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A theoretical framework to study potassium utilization efficiency in response to withdrawal of potassium

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Abstract

An important objective of plant research is to improve the efficiency in the utilization of major nutrients, particularly nitrogen, phosphorus, and potassium. Several definitions of internal nutrient utilization efficiency (NUE) have been proposed, but the theoretical consistence of their use has been poorly explored. Here, a non-mechanistic approach was developed to theoretically examine the dynamics of commonly used NUE indicators following complete potassium deprivation. This approach was used to study the sensitivity of NUE indicators to changes in the actual NUE (NUEa) of K+ in virtual plants. Three empirically based models that differ in the relationship between NUE and the internal K+ concentration were examined. Frequently used indicators (potassium use efficiency, utilization efficiency, physiological efficiency, and nutrient productivity) and two additional ones introduced here (accumulated productivity and physiological ratio) differed in their capacity to reflect differences in NUEa. They also exhibited large disparities in their temporal variation and in their responsiveness to the concentration of K+ before the beginning of the deprivation period. According to this analysis, the simultaneous use of several indicators could help to refine plant breeding for high NUE. The data also suggest that a trade off between plant productivity and the time necessary to reduce the concentration of K+ by half is inherent to the dynamics of plant systems. Finally, it is proposed that for some plant species selection for high NUEa would not always be in conflict with selection for improved relative plant performance in low K+ environments.

Key words: Growth, nutrient utilization, plant breeding, potassium, productivity, theory, use efficiency.

Introduction

A major challenge faced by humanity is to ensure food supply for a growing population, which will require high input agro-ecosystems. In these highly productive environments, an important fraction of the mineral nutrients exported with crop products needs to be replenished. This means that the use of fertilizers cannot be easily avoided, at least with current technologies (Denison and Kiers, 2005). Unfortunately, fertilizer utilization greatly augments the costs of agricultural practices, which may be critical in poor regions, while exerting important detrimental effects on the dynamics of both terrestrial and aquatic ecosystems

when used improperly (White and Brown, 2010). In this context, an important objective of current research in plant nutrition is to optimize the application of fertilizers by improving their agronomic use. This could be achieved by optimizing the efficiency of both nutrient capture and nutrient utilization.

The idea to select plants with improved efficiency in the acquisition and utilization of mineral nutrients is certainly not new (Siddiqi and Glass, 1981) and continues to be an important objective of plant breeding programmes (Good *et al.*, 2004; Rengel and Damon, 2008; Rose and Wissuwa,

2012). Breeding for enhanced internal nutrient utilization efficiency (NUE), defined as the amount of biomass that could be generated by each unit of nutrient present in plant tissues (Chapin III, 1980), requires the ability to identify genetic loci that contribute to set plant growth at low internal nutrient concentrations. While the concept of NUE appears prima facie a simple one, its precise meaning as well as its instrumental use have been widely debated (Siddiqi and Glass, 1981; Berendse and Aerts, 1987; Gourley *et al.*, 1994; Baligar *et al.*, 2001; Good *et al.*, 2004; Hirose, 2011). This situation is illustrated by the presence of contrasting definitions in the scientific literature and by the fact that methodologically it is difficult to separate the efficiency in nutrient utilization from the efficiency in nutrient capture (Rose *et al.*, 2011). This difficulty is thought to have contributed to the slow progress in the selection of crops with improved NUE, particularly for phosphorus (Rose and Wissuwa, 2012). It also remains unclear to what extent the definitions currently in use to describe NUE provide unambiguous and meaningful results. Thus, an examination of the theoretical validity of the concepts to assess NUE seems necessary. Besides, the use of a given operational tool for breeding programmes requires the corresponding algorithm to be adequately sensitive to actual changes in NUE, while minimizing the noise imposed by other factors.

Plants, as well as other cell-walled eukaryotic organisms, require large quantities of potassium ions to sustain growth and alleviate the effect of some environmental stresses (Maathuis and Amtmann, 1999; Shabala, 2003; Tester and Davenport, 2003; Cakmak, 2005; Mangano *et al.*, 2008). Since soils of large portions of the agricultural land areas are deficient in K^+ (Römheld and Kirkby, 2010) this element is included in many fertilizer formulations. In order to improve the agronomic utilization efficiency of the K^+ available in the soil solution, valuable efforts have been made to identify genetic loci involved in determining the acquisition and utilization of this element in model plants and crops (Yang *et al.*, 2003; Harada and Leigh, 2006; Damon *et al.*, 2007; White *et al.*, 2010). These works have been accompanied by the identification of structural components that determine K^+ acquisition (Sentenac *et al.*, 1992; Santa-María *et al.*, 1997; Pyo *et al.*, 2010; Rubio *et al.*, 2010). Although some progress has been made in understanding of the molecular determinants of plant growth at low internal K^+ concentrations (Armengaud *et al.*, 2009; Jung *et al.*, 2009; Moriconi *et al.*, 2012), an adequate knowledge of the mechanisms involved in this process remains elusive.

Pioneering work by Leigh and Wyn Jones (1984) offered a foundation to understand, at a physiological level, the complex relationship between growth and K^+ concentration within plant tissues. Early on, evidence has been offered for a linear relationship below a suboptimal concentration between growth and the internal concentration of several elements, including K^+ (Table 1). Other studies demonstrated that, in some cases, there is a curvilinear relationship between growth and the internal K^+ concentration (Table 1). Simple and empirically based mathematical models could account for those relationships and can be used to describe vegetative plant growth within the suboptimal range of tissue K^+ concentrations. This work explored the consequences of three of those non-mechanistic models, which differ in the relationship between NUE and the internal nutrient concentration, on the dynamics of the actual NUE (NUEa) of K^+ and commonly used NUE indicators when plants are suddenly exposed to a complete deprivation of K^+. Although this extreme situation is unlikely to occur in nature, it allows to build up a simple theoretical framework, which helps to estimate the otherwise unknown NUEa and to make an assessment of the theoretical validity and limitations of each K^+ NUE indicator, which could be useful in breeding programmes.

