

## Sugars in watermelon root exudates and their effects on *Fusarium oxysporum* f. sp. *niveum*

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### ABSTRACT

Sugar contents in root exudates from different watermelon varieties (susceptible or resistant to *Fusarium oxysporum* f.sp. *niveum*) and their grafted seedlings (with bottle gourd or pumpkin as rootstock) were measured. Effects of sugars on the growth and development of *Fusarium oxysporum* f.sp. *niveum* were also evaluated in this experiment. Glucose, sucrose and total soluble sugar in root exudates of ungrafted resistant watermelon seedling were significantly lower than in ungrafted susceptible cultivar ( $P<0.05$ ); but there was no significant difference in sugar contents of bottle gourd and pumpkin rootstocks. Glucose, sucrose and total soluble sugar contents in root exudates of grafted susceptible cultivar were significantly lower than in ungrafted susceptible cultivar ( $P<0.05$ ). Resistant cultivar seedling grafted on pumpkin rootstock had higher glucose, sucrose and total soluble sugar contents compared with ungrafted watermelon seedling ( $P<0.05$ ). Total soluble sugar content in resistant cultivars grafted on bottle gourd was significantly higher than in ungrafted watermelon seedlings ( $P<0.05$ ). Glucose and sucrose promoted the growth and development of *Fusarium oxysporum* f.sp. *niveum*, and the stimulatory effect increased with sugar concentration. Our results suggested that the resistance of grafting and resistant cultivars may be due to the differences in sugar contents in root exudates.

**Key words:** Bottlegoured, *Fusarium oxysporum* f.sp. *niveum*, grafting, pumpkin, root exudates, root stock, sugars, watermelon.

### INTRODUCTION

Watermelon (*Citrullus lanatus* Thunb.) is cultivated crop in many countries. However, the watermelon Fusarium wilt (caused by *Fusarium oxysporum* f.sp. *niveum*) first found in North Carolina, USA, has become a worldwide soil-borne disease (8,11). It severely reduces the watermelon yield and quality, especially in continuously monocropped field (8,9). Many researches indicated that grafting of watermelon can enhance the resistance to Fusarium wilt and increase watermelon yield (3,6). Bottle gourd (*Lagenaria siceraria* Standl.) and pumpkin (*Cucurbita moschata* Duch) are resistant to *Fusarium oxysporum* f.sp. *niveum* and are usually used as grafting rootstocks in watermelon production.

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It is suggested that differences in biochemical characters between resistant and susceptible cultivars might contribute to the resistance of the crop, and these differences could be reflected in the allelopathic effects of root exudates (1,4,5). The root exudates composition of different crops is variable i.e. contained different types and quantities of sugar and the root exudates compositions from different varieties of same crop also differs (9, 11,17,19). Sugars contents in root exudates had been determined in cotton (10,20), cucumber (16), and wheat (23), but there were relatively few reports on the composition of watermelon root exudates, especially the sugar contents in root exudates.

In this study, root exudates from previously selected resistant and susceptible watermelon cultivars (11) and from their grafted seedlings were collected. The sugar content of root exudates from these ungrafted and ungrafted seedlings was analyzed. The effects of glucose and sucrose on the growth and development of *Fusarium oxysporum* f.sp. *niveum* were then investigated. This study aimed to clarify the relationship between the sugar contents of watermelon root exudates and *Fusarium oxysporum* f.sp. *niveum* resistance.

## MATERIALS AND METHODS

An isolate of *Fusarium oxysporum* f.sp. *niveum* was obtained from the Pathology Laboratory of Northeast Agricultural University. Watermelon cultivars used were: susceptible cultivars: ‘Tianshi’, resistant cultivar ‘Tianniu’ (Hefei Fengle Seed Company Limited) (11); rootstocks: bottle gourd ‘Tiezhenyihao’ (Longda Seed Company Limited, Changyi, Shandong) and pumpkin ‘Deruite’ (Tianjing Deruite Seed Company Limited). Detailed experimental treatments are shown in Table 1.

