other crops. It is widely spread in the world, especially in China, Africa, Japan, the United States and so on (Chang *et al.*, 2011; Sharma *et al*., 2013; Bowling *et al*., 2016). Sugarcane aphids mainly aggregate on the dorsal surface of lower leaves (Brewer *et al*., 2017). They are highly fertile and numerous; and they also secrete honeydew, which causes mould infestation, which in turn impairs leaf photosynthesis (Yang *et al*., 2020; Mercer *et al*., 2020). As a result, the yield and quality of crops such as sorghum were declined (Wang *et al.*, 2013; Lama *et al*., 2019). Chemical control is reliable, but it is also prone to causing drug resistance, so screening and planting aphid-resistant crop varieties is the best option (Nagaraj *et al*., 2002; Nagaraj *et al*., 2005). Some aphid resistant varieties have been reported (He *et al*., 1996; Limaje *et al*., 2017; Paudyal *et al*., 2019;Yang *et al*., 2020), such as 'HeNong No.16' (reported by Jin-Hua Chang's team at Hebei Agricultural University) (Chang *et al*., 2011), and 'JiNiang No.2' (reported by the grain Institute of Hebei Academy of Agriculture and Forestry Sciences) (Wang *et al*., 2019).

Aphid stress has been shown to influence plant physiological processes (photosynthesis, stomatal conductance, respiration, and chlorophyll content), resulting in chlorophyll depletion and photosynthesis inhibition in sorghum leaves (Paudyal *et al*., 2020; Macedo *et al*., 2003). Aphid stress hinders the transport of nutrients and blocks the stomata of leaves through the accumulation of honeydew, thereby limiting CO_2 absorption. These physiological processes ultimately affect plant growth, development, and yield. However, some varieties are able to tolerate aphid stress and are trend to compensate by changing the rate of photosynthesis (Uchimiya & Knoll, 2019; Diaz-montano *et al*., 2007; Pierson *et al*., 2011). Aphid stress alters agronomic traits (plant height, fresh weight, dry weight) of sorghum. Sugarcane aphids reproduce very fast and occupy a large area of the leaf in a short time, which limits the functionality of the leaf, which in turn reduces the efficiency of photosynthesis and ultimately reduces the biomass and yield of sorghum (Macedo *et al*., 2003). In this paper, the effects of aphid density and infestation time on photosynthesis, chlorophyll fluorescence, and agronomic traits on the seedlings of resistant and sensitive sorghum varieties were investigated to provide a basis for the identification and screening of resistant sorghum varieties in the future.

Materials and Methods

Sorghum varieties and aphids: 'JiNiang No.2', an aphid resistant variety that was identified by Hebei grain Institute, and 'SanChiSan', an aphid sensitive variety were used in this experiment (Wang *et al*., 2019; Yang *et al*., 2020). Sugarcane aphid was collected in 2014 from a sorghum field in our campus, then they were raised on a sweet sorghum variety 'DaLiShi' seedlings which were covered with a mesh cage. The seedlings were then placed in a growth chamber with a photoperiod of 14L/10D, a temperature of 24 ± 1°C, a humidity of 60 ± 10%, and a light intensity of 8000Lx.

Aphid release treatments: 'JiNiang No.2' and 'SanChiSan' sorghum varieties were grown in an artificial climate chamber for germination at 25°C for 48 h, after which the germinated seeds were sown in plastic pots (20×30 cm) filled with nutrient soil. Five seeds per pot and a total of 120 pots per variety were prepared. All the pots were placed in the chamber with the conditions were set as follows: photoperiod at 14L/10D, the temperature at 24±1°C, humidity at 60±10% and light intensity at 8000lx. After sprouting, only one plant was left in each pot, and until the sorghum seedlings were grown to four leaves stage. The 3-4th instar aphids and adult aphids were transferred onto the sorghum leaves at a density of 0, 10, 20 and 40 per plant. The whole plants were covered with a mesh cage to prevent aphid escape.After 4d, 8d, 12d, and 16d treatments, photosynthetic indexes, chlorophyll content, and chlorophyll fluorescence parameters were calculated, and agronomic trait indexes were measured only when the treatment period was 16d.

Determination of photosynthetic indices and chlorophyll fluorescence parameters: The upper second leaf was selected to determine the photosynthetic induces of different sorghum varieties using a CI-340 photosynthetic apparatus (CID company, USA). Nine replicates for each treatment. The following indicators were measured: net photosynthetic rate (Pn, μ mol CO_2 $m^{-2}s^{-1}$), transpiration rate (Tr, mmol H_2O $m^{-2}s^{-1}$), stomatal conductance (Gs, mmol H_2O $m^{-2}s^{-1}$), and intercellular CO_2 concentration (Ci, ppm). The upper third leaf was selected and was firstly dark-adapted for 30 min before determination. Leaf chlorophyll fluorescence parameters were measured using MINI-PAM-II (Walz company, Germany). The following indicators were measured: initial fluorescence (Fo), maximum fluorescence (Fm), maximum photosynthetic efficiency (Fv/Fm), actual photosynthetic efficiency Y (II), photochemical quenching coefficient (qP), non-photochemical quenching coefficient (NPQ), and nine replicates for each treatment.

Determination of agronomic traits of Sorghum: The chlorophyll content was measured using a SPAD-502 (Japan) chlorophyll determination instrument, with the upper second leaf being chosen for analysis of chlorophyll content. The remaining agronomic traits including plant height, root length, root number, fresh weight, and dry weight were tested according to standard methods. Ten plants were assayed for each treatment in 3 replicates.

Data analysis: The experimental data were processed using Excel 2007. ANOVA was performed using SPSS17.0 software, and multiple comparisons were made using Duncan's method.

Results and Analysis

Effects of aphid on photosynthetic indexes of sorghum varieties

Effect of aphid on the photosynthetic rate (Pn) of sorghum: As shown in Fig. 1, the Pn values of both resistant and susceptible varieties showed a decline trend with increasing aphid density at 4, 8, 12 and 16d of aphids infestation. As the infestation time prolonged, the Pn value of susceptible variety decreased much more while that of resistant variety decreased less. At 4d, the Pn value of susceptible variety was bigger than those of the resistant variety at aphid densities of 0, 10, and 20 aphids per plant, while the Pn value of susceptible varieties was smaller than those of the resistant varieties at 40 aphids per plant, but no obvious differences were observed. The Pn value of susceptible varieties at 40 aphid/plant aphid density was greatly smaller (p<0.05) than that of the control (0 aphid/plant), but there was no obvious differences in the Pn values between resistant varieties at different aphid density. At 8d, no obvious difference was observed between the Pn values of each treatment within resistant varieties compared with the susceptible variety. The Pn value of 20 and 40 aphids/plant of susceptible varieties was smaller than that of the control (p<0.05), and there was a obvious difference between these two treatments. The Pn values within resistant variety showed no obvious differences at different aphid densities. At 12 d, the Pn values of the 10, 20 and 40 aphids/plant of susceptible variety were smaller than that of the control, and the Pn value of seedlings with 40 aphids was smaller than that with 10 aphids (p<0.05). The Pn value of the seedlings with 40 aphids aphid in resistant variety was greatly smaller than that of the control (p<0.05), while the other treatments were not greatly different. At 16d, the Pn value of susceptible variety was greatly smaller than that of the resistant variety at an aphid density of 20 and 40 aphids/ plant(p<0.05), but no obvious difference was observed within resistant variety(p>0.05). The Pn values of the susceptible variety in 10 aphids/plant were signifcantly smaller than that of the control but bigger than that of 20 and 40 aphids/plant(p<0.05). The Pn values of the susceptible variety between 20 and 40 aphids/plant were not notable.