Materials and methods

Theoretical framework

Early empirical evidence supported, for some plants, the existence of a linear relationship between plant growth and the internal nutrient concentration under conditions of suboptimum nutrient supply (Bouma, 1983). That linear relationship, depending on the plant species (Table 1), can be approximated by two alternative models:

$$\text{Model I}: RGR = (dW/dt)W^{-1} = Pj(cj - CjMin) \quad (1)$$

$$\text{Model II}: RGR = (dW/dt)W^{-1} = Pj\,cj \quad (2)$$

According to the first model, the relative growth rate (*RGR*) of plant biomass (*W*) is linearly related to the internal concentration of the element *j* (*cj*, *j* being in this case K^+) in plant tissues minus the minimal concentration of *j* (*CjMin*) (i.e. that above which growth is allowed to continue; Fig. 1A). *Pj* is a proportional factor known as nutrient productivity (Ingestad and Ågren, 1992). Instead, according to model II (which is a particular subcase of model I), the value of *CjMin* must be considered zero, which means that there is no need for a minimal structural concentration of the *j* element to initiate growth (Fig. 1A).

However, for some plants, there is evidence for a curvilinear relationship between *RGR* and *cj* (Table 1). In order to choose a function that can be used to describe that relationship for K^+, the detailed set of data provided early for two *Taraxacum* species (Hommels *et al.*, 1989) was graphically derived. Several functions gave an adequate fitness (data not shown) within the range of internal K^+ concentrations between *CjMin* and *CjCrit* (i.e. the internal concentration at which 90% of maximum growth is attained). Among the equations that provided an acceptable adjustment, within this range of internal K^+ concentrations, one was chosen, designated hereafter as model III, because it was the only one found to be amenable to exact mathematical treatment. The suitability of this model to adjust experimental data was further confirmed by using data derived from other studies (Smith *et al.*, 1982; Bailey and Laidlaw, 1998). Model III contains only two parameters: *CjMin*, also used in model I (Fig. 1), and a second one that has units of time^{-1} and is designated hereafter as *Aj*:

$$\text{Model III}: RGR = (dW/dt)W^{-1} = Aj(cj - CjMin)/cj \quad (3)$$

The three outlined models offer alternative approximations to the actual relationship between *RGR* and *cj* for a range of tissue concentrations of *j* within *CjMin* and the optimum concentration (*CjOpt*, i.e. the minimal concentration at which maximum *RGR* is attained). As shown in Fig. 1B, they also establish

Table 1. *A non-exhaustive bibliographic survey of the relationship between plant growth and internal K$^+$ concentration (cj)*

Data derived from the literature were adjusted to different models. *CjCrit* and *CjMin* estimated, correspond to those obtained after curve-fitting the data. *CjCrit* and *CjMin* are expressed in µmol (g FW)$^{-1}$ unless otherwise indicated.

Plant species	Type of model	Plant part	*CjCrit* reported	Model for adjustment	*CjCrit* estimated	*CjMin* estimated/ assumed	Reference
Betula pendula	Linear	Whole plant (DW)	1.06% DW	nd	nd	nd	Ericsson and Kähr (1993)
Bromus rigidus	Linear	Whole plant (FW)	nd	II	161	0	Asher and Ozzane (1967)
Cryptostemma calendula	Linear	Whole plant (FW)	nd	I	136	6	Asher and Ozzane (1967)
Erodium botrys	Linear	Whole plant (FW)	nd	I	118	2.5	Asher and Ozzane (1967)
Hordeum vulgare cv. *BT 334*	Linear	Whole plant (FW)	nd	I	113	14	Siddiqi and Glass (1981)
Hordeum vulgare cv. *Fergus*	Linear	Whole plant (FW)	nd	I	113	21	Siddiqi and Glass (1981)
Lycopersicum esculentum	Linear	Whole plant (DW)	4.30%	nd	nd	nd	del Amor and Marcelis (2004)
Medicago sativa (high Mg^{2+})	Curvilinear	Leaves (DW)	1.9% DW[c]	III	1.94[c]–3.22[b] % DW	0.29[c]–0.62[a] % DW	Smith *et al.* (1982)
Medicago sativa (low Mg^{2+})	Curvilinear	Leaves (DW)	2.8% DW[b]	III	2.17[b]–4.12[a] % DW	0.23[b]–0.64[a] % DW	Smith *et al.* (1982)
Medicago tribuloides	Linear	Whole plant (FW)	nd	I	156	12	Asher and Ozzane (1967)
Ornithopus sativus	Linear	Whole plant (FW)	nd	II	132	0	Asher and Ozzane (1967)
Secale cerale	Linear	Root (FW)	24	nd	nd	nd	White (1993)
Secale cereale	Curvilinear?	Shoot (FW)	86	nd	nd	nd	White (1993)
Taraxacum nordstedtii	Curvilinear	Whole plant (FW)	40	III	53	7	Hommels *et al.* (1989)
Taraxacum sellandii	Curvilinear	Whole plant (FW)	87	III	98	9	Hommels *et al.* (1989)
Trifolium hirtum	Linear	Whole plant (FW)	nd	I	150	15	Asher and Ozzane (1967)
Trifolium repens	Curvilinear[d]	Above ground (DW)	nd	III	1.21% DW	0.29% DW	Bailey and Laidlaw (1998)
Trifolium subterraneum	Linear	Whole plant (FW)	nd	I	147	11	Asher and Ozzane (1967)
Vulpia (festuca) myuros	Linear	Whole plant (FW)	nd	II	146	0	Asher and Ozzane (1967)

a, *b*, and *c* estimated from Figures 1, 3, and 4, respectively, from Smith *et al.* (1982). *d*Data are also consistent with a linear model within the suboptimum range. Model I, $RGR = (dW/dt) W^{-1} = Pj (cj - CjMin)$; Model II, $RGR = (dW/dt) W^{-1} = Pj cj$; Model III, $RGR = (dW/dt) W^{-1} = Aj (cj - CjMin)/cj$. DW, dryweight; FW, freshweight; nd, not determined.

three different kinds of functional dependence between the NUE, expressed in units of biomass nutrient-content^{-1} time^{-1}, and the internal nutrient concentration. According to model II NUE remains unaltered and equal to *Pj*, while for model I NUE increases progressively with the increase of internal nutrient concentration. In turn, for plants following model III, NUE increases with internal *cj* until it reaches a maximum and then declines. Taken together, these models could account for an important part of the variation among plants in NUE patterns. The expressions that describe the functional dependence of NUE on internal *cj* are formally introduced in Supplementary Appendix II (available at *JXB* online).

