Plant Stress ©2010 Global Science Books

Mineral Nutrition of Aeroponically Grown Subtropical and Temperate Crops in the Tropics with Manipulation of Root-Zone Temperature at Different Growth Irradiances

Jie He[*]

National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, 637 616 Singapore

Correspondence: * jie.he@nie.edu.sg

ABSTRACT

Plant growth and productivity are often limited by high root-zone temperatures (RZT) which restricts the growth of subtropical and temperate crops in the tropics. High RZT temperature coupled with low growth irradiances during cloudy days which mainly lead to poor root development and thus causes negative impact on the mineral uptake and assimilation. However, certain subtropical and temperate crops have successfully been grown aeroponically in the tropics by simply cooling their roots while their aerial portions are subjected to hot fluctuating ambient temperatures. This review first discusses the effects of RZT and growth irradiance on root morphology and its biomass, the effect of RZT on uptake and transport of several macro nutrients such as N [nitrogen, mainly nitrate, (NO_3^-)], P ($H_2PO_4^-$, phosphate), K (potassium) and Ca (calcium), and micro nutrient Fe (iron) under different growth irradiances. The impact of RZT and growth irradiance on the assimilation of NO_3^- (the form of N nutrient given to the aeroponically grown plants) and the site of NO_3^- assimilation are also addressed.

Keywords: mineral uptake and transport, nitrate assimilation, root morphology

Abbreviations: A-RZT, ambient root-zone temperature; **A→20°C-RZT**, plants grown initially at A-RZT then transferred to 20°C-RZT; **A→25°C-RZT**, plants grown initially at A-RZT then transferred to 25°C-RZT; **C-RZT**, cool-RZT; **DW**, dry weight; **FW**, fresh weight; **NR**, nitrate reducatase; **NRA**, nitrate reducatase activity; **RWC**, relative water content; **RZT**, root-zone temperature; **PPFD**, photosynthetic photon flux density; **20°C→A-RZT**, plants grown initially at 20°C-RZT then transferred to A-RZT; **25°C→A-RZT**, plants grown initially at 25°C-RZT then transferred to A-RZT

CONTENTS

INTRODUCTION

Advantages of an aeroponic growth system in studying root morphology with manipulation of RZT

Aeroponics is the cultivation of plants with their root systems suspended in the air while a nutrient solution is misted at the root system to maintain a constant film of nutrients and moisture on the roots. The top of each trough was insulated by polystyrofoam planks on which the plants were anchored. The nutrient solution is pumped through a series of discharge pipes installed at the base of the aeroponics trough (Lee 1993). The system was programmed to spray the nutrient maintained at fixed temperature by the thermostat for 30 s at every 30 s interval (**Fig. 1**).

According to Waisel (2002), plants grown with their roots in the moist air could be traced back to more than 120 years ago. For plants grown in soil, their roots are hidden underground, and therefore, they must be excavated before studies can be carried out. Practically, it is not possible to dig out the whole root systems without damaging them. Hence, most root studies have been limited to roots of seedlings (Waisel 2002). Today, aeroponic system has been developed for non-destructive and continuous studies of root behaviour. It constitutes an important tool for root research (Stoner 1983; Waisel 2002). It also allows more control of the rhizosphere (root-zone) environment especially the RZ temperature (RZT) (He and Lee 1998a, 1998b; He *et al*. 2001; Qin *et al*. 2002; He and Lee 2004; He *et al*.

Received: 26 February, 2009. Accepted: 8 December, 2009.

Invited Review

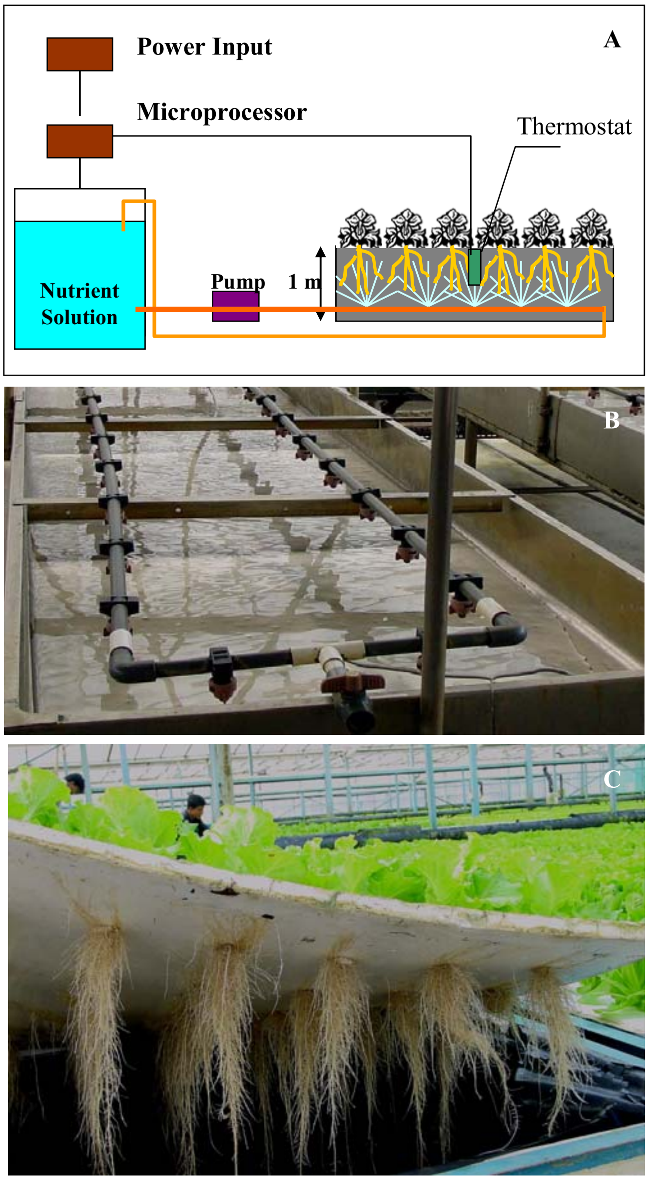

Fig. 1 Diagrammatic representation of the aeroponics system (**A**, Tan 2004), the inside of trough with nozzles arranged along the base (**B**, photo Jie He), lettuce anchored on polystyrofoam planks (**C**, photo Jie He).

2009). By using aeroponically-grown plants, it is easy to access the entire, intact root systems without destroying them. Our studies indicated that the aeroponics system can be a powerful tool for the study of RZT on photosynthesis, water relations, shoot development, root morphology, shoot-root interaction and mineral nutrition of subtropical and temperate vegetable crops grown in the tropics (He and Lee 1998a, 1998b; He and Lee 2001; He *et al*. 2001; Tan *et al*. 2002; Qin *et al*. 2002; He and Lee 2004; Qin *et al*. 2007; He *et al*. 2008, 2009).

Plant growth and productivity are often limited by high RZT which restricts the growth of temperate crops in the tropics. However, since 1997, certain temperate and subtropical crops have been successfully grown in Singapore by simply cooling their roots while their aerial portions are subjected to hot fluctuating temperatures. Today, the aeroponic system has been used for the commercial production of several high value vegetables in Singapore (He and Lee 1998a, 1998b). The other advantages that aeroponic provides for growing temperate and subtropical vegetables in the tropics for both research and commercial production are much smaller amount of nutrient solution needed to be cooled to a given optimal RZT and shorter growth durations compared to hydroponics system (Lee 1993; Lee and Chong 1996; He and Lee 1998a, 1998b).

Architecture of plant roots and mineral acquisition and assimilation

Plant roots growing in the soil are marvelously successful at foraging for nutrients and water in a hostile, competitive environment where supplies of them are very limited, local, and variable (McCully 1995). Plant root morphology is important for maximizing mineral uptake, because root systems have higher ratios of surface area to volume which more effectively explore a larger volume of soil (Lynch 1995). Root system functions like a community of individuals in which some of the roots contribute more directly to water and mineral uptake than others. Individual roots often vary widely in their ability to absorb water and minerals, depending on their order, age, and location in the soil (Volder *et al*. 2005). Similar to the plants grown in the soil, in an aeroponic system, the amount of water and nutrient available to a plant is determined by the root surface area and volume of nutrient solution with which its root are in contact with. The volume of nutrient solution depends on the amount of branching and the distances to which the root extend horizontally and vertically (Tan *et al*. 2002; Qin *et al*. 2007; He *et al*. 2009). Plant roots can alter their nutrient acquisition capacity by adjusting their longevity, morphological, architectural and/or physiological characteristics to meet changes in shoot nutrient demand in response to environmental stress (Forde and Clarkson 1999).

THE EFFECTS OF RZT AND GROWTH IRRADIANCE ON ROOT MORPHOLOGY AND PLANT PRODUCTIVITY

Root morphology

Low (suboptimal) and high (supraoptimal) soil temperatures often limit the root growth and physiological activities (Macay and Barber 1984; Moorby and Nye 1984; Huang *et al*. 1991a, 1991b). Manipulating soil or RZT alone, perhaps even has greater influence than shoot temperature manipulation (Cumbus and Ny 1982; Sattelmacher *et al.* 1990; Pardales *et al.* 1991; Menzel *et al.* 1994). Although the plant roots show very few distinctive external features there is variation of root morphology due to the variation of the root environments. Root morphology, especially root length, could be a more sensitive indicator of RZT impact than root weight. For instance, there was 5-fold difference in root length on rape (*Brassica napus* cv. 'Emeralk') between plant roots exposed to optimum RZT (30°C) and supra optimum RZT (35°C), but only 2-fold difference in root weight was found (Cumbus and Ny 1982). Such disproportionate differences between root length and root weight occurred as a result of greater development of lateral roots at the optimum temperatures. That was, at an optimum RZT, a significant higher root length/weight ratio was due to the smaller diameter of the successive orders of lateral roots. High RZT was reported to affect cellular growth of roots (Sattelmacher *et al.* 1990). It was found that in both heat tolerant and heat sensitive clones of potato (*Solanum tuberosum* L.) grown in solution culture, the size of the root system was reduced by supra-optimal RZT (30°C). This was principally as a result of the decreased number and length of lateral roots of potato clones grown at 30°C-RZT, compared with the optimal 20°C-RZT. The researchers also observed that the first symptom of heat damage was a reduction in the rate of cell division, followed by cessation of cell elongation in roots (Sattelmacher *et al.* 1990). Sorghum (*Sorghum bicolo*) grown in nutrient solution at three RZTs (constant 25°C, 40°C, or alternating 40°C/25°C day/night), Pardales *et al.* (1991) found that maximum seminal root elongation and first order lateral root initiation and elongation occurred at 25°C (control) but they were severely inhibited at 40°C. Less inhibition in root growth occurred at RZT of 40°C/ 25°C (day/night) than of 40°C (24 h/day). The reduction in both number and length of the first order lateral roots by 40°C RZT was due to the fact that many lateral roots pro-

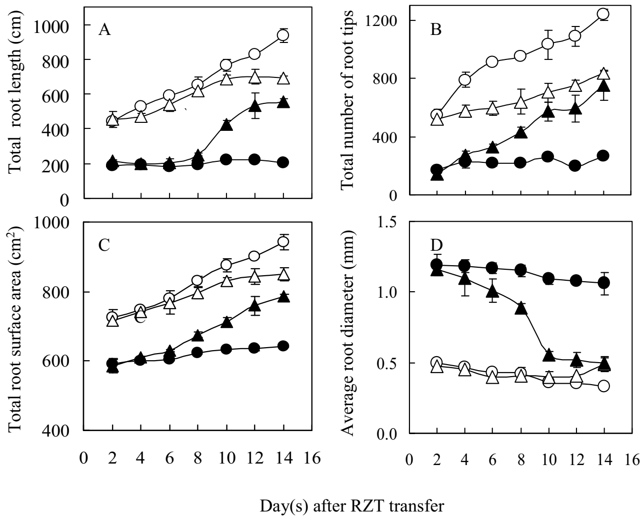

Fig. 2 Changes in total root length (**A**), total number of root tip number (**B**), total root surface area (**C**) and average root diameter (**D**) of *L. sativa* cv. 'Panama' grown at 20°C-RZT (O), A-RZT (●), 20°C→A-RZT (Δ) and A→20°C-RZT (▲) from day 0 to 14 after RZT transfer. Each point is a mean of 4 measurements from 4 different plants. Vertical bars represent standard errors (redrawn from Tan *et al.* 2002).

duced before and shortly after exposure to hot RZT treatment died off. Hence, the hot-RZT stressed plants would have a reduced ability to acquire water and nutrients from the soil. Pardales *et al.* (1991) also suggested that the better seminal root and lateral root growth at 40°C/25°C RZT than at constant 40°C was due to the fact that 25°C night temperature offset the detrimental effect of the hot day temperature (40°C). The 25°C night temperature in the 40°C/25°C RZT cycle alleviated the excessive loss of carbohydrates occurred in constant 40°C-RZT treated plants. A sufficient amount of carbohydrates were immobilized to stimulate continued root growth, including the continued increase in the length of the seminal root and the production and elongation of its first order lateral roots with time.

In the studies of temperate crops grown in the tropics, our root morphological analyses showed that, three weeks after transplanting, for *Lactuca sativa* cv. Panama grown at 20°C-RZT, the roots were longer (**Fig. 2A**) with more root tips (**Fig. 2B**) and lager total root surface area (**Fig. 2C**), and small average root diameter (**Fig. 2D**) as compared with those of A-RZT grown plants (Tan *et al.* 2002). In 20°C→A-RZT plants, there was root thickening but the increases in its length, tip number and surface area decreased over a period of 14 days of RZT transfer. The reverse was observed for A→20°C-RZT plants. Similar results were also found from our other studies with different cultivars of lettuce (Qin *et al.* 2002, 2007; He *et al.* 2009) and also the subtropical vegetable Chinese broccoli (He *et al.* 2008). Pierik *et al.* (1999) observed that root thickening, or an increase in diameter, is controlled through signals emanating from shoot apices and root tips and root thickening may also be accompanied by associated changes in microfibril angles within expanding cell walls (Pierik *et al.* 1999). One of the chemical signals may be ethylene produced in the roots caused by oxygen shortages, flooding and hot temperatures (Arshad and Frakenberger 2002). The role of ethylene in the thick root syndrome and inhibition of root elongation has been investigated by our research team. Our results indicated that the presence of an ethylene inhibitor promoted root elongation at high RZT of 38°C. In the absence of ethylene inhibitor, root elongation at high RZT was significantly inhibited (Qin *et al.* 2007). In a study with young barley, Minchin *et al.* (2002) reported that phloem loading of carbohydrate within a mature exporting leaf is shown to respond quickly to a change in temperature of the root and shoot meristems. In our recent $^{14}CO_2$-feeding experiments (He *et al.* 2009), it was found that the developing leaves of 20°C-RZT lettuce plants (*L. sativa* cv. 'Panama') had higher ^{14}C-photoassimilate than that of A-RZT plants. A→20°C-RZT plants also had higher ^{14}C-photoassimilates in their developing leaves than A-RZT plants. However, more ^{14}C-photoassimilate was translocated to the roots of A-RZT plants than those of 20°C→A-RZT plants, but it was mainly used for root thickening than for its elongation. Lower root/shoot ratio in 20°C-RZT than in A-RZT plants, also indicated that more photoassimilate was channelled to the shoots than the roots of 20°C-RZT plants. These results further supported that newly fixed photoassimilates were mainly for root thickening than for root elongation in A-RZT and 20°C- to A-RZT plants (He *et al.* 2009).

The plant growth and its metabolism are supported by the process of photosynthesis occurring in the leaves. Hence, light is another environmental factor that influences the growth of plant root systems. In soybean [*Glycine max* (L.) Merr.], Buttery and Stone (1988) and Del Castillo *et al.* (1989) noticed that the number of lateral roots was reduced under C-limited conditions. Demotes-Mainard and Pellerin (1992) observed a reduction in the number of internodes bearing elongated roots, together with a lower number of roots on the upper root internodes when a single maize genotype cultivated under reduced light conditions. Changes in intercepted light could affect the root elongation rate of sunflower (*Helianthus annuus* L. hybrid 'Ludo') plants in field conditions (Aguirrezabal *et al.* 1994). It was observed that after the 2-leaf stage, i.e. when the contribution of photosynthetic carbon became appreciable in root growth, daily root elongation rate was closely linked to the photosynthetic photon flux density (PPFD) intercepted from 12 to 36 h before the measurement of root elongation. For an intercepted PPFD of 275 or 550 µmol m^{-2} s^{-1}, the taproot elongated faster than secondary roots, and secondary roots originating near the base of the taproot, elongated faster than those originating near the apex. There was a relationship between the elongation rate of any secondary root apex and the ratio of intercepted PPFD to the distance between the apex and the base of the taproot ($r^2 = 0.77$). No relationships between intercepted PPFD and elongation rate were found before the 2-leaf stage, when the CO_2 labelling experiment suggested that carbon essentially originates from the seed. These findings suggested a role for source-sink relations in the root elongation among the apices and also a role for carbon nutrition in day-to-day variations of root elongation rate (Aguirrezabal *et al.* 1994).

