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REGULAR ARTICLE

Characterization of fluoride uptake by roots of tea plants (*Camellia sinensis* (L.) O. Kuntze)

Lei Zhang · Qiong Li · Lifeng Ma · Jianyun Ruan

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Abstract
Backgrounds and aims Tea plants (*Camellia sinensis* (L.) O. Kuntze) accumulate high fluoride in the leaves whereas the mechanism on its uptake is poorly understood. The measured F^- uptake was compared to calculated uptake from transpiration rates assumuing no discrimination between F^- and water to characterize the property of F^- absorption by tea plant roots.
Methods The F^- uptake was examined by depletion method under variable external F^- concentrations, pH, temperature, relative air humidity, anion channel blockers and metabolism inhibitors in solution experiments.
Results Measured F^- uptake rates were significantly larger than those calculated from transpiration rates regardless of external F^- concentrations, uptake durations, relative humidity, and solution pH. The measured and net F^- uptake (subtracting that calculated from transpiration rate from the measured uptake) were reduced by low temperature and inhibited by anion channel and metabolism inhibitors anthracene-9-carboxylic acid (A-9-C), niflumic acid (NFA), and carbonylcyanide m-chlorophenylhydrazone (CCCP) but not by dihydro-4, 4′ diisothiocyanostilbene-2, 2′-disulphonic acid (DIDS). The F^- uptake showed biphasic response patterns, following saturable Michaelis–Menten kinetics in the range of low external F^- (below 100 $\mu mol L^{-1}$) while increased linearly with external supply in the range of high concentrations.
Conclusion The uptake of F^- by roots of accumulator tea plants was likely an active process and energy-dependent. This helps to explain why tea plants are able to accumulate considerably high F^-.

Keywords *Camellia sinensis* · Tea · Fluoride · Active uptake · Passive uptake · Kinetics · Root · Anion channel · Metabolism inhibitor · Fluoride accumulating plants

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Introduction

Fluoride is a common phytotoxic air and soil pollutant. Fluoride toxicity causes visible injuries and reduction in growth by inhibiting the activity of enzymes (Facanha and Meis 1995; Reddy and Kaur 2008) and

photosynthesis (Kamaluddin and Zwiazek 2003). The respiration rate and metabolism of carbohydrate, amino acids, nucleotides and lipids were also altered by F^- (Zwiazek and Shay 1988a, b). Fluoride metabolically inhibits root water hydraulic conductivity or water flow affecting gas exchange and leaf expansion (Kamaluddin and Zwiazek 2003). Fluoride can be absorbed by leaves from air or by roots from the soil. Solution experiments have shown that F^- uptake by plant roots was affected by its availability, pH and speciation in the substrates, as well as other elements such as Al, Ca and B (Takmaz-Nisancioglu and Davison 1988; Horner and Bell 1995; Arnesen 1997; Stevens et al. 1997, 1998a, b; Ruan et al. 2003, 2004). Many works have been done with non-accumulating plants (Venkateswarlu et al. 1965; Bar-Yosef and Rosenberg 1988; Takmaz-Nisancioglu and Davison 1988; Stevens et al. 1998a, 2000; Mackowiak et al. 2003) and it was suggested that F^- was passively or rejectively taken up by plants due to the endodermal barrier and very low permeability of cell membranes to F^- (Mackowiak et al. 2003; Weinstein and Davison 2004). A recent study suggested that aquaporins may be involved in the transmembrane transport of F^- (Calvo-Polanco et al. 2009). Plant species vary by several-fold in their sensitivities to environmental F^- (Doley et al. 2004). By contrast to other plants, tea plants nevertheless accumulate large amount of F^- in mature leaves from soils of normal F^- availabilities without toxicity symptom (Ruan and Wong 2001). Our previous works revealed that F^- was readily taken up by tea roots and most of the F^- was finally transported in particularly to the leaves (Ruan et al. 2003, 2004). Fluoride is an essential element to mammals and a small amount of F^- helps bone development whereas excessive ingestion may lead to fluorosis. Therefore, ingestion of F^- through food and beverage by human and animal has been a great concern, attracting extensive studies on F^- uptake by plants or crops (e.g. Fung et al. 1999; Jha et al. 2008; Stevens et al. 2000).

When tea plants accumulate very high F^- in leaves, the property of F^- absorption by roots remains poorly understood. In this work, the F^- uptake by tea plants was measured in a series of solution experiments by depletion method and was compared to that of water uptake to demonstrate the characteristics of F^- absorption by the roots of tea plants.

Materials and methods

Experiments of F^- uptake from nutrient solutions under varied conditions

Tea seedlings with 4–5 leaves germinated from seeds (cv. Longjing 43) in perlite were transplanted to 20-L nutrient solutions (pH 4.8) containing macronutrients ($mmol \cdot L^{-1}$) of N 1.7, P 0.07, Mg 0.67, Ca 0.53, K 0.67, S 0.7 and micronutrients ($\mu mol \cdot L^{-1}$) of B 7, Mn 1.0, Zn 0.67, Cu 0.13, Mo 0.33, Fe 4.2 (Ruan et al. 2007). The nutrient solution was replenished every week and completely renewed every 2 weeks. Tea plants were cultivated in a glasshouse with natural light conditions supplemented with additional high pressure sodium lamps (light/dark 16/8 h) to ensure a minimum intensity of 200 $\mu mol\, m^{-2} s^{-1}$ at canopy level. The air temperature was 30/20 °C day/night controlled by air conditioners. The relative humidity was maintained at about 70 % by humidifier. The following F^- uptake experiments were conducted under similar conditions except otherwise described.