To evaluate the changes of growth and internal concentration during the course of K$^+$ deprivation it is necessary to consider that the content of *j* (*Qj*) in plant tissues can be expressed as the product between *cj* and *W*. The first derivative of *Qj* against time (*t*) is:

$$(dQj/dt) = (dcj/dt)W + (dW/dt)cj \qquad (4)$$

When no sources of *j* are available in the medium (i.e. complete deprivation), the uptake of the nutrient becomes negligible. This means that no further variation in the internal content of *j* occurs. Thus, the term at the left in equation 4 becomes zero and the equation may be reshaped as:

$$-(dcj/dt)cj^{-1} = (dW/dt)W^{-1} = RGR \qquad (5)$$

A

B
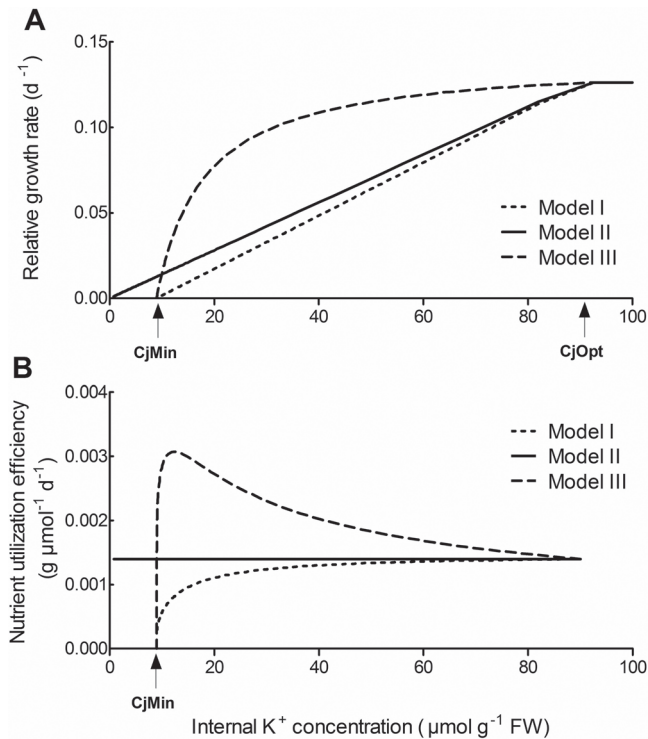
Fig. 1. Theoretical relationships between relative growth rate (*RGR*) and internal potassium concentration (*cj*) (A) and nutrient utilization efficiency (NUE) and *cj* (B) according to three alternative models within the suboptimal range of *cj*. For the three models, the value of *CjOpt* was set at 90 µmol (g FW)$^{-1}$ and *CjMin* was set at 9 µmol (g FW)$^{-1}$ for models I and III. Values of *Pj* were 0.00155 and 0.0014 g FW µmol^{-1}d^{-1} for models I and II, while *Aj* for model III was 0.14 d^{-1}, respectively. The initial concentration of K$^+$ was set at 135 µmol (g FW)$^{-1}$.

By combining equations 1, 2, or 3 with equation 5, differential equations can be obtained for models I, II, and III, respectively (Supplementary Appendix I). This requires assuming that during the course of a nutrient interruption procedure, the relationship between *RGR* and internal concentration can be approximated by equations 1, 2, or 3. To solve the resulting differential equations, specific assumptions should be made regarding the possible changes suffered by *CjMin*, *CjOpt* (or *CjCrit*), and *Aj* (or *Pj*) for K$^+$ during the course of K$^+$ deprivation. Evidence obtained in experiments with plants grown at different K$^+$ supplies, including no K$^+$ addition, for which successive harvests were made (Hommels *et al.*, 1989), suggests that variations in *CjMin* and *CjCrit* following a change of K$^+$ supply could be of low magnitude. Given this evidence, and in order to maintain the mathematics of the approach as simple as possible, this work assumed temporal invariance of those parameters as well as of *Pj* (or *Aj*). In order to avoid the interaction with other variables, it was also assumed that K$^+$ was the only constraint to simulated plant growth. The solutions obtained for those equations are given in Supplementary Appendix I. Through their use, it was possible to simulate the time dependence of growth and internal nutrient concentration once *cj* < *CjOpt*.

Outline of calculations

In order to simulate the decline of *cj* and growth it is necessary to distinguish two main phases during the course of nutrient deprivation (Supplementary Fig. S1). The first one takes place when *cj* ≥ *CjOpt*, while the second one occurs when *CjOpt* > *cj* > *CjMin*. During the first phase, plant growth does not depend

on *cj*, and maximum *RGR* (*RGRmax*) is maintained. As shown in Supplementary Appendix I, the decline of *cj* over such range can be estimated as:

$$cj = cji\, e^{-RGRmax\, t}$$

where *cji* is the initial concentration of *j* in plant tissues. During the second phase, once *cj* drops just below *CjOpt*, growth becomes dependent on *cj*, and any further decline of both *cj* and growth must be calculated through the solutions of the differential equations shown in Supplementary Appendix I, which are particular for each model. Exact solutions were obtained for the time course of internal nutrient concentration and growth according to each one of those models. However, no expressions in terms of standard mathematical functions were found to estimate the time required to reduce the biomass of K$^+$ starved plants to a half of the value observed in plants well supplied with K$^+$ (T½BIOM). In order to find it, the quotient of biomass between K$^+$-deprived and K$^+$-well-supplied plants was estimated at different times since the beginning of the deprivation period until to find the time at which the quotient reached a 0.50 ± 0.01 value. Calculations were performed using Microsoft Excel Office 2003. A script illustrating the calculations made is provided in Supplementary Appendix III.

