# Physiological basis of drought tolerance in potato grown under long-term water deficiency

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## Abstract

Coping with water shortages without compromising tuber yield is a major challenge for potato (*Solanum tuberosum* L.) production in northern China. In this study, we used three potato cultivars with different sensitivities to drought to evaluate the effect of long-term drought stress on morphological and physiological characteristics under field conditions during three growing seasons (2009-2011). Our aim was to identify the specific drought tolerance traits of the potato cultivars to enable their cultivation in arid and semi-arid regions. Our results show that the drought-tolerant cultivars had higher tuber yields under conditions of drought due an increased number of tubers per hill and increased individual tuber weight. Compared with the drought-sensitive cultivar Neishu no. 7, the drought-tolerant cultivars Kexin no. 1 and Konyu no. 3 had stronger root systems and a higher capacity for water absorption at later developmental stages and a higher leaf relative water content, which conferred enhanced water retention under drought conditions. Moreover, the levels of superoxide radical and hydrogen peroxide were lower and the activities of antioxidative enzymes, including superoxide dismutase, peroxidase and catalase, were higher in the drought-tolerant cultivars under drought stress conditions. These combined characteristics allowed the cultivars to exhibit different levels of water deficiency. The above agro-morphological and physiological parameters could be used as a basis for breeding potato varieties with enhanced drought tolerance.

*Keywords:* Potato; Drought; Physiological response; Reactive oxygen species.

***Running title:*** Tolerance of potato to long-term drought

## Introduction

As the fourth most important food crop in the world, *Solanum tuberosum* L. (potato) plays an increasingly essential role in ensuring food safety worldwide (Vreugdenhil, 2007). However, water deficiency threatens potato production due to the massive water requirement of potato cultivation and the sensitivity of potato to water availability during the growing season. Previous studies have shown that the responses of potato to drought vary among varieties and some drought-resistant potato cultivars produce reasonable yields under conditions where grain crops fail, particularly when drought coincides with flowering and seed set (Iwama and Yamaguchi, 2006). Potato is often grown under water-limited conditions; thus, understanding drought tolerance or adaption in potato is important for its production.

Adaptation to drought may involve several different morphological and physiological characteristics, the relative importance of which may vary with plant genotype and growth stage. Stem height, number of green leaves and leaf length are considered to be the parameters most sensitive to moderate drought conditions (Deblonde and Ledent, 2001). The depth to which potato roots penetrate the soil is directly related to water absorption and closely related to drought tolerance (Lahlou and Ledent, 2005). Drought tolerance has also been associated with the control of growth and carbon transfer under water stress (Tourneux et al., 2003), enhanced water use efficiency (Alva et al., 2012) and osmotic adjustment (Heuer and Nadler, 1998).

Drought stress also leads to the increased accumulation of reactive oxygen species (ROS) in plants, including superoxide radical ($O_2^-$) and hydrogen peroxide ($H_2O_2$). Overproduction of ROS can disrupt normal plant metabolism through impaired enzyme activity due to oxidative damage, protein degradation, DNA and RNA damage and membrane lipid peroxidation, which can ultimately culminate in cell death (Finkel and Holbrook, 2000). To counteract ROS, plants produce various types of antioxidants. The activation of antioxidants in response to oxidative stress induced by drought is associated with the degree of drought stress tolerance of various plant species (Sunkar et al., 2006). Partial root zone drying has been shown to enhance antioxidant activity in potato tubers (Jovanovic et al., 2010).

The objective of this study was to investigate the effects of long-term drought stress on potato morphological and physiological parameters and to explore specific parameters related to drought tolerance, including total root growth, leaf relative water content (RWC) and enzymatic antioxidant capacity as represented by the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in the leaves of potato cultivars exhibiting different levels of drought resistance. Our results may provide guidance for the breeding of drought-tolerant potato varieties and for improving potato cultivation in arid and semi-arid areas.

## Materials and Methods

### Plant materials

Potato cultivars exhibiting different levels of drought resistance were grown in a field and supplied with an optimal amount of water by irrigation (control) or subjected to drought by withholding irrigation (drought treatment). Two drought-tolerant potato cultivars (Konyu no. 3 and Kexin no. 1) and one drought-sensitive potato cultivar (Neishu no. 7) were used. Konyu no. 3 was provided by Dr. Iwama Kazuto of the Research Faculty of Agriculture (Iwama, 2008), Hokkaido University (Sapporo, Japan). The other two cultivars were provided by the Research Institute of Potato Propagation (Hohhot, China).

