1.5 Theses of Graduate Courses

(1) Doctoral Theses

Sonobe, K.

Physiological mechanisms related to silicon-induced improvement of water uptake in sorghum seedlings under water stress

Summary:

Silicon application can alleviate both biotic and abiotic stresses, including water stress. It has been reported that silicon application enhanced drought tolerance of sorghum seedlings [*Sorghum bicolor* (L.) Moench]. This enhancement was ascribed to higher photosynthetic rate and stomatal conductance due to improvement of leaf water status under water stress. In the most past studies, water relation characteristics related to this effect of silicon were measured only at midday after a specific period of water stress. However, these characteristics closely interact, and vary both diurnally and over the course of an extended period of the stress. Therefore, it is important to consider the dynamics of the changes in the characteristics for better understanding of the silicon-induced improvement of leaf water status under water stress. The objective of this study was to determine the effect of silicon application on dynamics of the changes in water relation characteristics related silicon-induced improvement of leaf water status, and elucidate the relevant physiological mechanisms. To meet these objectives, two experiments were conducted.

1) Diurnal variations in photosynthesis, stomatal conductance and leaf water relations in sorghum grown with or without silicon, under water stress.

Sorghum seedlings (cv. Gadambalia) were grown hydroponically in two different silicon concentrations (0 and 1.78 mM) and two levels of water stress (with and without polyethylene glycol 6000). Water stress was imposed to the seedlings from 10 days after sowing (DAS) with increasing its level in response to the seedling growth. Water stress reduced dry weights of the seedlings at 15 DAS. The reduction in dry weight became more pronounced at 23 DAS, but this was ameliorated by silicon. Similar effects of silicon application were also observed in photosynthetic rate and stomatal conductance; the silicon-induced reduction of these physiological traits was ameliorated by silicon application. Silicon-applied seedlings showed higher photosynthetic rate and stomatal conductance throughout the daytime compared with the seedlings grown without silicon under water stress. However, leaf water potential changed little throughout the daytime, and was little influenced by silicon application. In the relationship between stomatal conductance and leaf water potential under water stress, leaf water potential was maintained by closure of stomata in seedlings grown without silicon and by opening of stomata in silicon-applied seedlings. The reduction of water uptake was also ameliorated by silicon application under water stress. These results suggested that the application of silicon ensured provision of water to the leaf by enhancing water uptake rate under water stress. These effects of silicon occurred soon after exposure to water stress.

2) Effect of silicon application on sorghum root responses to water stress

Sorghum seedlings were grown under similar growth conditions and using the same treatments described above. To analyze how silicon improves water uptake rate under water stress, we investigated the root responses of sorghum seedlings to silicon application. The reduction in dry weight due to stress was alleviated by silicon application, accompanied by an increase in root water uptake. Silicon application decreased the osmotic potential of the roots without affecting their water content. These results showed that silicon application positively induced osmotic adjustment in sorghum roots. This silicon-induced root osmotic adjustment could be linked to the increase water uptake under the water stressed condition. From an assessment of root solutes, the osmolytes responsible for this osmotic adjustment were soluble sugar and amino acids (alanine and glutamic

acid), not minerals such as potassium. Root anatomical traits such as the diameter and number of the xylem vessels, which are related to water transport, were not affected by the silicon application. These results suggested that the effect of silicon application on water uptake was ascribed to silicon-induced root osmotic adjustment by soluble sugar and several amino acids (alanine and glutamic acid).

In this thesis, it was demonstrated that silicon application increased stomatal conductance through the alleviation of reduction in water uptake rate throughout the daytime under water stress. This ameliorative effect of silicon application on water uptake was ascribed to root osmotic adjustment by soluble sugar and several amino acids (alanine and glutamic acid).

Yin, L.

Improvement of aluminum tolerance through scavenging reactive oxygen species and lipid peroxide-derived aldehydes

Summary:

Aluminum (Al) toxicity is a major factor limiting plant growth and productivity in acid soils. Al ions inhibit plant growth partly by causing oxidative damage that is promoted by reactive oxygen species (ROS) and can be prevented by improving antioxidant capacity. Ascorbic acid (AsA) and glutathione (GSH) are the major antioxidants in plants, which are regenerated by the action of monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). However, the functions of DHAR, MDAR and GR in Al tolerance have not been characterized. In the present study, I investigated the role of MDAR, DHAR and GR in AsA and GSH regeneration during Al stress using transgenic tobacco (*Nicotiana tabacum*) plants overexpressing AtMDAR (MDAR-OX) or AtDHAR (DHAR-OX), and transgenic Arabidopsis (*Arabidopsis thaliana*) overexpressing AtGR (GR-OE), and the wild-type tobacco SR-1 and Arabidopsis Columbia (Col) plants were used as control plants.