Table 1. Experimental treatments

Experimental treatments
A. Watermelon ‘Tianniu’ ungrafted seedling
B. Watermelon ‘Tianshi’ ungrafted seedling
C. Bottle gourd seedling
D. Pumpkin seedling
E. Bottle gourd (rootstock) + ‘Tianniu’ (Scion) grafted seedling
F. Bottle gourd (rootstock) + ‘Tianshi’ (Scion) grafted seedling
G. Pumpkin (rootstock) + ‘Tianniu’ (Scion) grafted seedling
H. Pumpkin (rootstock) + ‘Tianshi’ (Scion) grafted seedling

### Collection and treatment of root exudates

Root exudates were collected and treated as per Wang *et al.* (16). Seeds of every watermelon cultivar and rootstock were rinsed three times with sterile water and then stirred in water at 55°C for 15 min. Watermelon, bottle gourd and pumpkin seeds were soaked in water for 6,10 and 12 h at 30°C, respectively. After rinsing, these seeds were germinated in a growth cabinet [30°C, relative humidity: 75-85% and photoperiod: 16 h with a photosynthetic flux of 1000  $\mu\text{mol}/\text{m}^2\text{s}$ ]. When the radicles were 0.5 cm long, the seedlings were planted in sterile vermiculite in 50-well agricultural seeding trays. For each cultivar, 500 seedlings were planted.

The top wedge grafting method was adopted. One week after bottle gourd and pumpkin seedlings were planted, watermelon seeds were soaked for germination. When rootstock seedlings began to form true leaves and the cotyledons of the watermelon seedlings expanded, watermelon scions were grafted onto the bottle gourd and pumpkin rootstocks. Each treatment included an extra 300 plants to ensure an adequate survival rate of grafted plants. When the second true leaf of all seedlings developed, the seedlings trays were placed into cultivation channel (150 cm × 100 cm × 12 cm) containing 100 L sterilized water for hydroponic culture. The culture solution was collected 3-days later, then filtered thrice in a Buchner's filter and finally through a membrane (pore size 0.45 µm). Finally, 200 ml of each culture solution were condensed to 10 ml with a rotary evaporation, which were termed as the concentrated root exudates solution and were stored at -20°C until use.

#### **Analysis of sugar composition in root exudates**

The content of glucose and total soluble sugar for each treatment were analyzed with the spectrophotometric method, and the sucrose content was analyzed by the 4-(2-pyridylazo)-resorcinol photometric method as previously described (11,18).

#### **Spore germination**

The concave glass slide method was used to determine the spore germination (11,18). Five concentrations [0 (control), 5, 10, 15 and 20 g/L] of glucose or sucrose solutions were placed in the hollow and then the spore suspension ( $1 \times 10^7$  spore/ml) was added. After mixing, the glass slide was placed into a porcelain dish with a cover and incubated at 25°C for 12 h. Spore germination was determined by microscope. Every treatment had 6 replicates, and spore germination counts were made in 10 visual fields for every replicate.

#### **Mycelial growth and biomass**

Five concentrations [0 (control), 5, 10, 15 and 20 g/L] of glucose or sucrose solution were added to the improved Czapek medium ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.3 g,  $\text{KH}_2\text{PO}_4$  1.25 g, KCl 0.25 g,  $\text{FeSO}_4$  0.01 g,  $\text{KNO}_3$  1.0 g,  $\text{NaNO}_3$  0.5 g, Agarose 20.0 g, distiller water 1000 ml). *Fusarium oxysporum* spores suspension ( $1 \times 10^7$  spore/ml) of the same age was inoculated in each culture medium. The culture plates were placed upside down and incubated at  $25 \pm 1^\circ\text{C}$ . The mycelial length was measured with a ruler after 120 h. The mycelial biomass was determined by the colorimetric method (11,18) after 120 h.

#### **Statistical Analysis**

Data were processed in Microsoft Excel 2003. The significance of differences between treatments was tested by ANOVA using SAS 6.12 (SAS, Inc., Cary, USA).