Range of primary parameters

In order to analyse the sensitivity of indicators of NUE and plant performance to the variation of each one of the parameters contained in the models, parameters were allowed to change within the range of values introduced in Table 1. Given that for model III growth converges asymptotically to *RGRmax*, *CjOpt* cannot be reached. Therefore, for operational purposes *CjOpt* was equated to *CjCrit* and allowed to change between 55 and 150 µmol K$^+$ (g FW)$^{-1}$. For model III, *CjCrit* and *CjMin* are interconnected. Therefore, patterns obtained for this model when either *CjOpt* or *CjMin* vary should be attributed to the simultaneous variation of both *CjCrit* and *CjMin*. The pair *CjOpt*/*CjMin* covered the following combinations of 55/5.5, 90/9, 125/12.5, and 150/15 µmol K$^+$ (g FW)$^{-1}$. For model I, except where indicated, *CjMin* varied between 1 and 25 µmol K$^+$ (g FW)$^{-1}$. Values of *Pj* were allowed to vary between 0.0006 and 0.0054 g FW d^{-1} µmol^{-1} K$^+$ for model I, between 0.0004667 and 0.0042 g FW d^{-1} µmol^{-1} K$^+$ for model II, while for model III *Aj* varied between 0.046667 and 0.42 d^{-1}. The combinations of parameters used in this work yielded *RGR* values that spanned over a 9–20-fold range (depending on the model), which resemble those determined in large-scale studies (Hunt and Cornelissen, 1997). Values of initial K$^+$ concentration (*cji*) essentially covered the range emerging from the data offered by Asher and Ozanne (1967) and Broadley *et al.* (2004) and were allowed to vary between 90 and 180 µmol K$^+$ (g FW)$^{-1}$.

Indicators used

Three kinds of indicators were used, as summarized in Table 2. The first class corresponds to those that describe the dynamics of K$^+$ concentration during the course of K$^+$ deprivation; the second one describes plant relative performance (Supplementary Appendixes I and III for a description of the algorithms used for both categories), while the third one corresponds to those that attempt to describe NUE. The algorithms used to calculate NUE indicators are given in Table 2.

Comparing NUE indicators

A main objective of this work was to estimate the NUEa and determine to what extent the NUE indicators defined in Table 2 are able to reflect it. Therefore, a precise formula for each model was obtained to calculate NUEa for a given K$^+$ deprivation period (Supplementary Appendix II).

Table 2. *Indicators used to describe the dynamics of internal K⁺ concentration, relative plant performance and nutrient utilization efficiency*

Formula used to calculate the parameters corresponding to K⁺ concentration dynamics are given in Supplementary Appendix I.

Category	Indicator	Unit
Dynamics of internal K⁺ concentration		
TCjOpt	Time of K⁺ deprivation required for the internal concentration to reach *CjOpt*	d
T½cj	Time of K⁺ deprivation required to reduce the internal concentration of K⁺ to half of its initial value	d
Plant relative performance		
T½BIOM	Time of K⁺ deprivation required to decrease the quotient of biomass between K⁺ deprived and K⁺ well supplied plants to 0.5	d
Nutrient utilization efficiency		
KUE (K⁺ use efficiency)	W/Qj	g FW µmol⁻¹
UE (utilization efficiency)	W/cj	(g FW)² µmol⁻¹
PE (physiological efficiency at the vegetative stage)	$(W^s - W^d)/(Qj^s - Qj^d)$	g FW µmol⁻¹
PR (physiological ratio)	$(W^s - W^d)/(cj^s - cj^d)$	(g FW)² µmol⁻¹
NP (nutrient productivity)	$(dW/dt)/Qj^f$	g FW µmol⁻¹ d⁻¹
AP (accumulative productivity)	$(Ln(W^f) - Ln(W^i))/(cj^f\ dt)$	g FW µmol⁻¹ d⁻¹

cj, internal K⁺ concentration; *CjOpt*, optimum K⁺ concentration; *dt*, time elapsed between the final and initial harvest; *dW*, difference in biomass between the final and initial harvest; *Qj*, internal content of K⁺; *W*, plant biomass on a fresh weight basis. ^{d}, Deprived of K⁺; ^{f}, final; ^{i}, initial; ^{s}, supplied with K⁺.

Results

Dynamics of growth and K⁺ concentration

The time course of growth and internal K⁺ concentration was examined for virtual plants obeying to each of the three models at approximately intermediate values of the parameters mentioned above. The plants had the same *RGRmax* (0.126 d⁻¹), *cji* (135 µmol K⁺ (g FW)⁻¹), and *CjOpt* (90 µmol K⁺ (g FW)⁻¹). *CjMin* was the same (9 µmol K⁺ (g FW)⁻¹) for models I and III (Supplementary Fig. S1). Internal K⁺ concentration steadily declined for the three models. During the first phase of K⁺ deprivation, *RGR* remained constant until *cj* reached *CjOpt*. The decrease of *RGR* during the second phase occurred initially fast for the linear models I and II. For model III, *RGR* declined slowly at first but finally faster than for the other two models and was almost nil at day 40 since the beginning of the stress (Supplementary Fig. S1).

Time course of K⁺ utilization efficiency indicators

Although it is well known that some NUE indicators are affected by the duration of the growth period at suboptimal supplies (Rose and Wissuwa, 2012), a theoretical prediction of their temporal variation has not been developed so far. For virtual plants it was found that all the NUE indicators showed a clear dependence on time, although to varying degrees (Supplementary Fig. S2). In this context, an important question is whether or not the temporal variation of NUE indicators accompanies temporal changes in the NUEa. An important consequence of the assumption *CjMin* = 0 in the linear model II, was that NUEa remained constant, while for the linear model I, for which *CjMin* > 0, NUEa progressively decreased. In turn, NUEa firstly increased and then decreased for model III (Supplementary Fig. S2). The capacity of each NUE indicator to be associated with NUEa can be estimated by the relative invariance of each one of them corrected by the relative variation of NUEa since TCjOpt has been reached. It was observed that, regardless of the model used, the indicators that better reflected temporal changes in NUEa were in the order of nutrient productivity (NP), accumulated productivity (AP), and K⁺ utilization efficiency (KUE), while the other indicators essentially failed to reflect these changes (Supplementary Fig. S3).

Relative plant performance indicators

Next, this work examined the effect exerted by the factorial combination of the parameters contained in each model on the dynamics of growth and internal K⁺ concentration. It was observed that TCjOpt decreased as *Aj* (or *Pj*) and *CjOpt* increased, while TCjOpt increased with an increase of *cji* (Supplementary Fig. S4). Therefore, low TCjOpt values could result from fast growth as well as from a short difference between *cji* and *CjOpt*. An important consequence of a short TCjOpt is that growth becomes quickly restricted by internal K⁺ concentration, which is accompanied by important effects on the subsequent relative growth performance. It was found that the effect exerted by the parameters mentioned above on T½BIOM for all the models tested here (Fig. 2) resembles the pattern found for TCjOpt.