Seedlings of similar sizes were selected for the uptake experiments. Each of two seedlings were transplanted to glass tubes (enwrapped by black film) filled with 80 mL continuously aerated uptake nutrient solutions containing F concentrations at 8, 44, 107 and 406 $\mu mol L^{-1}$ prepared from NaF. The concentrations of nutrients in the uptake solutions were the same as mentioned above while Ca and Mg were omitted to avoid any precipitation with F^-. The pH of uptake solutions was adjusted to 4.8 with 0.1 $mol L^{-1}$ H_2SO_4 or NaOH except otherwise stated. The depletion of F^- concentrations in the uptake nutrient solutions were monitored 12 and 36 h after the start of uptake. Water loss from transpiration was recorded by weighting the uptake tubes between experimental intervals. The same volumes of uptake nutrient solution without F^- (assuming 1 $g mL^{-1}$) were added to the uptake solutions to compensate water loss by transpiration every 12 h. Roots were sampled for measurement of dry weight (oven-dried at 60 °C) after termination of uptake. There were 4–5 replicates for each F^- concentration.

In the experiment investigating the effect of pH on F^- uptake, the pH of uptake nutrient solutions were adjusted to 2.0, 4.0, 5.0, and 6.0 (using 0.1 $mol L^{-1}$ H_2SO_4 or NaOH) and the F^- concentrations in uptake nutrient solutions were 18, 43 and 150 $\mu mol L^{-1}$. The

uptake was conducted for 12 h and there were 4 replicates for each combination of F^- concentration and solution pH.

For investigating the effect of temperature, the seedlings with preconditioned uptake nutrient solutions were transferred to lighting growth chambers (GXZ-260B, Jiangnan Instrumental Company, Ningbo, China). The temperatures of chambers were set at 5 °C, 15 °C, 25 °C and 35 °C. The concentrations of F in the uptake nutrient solutions were 11, 53, and 211 $\mu molL^{-1}$. The uptake was carried out for 24 h and there were 4 replicates for each treatment of temperature and F concentration.

To examine the influence of relative humidity on F^- uptake, the plants with uptake nutrient solutions were transferred to two neighbouring green houses with the same conditions except relative humidity which was set at 20 % and 90 %. The F^- concentrations in the uptake solutions were 28, 64 and 602 $\mu molL^{-1}$. The uptake was conducted for 48 h and the water loss by transpiration was measured 24 h after the onset of uptake by weighting and same volumes of uptake nutrient solution without F^- were supplemented. Each treatment of humidity and F^- concentration was replicated for 4 times.

To investigate the effect of anion channel and metabolism inhibitors, anthracene-9-carboxylic acid (A-9-C, 100 $\mu molL^{-1}$), niflumic acid (NFA, 100 $\mu molL^{-1}$), carbonylcyanide m-chlorophenylhydrazone (CCCP, 500 $\mu molL^{-1}$), and dihydro-4, 4′ diisothiocyanostilbene-2, 2′-disulphonic acid (DIDS, 100 $\mu molL^{-1}$) were added to uptake nutrient solutions. Inhibitors were pre-dissolved in 70 % (v/v) ethanol solutions. The F concentrations in uptake nutrient solutions were 22, 56, and 191 $\mu molL^{-1}$. There were 4 replications for each treatment including a control (CK) without inhibitors and the uptake duration was lasted for 6 h.

For the experiment measuring kinetics of F^- uptake, the concentrations of F^- in the uptake nutrient solutions were 8, 11, 23, 37, 45, 55, 107, 196 and 406 $\mu molL^{-1}$. A volume (determined by weighting) of uptake nutrient solution (without F^-) was added to replenish nutrients and water loss by transpiration after 12 h. A 2.0-mL aliquot of uptake nutrient solution was taken for measurement of F^- concentrations 24 h after the onset of uptake. The roots were sampled for dry weight measurement. There were 4–5 replicates for each F^- concentration. In another two sets of experiments, each of three tea plants were exposed to pots filled with 1.5 L nutrient solutions containing a set of F^- concentrations at 0, 26, 53, 105, 211 $\mu molL^{-1}$ and at 0, 211, 421 and 842 $\mu molL^{-1}$. After uptake for 6 h the tea plants were sampled and the leaves were oven dried and ground subjected to F^- measurement. All treatments were replicated for 3 times.

Measurement of F^- in nutrient solutions and plant leaves

The concentrations of F^- in nutrient solutions (at both onset and termination) were measured by ion chromatograph (ICS-200, Dionex Corp, USA) with IonPac AS18 column and AG18 protection column. An aliquot of 25 μl was injected and the rate of eluent (KOH and pure water) was at 1.0 $mLmin^{-1}$. The gradient of KOH was 5 $mmolL^{-1}$ at time zero, adjusted to 3 $mmolL^{-1}$ at time 1 min and maintained until 5 min, then increased to 60 $mmolL^{-1}$ at 5.01 min and maintained until 10 min and decreased to 5 $mmolL^{-1}$ at 10.1 min and kept thereafter until 13 min. The temperatures of column and detector were 35 °C and 30 °C, respectively. The recovery rate was 102.5±3.6 % and the detection limit was 0.13 $\mu molL^{-1}$. The concentrations of F^- in plant leaves were measured by a combination F^- selective electrode (Orion 9609BNWP, Themo Scientific, USA) with total ionic strength adjusting buffer after digested (Ruan et al. 2003).

