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Full Length Research Paper

Differential nitrate accumulation, nitrate reduction, nitrate reductase activity, protein production and carbohydrate biosynthesis in response to potassium and sodium nitrate

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For most of the cultivated crops, nitrate is the major source of nitrogen. Most steps in the nitrate assimilatory pathway are nitrate inducible. In this study, *Cucurbita pepo* were grown in washed sand per pot at three potassium and sodium nitrate supplies (25, 50 and 100 mM) to investigate the effects of nitrate salts supply on nitrate accumulation, amino acid biosynthesis, total protein production, nitrate reductase activity and carbohydrate biosynthesis in the roots and leaves of the plants. The results indicate that both sodium and potassium nitrate supplementation had stimulatory effects on all of the mentioned factors in a dose dependent manner. In low concentration ranges (25 and 50 mM), nitrate stimulated nitrate assimilation pathway, but at 100 mM nitrate, this pathway was suppressed. However, potassium nitrate supplementation increased all of these parameters more than sodium nitrate supplementation. Both sodium and potassium nitrate, as inducers, had significant effects on both the nitrate assimilation and metabolism in low concentrations. However, the effects of potassium nitrate were higher than sodium nitrate, which was due to the positive effects of potassium on the enzyme activity, sugars transport, water and nutrient transport, protein synthesis and carbohydrate metabolism. In conclusion, potassium nitrate has better effect on the nitrate assimilatory pathway and protein and carbohydrate metabolisms.

Key words: Nitrate salts supply, nitrate accumulation, nitrate reductase activity, amino acid, protein, carbohydrate, potassium nitrate, sodium nitrate.

INTRODUCTION

Nitrate is a major source of inorganic nitrogen utilized by most plants. Nitrate in soil after being taken up by plant roots is: (1) reduced in root cells, (2) stored in the root cells vacuoles, (3) transported in the xylem through transpiration stream to be reduced in the leaves, or (4) storage in the leaf vacuole cells to be released later and reduced in the cytosol (Jackson et al., 2008). The nitrate assimilatory pathway is mediated by two enzymes, nitrate reductase (EC 1.6.6.2) and nitrite reductase (EC 1.7.7.1), which catalyze the stepwise reduction of nitrate to nitrite and nitrite to ammonia, respectively. Nitrate is first reduced to nitrite in the cytosol by nitrate reductase. Nitrite is then transported to the chloroplasts where it is reduced to ammonium by nitrite reductase. Ammonia itself can enter amino acids, proteins and nucleic acids using carbon skeletons derived from metabolites, synthesized by photosynthesis. Nitrate is reduced more efficiently in the leaves because of the readily available reductants, energy supply and carbon skeletons provided by photosynthesis (Oaks, 1994). Most steps in the nitrate assimilatory pathway are nitrate inducible. Nitrate strongly stimulates the transcription of nitrate transporters and nitrate reductase genes (Chen et al., 2004; Skrdleta et al., 1979; Stohr, 1999; Sivasankar et al., 1997).

The differential effects of nitrate salts on the nitrate and nitrite accumulation, amino acid, protein, carbohydrate and other biomass production is less studied. Thus, in

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this study, to gain more insight of these effects, we examined the effects of potassium and sodium nitrate supplies on nitrate accumulation, nitrite production, amino acid biosynthesis, total protein production, nitrate reductase activity and carbohydrate biosynthesis in the roots and leaves of *C. pepo* as a model. The different effects of sodium and potassium nitrate at various concentrations were investigated. We examined two subjects: (1) stimulatory effects of nitrate metabolism pathway dependent on nitrate ion concentration, and (2) potassium and sodium nitrate supply have different effects on nitrate assimilation.

MATERIALS AND MATHODS

Plant materials and treatments

C. pepo plants were grown in standard nutrient solution (Hoagland's solution) for three weeks. Plants were then divided into two groups: (1) grown in standard nutrient solutions (control), and (2) grown in media without nitrate (starved plants) for one week. Starved plants were subdivided into three groups: (1) grown in media without nitrate for additional four days, (2) grown in various concentrations of potassium nitrate (25, 50 and 100 mM) for three days, and (3) grown in various concentrations of sodium nitrate (25, 50 and 100 mM) for three days. 24 h after nitrate supplementation, the roots and leaves were harvested and frozen in liquid nitrogen.

