Empirical Modeling of Hormone Flows in Reciprocally Grafted Tomato Plants

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Grafted vegetable transplants represent a significant component of vegetable industries throughout the world. Control of rootstock traits can be utilised to improve crop quality and resistance to a variety of biotic and abiotic stresses. The challenge of optimising vegetable production by rootstock-mediated crop improvement is, in part, due to the inconsistency between the expected phenotypes of grafted plants based on particular hormone traits and the varying compatibility between the scion and the rootstock. Abscisic acid (ABA) deficient mutant *flacca* provides a useful tool to illuminate the scion/rootstock interaction and communication. Empirical flow models have been used previously to illuminate the hormone flow and metabolism within plants. This project produced empirical models for self and reciprocally grafted wild type and *flacca* plants. Wild type rootstocks are sufficient to phenotypically revert *flacca* scions, while *flacca* rootstocks have no effect on wild type scion phenotype with regards to leaf area, biomass and transpiration. In addion empirical flow models were used to illuminate the scion and rootstock contribution, to xylem ABA concentration revelling that the addition of wild type plants either as a scion or rootstock stimulates ABA production in the reciprocal *flacca* tissue contributing to an intermediate concentration of ABA within xylem sap. However, care must be drawing conclusions when using these models which can be affected by changes in nutrient uptake.

Keywords — ABA, Empirical Models, Grafting, Xylem, Phloem, Flacca

Word count — 9274.

INTRODUCTION

Global Agriculture

The global food system is expected to experience an unprecedented mix of pressures over the next 40 years. The global population is estimated to surpass 9 billion by 2050 (UN-DESA 2013), whilst growing wealth in developing countries like China will lead to an increased demand for high-quality and more varied diets. This, in turn, will require additional resources for the production of foodstuffs, putting current agricultural systems under pressure (Foresight 2011). Meanwhile, the effects of climate change are likely to reduce crop yields and the total area available for agriculture, intensifying competition for land, water and energy. In combination, these factors have come to be referred as the 'perfect storm' by Professor Sir John Beddington (the previous Government Chief Scientific Advisor). In a recent report he stated "Agriculture will have to adapt to increasingly variable and unpredictable growing conditions. This will require farmers and other food producers to learn new skills, from traditional agronomy and husbandry" (Foresight 2011). Responding to these pressures, there is increasing focus on 'sustainable intensification' as a means to increase yields on underperforming landscapes while simultaneously decreasing the environmental impacts of agricultural systems (Davies et al. 2009; Tilman et al. 2011; Foley et al. 2011).

For many years, science has been applied to enhance agriculture. In the past, and as part of the 'green revolution', yields increased dramatically with optimum fertiliser application, and the development and introduction of more robust dwarf cereal varieties. However, it is unlikely that large scientific developments such as these will be established and implemented at a sufficient rate in future to match rising demand. Therefore, science must seek to focus on maximising the efficiency of growth and improving the tolerance of crop plants to sub-optimal growing conditions; For example, grafted plants have been used recently to induce resistance to low and high temperatures (Zhou et al. 2009; Rivero et al. 2003), salinity, enhance nutrient uptake(Ahmedi et al. 2007), induce resistance against heavy metal toxicity (Rouphael et al. 2008) and increase synthesis of endogenous hormones (Dong et al. 2008). Furthermore, with the current opposition to the use of genetically modified organisms within the food production system – particularly in Europe – this necessitates the development of 'natural' solutions to the problems.

In the field, plants may experience a number of different abiotic stresses that significantly impact plant yields; including: extremes of temperature, higher concentration of salt (salinity), and water shortage (Mahajan & Tuteja

2005). Theses stresses affect a large proportion of land globally, for example, high salinity impairs crop production on at least 20% of irrigated land worldwide (Tuteja 2007) resulting in up to 50% land loss by the middle of 21st century. This is predicted to have devastating global effects on food production (Mahajan & Tuteja 2005). With increasing climatic changes and competition for limited resources such as freshwater, these stresses will be a serious and growing problem. Plants have the capacity to perceive and respond adaptively to abiotic stresses such as those imposed by salt, cold, drought and wounding. This adaptive process to stress is controlled, mediated and regulated by plant hormones; particularly in the examples above, by the phytohormone, abscisic acid (ABA), which acts as a messenger in the regulation of the plant's water status (Huang et al. 2012; Chinnusamy et al. 2004).

Use of ABA signalling responses in Agriculture

Successful manipulation of the root to shoot signalling of ABA has already been demonstrated through the technique of partial root zone drying, which has been applied to improving crop quality and water use efficiency (Dodd et al. 2006; Jacobsen et al. 2012; Sun et al. 2013). This technique involves the alternation between drying and re-wetting of areas of the root zone, stimulating the production of ABA as the soil dries. This causes the plant to grow more conservatively, reducing vegetative growth and improving water use efficiency, without affecting yield(Santos et al. 2003).

Plant ABA responses

Plant hormones are important endogenous factors which regulate all aspects of plant vegetative and reproductive development, and thus, are believed to be key factors in root–shoot communication. The "hormone message concept" states that hormones are produced in one part of the plant, translocated, and have physiological effects elsewhere in the plant, affecting a remote tissue (Aloni et al. 2010). A number of hormones have been identified within plants which collectively affect nearly all aspects of development, morphology and functioning (Davies 2004). It has also been demonstrated that feedback loop exists between hormones, for example the feedback between ABA and gibberellin controlling seed germination. These integrated feedback loops allow plants to integrate incoming signals from number of stimuli, mean that a hormone signal can be amplified or muted depending on the whole plant environment, enabling adaption to the most pressing need in a changing environment. This allows few core chemicals to dramatically affect gene expression and growth (Besnard et al. 2011). Plant hormones also act as a sensor and regulator of a particular stress. For example, ABA acts as both a

sensor and regulator of water status being produced in response to drying soil and acting on the guard cells in the leaf to reduce transpiration (Dodd 2005).

ABA production is dynamic and used as a signal throughout a plant's life cycle, controlling seed germination and a number of developmental processes. However, ABA also plays an important role in integrating various stress signals and controlling downstream stress responses(Urano et al. 2009); which it is now more commonly recognised for. Plants are capable of adjusting ABA levels continuously in response to changing environmental and physiological conditions through systemic signalling, including in cases of severe stress (Urano et al. 2009). For example, water deficit has been previously shown to stimulate both within-leaf ABA accumulation (initially due to decreased catabolism and later due to increased synthesis) and ABA delivery to the leaf through the xylem sap (due to increased root ABA synthesis) (Kim et al. 2012; Speirs et al. 2013). This is one of the main actions of ABA, specifically targeting guard cells for induction of stomatal closure (Israelsson et al. 2006). To date, the mechanisms for fine-tuning of ABA levels remain elusive as the mechanisms by which plants respond to stress include both ABA-dependent and ABA-independent processes (Qin et al. 2011).

The importance of ABA in the signalling and in response to many of the yield limiting stresses, and its action to reduce vegetative growth allowing increased resources to be allocated to the fruits, makes it an attractive target for manipulation to improve crop growth and yield in both agriculture and horticulture.

Plant Grafting

Grafted vegetable transplants represent a significant component of vegetable industries throughout the world. Control of rootstock traits can be utilised to improve crop quality and resistance to a variety of biotic and abiotic stresses (Trionfetti Nisini et al. 2002; Davis & Perkins-Veazie 2008; Pérez-Alfocea et al. 2010; Ghanem et al. 2011). Furthermore, the use of grafted rootstock allows the quick exploitation of a particular plant trait, providing the benefits of improved tolerance, without the need for genetic modification or lengthy breeding times. However, the use of grafted plants in the vegetable sector has not reached its full potential. This is mostly due to high pricing of the grafted seedlings, development of stable graft unions between the scion and the rootstock (especially at the late stages of fruit bearing plants), and a lack of adequate information about the factors and the processes that determine the communication between the grafted partners under diverse environmental conditions (Aloni 2010).

Over recent years, growing understanding of the impact of hormone signalling within stress responses have been used to identify novel grafting combinations and target selection of new rootstocks of interest. This has led to some success in attenuating stress-induced limitations to crop productivity (Ghanem et al. 2011), highlighting the usefulness of exploiting the root to shoot signalling pathways.

The challenge of optimising vegetable production by rootstock-mediated crop improvement is, in part, due to the inconsistency between the expected phenotypes of grafted plants based on particular hormone traits and the varying compatibility between the scion and the rootstock (Edelstein et al. 2004; Aloni et al. 2010). The causes of these inconsistencies are not always clear and there are some discrepancies in the literature on the contribution of the rootstock and scion to the hormone signalling within the plant.