For model I, it was also found that increasing *CjMin* led to a concomitant increase of T½BIOM (Fig. 2A and B).
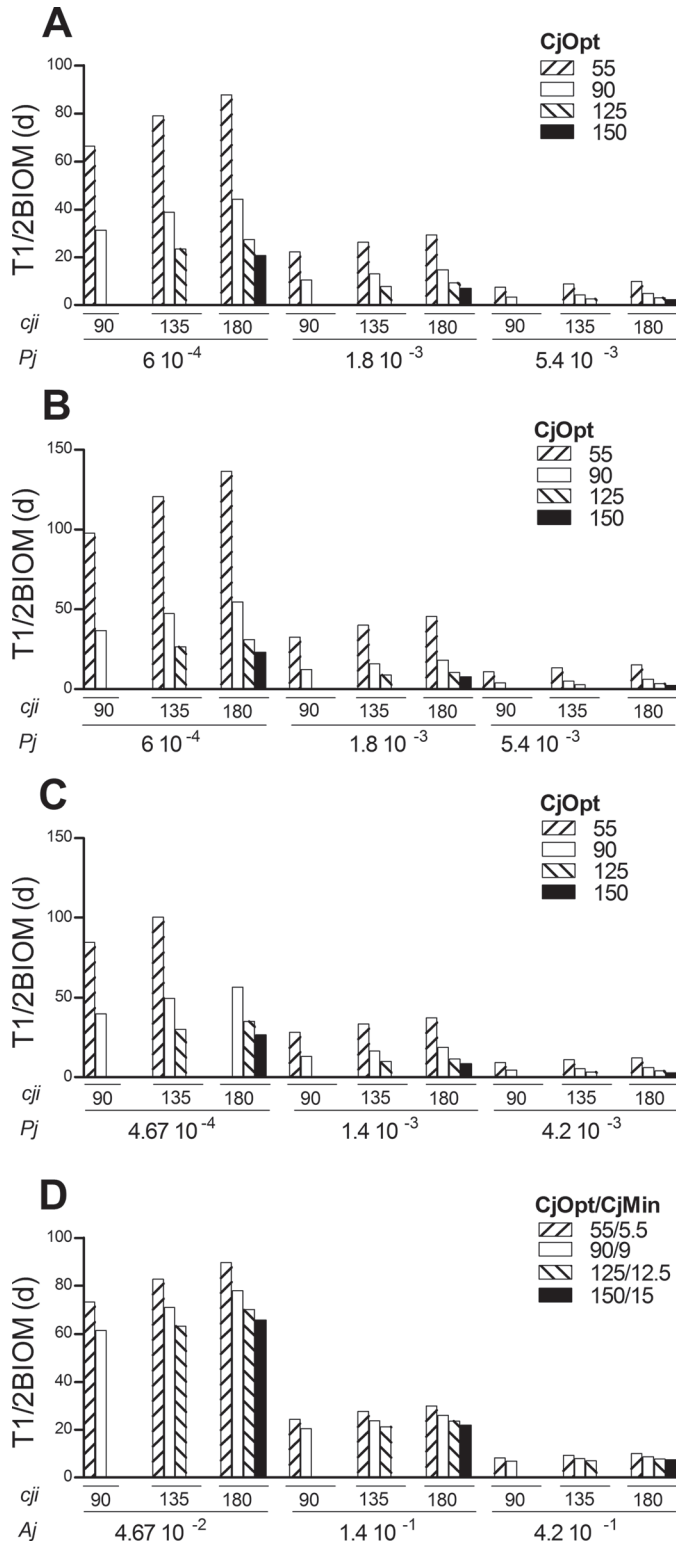
Fig. 2. Effect of variation in productivity (*Pj*), optimum concentration (*CjOpt*), minimal concentration (*CjMin*), the initial concentration (*cji*), and *Aj* on the time necessary to reduce the biomass of K⁺ deprived plants to half of the value estimated for K⁺-well-supplied plants (T½BIOM). (A) Model I with *CjMin* set at 1 μmol (g FW)⁻¹. (B) Model I with *CjMin* set at 25 μmol (g FW)⁻¹. (C) Model II. (D) Model III. *cji* and *CjOpt* are expressed in μmol (g FW)⁻¹ and *Pj* and *Aj* are expressed in g FW μmol⁻¹ d⁻¹ and d⁻¹, respectively.

However, it must be noted that for this model, a change of *CjMin* while *CjOpt* is kept constant, or the reciprocal, results in a concomitant change of the difference between *CjOpt* and *CjMin* (hereafter named as Δc) as well as in *RGR*. Therefore, it was next explored the effect of changing both *CjOpt* and *CjMin* while keeping Δc constant. By maintaining the same value of *Pj* for all the *CjOpt*/*CjMin* pairs, the *RGR* becomes also the same. Under this condition, it was observed that for a given Δc value, T½BIOM declined as the values of *CjOpt* and *CjMin* simultaneously increased (Supplementary Fig. S5).

Paradoxically, as shown in Supplementary Fig. S6, virtual plants with low values of *Pj* and high T½BIOM values displayed a lower accumulation of biomass after a 40-d deprivation period than plants with high *Pj* (Supplementary Fig. S6) and a worst relative performance (Fig. 2). In turn, increasing the value of *Aj* from low to intermediate values in model III, led to a strong enhancement of biomass accumulation, but a further increase of *Aj* led only to a modest increase of plant weight (Supplementary Fig. S6).

In spite of the strong relative effect of *CjOpt* on T½BIOM in models I and II, it was not translated into a large change of biomass accumulation (Supplementary Fig. S6). In turn, for model I, reducing only *CjMin* strongly influenced biomass accumulation. Consistently, a simultaneous reduction of the pair *CjOpt*/*CjMin* exerted a major positive effect on plant weight for models I and III (Supplementary Figs. S5B and Fig. S6D, respectively). Increasing *cji* led to an increase of T½BIOM (Fig. 2) as well as to improved biomass accumulation for the three models (Supplementary Fig. S6).

A valuable conclusion emerging from this analysis is that selection for improved relative performance in nutrient deficient environments, should not necessarily lead to enhanced biomass accumulation; since the later dramatically depends on the variable parameter (*CjOpt*, *CjMin*, *cji*, *Pj*, or *Aj*).

