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Influence of early nitrogen application on physiological properties and ultrastructures of functional leaves of potatoes under drought stress

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The changes in physiological properties and leaf ultrastructures caused by early nitrogen application for potatoes under drought stress were explored. Potato variety Chuanyu117 was used to conduct a controlled experiment in a sand medium. Nutrient solution treatments (0.84, 1.26, 1.68 and 2.10) were administered, followed by severe drought, moderate drought, and normal (control) water treatments (45, 60, and 75% of soil saturation moisture capacity) in the seedling stage of potatoes. Early additional nitrogen application maintained chlorophyll content in potato leaves under drought stress and increased activities of protective enzymes in the plants. Severe and moderate drought stress treatments resulted in the smallest reductions in chlorophyll content compared with the control and largest increases in POD and CAT activities. Under the same water conditions, potato with high nitrogen treatment (1.68 g N L^{-1}) had significantly higher net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) than other nitrogen treatment levels; the highest levels of these photosynthetic parameters were obtained under the normal water condition. As drought stress increased, the degree of damage to chloroplasts and mitochondria in potato leaves gradually increased and the number of starch grains increased; however, with increases in the nitrogen application level, the degree of damage to chloroplasts and mitochondria gradually decreased. Early additional nitrogen application had mitigative effects on the damage to cellular structures of potato leaves under drought stress and could improve gas exchange and increase absorption and utilization of light energy in potato leaves. Further, the adaptive capacity of photosynthetic structures in potato leaves increased, leading to greater drought resistance in potato. However, with increases in the nitrogen levels, drought resistance of potato showed an initially increasing trend that decreased subsequently, suggesting that over-application of nitrogen reduced drought resistance in potato.

Key words: Early nitrogen application, drought stress, physiological properties, photosynthetic parameters, ultrastructure.

INTRODUCTION

China is the world's largest potato-producing country and launched the "Potato as a Staple Food" strategy in 2015, whereby over 50% of potatoes produced were expected to be consumed as a staple food by 2020 and the planting area of potato would gradually increase annually. However, as a typical temperate crop, potato is relatively sensitive to insufficient water (Stefani et al., 2016). 60% of China's potato planting area is in arid or semi-arid regions, thus drought stress is severely restricting China's potato production and the development of the potato industry (Xu et al., 2011).

The physiological drought-responding morphological structures of plants under drought stress and the molecular regulations have been extensively studied, and it has been found that drought stress cannot only reduce photosynthesis (Liu et al., 2016; Zhang et al., 2015b) and damage metabolic pathways (Zhou et al., 2013), but also changes ultrastructures of tissues and organs are mainly manifested as damage to cytoplasmic and chloroplast membranes, deformations of chloroplast and mitochondrion ultrastructures (Xu et al., 2013), the disordered arrangement of stroma lamella, expanded granum thylakoids, and the appearance of starch grains in chloroplasts. Chloroplasts are most sensitive to, and more damaged by, drought stress than mitochondria, whereas the nucleus is less affected (Zhang et al., 2015a). Recent studies revealed that nitrogen application had significant compensating effects on reduced photosynthesis caused by water stress. Nitrogen can significantly improve gas exchange in plant leaves, increase absorption and utilization of light energy, and reduce photorespiration (Zhang and Shang-Guan, 2006). Nitrogen is associated with plant growth and yield and plays an important role in regulating crop growth and development under stress (Saneoka et al., 2004). A sufficient amount of nitrogen can increase the adaptive capacity of the leaf's photosynthetic structures in drought environments (Gimeno et al., 2014), and can, to some extent, increase a crop's drought resistance by improving its nitrogen nutritional status (Zhang and Shan, 2003). However, under different soil water supply conditions, it is still unclear whether nitrogen nutritional status affecting growth and photosynthetic physiological responses of plants is associated with cell structure damage. China's major potato planting regions often experience early stage (seedling stage) drought, which causes poor growth that seriously affects tuber yield in the late stage, while the relationship among early nitrogen application, photosynthesis, ultrastructures, and drought resistance of potatoes still needs to be investigated further.

In this study, through an examination of the influence of early nitrogen application on physiological properties and ultrastructures of potato leaves under drought stress, this study aimed to reveal the mechanism underlying the improvement in the drought resistance of the potato plants through early nitrogen application, so as to provide a theoretical basis for China's drought resistant potato cultivation.