Effect of RZT and growth irradiance on root morphology of temperate lettuce (*L. sativa* cv. 'Baby Butterhead') grown in the tropics was carried out by our research team. All plants were first grown at 20°C-RZT or A-RZT under a maximal PPFD of 1200 µmol m^{-2} s^{-1} in the greenhouse for three weeks before transferring to different PPFDs at each RZT. Analysis of root morphology was carried out every 2 days after growth irradiance transfer over a period of 8 days. At 20°C-RZT, among the three growth irradiances, plants grown under a PPFD of 1200 µmol m^{-2} s^{-1} had the longest total root length (**Fig. 3A**) with most root tips (**Fig. 3B**) and greater total root surface area (**Fig. 3C**). At 20°C-RZT, plants grown under a PPFD of 300 µmol m^{-2} s^{-1} had the shortest total root length (Fig. 3A) with smallest number of root tips (**Fig. 3B**) and total root surface area (**Fig. 3C**). The values of these parameters found in 20°C-RZT plants under a PPFD of 600 µmol m^{-2} s^{-1} were between those grown at a PPFD of 300 and 1200 µmol m^{-2} s^{-1} (**Fig. 3**). However, there were no significant differences in root diameter among the different light treatments. Similar to those plants grown at 20°C-RZT, although root growth was very slow at A-RZT, significant differences in total root length, total number of root tip and root surface area were also found among the different growth irradiances (unpublished results). Our works on the subtropical vegetable *Brassica chinensis* L. (Qin 2004) and capsicum (*Capsicum annum* Indra F1-hybrid) (Tan 2004) showed the similar results. These findings confirm our observation that although shading lowered midday ambient temperatures by 3 to 5°C as compared to

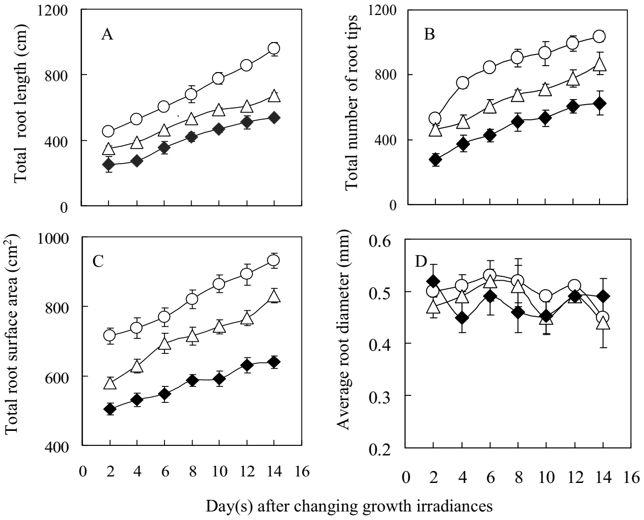

Fig. 3 Changes in total root length (**A**), total root tip number (**B**), total root surface area (**C**) and average root diameter (**D**) of *L. sativa* cv. 'Baby Butterhead' grown at 20ºC-RZT under PPFD of 1200 (O), 600 (Δ) 300 μmol photons m^{-2} s^{-1} (◆), respectively over a period of 14 days. All plants were first grown at 20ºC-RZT under PPFD of 1200 μmol photons m^{-2} s^{-1} for three weeks before transferring to the different grown irradiances. Each point is a mean of 6 measurements from 6 different plants. Vertical bars represent standard errors (He *et al.* unpublished data).

full sunlight condition (He *et al.* 2001), low growth irradiances during cloudy days coupled with hot RZT mainly leads to poor root development and thus, may cause negative impact on the mineral uptake and assimilation (discussed next). With sunflower (*H. annuus* L. hybrid 'Ludo') plants, Aguirrezabal and Tardieu (1996) also investigated the effects of PPFD and soil temperature on the elongation rate of root system. They showed that the direct effect of root apex temperature on individual root elongation rate was surprisingly low in the range from 13-25°C. Root elongation rate was related to intercepted PPFD and to distance to the source, both in the field and in the growth chamber. Recently, our research showed that under low growth irradiance, root length, root surface area, root volume and number of root tips obtained from *L. sativa* cv. 'Baby Butterhead' significantly increased when Ca, K and P concentrations in the nutrient solution were increased by 25% at 25°C-RZT (Luo 2008; Luo *et al.* 2009). The effects of different Ca, K and P concentrations on the development of root morphology under different growth irradiances could be through their effect on altering assimilate partitioning between starch and soluble sugar (Luo 2008). P availability under the different RZTs could be an important factor modifying root architecture (Pellerin *et al.* 2000; Williamson *et al.* 2001; Fitter *et al.* 2002; Wissuwa *et al.* 2005; Lambers e*t al.* 2006; Zhou *et al.* 2008). Fe availability under the different RZTs and its interaction with other mineral such as P could also be associated with root morphology (Ward *et al.* 2008).

Plant productivity

The importance of temperature for plant productivity has been demonstrated by exposing the whole plant to various temperatures (Wurr *et al.* 1992; Teskey and Will 1999). However, manipulating soil or RZT alone also has a great influence on plant productivity, perhaps even greater than shoot temperature manipulation (Gosselin and Trudel 1984; Janes *et al.* 1988, Kuroyanagi and Paulsen 1988; Sattelmacher *et al.* 1990; Pardales *et al.* 1991; Bode Stoltzfus *et al.* 1998; He and Lee 1998a, 1998b; Qin *et al.* 2002; Qin 2004; Tan 2004; Luo 2008). Wurr *et al.* (1992) found that larger and denser heads of crisp lettuce (*L. sativa* cv. 'Saladin') were associated with low temperatures during the period up to and around hearting. Less dense and smaller heads were primarily associated with higher temperatures in the period up to hearting. Gosselin and Trudel (1984) subjected greenhouse-grown *Lycopersicon esculentum* to the same shoot temperature of 25°C but different RZTs of 12.7, 18.0, 23.6, 29.4 and 34.9°C. They reported that maximum shoot DW, leaf area and fruit weight were attained at 23.6°C-RZT when light is not limiting. However, the fruit and shoot DWs as well as leaf area were significantly lower at 34.9°C than 23.6°C-RZT. When roots of *L. esculentum* were grown in tissue cultures held at various RZT (ranging from 7 to 45°C) but similar shoot temperature (17-25°C), it was found that root fresh weight (FW) and DW increased up to 28°C and declined thereafter (Janes *et al.* 1988). This study also found that aerobic respiration, providing energy for growth and nutrient uptake, was the highest at RZT between 40 and 45°C. There was also higher cell-membrane bound K$^+$-stimulated ATPase activity with increasing RZT (from 7 to 37°C). It was interesting that the higher respiration and ATPase activity were not accompanied by higher root growth at higher RZT (28-37°C). Thus, this suggests that root growth and development may be inhibited at the expense of higher respiration and ATPase activity at high RZT. In their study using various RZT (25, 30, 35, 40 and 45°C), Bode Stoltzfus *et al.* (1998) found that RZT greater than 35°C resulted in sharp linear decrease in FW, DW and leaf number of *Cucumis melo* L. 'Gold Star'. Three aeroponically grown lettuce plants, we found that the aerial parts of lettuce plants maintained at hot A-RZT while their

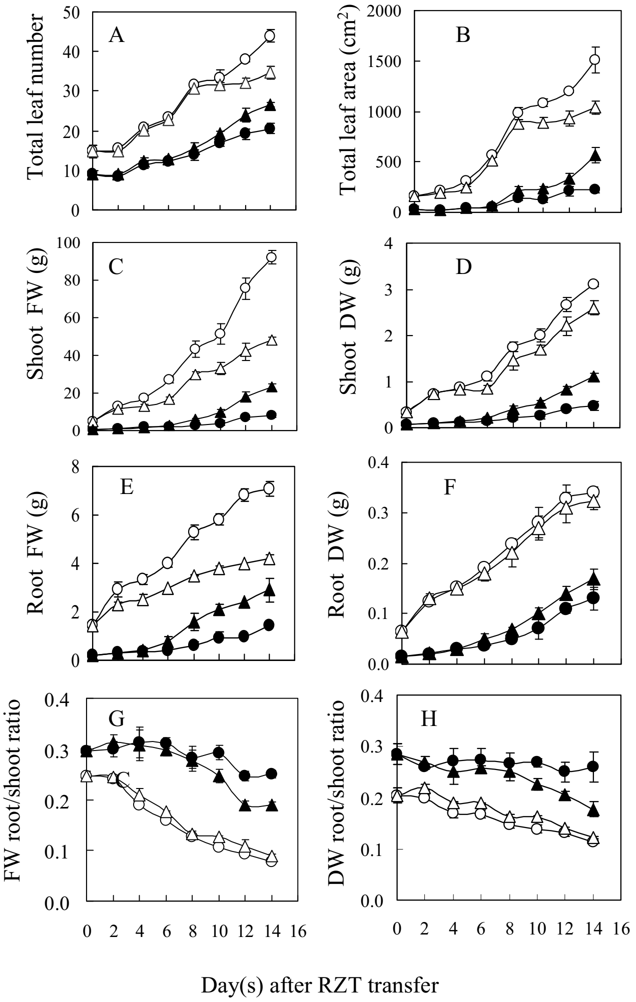

Fig. 4 Changes in total leaf number and leaf area (**A, B**), shoot FW and DW (**C, D**), root FW and DW (**E, F**), and FW and DW root/shoot (**G, H**) of *L. sativa* cv. 'Panama' plants grown at 20ºC-RZT (O), A-RZT (●), 20ºC→A-RZT (Δ) and A→20ºC-RZT (▲). Each point is the mean of six measurements. Vertical bars represent the standard errors (redrawn from He *et al.* 2009).

RZ was cooled to 20°C, productivity increased by more than 50% as compared to those whole plants that were exposed to hot A-RZT (He and Lee 1998a). Studying *L. sativa* cv. 'Panama', He *et al.* (2009) reported that increases in leaf area, shoot and root FW were slower in 20°C→A-RZT than in 20°C-RZT plants. Conversely, A→20°C-RZT plants had higher increases in these parameters than A-RZT plants. The younger developing leaves of 20°C-RZT lettuce exhibited greater sink strength and thus higher growth rate of leaves. Total leaf number and leaf area data revealed that 20°C-RZT lettuce had greater leaf initiation and expansion (leaf area) than A-RZT plants (**Figs. 4A, 4B**; He *et al.* 2009). This was in accordance with other observations that RZT influenced leaf growth in *L. esculentum* (Gosselin and Trudel 1984), *Triticum aestivum* (Kuroyanagi and Paulsen 1988) and *C. annuum* (Dodd *et al.* 2000). 20°C→A-RZT resulted in declining the total leaf expansion rates while A→20°C-RZT increased this parameter (**Fig. 4B**). Other than leaf expansion and initiation, both shoot and root FW and DW were negatively affected by A-RZT (**Figs. 4C-F**). This has provided further evidence that RZT has a significant impact on shoot and root development (Sattelmacher *et al.* 1990; Pardales *et al.* 1991; DeLucia *et al.* 1992; Bode Stoltzfus *et al.* 1998). Lower root/shoot ratio in 20°C-RZT than in A-RZT plants suggested that more photoassimilates were channelled to the shoots than to the roots of 20°C-RZT plants (**Figs. 4G, 4H**) (He *et al.* 2009). Qin *et al.* (2002) and Tan *et al.* (2002) reported that similar results from the studies of different cultivars of lettuce plants. Results from our team on aeroponically grown subtropical plants in the tropics with manipulation of RZT, we also found cooling the RZ of *C. annuum* L (Dodd *et al.* 2000), *B. chinensis* L. (Qin 2004), *Brassica alboglabra* L. (He *et al.* 2008) enhanced their productivity. Studied with three aeroponically grown *L. sativa* cultivars, also showed that both FW and DW were much higher in plants grown under 100% of prevailing solar radiation than under 70% of prevailing solar radiation (He and Lee 1998b) and Luo (2008) with other *L. sativa* cv. Baby Butterhead (**Fig. 5**). Shoot and root FW and DW increased with increasing PPFD for both 20°C-RZT and A-RZT plants (**Figs. 5A-D**). However, FW and DW shoot/root ratio (**Figs. 5E, 5F**) deceased with increasing PPFD. The decreases in shoot/root ratios with increasing PPFD were much more drastic at A-RZT than at 20°C-RZT. High shoot/root ratio is an important mechanism in plant adaptation to environmental stresses (King *et al.* 1995). The shoot/root ratio is often decreased by soil factor such as water stress (Chaves *et al.* 2002; Carvalho *et al.* 2006; Izanloo *et al.* 2008), low availability of phosphorus, P (Brouwer 1983; Fredeen *et al.* 1989; Mollier and Pellerin 1999; Varadarajan *et al.* 2002; Vance *et al.* 2003; Wissuwa *et al.* 2005) or N (Huber *et al.* 1989; Durieux *et al.* 1994; Paterson *et al.* 2006) or shoot environments such as high light and high atmospheric CO_2 (Schenk 1995; Crookshanks *et al.* 1998; Gavito *et al.* 2001). The effect of low RZT (Engels *et al.* 1992; Macuff *et al.* 1994; Zhang and Smith 1994; Gavito *et al.* 2001) or high RZT temperature (Johnson and Ingram 1984; Graves *et al.* 1989; He and Lee 1998a, 1998b) on dry matter partitioning between shoot and root is not easy to predict as low RZT or high RZT may directly affect the nutrient and water uptake. According to Brouwer (1983), a general principle of the functional equilibrium that exists between shoots and roots is that when shoot growth is favoured, the limiting substance is absorbed by the shoots, while growth is limited by an essential substance absorbed by the roots, root growth is favoured. Under light-limited conditions at optimal 20°C-RZT, the growth of lettuce roots is reduced more than the growth of the aerial parts, which leads to an increase in the shoot/root ratio. However, when there was interaction between limited light and unflavoured A-RZT, the shoot/root ratio was even greater than a single factor of limited light (**Figs. 5E, 5F**). These findings suggest that light is primary factor in partitioning the new assimilates that provide optimal proportions of shoot and root as plant production is driven by photosynthesis (Loomis and Amthor 1999).

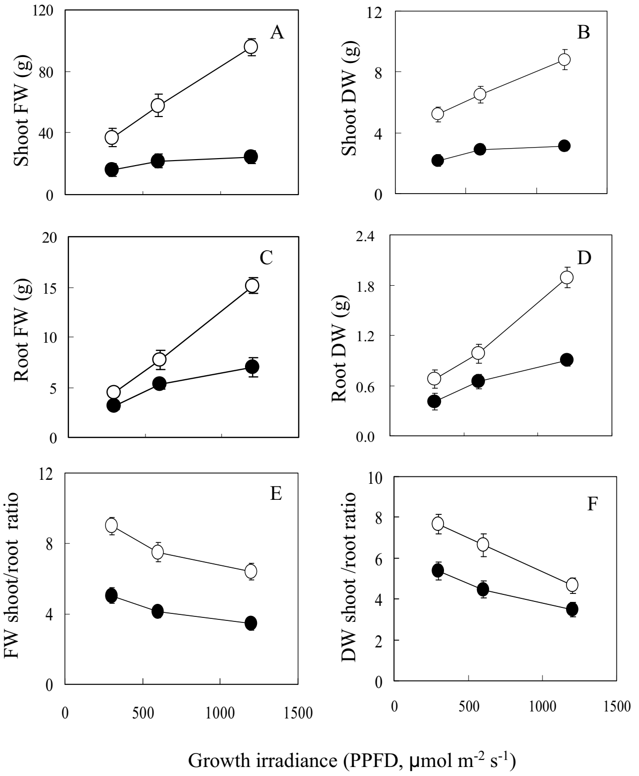

Fig. 5 Shoot FW and DW (**A, B**), and Root FW and DW (**C, D**), and FW and DW shoot/root ratio (**E, F**) of *L. sativa* cv. 'Baby Butterhead' grown at 20°C-RZT (○) and A-RZT (●) under different growth irradiances for 35 days after transplanting. Each point is the mean of 6 measurements. Each point is the mean of 6 measurements (modified from Luo 2008).