### Experimental design

All experiments were conducted at the Experimental Station of Inner Mongolia Agricultural University (Hohhot, China) using a randomized complete block design with four replications during the three growing seasons of 2009-2011. The plots were fertilized with 100:160:120 kg ha$^{-1}$ of nitrogen:phosphate:potassium before planting and with 100 kg ha$^{-1}$ of nitrogen 40 days after planting. Weeds, insects and diseases were controlled according to standard practices and local prevention and care recommendations. The row spacing was 80 cm, the seed spacing was 30 cm and the planting depth was 10 cm.

Three field plots (5×20 m) were prepared with roofs made of transparent plastic to shield the plots from rain. The soil water potential ($\Psi_s$) was measured once per week at four depths (20, 30, 50 and 100 cm below the

hill surface) using a WP4 Dewpoint Potentiometer (Decagon Devices, Inc., Pullman, WA, USA).

Water was supplied with subsurface drip irrigation systems. In each experiment, the control plants were watered throughout the growing season to maintain optimal soil moisture conditions. For the drought stress treatments, soil water was first depleted until wilting symptoms appeared, with the symptoms being apparent in the morning under good weather conditions (the $\Psi_s$ values in the 30-cm-deep root zone were approximately-0.9 MPa based on our previous experiments). Potato plants are susceptible to $\Psi_s$ values less than-0.05 MPa in the plow layer (Epstein and Grant, 1973; Mackerron and Jefferies, 1986), indicating that the plants in the drought treatment plots in this study were subjected to water deficiency stress. Beginning with the commencement of irrigation, the drought stress plots received approximately half as much water as the control plots based on the measured $\Psi_s$. As a result, the plant water status fluctuated continuously throughout the experimental period, similar to the natural variation in rainfall (Figure 1).
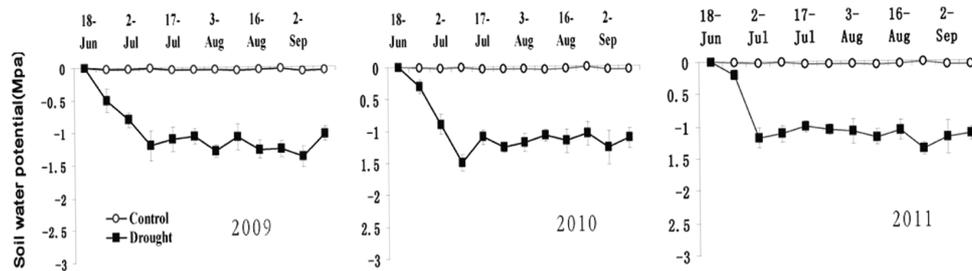


Figure 1. Soil water potentials to a depth of 30 cm during the potato growing season in 2009, 2010 and 2011. Vertical bars represent values ± the standard error of the mean (n=6).

*Measurements*

*Tuber yield*

For each treatment, the entire plot was harvested and the tuber yield (kg m$^{-2}$) was calculated based on the fresh weight. The number of tubers per plant was also recorded for each treatment. Tuber yield and yield traits were measured at harvest each year, while other physiological parameters were measured only in 2011.

*Total root length (TRL)*

The TRL per plant (cm) was determined using an image analysis system (WinRHIZO; Regent Instruments Inc., Québec, Canada) as described by Gopal and Iwama (2007).

*Leaf RWC*

The leaf RWC per plant was estimated gravimetrically according to the method of Tambussi et al. (2005).

*Antioxidant enzyme activities*

Six terminal leaflets, each from different fully expanded intact leaves at the third leaf node from the apex, were collected for biochemical analyses from stressed and control plants after 7 days of stress treatment (Fidalgo et al., 2004). The leaf samples, with three replicates each, were analyzed immediately for SOD (EC1.15.1.1) activity using the spectrophotometric method described by Santos et al. (1999), CAT (EC 1.11.1.6) activity using the method described by Kumar and Knowles (1993) and POD (EC 1.11.1.7) activity using the method of Fu and Huang (2001). The protein contents of the extracts were measured using the method of Sedmak and Grossberg (1977) with bovine serum albumin serving as a standard.

*Determination of leaf ROS production*

The production of $O_2^-$ was measured as described by Able et al. (1998) The $H_2O_2$ content was measured by monitoring the absorbance of titanium-peroxide complexes at 415 nm according to the method of Brennan and Frenkel (1977).

*Statistical analysis*

Each data point represents the mean of twelve replications. Comparisons with *P* values < 0.05 were considered to be significantly different. In all figures, the variation in values is indicated by error bars, which represent standard errors of the means. All calculations were made using SPSS (version 20.0 for Windows; IBM Corp., Armonk, NY, USA) and Excel 2010 (Microsoft Corp., Redmond, WA, USA) software.