DHAR-OX plants showed rapid root growth than wild-type (SR-1) plants after exposure to $AlCl_3$ for 14 d on agar plate, but MDAR-OX plants did not. There was no difference in Al distribution and accumulation in the root tips among SR-1, DHAR-OX and MDAR-OX plants after treatment with 500 μM $AlCl_3$ for 24 h in hydroponic medium. However, DHAR-OX plants showed lower hydrogen peroxide content, less lipid peroxidation and lower level of oxidative DNA damage than wild-type SR-1 plants, whereas MDAR-OX plants showed the same extent of damage as SR-1 plants. Compared with SR-1 plants, DHAR-OX plants consistently maintained a higher AsA level both with and without Al exposure, while MDAR-OX plants maintained a higher AsA level only without Al exposure. Also, DHAR-OX plants maintained higher ascorbate peroxidase (APX) activity under Al stress. The higher AsA level and APX activity in DHAR-OX plants contributed to their higher antioxidant capacity and higher tolerance to Al stress. These findings show that the overexpression of DHAR, but not of MDAR, confers Al tolerance, and that maintenance of a high AsA level is important to Al tolerance.

Arabidopsis plants overexpression GR also showed Al tolerance as compared with wild-type Col plants. Under Al stress, GR transgenic plants exhibited better root elongation, lower hydrogen peroxide content and less lipid peroxidation compared to wild-type plants. Although no difference in Al accumulation and the activities of superoxide disumutase (SOD), catalase (CAT) and dehydroascorbate reductase (DHAR) were observed in roots of transgenic and wild-type plants after 24-h Al treatment, GR transgenic plants showed higher activities of GR and ascorbate peroxidase (APX), and higher levels of GSH and AsA than wild-type plants. Thus, overexpression of GR did not affect Al accumulation and the activities of other antioxidant enzymes. This results demonstrate

that overexpression of GR improves the antioxidant capacity of Arabidopsis through increasing GSH and AsA level in the cell, leads to suppression of H_2O_2 generation and lipid peroxidation, and results in enhanced tolerance to Al stress.

Lipid peroxidation, in the downstream of ROS, is a common symptom of Al toxicity, and it increases with increasing Al concentration. From animal cell studies, it is now recognized that the toxicity of lipid peroxide (LOOH) is largely ascribed to LOOH-derived aldehydes. In plants, a close correlation between the level of LOOH-derived aldehydes (determined as thiobarbituric acid-reactive substances (TBARS)) and cellular damage has been shown in environmental stresses caused by heat, chilling, UV-B radiation, salinity, heavy metals and Al. Thus, it is possible that LOOH-derived aldehydes are involved in Al toxicity. In this study, I verified the hypothesis that LOOH-derived aldehydes, especially highly electrophilic *α,β*–unsaturated aldehydes (2-alkenals), participate in Al toxicity. Transgenic tobaccos overexpressiong *Arabidopsis thaliana* 2-alkenal reductase (AER-OE plants), wild-type SR-1, and an empty vector-transformed control line (SR-Vec) were exposed to Al on their roots. Compared with the two control plants, AER-OE plants suffered less retardation of root elongation under Al treatment and showed rapid regrowth upon Al removal. Under Al treatment, the roots of AER-OE plants accumulated Al and hydrogen peroxide (H_2O_2) to the same levels as did the sensitive controls, while they accumulated lower level of aldehydes and suffered less cell death than SR1 and SR-Vec roots. In SR1 roots, Al treatment markedly increased the contents of the highly reactive 2-alkenals acrolein, 4-hydroxy-(*E*)-2-hexenal, and 4-hydroxy-(*E*)-2-nonenal and other aldehydes such as malondialdehyde and formaldehyde. In AER-OE roots, accumulation of these aldehydes was significantly less. Growth of the roots exposed to 4-hydroxy-(*E*)-2-hexenal and (E)-2-hexenal were retarded more in SR1 than in AER-OE plants. Thus, the lipid peroxide-derived aldehydes, formed in the downstream of ROS, injured root cells directly. Their suppression by AER provides a new defense mechanism against Al toxicity.