THE EFFECTS OF RZT ON MINERAL UPTAKE AND TRANSPORT UNDER DIFFERENT GROWTH IRRADIANCES

The temperature optimum for plant growth varies from species to species, and tends to be lower for roots than for shoots (Brouwer 1983). Changes in plant growth rate induced by alteration of RZT are reflections of changes in uptake and transport of nutrient elements under different RZTs in many species (Cooper 1973; DeLucia *et al.* 1992; Tan *et al.* 2002; He *et al.* 2008; Luo 2008). Several studies have indicated that root or soil temperature plays an important role in plant mineral nutrition (DeLucia *et al.* 1992; Hood and Mills 1994; Menzel *et al.* 1994; Bode Stoltzfus *et al.* 1998). A temperature-induced reduction in the nutrient supply through the roots can be due to effects on (1) the supply of nutrients by the soil (Marschner 1995) (2) the growth, morphology, or distribution of the roots (Engels and Marschner 1992); and (3) the uptake efficiency per unit of root length (Bravo and Uribe 1981; Mackay and Barber 1984). For soil grown plants, unfavourable RZT causes not only poor root growth and development (Engels and Marschner 1992) but also results in lower chemical (Singh and Sharma 1972) and spatial (Mackay and Barker 1984) nutrient availability in soil and reduce uptake efficiency per unit root length (Brovo and Uribe 1981; Mackay and Barber 1984). However, the apparent effects of temperature on ion uptake vary with the ion, experimental procedure, species and age of plants (Josh 1983; Engles and Marschner 1990; Bingham and Cumpus 1991). In theory, the growth-limiting factors in soilless culture, including aeroponic systems are the availability of photosynthetic light and CO_2, as aeroponic systems supplying plants with a misted nutrient solution could reduce water and nutrient stress. The root morphological analyses revealed that hot A-RZT inhibited root elongation, hair formation and increased root diameter of temperate and subtropical crops grown in the tropics as discussed above. Hence, the root morphologies of these aeroponically grown crops could be the main factors that influence the ability of these plants to uptake and transport nutrient under different RZTs (Qin *et al.* 2002; Tan *et al.* 2002; He *et al.* 2009). This section discusses the effects of RZT and growth irradiance on both mineral uptake and transport.

Macronutrients - NO_3^-, P, K and Ca

Macronutrients used in soilless culture are the dissolved cations and anions including primary macronutrients such as N (mainly NO_3^-), P ($H_2PO_4^-$, phosphate) and K^+ (potassium). Other macronutrients are Ca^{2+} (calcium), Mg^{2+} (magnesium), and SO_4^{2-} (sulfate). This paper mainly focuses on the effects of RZT and light on the uptake and transport of NO_3^-, P, K and Ca.

NO_3^- is the major N source available in aerobic soils and is the mineral nutrient that most frequently limits plant growth (Marschner 1995). Once absorbed by root cells, it can be redirected out of the root cell, either by unloading into the xylem vessels to reach the aerial organs or by extrusion into the external medium (Forde and Clarkson 1999). The third possible fate for NO_3^-, in roots as well as in leaves, is its uptake by the vacuole where it can be of considerable importance for osmoregulation (Smirnoff and Stewart 1985) or serves as a reservoir to sustain the growth process when the external N supply becomes limiting (der Leij *et al.* 1998). Although it is generally agreed that RZT can affect N nutrition, there is no clear consensus whether high or low RZT decreases or increases NO_3^- uptake and transport in plants (BassiriRad *et al.* 1991; Atkin and Cummins 1994; Ali *et al.* 1998). The effect of low RZTs on NO_3^- contents in *Dryas integrifolia* and *Oxyria digya* plants were assessed by Atkin and Cummins (1994). For example, following 4 weeks of growth in a nutrient solution (with 0.5 mM NO_3^-) at 20°C, individuals of both species were transferred to the same solution with temperatures set at 20 or 3°C, for a further 14 days with shoots maintained at 20°C. For *D. integrifolia* plants, little change in tissue NO_3^- content was observed following 14 days of 3°C RZT treatment. However, a significant reduction in the absolute tissue NO_3^- content were observed in roots and shoots of *Oxyria digya* plants exposed to 3°C RZT. By growing oilseed rape plants at three RZT regimes (20/20, 16/8, or 12/12°C, day/night) with shoot maintained at constant 20°C, Ali *et al.* (1998) showed that with decreased RZT, NO_3^- contents increased in the shoot and decreased in the roots. The total N content in both shoot and roots was not affected by the RZT regimes. However, the concentration of metabolized N (the difference between total N and N-NO_3^-) was significantly higher in shoots but lower in roots when plants are grown at RZT of 20/20°C than at 12/12°C. Ali *et al.* (1998) suggested that low RZT retarded the upward transport of the metabolized N due to its lower demand as a result of the reduced shoot growth at the low RZT and/or as a consequence of the reduction in the upward water flow. Contradicting these results, BassiriRad *et al.* (1991) reported that a high RZT of 40°C greatly decrease ion flux, particularly NO_3^- to the xylem of detached roots of both *Hordeum vulgare* and *S. bicolor* seedlings. Hood and Mills (1994) also reported that high RZT of 36°C decreased the N uptake rate of *Antirrhinnum major*. With aeroponically grown temperate lettuce in the tropics, it was generally noted that the total shoot and root NO_3^- contents (concentration × DW) of plants grown under all RZT treatments increased during the experimental period (**Figs. 6A, 6B**). However, the rate of increase was the fastest for lettuce grown at 20°C-RZT and the slowest for plants grown at A-RZT. When the plants were transferred from 20°C-RZT to A-RZT, the rates of increase in shoot and root NO_3^- contents decreased. Therefore, on day 12th after RZT transfer, the shoot NO_3^- of 20°C →A-RZT plants were significantly lower than those grown at 20°C-RZT. Conversely, A→20°C-RZT plants demonstrated a relatively rapid rise in the rate of increase of total shoot and root NO_3^- contents as compared to their control (A-RZT plants). The reduction of NO_3^- in A-RZT and 20°C→A-RZT plants may be due to inhibition of NO_3^- uptake at hot A-RZT. Bode Stoltzfus *et al.* (1998) suggested that RZT greater than 35°C most likely restricted the absorption of mineral nutrients. This may be associated with poor root development at high temperature which inevitably reduced plants ability to uptake certain nutrients (Clarkson *et al.* 1986; Janes *et al.* 1988; Du and Tachibana 1994). As NO_3^- is not only a major N source for nutrition of plants, but also acts as a signal to modulate plant development this had led to the hypothesis that plant cells must have a sensor for NO_3^- availability (Crawford and Glass 1998). The initiation and elongation of *Arabidopsis* lateral root development is stimulated by local availability of NO_3^- (Little *et al.* 2005). However, when the results of NO_3^- content from lettuce plant was plotted against the root tip number and total root length over the 12 days of RZT transfer, no significant relationships were established (Tan *et al.* 2002). This finding suggested that the uptake of NO_3^- might also be due to the pronounced effects of temperature on enzymatic reactions and ion transport (DeLucia *et al.* 1992; Cruz *et al.* 1993). This is evident from their lower shoot/shoot ratios in A-RZT, 20°C→A-RZT and A→20°C-RZT plants (**Fig. 6C**) which imply that once the plants were grown under high A-RZT may affect enzymatic activity such as nitrate reductase (NR) for NO_3^- and/ion transport (Cruz *et al.* 1993). This may be, however a sign of lower internal demand for growth when plants were grown at high A-RZT as N uptake usually has been based on the balance of demand and supply (Engels and Marschner 1990; Dodd *et al.* 2000; He *et al.* 2001; Dodd *et al.* 2003). Higher plants acquire NO_3^- in soil through the activity of both high-affinity transport systems (HATS) and low-affinity transport systems (LATS), respectively. It is generally assumed that root NO_3^- uptake is mostly determined by the activity of the HATS (Crawford and Glass 1998; von Wirén *et al.* 2000, Malagoli *et al.* 2004; Krouk *et al.* 2006). In an aeroponic system, although all plants were supplied with full NO_3^- continuously, reduction of NO_3^- uptake and transport of NO_3^- suboptimal or supraoptimal RZT could result from inactivity of both HATS and LATS (Malagoli *et al.* 2004). The shoot/root ratios of NO_3^- were calculated from the total shoot and root NO_3^- contents of lettuce plants at day 12 after RZT transfer (**Fig. 6C**). It was found that NO_3^- shoot/root ratios of A-RZT, 20°C→A-RZT and A→20°C-RZT plants were all significantly lower than that of 20°C-RZT plants (**Fig. 6C**). The highest shoot/root ratio of NO_3^- found in 20°C-RZT plants may be due to the large amount of organic N are continuously recycled from the root to the shoot and then back to the root and so on (Marschner 1995). Therefore, the lower shoot/root ratios of NO_3^- in A-RZT, 20°C→A-RZT and A→20°C-RZT plants may affect the translocation of the absorbed NO_3^- (DeLucia *et al.* 1992; Cruz *et al.* 1993). Similar results were found in our studies with tropical plants *C. annuum* (Tan 2004), *B. chinensis* L. (Qin 2004), *B. alboglabra* (He et *al.* 2008).

Other than temperature, NO_3^- uptake and transport are also affected by light intensity. Modulation of NO_3^- uptake and content by light/dark transition and light intensities has been reported (Li and Oats 1994; Delhon *et al.* 1995a, 1995b, 1995c; Ferretti *et al.* 1995; Zhao and Oosterhuis 1998; Aslam *et al.* 2001). A two-year study was conducted by Zhao and Oosterhuis (1998) to determine the effect of shade (63% light reduction) on the mineral nutrient status of *Gossypium hirsutum* plants. They found that an 8-day period of shade increased petiole NO_3^- by 145%. Total N in the leaf blades of the sampled petioles increased 19% compared with those of unshaded control plants. However, bracts and floral buds of 20 day old shaded *G. hirsutum* showed a slight decrease (6%) in total N concentration. Zhao and Oosterhuis (1998) indicated that light intensity at the time of sampling must be considered when sampling *G. hirsutum* petioles and other tissues for nutrient diagnosis. In our study with *L. sativa* cv. 'Nanda', when grown under a

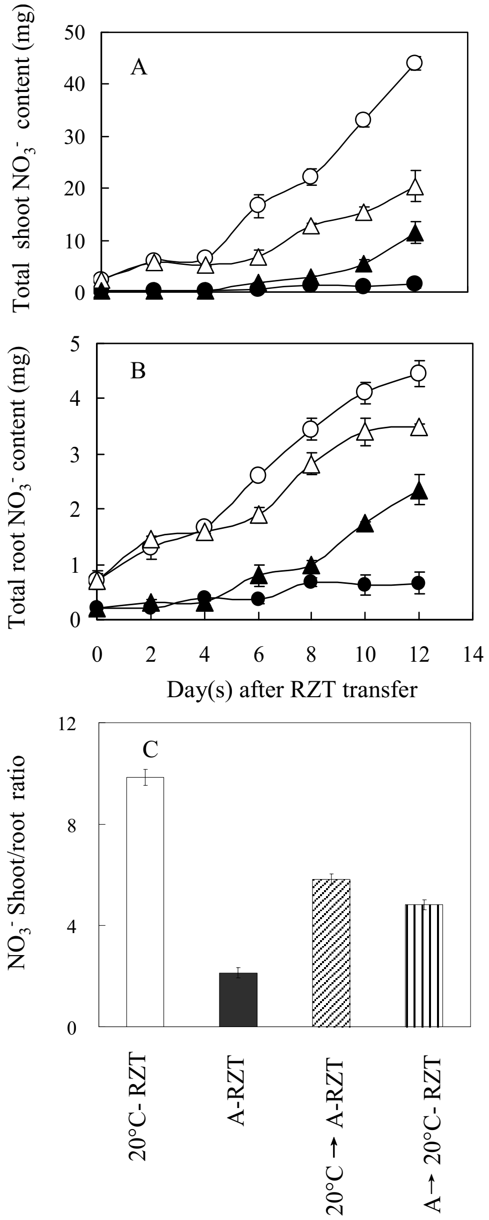

Fig. 6 Changes in total shoot and root NO$_3^-$ content (**A, B**) of *L. sativa* cv. 'Panama' grown at 20°C-RZT (○), A-RZT (●), 20°C→A-RZT (Δ) and A→20°C-RZT (▲) from day 0 to 12 after RZT transfer. Total shoot/root ratios NO$_3^-$ contents (**C**) on day 12 after RZT transition (calculated from **Figs. 6 A, B**). Each value is a mean of 4 measurements from 4 different plants. Vertical bars represent standard errors (redrawn from Tan *et al.* 2002).

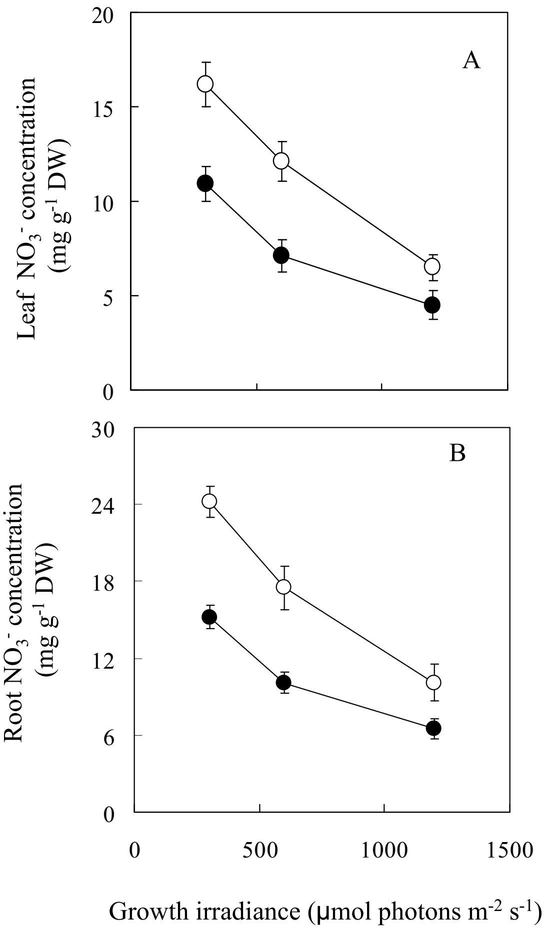

Fig. 7 Leaf (**A**) and root NO$_3^-$ concentration (**B**) of *L. sativa* cv. 'Nanda' grown at 20°C-RZT (○) and A-RZT (●) under different growth irradiances for five weeks after transplanting. Each point is the mean of 6 measurements. Each point is the mean of 6 measurements (He *et al.* unpublished).

low PPFD of 200 µmol m^{-2}s^{-1}, there was significant higher NO$_3^-$ concentration not only in root (**Fig. 7B**) but also in leaves (**Fig. 7A**) compared to other plants grown under higher PPFDs at both 20°C- and A-RZT. NO$_3^-$ could possibly induce carcinogenic effects on humans when eaten in high quantity since the leaf is the edible part of lettuce plants. Based on DW, the total accumulation (total content = concentration × DW) of NO$_3^-$ was also higher in both leaves (**Figs. 8A, 8B**) and roots (**Figs. 8C, 8D**) of *L. sativa* cv. 'Nanda' grown under lower PPFD than under higher PPFD. It was also found that plants grown under lower PPFD had much higher Leaf/root NO$_3^-$ content than that of plants grown under higher PPFD at both RZTs especially at 20°C-RZT (**Figs. 8E, 8F**). Similar results were found in our studies with tropical plants *C. annuum* and *B. alboglabra* (He *et al.* unpublished). Many hypothesis have been proposed to account for the adverse effect of light on NO$_3^-$ uptake (Delhon *et al.* 1995a, 1995b). Based on Dijkshoorn-Ben Zioni's model (Dijkshoorn *et al.* 1968; Ben Zioni *et al.* 1971), the dark inhibition of NO$_3^-$ uptake in the roots results from NO$_3^-$ assimilation in the shoots. Dijkshoorn-Ben Zioni model postulates that the OH$^-$ released by the reduction of NO$_2^-$ to NH$_4^+$ in the shoots is neutralized by the synthesis of malate. The downward phloem transport of malate with K$^+$ to the roots is decarboxylated there to generate HCO$_3^-$ which excretes out of the root and results in a stimulation of NO$_3^-$ uptake through the enhancement of a putative "HCO$_3^-$/NO$_3^-$" antiport (Ben-Zioni *et al.* 1971; Jackson *et al.* 1986; Touraine *et al.* 1992). In the study with soybean plants (*G. max* L. Merr. cv. 'Kingsoy'), the conclusion of Delhon and her co-workers was that the mechanisms evoked in the Dijkshoorn-Ben Zioni model do not play an important role in the diurnal variations of NO$_3^-$ uptake in soybean plants (Delhon *et al.* 1995c). Our data on lettuce (**Figs. 6-8**) and subtropical vegetable crops grown in the tropics also suggested that low light did not inhibited the uptake and transport of NO$_3^-$ as under low PPFD, concentration, total content and leaf /root ratio were higher than under high PPFD at both 20°C-RZT and A-RZT. The increases in NO$_3^-$ concentration, content and leaf/root ratio under low PPFD may result from several causes, namely, limitation by the availability of reducing power (Abrol *et al.* 1983; Aslam and Huffaker 1984) or inactivation of NR (to be discussed next) (Kaiser *et al.* 1992; Riens and Heldt 1992; Kaiser and Huber 1994).