## Results

### Potato tuber yield

Table 1 shows the calculated *F* values for the differences in tuber yield and yield traits of potato plants between and among years, cultivars and irrigation treatments. Significant differences existed among the cultivars and between irrigation treatments. The differences in data across years and in interactions between year and cultivar, year and irrigation treatment and cultivar and irrigation treatment were not significant. The average yields over the three years were 2031.5 kg, 1938.6 kg and 1713.5 kg for Neishu no. 7, Kexin no. 1 and Konyu no. 3, respectively, under full irrigation (Figure 2).

Drought caused a significant decline in yield from all three cultivars relative to the controls. The yields from Konyu no. 3, Kexin no. 1 and Neishu no. 7 decreased approximately 37%, 42% and 64%, respectively, in response to drought stress treatment. The decreased yield from Neishu no. 7 was caused by a decreased number of tubers per plant and a decreased average weight of individual tubers, both of which were significantly less than those of Kexin no. 1 and Konyu no. 3 (Figure 2).

Table 1. Analysis of variance in *F* values for tuber yield and major traits of potato between/among years, cultivars and irrigation treatments.

| Source of variation | df | Tuber number per hill | Average tuber weight | Rate of commercial tuber production | Yield |
|---|---|---|---|---|---|
| Year (Yr) | 2 | $0.72^{ns}$ | $19.61^{ns}$ | $13.05^{ns}$ | $1.61^{ns}$ |
| Cultivar (C) | 2 | $3.67^{**}$ | $11.74^{**}$ | $169.53^{**}$ | $0.62^{**}$ |
| Irrigation treatments (I) | 1 | $195.05^{**}$ | $1081.20^{**}$ | $2377.47^{**}$ | $598.51^{**}$ |
| Yr × C | 4 | $1.72^{ns}$ | $1.93^{ns}$ | $4.55^{ns}$ | $0.39^{ns}$ |
| Yr × I | 2 | $0.12^{ns}$ | $0.45^{ns}$ | $3.23^{ns}$ | $0.71^{ns}$ |
| C × I | 2 | $17.13^{*}$ | $138.05^{ns}$ | $180.55^{ns}$ | $22.34^{*}$ |

df, degrees of freedom.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
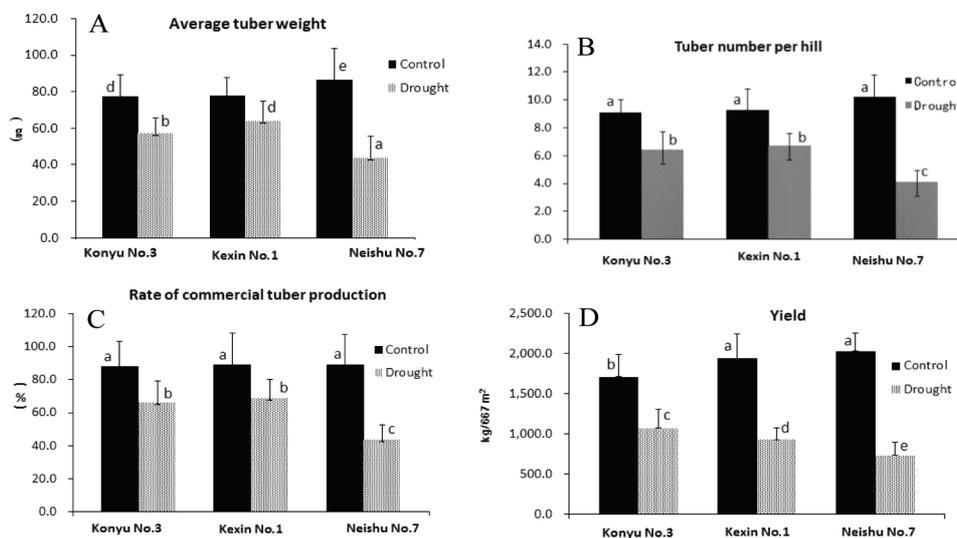ns Not significant at the 0.05 probability level.

Figure 2. Effects of drought on the tuber number and weight of various cultivars. The data are the averages of values observed during the three study years, Vertical bars represent values ± the standard error of the mean (n=6). Values with the same letter are not significantly different at the P=0.01 level.

## Water status in the soil and leaves

The $\Psi_s$ was maintained at a level greater than -1.0 MPa in the upper 30-cm-deep soil layer under control conditions throughout the growing season in all three years. Under drought conditions, the $\Psi_s$ decreased gradually to approximately -1.3 MPa on July 10th in 2009 and to approximately -1.5 MPa in 2010 and 2011 (Figure 1).

