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# Alteration of physiological parameters of milkvetch (*Astragalus adsurgens*) by the pathogen *Embellisia astragali*

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**Keywords:** Milk vetch, disease resistance, photosynthesis, stomatal conductance, water use efficiency.

## Introduction

Milkvetch (*Astragalus adsurgens*) is sown on >1 M ha in China as a forage legume and to control soil and water erosion. It is drought-tolerant and has high herbage yield, wide adaptability and medium forage quality. However, fungal disease is a major factor affecting its persistence and productivity. *Embellisia astragali* is a highly virulent seed-borne pathogen which commonly causes stunt and root rot. The objective of this study was to characterize the impact of *Embellisia astragali* on the physiological status of milkvetch by monitoring changes through time in net photosynthesis, stomatal conductance and other parameters of milkvetch inoculated with the pathogen and to compare the data between resistant and susceptible varieties to identify the disease resistance physiological mechanism.

## Materials and Methods

The disease resistance of two milkvetch varieties, Shanxi (a resistant variety) and Neimeng (a susceptible variety) was evaluated. One isolate of *E. astragali* (MHLZU-HX0401) from the infected stems of milkvetch growing in Huan County, Gansu province, China was used throughout the study.

### *Stomatal conductance (Gs), photosynthesis (A) and water use efficiency (WUE)*

To study stomatal conductance (Gs), photosynthesis (A) and water use efficiency (WUE) of milkvetch to *E. astragali*, 40 seedlings (2 years old) were inoculated in the stem with a 5-mm-diameter hyphal plug cut from a 4-week-old *E. astragali* culture growing on WHDA. 40 plants designated as controls were inoculated with a 5-mm-diameter plug of sterile WHDA.

Stomatal conductance (Gs, mol/m<sup>2</sup>/s) and net photosynthetic rate (A, CO<sub>2</sub> assimilation, μmol/m<sup>2</sup>/s) were monitored weekly, 1–4 weeks after inoculation, with a LICOR 6400 portable photosynthesis system. Duplicate measurements were made on an area basis in 10 milkvetch plants per time and treatment. At the same times, photosynthetic photon flux density (PPFD, μmol/m<sup>2</sup>/s) was measured with the radiation sensor provided with the same equipment. Values of net photosynthesis and stomatal conductance were recorded at PPFD > 300 μmol μmol/m<sup>2</sup>/s. WUE was estimated as the ratio between A and Gs when

PPFD was higher than 500 μmol μmol/m<sup>2</sup>/s (light saturation). Comparisons between inoculated and control plants for each period of time were performed using t-tests.

Whole leaves and stem portions of non-inoculated plants were used to study the effect of the culture filtrate (CF) of *E. astragali* on membrane permeability.

To obtain the CF, ten tubes containing 10 ml fluid nutrient medium (wheat hay decoction) and one 5-mm-diameter *E. astragali* hyphal plug were prepared per isolate. After the incubation, the cultures were filtered through a sterile 0.2 μm membrane. Equal parts of CF from each isolate were then combined. The leaves and stems of ten plants were used in the assay; control and CF-treated samples were obtained from each plant. 100 mg leaves and 25 mg stems of each plant were placed in sterile tubes containing 3 ml CF or for the control 3 ml sterile liquid medium. Conductivity and pH were measured in each tube before (zero time point) and immediately after the addition of the leaves and stems (initial level). Tubes were incubated for 72 h at 22°C, with 16 h light/8 h dark, then put for 30 min on a shaker set at 150 strokes per min. After resting the tubes for 5 h, conductance (in μS) and pH of the solution were determined with a conductivity meter and pH meter. The increase in electrolyte leakage and change in pH induced by the CF was determined. All tests were conducted in three replications.

## Results

### *Stomatal conductance (Gs), photosynthesis (A) and water use efficiency (WUE)*

Stem inoculations of *E. astragali* were successful in 89% of the cases judged by reisolation. Lesions reached an average length of 20.8±4.3 mm at the end of the 4-week experiment. Net photosynthesis (A) was the first parameter affected by the inoculation. By the first week it was reduced by 18.9% and 8.9% of Neimeng and Shanxi, respectively. The declines continued in the following weeks, reaching 65.9% and 31.4% reduction in the fourth week of Neimeng and Shanxi, respectively (Fig. 1a, b;  $P < 0.05$  vs. control).