Klock *et al.* (1997) found that shoot P concentration of *L. esculentum* plants grown at RZT of 36°C decreased linearly over time, while root phosphatase activity increased linearly over time. Decreased shoot growth and demand for P, along with decreased root respiration probably resulted in the decreased P uptake and shoot P concentration in *L.*

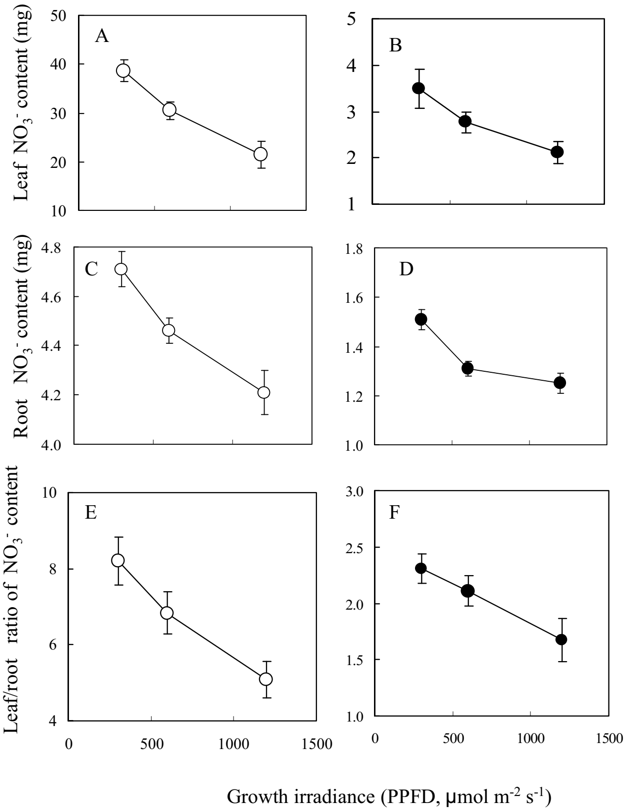

Fig. 8 Leaf (**A, B**) and root NO$_3^-$ content **(C, D)**, and shoot/root ratio of total NO$_3^-$ content (**E, F**) of *L. sativa* cv. 'Nanda' grown at 20°C-RZT (○) and A-RZT (●) under different growth irradiances for five weeks after transplanting. Each point is the mean of 6 measurements. Each point is the mean of 6 measurements (He *et al.* unpublished).

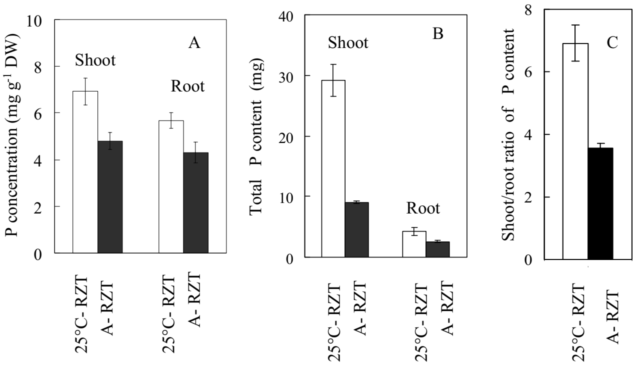

Fig. 9 Shoot and root P concentrations (**A**) and contents (**B**), and shoot/root ratio of P content (**C**) of *L. sativa* cv. 'Baby Butterhead' plants grown at different RZTs. Data were obtained 35 days after transplanting. Data are mean ± SE of 5 plants (modified from Luo 2008).

esculentum grown at 36°C-RZT (Klock *et al.* 1997). Similar result was reported by Bode Stoltzfus *et al.* (1998) in *C. melo* L. 'Gold Star'. However, Cumbus and Nye (1985) found a decrease in shoot total P but an increase in root total P at 35°C-RZT compared with optimal 25°C-RZT in rape *B. napus*. Although this high RZT severely restricted root growth there was a higher inflow of P. High 36°C-RZT did not decrease the P content of *C. annum* shoots (Gosselin and Trudel 1986). Instead, P content in shoot was increased when 10-week old *C. annum* plants were grown at 36°C-RZT for a period of 8 weeks. It was believed that the increased P content at high RZT was due to a greater amount of respiratory energy available for P uptake at the higher RZT. Therefore, whether high RZT may cause an increase or decrease in P nutrition is very much dependent on the plant species. In a study of *L. sativa* cv. 'Palma' under aeroponical conditions, our research team reported that plants grown at optimum 20°C-RZT had higher leaf P concentration compared to the plants grown at hot A-RZT (Tan *et al.* 2002). With muskmelon (*C. melo* L. 'Gold Star'), Stoltzfus *et al.* (1998) obtained similar results. These results suggested that uptake of P by plants and its accumulation within plants were strongly affected by RZTs.

It is commonly observed that high RZT caused decreases in K and Ca concentration in plant tissues (Gosselin and Trudel 1984; Menzel *et al.* 1994). A-RZT of 36°C caused reductions in K and Ca concentrations of *L. esculentum* shoots (Gosselin and Trudel 1984). There was a similar effect of high RZT on shoot K and Ca concentrations reported by Menzel *et al.* (1994). For instance, high RZT of 34.9°C increased K and Ca uptake of *C. annum* when compared to the optimum RZT of 30°C. This could be due to increased cell permeability and reduced cytoplasm viscosity (Gosselin and Trudel 1984). Menzel *et al.* (1994) found that increasing RZT up to 38°C decreased *Passiflora edulis* shoot K and Ca concentration when compared to the optimum RZT of 24°C. Higher shoot K and Ca concentration in *Passiflora edulis* may be due to increased uptake or translocation of nutrients by the root system. However, they cannot determine if uptake or translocation were involved because roots mineral composition was not analysed BassiriRad *et al.* (1991) reported that extreme RZT of 40°C greatly increased ion flux of K$^+$ to the xylem of detached roots of *H. vulgare* and *S. bicolor*. They also discovered that the removal of external K$^+$ did not alter short-term K$^+$ flux to the xylem of *S. bicolor* but strongly inhibited the K$^+$ flux in *H. vulgare* at 40°C-RZT. Therefore, they suggested that there were probably differences in the sites of temperature effects in *H. vulgare* and *S. bicolor*. Meanwhile, a RZT of 36°C decreased the total amount of K per *A. major* plant (Hood and Mills 1994). The highest amount of K per plant was recorded at 22°C-RZT and this corresponded to the optimum growth RZT of 22°C which was based on DWs of stem, leaves and roots (Hood and Mills 1994).

With aeroponically grown temperate *L. sativa* cv. 'Baby Butter' in the tropics, we studied that the effect of RZT on P concentration of shoot and root as well as its partitioning between shoot and root are summarized in **Fig. 9** (Luo *et al.* 2009). P concentrations, total P content of shoot and root were significantly reduced when lettuce plants were grown at hot A-RZT compared to those of 25°C-RZT plants (**Figs. 9A, 9B**). Klock *et al.* (1997) reported P uptake and leaf P concentration decreased when tomato (*L. esculentum*) seedlings were maintained at high 36°C-RZT. Huang and Xu (2000) reported that in creeping bentgrass (*Agrostis palustris*), when roots were exposed to 36°C-RZT, while shoots were kept at normal 20°C, both shoot and root P concentrations decreased. Our results also indicated that lettuce plants grown at 25°C-RZT had higher shoot and root P concentrations than at A-RZT. In **Fig. 9C**, results also showed that shoot/root ratio of total P content were significantly higher at 25°C-RZT than at A-RZT. Our data suggested that hot A-RZT inhibited not only the uptake but also the transport of P to the shoot. The main factors that caused the reduction in the translation of P from roots to shoots could be related to the changes in root morphology under hot A-RZT (**Fig. 3**). Our results indicate that the development of root systems of lettuce plants was severely restricted when grown at hot A-RZT (Qin *et al.* 2002; Tan *et al.* 2002; He *et al.* 2009). He *et al.* (2001) also reported that the uptake of water for lettuce plant grown at A-RZT was severely restricted. Graves *et al.* (1991) and Dodd *et al.* (2000) observed that root hydraulic conductivity decreased when lettuce plants were transferred from cool-RZT (C-RZT) to hot A-RZT. DeLucia *et al.* (1992) grew *Andropgon geradii* plants at soil temperatures ranging from 5.9 to 35.5°C. They found that plants grown at lower or higher than 24.5°C had lower shoot P concentrations. They suggested that translocation of P from roots to shoots may have been impaired. Similar results were found in our studies with tropical plants *C.*

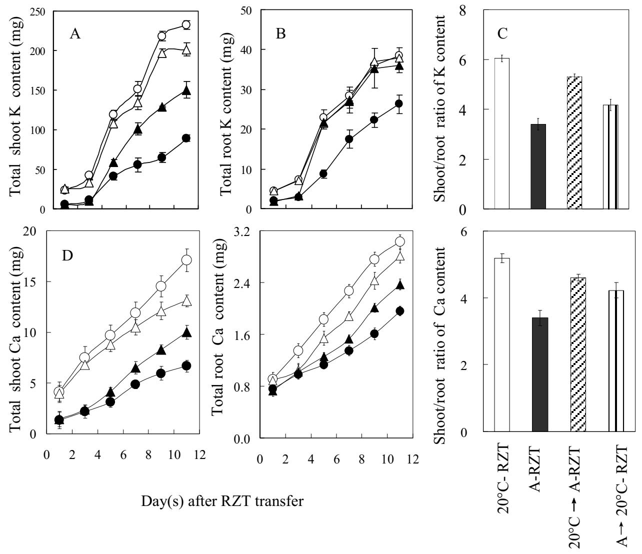

Fig. 10 Changes in total shoot and root K content (**A, B**; redrawn from Tan *et al.* 2002) and total shoot and root Ca content (**D, E**; He *et al.* unpublished), of *L. sativa* cv. 'Panama' grown at 20°C-RZT (○), A-RZT (●), 20°C→A-RZT (Δ) and A→20°C-RZT (▲) from day 0 to 11 after RZT transfer. Total shoot/root ratios of K and Ca contents (**C, F**) on day 11 after RZT transfer (calculated from **Figs. 11 A, B, D, E**). Each value is a mean of 4 measurements from 4 different plants. Vertical bars represent standard errors.

annuum (Tan 2004).

In our study with *L. sativa* cv. 'Panama', it was found that the rate of increase in shoot K (**Fig. 10A**) and Ca (**Fig. 10D**) during the active vegetative growth was the fastest for lettuce grown at 20°C-RZT and the slowest for plants grown at A-RZT. In 20°C →A-RZT plants, significant decrease in the increase rate of shoot K and Ca contents was only observed on day 11 after RZT transfer. Conversely, A→20°C-RZT plants demonstrated a relatively rapid rise in the rates of increase of total shoot K and Ca contents as compared to their control (A-RZT plants, **Figs. 10A, 10D**). However, there were no significant differences in the root K content among 20°C-RZT, 20°C→A-RZT and A→20°C-RZT plants during the whole RZT transfer period. These plants had much higher root K content than that of A-RZT plants from 5 day (**Fig. 10B**) On 11th day after RZT transfer, however, 20°C-RZT plants had significant higher root Ca content than those of 20°C→A-RZT and A→20°C-RZT and A-RZT plants (**Fig. 10E**). The shoot/root ratios of K and Ca were calculated from the total shoot and root K content of lettuce plants at day 11 after RZT transfer. It was found that K shoot/root ratio was significantly lower in A-RZT, 20°C→A-RZT and A→20°C-RZT plants than in 20°C-RZT plants (**Fig. 10C**). Therefore, the lower shoot/root ratio of K in plants that grown or once exposed to A-RZT may imply that high RZT had no inhibition on the root absorption of K. However, the high RZT may affect the translocation of the absorbed K (DeLucia *et al.* 1992; Cruz *et al.* 1993). The transport of K by plant roots may be dependent upon the concentration of other macronutrients in the solution, for example, NO_3^- (**Fig. 6**, Yanai *et al.* 1996; Ashley *et al.* 2005). The shoot/root ratio of Ca was also lower in those of A-RZT, 20°C→A-RZT and A→20°C-RZT than in 20°C-RZT plants. Higher proportion of Ca accumulated in the roots of plants that grown or once exposed to A-RZT may imply that low transpiration of lettuce leaves under hot A-RZT could lead to low leaves Ca (Ho *et al.* 1993). Ca appears to be virtually immobile in the phloem and is transported through the plant almost entirely via the xylem (Raven 1977; Adams and Ho 1993; Malone *et al.* 2002). Long-distance movement of Ca through plants is predominantly by bulk flow in the xylem (Atkinson *et al.* 1992). This means that plant organs of rapid transpiration, such as mature leaves, will accumulate high levels of Ca while organs of low transpiration will receive little. Larger amount of Ca is normally found in the leaves with a higher rate of transpiration than those leaves with low transpiring (White 2001; White and Broadley 2003; Kerton *et al.* 2009). 'Tipburn' of leafy vegetables is one of the Ca deficiency symptoms in young expanding leaves (White and Broadley 2003). In our commercial farm, tip burn is a common problem in leaves of temperate lettuce grown in the tropics plants and Ca has been recognized as a possible cause of this tip burn (Luo 2008). Although all plants were sprayed with nutrient solution regularly, drought stress could be caused by hot A-RZT resulting from poor root growth and development (Qin *et al.* 2002; Tan *et al.* 2002; Qin *et al.* 2007; He *et al.* 2009). It is previously reported that stomatal inhibition of photosynthesis and leaf relative water content (RWC) occurred immediately after 20°C-RZT lettuce plants transfer to hot A-RZT. Similar results were also found in tropical vegetable crops grown in the tropics such as *B. alboglabra* (He and Lee n2004) and *C. annuum* (Tan 2004). Our studies also showed that the uptake and transport of other macro nutrients such as Mg and S were also reduced by A-RZT in temperate *L. sativa* cultivars (Tan *et al.* 2002; Qin 2004; Luo 2008), subtropical *C. annuum* (Tan 2004) and *B. chinensis* L. (Qin 2004).

Light also has significant effect of the uptake of P, K and Ca by plants. In a study with *L. sativa* L. plants, Stefanson and Collis-George (1974) noted that the uptake and concentration of P, K and Ca in plant tissue were significantly affected by incident light. High incident light resulted in high P, K and Ca concentrations in plant tissue. Under high light condition, Hall (1977) found that for *L. esculentum*, the supplement of Ca stimulated growth of plants, but when the plants received low light, supplement Ca did not have any effect on plant growth. The effect of light intensity on the uptake of Ca by plants and their interaction on plant growth could be related to growth and development of roots (Hall 1977). Massimino *et al.* (1981) demonstrated that P uptake was reduced by 27% when plants were put under artificial shading for 4 hours. Meanwhile, root respiration was reduced by 55%. Jackson and Caldwell (1992) observed that P uptake by unshaded *Agropyron desertorum* plants was 73% higher than by shaded plants. They attributed to the uptake capacity of the root that was significantly reduced by shading. In *L. esculentum*, Adams and Ho (1993) found that water uptake was stimulated as transpiration increased with irradiance. Water uptake was closely related to solar radiation and the uptake of Ca was linearly related to that of water while, Zhao and Oosterhuis (1998) studied on *G. hirsutum* and revealed that when plants were treated by shading (700 μmol quanta m^{-2} s^{-1}) and unshading (1900-2000 μmol quanta m^{-2} s^{-1}), the uptake of P, K and Ca were significantly affected by shading. Leaf, floral buds and bracts of shaded *G. hirsutum* plants, P and Ca concentration was significantly higher than unshaded plants, and the differences increased as the length of shade period increased. Starkey and Andersson (2000) demonstrated that when plants were grown under three levels of supplementary PPFDs: 0, 50, and 100 μmol m^{-2} s^{-1} for 10 h per day, the uptake of Ca in the shoot increased with the increasing light. However, Zhao and Oosterhuis (1998) showed that shaded plants had significantly lower leaf K content than unshaded plants in different tissue at different growth stages in *G. hirsutum*. For example, 93 days after planting, the K content in the petiole and leaves of shaded plant is only 26% of the plant grown under normal light condition. But in bracts and floral buds, the change of K showed contradictory pattern, is higher in shaded plant. The increased mineral nutrients in the bracts and floral of shaded cotton plants were mainly associated with the decreases in both photosynthesis and non-structural carbohydrate accumulation (Zhao and Oosterhuis 1998), rather than an increased uptake of mineral nutrients as leaf total non-structural carbohydrate of shaded plants decreased by 56% compared to the unshaded plants.