The RWC of all three cultivars declined markedly in the leaves throughout the entire growing period under drought stress (Figure 3). The decrease in leaf RWC was lowest in Konyu no. 3 and highest in Neishu no. 7. With prolonged drought stress, the RWC values of the Kexin no. 1 and Neishu no. 7 cultivars decreased significantly compared with the control plants; however, the Konyu no. 3 plants exhibited slow, small decreases under both treatments.

## TRL

Under control conditions, the TRL value per plantlet differed among the three cultivars. The TRL values were nearly 400 cm in Kexin no. 1, greater than 500 cm in Konyu no. 3 and less than 300 cm in Neishu no. 7 before August 3 followed by decreases in the TRL values. A similar trend was

exhibited under drought stress conditions. As drought stress increased, the TRL values of all three cultivars increased significantly compared with the control plants. The extent of the root length increase differed among the three cultivars. The TRL values of Kexin no. 1, Konyu no. 3 and Neishu no. 7 were 498 cm, 643 cm and 389 cm, respectively, on August 3rd, representing increases of 20.9%, 13% and 2.9%, respectively, relative to the controls (Figure 4).
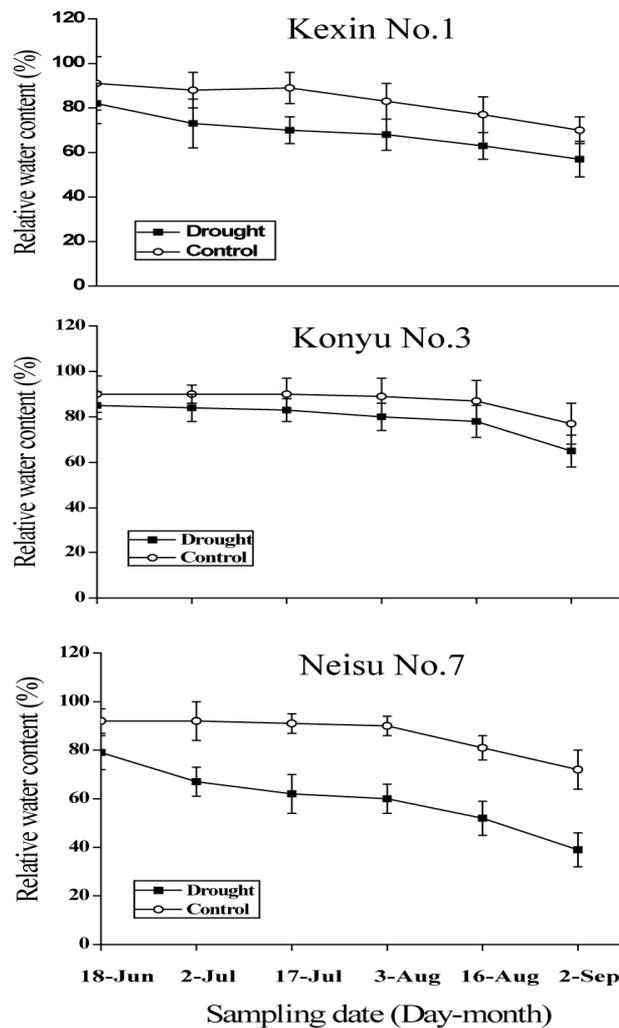


Figure 3. Leaf relative water content in the potato cultivars Kexin no. 1, Konyu no. 3 and Neishu no. 7 under conditions of drought stress. The data are the averages of twelve independent measurements. Vertical bars represent values ± the standard error of the mean (n=12).