Calculation of F^- uptake rates and statistical analysis

The measured F^- uptake rate was calculated from the difference of F^- amounts in the uptake solutions between the onset and after specified time of uptake. The F^- uptake calculated from transpiration rate (UCFT) was estimated from the product of the averaged F^- concentrations in the uptake solutions at the onset and after uptake and the volume of water loss (by weighting method assuming 1 gmL^{-1}) via transpiration streams within a given period. The net F^- uptake rate was calculated by subtracting uptake calculated from transpiration rate from the measured uptake. The rate of F^- uptake was express as per dry root mass (g) per hour.

Data were subjected to analysis of variance and Fisher's least significant difference (LSD) if initial F test shows significant ($p<0.05$) difference.

Results

Measured accumulated F^- uptake by tea plants increased significantly with increasing external F^- concentrations and uptake time (Fig. 1a, b). The water uptake rates (based on root mass) were unaffected by F^- concentrations (data not shown) while the F^- uptake calculated from the transpiration rate (UCFT) increased with F^- concentrations in the uptake solutions (Fig. 1a, b). The measured uptake was significantly larger than UCFT irrespective of F^- levels and uptake times. For example, the ratios of measured F^- uptake to UCFT were 7.3 and 4.9 at low (8 and 44 $\mu molL^{-1}$), 2.8 at moderate (107 $\mu molL^{-1}$) and 2.2 at high external F^- levels (406 $\mu molL^{-1}$), respectively, during the first 12 h (Fig. 1c). The corresponding ratios were 7.9, 6.2, 3.3 and 2.0 during 36 h, respectively for the low, moderate and high external F^- levels, respectively. The ratios of measured F^- uptake to UCFT decreased with increasing external F^- concentrations for both 12 h and 36 h uptake times.

The measured, UCFT and net F^- uptake were significantly affected by solution pH only at low external F^- concentration (18 $\mu molL^{-1}$) (Fig. 2). In such case, the measured and net F^- uptake rates were significantly larger at pH 2.0 and pH 5.0 than those at pH4.0 and pH 6.0 (Fig. 2 a, c). The UCFT was significantly lower at pH 2.0 than at other pHs although the water uptake rates (0.68–0.75 mLg^{-1} root h^{-1}) were unaffected by pH at this external F^- concentration (Fig. 2b). The measured F^- uptake rates were significantly larger than UCFT, regardless of nutrient solution pH. The ratios of measured to calculated F^- uptake were significantly larger at pH 2.0 and smaller at pH 6.0 for the low external F^- (18 $\mu molL^{-1}$) while was unaffected by pH at higher external F^- supplies (43 and 150 $\mu molL^{-1}$, Fig. 2d).

Exposure to low temperature considerably reduced measured F^- uptake and UCFT (Fig. 3a, b). The measured F^- uptake at 5 °C were only 37 %, 12 % and 55 % of those at 25 °C for the three F^- concentrations of 11, 53 and 211 $\mu molL^{-1}$ in the uptake solutions, respectively. On the contrary, increasing temperature to 35 °C increased measured F^- uptake by 52 %, 94 %, 165 % as compared to 25 °C for the three F^- levels, respectively. The average water uptake rates pooled over external F^- concentrations were significantly different, being largest (0.50 mLg^{-1} root h^{-1}) in high (35 °C) and smallest (0.19 mLg^{-1} root h^{-1}) in low (5 °C) temperature treatments. Accordingly, the UCFT were significantly lower at 5 °C than at other temperatures (Fig. 3b). The net F^- uptake was smallest at 5 °C and largest at 35 °C (Fig. 3c). In spite of temperature treatments, the measured F^- uptake always surpassed UCFT. The ratios of measured to calculated uptake were considerably larger at high temperature (35 °C) (Fig. 3d).

The measured, calculated and net F^- uptake were affected by relative humidity significantly only at high external F^- concentration (602 $\mu molL^{-1}$) (Fig. 4a, b, c). Regardless of relative humidity, the measured F^- uptake was generally much greater than UCFT. The ratios of measured to calculated F^- uptake were considerably lower at high (602 $\mu molL^{-1}$) than at other two external F^- concentrations and significantly affected by relative humidity at high external F^- concentrations (Fig. 4d). The water uptake rates were significantly different

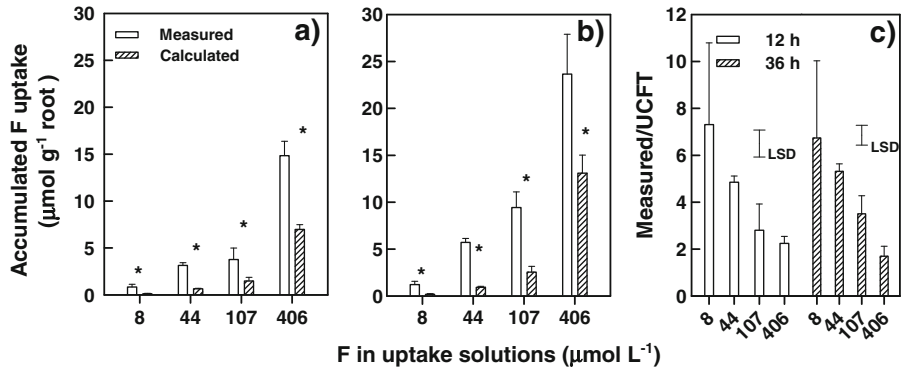

Fig. 1 Accumulated fluoride uptake measured by depletion method, calculated from transpiration (**a**, **b**), and ratio of the measured to calculated (**c**) by tea plants from uptake solutions containing variable F^- concentrations for 12 (**a**) and 36 (**b**) hours. *Asterisks or LSD bars above columns* indicate significant differences between the measured and calculated uptake (**a**; **b**) at each external F^- concentration or among F^- concentrations (**c**) at two uptake durations, respectively