With aeroponically grown temperate *L. sativa* cv. 'Baby Butterhead', we have found that the effects of light intensity

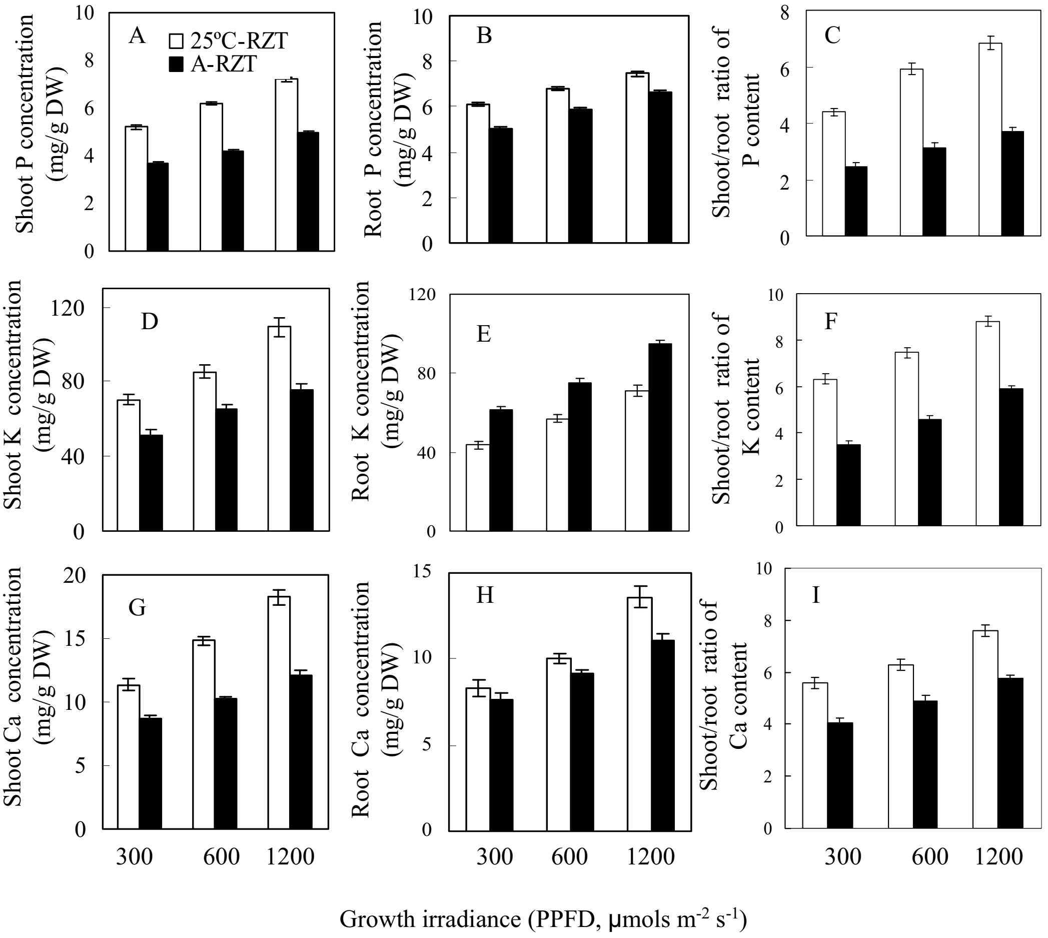

Fig. 11 Shoot, root P (**A, B**) , K (**D, E**) and Ca (**G, H**) concentrations and shoot/root ratio of P, K and Ca content (**C, F, I**) of *L. sativa* cv. 'Baby Butterhead' plants grown under different growth irradiances 35 days after transplanting. Data are mean ± SE of 5 plants (modified from Luo 2008).

on P concentrations of shoot and root as well as its partitioning between shoot and root (**Figs. 11A-C**). High PPFD resulted in high shoot and root P concentration as well as high shoot/root P content at both 25°C-RZT and A-RZT (Luo *et al.* 2009). From the same study, Luo (2008) also found that K (**Figs. 11D, 11E**) and Ca (**Figs. 11G, 11H**) concentrations were significantly higher under high PPFD than under low PPFD at both RZTs. At each given PPFD, 25°C-RZT plants had higher shoot K (**Fig. 11D**), and higher shoot and root Ca (**Figs. 11G, 11H**) concentrations than that of A-RZT plants. However, A-RZT plants had higher root K concentration than that of 25°C-RZT plants at each given PPFD (**Fig. 11E**). The shoot/root ratios of K content from A-RZT plants were lower than that of 25°C-RZT plants (**Fig. 11F**) at all PPFDs was mainly due to its higher root K concentration (**Fig. 11E**) and also its content (unpublished results, Luo 2008). Similar results were found in our studies with tropical plants *C. annuum* (Tan 2004). Dodd *et al.* (2000) reported that root hydraulic conductivity of *C. annuum* decreased when the plants grown at A-RZT and then were transferred from 25°C-RZT to hot A-RZT. He *et al.* (2001) reported that RWC, stomatal conductance and photosynthetic gas exchange were reduced in A-RZT and 20°C→A-RZT lettuce plants. These findings lead to the conclusion that lettuce plants grown at hot A-RZT had higher root K concentration compared to the plants grown at 25°C-RZT (**Fig. 11B**) may imply that transport of the absorbed K to shoots was impaired. Therefore, a large part of it remained in roots when the plants were grown at hot A-RZT. The lower shoot/root Ca content in A-RZT plant under high PPFD (**Fig. 11I**) further confirm that high level of Ca found in the roots of these plants are mainly due to the low transpiration of their leaves caused by hot RZT and lower PPFD (White 2001; White and Broadley 2003; Kerton *et al.* 2009). The combination of high light and C-RZT usually results in rapid transpiration in the temperate and subtropical crops grown in the tropics (Dodd *et al.* 2000; He *et al.* 2001) could explain the higher shoot/root Ca in these plants.

Micronutrients – Iron (Fe)

Various essential micronutrients namely, iron (Fe), boron (B), chloride (Cl), copper (Cu), manganese (Mn), molybdenum (Mo), zinc (Zn) and nickel (Ni) are typically added to soilless culture solutions in very small quantities. However, when they are made unavailable, it can adversely affect quality; reduce crop production (Rashid and Ryan 2004). This paper only focuses on the micronutrient Fe.

Several studies have shown that RZT plays an important role in micronutrients of plants (Marschner 1995; Bode Stoltzfus *et al.* 1998). Extremely high RZT can strongly affect various micronutrients uptake and transport in *C. melo* L. ('Gold Star' muskmelon) (Bode Stoltzfus *et al.* 1998), *Zea mays* (Engels and Marschner 1990) and *A. major* (Hood and Mills 1994). We observed that *L. sativa* grown at high RZT suffered from Fe deficiency symptoms (He and Lee 1998b). This was despite the fact that these aeroponically grown plants were constantly supplied with a balanced nutrient solution in the form of a mist. It was believed that the plants grown under hot A-RZT showed symptoms of Fe deficiency because of their poor root development (He and Lee 1998b; Tan *et al.* 2002; He *et al.* 2009). When grown under limited Fe supply, many plant species develop Fe-acquisition mechanisms. The most widespread Fe-acquisition mechanism in plants, Strategy I, has been found in dicotyledonous species (Marschner *et al.* 1986; Bienfait 1988; Brown and Jolley 1988; Schmidt 1999;

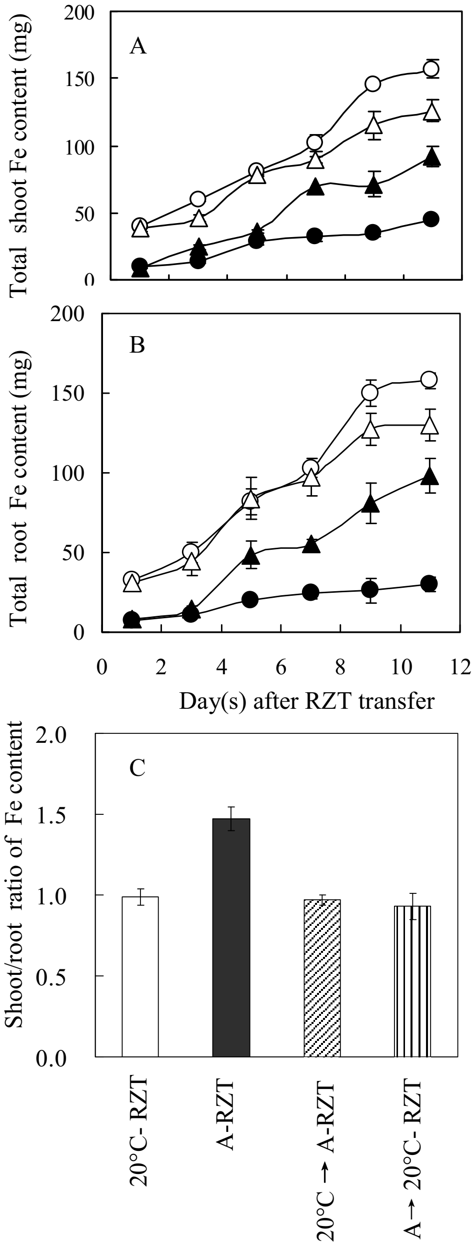

Fig. 12 Changes in total shoot and root Fe content (**A, B**) of *L. sativa* cv. 'Panama' grown at 20°C-RZT (O), A-RZT (●), 20°C→A-RZT (Δ) and A→20°C-RZT (▲) from day 0 to 12 after RZT transfer. Total shoot/root ratios of Fe content on day 12 after RZT transition (calculated from Figs. 12 A and B). Each value is a mean of 4 measurements from 4 different plants. Vertical bars represent standard errors (redrawn from Tan *et al.* 2002).

Zocchi *et al.* 2007). This Strategy involves morphological changes, such as increased formation of lateral roots, root hairs, and transfer cells, all of them increasing root surface for Fe uptake (Schmidt 1999). However, in our studies with temperate and subtropical crops grown in the tropics under hot A-RZT, none of these plants were observed to have developed Fe-acquisition mechanisms. Fe deficiency leads to visible morphological and physiological responses in many plants and micro-organisms (Timperio *et al.* 2007). As Fe is highly immobile and the primary visible symptom of Fe deficiency is the development of interveinal chlorosis on young leaves (Marschner 1995). Leaf chlorosis occurs because Fe is required at several steps in the pathway for chlorophyll synthesis. Fe chlorosis results from decreased concentrations of photosynthetic pigments (Abádia and Abádia 1993; Tan 2004; He *et al.* 2008). In our further studied on the RZT effects on Fe uptake and transport in *L. sativa* cv. 'Panama' plants it was found hot A-RZT inhibited both the uptake and transport of Fe (Tan *et al.* 2002). Three weeks after transplanting, Fe uptake at 20°C-RZT plants was more than A-RZT as both shoot and root concentration and content were lower in A-RZT plants than in 20°C-RZT. In their RZT transfer treatment from 20°C to A-RZT, plants suffered reduction of total concentration and accumulation of Fe, but when plants were transferred from A-RZT to 20°C RZT, these parameter were increased (**Fig. 12**; Tan *et al.* 2002). It was found that Fe shoot/root ratio was much lower than shoot/root ratios of NO_3^-, P, K and Ca (**Figs. 6C, 9C, 10C, 10F**) as Fe are less mobile compared to the other minerals. On the other hands, it is very likely that uptake of Fe was more strongly inhibited than its translocation for lettuce plants grown at A-RZT since there was higher concentrations and content of Fe in the shoot than in the root and thus a higher shoot/root ratio of Fe (**Fig. 12C**). Our studies also showed that the uptake and transport of other micronutrients such as Cu, Mn and Zn were also reduced by A-RZT in temperate *L. sativa* cultivars (Tan *et al.* 2002; Qin 2004; Luo 2008), subtropical *C. annuum* (Tan 2004) and *B. chinensis* L. (Qin 2004).

Light intensity also impacts on Fe uptake by plants. According to our observation, Fe chlorosis is more obvious common in our commercial temperate lettuce and other subtropical vegetables at A-RZT under low light conditions. Chlorotic symptoms in younger leaves were more evident after plants transferred from full Fe to Fe starvation for 2 to 3 days with chlorophyll content being dramatically decreased at A-RZT under low light condition (unpublished results). Compared to the studies carried out on the light effects on the uptake of other minerals discussed above, there are not many literatures available on the effect of light on the uptake of Fe by plants. Recently, through monitoring the Arabidopsis high-affinity root Fe uptake two genes, *FRO2* and *IRT1* gene expression, Vert *et al.* (2003) reported plants carrying only one copy of the *IRT1* gene take up Fe more efficiently during the light period, which raises the question of whether Fe uptake activity occurs mainly during the day. That was the first report of such a diurnal control of the Fe deficiency response genes in strategy I plants. Photosynthetic products may mediate this diurnal regulation given that variation of sugar, and particularly sucrose, during the day/night variations parallels the one of *IRT1* and *FRO2* expression (Kerr *et al.* 1985). The link between Fe and sugar metabolisms was revealed by Thimm *et al.* (2001) and starch degradation was up-regulated by Fe starvation in *Arabidopsis*. A role of sugar as activator of the Fe uptake activity may explain why roots of A-RZT lettuce plants in our studies (Qin 2004; Luo 2008; He *et al.* 2009) had higher soluble carbohydrates, which were related to higher concentration of starch. Interestingly, Vert *et al.* (2003) also found that in Fe starving plants, the diurnal regulation of *IRT1* and *FRO2* was overruled. They concluded that the root Fe uptake machinery responds primarily to Fe nutrition. Under Fe deficient conditions, it is additionally regulated by external signals, which could be sugars, other metabolites, or even light or circadian rhythm.

THE IMPACT OF RZT AND GROWTH IRRDIANCE ON THE ASSIMILATION OF NO_3^- AND THE SITE OF ASSIMILATION

NO_3^- is the only form of N available to roots in the soilless culture solution. NO_3^- incorporation into organic N containing compounds involves the reduction of NO_3^- to NO_2^- via the cytosolic enzyme NR. NO_2^- is then translocated to the root plastid or the chloroplast of leaves where it is reduced into NH_4^+ by nitrite reductase (NiR) (Stitt *et al.* 2002; Cookson *et al.* 2005). The rate-limiting and regulated step of NO_3^- assimilation appears to be the initial reaction, catalyzed by NR (Solomonson and Barber 1990; Orsel *et al.* 2002). NR is known to be under complex regulation and it is NO_3^--inducible enzyme (Riens and Heldt 1992; Savidov *et al.* 1997; Cookson *et al.* 2005).

The reduction of NO_3^- could take place either in roots or in leaves or both (Gojon *et al.* 1994; Savidov *et al.* 1997;

Scheurwater *et al.* 2002). Gojon *et al.* (1994) calculated the shoot/root ratio of NRA for several herbaceous and woody species. On average, this ratio was more than four times higher in herbaceous plants than in woody plants. Gojon *et al.* (1994) proposed that, when supplied with adequate of NO_3^-, slow-growing woody plant species carried out a greater proportion of total plant NO_3^- reduction in roots than in shoots, when compared with fast-growing herbaceous species. This was based on the hypothesis that NO_3^- reduction in roots competed with delivery of NO_3^- to xylem for transport to the shoot, which thus determined the proportion of NO_3^- reduced in the roots versus that in the shoots. Significant translocation of NO_3^- to the shoot would occur only when the net NO_3^- uptake rate was fast enough to saturate the reduction process in the roots. In slow-growing species with a low net NO_3^- uptake rate, xylem loading of NO_3^- would be relatively slow when compared with fast-growing species with a high net NO_3^- uptake rate. Consequently, slow-growing species might carry out a greater proportion of total NO_3^- reduction in their roots than do fast-growing species (Gojon *et al.* 1994). Scheurwater *et al.* (2002) grew eight monocotyledonous grass species that differed in maximum relative growth rate and net NO_3^- uptake rate with free access to nutrients in hydroponics under controlled environment conditions. They found that *in vivo* NRA of roots and shoots increased significantly with increasing net NO_3^- uptake rate and maximum relative growth rate. The proportion of total NO_3^- assimilation that occurs in roots was independent of maximum relative growth rate and net NO_3^- uptake rate, with the shoot being the predominant site of NO_3^- assimilation in all tested species. The results indicate that slow-growing grass species perform a similar proportion of total plant NO_3^- reduction in their roots to that exhibited by fast-growing grasses (Scheurwater *et al.* 2002). In soybean (*G. max* L. Merr. cv. 'Maple glen') plants, Cen and Layzell (2003) showed that neither roots nor shoots were the dominant site of NO_3^- reduction. Rather, both organs shared the responsibility, with roots being proportionally more important during the dark, whereas shoots were more important during the light. The light-dark differences of the sites of NO_3^- assimilation on a single plant may explain the controversy in previous studies over whether the site of NO_3^- reduction is in roots (Crafts-Brandner and Harper 1982; Vessey and Layzell 1987) or in shoots (Rufty *et al.* 1982; Andrews *et al.* 1984; Andrews 1986).

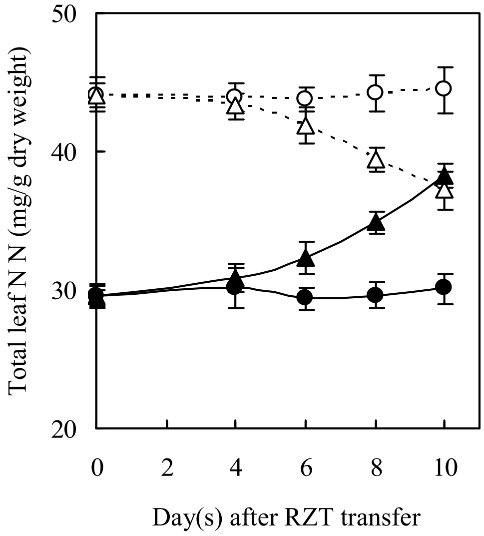

Fig. 13 Changes in leaf N of *L. sativa* cv. 'Panama' plants grown and maintained at 20°C-RZT (○) and A-RZT (●), 20°C→A-RZT (Δ) and A→20°C-RZT (▲) from day 0 to 10 after RZT transfer. Each point is the mean ± standard error of 5 measurements (redrawn from He *et al.* 2001).