This study indicates that Al toxicity induced irreversible oxidative damage in tobacco and Arabidopsis. Plants with overexpressed antioxidant enzyme genes DHAR and GR showed enhanced Al tolerance in tobacco and Arabidopsis. However, MDAR showed no protective effect on improving Al tolerance in tobacco. Both DHAR-OX and GR-OE plants showed increased AsA level and APX activity in their roots as compared with wild-type plants, indicating AsA and APX play a paramount role in Al tolerance. Furthermore, tobacco plants with overexpressed AER gene showed improving tolerance to Al. AER-OE plants accumulated less LOOH-derived aldehydes, especially 2-alkenals, than that in wild-type plants, indicating the LOOH-derived aldehydes are the cause of Al-induced injury, and enhanced aldehydes scavenging capacity could alleviate Al toxicity. Taken together, oxidative injuries caused both by reactive oxygen species and LOOH-derived aldehydes, are the important causes of Al toxicity. Our study provide a new mechanism for understanding Al toxicity in plants, meanwhile, new strategies for breeding Al tolerant plants are suggested. This will benefit improving plant productivity on acid soils in the world.

Zhang, Q. T.

Study on evaluation of mulching effect and establishment of irrigation threshold for water-saving production

This study was carried out to evaluate mulching effect for sustainable agriculture and establish irrigation threshold (based on a new photogrammetry system) for water-saving production.

Water-saving is important since freshwater resources have been over-exploited in many areas. Therefore a pot experiment was conducted to evaluate the effects of three mulching types (gravel, pine-needles and rice-straw with 3 cm thickness) together with diluted seawater irrigation during winter season. Seawater was diluted to achieve the electrical conductivity of irrigated water as 4.8

and 7.4 dS m^{-1}. High diluted seawater irrigation could be used under mulch condition without serious salinity-damage to Swiss chard. Gravel mulching enhanced soil temperature, biomass and water use efficiency (WUE) under saline irrigation.

From November to June, the effect of gravel mulch (G) and rice-straw mulch (R) (3 cm thickness) on Swiss chard were investigated. Three weighing lysimeters were irrigated with diluted seawater (6.9 dS m^{-1}) from below. The cumulative ET and soil salinity were higher with no-mulch than mulches. The dry yield was 113% higher under R and 64% higher under G than under no-mulch. R increased WUE by 143% and 10% as compared to no-mulch and G, respectively. Thus mulching using R is recommended for reducing salinity under shallow water table of saline water and improving WUE.

The grapevine plays an important role in enhancing income in drylands with the problems of water scarcity and improper irrigation, hence water-saving production is on urgent business. Based on the results of above-mentioned experiment, effects of rice-straw mulching and sub-surface seepage irrigation (at 15 cm soil depth) on growth of grapevines (*Vitis vinifera* L.) in weighing lysimeters were investigated from June to September. Mulching combined with surface irrigation gave the highest berry size, fresh yield and WUE, while mulching combined with sub-surface irrigation gave the highest sugar content.

It is also essential to determine the critical timing for starting irrigation in drylands. When the berries were in the last phase of Stage 1, a potted grapevine was placed in an environmentally controlled growth chamber. Berry diameter was monitored by a new photogrammetry-system that consists of two digital cameras attached to a computer having an image analysis program. Soil water potential was measured by a pressure transducer system for tensiometers. Berry diameter increased rapidly after irrigation till ψ became –5.4 kPa, beyond which the berry started shrinking and the shrinkage showed a strong linear relationship with decreasing ψ. Berry diameter was a more sensitive indicator of moisture stress than photosynthesis and –5.4 kPa should be considered as the threshold ψ for scheduling irrigation in this growth stage.

In the similar way, when the berries were in the ripening stage (Stage 3), leaf photosynthesis and stomatal conductance were monitored from a potted grapevine in a growth chamber. When soil water potential (at 10 cm soil depth) decreased from -13.2 kPa to -14.7 kPa, photosynthesis, stomatal conductance and transpiration decreased rapidly and did not recovery thereafter. Afterwards the berry shrunk significantly as soil water potential became -16.2 kPa. Thus, photosynthesis was more sensitive to water stress than berry size during the ripening stage.

In conclusion, rice-straw mulch was a good option in several mulching materials for preventing soil salinity and improving WUE. Furthermore, the rice-straw mulch combined with seepage irrigation could influence yield and quality of grapevines. A new photogrammetry system is suitable for measuring berry diameter for irrigation timing scheduling, therefore this effective method could be used to get cost-effective use of water resources, especially for fruit trees under stressful conditions. The valuable information provided here will benefit the sustainable agriculture, especially in arid and semi-arid areas.

(2) Master's Theses

Division of Afforestation and Land Conservation

Kidou, A.

Percolation control layer for water-saving vegetable cultivation in sandy soil

Fujii, S.

Interception of capillary rise to prevent salt accumulation

(3) Graduation Theses

Division of Afforestation and Land Conservation

Okano, A.

Development of new fruit/vegetable cultivation under controlled constant water-level in limited root zone