ABA signalling, metabolism and recycling

It is mainly thought that long distance stress signalling of ABA is predominately elicited from ABA produced by the root system (Wilkinson & Davies 2002; Wilkinson & Davies 2008), however, studies have shown that leaf cells can also synthesize ABA (Cutler & Krochko 1999; Christmann et al. 2007). An ABA signal from the shoot, generated in response to hydraulic signal, is thought to be sufficient to facilitate a stomatal response in isolation, ruling out a function for root-sourced ABA in the communication of a water deficit from the root to the shoot (Christmann et al. 2007). However, it is also reported that the recirculation of ABA originally synthesized in the shoot back into the transpiration stream, via the root, represents a substantial proportion of the root-sourced signal (Wolf 1990; Neales & McLeod 1991). In addition, stomatal reactions are usually much better correlated with ABA xylem concentration than with leaf bulk ABA concentration (Jiang et al. 2007), even though there is often great variation in the apparent sensitivity of leaf conductance to a given concentration of ABA in the xylem stream (Correia & Pereira 1995). This suggests that there is likely modification of the ABA signal within the leaf tissue before stomatal response is observed (Trejo et al. 1993; Trejo et al. 1995; Wilkinson & Davies 2008). Therefore, it is important to be able to differentiate deposition resulting from changes between the catabolic degradation of ABA in the leaf, and those that arise due to changes in ABA import from the root.

These previous findings suggest that shoots may generate and respond to ABA-based signals produced within shoots themselves, independently of those sourced from the root, or, ABA-based signals from both sources. Therefore,

interactions between them are likely to be important in determining plant responses to various signals(Wilkinson & Davies 2002). In turn, rather than estimating these effects from point measurements, complete models of ABA flows within plants are necessary to fully understand the responses of this hormone to signalling.

In a recent case, it was demonstrated that external ABA application can be used to improve the production of tomato by reducing the incidence of blossom end rot, a common issue caused by the high transpiration conditions used to drive growth in commercial glasshouses by reducing the transpiration of the leaves, a subsequent increase in the transpiration flow to the developing fruits is caused (Tonetto de Freitas et al. 2013). Unfortunately, this method is not commercially viable as the cost of application is extremely high, so a method to deliver the desired signal response without external intervention would be desirable.

It is apparent that to successfully improve crop tolerance to the stresses described, further work must be undertaken to understand mechanism by which plant root and shoot systems communicate and integrate increasing environmental signals. This may be achieved through the observation of communication between shoot and root stocks in hormone over or under producing stains; which may, in future, be applied to uses within agriculture and horticulture.

One example of this is shown in the literature of grafting between near isogenic genotypes of tomato. *Flacca* is a ABA deficient mutant of tomato, impaired in the biosynthesis pathway, grafts using this genotype were used to investigate the effects of ABA deficiency on shoot growth. The shoots of wild-type/*flacca* (scion/rootstock) plants have been shown to be indistinguishable from wild-type self-grafts with regards to biomass, leaf area, stomatal behaviour or transpiration and leaf water potential (Chen et al. 2003; Holbrook et al. 2002; Jones et al. 1987; Fambrini & Vernieri 1995), indicating that shoot ABA biosynthesis is able to maintain a wild-type shoot phenotype, independently of root ABA biosynthesis. However, *flacca*/wild-type plants display phenotypic reversions of leaf area, stomatal conduction, transpiration, leaf water potential and leaf ABA concentration (Jones et al. 1987; Fambrini & Vernieri 1995; Chen et al. 2003) demonstrating the importance of rootstock derived ABA. This data highlights the difficulty in using rootstocks to elicit a particular phenotype in the shoot.

Furthermore, it has been reported that within that wild type/*flacca* plants, the xylem ABA concentration was similar to that of wild type. Indicating that the

xylem sap ABA concentration is determined solely by the scion (Dodd, Theobald, Richer, &Davies, 2009). However in other studies it has been shown to be an intermediate concentration between wild type and *flacca* self-grafts respectively (Holbrook 2002) indicating that xylem concentration is determined by both the scion and the rootstock. The relative contributions of scion and rootstock derived ABA to xylem sap is unknown. Clearly, further investigation into the contribution of both the rootstock and scion to the hormone status of the xylem sap within grafted plant is required, to maximise the benefit from future graft combinations.

One possible explanation for this is a disruption of the natural signalling response caused by the grafting process. Sorce, et al. (2002) demonstrated that in non-grafted Prunus species, the plant hormones auxin and cytokinin are in similar concentrations. However, in grafted plants the auxin and cytokinin balance is upset, therefore the invigorating properties of the rootstocks induce a higher growth rate in the scion, possibly by increasing the supply of cytokinin to the shoot and decreasing that of IAA. However, it has also been shown that the graft union has no effect on xylem ABA (Dodd et al. 2008). Alternatively, shoot derived ABA could be recirculated to the roots via the phloem causing an increase in xylem ABA driven by the shoot (Wolf 1990) .Therefore creating an empirical model applicable to the rootstock–scion relationship within grafted plants is worth investigating.

Empirical incremental flow models, are based on the measured increase in water, mineral nutrients, carbon assimilates or phytohormones between two defined time points. They have been used previously to model the flow of nutrients and hormones to parasitic plants through the union, or changes to hormone flows within plants due to rhizobacteria (Jeschke & Pate 1991; Jiang et al. 2007; Jiang & Chen 2012).

Since these models have never been used to illuminate the root to shoot signalling within grafted plants, the objectives of this study were to develop empirical models to highlight the ABA hormone cycling within the whole plant. This is intended to allow insight to the scion and rootstock contribution to root xylem ABA composition, to identify mechanisms of ABA re-cycling or metabolism within scion in response to rootstock genotype in grafted tomato, and to test the validity of the models based on measurements of transpiration rates. These experiments and models will test the hypothesis that the phenotypic reversion observed is due to increased wild type contribution to xylem sap ABA in grafted plants.

Materials and Methods

Plant material and growth conditions

Isogenic wild type (WT, Solanumlycopersicum cv. Ailsa Craig) and the ABA-deficient mutant *flacca* genotypes of tomato were used in all experiments.

The flc genotype is an ABA-deficient mutant of tomato impaired in the oxidation of ABA-aldehyde to ABA (Taylor et al., 1988), giving leaf ABA concentrations only 26–33% of the wild type (Neill and Horgan, 1985; Sharp et al., 2000).

All plants were grown from seed in a well-watered loam-based substrate (John Innes No 2, J Arthur Bowers, Lincoln, UK) in seedling trays, with a single seed in each separate compartment (3 cm deep x 2 cm x 2 cm).

Plants were grafted at the emergence of the second true leaf with graft unions established just below the cotyledonary node as previously described (Chen et al., 2002, Dodd et al., 2009). A vertical cut was made in the rootstock, into which the shoot tip inserted, and the union bound with grafting clips. Plants were immediately covered with transparent plastic bags, secured with elastic bands around the pots and left to establish in semi-darkness for 2–3 weeks. Towards the end of this period, plastic bags were removed for short intervals (4–6 h) each day, to harden the plants to the growth environment and were then available for use in experiments.

Plants were grown in a controlled environment greenhouse at the Lancaster Environment Centre: 16 h photoperiod, with air temperature ranging from 20 to 26 ºC, relativity humidity from 37 to 61%, and a PPFD at plant height of 1000 µmol m-2 s-1 unless otherwise stated.

Modelling experiment

Self- and reciprocally-grafted wild-type (WT; Ailsa Craig) and ABA deficient *flacca* (flc) were grown; and four scion/rootstock plant combinations were assayed: Wt/Wt, Wt/Flc, Flc/Wt, Flc/Flc (n=9).

Grafted plants were transplanted into enclosed cylindrical pots designed to fit within a pressure chamber (height 20cm, diameter 9cm) in a sand substrate. Plants were covered with transparent plastic bags for 1 week to establish. After 1 week, plastic bags were removed for short intervals (2-3h) each day to harden plants to the growth environment. Plants were watered daily with Hoaglands solution (Hoagland and Arnon, 1933) to drained capacity and randomly distributed within the controlled environment room, with the position of each plant re-randomized daily. Plants were allowed to grow in this environment for 3 weeks prior to harvest. Whole plant transpiration was measured gravimetrically 1 day prior to harvest for 10 hours in the light.

Plants were randomly split into two groups with the second group of plants harvested 7 days after the first to allow the growth interval to be calculated. Plants were watered to capacity before being placed in the pressure chamber which was then sealed. Root zone pressure was incrementally increased until exudation of xylem sap from cuts in the leaf central vein; sap was harvested sequentially at four points, leaves, petiole, stem and below graft union (figure 1). The initial exudate was washed off with deionised water and dried using filter paper, xylem sap was then sampled to collect 150 μL into pre-weighed 1.5ml eppendorfs. Xylem sap was collected from the cut below the graft union collection point at decreasing pressures of 9, 6 and 4 bar in order to calculate the root hydraulic conductance. Hydraulic conductance was calculated from the gradient of the linear regression between flow rate per gram of root fresh weight plotted against external pressure applied.