## **RESULTS**

#### **Sugars contents in root exudates**

Glucose, sucrose and total soluble sugar contents in root exudates from the ungrafted resistant cultivar were significantly lower than in ungrafted susceptible cultivar

( $P<0.05$ ), but there were no significant differences between the two rootstocks (Table 2). Glucose, sucrose and total soluble sugar contents were significantly lower in root exudates of grafted susceptible cultivar than in ungrafted susceptible cultivar ( $P<0.05$ ). Whereas, resistant cultivar grafted on pumpkin rootstock had higher glucose, sucrose and total soluble sugar contents compared to ungrafted watermelon seedlings. Glucose and sucrose contents in the resistant cultivar grafted on bottle gourd rootstock were similar to ungrafted watermelon seedlings, but the total soluble sugar content was higher than ungrafted seedlings ( $P<0.01$ ).

Table 2 Concentration of sugars in watermelon root exudates of different treatments ( $\mu\text{g/plant}$ )

Treatment	Glucose	Sucrose	Soluble sugar
A	45.34 $\pm$ 0.16 fF	2.25 $\pm$ 0.14 dD	65.22 $\pm$ 0.12 fF
B	76.52 $\pm$ 0.40 aA	5.15 $\pm$ 0.12 aA	95.18 $\pm$ 0.06 aA
C	67.45 $\pm$ 0.11 cC	1.98 $\pm$ 0.06 eE	66.74 $\pm$ 0.09 eE
D	67.23 $\pm$ 0.08 cC	1.99 $\pm$ 0.09 eE	66.79 $\pm$ 0.11 eE
E	45.24 $\pm$ 0.13 fF	2.23 $\pm$ 0.04 dD	67.81 $\pm$ 0.12 dD
F	46.23 $\pm$ 0.11 eE	3.01 $\pm$ 0.05 cC	69.61 $\pm$ 0.15 cC
G	51.35 $\pm$ 0.32 dD	2.98 $\pm$ 0.04 cC	67.83 $\pm$ 0.13 dD
H	73.98 $\pm$ 0.11 bB	3.51 $\pm$ 0.04 bB	89.22 $\pm$ 0.13 bB

A to H denote the treatments. Values (mean  $\pm$  standard error) with different capital and small letters are significantly different at 0.01 and 0.05 probability level according to least significant difference (LSD) test, respectively.

Glucose, sucrose and total soluble sugar contents in root exudates of two rootstocks were not significantly different (Table 2). Glucose, sucrose and total soluble sugar contents in root exudates of susceptible watermelon cultivar grafted on bottle gourd rootstock were lower than in susceptible watermelon cultivar grafted on pumpkin ( $P<0.05$ ). Glucose and sucrose contents in root exudates from resistant watermelon cultivar grafted on bottle gourd rootstock were significantly lower than resistant watermelon cultivar grafted on pumpkin rootstock in resistant cultivars ( $P<0.05$ ), but the total soluble sugar was identical.

### Spore germination

The spore germination rate of *Fusarium oxysporum* was significantly stimulated by different concentrations of glucose or sucrose (Fig. 1). The glucose application was more stimulatory than sucrose. The spore germination rate increased as the concentration increased. At the highest concentration (20 g/L) of glucose and sucrose, the spore germination rate of *Fusarium oxysporum* was maximum and was 58.46% and 52.52%, respectively.

### Mycelial growth

Figure 2 showed the effects of root exudates from different cultivars on the mycelial length of *Fusarium oxysporum*. In each culture stage, the mycelial length of *Fusarium oxysporum* also increased with glucose or sucrose concentrations. There was significant difference between each treatment. There was positive relationship between the glucose and sucrose concentration in culture medium and the mycelial length.