Effect of aphid on transpiration rate (Tr) of sorghum: As shown in Fig. 2, at different disoperation times, a decreasing trend was observed in the Tr of both resistant and susceptible varieties as the increase of aphid density. In the Tr, the susceptible variety showed a greater decline. At 4d, the Tr of susceptible variety was bigger than that of resistant variety at different aphid densities, and the Tr of susceptible variety was greatly bigger than that of the resistant variety at aphid densities of 0 and 10 aphids/ plant (p<0.05), but there was no obvious difference between susceptible variety and resistant variety at aphid densities of 20 and 40 aphids/plant (p>0.05). At aphid densities of 0 and 10 aphids/plant, the Tr of the susceptible variety was greatly bigger than that of 20 and 40 aphids/plant. There was no obvious difference in Tr within resistant variety under different aphid densities (p>0.05). At 8d, In the control group, the Tr of the susceptible variety was greatly bigger than that of the resistant variety (P < 0.05), but there was no substantial difference between the susceptible and resistant varieties with 10, 20, and 40 aphids/plant (p>0.05). The Tr value of susceptible variety with 20 and 40 aphids/plant was greatly smaller than control (p<0.05), but no obvious difference was noticed within resistant varieties under different aphid densities (p>0.05). At 12d, the Tr value of susceptible varieties at 20 and 40 aphids/plant was greatly smaller than that of 0 and 10 susceptible varieties (p<0.05), the Tr value of all treatment of resistant varieties was no obvious difference. and there was no obvious difference between the Tr of susceptible varieties and resistant varieties (p>0.05). At 16d, the Tr value of susceptible varieties with aphid densities of 20 and 40 per plant was obviously smaller than that of resistant varieties (p<0.05), but there was no substantial difference between susceptible and resistant varieties with aphid densities of 0 and 10 per plant (p>0.05).

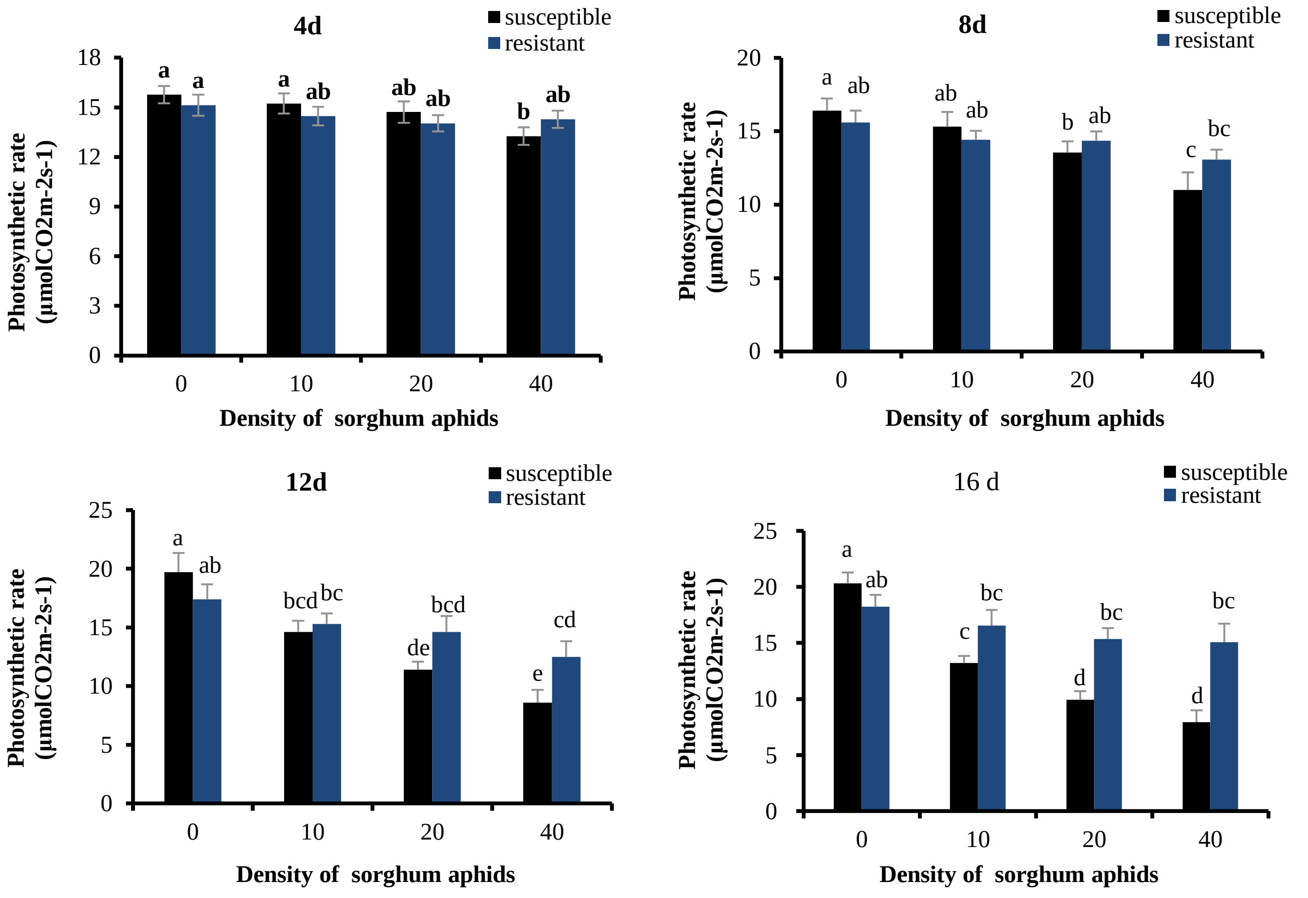

Fig. 1. Effect of density and damaged time of aphid on the photosynthetic rate (Pn) of sorghum.