MATERIALS AND METHODS

Test material and experimental design

The experiment was carried out in a rainproof shed at Sichuan Agricultural University in 2014 and 2015. The rainproof was made by lucency plastic film and other environmental factors depend on natural conditions, the potato variety Chuanyu117 was used as the test material. To ensure consistency in the test material, 9–11 g small seed stock potatoes were carefully selected. Each 15-cm diameter PVC pot was filled with 1.0 kg air-dried river sand and 3 seed stock potatoes were planted at a depth of 5 cm. MS nutrient solution (removed sucrose, agar, activated carbon and other additional components from MS medium formula, containing only a large number of elements, trace elements, iron salt, vitamins) was used as the base nutrient solution. A two-factor completely randomized experimental design was used with 4 nitrogen application levels (N0: 0.84 mg N L^{-1}; N1: 1.26 mg N L^{-1}; N2: 1.68 mg N L^{-1}; N3: 2.10 mg N L^{-1}) and 3 drought stress levels (B1: severe water stress, 45% of the field's maximum water holding capacity; B2: moderate water stress, 60% of the field's maximum water holding capacity; B3: normal water supply (control), 75% of the field's maximum water holding capacity); all treatments were watered with nutrient solution. The soil water holding capacities of different treatments were maintained at the required water stress levels using a weighing method every second day. Different treatment levels of the two factors generated 12 treatment combinations where 6 pots were planted for each combination to achieve a total of 72 pots. Nutrient solution was applied once every 4 days at 400 ml per pot after potato seedlings emerged. Pots were watered 5 times, thereafter, a 14-day period of drought stress, during which no nutrient solution was given, was applied to potato plants 20 days after they entered the seedling stage.

Determination of indices and parameters

Determination of physiological indices

After the drought the period of stress, the 3rd or 4th fully expanded leaf below the apical meristem of the stem tip was utilized to determine physiological indices. Chlorophyll content was measured using the ethanol-acetone mixture method, peroxidase (POD) activity was determined using the guaiacol colorimetric method, and catalase (CAT) activity was measured using the H_2O_2 UV absorption method.

Determination of photosynthetic parameters

After drought stress, potato plants with consistently vigorous growth from different treatments were selected, and 3 measurements were randomly performed for each treatment. Stomatal conductance (Gs), net photosynthesis rate (Pn), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) of the 3rd to 4th compound leaf of the stem tip were measured between 9 and 11 am on sunny days using a LI-6400 portable photosynthesis system (Li Cor imported

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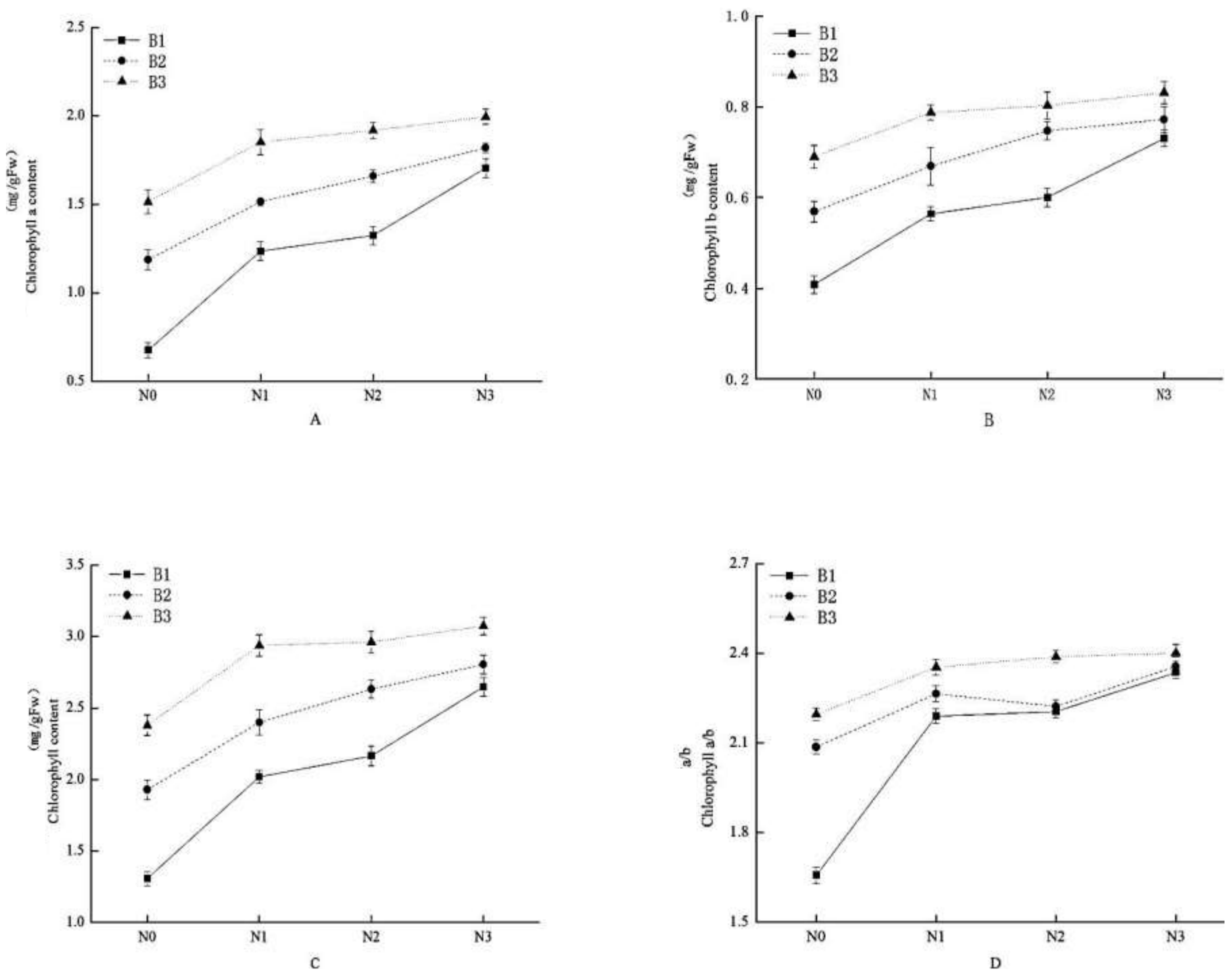