Figure 4. Total root length in Kexin no. 1, Konyu no. 3 and Neishu no. 7 potato plants subjected to drought stress. The data are the averages of twelve independent measurements. Vertical bars represent values ± the standard error of the mean (n=12).
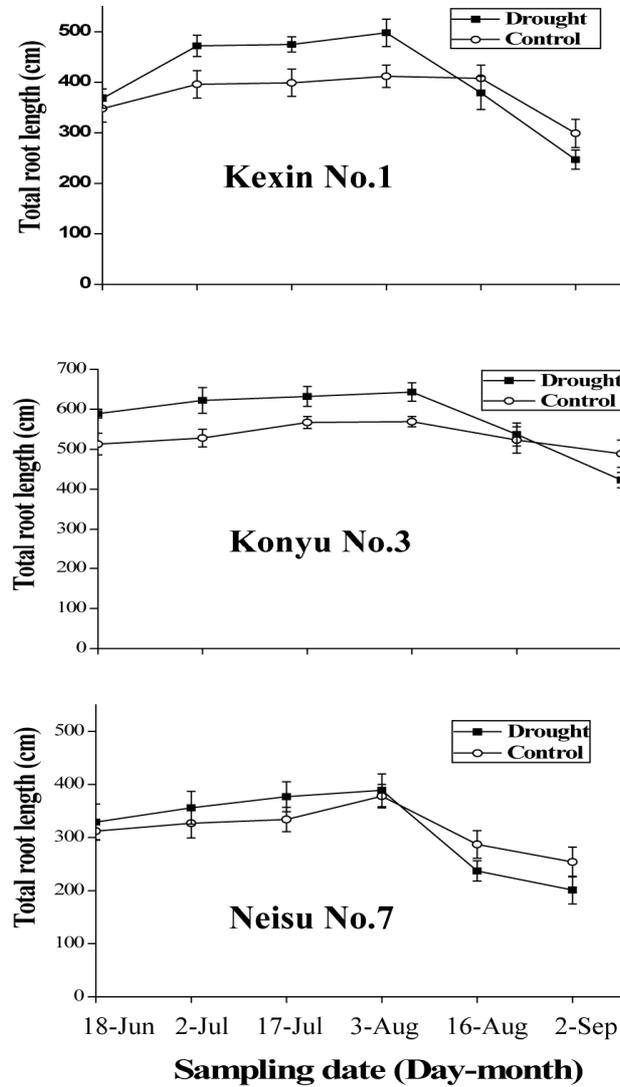
## ROS and antioxidative enzyme activity

Throughout plant development, the $H_2O_2$ content increased in all three cultivars under both treatments. Drought stress increased the production of

$H_2O_2$ significantly relative to the control treatment. On August $3^{rd}$, the Neishu no. 7 plants exhibited the greatest relative increase in $H_2O_2$ content (111.2%) relative to the control plants, whereas the smallest increase (26.6%) was observed in Konyu no. 3 plants (Table 2). The measured changes in $O_2^-$ generation in leaves are shown in Table 2. Under well-watered conditions, the rates of $O_2^-$ generation increased gradually to a maximum value on September $2^{nd}$ in all three cultivars. Drought stress caused the rates of $O_2^-$ generation to increase gradually at first and then to decrease gradually during the remaining growth period in the three cultivars. The rates of $O_2^-$ production were significantly different between the control and drought treatments (Table 2). Drought caused the greatest relative increase in $O_2^-$ production relative to the control plants in Neishu no. 7 (average of 19.4%), whereas the smallest increase was observed in Konyu no. 3 (average of 1.2%).

The changes in antioxidative enzyme activity are presented in Table 3. Under well-watered conditions, SOD activity increased gradually for all three cultivars during plant growth and development. Drought stress caused increased SOD activity relative to the control treatment before August $3^{rd}$, followed by a gradual decrease. SOD activity in drought stress-treated Kexin no. 1 and Konyu no. 3 plants increased significantly compared with the control plants. Conversely, the drought stress-treated Neishu no. 7 plants exhibited lower SOD activity than the control plants after August $3^{rd}$. The levels of CAT activity in the Kexin no. 1 and Konyu no. 3 plants increased gradually under both treatment conditions during each growing season, but they were significantly higher under drought than under control conditions. Neishu no. 7 exhibited increased CAT activity during the early growth season, which then decreased from August $16^{th}$ under drought stress. During the entire experimental period, the average levels of CAT activity in the drought stress-treated Kexin no. 1, Konyu no. 3 and Neishu no. 7 plants increased by 56.8%, 30% and 28.6%, respectively, relative to the control plants. The changes in POD activity under control conditions were similar to those in SOD activity for all three cultivars. Unlike the changes in SOD activity, POD activity increased in Kexin no. 1 and Konyu no. 3 during the entire growth period in response to drought stress treatment.

Table 2. Effects of drought on reactive oxygen species production in three potato cultivars during the growing season.