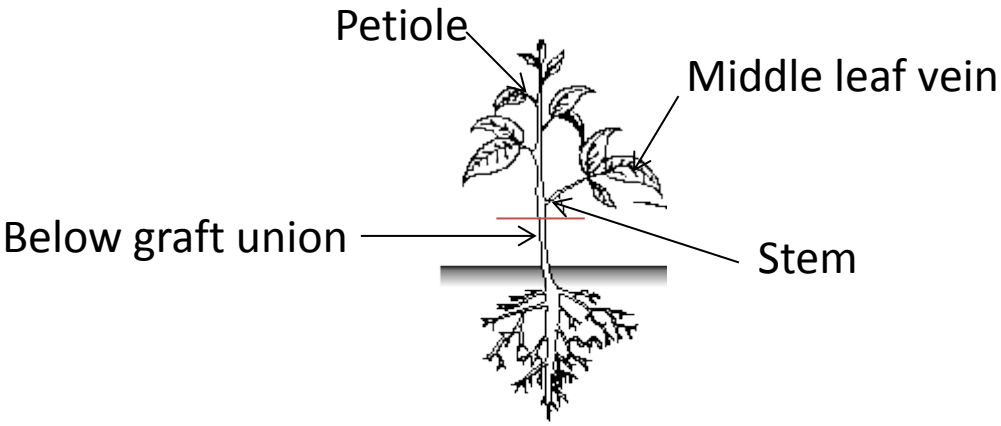

Figure 1. *A diagrammatic representation of a grafted plant used in the experiments, red line indicates graft union and arrows indicate the points at which xylem sap was harvested, grey bar represents top of pressure pot.*

Samples were frozen in liquid nitrogen and stored at -80oC prior to ABA and nutrient analysis. Leaf, stem and root fresh weight and leaf area were recorded. A sub-sample was taken, weighed and frozen in liquid nitrogen and stored at -80oC with the remainder oven dried at 60oC for at least 48 hours. Phloem exudates were obtained by placing a single detached leaf in vials containing 1.5ml of 5mM Na2-EDTA solution and maintained in a humid environment for a period of 5h, previously described (Wolf 1990; Jiang & Chen 2012). Sap samples were then frozen in liquid nitrogen and stored at -80oC prior to analysis.

For mineral analysis, dry samples of root, stem and leaf tissue were ground to a fine powder. The mineral concentrations were determined by inductively coupled plasma emission optical spectrometry (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA) after an acid digestion in HNO3:H2O2 (5:3 by volume) in a microwave that reached 190ºC in 20 minutes and held at this temperature for 2 hours (CEM Mars Xpress, North Carolina, USA). The nitrogen concentration was determined using a Thermo- Finnigan 1112 EA elemental analyser (Thermo-Finnigan, Milan, Italy) at the facilities in CEBAS Murcia, Spain.

For ABA analysis frozen plant material was freeze dried and ground to powder; deionised water was added and samples were shaken in a cold room overnight to extract ABA, with a dilution factor 1:25. Liquid samples required no further processing before ABA analysis. Samples were then subjected to a Radio-Immunoassay (Quarrie et al. 1988) using the monoclonal Tritium

labelled ABA and antibody MAC252, precipitated with ammonium sulphate. Sap volumes used in the assay were typically 50µl.

Relative growth rate (RGR), mean net assimilation rate (NAR) and leaf area ratio (LAR) were calculated using the equations below:

$$\text{RGR}=\frac{\ln(M_{D2})-\ln(M_{D1})}{t}$$

Where MD1 and MD2 are the total dry mass of each organ (leaf, shoot, root) at the first harvest point and second harvest point respectively and t is the growth period between harvests.

$$NAR=\frac{(M_{T2}-M_{T1})(\ln(L_{A2})-\ln(L_{A1}))}{(t)(L_{A2}-L_{A1})}$$

Where MT1 and MT2 are the total dry mass of each plant at the first and second harvest point with LA1 and LA2 representing the total leaf area at the first and second harvest respectively.

$$LAR=\frac{Total\ Leaf\ Area}{Total\ Biomass}$$

To indicate whether the variation in RGR may be achieved by either modifications in leaf allocation and leaf morphology or by increases in foliage physiological potentials (NAR)

The growth requirement for each element was calculated as the sum of the RGR multiplied by the element content (%) of each organ.

Modelling plant internal flows

Empirical models of ABA flow were built from net potassium flow models. These K models were calculated based on the assumption that (i) calcium is only transported in the xylem. Therefore, you can conclude that the Ca increments (ΔCa) in the tissue were exclusively caused by uptake in the roots and subsequent transport in the xylem. The second assumption was that (ii) mass flow occurs in the xylem and phloem; hence solutes are trans-located according to their relative concentrations.

From these assumptions the net xylem potassium flow (moles per plant) from root to shoot (Jk,x) was calculated from the ratio of potassium to calcium [K/Ca]x in xylem sap and the increment of calcium in the shoot, ΔCa between harvests (Armstrong and Kirkby, 1979):

$$\mathbf{J_{k,x}=[K/Ca]_x \times \Delta Ca \quad (1)}$$

Net potassium flow in the phloem (Jk,p) was calculated from the difference between the total potassium increment (ΔK) in each organ between harvests and the net xylem import to that organ, Jk,x:

$$\mathbf{J_{k,p}=\Delta K - J_{k,x} \quad (2)}$$

The total element content in each organ was calculated from the mineral concentrations and the dry weights. (See table 1)

The potassium flows form the basis of the estimation of ABA flow, with net xylem and phloem flow of ABA (JABA,x and JABA,p respectively) given by the associated ratio of ABA to K [ABA/K] and K flow within the xylem sap or phloem excaudate:

$$\mathbf{J_{ABA,x}=J_{k,X} \times [ABA/K]_X \quad (3)}$$

$$\mathbf{J_{ABA,p}=J_{k,p} \times [ABA/K]_p \quad (4)}$$

The differences between the total ΔABA and the import and export to a particular organ (with an influx into, or an efflux from, an organ being a positive or negative flow, respectively) was used to estimate the metabolic changed of ABA (JABA,met); indicating synthesis (where JABA was negative) or degradation (if JABA,met was positive) within the tissue:

$$\mathbf{J_{ABA,met} = \Delta ABA - J_{ABA,p} - J_{ABA,x} \quad (5)}$$

The flow modelling approach presented, has been used previously(Jeschke & Pate 1991; Jiang et al. 2007; Jiang & Chen 2012) and depends on increments of nutrient and ABA contents between first and second harvest, the standard errors of which are presented below (Table 1).

Results

Physiological Measurements

Wild type rootstock restored flacca scion biomass, leaf area and transpiration rate to similar levels observed in wild type plants.

Flacca plants had significantly reduced leaf dry weight, stem dry weight and leaf area compared to wild type self-grafts, but similar root dry weights (Figure 2, ANOVA, $p=0.02$). WT/flc graft combination had increased root growth compared to all others with a 3 fold increase in root biomass, but similar shoot biomass and leaf area to the wild type plants (ANOVA, $p<0.001$). Yet, this increased root biomass does not increase root hydraulic conductance or whole plant transpiration rate, therefore, the wild type scion was able to control transpiration even when grafted on an ABA deficient rootstock (figure 3). Wild type rootstock restored leaf area, leaf and stem biomass in *flacca* scions to levels similar to those observed in wild type scions (ANOVA, ns). The rootstock also was able to reduce the transpiration per unit leaf area of the *flacca* scion to levels of those with a wild type scion.

Over the growth period, *flacca* plants had a 2 fold increase in relative growth rate in the leaf, stem and root compared to wild type plants (Figure 3). However, this could be dues to the small size of the *flacca* plants. Reciprocal grafts had an intermediate growth rate in the leaves and root compared to wild type self-grafts. However, net assimilation rate and leaf area ratio was determined by the rootstock (Anova $p<0.001$). Meanwhile, *flacca* rootstock only partially increased the net assimilation rate.

Flacca self-grafts, displayed a 4 fold increase in Ca uptake compared to wild type plants (ANOVA $p<0.05$); though this increased uptake did not correspond with an increase in the calcium proportion in the leaves, stem or roots. The growth requirement for each element was calculated. It was found that there was in fact a significant increase in the demand for Ca, K and P within *flacca* scions over the growth period (ANOVA, $p<0.05$). This corresponds with the increased relative growth rate of *flacca* plants over the experimental period, indicating that this uptake in *flacca* plants was driven by the growth requirements of the plant, rather than a specific affinity for these elements.

Wild type rootstock significantly increased calcium uptake in the flc/WT plants (Anova $p<0.05$) this corresponded with higher calcium proportion in the leaf and stem tissue within the plants and a 4 fold increase in the incremental calcium change over the growth period (ANOVA $P<0.001$). However, this did

not correspond with an increased growth requirement of this element which was similar to that of wild type plants; suggesting an increased affinity of this graft combination to calcium.

There was no effect of the WT/flc graft combination showed similar calcium uptake to wild type plants.