Stomatal conductance (Gs) decreased by 22.2% and 10.3% relative to control values in the second week of Neimeng and Shanxi, respectively. The declines continued in the following weeks, reaching 33.9% and 19.7% reduction in the fourth week of Neimeng and Shanxi,

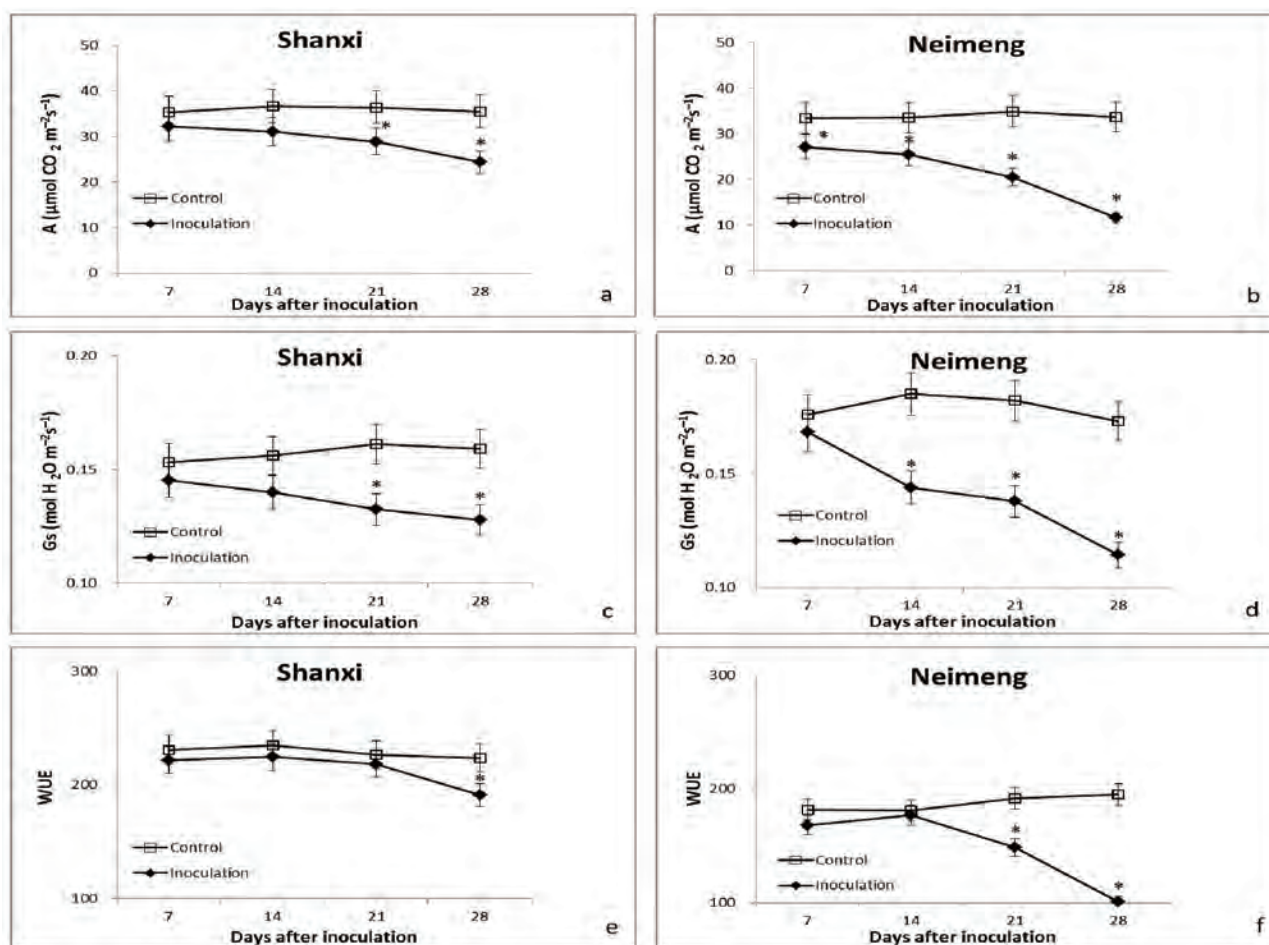


Figure 1. (a, b) Weekly measurements of net photosynthetic rate (A);(c, d) stomatal conductance (Gs); and (e, f) water use efficiency (WUE). Each point represents the mean  $\pm$  standard error. \* $P < 0.05$  vs. control