Effects of Pj (Aj), CjOpt, CjMin and cji on NUEa

In order to compare the absolute and relative theoretical performances of plants in the previous analysis, this work simulated deprivation periods as long as 40 d. However, for real fast-growing-plants, starvation periods longer than 15–30 d are likely to result in a considerable damage to leaves (Moriconi *et al.*, 2012). In turn, starvation periods shorter than 10 d could be not long enough to ensure the observation of a marked effect of K⁺ deprivation on the growth pattern. Thus, the effect exerted by intrinsic differences in *CjOpt*, *CjMin*, *Pj* (or *Aj*), and *cji* on NUEa was explored for virtual plants deprived of K⁺ for 20 d. Given that operational algorithms to calculate NUEa are defined for deprivation periods when growth is actually limited by internal K⁺ concentration (Supplementary Appendix II), only the combinations of parameters for which TCjOpt < 20 d were considered for each model.

It was observed (Fig. 3) that NUEa increased as *Pj* or *Aj* increased regardless of the model considered. In fact for model II, changes in NUEa were only associated to changes in *Pj* (Supplementary Appendix II, Fig. 3). For model I,
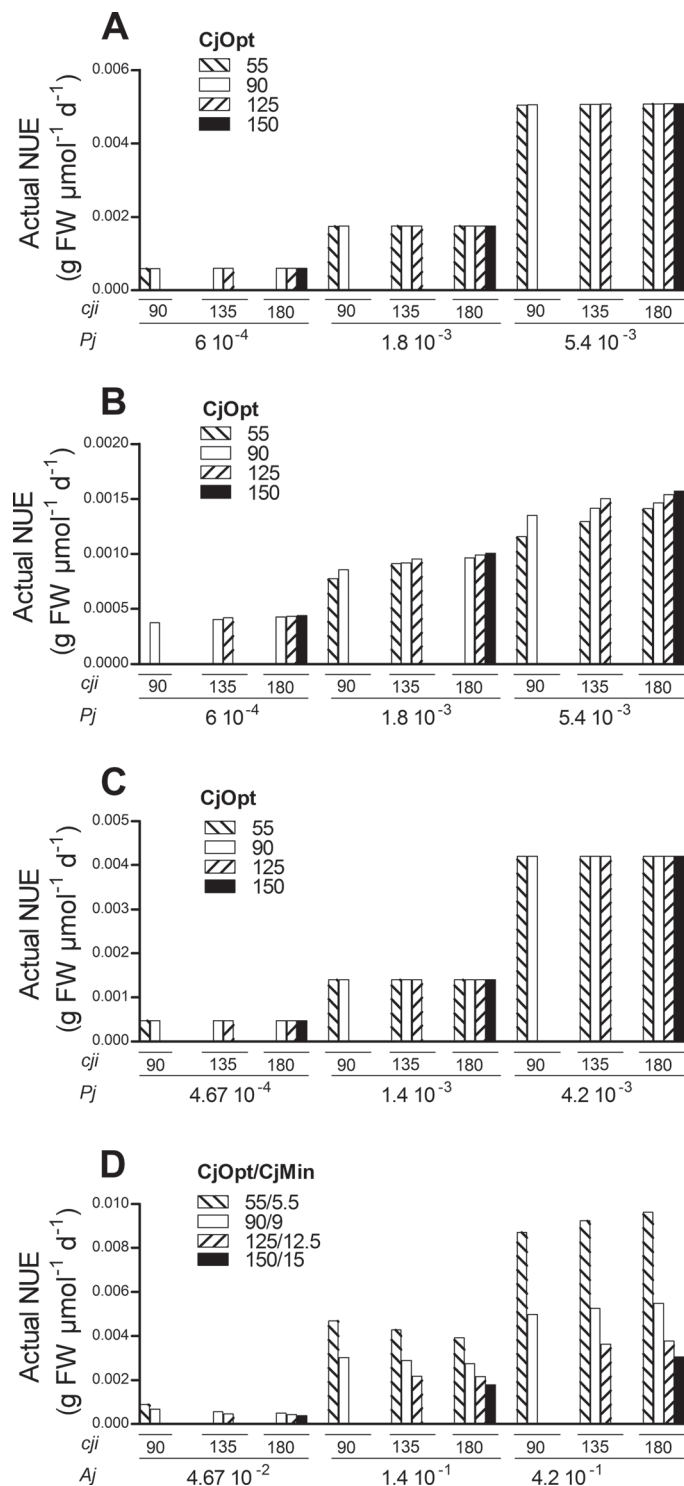
Fig. 3. Effect of variation in productivity (*Pj*) and *Aj*, optimum concentration (*CjOpt*), minimal concentration (*CjMin*), and the initial concentration (*cji*) on the actual nutrient use efficiency (NUEa). (A) Model I with *CjMin* set at 1 μmol (g FW)$^{-1}$. (B) Model I with *CjMin* set at 25 μmol (g FW)$^{-1}$. C) Model II. (D) Model III. Note that at low *CjMin*, variation of NUEa associated to *cji* is very small (columns corresponding to different *cji* values have similar height in panel A), while at high *CjMin*, the effect of *cji* becomes evident (panel B). *cji* and *CjOpt* are expressed in μmol (g FW)$^{-1}$ and *Pj* and *Aj* are expressed in g FW μmol^{-1}d^{-1} and d^{-1}, respectively.

NUEa correlated positively with an increase of *cji* as well as with a decrease of *CjMin* in addition to increase with *Pj* (Fig. 3, Supplementary Table S1A). For this model, the effect exerted by *CjOpt* alone on NUEa tended to be positive, but it was usually weaker than that exerted by other parameters. A sensitivity analysis showed that the mean slopes obtained for the linear regression between the variation of NUEa and the variation of *Pj*, *CjMin*, *cji*, and *CjOpt* were 0.689, –0.134, 0.072, and 0.036, respectively. Decreasing *CjOpt* and *CjMin* simultaneously always resulted in an increase of NUEa (Supplementary Fig. S5). Similarly for model III, a decrease of the pair *CjOpt*/*CjMin* also led to a major concomitant increase of NUEa (Fig. 3, Supplementary Table S1C). For plants following this model, the effect exerted by *cji* on NUEa depended also on the value of *Aj*: NUEa and *cji* were negatively associated at low but positively associated at high *Aj* values. For this model, a sensitivity analysis showed that the mean slopes for *CjOpt*/*CjMin*, *Aj*, and *cji* were –0.911, 0.473, and –0.116, respectively, being the last value affected by the dual effect of *Aj* mentioned above.