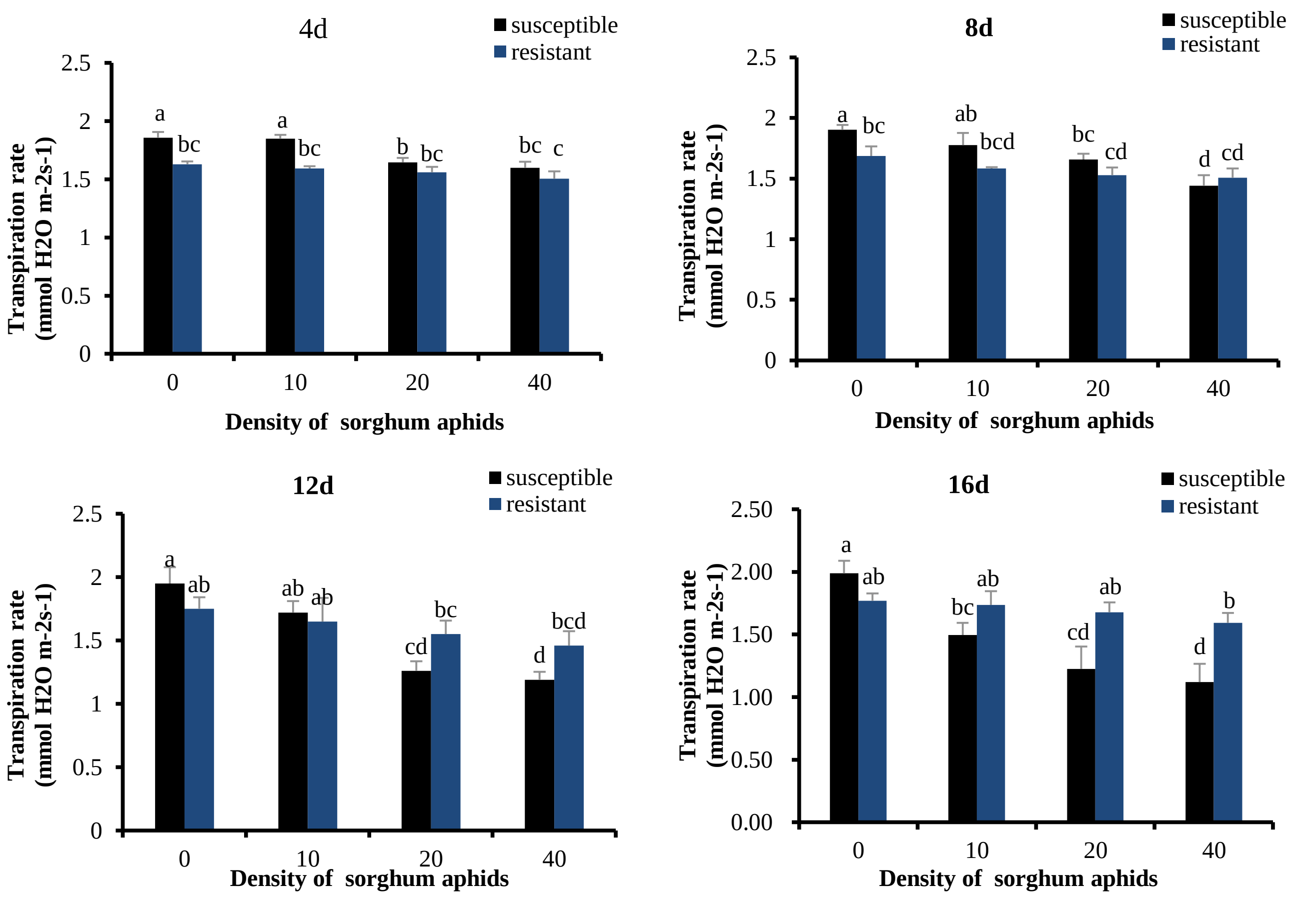

Fig. 2. Effect of density and damaged time of aphid on transpiration rate (TR) of sorghum.

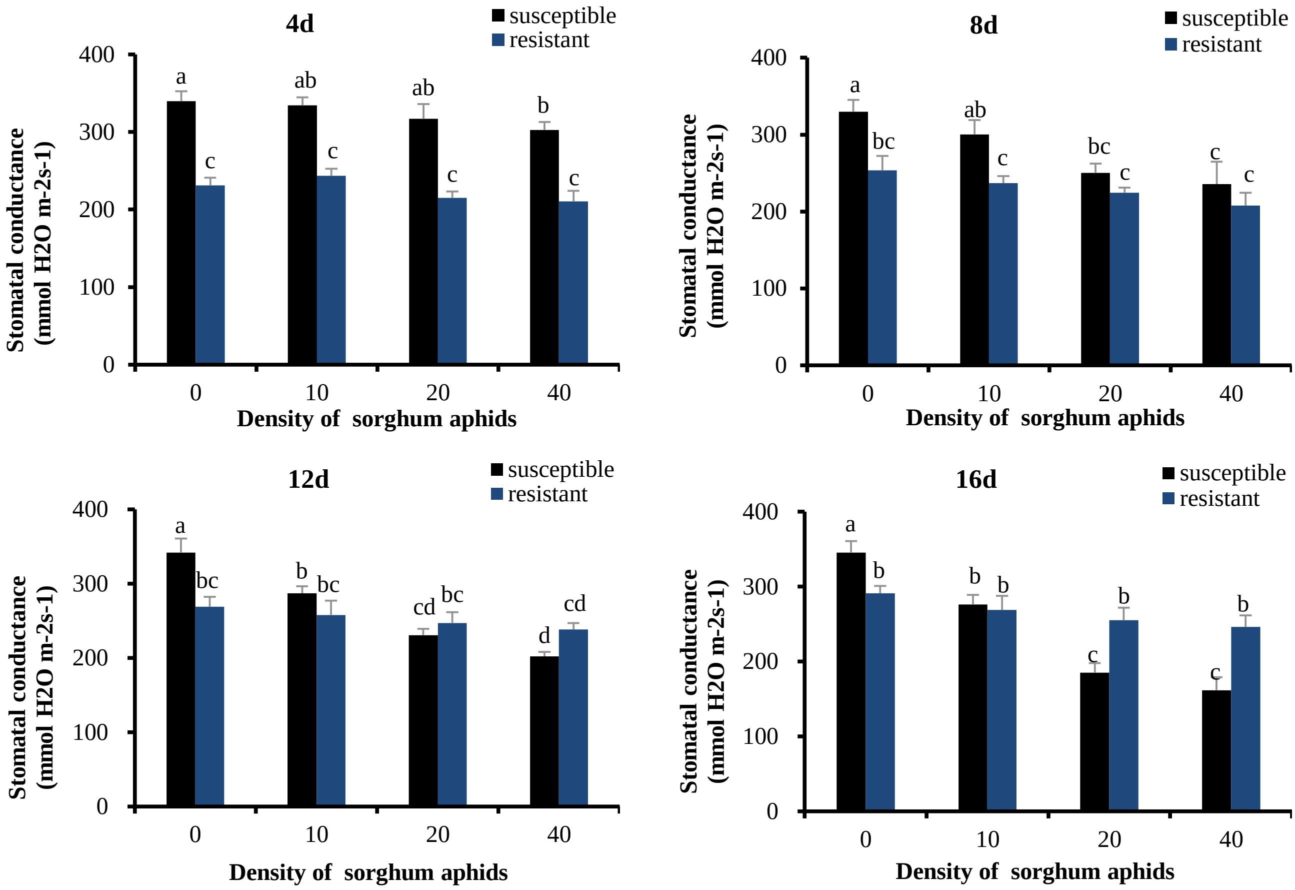

Fig. 3. Effect of density and damaged time of aphid on **Gs** of sorghum.