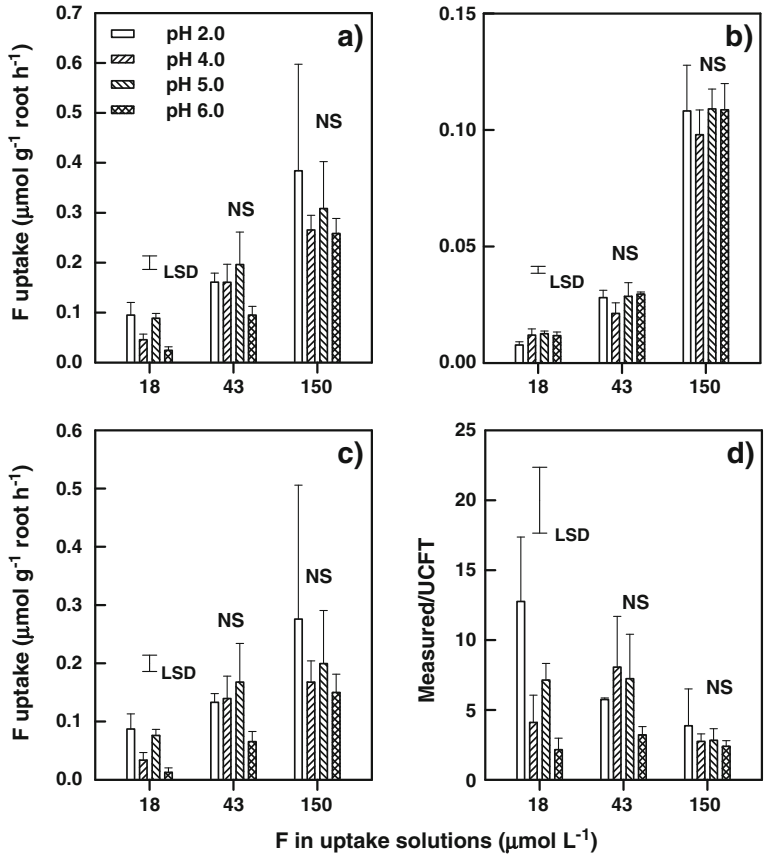

Fig. 2 Fluoride uptake measured by depletion method (**a**), calculated from transpiration (UCFT) (**b**), net uptake by subtracting that calculated from the measured (**c**), and ratio of measured to calculated (**d**) by tea plants from uptake solutions containing variable F^- concentrations with different solution pH. The uptake was carried out for 12 h. *LSD bars or NS above columns* indicate significant or insignificant difference between pH treatments at each external F^- concentration, respectively

between the two relative humidity at low (28 $\mu mol L^{-1}$) but insignificantly at other external F^- concentrations (Fig. 4e). However, when pooled over external F^- concentrations the water uptake rates were significantly ($p<0.01$) larger at low (20 %) than at high (90 %) relative humidity (1.01 vs 0.85 $ml g^{-1}$ root h^{-1}).

Measured and net F^- uptakes were strongly inhibited by inhibitors A-9-C, NFA, and CCCP (Fig. 5 a, c). The UCFT was affected by inhibitors significantly only at low external F^- concentrations (Fig. 5b). Water uptake rates were inhibited by NFA and A-9-C significantly only at low F^- (22 $\mu mol L^{-1}$) while unaffected by all inhibitors at high external F^- concentrations (56 and 191 $\mu mol L^{-1}$) (Table 1). The net F^- uptake was inhibited by 83.5–88.5 % by NFA, by 72–84.6 % by A-9-C and by 70.4–92.4 % by CCCP for the three F^- concentrations in uptake nutrient solutions (Table 1). The inhibition by NFA was indifferent among the three levels of external F^- concentrations whereas the inhibition by A-9-C was significantly stronger at low (22 $\mu mol L^{-1}$) and weaker by CCCP at higher (191 $\mu mol L^{-1}$) than at other external F^- concentrations. The ratios of the measured uptake to UCFT decreased from 9.4 to 15.9 in the CK plants without inhibitors to 3.1–6.0, 1.8–3.9 and 2.8–3.4 in the plants treated with A-9-C, NFA and CCCP, respectively. DIDS had no significant effect on F^- uptake and the ratio of measured to calculated uptake.