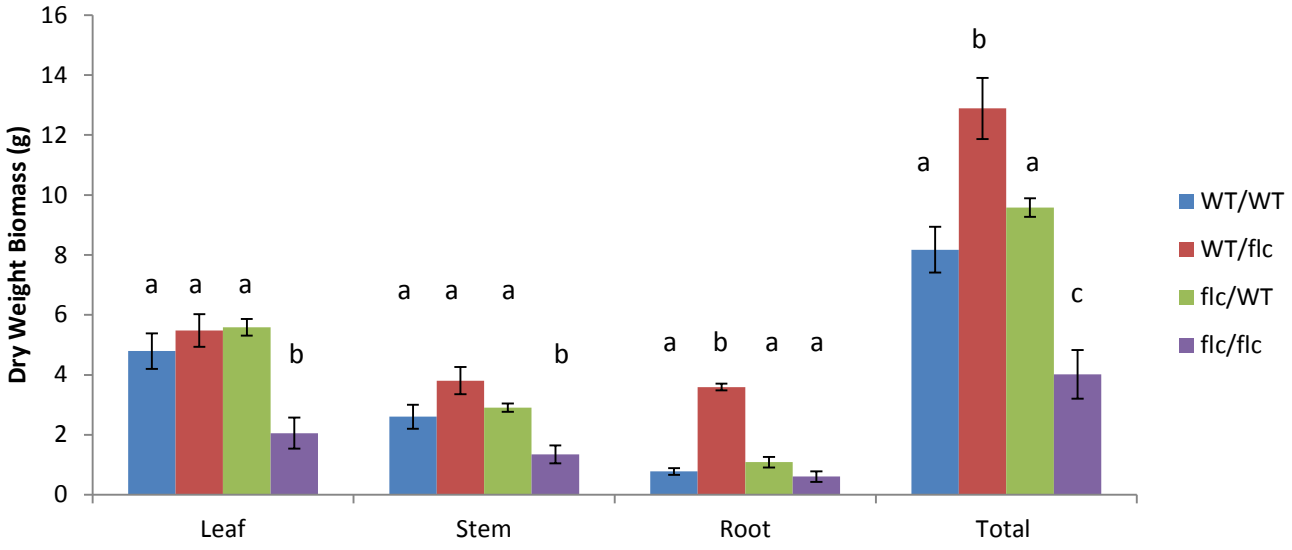

Figure 2. *Dry weights of leaf, stem, root and total tissue of WT/WT, WT/flc, flc/WT, and flc/flc plants at the second harvest point. Data are means ±SE of 5 plants, with significant (P <0.05) differences between graft combinations within tissue groups according to Tukey's HSD test denoted by different letters above the bars.*

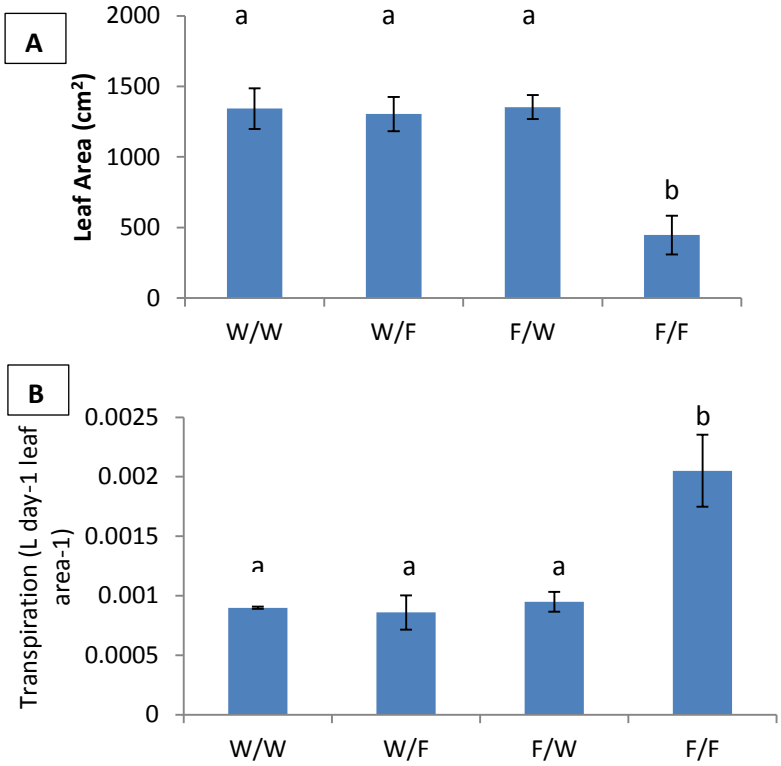

Figure. 3 *Whole plant leaf area (A) and transpiration rate (B) of WT/WT, WT/flc, flc/WT, and flc/flc plants at the second harvest point. Data are means ±SE of 5 plants, with significant (P <0.05) differences between graft combinations according to Tukey's HSD test denoted by different letters above the bar.*

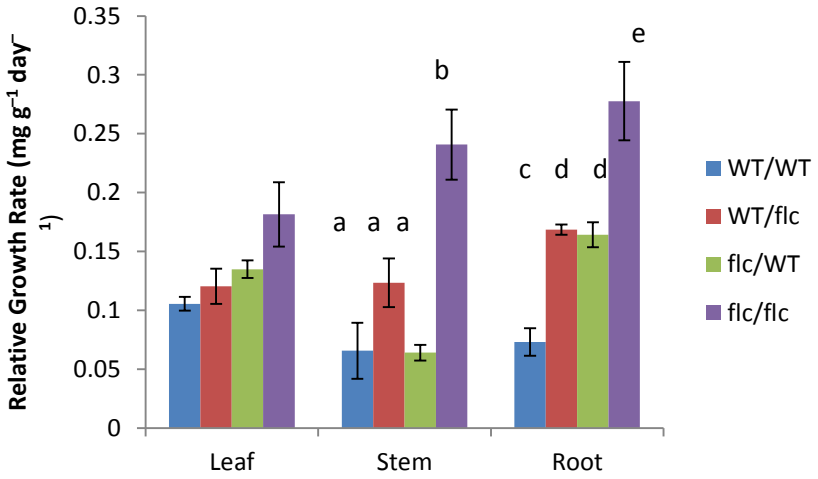

Figure 4. *Relative growth rates of leaf, stem and root tissue of WT/WT, WT/flc, flc/WT, and flc/flc plants over the growth period (t=7 days). Data are means ±SE of 5 plants, with significant (P <0.05) differences between graft combinations within tissue groups according to Tukey's HSD test denoted by different letters above the bars.*

Flow models

Grafting effects on nutrient budgets and ABA flows were investigated by constructing flow models of calcium and potassium using data from tables 1 and 2.

Calcium

Across all plants, the majority of calcium uptake was deposited in the leaves. However, there was some variation between the calcium distributions across organs over the growing period (figure 3a). Wild type plants almost exclusively deposited calcium in the leaves, with relatively little deposited in the stem (96% and 4% respectively).In addition, calcium was exported from the roots and relocated to the scion. *Flacca* plants deposited 5 times more calcium into the stem tissue than wild type plants, accounting for 25.5% of total uptake, with leaf deposition only accounting for 71% of uptake.

The effect of the wild type rootstock increased the calcium deposition in the *flacca* leaves compared to *flacca* self-grafts, accounting for 80% of the deposition. However, there was still significant deposition in the stem tissue (18%).

The influence of the *flacca* rootstock drastically changed the calcium deposition within the plants over the growth period, with a quarter of calcium uptake being deposited within the stem, and 15% being retained in the roots, the raining 60% was deposited in the leaves.

Potassium

The major sinks for potassium deposition were the shoots and leaves (figure 3b). In wild type plants 70% of the uptake was deposited in the leaves with the remaining 30% being deposited in the shoot. However, there was significant recycling within the phloem, with 50% of xylem influx to the leaf being recirculated via the phloem. Interestingly, it was calculated that the majority of potassium deposition in the stem occurred from re-uptake from the phloem, accounting for 70% of the deposition.

Within *flacca* plants, the deposition between the stem and leaves was similar (46% and 49% of uptake respectively). In addition, there was reduced recycling, with only 30% of leaf xylem influx being recirculated within the phloem. Furthermore, only 34% deposition within the stem resulted from potassium transport in the phloem.

Wt/Flc grafts had a two fold increase in potassium uptake compared to all other graft combinations. This was probably due to the larger root biomass in this graft combination. In these plants the majority of potassium deposition occurred in the stem accounting for 49% of uptake with deposition in the roots and leaves accounting for 23% and 28% respectively. Similar to the *flacca* plants 33% of leaf xylem import was recycled in the phloem.

Flacca/wild type grafts had a similar distribution of potassium deposition to *flacca* plants. However, the calculated model had a 6 fold increase in xylem flow and an 8 fold increase in phloem flow compared to the other models. 94% leaf xylem influx was recirculated in the phloem.

To assess the relationship between xylem and phloem flows and the deposition within the stem the ratios of transport to deposition was calculated (figure 4) were calculated. There was no difference within the ratio of depostition to xylem concentration. However, the ratio of the potassium flows in the phloem to the roots was lower in grafts with *flacca* as the rootstock; meaning that when *flacca* is the rootstock more potassium was deposited in the stem tissue from the phloem rather than being recycled to the roots.

ABA

The inclusion of *flacca* genotype as either rootstock or scion decreased the levels of ABA deposited in the leaves of plants.

Wild type plants had tenfold increase in ABA deposition within the leaves over the growth period, with a corresponding tenfold increase of ABA synthesis in the roots in comparison to *flacca* plants (figure 3c). The predominant ABA flow within these plants resulted from biosynthesis in the roots and net catabolism in the stem and leaves. Only 34% of leaf xylem influx was transported in the phloem at the leaves and 25% was recirculated to the roots.