Nitrate/nitrite assay

Frozen roots and leaves were powdered with a mortar and pestle, solubilized in phosphate buffered saline pH 7.4 and the extract was centrifuged at 9,000 rpm for 15 min. The supernatant was stored at -70°C. Nitrate was determined by Griess reagent using sodium nitrate as standards (Miranda et al., 2001). Briefly, to 100 µl of the culture medium, 100 µl of vanadium chloride (III) (8 mg/mL) was added. After 40 min, 50 µl of Griess reagents [1:1 (v/v) of 0.1% naphthylethylenediaminedihydrochloride (NED) in H_2O + 2% sulphanilamide in 5% H_3PO_4] was added and incubated at 37°C for 10 min and the absorbance was read at 540 nm. For nitrite assay, vanadium chloride (III) was omitted from the test. Results were reported as percentage of starved plants.

Total amino acid assay

Total amino acids were determined by reaction with ninhydrin using glycine as standard (Sun et al., 2006). Results were reported as percentage of starved plants.

Total protein assay

Total protein content was determined by Bradford method using bovine serum albumin (BSA) as standard (Bradford, 1976). Results were reported as percentage of starved plants.

Nitrate reductase activity assay

Nitrate reductase assays were performed by the reduction of nitrate to nitrite (Brunetti and Hageman, 1979). One unit of enzyme activity is defined as the production of 1 µM nitrite per min. Results were reported as percentage of starved plants.

Total carbohydrate determination

Carbohydrate contents were determined by phenol-sulfuric acid method (Rao and Pattabiraman, 1989). Briefly, to 1 ml of sugar solution, 50 µl 80% phenol and then 3 ml 98% sulfuric acid was added. The mixture vortexed were kept at room temperature for 30 min and the absorbance read at 490 nm. Results were reported as percentage of starved plants.

Statistical analyses

All of data were analyzed as a completely randomized design with three replications. Data were expressed as means ± standard deviation (SD). The statistical significance of differences between treatments was determined by analysis of variance (ANOVA) and then testing for differences between means was by Duncan's new multiple range test and SPSS software version 16 at $p < 0.05$.

RESULTS

Effects of nitrate supply on the nitrate accumulation in the roots and leaves

The concentration of nitrate in the roots and leaves (nitrate accumulation) of starved plants was taken as 100%. In plants supplied with potassium nitrate at 25, 50 and 100 mM, the nitrate concentrations in the roots were 366, 470 and 370%, respectively. Corresponding values in plants supplied with sodium nitrate at 25, 50 and 100 mM were 324, 430 and 368%, respectively ($p < 0.05$, $n = 3$). However, in plants supplied with potassium nitrate at 25, 50 and 100 mM, the nitrate concentrations in the leaves were 388, 621 and 509%, respectively. Corresponding values in plants supplied with sodium nitrate at 25, 50, and 100 mM were 286, 551, and 461%, respectively ($p < 0.05$, $n = 3$). Thus, both sodium and potassium nitrate supplementation increased nitrate accumulation in a dose dependent manner. However, in plants supplemented with potassium nitrate, the concentrations of nitrate in the leaves were higher than in the plants supplemented with sodium nitrate (Table 1).

Effects of nitrate supply on the nitrite production in the roots and leaves

The concentration of nitrite in the starved plants was taken as 100%. In plants supplied with potassium nitrate at 25, 50 and 100 mM, the nitrite concentrations in the roots were 209, 270, and 246% of the control plants, respectively. However, in plants supplied with sodium nitrate at 25, 50 and 100 mM, the corresponding values were 142, 249, and 242%, respectively ($p < 0.05$, $n = 3$). In plants supplied with potassium nitrate at 25, 50, and 100 mM, the nitrite concentrations in the leaves were 160, 181 and 150% of the control plants, respectively. However, in plants supplied with sodium nitrate at 25, 50 and 100 mM, the corresponding values were 137, 157,

Table 1. Effects of nitrate concentration on nitrate accumulation in starved plants.

Treatment	Nitrate in root (%)	Nitrate in leaf (%)
Control	322 ± 20^{d}	488± 15^{c}
Starved	100± 16^{e}	100± 11^{f}
KNO_3 (25 mM)	366± 14 ^{c}	388± 17^{d}
KNO_3 (50 mM)	470± 18^{a}	621± 15^{a}
KNO_3 (100 mM)	370± 20^{c}	509± 21^{bc}
$NaNO_3$ (25 mM)	324± 12^{d}	286± 13^{e}
$NaNO_3$ (50 mM)	430± 10^{b}	551± 30^{b}
$NaNO_3$ (100 mM)	368± 30^{c}	461± 42^{c}

Means with the same letter within an assay are not significantly different as determined by SPSS software version 16 at $p< 0.05$.