Figure 1. Influence of early nitrogen application on chlorophyll content of potato leaves under drought stress in the seedling stage.

from the United States). Averages were calculated from 3 replicate measurements.

Preparation and observation of electron microscopy samples

After drought stress, the 3rd to 4th compound leaf of the stem tip were harvested, cut into 1 mm × 3 mm rectangular pieces, placed into 2.5% glutaraldehyde (pH 6.7), and fixed at 4°C for 24 h. Samples were washed 3 times with phosphate buffer solution (PBS) (pH 6.8), fixed with 2% osmium tetroxide for 1.5 h, and washed 3 times with PBS (pH 7.2). Gradient ethanol dehydration (50, 70, 90 and 100%) with a transition from 100% ethanol to 100% acetone (1:1) at 4°C was used to develop samples. Acetone (100%) was added to each sample and allowed to equilibrate at room temperature (28 - 30°) for 5 min. Samples were embedded in epoxy resin 812 and placed in a thermostat for polymerization. Embedded samples were sectioned using an ULPRACUT EXINXIN microtome, stained with uranyl acetate and lead citrate solution at 25°C, and then observed and photographed using a Hitachi H-600IV TEM.

Data analysis

ANOVAs were performed and significant differences among means were determined (LSD method at the 0.05 probability level) using DPS 7.5 and SPSS 11.5.

RESULTS

Influence of early nitrogen application on chlorophyll content of potato leaves under drought stress in the seedling stage

Drought stress led to decreased levels of chlorophyll a, chlorophyll b, total chlorophyll, and chlorophyll a/b in seedling stage potato leaves, with severe drought stress causing the largest decrease in chlorophyll content (Figure 1). Under different water stress conditions, chlorophyll a, chlorophyll b, and total chlorophyll content of potato leaves all significantly increased as the quantity of nitrogen applied increased, while chlorophyll a/b also increased but not significantly. For different nitrogen application treatments, the levels of chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b of the