The response of F uptake to external F^- concentrations in the solutions showed biphasic patterns and differed in low and high external F^- concentration ranges (Fig. 6). The measured F^- uptake appeared saturated in the range of low external F concentrations (up to 100 $\mu mol L^{-1}$) but increased linearly in the high range. In the low F^- concentration range, the measured F^- uptake followed Michaelis–Menten kinetics and the V_{max} and K_m were estimated to be 0.41 $\mu mol g^{-1}$ root (DW) h^{-1} and 50.2 $\mu mol L^{-1}$, respectively. The F^- concentrations in the mature leaves increased linearly with increasing external F^- in the uptake solutions within the high range (0–800 $\mu mol L^{-1}$) but showed curving trend within the low range (0–200 $\mu mol L^{-1}$) (Fig. 7), following similar patterns as F^- uptake rate by depletion method in response to low and high external F^- supplies in nutrient solutions (Fig. 6).

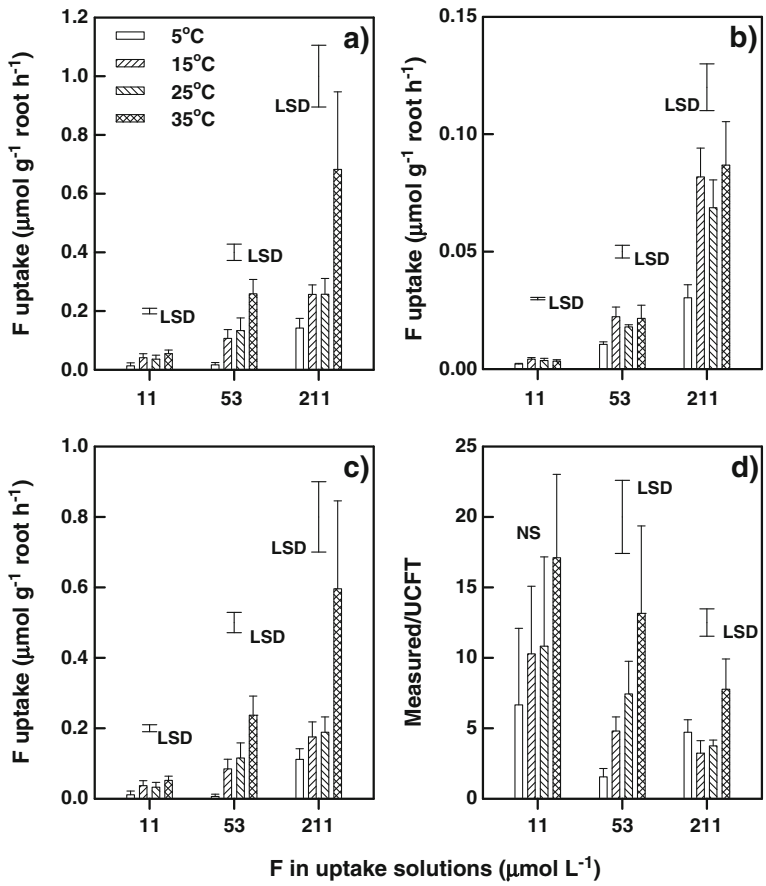

Fig. 3 Fluoride uptake measured by depletion method (**a**), calculated from transpiration (UCFT) (**b**), net uptake by subtracting calculated from the measured (**c**), and ratio of measured to calculated (**d**) by tea plants from uptake solutions containing variable F^- concentrations at 5 °C, 15 °C, 25 °C, and 35 °C for 24 h. *LSD bars or NS above columns* indicate significant or insignificant differences between temperature treatments at each external F^- concentration, respectively

Discussion

The purpose of the present study was to examine the characteristic of F^- uptake by roots of accumulating tea plants. The F^- uptake was measured by depletion method in the environments of variable external F^- concentrations, solution pH, temperature, relative humidity, and was compared to those calculated from transpiration rates. Such experimental approach provided important evidence of the absorption characteristic of elements such as F^- or others (e.g. Si) assuming no discrimination between F^- and water in uptake (Bar-Yosef and Rosenberg 1988; Cornelis et al. 2010; Liang et al. 2005; Mackowiak et al. 2003). If the measured uptake was greater than the amount supplied from mass flow of transpiration, F^- uptake was likely active (Cornelis et al. 2010; Liang et al. 2005; Mackowiak et al. 2003). Vice versa, if the latter was greater than the measured uptake, the uptake was likely passive. The present data (Fig. 7) and the previous experiments have shown that most F^- taken up by tea roots was readily transported to the above-ground organs particularly the leaves even within a relatively short uptake time (Ruan et al. 2003), excluding the possibility that F^- was adsorbed in extracellular compartment (apoplast) of roots as observed in other non-accumulating plants (Venkateswarlu et al. 1965; Takmaz-Nisancioglu and Davison 1988). In all cases the measured F^- uptake was significantly larger than that UCFT, indicating that there was likely an active component of F^- uptake in tea plants. Meanwhile the measured F^- uptake followed Michaelis–Menten kinetics and became saturated in the range of low F^- concentrations. The F^- concentrations in the mature leaves responded to external F^- supply in nutrient solutions in similar patterns (Fig. 7). Such patterns were largely different from the previously observed uptake with barley (Venkateswarlu et al. 1965), rice (Mackowiak et al. 2003), tomato and corn (Bar-Yosef and Rosenberg 1988), bean (Takmaz-Nisancioglu and Davison 1988), oats and tomato (Stevens et al. 1998a, b), and some pasture species (Stevens et al. 2000). It was believed that F^- was passively or rejectively taken up by these