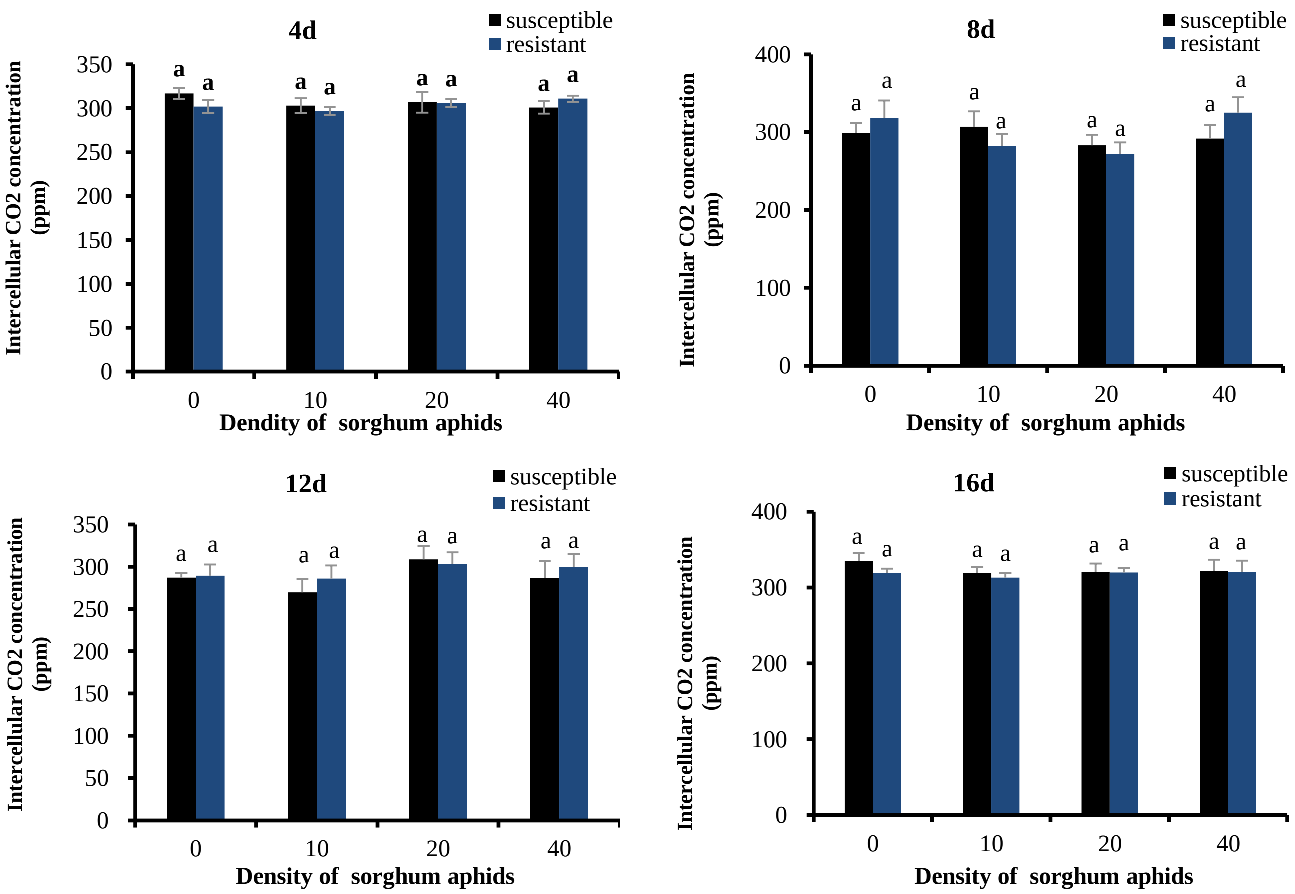

Fig. 4. Effect of density and damaged time of aphid on the Ci of sorghum.

Effects of aphids on the Ci of sorghum: As shown in Fig. 4, aphid density and infestation time had no obvious effects on the Ci of both sorghum varieties. At 4d, 8d,12d and 16d, the Ci of susceptible variety was similar to that of resistant variety under different aphid density(p>0.05). The Ci of both varieties at 10, 20, 40 aphids/plant were not greatly different from that of the control (p>0.05).

Effects of aphids on Gs of sorghum: As shown in Fig. 3, the Gs of susceptible variety was greatly altered, while the Gs of resistant variety was not greatly altered at different aphid density and different stress time. At 4d, the Gs of susceptible variety were bigger than that of the resistant variety (p<0.05), and the Gs of seedlings with 40 aphids in susceptible variety were greatly lower than that of control (p<0.05), while Gs of seedlings with 10, 20 and 40 in susceptible variety had no obvious difference to each other(p>0.05). The Gs of resistant variety had no obvious difference under different aphid densities (p>0.05). At 8d, the Gs of 0 and 10 aphids/plant of susceptible variety was greatly bigger than those of resistant variety (p <0.05), whereas the Gs of the 20 and 40 aphids/plant of susceptible variety were similar to that of the resistant variety (p<0.05). the Gs of the 20 and 40 aphids/plant of susceptible variety were greatly lower than the control (p<0.05). The Gs of resistant variety were not greatly different from each other under different aphid densities(p>0.05). At 12d, the Gs of susceptible variety were bigger than those of resistant varieties(p<0.05) at different aphid density, whereas no obvious differences were observed between them at 10, 20 and 40 aphids/plant (P > 0.05). At 16d, the Gs of susceptible varieties were bigger than those of the resistant varieties without aphids, whereas the Gs of susceptible varieties were not greatly different from those of the resistant varieties at the presence of 10 aphids/plant (p<0.05). When 20 or 40 aphids/plant were present, the Gs of susceptible varieties were lower than those of resistant varieties (p<0.05). The Gs of the susceptible varieties were bigger in 0 or 10 aphids/plant than in 20 or 40 aphids/plant (p>0.05), but they were identical in 20 and 40 aphids/plant. At different treatments, the Gs of resistant variety at different aphid density barely changed.

Effects of aphids on chlorophyll SPAD value in sorghum: As shown in Fig. 5, chlorophyll SPAD value showed a obvious decline in susceptible variety under different aphid density, while it stayed unchanged in resistant varieties. At 4d, the SPAD value of 0, 10, 20 aphid/plant in susceptible variety was no obvious difference compared than those of resistant variety (p>0.05), but were greatly smaller than those of resistant variety under the presence of 40 aphids/plant (p<0.05). The SPAD values were similar between both varieties under 10 and 20 aphids/plant (p>0.05). Under 20 and 40 aphids/plant conditions, SPAD value was smaller in susceptible varieties compared with the control (p<0.05). None of the SPAD values was hardly changed within resistant variety under all aphid density conditions (p>0.05). At 8d, SPAD values of 0 and 10 aphids/plant in susceptible variety were greatly bigger than resistant variety under similar aphid density (p<0.05), whereas those of the 20 and 40 aphid/plant in susceptible variety were greatly smaller than those of resistant variety (p<0.05). At 12d, SPAD values of 0 aphids/plant in susceptible variety were greatly bigger than those of the aphid resistant varieties (p<0.05), whereas at 16d, SPAD values of susceptible variety was similar to that of resistant variety without aphids presence (p>0.05). At 12 and 16d, the SPAD values of the susceptible varieties were greatly smaller than those of resistant variety under 10, 20 and 40 aphids/plant. Chlorophyll SPAD values were greatly bigger (p<0.05). At 8, 12 and 16d, SPAD values of susceptible variety under 0 or 10 aphids/plant were bigger than that of 20, 40 aphids/plant. However, there were no obvious differences within resistant variety under different aphid density conditions (p>0.05).