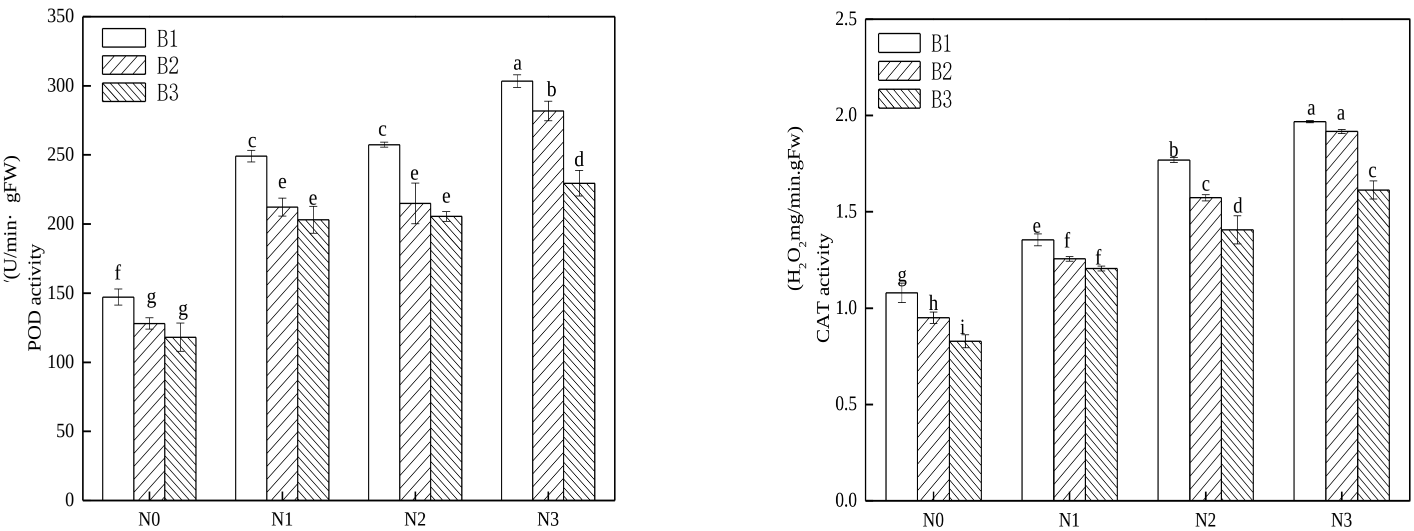

Figure 2. Influence of additional nitrogen application on POD activity and CAT activity of potato leaves under drought stress in seedling stage.

severe water stress treatment (B1) and normal water supply treatment (B3) decreased by 14.54 – 55.38, 12.15 – 40.87, 13.91 – 45.07, and 2.71 – 24.54%, respectively. The levels of chlorophyll a, chlorophyll b, total chlorophyll, and chlorophyll a/b of the moderate water stress treatment (B2) and the normal water supply treatment (B3) decreased by 8.82 – 21.65, 7.1 – 17.54, 8.79 – 18.96, and 1.85 – 4.99%, respectively, with the N3 treatment having the smallest decrease in chlorophyll content. Chlorophyll a/b decreased as drought stress increased, but increased as nitrogen application increased, indicating that early nitrogen application changed the allocation of photosynthetic pigments in potato seedlings under drought stress and drought stress had a more significant influence on the decrease of chlorophyll a content.

Influence of additional nitrogen application on protective enzymes activity of potato leaves under drought stress in seedling stage

In the different drought stress treatments, potato POD activity showed an initially increasing trend and then a decreasing trend with increases in nitrogen application (Figure 2). For different nitrogen application levels, all drought stress treatments resulted in higher POD activity than normal water treatment, which increased as the drought degree aggravated, severe water stress (B1) > moderate water stress (B2) > normal water supply (B3), POD activity was extremely significantly higher in severe drought stress treatment than in moderate water stress treatment and control treatment. Compared with that of the control, POD activity of B1 treatment increased by 34.14, 47.51, 32.20, and 24.64%, and POD activity of B2 treatment increased by 20.90, 33.71, 9.68, and 8.43%. The increasing amplitude of POD activity caused by drought stress compared with normal water treatment increased and then decreased as nitrogen application increased, where A1 treatment had the greatest increasing amplitudes, which were 47.51 and 33.71%, respectively.

In different drought stress treatments, CAT (catalase) activity all showed an increasing trend as nitrogen application increased, N3 > N2 > N1 > N0. In different nitrogen application treatments, CAT activity all increased as the drought degree aggravated, B1 > B2 > B3. Under N1 treatment, CAT activity of B1 treatment was significantly higher than that under B2 and B3 treatments; in addition to this, CAT activities of drought stress treatments with other nitrogen application levels were all extremely significantly higher than that of normal water supply, where CAT activities of different treatments under N3 were all significantly higher than that of other nitrogen application levels, and drought stress treatments resulted in the most increasing amplitude comparing with normal water supply, which were 22.0 and 18.85%, respectively.

Influence of additional nitrogen application on photosynthetic parameters of potato leaves under drought stress in seedling stage

As the degree of drought stress aggravated, potato's Pn, Gs, Tr and Ci of different nitrogen application levels all showed decreasing trends (Table 1), and B1 < B2 < B3. Under different water stress conditions, the photosynthetic parameters all showed a trend that increased first then decreased as nitrogen application increased. Particularly, photosynthetic parameters in N1 treatment

Table 1. Effects of additional nitrogen application and drought stress on photosynthetic characteristics of potato seedlings