| Reactive oxygen species | Cultivar | Treatment | Sampling date (day-month) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 18-June | 2-July | 17-July | 3-August | 16-August | 2-September |
| $H_2O_2$ ($\mu$mol g$^{-1}$ FW) | Kexin no. 1 | Control | $5.78 \pm 0.57^c$ | $6.10 \pm 0.52^b$ | $6.79 \pm 0.65^b$ | $7.76 \pm 0.48^c$ | $12.17 \pm 0.74^c$ | $13.56 \pm 0.68^{cd}$ |
| | | Drought | $6.62 \pm 0.49^{bc}$ | $6.72 \pm 0.64^b$ | $8.25 \pm 0.59^b$ | $12.98 \pm 0.56^b$ | $16.90 \pm 0.63^b$ | $17.47 \pm 0.85^b$ |
| | Konyu no. 3 | Control | $6.80 \pm 0.62^{bc}$ | $6.90 \pm 0.37^b$ | $7.02 \pm 0.49^b$ | $7.13 \pm 0.78^c$ | $8.17 \pm 0.62^d$ | $10.89 \pm 0.69^d$ |
| | | Drought | $7.90 \pm 0.54^{ab}$ | $7.20 \pm 0.47^b$ | $7.44 \pm 0.58^b$ | $9.03 \pm 0.69^c$ | $10.09 \pm 0.58^{cd}$ | $11.41 \pm 0.45^d$ |
| | Neishu no. 7 | Control | $8.23 \pm 0.93^a$ | $8.27 \pm 0.62^b$ | $8.77 \pm 0.83^b$ | $9.78 \pm 0.88^{bc}$ | $10.89 \pm 0.85^{cd}$ | $15.87 \pm 1.12^{bc}$ |
| | | Drought | $9.08 \pm 0.87^a$ | $11.18 \pm 0.95^a$ | $14.27 \pm 0.91^a$ | $20.66 \pm 1.20^a$ | $23.78 \pm 1.31^a$ | $26.78 \pm 1.26^a$ |
| $O_2^{\cdot}$ (nmol min$^{-1}$ g$^{-1}$ FW) | Kexin no. 1 | Control | $8.48 \pm 0.47^b$ | $8.60 \pm 0.87^b$ | $8.72 \pm 0.67^b$ | $11.90 \pm 0.20^{cd}$ | $12.45 \pm 0.30^c$ | $12.56 \pm 0.98^b$ |
| | | Drought | $9.62 \pm 0.90^b$ | $9.90 \pm 0.23^b$ | $10.75 \pm 0.80^b$ | $13.98 \pm 0.77^b$ | $14.10 \pm 0.73^{bc}$ | $10.47 \pm 0.69^{bc}$ |
| | Konyu no. 3 | Control | $8.80 \pm 1.29^b$ | $8.90 \pm 1.12^b$ | $8.87 \pm 1.15^b$ | $11.67 \pm 1.03^d$ | $12.64 \pm 1.35^c$ | $12.76 \pm 1.05^b$ |
| | | Drought | $9.39 \pm 1.11^b$ | $9.70 \pm 1.13^b$ | $10.12 \pm 1.25^b$ | $12.58 \pm 0.83^{bcd}$ | $13.30 \pm 1.09^c$ | $9.22 \pm 1.17^c$ |
| | Neishu no. 7 | Control | $10.23 \pm 0.86^b$ | $10.27 \pm 1.28^b$ | $11.37 \pm 1.32^b$ | $13.78 \pm 1.02^{bc}$ | $15.89 \pm 1.56^{ab}$ | $15.90 \pm 1.68^a$ |
| | | Drought | $13.08 \pm 1.25^a$ | $14.18 \pm 1.21^a$ | $15.27 \pm 1.38^a$ | $19.52 \pm 1.27^a$ | $18.01 \pm 1.23^a$ | $12.33 \pm 1.19^b$ |

The data are the means ± standard error of twelve independent measurements. Values within a column with the same letter are not significantly different at the P=0.01 level. FW, fresh weight.

Table 3. Effects of drought on antioxidative enzyme activity in three potato cultivars during the growing season.