Effects of aphids on chlorophyll fluorescence parameters of sorghum

Effects of aphids on Fo of sorghum: At 4d, the Fo values of both sorghum varieties increased compared with the control, but no obvious differences among all the treatments were observed (p>0.05). The Fo value of the resistant variety was slightly bigger than that of the susceptible variety (p>0.05) (Table 1). At 8d, the Fo value of susceptible varietyincreased with increasing aphid density, the Fo value of susceptible variety at aphid densities of 40 aphids/plant was greatly bigger than that of the control and resistant variety. Within resistant varieties, the Fo values barely changed (Table 2). At 10, 20, and 40 aphids/plant, the Fo value of the susceptible variety was bigger than that of the resistant variety at 12d. With the increasing of aphid density, the Fo value of susceptible variety was bigger in 40 aphids/plant than that in 10 aphids/plant. The Fo value of susceptible variety was bigger than that of resistant variety under 20 or 40 aphids/plant but stayed unchanged within resistant variety(Table 3). At 16d, the Fo values of susceptible variety were slightly bigger than that of resistant variety under 10, 20 and 40 aphids/plant. whereas decreased with the duration of aphid infestation in resistant variety under 10, 20 and 40 aphids/plant.

Effects of aphids on Fm value of sorghum: At 4d, in comparison to the control, the Fm value of the susceptible variety was slightly smaller, while the Fm value of the resistant variety was slightly bigger, but there was not obvious difference between the treatments of variations (p>0.05). (See Table 1). At 8d, the Fm values of both aphid varieties decreased slightly but not greatly altered within variety under different treatments. Only the Fm value of the resistant variety at 0 aphid/plant was greatly bigger than the Fm value of the susceptible variety at 40 aphids/plant (Table 2). At 12d, the Fm value decreased in both varieties under aphid disoperation, and the Fm value decreased more in susceptible varieties under the 40 aphids/plant, which greatly smaller than that of the control, and also greatly smaller than that in resistant variety under 0 or 10 aphids/plant. However, no obvious difference in Fm value within resistant variety under all treatments (p>0.05) (Table 3). At 16d, the Fm value of 10, 20 and 40 aphids/plant in susceptible variety decreased and was greatly smaller than that of the control in both varieties. In both varieties, the Fm values for 10, 20, and 40 aphids/plant were comparable. The difference between them, as well as the Fm of each treatment among the resistant varieties (p>0.05), was not obvious (See Table 4).

Table 1. Effects on chlorophyll fluorescence parameters of resistant and susceptible sorghum damaged by aphid after 4 day.

Density of aphid (aphid/plant)	Variety of sorghum	Fo	Fm	Fv/Fm	Y (II)	qP	NPQ
0	Susceptible	226.9±7.1 a	1153±45 a	0.804±0.013 a	0.528±0.023 a	0.725±0.023 a	0.258±0.019 b
	Resistant	233.4±6.5 a	1143±29 a	0.796±0.011 a	0.519±0.017 ab	0.736±0.028 a	0.306±0.030 ab
10	Susceptible	229.0±6.5a	1151±43 a	0.801±0.017 a	0.500±0.026 ab	0.703±0.021 ab	0.278±0.025 ab
	Resistant	235.4±6.6 a	1146±42 a	0.795±0.012 a	0.510±0.066 ab	0.714±0.014 a	0.291±0.019 ab
20	Susceptible	229.4±4.5a	1092±42 a	0.790±0.007 a	0.460±0.018 bc	0.646±0.016 bc	0.272±0.022 ab
	Resistant	234.6±6.0 a	1146±41 a	0.796±0.009 a	0.505±0.015 ab	0.711±0.025 a	0.294±0.018 ab
40	Susceptible	230.9±6.4 a	1086±71 a	0.788±0.007 a	0.425±0.012 c	0.623±0.024 c	0.343±0.037 a
	Resistant	236.3±4.9 a	1144±50 a	0.793±0.008 a	0.487±0.025 bc	0.697±0.015 ab	0.327±0.026 ab

Table 2. Effects on chlorophyll fluorescence parameters of resistant and susceptible sorghum damaged by aphid after 8 day.

Density of aphid (aphid/plant)	Variety of sorghum	Fo	Fm	Fv/Fm	Y (II)	qP	NPQ
0	Susceptible	217.2±4.2 d	1155±39 ab	0.812±0.012 a	0.538±0.022 a	0.724±0.013 a	0.244±0.020 b
	Resistant	224.3±6.2 cd	1160±61 a	0.805±0.024 ab	0.536±0.012 a	0.746±0.020 a	0.296±0.034ab
10	Susceptible	236.8±5.8 abc	1135±41 ab	0.791±0.013 ab	0.476±0.014 bc	0.674±0.019 ab	0.302±0.032 ab
	Resistant	228.0±5.5 bcd	1141±48 ab	0.800±0.006 ab	0.523±0.024 ab	0.744±0.032 a	0.305±0.025 ab
20	Susceptible	242.4±3.3 ab	1079±32 ab	0.774±0.020 ab	0.433±0.018 cd	0.634±0.028 bc	0.306±0.022 ab
	Resistant	228.6±3.7 bcd	1136±30 ab	0.797±0.022 ab	0.510±0.028 ab	0.717±0.015 a	0.298±0.026 ab
40	Susceptible	245.9±4.6 a	1073±35 b	0.769±0.023 b	0.400±0.015 d	0.596±0.024 c	0.349±0.033 a
	Resistant	225.9±5.8 cd	1127±54 ab	0.799±0.015 ab	0.495±0.021 ab	0.704±0.026 a	0.317±0.024 ab

Table 3. Effects on chlorophyll fluorescence parameters of resistant and susceptible sorghum damaged by aphid after 12 day.

Density of aphid (aphid/plant)	Variety of sorghum	Fo	Fm	Fv/Fm	Y (II)	qP	NPQ
0	Susceptible	209.1±6.1 d	1182±41 a	0.822±0.013 a	0.561±0.006 a	0.748±0.027 a	0.235±0.021 d
	Resistant	219.9±6.6 cd	1186±29 a	0.815±0.006 ab	0.542±0.024 a	0.740±0.018 a	0.275±0.017 cd
10	Susceptible	241.4±5.4 bc	1089±44 ab	0.777±0.016 bc	0.452±0.023 b	0.676±0.026 ab	0.317±0.022 ab
	Resistant	214.7±8.0 d	1140±70 a	0.809±0.018 ab	0.506±0.010 ab	0.720±0.016 a	0.287±0.030 abc
20	Susceptible	256.9±10.7 ab	1090±34 ab	0.765±0.015 bc	0.412±0.016bc	0.631±0.014 b	0.348±0.012 a
	Resistant	223.0±3.9 cd	1106±51 ab	0.796±0.021 ab	0.477±0.015 ab	0.680±0.020 ab	0.296±0.020 ab
40	Susceptible	267.0±14.3 a	998±30 b	0.733±0.024 c	0.347±0.020 d	0.579±0.011 c	0.355±0.034 a
	Resistant	226.7±4.9 cd	1091±36 ab	0.792±0.008 ab	0.449±0.026 b	0.639±0.021 b	0.304±0.017 ab

Table 4. Effects on chlorophyll fluorescence parameters of resistant and susceptible sorghum damaged by aphid after 16 day.