Capacity of NUE indicators to reflect NUEa at different CjMin, CjOpt, Pj (Aj), or cji values

In order to examine the capacity of each NUE indicator to reflect NUEa for 20-d K$^+$-deprived plants when NUEa varies as a result of changes in *Pj* (or *Aj*), *CjOpt*, *CjMin*, or *cji*, the variation of each NUE indicator was estimated and plotted against the variation suffered by NUEa. Variation of NUEa associated to a change in *Pj* or *Aj* positively correlated with all the NUE indicators, regardless of the model considered. However, the capacity to reflect the variation of NUEa largely differed among NUE indicators (Supplementary Table S1). Clearly NP and KUE tended to accurately reflect that variation, while AP tended to slightly amplify it, although at a lower extent than utilization efficiency (UE), which, with the exception of model III, tended to reflect the variation of NUEa associated with *Pj* (or *Aj*) better than physiological efficiency (PE), and this one better than physiological ratio (PR).

For model I, the variation of NUEa associated with variation in *CjMin* (Supplementary Table S1A) was adequately reflected by NP and to a lower extent by AP, KUE, and UE. For several combinations of parameters, PE poorly reflected the variation of NUEa associated with *CjMin*. It was observed that in model I almost all the indicators tended to excessively amplify the variation of NUEa associated with changes in *CjOpt*. As a general statement the degree of bias introduced by the NUE indicators was less pronounced following the order KUE, NP, AP, UE, PE, PR.

For model III, it was found that the important variation of NUEa associated with a change in the pair *CjOpt*/*CjMin* can be accounted by the following sequence of indicators: AP, UE, NP, KUE (Supplementary Table S1C). In this case PE and PR negatively correlated with NUEa. For plants obeying model II, where NUEa is not responsive to changes in *CjOpt*, variation in *CjOpt* resulted in a significant effect on NUE indicators. In this situation, it must be noted that the lower

the responsiveness, the better the capacity of the indicator to avoid false positives. This capacity followed the sequence KUE, NP, AP, UE, PE, PR (Supplementary Table S1B).

The last source of variation here considered, was *cji*. It was observed that for model I, for which NUEa was positively associated with *cji*, some indicators always negatively correlated with NUEa: KUE, PE, and PR. Regarding the other three, their capacity to reflect the actual variation accurately, as well as reduce the response when negatively correlated, were simultaneously evaluated. Considering both effects, a possible sequence may be AP, UE, NP (Supplementary Table S1A). For model III, only AP and NP always positively correlated with the variation of NUEa associated with a change in *cji* (Supplementary Table S1B). In turn, for model II, the capacity to avoid false correlations due to the effect of *cji* followed approximately the order AP, NP, UE, KUE, PE, PR (Supplementary Table S1B).

Trade off between relative growth performance and nutrient productivity

It has been proposed that NUE could be alternatively estimated as the product between NP and the mean residence time, the latter being an estimator of the mean period during which a given nutrient is retained within the plant (Berendse and Aerts, 1987). In an analogous way, this work examined the relationship between NUEa and T½cj for all the combinations of parameters formerly analysed. As a general trend an inverse relationship between NUEa and T½cj was found regardless of the model used, which was evident when the source of variation were *Pj* or *Aj* (Supplementary Fig. S7). In that context, the next question addressed was the existence of a potential conflict between selection for high NUEa and enhanced relative plant performance. In order to make an assessment on this subject, the relationship between T½BIOM and NUEa was examined (Supplementary Figs. S5 and S8). It was found that T½BIOM was always inversely related with NUEa when the sources of variation were *Pj* or *Aj*. A further analysis indicated that for models I and III this negative association disappeared when the source of variation was the simultaneous reduction of *CjOpt* and *CjMin* (Supplementary Figs. S5D and S8C). In that case T½BIOM and NUEa were positively correlated.

Discussion

The criteria used to estimate NUE by plants have been a subject of discussion for the last 30 years (Chapin III, 1980; Siddiqi and Glass, 1981; Berendse and Aerts, 1987; Gourley *et al.*, 1994; Baligar *et al.*, 2001; Good *et al.*, 2004; Hirose, 2011). Debate has focused on three main questions: (i) to what extent the indicators commonly used could be accurate predictors of the actual NUE; (ii) whether the variation in these indicators is high enough for successful crop improvement; and (iii) whether these indicators could offer an adequate estimation of the overall plant performance under limiting nutrient supply. The approach outlined in this work, which lays on the use of empirical relationships, provides a simple theoretical framework to estimate the otherwise unknown actual NUE value for plants growing in the complete absence of K^+. This, in turn, enabled a quantitative assessment of the previous issues in plants obeying models that differ in the relationship between NUE and internal K^+ concentration.

Conceptually, NUE should inform on the rate of biomass generated by each unit of the nutrient present in plant tissues. This analysis shows that several sources of variation contribute to determine NUEa, which corresponds to the actual efficiency of nutrient utilization once plant growth becomes dependent on the internal nutrient concentration (Supplementary Appendix II). Although certain inferences are common to the three models, the magnitude and direction of the effect exerted by each specific source depend in some cases on the model used. While for model II the only source of variation of NUEa was *Pj*, it was found that in addition to *Pj* (or *Aj*) the pair *CjOpt/CjMin* can also exert a significant effect on NUEa for models I and III. Importantly, for these two models, the concentration of K^+ at the onset of the deprivation period also exerted a substantial, yet less pronounced, effect on NUEa. Therefore selection for high NUEa, could involve selection for high *Pj* (or *Aj*), low *CjOpt/CjMin* pair (or low *CjMin* alone for model I), and high (for model I and sometimes model III) or low (for model III at low *Aj* values) *cji*.