Growth temperatures have been reported to affect NRA (Laurie and Stewart 1993; Jones *et al.* 1998). In a study using tomato (*L. esculentum* Mill. cv. 'Floramerica'), Jones *et al.* (1998) revealed that overnight low temperature exposure delayed NRA. They reported that low temperature has direct effects on NR expression and thus delays NRA, resulting in a mistiming in the regulation of NR and perhaps other key metabolic enzymes under circadian regulation. Laurie and Stewart (1993) grew chickpea (*Cicer arietinum* L. ICARDA cultivar no. 'ILC 482') plants at high (40/25°C, day/night) and moderate (25/17°C, day/night) temperature regimes. The results showed that high temperature (40/25°C) reduced shoot NRA (*in vivo*) but had little effect on root NRA. On a total plant basis, high temperature growth shifted NRA from shoot to root, particularly in the young plant. There was a greater decline in leaf NRA with age. Several other researches showed that total N contents were reduced in high RZT treated plants (Du and Tachibana 1994; He *et al.* 2001). For example, Du and Tachibana (1994) grew cucumber (*C. sativus* cv. 'Sharp I') plants hydroponically at RZTs of 25 (control), 30, 35, or 38°C, with shoot subjected to 26/23 ± 3°C (day/night). Total N concentration in leaf was reduced as the RZT was raised to 35°C and to 38°C in particular. The reduced of total N could result from the inhibition of NR at high RZT. Similar results were obtained by our research team with *L. sativa* L. cv. 'Palma' plants (He *et al.* 2001). Aerial parts of lettuce plants were grown under natural tropical ambient temperatures, but with their roots exposed to two different RZTs: a constant 20°C-RZT and a fluctuating A-RZT from 23-40°C. Three weeks after transplanting, leaf organic N content was 32% lower in A-RZT plants than 20°C-RZT plants. Transfer of plants between these two RZTs altered leaf N content after 6 days. Leaf N content increased and decreased, respectively, in A→20°C-RZT and 20°C→A-RZT plants after RZT transfer. After 10 days of reciprocal RZT transfer, A→20°C-RZT plants and 20°C →A-RZT plants had a similar leaf N content (**Fig. 13**). The results also showed tight temporal coupling of leaf N content, and light- and CO_2-saturated photosynthetic O_2 evolution rate throughout the reciprocal temperature transfers. He *et al.* (2001) suggested that the decreased nutrient status of lettuce plants caused non-stomatal limitation of photosynthesis under high A-RZT conditions. Therefore, it was very important to investigate the role of NR and its activity in our studies to elucidate the mechanisms of how RZT alters N metabolism in temperate and subtropical plants grown in the tropics. NO_3^- concentrations and NRA of leaf and root, root/leaf ratios of NO_3^- concentration of *L. sativa* cv. 'Baby Butterhead' plants determined after 10 days at each RZT transfer are shown in **Fig. 14**. Leaf and root NO_3^- concentration of 20°C-RZT plants was significantly higher than that of A-RZT plants. After 10 days of RZT transfers, the leaf and root NO_3^- concentrations of 20°C→A-RZT plants decreased significantly compared to those of 20°C-RZT and A-RZT plants. However, the leaf and root NO_3^- concentrations of A→20°C-RZT plants increased significantly compared to the A-RZT plants, and reached the similar levels to those of 20°C-RZT plants (**Figs. 14A, 14B**). Root/leaf ratios of NO_3^- concentration of A-RZT plants were significant higher than that of 20°C-RZT plants. There was no significant difference in root/leaf ratios of NO_3^- concentration between 20°C→A-RZT and A-RZT plants, but they were much higher than that of 20°C-RZT plants. Root/leaf ratios of NO_3^- concentrations of A→20°C-RZT plants were lower than those of A-RZT and 20°C→A-RZT plants, but higher than that of 20°C-RZT plants. After 10 days of RZT transfer, leaf maximum NRA of 20°C→A-RZT and A→20°C-RZT plants were much lower than those of 20°C-RZT plants, but significantly higher than those of A-RZT plants (**Fig. 14D**). However, root maximum NRA of 20°C-RZT plants were much lower compared to those of A-RZT plants. After 10 days of RZT transfers, root maximum NRA of 20°C→A-RZT plants were higher than those of 20°C-RZT plants, but lower than those of A-RZT plants. Root maximum NRA of A→20°C-RZT plants were much lower than those of A-RZT plants, but did not show significant difference to those of 20°C-RZT plants (**Fig. 14E**). Differences in root/leaf

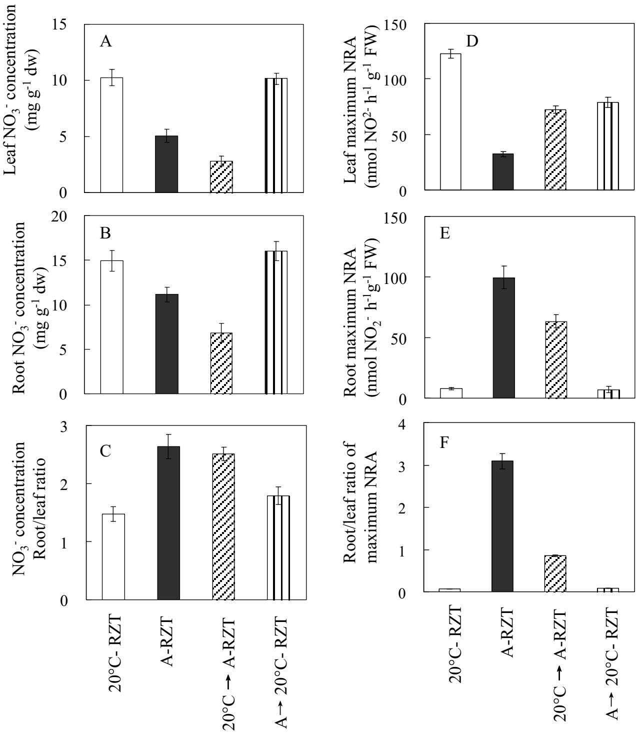

Fig. 14 Leaf NO_3^- concentration (**A**), root NO_3^- concentration (**B**), root/leaf ratio of NO_3^- concentration (**C**), leaf maximum NRA (**D**) root maximum NRA (**E**) and root/leaf ratio of maximum NRA (**F**) of *L. sativa* cv. 'Baby Butterhead' 30 days after transplanting. □, grown at 20°C-RZT; ■, grown at A-RZT; ▨, first grown at 20°C-RZT for 20 days but transferred to A-RZT for another 10 days; ▥, first grown at A-RZT for 20 days but transferred to 20°C-RZT for another 10 days (He *et al.* unpublished).

ratios of maximum NRA among different RZT plants (**Fig. 14F**) were similar to that of the root maximum NRA (**Fig. 14E**), indicating that high or low values of root/leaf ratio of maximum NRA was due to the high or low values of the root maximum NRA grown at the same RZT. Tan (2004) from our research team obtained the similar results using *L. sativa* cv. 'Panama' and *C. annum* Indra F1-hybrid plants. Controversial results of high RZT effects on N metabolism in shoots and roots have been reported by others (Johnson and Ingram 1984; Cruz *et al.* 1993; Du and Tachibana 1994). In our studies with temperate and subtropical plants grown in the tropics, high RZTs significantly inhibited N metabolism both in leaves and roots (He *et al.* 2001; Tan *et al.* 2002; Qin 2004; Tan 2004). NR is a key enzyme of the NO_3^- assimilation pathway and its activity is controlled at both the transcriptional and post-transcriptional level by number of metabolites and stimuli, such as light, NO_3^- availability and sucrose content (Vincentz *et al.* 1993; Kaiser and Huber 1994). However, higher NRA in roots of A-RZT plants than that of 20°C-RZT plants (**Fig. 14D**) imply that root NO_3^- concentrations is not responsible for the increases of root NRA of A-RZT lettuce plants (**Fig. 14E**). The explanation for the NRA increase in root of A-RZT plants could be due to lower end-product concentration (organic N) in the root (unpublished results). Amino acid and amide such as glutamine and glutamate and their relationship to NRA has been reported by others (Scheible *et al.* 1997). The reduction of NO_3^- could take place either in roots or in leaves or both (Cen and Layzel 2003; Scheurwater *et al.* 2002). For lettuce plants grown at optimum 20°C-RZT, shoot is the predominant site of NO_3^- reduction. Hot A-RZT treatment switched NO_3^- reduction from shoot to root, evidenced by the higher NRA in A-RZT roots than in leaves (**Fig. 14D**). This maybe due to low content of reduced N-components in A-RZT roots (unpublished results). By growing chickpea (*C. arietinum* L. ICARDA cultivar no. 'ILC 482') plants at high (40/25°C, day/night) and moderate (25/17°C, day/night), Laurie and Stewart (1993) found that 40/25°C reduced shoot *in vivo* NRA but had little effect on root NRA. On a total plant basis, high temperature growth shifted NRA from shoot to root, particularly in the young plant. This was in accordance with our findings that leaf maximum NRA of 20°C-RZT lettuce plants was the highest. Although root maximum NRA were higher in A-RZT plants compared to that of 20°C-RZT plants, the lower NO_3^- uptake and transport capacity (lower root and leaf NO_3^- concentration) still restricted NO_3^- reduction of A-RZT plants. This was evidenced by the lower total reduced N concentration of leaf and root of A-RZT plants compared to 20°C-RZT plants (unpublished results). In the reciprocal RZT transfer studies, the results indicate that 20°C→A-RZT plants suffered from decreased capacities of NO_3^- uptake, transport and assimilation. The data of reciprocal RZT transfer also showed that transfer lettuce plants from A-RZT to 20°C-RZT could completely overcome the negative effect on NO_3^- uptake and transport caused by A-RZT. This was supported by the fact that NO_3^- and N concentrations of leaf and root in A→20°C-RZT plants were much higher than those of A-RZT plants, and reached similar level to those of 20°C-RZT plants (unpublished results).

NRA has been showed to be modulated not only by temperature but also light intensity (De Cires *et al.* 1993; Vincentz *et al.* 1993; Ferretti *et al.* 1995; Meyer and Stitt 2001; Huber *et al.* 2002; Lillo *et al.* 2004). In higher plants, NR is regulated at the transcriptional as well as posttranslational level by light (Meyer and Stitt 2001) through the activation of photosynthesis and the production of sugars, stimulates the NR promoter (Vincentz *et al.* 1993) and NRA (Huber *et al.* 2002; Lillo *et al.* 2004). For example, NR from barley (*H. vulgare* cv. 'Hassan') leaves was inactivated during a light-dark transition (De Cires *et al.* 1993). NRA was observed losing approximately 50% after 30 min of darkness. The dark inactivation was reversed by illumination of the seedlings, the kinetics of reactivation being similar to those of inactivation. The activation state of NR was higher in high-light leaves than in low-light ones. Geiger *et al.* (1998) found that in the leaves and roots of 35-d-old tobacco (*Nicotiana tabacum* L. cv. 'Gatersleben') plants grown in a 12 h/12 h light/dark cycle at 25°C, NR protein and maximum activity were low at the end of the night and increased to a maximum 2–3 h into the photoperiod, and declined during the second part of the photoperiod. In roots, maximum NRA declined slightly during the day and recovered during the night. No major changes in NR activation in roots were found during the photoperiod. Kaiser and Huber (1997) described that NRA was rapidly inactivated to a very low level while detached spinach leaves was transferred from light to dark. Initially, NR content hardly affected. But during continuous darkness, NR content declined with a half-time of about 21 h, indicating slow NR protein degradation. Our results of leaf and root NRA, and root/leaf ratio of total NRA of *L. sativa* cv. 'Nanda' shown in **Fig. 15** were obtained from the same experiments, which data of shoot and root NO_3^- concentration, content and ratio are shown in **Figs. 7** and **8**. Plants had significantly higher leaf and root maximum NRA under high PPFD than under low PPFD (**Figs. 15A-D**) at both 20°C-RZT and A-RZT. However, under low PPFD both leaf and root NO_3^- concentration and content were higher than high PPFD (**Fig. 7**). These results indicate that high concentration and content of NO_3^- in plants grown at low PPFD resulted from the reduction of NRA (**Figs. 15A-D**). Higher root/leaf ratios of maximum NRA in low PPFD grown plants than the high PPFD ones (**Figs. 15E, 15F**) imply that the assimilation site of NO_3^- had shifted to root under low PPFD. Similar results were found in our studies with tropical plants *C. annuum* and *B. alboglabra* (He *et al.* unpublished). In the leaves of 10 day old maize (*Zea may* cv. 'Adon') plants grown under low (150 µmol photons m^{-2} s^{-1} and high (450 µmol photons m^{-2} s^{-1}) irradiance at normal temperature (27/21°C, day/night) conditions, Ferretti *et al.* (1995) found that leaf NRA was higher in high-light plants

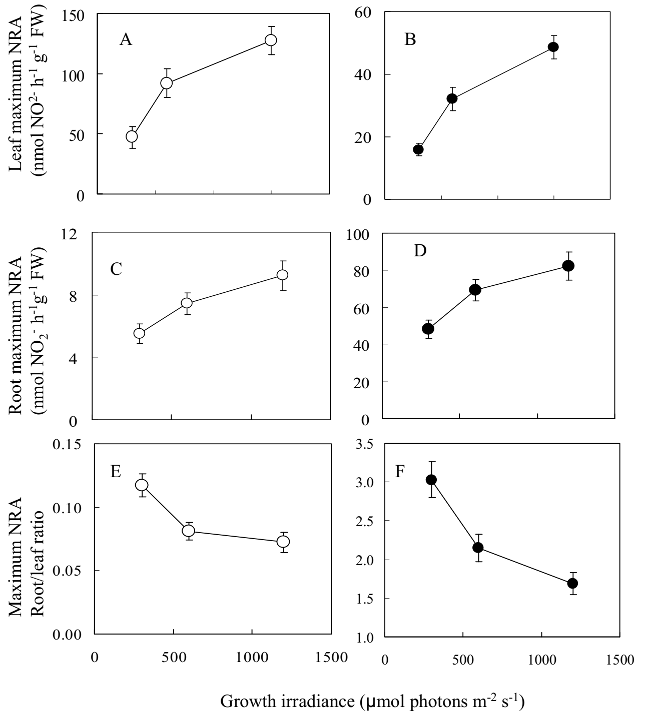

Fig. 15 Leaf (**A, B**) and root NRA (**C, D**), and root/leaf ratio of total NRA (**E, F**) of *L. sativa* cv. Nanda grown at 20°C-RZT (○) and A-RZT (●) under different growth irradiances for five weeks after transplanting. Each point is the mean of 6 measurements. Each point is the mean of 6 measurements (He *et al.* unpublished).

than in low-light ones. The assimilation of N in the leaves of higher plants requires both energy and C skeletons (Foyer *et al.* 1998). C metabolism is inextricably linked to N metabolism and any effect of a change in C abundance impacts on N metabolism and vice versa. Co-ordination of C and N metabolism is essential to prevent uncontrolled competition for energy and C skeletons among the pathways of N assimilation, carbohydrate production and CO_2 assimilation (Paul and Foyer 2001). In our previous studies, we suggested that the non-stomotal limitation of photosynthesis under prolonged growth at hot A-RZT could be due to a decrease in Ribulose-1,5-Bisphosphate Carboxylase (Rubisco) protein in response to a decrease in leaf N levels (He and Lee, 2001; He *et al.* 2001). Decreasing NO_3^- uptake and transport capacity under A-RZT resulted in the low concentrations of NO_3^- and total N in leaves. N-deficiency may cause decrease in Rubisco contents (He and Lee 2004; Qin 2004; Tan 2004). Paul and Foyer (2001) stated that the decline in Rubisco content that accompanied N deficiency can be prevented in leaves that are shaded to prevent carbohydrate accumulation. This result indicates that there is a close interaction between C and N signaling that regulates Rubisco levels. Scheible *et al.* (1997) proposed that NO_3^- acts as a signal to initiate coordinated changes in C and N metabolism. In their study, Nia 30(145) transformants synthesize small amounts of starch at low NO_3^- condition. After increasing NO_3^- to 12 mM, starch quickly decreased. Scheible *et al.* (1997) suggested that NO_3^- may also stimulate starch degradation. The accumulation of starch in A-RZT lettuce leaves could be caused by low leaf NO_3^- concentrations. However, in A-RZT *L. sativa* cv. 'Panama' leaves, the low NO_3^- concentrations are not companied by high starch concentration (He *et al.* 2009). This maybe due to that starch is not the main store carbohydrate in lettuce leaves.