Table 2. Effects of nitrate concentration on the nitrite concentration in the starved plants.

Treatment	Nitrite in root (%)	Nitrite in leaf (%)
Control	215± 8^{c}	152±6^{b}
Starved	100 ± 15^{e}	100±4^{d}
KNO_3 (25 mM)	209±9^{c}	160±6^{b}
KNO_3 (50 mM)	270±8^{a}	181±6^{a}
KNO_3 (100 mM)	246±8^{b}	150±5^{b}
$NaNO_3$ (25 mM)	142±12^{d}	137±3^{c}
$NaNO_3$ (50 mM)	249±13^{b}	157±7^{b}
$NaNO_3$ (100 mM)	242±11^{b}	129±9^{c}

Means with the same letter within an assay are not significantly different as determined by SPSS software version 16 at $p< 0.05$.

and 129%, respectively ($p < 0.05$, $n = 3$). Thus, both sodium and potassium nitrate supplementation increased nitrite accumulation in a dose dependent manner. However, in plants supplemented with potassium nitrate the concentrations of nitrite in the leaves were higher than in plants supplemented with sodium nitrate (Table 2).

Effects of nitrate supply on the amino acid production in the roots and leaves

The concentration of amino acids in the starved plants was taken as 100%. In plants supplied with potassium nitrate at 25, 50 and 100 mM, the amino acid concentrations in the roots were 106, 106 and 106%, respectively but in plants supplied with sodium nitrate at 25, 50 and 100 mM, the amino acid concentrations were 106, 105 and 106% relative to the control plants, respectively ($p < 0.05$, $n = 3$). In plants supplied with potassium nitrate at 25, 50 and 100 mM, the amino acid concentrations in the leaves were 174, 227 and 188%, respectively but in plants supplied with sodium nitrate at 25, 50 and 100 mM, the amino acid concentrations were 113, 178 and 164% relative to the control plants, respectively ($p < 0.05$, $n = 3$). Thus, both sodium and potassium nitrate supplementation increased amino acid accumulation in a dose dependent manner. However, in plants supplemented with potassium nitrate, the concentrations of amino acids in the leaves were higher than in plants supplemented with sodium nitrate (Table 3).

Effects of nitrate supply on the total protein production in the roots and leaves

The concentration of proteins in the starved plants was taken as 100%. In plants supplied with potassium nitrate at 25, 50 and 100 mM, the protein concentrations in the roots were 141, 154 and 158%, respectively but in plants supplied with sodium nitrate at 25, 50, and 100 mM, the proteins concentrations were 132, 149 and 150%, respectively ($p < 0.05$, $n = 3$). In plants supplied with potassium nitrate at 25, 50 and 100 mM, the protein concentrations in the leaves were 123, 138 and 129%, respectively but in plants supplied with sodium nitrate at 25, 50 and 100 mM, the proteins concentrations were

Table 3. Effects of nitrate concentration on the amino acid concentration in the starved plants. Means with the same letter within an assay are not significantly different as determined by SPSS software version 16 at p< 0.05.

Treatment	Amino acid in root (%)	Amino acid in leaf (%)
Control	105 ± 0.7^{a}	171± 7.6^{c}
Starved	100 ± 1^{a}	100± 8^{d}
KNO_3 (25 mM)	106 ± 1.4^{a}	174± 7.6^{bc}
KNO_3 (50 mM)	106 ± 1.3^{a}	227± 5.6^{a}
KNO_3 (100 mM)	106 ± 0 .7^{a}	188± 15.4^{b}
$NaNO_3$ (25 mM)	106 ± 1.1a	113± 6^{d}
$NaNO_3$ (50 mM)	105 ± 1.4^{a}	177± 6.4^{bc}
$NaNO_3$ (100 mM)	106 ± 1.1^{a}	164± 9^{c}

Table 4. Effects of nitrate concentration on the protein concentration in the starved plants. Means with the same letter within an assay are not significantly different as determined by SPSS software version 16 at p< 0.05.