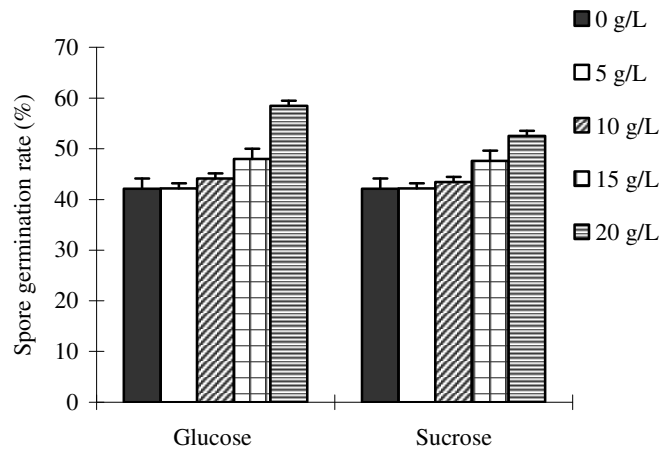


Figure 1. Effects of glucose and sucrose on spore germination rate of *Fusarium oxysporum* f.sp. *niveum*.

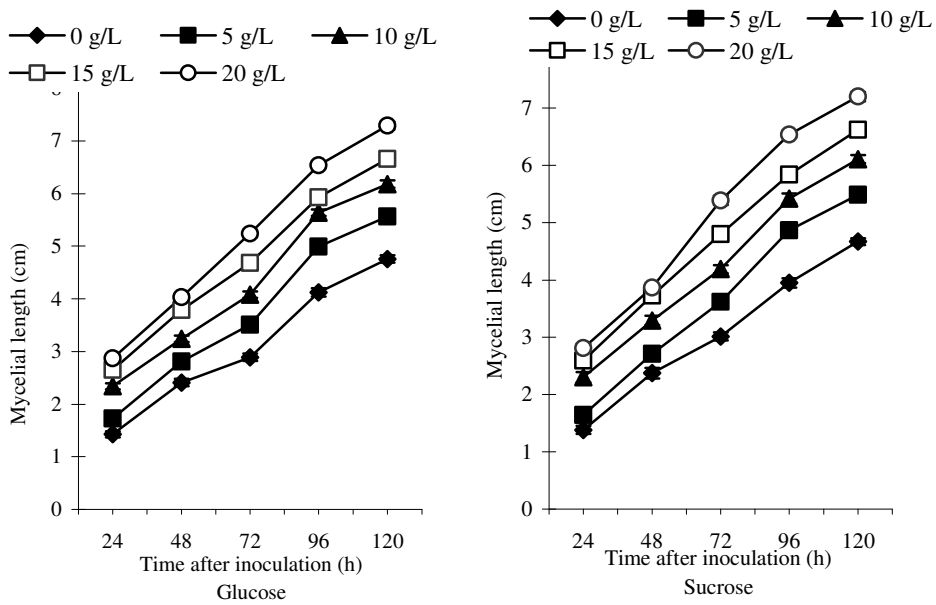


Figure 2. Effects of glucose and sucrose on mycelium length of *Fusarium oxysporum* f.sp. *niveum*

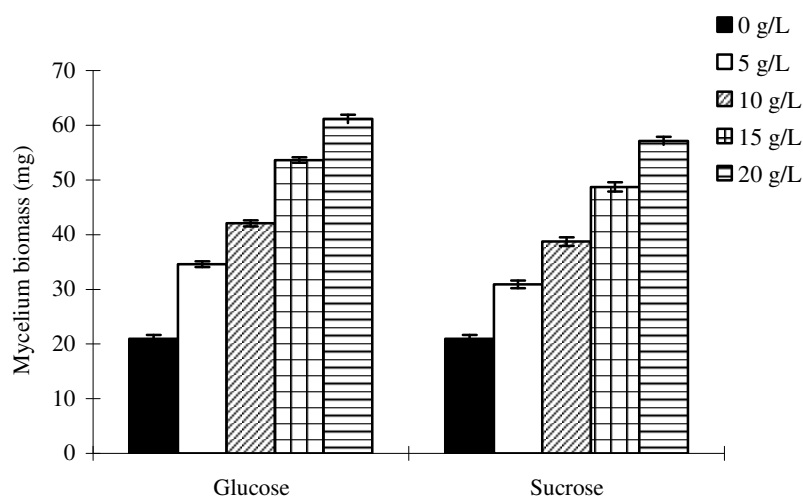


Figure 3. Effects of glucose and sucrose on mycelium biomass of *Fusarium oxysporum* f.sp. *niveum*.