Treatment		$Pn/(\mu mol \cdot m^{-2} \cdot s^{-1})$	$Gs/(m\ mol \cdot m^{-2} \cdot s^{-1})$	$Tr/(m\ mol \cdot m^{-2} \cdot s^{-1})$	$Ci/(\mu mol \cdot mol^{-1})$
	B1	6.97 ± 0.53^h	0.096 ± 0.006^g	0.60 ± 0.05^f	131.41±5.98
N0	B2	8.31 ± 0.45^g	0.132 ± 0.009^f	1.53 ± 0.39^d	141.64±2.42
	B3	12.62 ± 0.66^f	0.267 ± 0.023^c	3.14 ± 0.25^c	189.81 ± 9.86^b
	B1	19.78 ± 0.65^c	0.134 ± 0.004^f	1.00 ± 0.10^{def}	190.17 ± 5.83^b
N1	B2	22.60 ± 0.89^b	0.265 ± 0.013^c	4.62 ± 0.43^a	208.55 ± 3.00^a
	B3	25.82 ± 0.74^a	0.322 ± 0.010^a	4.90 ± 0.16^a	222.16 ± 4.02^a
	B1	15.55 ± 0.62^e	0.132 ± 0.005^f	0.90 ± 0.09^{ef}	155.45 ± 6.79^c
N2	B2	17.63 ± 0.50^d	0.210 ± 0.008^d	3.71 ± 0.52^b	188.63 ± 7.33^b
	B3	20.85 ± 1.04^c	0.292 ± 0.005^b	4.73 ± 0.51^a	207.35 ± 7.49^a
	B1	2.96±0.37j	0.092 ± 0.002^g	0.76 ± 0.11^{ef}	70.67 ± 4.67^g
N3	B2	4.37±0.31i	0.110 ± 0.006^g	1.29 ± 0.05^{de}	93.80 ± 3.71^f
	B3	7.86 ± 0.12^{gh}	0.192 ± 0.008^e	2.65 ± 0.17^c	121.37 ± 5.28^e

Different superscript letters indicate significant differences at $P < 0.05$ ($n = 12$); data represent mean ± standard deviation (LSD) of 3 replicates. Pn-net photosynthetic rate; Gs-stomatal conductance; Tr-transpiration rate; Ci-intercellular CO_2 concentration.

were significantly greater than that of other nitrogen application levels, among which normal water supply treatment N1B3 had the greatest parameters. With different nitrogen application levels, Pn, Gs, Tr and Ci of B1 respectively decreased by 23.39–62.36%, 52.74–61.15%, 71.19–80.83% and 14.40–41.77% compared with normal water supply treatment. The decreasing amplitudes of Pn and Ci decreased initially, and then increased with increase in nitrogen, where the smallest decreasing amplitudes were observed in N1, which were 23.39 and 14.40%, respectively. Although both Gs and Tr significantly decreased, the decreasing amplitudes of different nitrogen levels were close. Pn, Gs, Tr, and Ci of B2 decreased by 12.47–44.43%, 13.25–47.63%, 5.69–51.41%, and 6.12–25.37%, respectively. As compared to those in the normal water supply treatment, the decreasing amplitudes of photosynthetic parameters decreased first then increased with the increase of nitrogen application, where the smallest decreasing amplitudes were observed in N1, which were 12.47, 13.25, 5.69 and 6.12%, respectively.

Influence of additional nitrogen application on ultrastructures of potato leaves under drought stress in seedling stage

With different nitrogen application levels, both drought stress treatments caused more severe damages to chloroplasts than normal water treatment. As the degree of drought stress aggravated, the damage of the mesophyll cells gradually increased, chloroplasts showed obvious changes, and the damage became increasingly worse. As nitrogen application increased, the damage degrees of chloroplasts in different drought stress treatments mitigated then aggravated. Under similar drought stress condition, chloroplast damage degrees showed that N1 < N2 <N0< N3, and the degree of chloroplast damages in N1 treatment was relatively low.

Under normal water condition, chloroplasts in potato leaves of different nitrogen application treatments were all in oval or fusiform shape, great in number, with clear envelope structure and regular shapes. Grana lamellas were arranged along the major axis of chloroplasts and closely attached to cell membrane. Also, grana and granum thylakoid membranes were orderly arranged with clear structures, along with dense stroma (Figure 3a1, b1, c1 and d1).

Under moderate water stress, chloroplasts in potato leaves of different nitrogen application treatments slightly deformed with obvious plasmolysis, chloroplasts were stretched or became nearly spherical and contained a small amount of starch grains. Chloroplasts in N0 treatment were wrinkled; other organelles showed local wall-adhering distribution, grana lamellas of chloroplasts were slightly distorted, and the number of starch grains changed insignificantly (Figure 3a2). Chloroplasts in N1 treatment were less damaged and showed basically complete structures (Figure 3b2). Chloroplasts in N2 treatment were swollen, and became spherical; also, the lamellas were loose with expanded spaces in between (Figure 3d2).