Density of aphid (aphid/plant)	Variety of sorghum	Fo	Fm	Fv/Fm	Y (II)	qP	NPQ
0	Susceptible	210.2±4.1 d	1216±39 a	0.826±0.016 a	0.583±0.021 a	0.767±0.028 a	0.237±0.024 c
	Resistant	214.3±9.3 d	1205±30 a	0.823±0.019 ab	0.585±0.028 a	0.775±0.024 a	0.225±0.012 c
10	Susceptible	244.2±6.1 bc	1054±65 ab	0.761±0.015 c	0.414±0.023 c	0.644±0.027 c	0.325±0.034 ab
	Resistant	221.0±8.9 cd	1146±46 a	0.806±0.024 ab	0.539±0.014 ab	0.751±0.018 ab	0.251±0.019 c
20	Susceptible	258.0±10.5 ab	1025±51 ab	0.746±0.025 c	0.366±0.016 cd	0.580±0.029 d	0.340±0.020 a
	Resistant	223.9±11.9 cd	1119±52 ab	0.800±0.008 ab	0.516±0.012 b	0.721±0.016 ab	0.267±0.021 bc
40	Susceptible	281.4±13.8 a	970±32 b	0.711±0.020 d	0.318±0.018 d	0.568±0.022 d	0.364±0.013 a
	Resistant	225.0±3.6 cd	1097±37 ab	0.793±0.027 b	0.494±0.020 b	0.702±0.015 bc	0.268±0.016 bc

Table 5. Effects of agronomic traits of sorghum seeding damaged by different densities sugarcane aphid.

Density of aphid (aphid/plant)	Variety of sorghum	Height of plant (cm)	Fresh weight per plant (g/plant)	Dry weight per plant (g/plant)	Root length per plant (cm/plant)	Number of roots per plant (cm/plant)
0	Susceptible	44.10±0.536a	15.533±0.3102 a	1.740±0.0314 a	41.61±0.956 a	11.60±0.393 a
	Resistant	42.58±0.693 ab	15.113±0.2936 a	1.715±0.0283 ab	41.36±1.267 a	11.45±0.344 ab
10	Susceptible	41.72±0.564 b	14.867±0.2514 a	1.557±0.0313 c	39.47±0.988 abc	10.85±0.466 abc
	Resistant	42.13±0.663 ab	14.973±0.3986 a	1.702±0.0287 ab	40.61±1.138 ab	11.25±0.397 ab
20	Susceptible	39.42±0.805 c	13.733±0.2652 b	1.456±0.0540 c	37.82±1.223 bc	10.20±0.536 bc
	Resistant	41.88±0.662 b	14.707±0.4258 ab	1.650±0.0326 abc	40.80±0.973 ab	11.30±0.317 ab
40	Susceptible	38.33±0.646 c	12.267±0.3059 c	1.311±0.0422 d	36.75±1.182 c	9.9±0.475 c
	Resistant	41.41±0.787 b	14.640±0.3602 ab	1.627±0.0329 bc	40.49±1.159 ab	11.20±0.345 ab

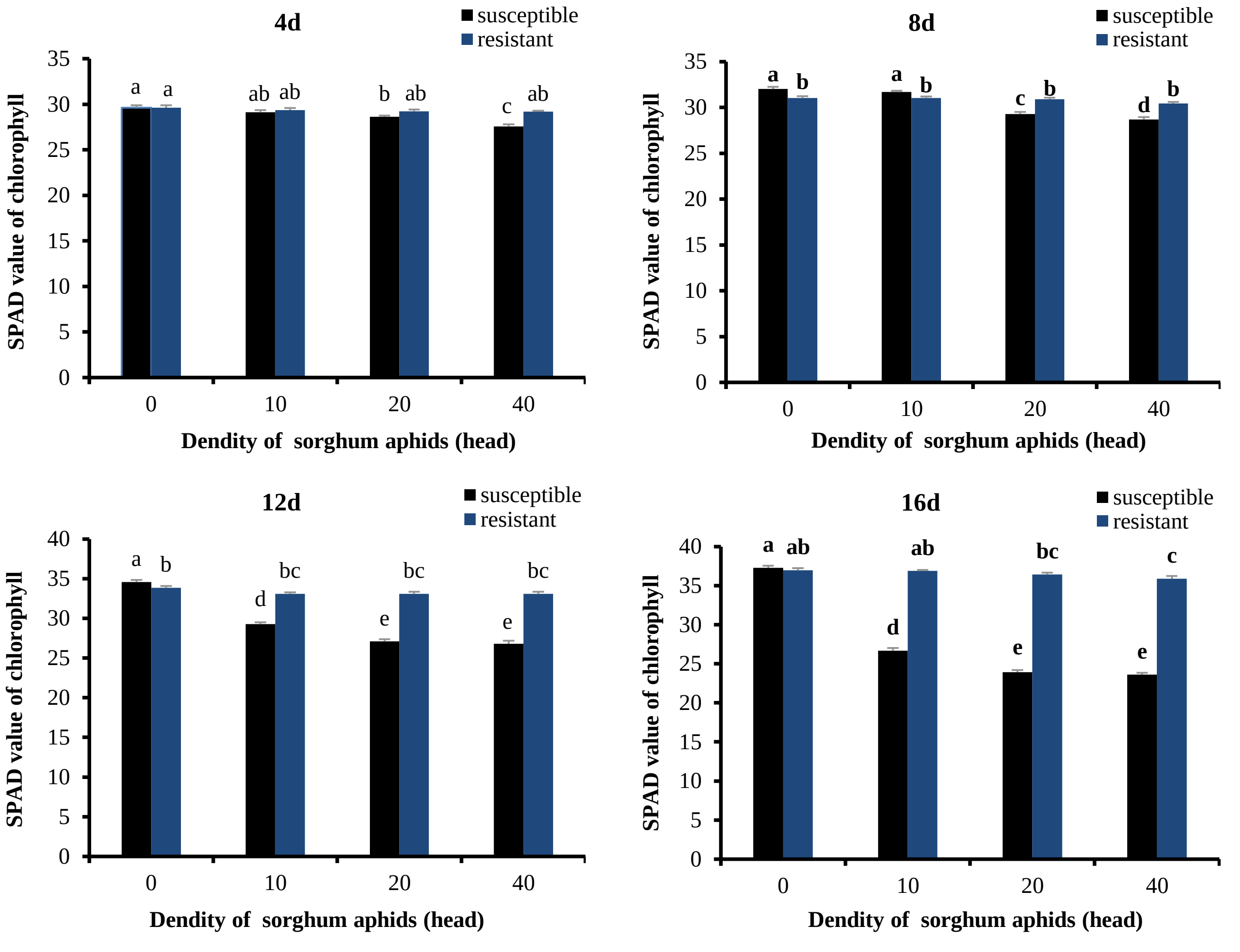

Fig. 5. Effect of density and damaged time of aphid on chlorophyll SPAD value in sorghum.

Effects of aphids on Fv / Fm value of sorghum: At 4d, the Fv/Fm value decreased slightly in both varieties, and the biggest decrease occurred in 40 aphids/plant susceptible variety. The Fv/Fm values stayed unchanged within all the treatments in the resistant variety(Table 1), but Fv/Fm values were ingreatly (p>0.05) decreased in both varieties compared with the control (Table 2). At 12d, Fv/Fm value decreased in both varieties compared with the control and was greatly smaller in susceptible variety at 10, 20 and 40 aphids/plant than that of the control. No obvious difference was found within each treatment in the susceptible variety and resistant variety (Table 3). At 16d, the Fv/Fm value of each treatment in susceptible variety was greatly smaller than that of the control. The Fv/Fm value of 40 aphids/plant was greatly smaller than that of the 10 and 20 aphids/plant in the susceptible variety. At 16d, the Fv/Fm value of each treatment in the resistant variety was smaller than that of the control but slightly elevated compared with that at 12d, and the Fv/Fm value of each treatment within resistant variety was not greatly different (Table 4).