CONCLUDING REMARKS

Cool-season crops grew poorly under the hot tropical conditions. Although shading lowered midday ambient temperatures by 3 to 5°C as compared to full sunlight condition (He *et al.* 2001), productivities of root and shoot decreased significantly with decreasing growth irradiance in both temperate and subtropical crops grown in the tropics. RZT also interacted strongly with light levels on productivity. The increases in shoot and root productivity with increasing light levels were more pronounced under C-RZT (either 20 or 25°C-RZT) than under hot A-RZT. Although the roots of aeroponically grown plants are continuously sprayed with nutrient mist, water deficit and mineral nutrient deficiency occurred at hot A-RZT due to poor root system development. The main mechanism responsible for achieving high productivity is mainly due to the well developed root architecture at C-RZT, which enhances water and mineral uptake and thus, preventing water deficit and mineral nutrient deficiency. C-RZT also alternates the partitioning of mineral nutrients uptake by the roots, and the partitioning of photoassimilate produced in the leaves meet changes in shoot and root nutrients demand in response to environmental stress. C-RZT could also alleviate the negative effects of A-RZT on NO_3^- assimilation and the coordination of C and N metabolism.

ACKNOWLEDGEMENTS

All experiment work on temperate and subtropical vegetables grown aeroponically was supported by the Academic Research Fund, Ministry of Education and The National Science and Technology Board (NSTB) (It is now called A*STAR, Agency for Science, Technology and Research), Singapore. The author of this paper, personally, indebted to Professor Lee Sing Kong in the National Institute of Education, Nangyang Technological University, Singapore for the opportunities he wisely and widely made available in plant physiology research of aeroponically grown temperate and subtropical crops in the tropic with manipulation of RZT.

REFERENCES

Abádia J, Abádia A (1993) Iron and plant pigments. In: Barton LL, Hemming BC (Eds) *Iron Chelation in Plants and Soil Microorganisms*, *Academic Press*, New York, pp 327-343

Abrol YP, Sawhney SK, Naik MS (1983) Light and dark assimilation of nitrate in plants. *Plant, Cell and Environment* **6**, 595-599

Adams P, Ho LC (1993) Effects of environment on the uptake and distribution of calcium in tomato and on the incidence of blossom-end rot. *Plant and Soil* **154**, 127-132

Aguirrezabal LAN, Deleens E, Tardieu F (1994) Root elongation rate is accounted for by intercepted PPFD and source-sink relations in field and laboratory-grown sunflower. *Plant, Cell and Environment* **17**, 443-450

Aguirrezabal LAN, Tardieu F (1996) An architectural analysis of elongation of field-grown sunflower root systems. Elements for modeling the effects of temperature and intercepted radiation. *Journal of Experimental Botany* **47**, 411-420

Ali IA, Kafkafi U, Yamaguchi I, Sugimoto Y, Inanaga S (1998) Response of oilseed rape plant to low root temperature and nitrate:ammonium ratios. *Journal of Plant Nutrition* **21**, 1463-1481

Andrews M (1986) Nitrate and reduced-N concentrations in the xylem sap of *Stellaria media*, *Xanthium strumarium* and six legume species. *Plant Cell Environment* **9**, 605-608

Andrews M, Sutherland JM, Thomas RJ, Sprent JI (1984) Distribution of nitrate reductase activity in six legumes: the importance of the stem. *New Phytologist* **98**, 301-310

Arshad M, Frakenberger WT Jr. (2002) *Ethylene: Agricultural Sources and Applications*, Kluwer, New York, pp 155-156

Ashley MK, Grant M, Grabov A (2005) Plant responses to potassium deficiencies: a role for potassium transport proteins. *Journal of Experimental Botany* **57**, 425-436

Aslam M, Huffaker RC (1984) Dependency of nitrate reduction on soluble carbohydrates in primary leaves of barley under aerobic conditions. *Plant Physiology* **75**, 623-628

Aslam M, Travis RL, Rains DW (2001) Diurnal fluctuations of nitrate uptake and *in vivo* nitrate reductase activity in *Pima* and *Acala* cotton. *Crop Science* **41**, 372-378

Atkin OK, Cummins WR (1994) The effect of root temperature in the induction of nitrate reductase activities and nitrogen uptake rates in arctic plant species. *Plant and Soil* **159**, 187-197

Atkinson CJ, Ruiz LP, Mansfield TA (1992) Calcium in xylem sap and the regulation of its delivery to the shoot. *Journal of Experimental Botany* **43**, 1315-1324

BassiriRad H, Radin JW, Matsuda K (1991) Temperature-dependent water and ion transport properties of barley and sorghum roots. *Plant Physiology* **93**, 426-432

Ben Zioni A, Vaadia Y, Lips SH (1971) Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. *Physiologia Plantarum* **24**, 288-290

Bingham IJ, Cumpus IP (1991) Effect of root temperature on the potassium requirements of young tomato plants. *Plant and Soil* **133**, 227-237

Bienfait HF (1988) Mechanisms in Fe-efficiency reactions of higher plants. *Journal of Plant Nutrition* **11**, 605-629

Bode Stoltzfus BM, Taber HG, Aiello AS (1998) Effect of increasing root-zone temperature on growth and nutrient uptake by 'Gold Star' Muskmelon Plants. *Journal of Plant Nutrition* **21**, 321-328

Bravo FP, Uribe EG (1981) Temperature dependence of the concentration kinetics of absorption of phosphate and potassium in corn roots. *Plant Physiology* **67**, 815-819

Brouwer R (1983) Functional equilibrium: Sense or nonsense? *Netherlands Journal of Agricultural Science* **31**, 335-348

Brown JC, Jolley VD (1988) Strategy I and strategy II mechanisms affecting iron availability to plants may be established too narrow or limited. *Journal of Plant Nutrition* **11**, 1077-1098

Buttery B, Stone J (1988) Some effects of nitrate and light intensity on soybean root growth and development. *Plant and Soil* **106**, 292-294

Carvalho DD, Irving LJ, Carnevalli RA, Hodgson J, Matthew C (2006) *Journal of Experimental Botany* **57**, 2015-2024

Cen YP, Layzell DB (2003) *In vivo* gas exchange measurement of the site and dynamics of nitrate reduction in soybean. *Plant Physiology* **131**, 1147-1156

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field? Photosynthesis and growth. *Annals of Botany* **89**, 907-916

Clarkson DT, Hopper MJ, Jones LHP (1986) The effects of root temperature on the uptake of nitrogen and the relative size of the root system in *Lolium perenne*. I. Solutions containing both NH_4^+ and NO_3^-. *Plant, Cell and Environment* **9**, 535-545

Cookson SJ, Williams LE, Miller AJ (2005) Light-dark changes in cytosolic nitrate pools depend on nitrate reductase activity in *Arabidopsis* leaf cells. *Plant Physiology* **138**, 1097-1105

Cooper AJ (1973) Root temperature and plant growth: A review. *Commonwealth Bureau of Horticulture and Plantation Crops*. Commonwealth Agriculture Bureaux, Farnham Royal, Slough, England, 73 pp

Crafts-Brandner SJ, Harper JE (1982) Nitrate reduction by roots of soybean (*Glycine max* [L.] Merr.) seedlings. *Plant Physiology* **69**, 1298-1303

Crawford N, Glass ADM (1998) Molecular and physiological aspects of nitrate uptake in plants. *Trends in Plant Science* **3**, 389-395

Crookshanks M, Taylor G, Dolan L (1998) A model system to study the effects of elevated CO_2 on the developmental physiology of roots: the use of *Arabidopsis thaliana. Journal of Experimental Botany* **49**, 593-597

Cruz C, Lips SH, Martins-Louҫão A (1993) Uptake of ammonium and nitrate by carob (*Ceratonia siliqua*) as affected by root temperature and inhibitors. *Physiologia Plantarum* **89**, 532-543

Cumbus IP, Nye PH (1982) Root zone temperature effects on growth and nitrate absorption in rape (*Brassica napus* cv. Emeralk). *Journal of Experimental Botany* **33**, 1138-1446

Cumbus IP, Nye PH (1985) Root zone temperature effects on gowth and phosphate absorption in rape *brassica napus* cv. Emerald. *Journal of Experimental Botany* **36,** 19- 227

De Cires A, de la Torre A, Delgado B, Lara C (1993) Role of light and CO_2 fixation in the control of nitrate-reductase activity in barley leaves. *Planta* **190**, 277-283

Del Castillo D, Acock B, Reddy V, Acock M (1989) Elongation and branching of roots on soybean plants in a carbon dioxide-enriched aerial environment. *Agronomy Journal* **81**, 692-695

Delhon P, Gojon A, Tillard, T, Passama L (1995a) Diurnal regulation of NO_3^- uptake in soybean plants. I. Changes in NO_3^- influx, efflux, and N utilization in the plant during the day/night cycle. *Journal of Experimental Botany* **46**, 1585-1594

Delhon P, Gojon A, Tillard T, Passama L (1995b) Diurnal regulation of NO_3^- uptake in soybean plants. II. Relationship with accumulation of NO_3- and asparagine in the roots. *Journal of Experimental Botany* **46**, 1595-1602

Delhon P, Gojon A, Tillard T, Passama L (1995c) Diurnal regulation of NO_3^- uptake in soybean plants III. Implication of the Dijkshoorn-Ben Zioni model in relation with the diurnal changes in NO_3^- assimilation. *Journal of Experimental Botany* **47**, 885-892

Delucia EH, Scott AH, Day TA (1992) Effects of soil temperature on growth, biomass allocation and resource acquisition of *Andropogon gerardii* Vatman. *New Phytologist* **10**, 543-549

Demotes-Mainard S, Pellerin S (1992) Effect of mutual shading on the emergence of nodal roots and the root: shoot ratio of maize. *Plant and Soil* **147**, 87-93

der Leij M, Smithe SJ, Miller AJ (1998) Remobilization of vacuolar stored nitrate in barley root cells. *Planta* **205**, 64-72

Dijkshoorn W, Latfawell DJ, Dewit CT (1968) Temporal changes in carboxylate content of rye grass with stepwise change in nutrition. *Plant and Soil* **29**, 369-90

Dodd IC, He J, Turnbull CGN, Lee SK, Critchley C (2000). Influence of supra-optimal root temperatures on growth and stomatal conductance in *Capsicum annuum* L. *Journal of Experimental Botany* **51**, 239-248

Dodd IC, Tan LP, He J (2003) Do increases in xylem sap pH and/or ABA concentration mediate stomatal closure following nitrogen deprivation? *Journal of Experimental Botany* **54**, 1281-1288

Du YC, Tachibana S (1994) Effect of supraoptimal root temperature on the growth, root respiration and sugar content of cucumber plants. *Scientia Horticulturae* **58**, 289-301

Durieux RP, Kamprath EJ, Jackson WA, Moll RH (1994) Root distribution of corn: The effect of nitrogen fertilization. *Agronomy Journal* **86**, 958-962

Engles C, Marschner H (1990) Effect of sub-optimal root zone temperature at varied nutrient supply and shoot meristem temperature on growth and nutrient concentration in maize seedlings. *Plant and Soil* **126**, 215-225

Engels C, Münkle L, Marschner H (1992) Effect of root zone temperature and shoot demand on uptake and xylem transport of macronutrients in maize (*Zea mays* L.). *Journal of Experimental Botany* **43**, 537-547

Ferretti M, Merlo L, Passera C, Ghisi R (1995) Influence of irradiance on enzymes of sulphate and nitrate assimilation pathways in maize leaves. *Plant Physiology and Biochemistry* **33**, 111-114

Fitter A, Williamson L, Linkohr B, Leyser O (2002) Root system architecture determines fitness in an Arabidopsis mutant in competition for immobile phosphate ions but not for nitrate ions. *Proceedings of the Royal Society London, Series B* **269**, 2017-2022

Forde BG, Clarkson DT (1999) Nitrate and ammonium nutrition of plants: Physiological and molecular perspectives. *Advances in Botanical Research* **30**, 1-90

Foyer CH, Valadier MH, Migge A, Becker TW (1998) Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. *Plant Physiology* **117**, 283-292

Fredeen AL, Rao IM, Terry N (1989) Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max*. *Plant Physiology* **89**, 225-230

Gavito ME, Curtis PS, Jakobsen I (2001) Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. *Journal of Experimental Botany* **52**, 1913-1923

Geiger M, Walcu-Liu P, Engels C, Harnecker J, Schulze ED, Ludewig F, Sonnewald U, Scheible W-R, Stitt M (1998) Enhanced carbon dioxide leads to a modified diurnal rhythm of nitrate reductase activity in older plants, and a large stimulation of nitrate reductase acitivity and higher levels of amino acids in young tobacco plants. *Plant, Cell and Environment* **21**, 253-268

Gojon A, Passard C, Bussi C (1994) Root/shoot distribution of NO_3^- assimilation in herbaceous and woody species. In: Roy R, Garnier E (Eds) *A Whole Plant Perspective on Carbon-Nitrogen Interactions*, SPB Academic Publishing, The Hague, pp 131-147

Gosselin A, Trudel MJ (1984) Interaction between root-zone temperature and light levels on growth, development and photosynthesis of *Lycopersicon esculentum* Mill. culticar 'vendor'. *Scientia Horticulturae* **23**, 313-321

Gossellin A, Trudel MJ (1986) Root-zone temperature effects on pepper. *Journal of the American Society for Horticultural Science* **111,** 220–224

Graves WR, Dana MN, Joly RJ (1989) Root-zone temperature affects water status and growth of red maple. *Journal of the American Society for Horticultural Science* **114**, 406-410

Graves WR, Joly RJ, Dana MN (1991) Water use and growth of honey locust and tree-of-heaven at high root-zone temperatures. *HortScience* **26**, 1309–1312

Hall DA (1977) Some effects of varied calcium nutrition on the growth and composition of tomato plants. *Plant and Soil* **48**, 199-211

He J, Lee SK (1998a) Growth and photosynthetic characteristics of lettuce (*Lactuca sativa* L.) grown under fluctuating hot ambient tempeatures with the manipulation of cool root zone temperature. *Journal of Plant Physiology* **152**, 387-391

He J, Lee SK (1998b) Growth and photosynthetic responses of three aeroponically grown lettuce cultivars (*Lactuca sativa* L.) to different root zone temperatures and growth irradiances under tropical aerial condition. *Journal of Horticultural Science and Biotechnology* **73**, 173-180

He J, Lee SK (2001) Relationship among photosynthesis, Ribulose-1,5-bisphosphate carboxylase (Rubisco) and water relations of subtropical vegetable Chinese broccoli grown in the tropics by manipulation of root-zone temperature. *Environmental and Experimental Botany* **46**, 119-128

He J, Lee SK (2004) Photosynthetic utilization of radiant energy by temperate lettuce grown under natural tropical condition with manipulation of root-zone temperature. *Photosynthetica* **42**, 457-463

He J, Lee SK, Dodd IC (2001) Limitations to photosynthesis of lettuce grown under tropical conditions: alleviation by root-zone cooling. *Journal of Experimental Botany* **52**, 1323-1330

He J, Chua NYA, Qin L (2008) Interaction between iron stress and root-zone temperature on physiological aspects of aeroponically grown Chinese broccoli (*Brassica alboglabra*). *Journal of Plant Nutrition* **31**, 1-20

He J, Tan LP, Lee SK (2009) Root-zone temperature effects on photosynthesis,

^{14}C-photoassimilate partitioning and growth of temperate lettuce (*Lactuca sativa* cv. 'Panama') grown in the tropics. *Photosynthetica* **47**, 95-103

Ho LC, Belda R, Brown M, Andrews J, Adams P (1993) Uptake and transport of calcium and the possible causes of blossom-end rot in tomato. *Journal of Experimental Botany* **44**, 509-518

Hood TM, Mills HA (1994) Root-zone temperature affects nutrient uptake and growth of snapdragon. *Journal of Plant Nutrition* **17**, 279-291

Huang BR, Taylor HM, McMichael BL (1991a) Behavior of lateral roots in winter wheat as affected by temperature. *Environmental Experimental Botany* **31**, 187-192

Huang BR, Taylor HM, McMichael BL (1991b) Growth and development of seminal and crown roots of wheat seedling as affected by temperature. *Environmental Experimental Botany* **31**, 471-477

Huang BR, Xu QZ (2000) Root growth and nutrient element status of creeping bentgrass cultivars differing in heat tolerance as influenced by supraoptimal shoot and root temperatures. *Journal of Plant Nutrition* **23**, 979-990

Huber SC, MacKintosh C, Kaiser WM (2002) Metabolic enzymes as targets for 14-3-3 proteins. *Plant Molecular Biology* **50**, 1053-1063

Huber SC, Sugiyama T, Alberte RS (1989) Photosynthetic determinants of growth in maize plants: Effects of nitrogen nutrition on growth, carbon fixation and photochemical features. *Plant Cell Physiology* **30**, 1063-1072

Izanloo A, Condon AG, Langridge P, Tester M, Schnurbusch T (2008) Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. *Journal of Experimental Botany* **59**, 3327-3346

Jackson RB, Caldwell MM (1992) Shading and the capture of localized soil nutrients: nutrient content, carbohydrates, and root uptake kinetics of a perennial tussock grass. *Oecologia* **91**, 457-462