As expected ABA concentration in *flacca* plants were reduced in the shoots compared to all other graft combinations (figure 5). However, net biosynthesis of ABA occurred in both the leaves and the roots with similar flow maintained within both the xylem and the phloem inside the shoots. There was also complete recycling of ABA between the leaves and stem, resulting in half of the ABA rootstock xylem flow being recirculated to the roots via the phloem.

As the phloem influx of ABA into the rootstocks is calculated it is possible to calculate the net increase in synthesis (or metabolism) of ABA within the root system assuming all phloem influx is recirculated. The wild type scion stimulated ABA production in the *flacca* rootstock leading to a 5 fold increase

in biosynthesis, compared to *flacca* plants, which resulted in a 3 fold increase in xylem transport. The model also suggests additional biosynthesis within the scion by the leaves resulting in only a 14% decrease in foliar ABA deposition than WT self-grafts. Furthermore, there was increased ABA recycling from the leaves to the stem with flow levels in the phloem 78% that of leaf influx xylem flows. However, this phloem recycling was not maintained as phloem transport from stem to root reduced to 10% of initial leaf efflux, indicating a large uptake of ABA from the phloem within the stem. In this graft combination there was slight deposition in the rootstock of 3.3% of xylem export.

In the case of the flc/WT grafts the models indicate ABA flow in the xylem is twice that of wild type plants, with a nine fold increase of flow rates in the phloem. However, the deposition rate in the scion does not follow this relationship. Despite the increased influx, compared to deposition in wild type plants flc/WT has a 2 fold reduction in the leaf deposition. In addition, model indicates net biosynthesis in the *flacca* leaves and net degradation in the wild type rootstock.

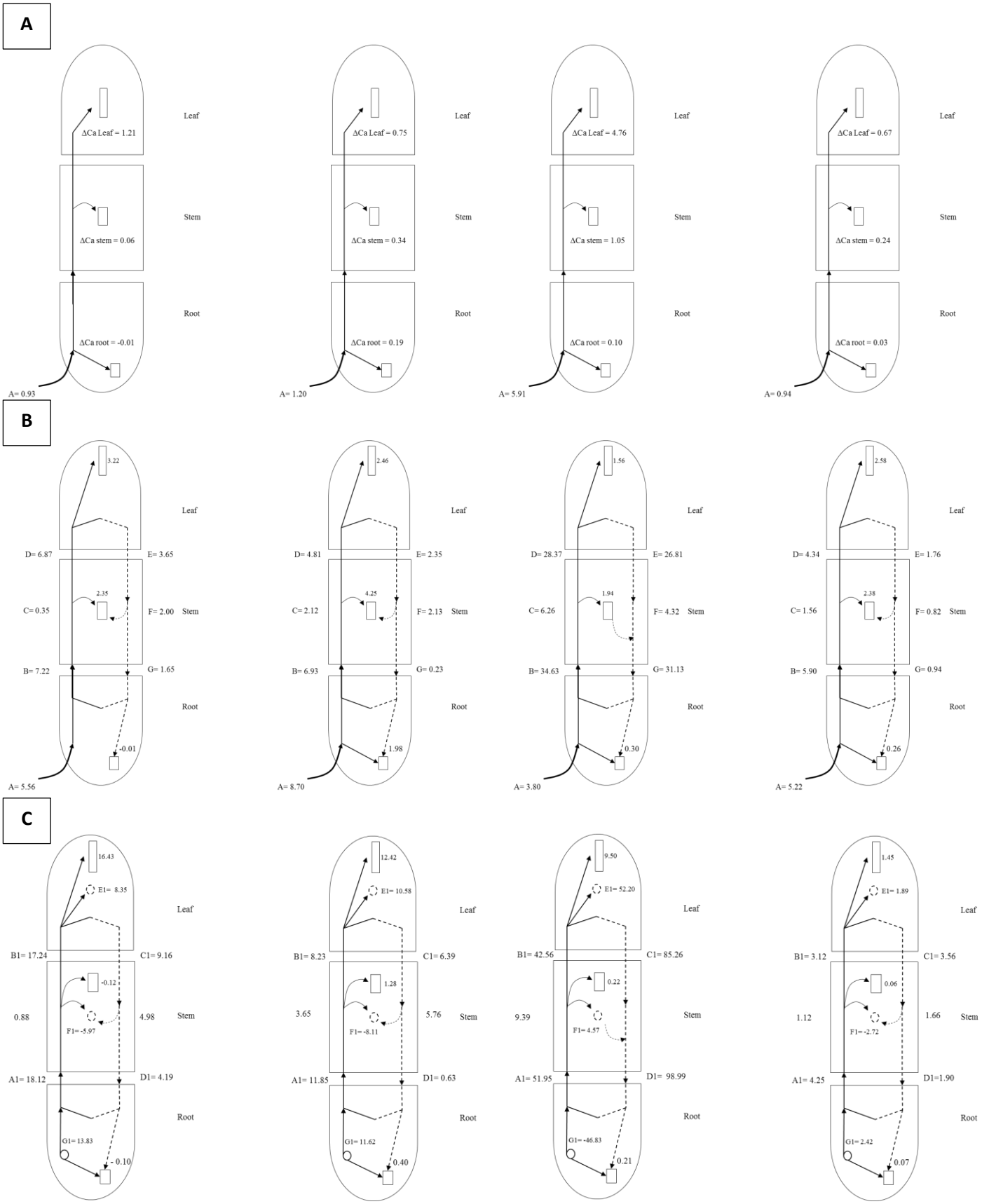

Figure 5. *Empirical models of the uptake, transport, and utilization of total calcium (A) and potassium (B), or the metabolism, transport, and deposition of ABA (C) in whole tomato plants (graft combinations WT/WT, WT/flc, flc/WT and flc/flc) over a 7 d study period. Net flows in xylem sap (black) or phloem (dotted), deposition (rectangle) and metabolism rates (circles) in each organ are drawn*

		Leaves				Stem				Roots				Total			
		WT/WT	WT/flc	flc/WT	flc/flc	WT/WT	WT/flc	flc/WT	flc/flc	WT/WT	WT/flc	flc/WT	flc/flc	WT/WT	WT/flc	flc/WT	flc/flc
Biomass (g)	**Harvest 1**	2.58±0.37	2.3±0.36	2.16±0.22	0.64±0.15	1.82±0.37	1.53±0.19	1.85±0.17	0.27±0.07	0.57±0.15	1.1±0.47	0.28±0.05	0.096±0.04				
	Harvest 2	4.79±0.66	5.48±0.61	5.58±0.31	2.05±0.58	2.6±0.44	3.81±0.51	2.91±0.16	1.35±0.34	0.78±0.12	3.59±0.12	1.09±0.20	0.61±0.20				
	Increment	2.21±0.76	3.18±0.71	3.42±0.38	1.41±0.60	0.78±0.58	2.28±0.54	1.06±0.23	1.08±0.34	0.21±0.19	2.49±0.49	0.81±0.23	0.514±0.20				
K (mmol)	**Harvest 1**	2.25±0.40	2.38±0.37	0.41±0.03	0.11±0.03	3.43±0.00	3.83±0.46	0.47±0.07	0.18±0.16	0.32±0.07	0.32±0.09	0.22±0.09	0.035±0.01				
	Harvest 2	5.48±0.24	4.84±0.55	1.97±0.11	2.69±0.45	4.77±0.33	8.09±1.34	2.41±0.01	2.56±0.97	0.3±0.09	2.3±0.55	0.53±0.01	0.29±0.10				
	Increment	3.23±0.47	2.46±0.66	1.56±0.03	2.58±0.46	1.34±0.33	4.26±1.44	1.94±0.07	2.38±0.98	-0.02±0.10	1.98±0.56	0.31±0.09	0.255±0.11				
	Uptake by roots													5.56	8.7	3.8	5.22
Ca (mmol)	**Harvest 1**	1.49±0.17	1.9±0.27	0.04±0.004	0.02±0.002	0.59±0.00	0.6±0.06	0.15±0.01	0.08±0.03	0.04±0.01	0.06±0.01	0.06±0.02	0.004±0.01				
	Harvest 2	2.69±0.26	2.65±0.21	4.8±0.47	0.69±0.17	0.66±0.08	0.94±0.11	1.2±0.16	0.32±0.06	0.04±0.01	0.25±0.07	0.16±0.02	0.033±0.01				
	Increment	1.2±0.31	0.75±0.34	4.76±0.47	0.67±0.17	0.07±0.08	0.34±0.13	1.05±0.16	0.24±0.07	0±0.02	0.19±0.07	0.1±0.03	0.029±0.01				
	Uptake by roots													1.26	1.27	5.91	0.94
ABA (nmol)	**Harvest 1**	6.99±0.51	6.79±1.02	6.1±0.84	0.56±0.11	1.92±0.85	1±0.22	1.19±0.35	0.14±0.06	0.24±0.05	0.21±0.04	0.051±0.01	0.0024±0.00				
	Harvest 2	23.42±5.52	19.21±2.96	15.6±1.72	2.01±0.39	1.8±0.27	2.28±0.48	1.41±0.31	0.2±0.04	0.15±0.01	0.61±0.12	0.26±0.05	0.071±0.001				
	Increment	16.43±5.54	12.42±3.13	9.5±1.91	1.45±0.41	-0.12±0.89	1.28±53	0.22±0.46	0.06±0.07	-0.09±0.04	0.4±0.13	0.21±0.05	0.069±0.01				