Under severe water stress, chloroplasts in potato leaves of different nitrogen application treatments were greatly deformed and carried a large amount of starch grains. Chloroplasts in N1 treatment still had clear yet

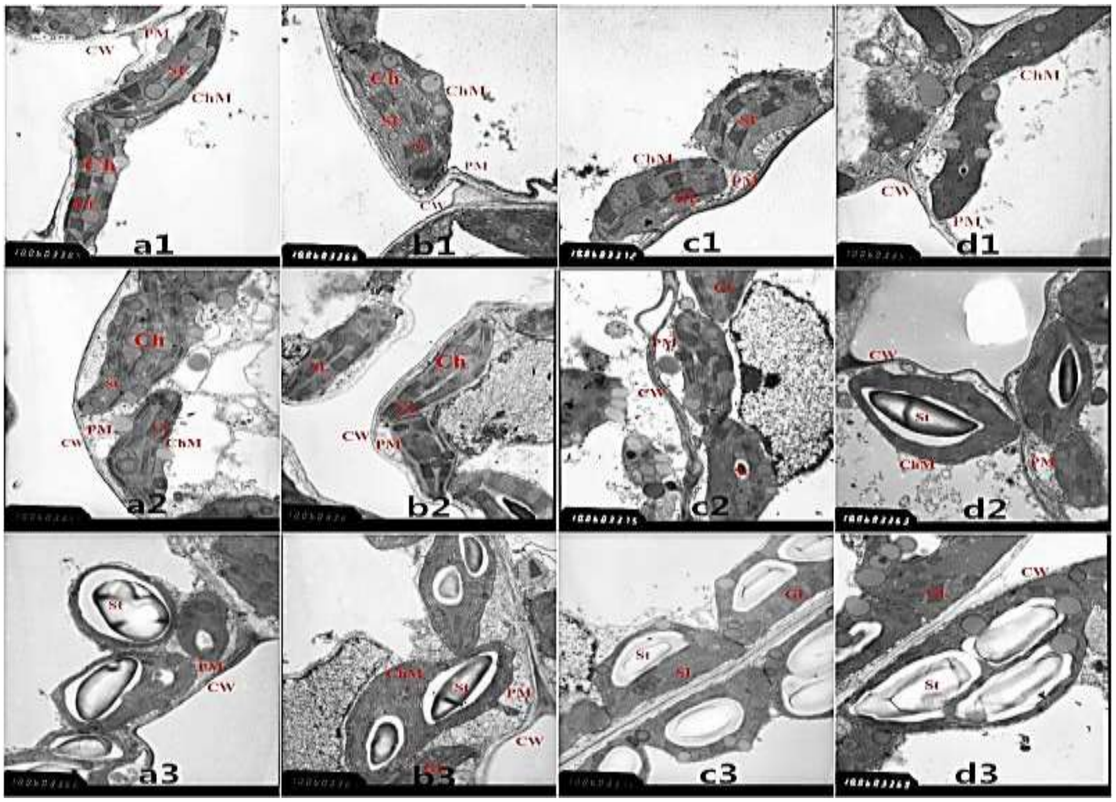

Figure 3. Influence of additional nitrogen application on ultrastructures of potato leaves under drought stress in seedling stage.

incomplete envelopes with local rupture, grana and granum thylakoid membranes showed blurred structures but were still orderly arranged (Figure 3b3). Chloroplasts in N2 treatment showed local envelope ruptures, grana and granum thylakoid membranes indicating blurred structures became wavy (Figure 3c3). Chloroplasts of potato leaves in N3 treatment had ruptured and dissolved envelopes, starch grains increased in number and size, the inclusions came out, and the lamellar structure showed disorganized arrangement (Figure 3d3).

Influence of additional nitrogen application on mitochondrion structure of potato leaves under drought stress in seedling stage

Under normal water condition, mitochondria in potato leave cells of different nitrogen application treatments were mostly in regular round or oval shape; their double-layer envelope had intact structure and sufficient cristae; inner cristae were small in size and low in number; the cytoplasm was dense, and mitochondria were distributed around chloroplasts (Figure 4a1, b1, c1 and d1).

Under moderate drought stress, mitochondria in the cells of potato leaves under different nitrogen application treatments increased in number, and became swollen, stretched, or spherical. Mitochondria in N0 treatment showed intact structure, but increased number of cristae, and were slightly swollen (Figure 4a2). Mitochondria in N1 treatment showed clear outer membranes. While some mitochondria showed blurred inner and outer membranes as well as cristae, the number of cristae decreased (Figure 4b2). Mitochondria in N2 treatment showed local outer membrane ruptures as well as blurred and unclear inner structures (Figure 4c2). Mitochondria in N3 treatment became swollen and spherical, with clear outer membranes, cristae inside mitochondria disappeared, presenting vacuolation (Figure 4d2).

Under severe stress, mitochondria in N0 treatment were swollen (Figure 4a3), mitochondria in N1 treatment showed clear outer membranes but blurred inner structures (Figure 4b3), mitochondria in N2 treatment began to degrade with inclusions coming out (Figure 4c3), mitochondria in N3 treatment showed ruptured outer and inner membranes, and cristae disappeared, presenting vacuolation (Figure 4d3).