Effects of aphids on Y (II) value of sorghum: At 4d, Y(II) value decreased in all the treatments in both varieties compared with the control, with the greatest decrease in 40 aphids/plant of susceptible variety, which was greatly smaller than that in the treatments of 0 and 10 aphids/plant of susceptible variety, while the difference in each treatment of the resistant variety was not obvious (Table 1). At 8d, Y (II) value was decreased in all treatments of susceptible variety compared with the control, and it was greatly smaller in the 10, 20 and 40 aphids/plant susceptible variety than the control and each treatment of resistant variety. The Y (II) value of susceptible variety at 40 aphids/plant was greatly smaller than that of susceptible variety at 10 aphids/plant. The Y (II) value of each treatment of resistant variety was smaller than that of the control but was slightly elevated compared to that at 4d (Table 2). At 12d, the Y (II) value was decreased in all treatments of both varieties compared with the control. The more density of aphids, the larger decrease in the Y(II) value. The greatest decrease observed in 40 aphids/plant of susceptible variety, which was greatly smaller than that in the other treatments. The Y (II) values in the 10 and 20 aphids/plant of susceptible variety were greatly smaller than the control and resistant varieties. By comparing the resistant variety to the susceptible variety, it can be shown that the decrease in Y (II) was less in each treatment of the resistant variety. The other treatments remained virtually unchanged, with the exception of the Y(II) value of 40 aphids/plant of resistant variety being slightly smaller than the control (Table 3). At 16d, the Y (II) values decreased in all treatments of susceptible variety compared with the control, with the greatest decrease in Y (II) value occurred in 40 aphids/plant of susceptible variety, which was greatly smaller than that in the other treatments. The Y (II) values of susceptible varieties with 10 and 20 aphids/plant were greatly smaller than those of susceptible varieties with 0 aphid/plant and resistant varieties with 0 aphid/plant. The Y(II) values in 20 and 40 aphids/plant of resistant variety were greatly smaller than that of 0 aphid/plant, but the difference in the Y (II) values among the treatments of 10, 20 and 40 aphids/plant of resistant variety was not obvious (Table 4).

Effects of aphids on qP value of sorghum: At 4d, the qP value was decreased in all treatments of both varieties compared with the control. The more density of aphids, the larger decrease in the qP value. The greatest decrease observed in 40 aphids/plant of susceptible variety, which was greatly smaller than that in the other treatments (except for 20 aphids/plant of susceptible variety). The qP value in 20 aphids/plant of susceptible variety was greatly smaller than that in 0, 10 and 20 aphids/plant of the resistant variety. The qP values of all the treatments in resistant variety decreased slightly and were similar among each treatment (Table 1). At 8d, the qP value of each treatment in susceptible variety decreased compared with the control, with the greatest decline occurred in 40 aphids/plant (except for 20 aphids/plant of susceptible variety). The qP value in 20 aphids/plant of susceptible variety was greatly smaller than that in 0 aphid/plant of susceptible variety and all the treatments of the resistant variety. Compared with that of 4d, the qP value of each treatment in resistant variety was slightly raised, although smaller than that of the control. The qP value among each treatment in resistant variety was similar (Table 2). At 12d, the qP values of both varieties decreased and smaller than that of the control. The greatest decline in qP value was observed in 40 aphids/plant of susceptible variety and smaller than that of other treatments. The qP value of 20 aphids/plant of susceptible variety and 40 aphids/plant of resistant variety was greatly smaller than that in 0 aphid/plant of susceptible variety and each treatment of 0 and 10 aphids/plant of resistant variety. The qP values of 0, 10 and 20 aphids/plant of the resistant variety were similar to each other (Table 3). At 16d, in comparison with 12d, the qP value decreased in each treatment of susceptible variety while elevated in each treatment of the resistant variety, although smaller than that of the control. The qP values in 20 and 40 aphids/plant of susceptible variety were smaller than that of other treatments. The qP value in 10 aphids/plant of susceptible variety was greatly smaller than that in 0 aphid/plant of susceptible variety and 0, 10 and 20 aphids/plant of resistant variety. The qP value in 10 aphids/plant of susceptible variety was greatly smaller than that of the control in both varieties. The qP values in 0, 10 and 20 aphids/plant of the resistant almost stayed unchanged (Table 4).

Effects of aphids on NPQ value of sorghum: At 4d, the NPQ value of each treatment in susceptible variety raised compared with the control, with the greatest elevation occurred in 40 aphids/plant. The NPQ value in each treatment of resistant variety was slightly changed (Table 1). At 8d, the situation was similar to that at 4d, except for a obvious elevation in 40 aphids/plant of susceptible variety (Table 2). At 12d, the NPQ values were elevated in all treatments of both varieties compared with the control. The more density of aphids, the larger elevation in the NPQ value. A larger increase was found in 10, 20 and 40 aphids/plant of the susceptible variety, which was greatly larger than that of the control. The NPQ value of each treatment in resistant variety was slightly raised and no obvious difference was found among them (Table 3). At 16d, the NPQ value increased in each treatment of susceptible variety and decreased in resistant variety, but all bigger than that of the control. A larger increase in 10, 20 and 40 aphids/plant of the susceptible variety was observed, which was greatly bigger than that of the control of both varieties and 10 aphids/plant of resistant variety. No obvious change was found in each treatment of resistant variety (Table 4).

Effects of aphids on the agronomic traits of sorghum seedlings

At 16d, the following indices including plant height, fresh weight, dry weight, root length and root number per individual plant of sorghum seedlings were determined. As shown in Table 5, the plant height in 20 and 40 aphids/plant of susceptible variety was smaller than that of the resistant variety. The plant height in all the treatments of susceptible was greatly smaller than that of the control (p<0.05), but it slightly changed in resistant variety. The fresh weight among 10, 20 and 40 aphids/plant of susceptible variety was greatly different from each other(p<0.05), and the larger density of aphids, the larger decrease in fresh weight. However, the fresh weight among each treatment of resistant variety stayed unchanged(p>0.05). It can be seen that the dry weight of 40 aphids/plant of susceptible variety were smaller than that of the other treatments, and the dry weight of 10 and 20 aphids/plant of susceptible variety was smaller than that of the control (p<0.05). No obvious change was found in the dry weight of each treatment of resistant variety. The root length and number in 20 and 40 aphids/plant of susceptible variety were smaller than that of the control (p<0.05), but there was no difference between 0 and 10 aphids/plant of susceptible variety and each resistant variety care.