Table 1. *Biomass (dry weight plant–1), total potassium and calcium contents (mmol plant–1) and ABA contents (nmol plant–1) and their increments in grafted tomato plants (WT/WT, WT/flc, flc/WT, and flc/flc) at the beginning and end of the study period (t=7 days). Data shown as means ± SE (harvest 1 n=4, harvest 2 n=5).*

	Graft Combination				
	WT/WT	**WT/flc**	**flc/WT**	**flc/flc**	
Xylem sap concentrations in leaves					
ABA (nM)	20.60±3.3	12.48±3.3	10.54±4.0	6.72±3.9	*
Leaf Xylem Ca (mM)	1.06±0.17	1.31±0.19	1.39±0.20	1.76±0.37	
Leaf Xylem K (mM)	5.74±0.83	8.34±1.10	8.29±1.27	10.37±1.17	
Phloem Concentrations					
ABA (nM)	1.58±0.10	1.76±0.20	2.00±0.20	1.00±0.17	
Xylem sap ratios					
K/Ca	5.68±0.34	6.42±0.59	5.96±0.13	6.48±0.85	
ABA/K	3.94±0.62	1.71±0.29	1.50±0.34	0.72±0.14	
Phloem ABA/K in phloem exudates	2.17±0.31	2.19±0.40	3.18±0.55	2.18±0.73	

Table 2. *The concentrations and ratios of Ca, K and ABA in xylem sap in different parts of the shoot, and the ratio of ABA to potassium (K) in phloem exudates obtained with the EDTA technique in tomato leaves. Data are shown as means ±SE; n=4–8. An asterisk indicates a significant difference at the $P < 0.05$ level.*

Discussion

Physiology and Phenotype

Plant growth is influenced by a number of factors, including, environmental conditions, plant nutritional status and hormone activities. These, in turn, are integrated into the different physiological processes of the plant, meeting their needs for growth within the environment. To date, research has tried to illuminate the role of root-synthesized ABA in regulating plant responses to stress - particularly soil drying - by correlating root (and more usually xylem) ABA concentrations with shoot physiology (reviewed in Dodd, 2005). The use of reciprocal grafts of wild-type plants and ABA-deficient mutants, such as *flacca*, has been particularly useful to the clarification of this relationship (Fambrini et al., 1995; Chen et al., 2002, 2003; Holbrook et al., 2002; Christmann et al., 2007).

Flacca ABA tomato mutants exhibit a marked tendency to wilt due to accelerated transpiration rates from lack of control of stomatal closure (Tal 1966; Sagi et al. 2002). It has been suggested that the primary reason for the wilty phenotype was the lower endogenous ABA content in leaves (Imber & Tal 1970) recorded to be 26–33% of the wild type in well watered conditions (Neill and Horgan, 1985; Linforth et al., 1987; Sharp et al., 2000). In addition, the *flacca* mutation often results in plants with reduced leaf area and stem biomass, compared to wild type plants (Jones et al. 1987; Cornish & Zeevaart 1988; Holbrook et al. 2002; Dodd et al. 2004; Dodd et al. 2009; Pérez-Alfocea et al. 2010), but, in another study the root growth was found to be unaffected with regard to biomass in well watered conditions (Prokić & Stikić 2011). The *flacca* self-grafts in this study conformed to these observations.

In other previous studies, it has been demonstrated that the effect of mutation can be reversed partially or entirely by exogenous ABA (sharp 2000) on endogenous ABA grafting (Cornish and Zeevaart, 1988, Holbrook et al., 2002 and Dodd et al. 2009, Pérez-Alfocea 2010).

It has also been observed in a number of studies that the shoot phenotype of mutant/WT plants can differ from that of mutant self-grafts, with partial phenotypic reversions of leaf area and growth, stomatal conductance and transpiration (Cornish and Zeevaart, 1988, Holbrook et al., 2002, Pérez-Alfocea 2010). However, this reversion is not always complete. Dodd et al (2009) reported that wild type rootstock resulted in only partial leaf area reversion, but transpiration and leaf ABA were determined by the scion with no rootstock influence, while Chen et al (2002) reported no effect of rootstock

on shoot growth but an increased xylem ABA concentration and a partial reduction in transpiration rate of the flc/WT graft compared to *flacca* self-grafts. This implies that that scion genotype is dominant, and its ABA level played the major role in the growth and phenotype of grafted plants, regardless of the rootstock genotype.

The results of this flc/WT plant study were more consistent with the findings of Holbrook et al, with a wild type rootstock restoring *flacca* leaf area, leaf and stem biomass, stomatal conductance and transpiration to wild type levels, i.e. full phenotypic reversion. This indicates that endogenous ABA from the rootstock is sufficient to restore growth to that of wild type plants.

Conversely, in this study and others, it was observed that in both WT self-grafts and WT/flc grafts, WT scions showed similar stomatal closure in response to drying soil (Holbrook et al. 2002), stomatal conductance and shoot growth in well-watered soil (Dodd et al. 2009) and shoot growth under control and saline (75 mM NaCl) conditions when grown hydroponically (Chen et al. 2003). This would indicate that the mutant rootstock had no impact on the phenotypic determination of the scion and that shoot derived ABA is sufficient to maintain the wild type phenotype.

Interestingly, reciprocal grafting of WT and ABA-overexpressing genotypes (Thompson) showed no influence of the ABA overexpressing rootstock on WT shoot growth under well watered conditions (only an increase in root hydraulic conductivity— (Ghanem et al. 2011; Thompson et al. 2007). In turn, this suggests that root-sourced ABA in isolation was not enough to change shoot ABA homeostasis or physiology, although this difference may become apparent under certain environmental conditions due to the increase in hydraulic conductivity.

This strongly suggests that there may be a threshold level of ABA that must be present in the shoot to effectively control growth and phenotype, and that shoot ABA synthesis is sufficient to meet this threshold. A recent paper by Giday supports this theory suggesting a growth [ABA]-related threshold for stomatal sensitivity to desiccation (Giday et al. 2014). This would be consistent with the wider ABA literature as even levels of ABA in xylem of well watered plants are sufficient to cause complete stomatal closure in isolated guard cells, implying that the plant mesophyll has a buffering and regulatory capacity of the ABA signal (Trejo et al. 1993; Trejo et al. 1995; Wilkinson & Davies 2008). Similarly, the lack of influence of ABA over-producing rootstocks on wild type may be due to increased modification of the signal and degradation of ABA in the mesophyll. Therefore, the reversion of phenotype of *flacca*

scions by wild type roots could be a result of an increased recirculation of ABA within the phloem increasing root xylem ABA export, a decreased modification of the signal in the mesophyll, or an increased production of… in the leaf tissue. These options will be discussed in the context of the flow models below.

Nutrition

Plants require a variety of minerals and nutrients to grow, including: Nitrogen, Phosphorous, Calcium and Potassium. These minerals and other nutrients are stored in different organs, such as roots, stems, leaves and/or fruits. Each organ has a significant influence on the uptake and translocation of mineral nutrients in plants, which, in turn, plays a crucial role in physiological processes, such as, growth, signal transduction and development (Wang et al. 2006; Flowers & Colmer 2008). The influence of the rootstock on the mineral content in aerial plant tissue has been attributed to physical characteristics of the root system, such as lateral and vertical development, which result in enhanced uptake of water and minerals (Yetisir et al. 2013; Jang 1992). These characteristics provided the main motives for the widespread use of grafted rootstocks (Lee 1994).

Past literature has seen debates over the relative influence of the rootstock and scion within grafted plants, particularly those of the melon(Rivero et al. 2003; Tagliavini et al. 1993; Kawaguchi & Backhouse 2008). However, there is agreement that changes in macro- and micronutrients are affected by rootstock and scion characteristics; these depend on the elemental and environmental conditions, thus changing the perceived influence of the rootstock and/or scion (Martínez-Ballesta et al. 2010).

In previous studies with tomato, some differences in nutrient content have been observed with significant increases of tissue Ca^{2+} and Mg^{2+} when grafted plants were compared with non-grafted plants under salt stress (Fernández-García et al., 2004, Martínez-Rodríguez et al., 2008). However, there have also been reports indicating no differences between mineral contents of grafted and non-grafted plants ((Santa-Cruz et al., 2002, Chen et al., 2003, He et al. 2009). The authors attribute the observed variations being caused by the smaller root systems and restricted xylem hydraulic conductivity of the rootstock relative to the scion.

In this study, calcium, potassium and phosphorous uptake and transport were dependent on the scion genotype, with significant increase in uptake in plants with *flacca* scions. However, this increased uptake did not lead to a change in

composition of the elements in any tissue in *flacca* self-grafts compared to wild type plants. Therefore, the increased uptake observed was most likely due to the growth of the plants rather than an increased affinity for the elements, which is supported by the significantly higher growth rate over the growth period.