Treatment	Protein in root (%)	Protein in leaf (%)
Control	138± 8.5^{cd}	131± 3^{ab}
Starved	100± 6.8^{e}	100± 4^{c}
KNO_3 (25 mM)	141± 4^{bdc}	123± 5.4^{b}
KNO_3 (50 mM)	154± 7.4^{ab}	138± 4^{a}
KNO_3 (100 mM)	158± 12^{a}	129± 3.2^{ab}
$NaNO_3$ (25 mM)	132± 5.4^{d}	131± 4.6^{ab}
$NaNO_3$ (50 mM)	149± 4.6^{abc}	136± 6^{a}
$NaNO_3$ (100 mM)	150± 5.3^{abc}	132± 5.7^{ab}

131, 136 and 132%, respectively ($p < 0.05$, $n = 3$). Thus, both sodium and potassium nitrate supplementation increased protein accumulation but there was no significant difference among various concentrations of nitrate and also between potassium and sodium nitrate (Table 4).

Effects of nitrate supply on the nitrate reductase activity in the roots and leaves

The activity of nitrate reductase in the leaves of starved plants was taken as 100%. In plants supplied with potassium nitrate at 25, 50 and 100 mM, the enzyme activity in the roots increased by 198, 249 and 194%, respectively. However, in plants supplied with sodium nitrate at 25, 50 and 100 mM, these increases were 176, 232 and 182%, respectively ($p < 0.05$, $n = 3$). In plants supplied with potassium nitrate at 25, 50 and 100 mM, the enzyme activity in the leaves increased by 170, 213 and 123%, respectively. However, in plants supplied with sodium nitrate at 25, 50 and 100 mM, these increases were 147, 163 and 119%, respectively ($p < 0.05$, $n = 3$). Thus, both sodium and potassium nitrate supplementation increased nitrate reductase activity in a dose dependent manner. However, in plants supplemented with potassium nitrate, the activity of nitrate reductase in the leaves was higher than in the plants supplemented with sodium nitrate (Table 5).

Effects of nitrate supply on the total carbohydrate content in the roots and leaves

The concentration of carbohydrate in the starved plants was taken as 100%. In plants supplied with potassium nitrate at 25, 50 and 100 mM, the concentrations of carbohydrates in the roots increased by 149, 155 and 206%, respectively but in plants supplied with sodium nitrate at 25, 50 and 100 mM, the respective values were 165, 170 and 195%, respectively ($p < 0.05$, $n = 3$). In plants supplied with potassium nitrate at 25, 50 and 100 mM, the concentrations of carbohydrates in the leaves increased by 172, 189 and 138%, respectively but in

Table 5. Effects of nitrate concentration on the nitrate reductase activity in the starved plants. Means with the same letter within an assay are not significantly different as determined by SPSS software version 16 at p< 0.05.

Treatment	Nitrate reductase activity in root (%)	Nitrate reductase activity in leaf (%)
Control	180±9.5^{b}	157± 6^{bc}
Starved	100±7^{c}	100± 9.6^{e}
KNO_3 (25 mM)	198± 13^{b}	170± 6^{b}
KNO_3 (50 mM)	249±18^{a}	213± 11.4^{a}
KNO_3 (100 mM)	194± 7^{b}	123± 9.3^{d}
$NaNO_3$ (25 mM)	176±8^{b}	147± 9.5^{c}
$NaNO_3$ (50 mM)	232± 15^{a}	163± 8.7^{bc}
$NaNO_3$ (100 mM)	182± 8.6^{b}	119± 5.8^{d}

Table 6. Effects of nitrate concentration on carbohydrate production in the starved plants. Means with the same letter within an assay are not significantly different as determined by SPSS software version 16 at p< 0.05.

Treatment	Carbohydrate in root (%)	Carbohydrate in leaf (%)
Control	155± 4de	160±9.5^{b}
Starved	100±3^{f}	100±5.5^{d}
KNO_3 (25 mM)	149±4.3^{e}	172± 10^{b}
KNO_3 (50 mM)	155± 2de	189± 13^{a}
KNO_3 (100 mM)	206± 8^{a}	138± 6.3^{c}
$NaNO_3$ (25 mM)	165±6.4^{cd}	143±8.3^{c}
$NaNO_3$ (50 mM)	170±3.5^{c}	162±10^{b}
$NaNO_3$ (100 mM)	195±10^{b}	104± 5^{d}

plants supplied with sodium nitrate at 25, 50 and 100 mM, the respective values were 143, 162 and 104%, respectively ($p < 0.05$, $n = 3$). Thus both sodium and potassium nitrate supplementation increased carbohydrate in a dose dependent manner. However, in plants supplemented with potassium nitrate, the content of carbohydrate in the leaves was higher than in plants supplemented with sodium nitrate (Table 6).