Discussion

Photosynthetic rate is an obvious reflection of pest infestation (Xu & Zhang, 1991). Therefore, it can be used as a crucial indication for evaluating plant resistance to pest. A decrease in chlorophyll content may be one of the many factors that lead to a reduced rate of photosynthesis upon pest damage. Upon pests damage, they may release some special chemicals such as pectinases in to plant that disturb chlorophyll content and photosynthetic processes (Ma *et al*., 1998), which will reduce photosynthesis. In this research, we discovered that as aphid density and disoperation time increased, the chlorophyll content and photosynthesis in susceptible varieties decreased, while they barely changed slightly in resistant varieties, indicating that it has much more resistance. Stomata are channels through which plant undergo gas and water exchange with the outside, and Gs indicates the extent of opening of stomata. CO_2 is the raw material for photosynthesis. Therefore, Gs and the concentration of Ci are very important to photosynthesis, respiration and transpiration(Naseer *et al*., 2017). In the present study, the Gs values in the treatments of susceptible variety decreased with the increasing aphid density and disoperation time, while Ci was hardly changed. These findings are consistent with Paudyal's report on sugarcane aphid in sorghum(2020) but in conflict with Kou's report on *Odontothrips loti* Haliday in *Medicago sativa* L.(Kou *et al*., 2014). This inconsistency might be due to the different pest and the way of damage. Transpiration (Tr) can maintain leaves at a relatively suitable temperature, which is conducive to the accumulation of photosynthetic products. In this study, the Tr of susceptible variety decreased gradually with increasing aphid density and disoperation time, probably due to the stomatal closure caused by aphid damage. The Fo was the minimum fluorescence of dark-adapted chloroplasts and the increase in Fo could be attributed to either a reversible inactivation or an irreversible disruption of the PSII or damage to the thylakoid membrane. The more severe the damage to the thylakoid membrane, the greater the increase in Fo (Burd *et al*, 1996; Xu *et al*, 1992). The Fo value was greatly increased in susceptible variety upon aphid damage (Table 2-4), suggesting that photoinhibition was induced by hypoxia due to blockage of electron movement at the PS II receptor site. The Fm and Fv/Fm values greatly decreased in susceptible variety upon aphid damage(Tables 2-4), and the decrease of Fv/Fm value might be caused by the decreasing of Fm and the increasing of Fv value.

The Fv/Fm value is directly proportional to the photochemical quantum yield of PSII and is highly related to the quantum yield of net photosynthesis. Therefore, investigating the Fv/Fm value provides a good indicator for measuring light efficiency. It is reported that Fv/Fm value$\geq$0.8(relative fluorescence units) is considered to indicate that plants have high photochemical efficiency and PSII electron transport capacity (Burd *et al*., 1996; Andrews *et al*., 1995). In this study, we found that Fv/Fm values were greatly smaller than 0.8 at 8d, 12d and 16d disoperation in susceptible variety after aphid damage, while bigger than 0.8 or slightly smaller than 0.8 in control and in resistant variety.

Y(II), the real photosynthetic efficiency, is a great indicator of radiation usage efficiency, reflecting the efficiency with which absorbed photon is converted into chemicals (Krause & Weis, 1984; Van and Sne, 1990), which can accurately reflect the actual primary light energy capture efficiency of the PS II reaction centre in the case of partial closure, is the actual efficiency of the PSII reaction centre to carry out photochemical reactions, and its value is closely related to the intensity of the carbon assimilation reaction (Genty *et al*., 1989). In the present study, Y(II) was found to be more sensitive in susceptible variety after aphid damage, which was greatly smaller than that of the control at the early stage of infection (4d) at 20 aphids/plant. As a result, it can be used to track the severity of aphid infection and assess aphid resistance in sorghum varieties.

Photochemical quenching (qP) represents the portion of radiation absorbed by PSII for electron transport (Zhang *et al*., 1999). NPQ is the share of radiation absorbed by PSII that fails to be used for electron transport but dissipated as heat (Bilger *et al*., 1990). If PSII absorbs too much radiation and does not dissipate it rapidly enough, the photosynthetic apparatus will be damaged. Therefore, NPQ is a protective mechanism of plants and plays a certain role in the photosynthetic apparatus (Zhang *et al*., 1999).

In this experiment, we found that the qP value in susceptible variety decreased rapidly and the NPQ increased rapidly with the increase of aphid density and disoperation time, indicating that aphid disoperation caused excess radiation of photosynthetic apparatus so that the CO_2 assimilation was inhibited and the proportion of radiation converting into chemicals decreased, which will lead to decrease in photosynthetic efficiency. However, the qP value and NPQ value were hardly changed in the resistant variety, indicating that the resistant variety had tolerance to aphid damage.

It is reported that the changes in chlorophyll fluorescence parameters were closely related to the resistance of plants (Chen *et al*., 2004; Huang *et al*., 2007; Kou *et al*., 2014; Wang *et al.,* 2017). Under brown planthopper damage, the Fo, NPQ, and qP values in resistant varieties decreased, while the Y(II) and Fv/Fm values increased. But in susceptible variety, all parameters decreased except for Fo (Chen *et al*., 2004). It was reported that the QP, Y(II) and Fv/Fm values experienced a larger decrease in high susceptible *Medicago sativa* variety, while those in medium and high resistance varieties only decreased less upon aphid damage. Interestingly, Fv/Fm value in high resistance variety was slightly elevated, and the NPQ value in different resistant varieties increased first and then decreased (Huang *et al*., 2007). The above findings were not fully consistent with our present results, which may be caused by either different damage way of pests or different infestation time. It is feasible to use chlorophyll fluorescence parameters such as Fo, Fm, Fv/Fm, Y(II), qP and NPQ to evaluate the pest tolerance of plants.

Upon aphid damage, the leaves of the plant become yellow, curly and crumpled, to the plant is dwarfed, growth arrested, and yield decreased, etc (Armstrong *et al.*, 2015; Brewer *et al*., 2017; Elliot *et al*., 2017; Du *et al*., 2018; Du *et al*., 2019). This study confirmed that there were large reductions in agronomic traits (plant height, fresh and dry weight, root length, and root number) in susceptible variety under aphid damage, while fewer reductions occurred in resistant variety. After aphid damage, the changes in agronomic traits of the two varieties were largely consistent with the changes in photosynthetic indicators, which explains the causal relationship between the symptoms and mechanisms involved in aphid infection in resistant and susceptible varieties, That is, different magnitudes of decline in susceptible and resistant varieties' agronomic traits were caused by different magnitudes of chlorophyll content and photosynthetic rate decline. According to the above results, we speculate that the main reasons responsible for the changes in agronomic traits of variety are chlorophyll content, photosynthetic rate, and actual photosynthetic efficiency Y (II), so these three parameters can be considered as key indicators for future study in aphid resistance of sorghum.

Conclusions

In this paper, different aphid density (0, 10, 20 and 40 aphids/plant) and infestation times (4d, 8d, 12d and 16d) were designed to evaluate the performance of different sorghum varieties upon aphid damage. Photosynthetic index (Pn, Tr, Gs, Ci), and chlorophyll fluorescence parameters (Fo, Fm, Fv/Fm and Y(II), qP and NPQ), as well as agronomic traits (chlorophyll content, plant height, fresh weight, dry weight, root length, root number), were determined to investigate the relationship between photosynthetic physiology and agronomic traits of different sorghum varieties.We discovered that changes in agronomic traits were generally consistent with changes in photosynthetic parameters (except Ci) and most chlorophyll fluorescence parameters (except Fo and NPQ), with the susceptible variety showing a greater decrease as aphid density increased and the disoperation period increased. However, the minor decrease was found in the resistant variety, suggesting a stronger resistance. The following indices, such as Pn, Tr, Gs, Y(II),qP and chlorophyll content were more sensitive to aphid damage, among which chlorophyll content, Pn and Y(II) play important roles in affecting the crop yield, therefore, they can be considered as key indicators for future study in aphid resistance of sorghum.

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