| Antioxidative enzyme | Cultivar | Treatment | Sampling date (day-month) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 18-June | 2-July | 17-July | 3-August | 16-August | 2-September |
| SOD (U mg⁻¹ protein) | Kexin no. 1 | Control | $3.30 \pm 0.42^{cd}$ | $3.52 \pm 0.38^{cd}$ | $3.68 \pm 0.28^{cd}$ | $4.82 \pm 0.35^{c}$ | $6.24 \pm 0.32^{ab}$ | $6.63 \pm 0.48^{a}$ |
| | | Drought | $5.82 \pm 0.35^{a}$ | $7.31 \pm 0.44^{a}$ | $7.74 \pm 0.42^{a}$ | $8.57 \pm 0.46^{a}$ | $6.60 \pm 0.43^{a}$ | $5.90 \pm 0.37^{b}$ |
| | Konyu no. 3 | Control | $2.90 \pm 0.18^{cd}$ | $2.92 \pm 0.28^{de}$ | $2.98 \pm 0.23^{de}$ | $3.80 \pm 0.25^{d}$ | $5.24 \pm 0.32^{c}$ | $5.83 \pm 0.47^{b}$ |
| | | Drought | $4.75 \pm 0.22^{b}$ | $4.90 \pm 0.26^{b}$ | $5.54 \pm 0.19^{b}$ | $7.17 \pm 0.26^{b}$ | $5.50 \pm 0.33^{bc}$ | $5.33 \pm 0.28^{b}$ |
| | Neishu no. 7 | Control | $2.47 \pm 0.12^{d}$ | $2.52 \pm 0.28^{e}$ | $2.65 \pm 0.30^{e}$ | $3.17 \pm 0.29^{d}$ | $3.88 \pm 0.22^{d}$ | $4.09 \pm 0.19^{c}$ |
| | | Drought | $3.65 \pm 0.25^{c}$ | $3.70 \pm 0.21^{c}$ | $4.24 \pm 0.38^{c}$ | $4.76 \pm 0.27^{c}$ | $2.91 \pm 0.23^{e}$ | $2.13 \pm 0.29^{d}$ |
| POD (U mg⁻¹ protein) | Kexin no. 1 | Control | $1.88 \pm 0.17^{ab}$ | $1.97 \pm 0.22^{ab}$ | $2.23 \pm 0.23^{cd}$ | $2.84 \pm 0.18^{b}$ | $3.18 \pm 0.27^{b}$ | $3.23 \pm 0.18^{a}$ |
| | | Drought | $2.52 \pm 0.12^{a}$ | $2.97 \pm 0.26^{a}$ | $3.47 \pm 0.19^{a}$ | $4.86 \pm 0.26^{a}$ | $4.89 \pm 0.13^{a}$ | $3.71 \pm 0.15^{A}$ |
| | Konyu no. 3 | Control | $1.32 \pm 0.13^{b}$ | $1.38 \pm 0.18^{b}$ | $1.45 \pm 0.18^{e}$ | $2.32 \pm 0.23^{b}$ | $2.88 \pm 0.12^{bc}$ | $3.46 \pm 0.16^{a}$ |
| | | Drought | $1.97 \pm 0.20^{ab}$ | $2.04 \pm 0.20^{ab}$ | $2.85 \pm 0.14^{b}$ | $4.56 \pm 0.12^{a}$ | $4.48 \pm 0.18^{a}$ | $3.56 \pm 0.18^{a}$ |
| | Neishu no. 7 | Control | $1.47 \pm 0.12^{b}$ | $1.57 \pm 0.12^{b}$ | $1.85 \pm 0.21^{de}$ | $2.07 \pm 0.08^{b}$ | $2.48 \pm 0.08^{c}$ | $2.64 \pm 0.06^{b}$ |
| | | Drought | $1.83 \pm 0.09^{ab}$ | $1.96 \pm 0.13^{ab}$ | $2.74 \pm 0.15^{bc}$ | $2.80 \pm 0.10^{b}$ | $2.33 \pm 0.05^{c}$ | $2.12 \pm 0.05^{b}$ |
| CAT (μmol $H_2O_2$ min⁻¹ mg⁻¹ protein) | Kexin no. 1 | Control | $1.13 \pm 0.17^{c}$ | $1.52 \pm 0.22^{cd}$ | $2.29 \pm 0.29^{c}$ | $3.95 \pm 0.38^{bc}$ | $4.76 \pm 0.27^{b}$ | $4.88 \pm 0.38^{b}$ |
| | | Drought | $2.72 \pm 0.39^{a}$ | $3.43 \pm 0.26^{a}$ | $4.97 \pm 0.59^{a}$ | $5.67 \pm 0.46^{a}$ | $6.19 \pm 0.33^{a}$ | $6.17 \pm 0.45^{a}$ |
| | Konyu no. 3 | Control | $1.32 \pm 0.28^{c}$ | $1.38 \pm 0.18^{cd}$ | $1.45 \pm 0.21^{d}$ | $3.52 \pm 0.23^{cd}$ | $4.94 \pm 0.27^{b}$ | $5.13 \pm 0.30^{b}$ |
| | | Drought | $1.93 \pm 0.32^{b}$ | $1.98 \pm 0.26^{bc}$ | $3.27 \pm 0.39^{b}$ | $4.17 \pm 0.49^{b}$ | $5.88 \pm 0.38^{a}$ | $6.13 \pm 0.34^{a}$ |
| | Neishu no. 7 | Control | $0.87 \pm 0.15^{c}$ | $1.01 \pm 0.16^{d}$ | $1.35 \pm 0.19^{d}$ | $1.97 \pm 0.27^{e}$ | $2.38 \pm 0.27^{c}$ | $2.39 \pm 0.20^{c}$ |
| | | Drought | $1.15 \pm 0.18^{c}$ | $2.12 \pm 0.17^{b}$ | $2.84 \pm 0.11^{b}$ | $3.07 \pm 0.19^{d}$ | $2.64 \pm 0.14^{c}$ | $2.22 \pm 0.19^{c}$ |

The data are the means ± standard error of twelve independent measurements. Values within a column with the same letter are not significantly different at the P=0.01 level. SOD, superoxide dismutase; POD, peroxidase; CAT, catalase.