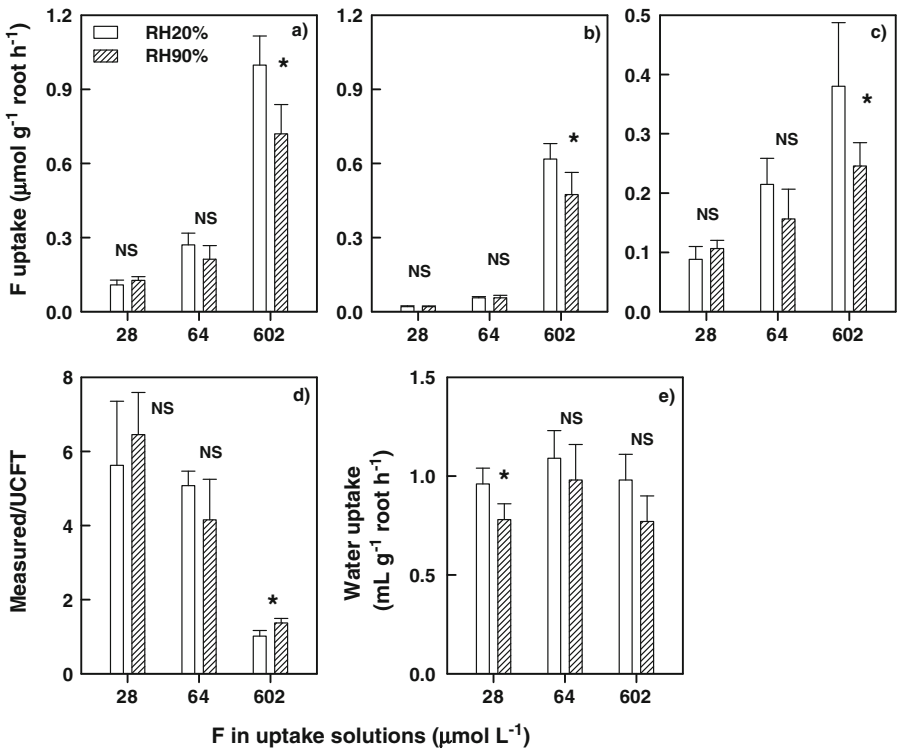

Fig. 4 Fluoride uptake measured by depletion method (**a**), calculated from transpiration (**b**), net uptake by subtracting calculated from the measured (**c**), ratio of measured to calculated (**d**), and water uptake rate (**e**) from solutions containing variable F^- concentrations by tea plants grown under two relative humidity. The uptake was conducted for 48 h. *Asterisks or NS above columns* indicate significant or insignificant difference between relative humidity treatments at each external F^- concentration, respectively

non-accumulating plants due to the endodermal barrier and very low permeability of cell membranes to F^-. In the present work, water uptake by tea plants was unaffected by F^- in the nutrient solutions. Therefore, the UCFT was not influenced by the mount of water uptake imposed by F^- treatments. In other study, F^- metabolically inhibits root water hydraulic conductivity or water flow in non-accumulating plants (Kamaluddin and Zwiazek 2003). The different observations of studies might be explained by the fact that tea plants are tolerant to F^-. However, this could also be due to the low F^- concentrations in the nutrient solution in the present study as compared to much higher levels in others (Kamaluddin and Zwiazek 2003). The present experiments showed that F^- uptake by tea plants was strongly inhibited when the plants were exposed to low temperature or when energy supply was reduced due to the presence of CCCP, the uncoupler of oxidative phophorylation in mitochondria. Early experiments showed that CCCP partially inhibits the pH gradient-activated Cl^- uptake and Cl^-/Cl^- exchange activities in brush-border membrane vesicles (Alvarado and Vasseur 1998) and active Cl^- uptake by plants (White and Broadley 2001).

The exact pathway of F^- transport across the membrane of plant roots is unclear. Due to a negative electrochemical potential gradient across root plasma membrane, the uptake of anions (e.g. Cl^-) is facilitated by transmembrane proteins such as transporters, channels or carriers (White 2012; Teakle and Tyerman 2010). It was suggested that anion channels in Cl^- transport or aquaporins may be involved in this process (Calvo-Polanco et al. 2009). Anion channels in plant membrane participate in various physiological functions, including for example Cl^- transport, NO_3^- storage and organic acid secretion (De Angeli et al. 2007; Roberts 2006; White and Broadley 2001). Treatment of A-9-C reduced Cl^- and NO_3^- uptake by inhibiting anion channels contributing to xylem loading of anions in barley plants and NIF (0.1 $mmol L^{-1}$) completely blocked the voltage-dependent Cl^- transport activity (Kawachi et al. 2002; Yamashita et al. 1996). It was reported that NIF was not a specific antagonist for one type of anion channel among the various anion channels in the plasma membrane and tonoplast (Yamashita et al. 1996). In the present study the uptake of F^- was strongly inhibited by anion blockers NFA and A-9-C irrespective of F concentrations in uptake nutrient solutions. The net F^- uptake was reduced by averagely about 86 % by NFA and 76 % by A-9-C, suggesting that anion channels might play a role in the absorption of F^- by tea plants.