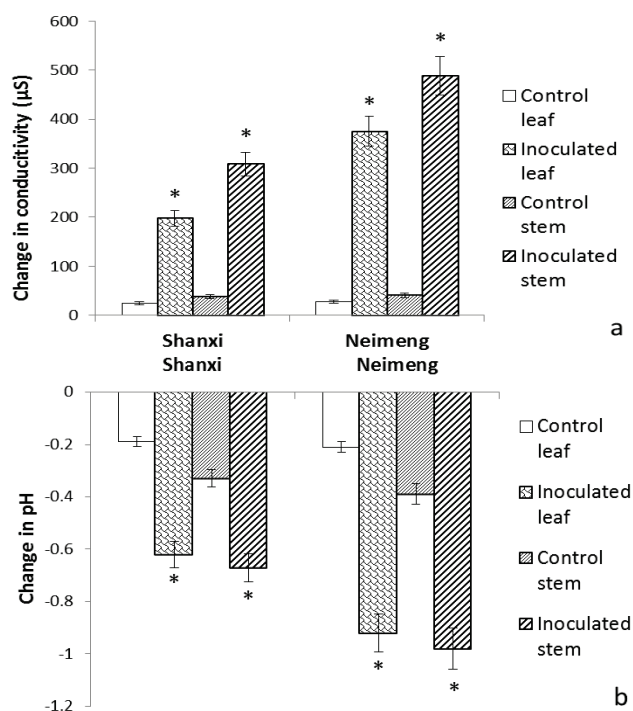


Figure 2. (a) Extracellular change in electrical conductivity; (b) extracellular change in pH, as described in materials and methods. Each bar represents the mean  $\pm$  standard error. \* $P < 0.05$  vs. control

respectively (Fig. 1c,d;  $P < 0.05$  vs. control).

No alteration in WUE was observed during the first and second week. From the third week WUE diminished, reaching 48.3% and 14.6% of the control level in the fourth week of Neimeng and Shanxi, respectively (Fig. 1e, f;  $P < 0.05$  vs. control).

Physiological changes appeared prior to the development of visible symptoms in the leaves, which were evident as foliage wilt after the third week.

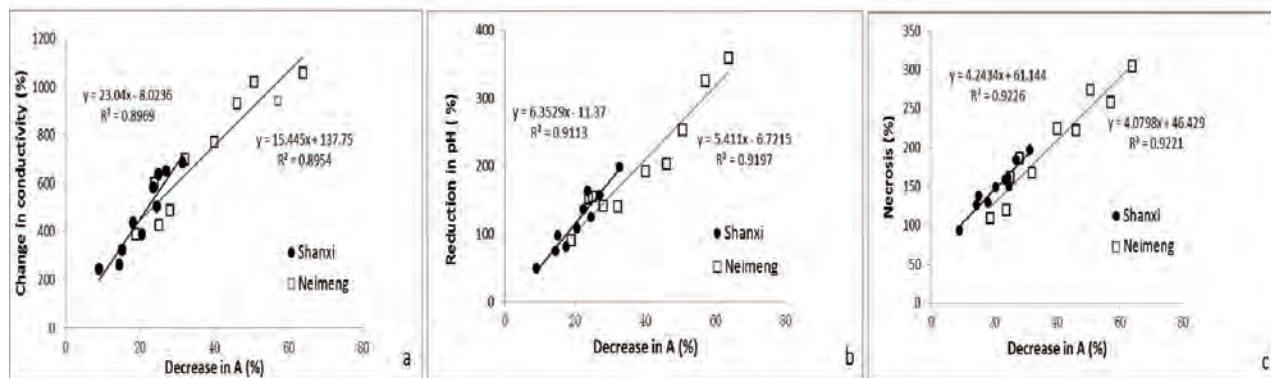
#### Toxic effect of *E. astragali* CF

Culture filtrate of *E. astragali* induced an increase in electrolytes liberated by the tissues, determined by an increase in conductivity of the solution (Fig. 2a;  $P < 0.05$  vs. control). Similarly, an alteration of  $H^+$  concentration regulation was evident by the decrease in external pH (Fig. 2b;  $P < 0.05$  vs. control).

The decline in net photosynthesis was strongly and positively correlated to the increase in electrical conductivity (Fig. 3a;  $R = 0.895$  and  $0.897$  of Neimeng and Shanxi, respectively,  $P < 0.001$ ), the decrease in pH (Fig. 3b;  $R = 0.920$  and  $0.911$  of Neimeng and Shanxi, respectively,  $P < 0.001$ ) and the increase in necrosis (Figure 3c;  $R = 0.922$  and  $0.923$  of Neimeng and Shanxi, respectively,  $P < 0.001$ ).

#### Discussion

Inoculation of milkvetch with *E. astragali* led to significant



**Figure 3. Relationship between the relative decrease in net photosynthetic rate (A; expressed as percentage of reduction) and (a) extracellular reduction of conductivity (expressed as percentage of reduction); (b) relative extracellular reduction of pH (expressed as percentage of reduction); and (c) percentage of necrosis**

declines in net photosynthesis, stomatal conductance and stem-specific hydraulic conductivity. The mechanism of action of the pathogen may involve a reduction in photosynthesis without significantly altering another physiological parameter, when the necrotic lesion in the stem is incipient. At a later stage, when the stem is extensively affected, down-regulation of all physiological functions may occur as a result of disruption of water transport. In this circumstance, a reduction of photo-

synthetic products and a drastic decrease in water supply may cause root necrosis and water stress, which may explain, at least in part, the functional decline.

### Conclusion

The mechanisms of the changes of these physiological parameters and the differences between susceptible and resistant varieties will be studied in the future.