Jackson WA, Pan WL, Moll RH, Kamprath EJ (1986) Uptake, translocation and reduction of nitrate. In: Neyra CA (Ed) *Biochemical Basis of Plant Breeding*, CRC Press, Boca Raton, pp 1-39

Janes HW, Chin CK, Bachmansky J (1988) Growth and metabolism of tomato roots grown in tissue cultures held at various temperatures. *HortScience* **23**, 773

Johnson CR, Ingram DL (1984) *Pittosporum tobira* response to container medium temperature. *HortScience* **19**, 524-525

Jones TL, Tucker DE, Ort DR (1998) Chilling delays circadian pattern of sucrose phosphate synthase and nitrate reductase activity in tomato**.** *Plant Physiology* **118**, 149-158

Josh H, Harry WJ (1983) Effect of altering the root-zone temperature on growth, translocation, carbon exchange rate and leaf starch accumulation in the tomato. *Plant Physiology* **73**, 46-50

Kaiser WM, Huber SC (1994) Posttranslational regulation of nitrate reductase in higher plants. *Plant Physiology* **106**, 817-821

Kaiser WM, Huber SC (1997) Correlation between apparent activation state of nitrate reductase (NR), NR hysteresis and degradation of NR protein. *Journal of Experimental Botany* **48**, 1367-1374

Kaiser WM, Spill D, Brendle-Behnisch E (1992) Adenine nucleotides are apparently involved in the light-dark modulation of spinach-leaf nitrate reductase. *Planta* **186**, 236-40

Kerton M, Newbury HJ. Hand D, Pritchard J (2009) Accumulation of calcium in the centre of leaves of coriander (*Coriandrum sativum* L.) is due to an uncoupling of water and ion transport. *Journal of Experimental Botany* **60**, 227-235

Kerr PS, Rufty TW Jr, Huber SC (1985) Changes in nonstructural carbohydrates in different parts of soybean (*Glycine max* [L.] Merr.) plants during a light/dark cycle and in extended darkness. *Plant Physiology* **78**, 576-581

King JR, Hill MJ, Willms WD (1995) Growth response of *Festuca altaica*, *Festuca hallii*, and *Festuca campestris* to temperature. *Canadian Journal of Botany* **73**, 1074-1080

Klock KA, Taber HG, Graves WR (1997) Root respiration and phosphorus nutrition of tomato plant growth at 36°C root zone temperature. *Journal of the American Society for Horticultural Science* **122**, 175-178

Krouk G, Tillard P, Gojon A (2006) Regulation of the high-affinity NO_3^- uptake system by NRT1.1-mediated NO_3^- demand signaling in *Arabidopsis*. *Plant Physiology* **142**, 1075-1086

Kuroyanagi T, Paulsen GM (1988) Mediation of high-temperature injury by roots and shoots during reproductive growth of wheat. *Plant, Cell and Environment* **11**, 517-523

Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006) Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits. *Annals of Botany* **98**, 693-713

Laurie S, Stewart GR (1993) Effects of nitrogen supply and high temperature on the growth and physiology of the chickpea. *Plant, Cell and Environment* **16**, 609-621

Lee SK (1993) Aeroponic system as a possible alternative for crop production in Singapore. *Commonwealth Agricultural Digest* **3**, 1-14

Lee SK, Cheong SC (1996) Inducing head formation of iceberg lettuce (*Lactuca sativa* L.) in the tropics through root-zone temperature control. *Tropical Agriculture (Trinidad)* **73**, 34-42

Li X-Z, Oats A (1994) Induction and turnover of nitrate reductase in *Zea mays*. *Plant Physiology* **106**, 1145-1149

Lillo C, Meyer C, Lea US, Provan F, Oltedal S (2004) Mechanism and importance of post-translational regulation of nitrate reductase. *Journal of Experimental Botany* **55**, 1275-1282

Little DY, Rao H, Oliva S, Daniel-Vedele F, Krapp A, Malamy JE (2005) The putative high-affinity nitrate transporter NRT2.1 represses lateral root initiation in response to nutritional cues. *Proceedings of the National Academy of Sciences USA* **102**, 13693-13698

Loomis MJ, Amthor JS (1999) Yield potential, plant assimilatory capacity and metabolic efficiencies. *Crop Science* **39**, 1584-1596

Luo HY (2008) Mechanisms of Ca, P and K uptake and partitioning characterristics of certain temperate vegetables in relation to different irradiance and root-zone temperatures. PhD Thesis, Nanyang Technological University, Singapore, 286 pp

Luo HY, Lee SK, He J (2009) Integrated effects of root-zone temperatures and phosphorus levels on aeroponically-grown lettuce (*Lactuca sativa* L.) in the tropics. *The Open Horticulture Journal* **2**, 6-12

Lynch J (1995) Root architecture and plant productivity. *Plant Physiology* **109**, 7-13

Macay AD, Barber SA (1984) Soil temperature on root growth and phosphorus uptake by corn. *Soil Science Society of America Journal* **48**, 818-823

Macuff JH, Jarvis SC, Cockburn JE (1994) Acclimation of NO_3^- fluxes to low root temperature by *Brassica napus* in relation to NO_3^- supply. *Journal of Experimental Botany* **45**, 1045-1056

Malagoli P, Laine P, Le Deunff E, Rossato L, Ney B, Ourry A (2004) Modeling nitrogen uptake in oilseed rape cv Capitol during a growth cycle using influx kinetics of root nitrate transport systems and field experimental data. *Plant Physiology* **134**, 388-400

Malone M, White P, Morales MA (2002) Mobilization of calcium in glasshouse tomato plants by localized scorching. *Journal of Experimental Botany* **53**, 83-88

Marschner H (1995) *Mineral Nutrition of Higher Plants* (2nd Edn), Academic Press, London, 889 pp

Marschner H, Römheld V, Kissel M (1986) Different strategies in higher plants in mobilization and uptake of iron. *Journal of Plant Nutrition* **9**, 695-713

Massimino D, André M, Richaud C, Daguenet A, Massiino J, Vivoli J (1981) The effect of a day at low irradiance of a maize crop. I. Root respiration and uptake of N, P and K. *Physiologia Plantarum* **51**, 150-155

McCully M (1995) How do real roots work? Some new view of root structure. *Plant Physiology* **109**, 1-6

Menzel CM, Turner DW, Doogan VJ, Simpson PR (1994) Root–shoot interactions in passionfruit (*Passiflora* sp.) under the influence of changing root volumes and soil temperatures. *Journal of Horticultural Science* **69,** 353–364

Meyer C, Stitt M (2001) Nitrate reduction and signalling. In: Lea PJ, Morot-Gaudry JF (Eds) *Plant Nitrogen*, Springer-Verlag, Berlin, pp 39-59

Minchin PEH, Thorpe MR, Frarrar JF, Koroleva OA (2002) Source-sink coupling in young barley plants and control of phloem loading. *Journal of Experimental Botany* **53**, 1671-1676

Mollier A, Pellerin S (1999) Maize root system growth and development as influenced by phosphorus deficiency. *Journal of Experimental Botany* **50**, 487-497

Moorby H, Nye PH (1984) The effect of temperature variation over the root system on root extension and phosphate uptake by rape. *Plant and Soil* **78**, 283-293

Orsel M, Filleur S, Fraisier V, Daniel-Vedele F (2002) Nitrate transport in plants: which gene and which control. *Journal of Experimental Botany* **53**, 825-833

Pardales JR, Yamauchi A, Kono Y (1991) Growth and development of sorghum roots after exposure to different periods of a hot root-zone temperature. *Environmental and Experimental Botany* **31**, 397-403

Paterson E, Sim A, Standing D, Dorward M, McDonald AJS (2006) Root exudation from *Hordeum vulgare* in response to localized nitrate supply. *Journal of Experimental Botany* **57**, 2413-2420

Paul MJ, Foyer CH (2001) Sink regulation of photosynthesis. *Journal of Experimental Botany* **52**, 1383-1400

Pellerin S, Mollier A, Plenet D (2000) Phosphorus deficiency affects the rate of emergence and number of maize adventitious nodal roots. *Agronomy Journal* **92**, 690-697

Pierik R, Verkerke W, Voesenek RACJ, Blom KWPM, Visser EJW (1999) Thick root syndrome in cucumber (*Cucumis sativus* L.): A description of the phenomenon and an investigation of the role of ethylene. *Annals of Botany* **84**, 775-762

Qin L (2004) Ontogeny, root-zone temperature and growth irradiance effects on temperate and subtropical vegetable crops grown in the tropics. PhD thesis, Nanyang Technological University, Singapore, 329 pp

Qin L, He J, Lee SK (2002) Response of lettuce (*Lactuca sativa* L.) growth to reciprocal root-zone temperature (RZT) transfer at different growth stages. *Journal of Horticultural Science and Biotechnology* **77**, 683-690

Qin L, He J, Lee SK, Dodd IC (2007) An assessment of ethylene mediation of lettuce (*Lactuca sativa*) root growth at high temperatures. *Journal of Experimental Botany* **58**, 3017-3024

Rashid A, Ryan J (2004) Micronutrient constraints it to crop production in soils with Meditarranean-type characteristics: A review. *Journal of Plant Nutrition* **27**, 959- 975

Raven JA (1977) H^+ and Ca^{2+} in phloem and symplast: relation of relative immobility of the ions to the cytoplasmic nature of the transport paths. *New Phytologist* **79**, 465-480

Riens B, Heldt HW (1992) Decrease of nitrate reductase activity in spinach leaves during a light-dark transition. Plant Physiology **98**, 573-577

Rufty TW, Volk RJ, McClure PR, Israel DW, Raper CD (1982) Relative content of NO_3^- and reduced N in xylem exudate as an indicator of root reduction of concurrently absorbed $^{15}NO_3^-$. *Plant Physiology* **69**, 166-170

Sattelmacher B, Marschner H, Kühne R (1990) Effects of the temperature of the rooting zone on the growth and development of roots of potato (*Solanum tuberosum*). *Annals of Botany* **65**, 579-579

Savidov NA, Tokarev BI, Lips SH (1997) Regulation of Mo-cofactor, NADH- and NAD(P)H-specific nitrate reductase activities in the wild type and two *nar*-mutant lines of barley (*Hordeum vulgare* L.). *Journal of Experimental Botany* **48**, 847-855

Scheible WR, Gonzalez-Fontes A, Lauerer M, Müller-Röber B, Caboche M, Stitt M (1997) Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *Plant Cell* **9**, 783-798

Schenk U, Manderscheid R, Hugen J, Weigel HJ (1995) Effects of CO_2 content and microbial biomass carbon in soil of perennial ryegrass and white clover. *Journal of Experimental Botany* **46**, 987-993

Scheurwater I, Koren M, Lambers H, Atkin OK (2002) The contribution of roots and shoots to whole plant nitrate reduction in fast- and slow-growing grass species. *Journal of Experimental Botany* **55**, 1635-1642

Schmidt W (1999) Mechanisms and regulation of reduction-based iron uptake in plants. *New Phytologist* **141**, 26

Singh OS, Sharma VK (1972) Alterations in growth and metabolism of potato plants by calcium deficiency. *Plant and Soil* **36**, 363-369

Smirnoff N, Stewart GR (1985) Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequences. *Physiologia Plantarum* **64**, 133-140

Solomonson LP, Barber MJ (1990) Assimilatory nitrate reductase: functional properties and regulation. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**, 225-253

Starkey KR, Andersson NE (2000) Effects of light and nitrogen supply on the allocation of dry matter and calcium in poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch). *Journal of Horticultural Science and Biotechnology* **75**, 251-258

Stefanson RC, Collis-George N (1974) The importance of environmental factors in soil fertility assessments. II. Nutrient concentration and uptake. *Australian Journal of Agricultural Research* **25**, 309-316

Stitt M, Muller C, Matt P, Gibon Y, Carillo P, Morcuende R, Scheible WR, Krapp A (2002) Steps towards an integrated view of nitrogen metabolism. *Journal of Experimental Botany* **53**, 959-970

Stoner RJ (1983) Aeroponics versus bed and hydroponic propagation. *Florists' Review* **1**, 4477

Stoltzfus RMB, Taber HG, Aiello AS (1998) Effect of increasing root-zone temperature on growth and nutrient uptake by 'Gold Star' muskmelon plants. *Journal of Plant Nutrition* **21**, 321-328

Tan LP, He J, Lee SK (2002) Effects of root-zone temperature on the root development and nutrient uptake of *Lactuca sativa* L. cv. 'Panama' grown in an aeroponic system in the tropics. *Journal of Plant Nutrition* **25**, 297-314

Tan LP (2004) Root-zone temperature effects on vegetable physiology in a commercial aeroponic system in Singapore. PhD thesis, Nanyang Technological University, Singapore, 263 pp

Teskey RO, Will RE (1999**)** Acclimation of loblolly pine (*Pinus taeda*) seedlings to high temperatures. *Tree Physiology* **19**, 519-525

Thimm O, Essigmann B, Kloska S, Altmann T, Buckhout TJ (2001) Response of Arabidopsis to iron deficiency stress as revealed by microarray analysis. *Plant Physiology* **127**, 1030-1043

Timperio AM, D'Amici GM, Barta C, Loreto F, Lello Z (2007) Proteomics, pigment composition, and organization of thylakoid membranes in iron-deficient spinach leaves. *Journal of Experimental Botany* **58**, 3695-3710

Touraine B, Muller B, Grignon C (1992) Effect of phloem-translocated malate on NO_3^- uptake by roots of intact plants. *Plant Physiology* **99**, 1118-1123

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* **157**, 423-447

Varadarajan DK, Karthikeyan AS, Matilda PD, Raghothama KG (2002) Phosphite, an analog of phosphate, suppresses the coordinated expression of genes under phosphate starvation. *Plant Physiology* ***1*****29**, 1232-1240

Vert G, Briat JF, Curie C (2003) Dual regulation of the Arabidopsis high affinity root iron uptake system by local and long-distance signals. *Plant Physiology* **132:** 796–804

Vessey JK, Layzell DB (1987) Regulation of assimilate partitioning in soybean: initial effects following change in nitrate supply. *Plant Physiology* **83**, 341-348

Vincentz M, Moureaux T, Leydecker M-T, Vaucheret H, Caboche M (1993) Regulation of nitrate and nitrite reductase expression in *Nicotiana plumbaginifolia* leaves by nitrogen and carbon metabolites. *Plant Journal* **3**, 315-324

Volder A, Smart DR, Bloom AJ, Eissenstat DM (2005), Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness. *New Phytologist* **165**, 493-502

von Wirén N, Gazzarrini S, Gojon A, Frommer WB (2000) The molecular physiology of ammonium uptake and retrieval. *Current Opinion Plant Biology* **3**, 254-261

Waisel Y (2002) Aeroponics: A tool for root research under minimal environmental restrictions. In: Waisel Y, Eshel A, Kafkafi U (Eds) *Plant Roots, the Hidden Half* (3rd Edn), Marcel Dekker, Inc., New York, Basel, pp 323-331

Ward JT, Lahner B, Yakubova E, Salt DE, Raghothama KG (2008) The effect of iron on the primary root elongation of *Arabidopsis* during phosphate deficiency. *Plant Physiology* **147**, 1181-1191

White PJ (2001) The pathways of calcium movement to the xylem. *Journal of Experimental Botany* **52**, 891-899

White PJ, Broadley MR (2003) Calcium in plants. *Annals of Botany* **92**, 487-511

Williamson LC, Ribrioux SPCP, Fitter AH, Leyser HMO (2001) Phosphate availability regulates root system architecture in Arabidopsis. *Plant Physiology* **126**, 875-882

Wissuwa M, Gamat G, Ismail AM (2005) Is root growth under phosphorus deficiency affected by source or sink limitations? *Journal of Experimental Botany* **56**, 1943-1950

Wurr DCE, Fellows JR, Hambidge AJ (1992) Environmental factors influencing head density and diameter of crisp lettuce cv. Saladin. *Journal of Horticultural Science* **67**, 395-401

Yanai J, Linehan DJ, Robinson D, Young IM, Hackett CA, Kyuma K, Kosaki T (1996) Effects of inorganic nitrogen application on the dynamics of the soil solution composition in the root zone of maize. *Plant and Soil* **180**, 1-9

Zhou K, Yamagishi M, Osaki M, Masuda K (2008) Sugar signalling mediates cluster root formation and phosphorus starvation-induced gene expression in white lupin. *Journal of Experimental Botany* **59**, 2749-2756

Zhang F, Smith DL (1994) Effects of low root zone temperatures on the early stages of symbiosis establishment between soybean [*Glycine max* (L.) Merr.] and *Bradyrhizobium japonicum*. *Journal of Experimental Botany* **45**, 1467-1473

Zhao D, Oosterhuis D (1998) Cotton responses to shade at different growth stages: Nonstructural carbohydrate composition. *Crop Science* **38**, 1196-1203

Zocchi G, Nisi PD, Dell'Orto M, Espen L, Gallina PM (2007) Iron deficiency differently affects metabolic responses in soybean roots. *Journal of Experimental Botany* **58**, 993-1000