With different nitrogen application levels, the damage degree of mitochondria was high in drought stress treatments. As the degree of drought stress increased, mitochondria were more and more severely damaged. With the increase of nitrogen application, the damage degrees to mitochondria of all drought stress treatments

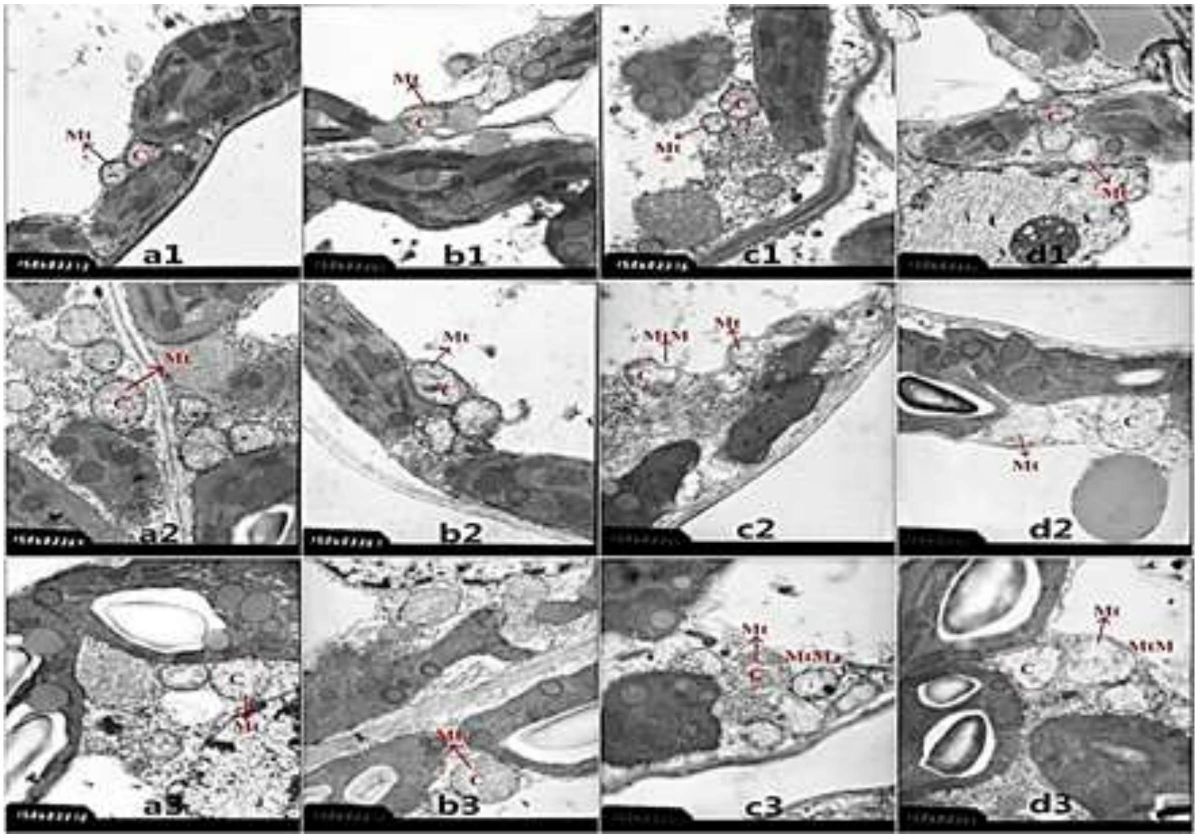

Figure 4. Influence of additional nitrogen application on mitochondrion structure of potato leaves under drought stress in seedling stage.

mitigated first and then aggravated; under similar drought stress condition, N1 treatment resulted in less damage to mitochondria.

DISCUSSION

Chloroplast and mitochondrion in mesophyll cells are the plant's organelles for photosynthesis and energy transfer, respectively, and are very sensitive to the environment (Endress and Sjoloud, 1976). Hence, comparing the changes in potato's physiological properties and ultrastructures of mitochondrion, chloroplast, and other organelles, caused by different drought stress conditions with 4 nitrogen application levels would help to determine whether additional nitrogen application promotes potato's drought resistance, and to identify a relatively suitable range of nitrogen application.