DISCUSSION

Nitrate in soil is absorbed by root cells mediated by nitrate transporter. Nitrate is reduced in the roots or stored in root cells vacuoles. However, the excess nitrate is transported to the leaves where it is reduced in the leaf cell cytoplasm and the excess of which is stored in leaves cells vacuoles. Thus, nitrate accumulation in the leaves increased with nitrate supply. The efficiency of net nitrate uptake is under negative feedback control by nitrate accumulation (Skrdleta et al., 1979; Chen et al., 2004). Therefore, when nitrate supply is higher than the plant demand, the decrease in nitrate accumulation might be due to the decrease of nitrate uptake as a result of the negative feedback regulation by accumulated nitrate (Stohr, 1999; Sivasankar et al., 1997). The rate of nitrate uptake relies on the activity of nitrate transport systems in the plasma membrane of root cells. External factors, such as nitrate concentration as well as internal factors such as nitrogen metabolites (ammonium and glutamine) all regulate the rate of nitrate uptake (Miller et al., 2007; Orsel et al., 2002). Exposure of roots to nitrate causes the induction of nitrate transport 2 transcripts, which leads to nitrate uptake by positive feed forward, whereas metabolites resulting from nitrate reduction, most likely ammonia and glutamine, down regulate NRT2 (Walch-Liu and Forde, 2008; Remans et al., 2006).

After uptake, nitrate can be reduced directly to nitrite by nitrate reductase. The nitrate reductase activity increased rapidly with the increase in internal nitrate within the lower nitrate concentration ranges and started to decrease when endogenous nitrate was high (Vidal et al., 2010; Debouba et al., 2006). In our experiments, the nitrate had a positive effect on nitrate reductase activity,

only at the lower nitrate supplies, while at the higher nitrate supplies, the nitrate reductase activity decreased considerably. The present results suggest that nitrate reductase activity and nitrite accumulation depend on the exogenous nitrate.

Nitrite itself is reduced to ammonium by palstidic nitrite reductase. Nitrite reductase is activated by both nitrate and nitrite ions by positive feed forward, whereas nitrate metabolites, most likely ammonium and glutamine; down regulate this enzyme by feedback inhibition strategy. Some results have indicated that nitrite reductase is co-regulated with nitrate reductase probably by similar mechanisms (Faure et al., 1991). Co-regulation may be required to prevent the deleterious accumulation of nitrite. High concentration of nitrite is transformed into the highly mutagenic nitrous acid and nitrosamine, which also destroy the tissues. Furthermore, nitrite can also prevent electron transfer between the two photosystems in photosynthetic electron transport. A concerted metabolic regulation of nitrate and nitrite reductase would prevent the accumulation of toxic metabolic intermediates and save energy for plant growth (Malolepsza, 2007; Meyer et al., 2005; Reddy and Menary, 1990).

Ammonium itself enters amino acid pools by glutamine synthetase, which catalyses the reaction of ammonia with glutamate in the presence of ATP. The glutamine synthetase has very high affinity for ammonium and is activated by both ATP and low concentrations of ammonium. The enzyme is subject to feedback inhibition by different products of glutamine metabolism, as well as by alanine and glycine (Miller et al., 2007; Jamtgard et al., 2008). The inhibition of glutamine synthesis leads to decrease in amino acids production.

Our results indicate that nitrate supply at high concentrations reduces amino acids production (Table 3). High nitrate supply increases the endogenous ammonium which leads to increase in glutamine production. Glutamine conversely inhibits both amino acids production and further nitrate uptake (Suzuki and Knaff, 2005; Vanoni et al., 2005).

Amino acids can enter to protein structure. Previous works have shown that high nitrate supply reduces protein production (Chen et al., 2004; Skrdleta et al., 1979; Stohr, 1999; Sivasankar et al., 1997). High nitrate supply exhibited toxicity symptoms on plants, which led to the decrease in plant biomass production, specially the proteins. High nitrate accumulation results in nitrite production which is converted into nitric oxide. Nitric oxide and superoxide ions could be rapidly combined by nitrate rductase to form peroxynitrite, which is highly toxic to plant. Peroxynitrite modifies tyrosine residues of protein (nitrosation), which inactivates several proteins and enzymes leading to reduced biomass production. Therefore, high nitrate accumulation in plants resulted from high nitrate supply is harmful to human and plants life (Malolepsza, 2007; Meyer et al., 2005; Reddy and Menary, 1990).