The precise model followed by a given plant species as well as the values of the parameters for a given growth condition are not known *a priori*. Therefore, a good NUE indicator should, regardless of the model assumed: (i) positively correlate with NUEa for all or at least most sources of variation; (ii) show a minimal responsiveness when a negative association cannot be avoided; and iii) proportionally reflect the actual variation in NUEa. An analysis of NUE indicators revealed that the capacity of each one of them to reflect NUEa was strongly influenced by the source of variation as well as by the time since the beginning of the K^+ deprivation period. While all NUE indicators positively correlated with NUEa when *Pj* or *Aj* varied, contrasting patterns among them were found when *cji*, *CjOpt*, and/or *CjMin* were the sources of variation. This finding helps to explain conflicting results arising in the literature from the use of different NUE indicators (Gourley *et al.*, 1994). In fact, the use of some indicators could paradoxically lead, under some circumstances, to the selection of low NUEa. In this regard, the current work unveils a formerly undetected problem with KUE, which has been the most extensively used NUE indicator in large screenings (Yang *et al.*, 2003; White *et al.*, 2010; Guo *et al.*, 2012). This indicator is prone to introduce an important noise when variation of NUEa is associated with changes in the initial K^+ concentration; which could lead to selection for low NUEa. Similarly, according to this analysis, PE tends to be an erratic NUEa predictor, which in some cases correlates negatively with NUEa. Unlike other indicators, AP, and even to a greater extent NP, have the capacity to reflect temporal changes in NUEa while displaying a lower temporal variation than other indicators. These indicators may allow proper comparisons between plants differing in their physiological age, which is

usual in many genetic screenings, or when comparing among different experiments performed under similar growth conditions. Taken together, the findings suggest that depending, on the source of variation, NP, AP, KUE, and in some cases UE can be appropriate predictors of actual differences in NUEa for plants grown in the complete absence of K⁺. Probably due to the difficulties associated with their estimation, the use of NP has been largely omitted in genetic studies of NUE, although its use has been highlighted as a part of the framework to understand NUE (Veneklaas *et al.*, 2012). However, the use of AP and NP in large screenings at the vegetative stage should not be discarded. Because non-destructive measurements of single plant total freshweight can be performed in hydroponic media and the errors derived from calculations can be reduced by appropriate sampling (Causton, 1991), reliable measurements of AP and NP could be obtained. In summary, the simultaneous use of several indicators, together with an adequate knowledge of their theoretical limitations, could improve the selection of plants with high NUEa.

It has been recently proposed that screenings for variation in NUE could be improved if plants are grown in individual pots containing a nutrient solution with a specific low amount of the nutrient under study (Rose *et al.*, 2011). This could help to reduce the masking effect of potential differences in nutrient acquisition (Rose and Wissuwa, 2012). The current results indicate that, when nutrient capture is equated to zero, initial differences in nutrient concentration exert a large effect on both NUEa and NUE indicators. The extent to which a similar phenomenon could affect NUE measurements performed according to the approach outlined by the referred authors needs to be assessed. A protocol based on the induction of a complete nutrient deprivation, when combined with the use of appropriate indicators (AP, UE, and NP) with adequate sensitivity to variation in *cji*, could be a valuable alternative tool for the selection of genotypes differing in NUEa at the vegetative stage.

Virtual, as well as real (Poorter *et al.*, 1995), plants with a high productivity tend to exhibit higher *RGR* values than plants with a low productivity. However, the latter tend to improve their relative growth performance under conditions of limiting nutrient supply due to a slow use of internal nutrient reserves, which could eventually help real plants to cope with nutritional shortage. Variations among plants in the decay of the nutrient accumulated have been usually ascribed to differences in the mean residence time (Berendse and Aerts, 1987; Silla and Escudero, 2004). The current work suggests that a low decay of internal nutrient concentration under conditions of limited nutrient supply obeys to the action of two components. One, accounted by any of the models here used, corresponds to reduced dilution effects, while the other that involves reduced nutrient loss is reflected by long mean residence time. Both components are likely to act cooperatively (Moriconi *et al.*, 2012) and should be taken into consideration for a full description of plant strategies to sustain growth during nutrient shortage. Besides, the analysis performed here for conditions of complete nutrient deprivation, provides further theoretical support for the existence of a trade off between plant productivity and reduced nutrient decay (Berendse and Aerts, 1987), indicating that it results from a conflict inherent to plant systems dynamics. In support of this assertion an inverse relationship between T½cj and the variation of NUEa associated to *Pj* (or *Aj*) was observed here. It operated in the same direction to that formerly reported for the relationship between NP and the mean residence time (Ecskstein and Karlsson, 1997; Aerts and Chapin III, 2000; Silla and Escudero, 2004).

A major objective of current research in plant nutrition is to generate crops that maximize the relative growth performance as well as NUE in nutrient poor environments or under conditions of deprivation induced by intensive nutrient extraction (White and Brown, 2010). An important question that needs to be addressed, particularly in the light of the abovementioned trade off, is whether or not selection for both traits can be pursued simultaneously. The current data indicate that the answer to this question depends both on the source of variation and on the model taken in consideration. A consequence of keeping *CjMin* = 0 for plants that follow model II is that the only source of variation of NUEa is *Pj*, which is always inversely related to T½BIOM. Therefore, in this case, selection for high NUEa should always lead to negative selection for improved relative performance. However, for plants following models I or III, selection for both high NUEa and high T½BIOM could be simultaneously pursued if there is enough genotypic variation to select for the simultaneous reduction of the pair *CjOpt*/*CjMin*. It is worth noticing that while the individual effect of each parameter can be isolated for some virtual plants (except when logically interconnected to another one), in real plants *CjOpt*, *CjMin*, *Pj*, or *Aj* are probably interdependent, introducing additional constraints to the breeding process.

The approach outlined in this work was built with the idea of obtaining a theoretical assessment of the questions raised above for K⁺. The extent to which a similar approach can be pursued for major metabolized nutrients, like nitrogen, phosphorus, or sulphur, remains to be explored. Even for K⁺, it must be stressed that this approach relies on the assumption that *CjOpt* and *CjMin* as well as *Pj* (or *Aj*) remain invariant during the course of K⁺ deprivation, which could be not necessarily valid for all plant species. Certainly, the models do not explicitly account for relevant acclimation processes that occur in real plants deprived of this element, which involve biochemical, physiological, and anatomical adaptations (White and Karley, 2010), the modification of biomass and nutrient partitioning between roots and shoots and within the shoot (Yang *et al.*, 2004) being probably the most important. Eventual interactions with other factors may also play an important role. Therefore, for a given plant species, the use of models that take into account the regulatory and detrimental effects that occur during K⁺ deprivation could help to further refine the conclusions of this work and confer operational value to the procedures here outlined for plant breeding.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Appendix I. Formula and calculations.
Supplementary Appendix II. Estimating theoretical NUE.

Acknowledgements

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