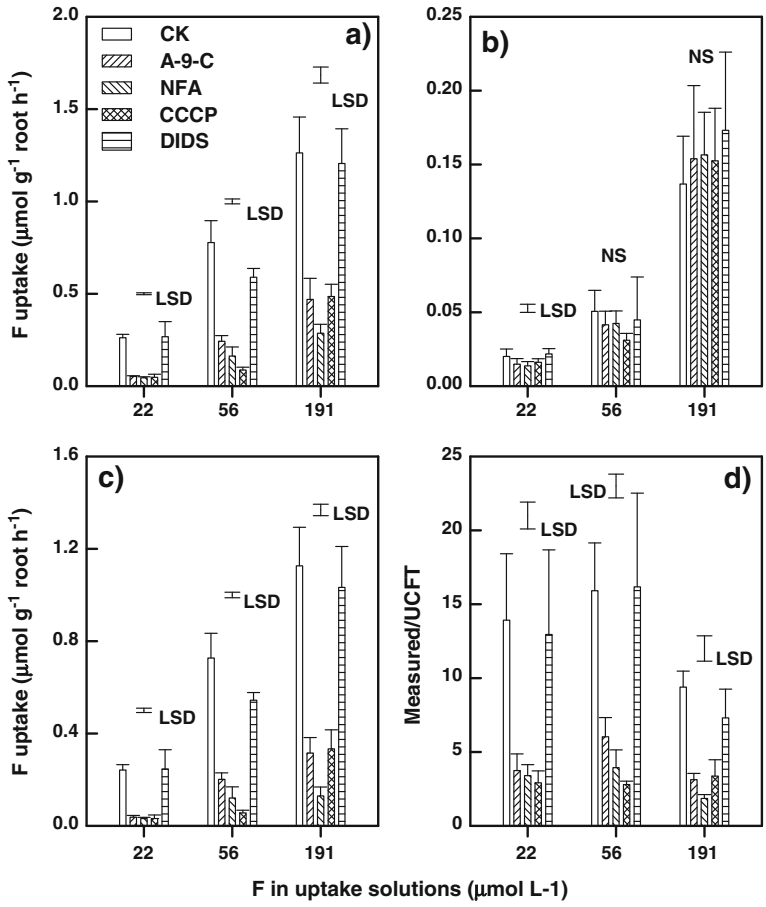

Fig. 5 Fluoride uptake measured by depletion method (**a**), calculated from transpiration (UCFT) (**b**), net uptake by subtracting calculated from the measured (**c**), and ratio of measured to calculated (**d**) from solutions containing variable F^- concentrations by tea plants grown with or without (CK) inhibitors. The uptake was conducted for 6 h. The concentrations of anthracene-9-carboxylic acid (A-9-C), niflumic acid (NFA), carbonylcyanide m-chlorophenylhydrazone (CCCP), and 4,4′-diisothiocyanatostilbene-2, 2′-disulphonic acid (DIDS) were 100, 100, 500 and 100 μmol L^{-1}, respectively. *LSD bars or NS above columns* indicate significant or insignificant difference between inhibitor treatments at each external F^- concentration, respectively

On the other hand, most F^- taken up by tea roots was readily transported via xylem to the leaves. The xylem loading after radial transport in roots is probably an important factor as well in determining F^- accumulation in the leaves. A number of channels or transporters involved in anion efflux to xylem have been identified (de Angeli et al. 2007; White and Broadley 2001; Teakle and Tyerman 2010). In the present study, decrease in F^- uptake could also be resulted from inhibition of xylem loading by channel blockers. More studies are therefore required to elucidate directly the role of channels or transporter in uptake and transport of F^- in tea plants. The biphasic responding patters of F^- uptake to external F^- concentrations possibly highlighted two transport systems in the roots as shown for other anions (Kochian et al. 1985; White and Broadley 2001).

The solution pH is an important factor influencing the species of F^- and therefore its bioavailability to plants. Previous experimental results showed that F^- can be taken up by plant roots as HF orders of magnitude over F^- ion for readily diffusion across the cell membrane (Horner and Bell 1995; Mackowiak et al. 2003; Stevens et al. 1998a, b). Mackowiak et al. (2003) suggested that F was absorbed by rice as HF^0 while F^- was likely restricted. In the present work, F uptake under different pH treatments (pH 4.0–6.0) was tested to measure the relative contribution of HF and F^- ion to tea plants. More alkaline treatment was not used for the reason that tea plants are sensitive to high pH above 6 (Ruan et al. 2007). Estimation of F species in solutions using modelling program GEOCHEM-EZ (Shaff et al. 2010) showed that the concentrations of HF ranged 0.01–1.30, 0.03–2.8 and 0.11–11.1 at pH 6.0–4.0 for external F concentrations of 18, 43 and 150 μmol L^{-1}, respectively. We did not observe significant pH effect on F uptake and the ratio of measured to UCFT for these F supplies regardless of largely different HF concentrations. In our work, an extremely acidic but unrealistic pH 2.0 was adopted in addition to other pHs. The corresponding concentrations of HF reached 9.6, 27 and 106.5 μmol L^{-1} at this pH value, respectively. The ratio of measured uptake

Table 1 Water uptake rate and relative net F^- uptake rate (CK= 100 %) by tea plants from solutions containing variable F^- concentrations and inhibitors