### Mycelial biomass

At 72 and 120 h after treatment, *Fusarium oxysporum* mycelial biomass under different concentration of glucose or sucrose treatments was significantly higher than control (Fig. 3). Mycelial biomass at 5 g/L concentration of glucose or sucrose was significantly lower than at higher concentrations ( $P < 0.05$ ). At the highest concentration (20 g/L) of glucose or sucrose, the mycelial biomass was maximum i.e. 61.15 mg and 57.15 mg, respectively. These results indicated that application of sugar stimulated the growth of *Fusarium oxysporum* and thus leading to its increased biomass. There was a positive relationship between the glucose and sucrose concentration in culture medium and the mycelial biomass.

## DISCUSSION

The root exudates are correlated to plant resistance to soil-borne diseases (1,4). Among organic carbons in root exudates, sugars are the most easily metabolized one. Sugars could supply nutrition for the growth and multiplication of microorganisms (including pathogens) in the rhizosphere (21). The root exudates of resistant cultivars may supply less nutrition to pathogen (14). This study indicated that root exudates of watermelon cultivars with difference in resistance to *Fusarium oxysporum* have the same types of sugars, but their quantities were different. The contents of glucose, sucrose and total soluble sugar in resistant cultivar were significantly lower than in susceptible cultivar. Previous studies found that root exudates of cucumber cultivars, resistant or susceptible to *Fusarium oxysporum*, had same types of sugars (glucose, sucrose) (16). The contents of glucose and sucrose were similar in two kinds of cultivars, but total sugar content of resistant cultivar was significantly lower than in susceptible cultivar. The sucrose and

soluble sugar contents of different cotton cultivars resistant to *Fusarium oxysporum* or *Verticillium* are not different, but the total sugar contents in resistant cultivars were significantly lower than in susceptible cultivars (12,22). Our results were consistent with these studies.

The soluble sugar contents in the root exudates were similar in two ungrafted rootstocks, but were different between the grafted root stocks. In susceptible cultivar, the glucose, sucrose and total soluble sugar contents were significantly lower in grafted cultivar than in ungrafted watermelon seedlings. In resistant cultivar, the glucose, sucrose and total soluble sugar contents were significantly higher in bottle gourd grafted seedlings than that in ungrafted watermelon seedlings. There was no significant difference between the resistant cultivars grafted on pumpkin rootstock and ungrafted seedlings. These results were contrary to that of Zhou *et al.* (24), who found that sugars in eggplant root exudates changed after grafting, the content of sugars in more resistant cultivars was lower than in ungrafted seedlings. Our results indicated that there was a positive relationship between the sugar content of root exudates and the resistance to *Fusarium oxysporum*. The possible reasons need to be further studied in the future.

The relationship between root exudates and plant resistance mainly depended on the effects of root exudates on rhizosphere microorganisms (2,4). The present study analyzed the effects of sugars on the growth and development of *Fusarium oxysporum*. Glucose and sucrose promoted the growth and development of *Fusarium oxysporum* and their promotion effect increased with the concentration. There was a negative relationship between the sugar concentration in root exudates and the resistance. This was different from results of Han *et al.* (7), who found that there was a positive relationship between the sugar concentration in root soybean exudates and the resistance to root rot pathogen. Our study was consistent with the study of Wu *et al.* (20), who showed that there was a significantly positive relationship between sugar content in root exudates of cotton cultivars and the incidence of *Verticillium dahlia*. It could be speculated that higher sugar content might supply more nutrition for spore germination and mycelial growth of soil-borne pathogens.

The resistance of watermelon to Fusarium wilt could be improved through regulating the sugar composition of root exudates in breeding programmes. The mechanism of plant resistance to pathogenic disease is complex (13,15). To reveal the mechanism of resistance to watermelon Fusarium wilt, amino acids, phenols, vitamins, growth regulators and other factors in watermelon root exudates should also be analyzed in future.

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