Conversely, the flc/WT graft showed a significant increase in calcium levels within the shoots and leaves yet had a similar growth rate to both wild type and WT/flc plants. This accumulation has not been seen previously as calcium concentrations within the shoot tissue were reported to be similar to the other graft combinations (by Chen (2003). The increased level of shoot calcium concentrations was still within normal range (between 0·1 and 5 % d. wt (Marschner & Rimmington 1996) and although the Ca physiotype of a plant determines its ability to accumulate Ca in the shoot (Kinzel & Lechner 1992; Broadley et al. 2001; White & Broadley 2003; Broadley et al. 2004). Genotypic differences in the activities of Ca^{2+} transporters in root cell membranes(Hirschi 2001; White et al. 2002) may contribute to phylogenetic variation in [Ca]shoot. This has been correlated with the cation exchange capacity (CEC) of plant roots (white 2003) which was not measured in this experiment, but may be considered for future studies.

ABA levels have also been linked to calcium uptake with ABA treatment increasing uptake in jojoba shoots (mills 2001), and increasing total tissue and apoplastic water-soluble Ca concentrations in tomato fruit ((de Freitas et al. 2011). However, as ABA concentration in the roots, stem leaves, xylem or phloem were not increased compared to wild type, and none of the plants had reached their fruiting stage, it is unlikely that this is a cause.

Further replication is required to determine if this is an isolated result or feature of this graft combination.

ABA relations

It is clear the full understanding of hormonal flow within grafted plants is far from being complete. ABA concentration is determined by its synthesis and re-distribution within the leaves, but also by the import via the xylem from the roots (Jiang & Hartung 2008; Daszkowska-Golec & Szarejko 2013). However, it has also been reported that the recirculation of ABA originally synthesized in the shoot back into the transpiration stream - via the root - represents a substantial proportion of the root-sourced signal (Wolf 1990; Neales& McLeod 1991). In studies investigating the ABA relationship in grafted plants, ABA

values are often based on instantaneous measurements, which provide little information of the temporal dynamics of these fluxes.

In this study, the xylem ABA concentration was found to be significantly lower in *flacca* self-grafts compared to wild type, while both reciprocal grafts had intermediate concentrations. Meanwhile, phloem ABA concentrations were shown to be similar across all plants (Table 2). Within the literature, the results of xylem ABA concentration are varied. Past studies have found that xylem ABA concentration correlate to: solely with shoot genotype (Dodd, et al 2009), shoot phenotype with an increase in xylem ABA in *flacca* with a wild type rootstock (Fambrini & Vernieri 1995), shoot phenotype with a decreased xylem ABA in wild type scions with a *flacca* rootstock (Chen et al. 2002) and reciprocal grafts having an intermediate xylem ABA concentration (Holbrook et al. 2002). These values also correlate with the expected physiological responses, for example stomatal conductance and transpiration in each experiment. However, this huge disparity is probably reflecting, the variation in experimental setups and the origin of plant material and the method of collection.

Looking at the models (summarised in figure 8) *flacca* self-grafts show a much lower rate of synthesis and deposition within the tissues, reflected by the xylem and phloem flows which are similarly reduced. The flow models also show that the proportion of ABA being recycled in the phloem is expected to be increased in the *flacca* self-grafts, though the observed concentrations are not as high, and that ABA synthesis is occurring within both the roots and the leaves. This correlates with the observation that xylem ABA concentration was significantly lower in *flacca* self-grafts ABA concentration compared to wild type grafts and the reduced shoot biomass, leaf area and increased transpiration rate.

Looking at the effects of the rootstock, *flacca* (figure 8) shows slightly decreased deposition but increased synthesis within the leaves compared to wild type self-grafts. Xylem flows are reduced but there is an increased proportion being transported in the phloem from leaves to stem even though total ABA concentrations are lower. This matches the observed decrease in xylem and leaf ABA concentration within this graft combination but explains the wild type phenotype observed in the scion.

Wild type rootstock (figure 8) causes a large increase in ABA deposition in both the stem and the leaves (3.7 and 6.6 fold increases respectively) with a large increase in both xylem and phloem transport from stem to leaves in the *flacca* scion. Interestingly, one effect of the wild type rootstock calculated by

this model is a 27.6 fold increase in ABA synthesis in the leaves compared to *flacca* self-grafts, with increased flow of ABA in the phloem. This would account for the observed increase in xylem ABA concentration and the phenotypic reversion to wild type.

Now considering the scion *flacca* causes a huge increase (23.6 fold) in phloem influx to the root (figure 8) as well as a tripling of ABA export in the Xylem and net metabolism of ABA in the rootstock. However, in this graft combination, the rootstock is the main source of ABA metabolism. This data suggests that the observed increase root xylem ABA concentration is a result of increased recycling of ABA within the plants rather than increased production by the rootstock.

Interestingly, wild type scions reduce the ABA influx to the root via the phloem (figure 8) but caused a 4.8 fold increase in ABA synthesis (to levels similar in wild type roots) resulting in a 2.8 fold increase in xylem export compared to *flacca* self-grafts. This increase in root xylem ABA export could also supports the wild type phenotype observed in the shoots. The reduction of phloem ABA influx is a particularly interesting result as this graft combination had an increased root biomass. This increase has been observed previously (Ian Dodd, personal communication). However the present model suggests that biomass is stimulated without ABA influx from the shoot which is a significant stimulator of root growth.

Based on these models and the relative flows shown, the intermediate ABA concentration observed within reciprocally grafted plants can be explained by the following contribution of scion and rootstock to xylem ABA concentration:

WT/flc : due to a combination of increased root and leaf synthesis, with some increased phloem reflux between the leaves and shoot, rather than increased recycling of ABA in the phloem to the roots of ABA produced in the wild type shoots.

flc/WT: due to the increased recycling of leaf produced ABA in the phloem to the roots, not increases in root ABA synthesis.

These conclusions reject the hypothesis and are puzzling. It seems that the intermediate xylem ABA concentration is achieved by different mechanisms depending on the graft combination. This could be due to changes in the hormonal signal coming from the root or shoot during the formation of the graft union. Hormone effects during this process can change the architecture of

the vascular connections, which may affect further signalling or flow from the roots to shoot.

It is interesting that in both cases there is increased synthesis in *flacca* either as a rootstock on scion. This suggests that grafting with the wild type plant increases ABA production in a genotype that should be impaired in the biosynthesis pathway. This could be caused by increased export of the precursors that bypass the mutant protein in *flacce* from the wild type plants. Further analysis of gene expression within the root and leaves might be useful in determining whether this calculation and hypothesis is correct.