Drought causes damage to the cellular structure of plants. The mitochondrial structure of plants may be damaged under mild drought stress, causing the release of hydrolase into the cytoplasm, while lipase and protease further damage the normal structure of cell membrane, leading to the degradation of vacuoles (Munne et al., 2001). Mitochondrion and chloroplast are also affected by severe drought stress, where the thylakoids become loose and distorted, stroma lamella and grana decrease in number, and cristae become blurred (Yao et al., 1993). The increase in alkaline lipase in chloroplast is closely related to the degradation of thylakoid structure (Giles et al., 1976). Plastoglobuli from thylakoid membrane increase in number and size (Steinmüller and Tevini, 1985). Severe drought stress results in appearance of fat granules in cytoplasm and rearrangement and condensation of chromatin around the nucleolus (Munne et al., 2001). According to the results of the present study, as the degree of drought stress increased, damages to chloroplasts and mitochondria became more severe, but the damage was mitigated initially, and was then aggravated as nitrogen levels increased. The damage was relatively low, with 1.26 g/L nitrogen concentration as over application of additional nitrogen caused increased damage to chloroplasts and mitochondria. The change of chloroplast's ultrastructure might be one of the factors that affected plant photosynthesis under nitrogen application and drought conditions.

In this study, as the applied nitrogen concentration increased, the chlorophyll content and photosynthetic rate of potato leaves increased first and then decreased, where with 1.26 g/L nitrogen concentration, the smallest decreasing amplitudes of chlorophyll content and photosynthetic rate in severe and moderate drought stress treatments were obtained compared with normal water treatment. This is consistent with the findings of study conducted by Loggini et al. (1999) in which nitrogen significantly increased the synthesis of photosynthetic pigments in leaf, and had positive significance on the leaf's luminous energy absorption, increasing the photosynthetic structure's self-protective ability (Andrea and Enrico, 1999). This is also consistent with the cell's ultrastructures. Chloroplast (the main site of chlorophyll synthesis and photosynthesis), as well as the photochemical process of photosynthesis mainly takes place on the granum membrane and stroma membrane, while correct arrangement of thylakoid can assure the maximum lighted area and photosynthetic rate (Trebst, 1974). As water stress aggravates, Gs and Tr get to relatively low levels to avoid further water loss, which is an adaptive water physiological adjusting phenomenon of plants to water deficit (Yang et al., 2004). In this study, with 1.26 g/L nitrogen concentration, Gs and Tr are the smallest decreasing amplitudes in severe and moderate drought stress treatments that were obtained compared with normal water treatment, suggesting a proper amount of nitrogen as positively significant to maintaining stomatal aperture and improving stomatal air exchange capacity (Zhang and Shang-Guan, 2006). On the contrary, over application of nitrogen inhibits potato leaf's photosynthetic rate.

It was found in this study that nitrogen had certain protective effects against the damage caused by drought stress to the potato leaf structure. One possible reason is that nitrogen application has certain mitigative effects on the damage caused by excess reactive oxygen species (ROS) in plants under drought stress. Peroxidase (POD) is a protective enzyme in plants, the increased activity of which under drought stress can control peroxidation of membrane lipids to reduce the damage of drought on membrane structures, and enhances plant's self-protective adjusting ability (Kong et al., 2004). In this study, with different nitrogen application levels, POD activities of potatoes under drought stress treatments were all significantly higher than that of normal water treatment. Compared with control, the increasing amplitudes of severe and moderate water stress treatments increased then decreased as nitrogen application increased, while the greatest increasing amplitude was obtained at 1.26 g/L nitrogen concentration. This is consistent with previous studies' findings. Several researchers believe that the damage of cellular structures under drought stress, especially the damage of membrane structures, is associated with the metabolic disorder of reactive oxygen species under drought stress, while nitrogen nutrients increase activities of protective enzymes in plants under drought stress, which helps to maintain the stability of cell membrane, and thus reduce the damage degree of plant's bio-membranes (Deng et al., 2007; Liu et al., 2008).

The results of this study suggest that additional nitrogen applications reduce the adverse influence of drought stress on photosynthetic physiology, improves gas exchange condition, increases absorption and utilization of luminous energy; while simultaneously enhancing the adaptive capacity of photosynthetic structures in drought environments, increases the activities of ROS metabolism related enzymes, thus reduces damages to cell structures and functions caused by ROS, and mitigates the damages of potato leaf's cellular structures under drought stress, and empirically confirming nitrogen fertilizers' effect of increasing drought resistance by protecting subcellular structures on subcellular level. Therefore, applying nitrogen fertilizers in a rational manner according to different periods and different drought degrees helps to exert positive effects on plant metabolism, so as to improve the crop's drought resistance and increase the utilization efficiencies of water and fertilizers.

Conclusion

Early additional nitrogen application had certain mitigative effect on the damage of potato leaf's cellular structures under drought stress, could improve potato leaf's gas exchange conditions and increase potato leaf's absorption and utilization of luminous energy, and meanwhile increase the adaptive capacity of photosynthetic structures in potato leaves, thus increasing the drought resistance of potato. However, with the increase of nitrogen application level, potato's drought resistance showed a trend that increased first and then decreased suggesting over-application of nitrogen could reduce potato's drought resistance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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