The reaction catalyzed by sucrose phosphate synthase (SPS) is an important control point in carbohydrate biosynthesis. Carbohydrate metabolism produces both the carbon skeletons and ferredoxin for nitrate assimilation. Inhibition of photosynthesis prevents the production of the reduced ferredoxin required for nitrite reduction in chloroplasts, which leads to nitrate and nitrite accumulation (Commichau et al., 2006; Kaiser, 1997). Two key enzymes, nitrate reductase and sucrose phosphate synthase, regulate nitrogen and carbon metabolism, respectively. Both of these enzymes are directly regulated by nitrate ions (Commichau et al., 2006; Kaiser, 1997). However, nitrate can become detrimental to the plant, if its cytosolic concentration increases. Therefore, nitrate assimilation has to be equilibrated with carbon availability. It is known that with increasing nitrate supply, the total amount of structural carbohydrates remains constant whereas sugar and starch contents decrease drastically. Enzymes of carbon metabolism that are directly regulated by nitrate and responsible for this decrease are phosphenolpyruvate carboxylase and sucrose phosphate synthase (Huber and Huber, 1992; Masumoto et al., 2010). Thus, carbon and nitrogen metabolisms equilibrated with each other to reduce toxic effects of nitrate metabolite.

One of the major findings of our studies is the different effects of potassium and sodium nitrate on the nitrate assimilation pathway. In all cases studied, potassium nitrate was more effective than sodium nitrate. As an essential macronutrient for higher plants, potassium has several functions such as enzymes' activation (more than 50 enzymes), neutralization of organic and inorganic anions, maintenance of cytosolic pH, cell turgidity and phloem transport. Thus, the rates of some reactions are controlled by the rate at which K enters the cell (Page and Cera, 2006; Mathis, 2009). The activation of some carbon reduction cycle enzymes by K and its involvement in ATP production is important in regulating the rate of photosynthesis. The ATP is used as the energy source for many other chemical reactions. The electrical charge balance at the site of ATP production is maintained with K ions. When plants are K deficient, the rate of photo-synthesis and the rate of ATP production are reduced, and all of the processes dependent on photosynthetic ATP production are slowed down (Winter and Huber, 2000; Mathis, 2009). On the other hand, sugars produced in photosynthesis must be transported through the phloem to other parts of the plant for utilization and storage. Plants transport systems use energy in the form of ATP. If K is inadequate, less ATP will be available and the transport system will be affected. An adequate supply of K helps to keep all of these processes and trans-portation systems function normally (Karley and White, 2009; Mathis, 2009). Potassium also plays a major role in the transport of solutes throughout the plant in the phloem. A sufficient supply of K is essential for efficient operation of these systems (Karley and White, 2009;

Mathis, 2009). Potassium is required for every major step of protein synthesis. The reading of the genetic code in plant cells to produce proteins and enzymes that regulate all growth processes would be impossible without adequate K. When plants are deficient in K, proteins are not synthesized despite an abundance supply of available nitrogen. Instead, protein precursors such as amino acids, amides and nitrate accumulate (Armengaud et al., 2009; Karley and White, 2009). The enzyme responsible for synthesis of starch (starch synthetase) is activated by K. Thus, with inadequate K, the level of starch declines while both soluble carbohydrates and nitrogen metabolites accumulate. Photosynthetic activity affects the rate of sugar formation for ultimate starch production. Under high K levels, soluble carbohydrates are efficiently moved from sites of production and will be converted to starch in storage organs (Li et al., 2009; Amtmann and Armengaud, 2009; Armengaud et al., 2009).

However, sodium is not an essential element and it cannot be expected to have a specific role in the metabolic activities of plants. In high concentration, sodium can wholly replace potassium. In such circumstances, sodium is ineffective as a substitute for potassium and reduces the positive physiological roles of potassium (Hasegawa et al., 2000).

Conclusion

In conclusion, our results indicate that nitrate supply stimulates nitrate uptake, nitrate reduction, nitrite reduction, amino acid production, protein production, nitrate reductase activity and carbohydrate production at low concentrations of potassium and sodium nitrate by positive effects of nitrate on the nitrate assimilation pathway and also on both protein and carbohydrate production. But at high nitrate supplies, nitrate metabolites including ammonium and glutamine suppress nitrate assimilation pathway, protein synthesis and carbohydrate production by inhibiting the related rate-limiting enzymes in the biosynthetic pathway as well as by reducing photosynthesis and ATP production. In addition, the positive effects of potassium are due to its effects on enzyme activity, photophosphorylatoion, sugars transport, water and nutrient transport, protein synthesis and carbohydrate metabolism.

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