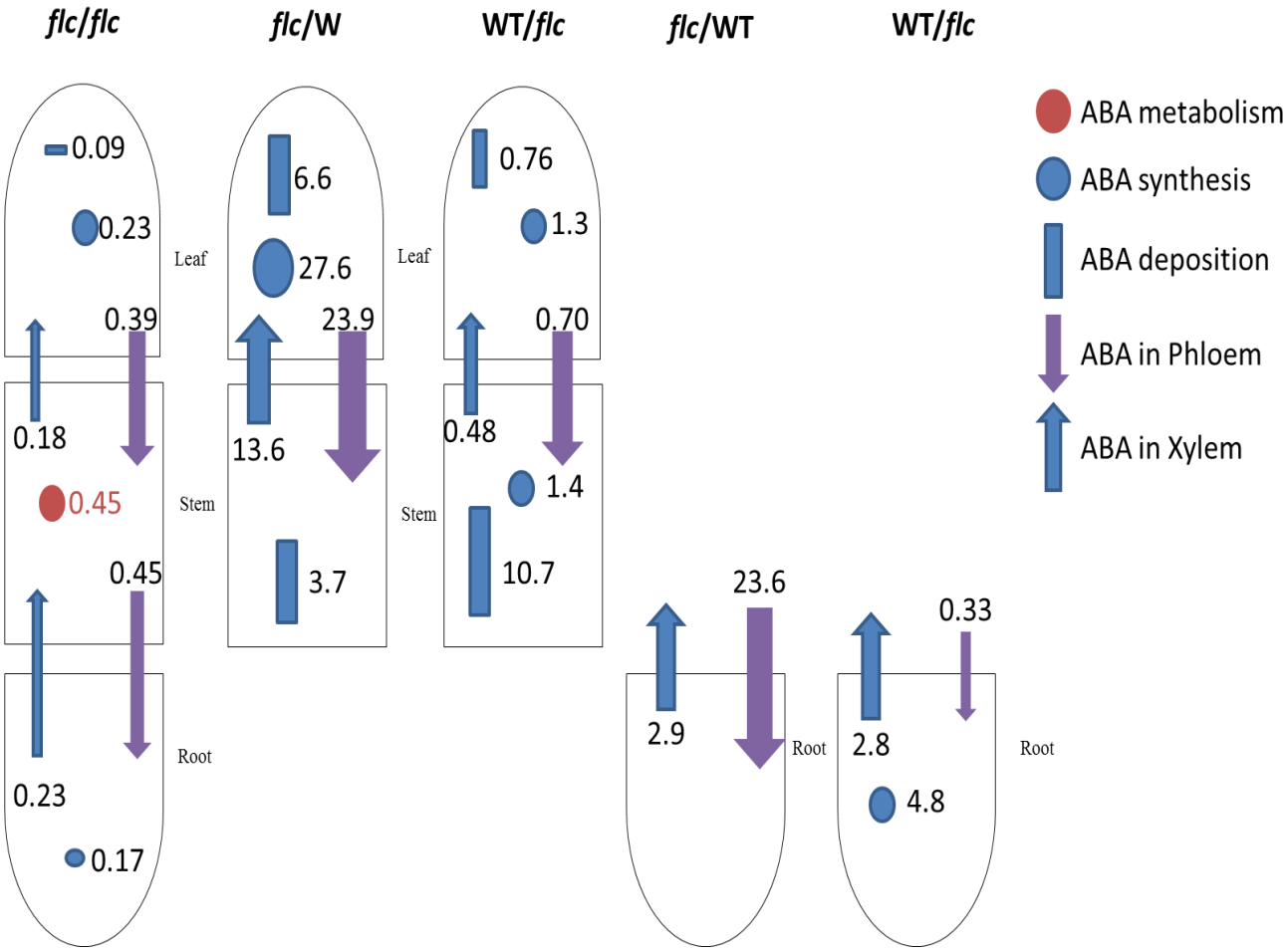

Figure 6. *Fold changes in xylem sap or phloem flows (arrows), deposition (rectangles), and metabolism (circles) of ABA induced by grafting.*

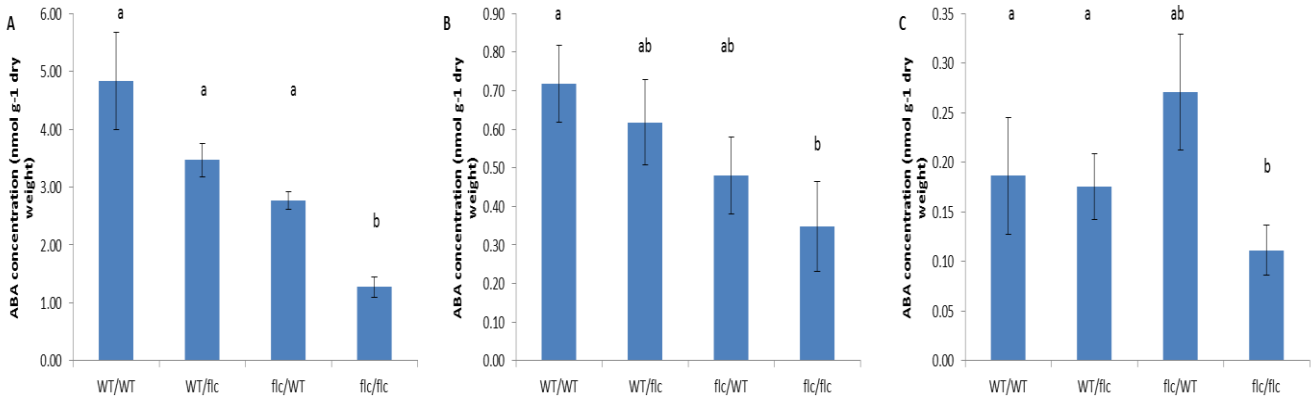

Figure 7. *ABA concentrations in the leaf (A), stem (B) and roots (C) of WT/WT, WT/flc, flc/WT, and flc/flc plants. Data are means ±SE of 9 plants, with significant (P <0.05) differences between graft combinations within graft combinations according to Tukey's HSD test denoted by different letters above the bars.*

Flow modelling, is it worth the effort?

Empirical incremental flow models, based on the increase in water, mineral nutrients, carbon assimilates or phytohormones between two defined time points, have been successfully employed in this study to investigate the changes plant physiology. However, there are some limitations that must be addressed.

In comparison to past literature, there are similarities between the ABA models that have been generated for a number of plant species; maize (Jiang et al. 2007), barley (Jiang et al. 2004), castor bean (Jaschke et al. 1997) and pea (Jiang & Chen 2012). Looking at the control models, in every case there was ABA recycling within the phloem. In the case of maize, barley and pea there was a clear relationship of net production in the root and net metabolism in the leaves. However, in the barley model this was reversed with net ABA production in the leaves, while metabolism took place in the roots. The percentages of leaf Xylem ABA import being recirculated in the phloem calculated in this current study's model were observed and similar to the proportions recorded other models in the literature - typically around 50%.

The data provided in the model discussed above was sufficient to provide a solution to the relative contribution of the scion and the rootstock to xylem sap, as well as the changes of the inter-organ ABA flows within the plant; raising some questions that would be interesting avenues for further research.

However, I there remain some concerns with the validity of the model calculated. Firstly, the magnitude of the increase in both the potassium and ABA flow calculated by the model for the flc/WT graft combination were not representative of the observed variation in the leaf xylem ABA. This discrepancy was likely to have been caused by the increased calcium increment within the tissue over the growth period, as the calcium increments for the base of the potassium flow model was subsequently used as the framework for the ABA flows. Looking back at the data from the first harvest point of the flc/WT graft, calcium concentrations were recorded to be very low. Reanalysis of the tissue samples from this time point may reveal an error increment. However, any models generated must be checked for discrepancies between the nutrient uptakes of graft combinations. Furthermore, point measurements may be sufficient to determine the phenotype (figure 7) so generation of models may not be necessary in most cases.

Secondly, using the transpiration flows and direct analysis of root xylem sap the actual potassium and ABA efflux from the root was calculated (figure 8). It is clear that although the model values follow a similar trend, the magnitude of these values were very different. As the transpiration flows are not usually calculated along with the models, conclusions drawn from these representations should be treated with caution. The accompaniment of values calculated from whole plant transpiration would be a useful addition to validate the model outputs.

On balance, although the phenotype can seemingly be determined by point measurements of tissue and xylem sap measurements alone; it is the mechanistic changes that need to be studied to optimise vegetable production in future. In addition empirical models can provide insight into more areas of root to shoot signalling rather than just hormone levels as evidenced by the increased synthesis of *flacca* predicted in the model above. Therefore, empirical models should be perused as a tool in future, in the research of grafted plants.

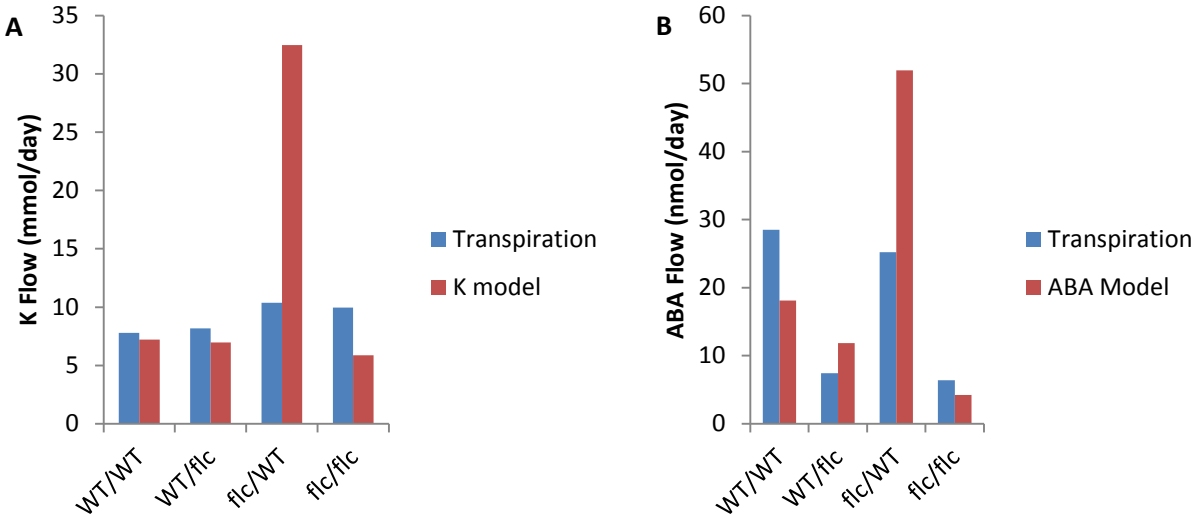

Figure 8. *Comparison of root export rates calculated by the model and estimated values based on transpiration rate for potassium (A) and ABA (B) of WT/WT, WT/flc, flc/WT, and flc/flc plants.*

Conclusion

Wild type rootstocks are sufficient to phenotypically revert *flacca* scions, while *flacca* rootstocks have no effect on wild type scion phenotype. Empirical flow models can be used to illuminate the scion and rootstock contribution to xylem ABA concentration in grafted plants, modelling the intermediate xylem ABA values observed despite an ABA deficient mutant component. The models suggest that the addition of wild type plants either as a scion or rootstock stimulates ABA production in the reciprocal *flacca* tissue contributing to an intermediate concentration rather than an increased contribution from wild type tissues. However, care must be drawing conclusions when using these models which can be affected by variation in nutrient uptake between graft combinations. Therefore, it is recommended that estimates of root export are calculated based on the whole plant transpiration flows to check the model values.

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