F concentration ($\mu mol \cdot L^{-1}$)	Inhibitor	Water uptake (ml g^{-1} root h^{-1})	Relative net F^- uptake (%)
22	CK	1.04±0.24b	100b
	A-9-C	0.73±0.19a	15.4±3.2a (A)
	NFA	0.65±0.14a	13.0±2.2a $^{(NS)}$
	CCCP	0.81±0.13ab	12.9±6.3a (A)
	DIDS	1.07±0.14b	101.6±34.6b $^{(NS)}$
56	CK	0.96±0.27^{NS}	100d
	A-9-C	0.78±0.17	27.6±3.9c (B)
	NFA	0.82±0.16	16.5±6.6b
	CCCP	0.61±0.09	7.6±1.6a (A)
	DIDS	0.87±0.57	74.8±4.5d
191	CK	0.76±0.17^{NS}	100c
	A-9-C	0.83±0.27	28.0±6.0b (B)
	NFA	0.85±0.16	11.5±3.4a
	CCCP	0.81±0.19	29.6±7.4b (B)
	DIDS	0.97±0.29	91.7±15.8c

Different lowercase letters or NS within the same columns indicate significant or insignificant difference among inhibitor treatments at each external F^- concentration, respectively. Different capital letters or NS in the brackets within the same columns indicate significant or insignificant difference among external F^- concentrations for each inhibitor, respectively

to UCFT increased remarkably as compared to other pHs only at low external F^- (18 $\mu molL^{-1}$) (Fig. 2d), suggesting that HF might contribute considerably to the total measured F uptake when F^- ion was very low

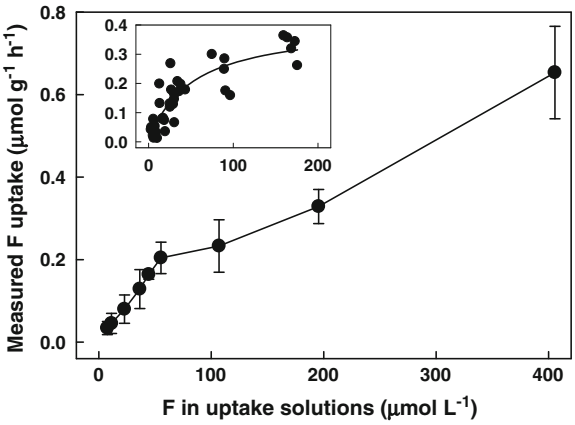

Fig. 6 Fluoride uptake measured by depletion method from nutrient solutions containing variable F^- concentrations for 24 h. The *inlet* shows saturation at range of low external concentrations and a two-parameter hyperbola nonlinear curve using the fitting regimes in Sigmaplot (SPSS, Michigan, USA)

under such extremely acidic conditions. The results showed that HF was likely not a major component taken up by tea roots under the conditions of prevailing pH range 4.0–6.0.

The overall data provided evidence supporting the F^- uptake by tea plant roots was an active process which was energy dependent and possibly mediated by a kind of anion transport system, in contradictory to passive or restrict absorption in other plants such as rice, barley, corn and tomato (Venkateswarlu et al. 1965; Bar-Yosef and Rosenberg 1988; Stevens et al. 1998a, 2000; Mackowiak et al. 2003; Weinstein and Davison 2004). The water soluble F^- concentrations of typical tea soils from China were 0.03–0.53 $mgkg^{-1}$ and averaged 0.14 $mgkg^{-1}$ (Ma et al. 2001). The F^- in soil solutions without considering its speciation were estimated to be 8–140 $\mu molL^{-1}$ and averaged 37 $\mu molL^{-1}$ assuming soil water content of 20 %. The measured K_m (50.2 $\mu molL^{-1}$) for F^- uptake by tea plants was therefore ecologically relevant to normal levels of F^- in soil solutions. Using the model of previous work (Fig. 1 of Bar-Yosef and Rosenberg 1988) and assuming external F^- concentration of 37 $\mu molL^{-1}$ (around the averaged solution F^- concentration in tea soils, Ma et al. 2002), the uptake rates of F^- of corn and tomato per unit root weight were 1.3 and 1.8 $nmolg^{-1}$(root FW) d^{-1}. Under the same external F^- concentration and using the Michaelis–Menten kinetics of the present work (Fig. 6) the F^- uptake rate for tea plants will be 0.74 $\mu molg^{-1}$ (root FW) d^{-1} when the calculated uptake rate by transpiration stream was 52 $nmolg^{-1}$ (root FW) d^{-1}. This rough estimation showed strong capability of tea plants to absorb F^- from substrate although such comparison

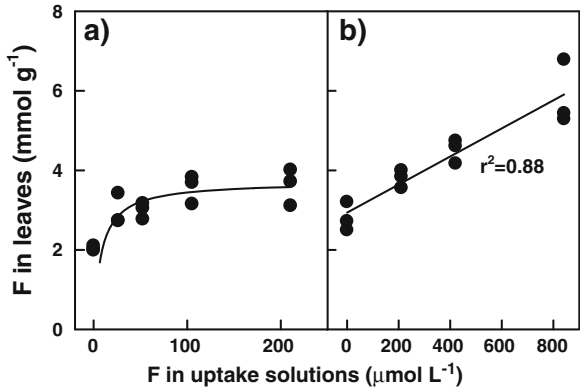

Fig. 7 Concentrations of F in the leaves of tea plants grown in nutrient solutions containing ranges of low (**a**) and high (**b**) external F^- concentrations for 6 h

cannot be very precise due to variations in experimental conditions. The coexisting of active with passive components during F^- uptake by tea plant roots therefore helps to explain the reason why tea plants are able to accumulate high F^-. The exact function of active F accumulation by tea plants is unclear from the present study. One possible explanation could be the transport in the xylem and detoxification of aluminium (Nagata et al. 1993), which however requires further investigation.

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