**Discussion**

Water deficiency threatens potato production in arid and semi-arid areas worldwide. Studies have investigated the physiological, biochemical and agro-morphological responses of potato to water stress at a specific stage of the growth period (Tourneux et al., 2003; Alva et al., 2012). Few reports have addressed the agro-morphological and physiological processes that occur under long-term drought stress conditions in which potato plants are subjected to water deficiency throughout the entire growth period. In this study, we demonstrated different physiological, biochemical and agro-morphological responses in three different potato cultivars subjected to long-term drought stress.

Drought-tolerant cultivars employ multiple strategies to survive under water-limited conditions and to produce higher yields than sensitive cultivars. One such strategy is to obtain as much water as possible from the soil by forming a well-developed root system. Previous studies have shown that drought stress not only affects root development and morphology, but also alters the growth of plant organs (Lahlou and Ledent, 2005). In this study, we found that all three cultivars developed longer roots under drought conditions. However, the drought-tolerant cultivars and Konyu no. 3 in particular, exhibited greater increases in root length under drought stress during early development (before August 16th) and smaller decreases in length thereafter. The well-developed roots guaranteed that more water was absorbed from the soil relative to the sensitive cultivars (Figures 1 and 2).

A second strategy is to retain more water in the plant. RWC values are one of the most reliable indicators for defining water retention in plants (Rampino et al., 2006; Sanchez-Rodriguez et al., 2010). Most studies have shown that the RWC decreases in response to drought stress (Bürling et al., 2013; Shaw et al., 2002). Our results show that, although the RWC decreased for both the tolerant and sensitive cultivars in response to drought stress, the decrease was significant only in the sensitive cultivar, Neishu no. 7 (Figure 3). Increased water retention in the leaves of tolerant cultivars is attributed partially to decreased water loss resulting from lower transpiration rates (Vos and Groenwold, 1989; Chaitanya et al., 2003; Flexas et al., 2002).

A third strategy of tolerant cultivars is to increase the capacity to defend against oxidative damage caused by drought stress. Oxidative damage is characterized by the overproduction of ROS such as $O_2^-$ and $H_2O_2$ resulting in lipid peroxidation and even cell death (Imlay, 2003; Ashraf, 2009; Ashraf, 2010). Water stress is associated inevitably with increased oxidative

stress due to enhanced ROS accumulation, particularly $O_2^-$ and $H_2O_2$, in chloroplasts, mitochondria and peroxisomes. As a result, the activities of antioxidant enzymes such as SOD, POD and CAT are induced as a general adaptation strategy to overcome oxidative stress (Foyer and Noctor, 2003).

We systematically examined the effects of water stress on the generation of $O_2^-$ and $H_2O_2$ and on the activities of the three main antioxidant enzymes (SOD, POD and CAT). Our results show a significant increase in the generation of ROS in the early developmental stages of potato leaves subjected to water stress compared with control leaves and a marked increase in the activities of the antioxidant enzymes following the increase in ROS (Tables 3 and 4). Although drought stress stimulated increased production of $O_2^-$ and $H_2O_2$ in the sensitive cultivar Neishu no. 7, it caused smaller increases in the activity of SOD, POD and CAT than in the drought-tolerant cultivars Konyu no. 3 and Kexin no. 1. This suggests that the antioxidant capacity of drought-tolerant potato cultivars is enhanced for protection against oxidative damage caused by drought stress. The stress-tolerant cultivars exhibited a higher antioxidant capacity than the sensitive cultivar under drought stress. These results are in agreement with reports of increased SOD activity in drought-resistant maize and bean cultivars (Turkan et al., 2005; Jagtap and Bhargava, 1995; Alscher et al., 2002).

By combining all three drought tolerance strategies, the drought-tolerant cultivars Konyu no. 3 and Kexin no. 1 were able to produce higher tuber yields under drought conditions than the sensitive cultivar, Neishu no. 7. The increased tuber yield was attributable not only to a higher tuber number per plant, but also to a higher average weight of individual tubers. Moreover, the rate of production of commercial tubers was significantly higher in the tolerant cultivars than in the sensitive cultivar under conditions of water stress, although the production rates were the same under well-watered conditions (Figure 2). Thus, direct screening of cultivars under water-deficient conditions using the above-described parameters should lead to increased productivity in potato cultivation (Sharp et al., 2004; Rytter and Rytter, 2012) and enhance the selection of drought-tolerant cultivars.

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