What is the accuracy of NaCl induced K^+ efflux in roots as an indicator of salt tolerance?

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ABSTRACT

There is an increasing interest in breeding salt tolerant plants due to salinization and a growing food demand. Since the current methods of identifying salt tolerant plants take rather long and are expensive, there is an upcoming interest in a new method of identification: measuring K^+ efflux. With a non-invasive ion flux measurement (MIFE) the K^+ efflux in roots is measured after introduction of salt (NaCl). Comparison of experiments which measured K^+ efflux in barley, wheat, grapevine rootstock and irises indicate that there is a relation between the capability of a plant to retain K^+ and salt tolerance. The higher the K^+ efflux, the lower the salt tolerance. To optimize measurements and compare outcomes, a universal method is proposed regarding measuring place, measuring time and NaCl concentration. Overall, K^+ efflux proves to be a solid indicator of salt tolerance and could therefore improve breeding programmes for salt tolerance.

Keywords:

Salt tolerance – K^+ efflux – MIFE

Introduction

Over the last decades, the growing world population led to a rapid increase in food demand. Meanwhile, salinization leads to a decrease of usable farmland and crop yield (Food and Agriculture Organization of the United Nations 2017). This decline in yield negatively impacts food availability and consequently leads to the clearing of land for agriculture. This clearing usually takes place at the expanse of uncultivated nature. A large scale example is the deforestation of the Amazon in Brazil (Watts 2019). This development has severe consequences for biodiversity and climate change in general. So, instead of claiming more land to meet the food demand, it could also prove fruitful to examine mechanisms to make plants more salt tolerant. Then, the yield per field would increase and the need for more land would decrease.

The first step to increase salt tolerance is identifying salt tolerant cultivars so that they can be used for further breeding. However, the current methods of identification are expensive and take rather long (Chen et al. 2005). Different ways of identification have been proposed such as ranking plants in order of growth rate or yield rate, plant survival in saline conditions, germination rate and many others (Chen et al. 2005). Recently, the focus shifted towards the interaction between natrium (Na^+) and potassium (K^+). High soil concentrations of Na^+, caused by salt, stimulate a K^+ efflux. Since Na^+ tends to compete with K^+ for binding sites in key metabolic processes, this efflux is considered harmful (S. Shabala and Cuin 2008). This relation between Na^+ influx, K^+ efflux and harmful consequences could provide a new indicator for salt tolerance, hence the recent interest. Different ways to find the best indicator are explored, focussing on the ability of the plant to exclude Na^+ from its roots, the capability to maintain an optimal K^+: Na^+ ratio and the ability to retain K^+. This paper will explore the accuracy or usefulness of measuring K^+ efflux in roots (and thus the capability of a plant to retain K^+) as an indicator or proxy of salt tolerance. The hypothesis: the smaller the amount of K^+ efflux in roots, the higher a plant its tolerance against saline conditions.

This K^+ efflux is measured by Non-invasive Measurements Techniques (NMT) such as the Micro-electrode Ion Flux Estimation (MIFE). This is a method which uses an ion-specific electrode which measures the efflux of the ion. In this case, the electrode measures K^+ and the effect NaCl has on the magnitude of this efflux. This method has been used in different experiments regarding barley, wheat, grapevine rootstock and Chinese irises (Chen et al. 2005; Cuin et al. 2009; 2008; Fu et al. 2019; Li and Zhang 2014).

The aim of this paper is to evaluate whether this method is effective. It will therefore answer the question: What is the accuracy of NaCl induced K^+ efflux in roots as an indicator of salt tolerance? It will do this by first looking at the effect Na^+ has on the functioning of K^+. Then, it will turn towards the mechanism which results in K^+ efflux. Next, it will look at the way K^+ efflux is measured. Followed by the results of experiments which used this method to identify salt tolerance in plants. Finally, an assessment will be given concerning the best use of this method.

What is the function of K^+ and how is this affected by high Na^+ concentration?

K^+ is the most abundant cation in plant cells and plays a key-role in many physiological processes (Isayenkov and Maathuis 2019). It is accumulated by roots and makes up for about 2-6% of dry weight. K^+ regulates cell homeostasis as an osmotic and functions as a PH-regulator (Pandey and Mahiwal 2020a). Furthermore, it is associated with defence against pests and diseases, immunity, signalling and transport processes (Demidchik 2014; Pandey and Mahiwal 2020b). Next to these functions, K^+ regulates two processes which are strongly affected by salt stress and will thus be addressed more deeply. Salt stress occurs when the salt concentration negatively effects a plant its functioning, this is often caused by the Na^+ in salt. The two K^+ regulated processes affected by salt stress are: regulation of the membrane gradient and regulation of enzymatic reactions.

K^+ plays an important role in the membrane voltage regulation due to its positive charge. The concentration of K^+ in plant cells is generally between 50-200 mM, this concentration is up to two or three times higher than the K^+ concentration of soil. To maintain this high level of positively charged K^+, the plasma membrane is charged negatively (Demidchik 2014). However, when the cell membrane is depolarized by the influx of positively charged ions such as Na^+, K^+ outward rectifier channels are activated (Assaha et al. 2017). This K^+ efflux results in a repolarization of the membrane potential (Demidchik 2014). Thus, when salt increases the Na^+ concentration in soil, this leads to Na^+ influx in the root cell which results in a membrane depolarization followed by K^+ efflux. Due to its role as voltage regulator K^+ is forced out of the cell and its cellular concentration decreases.

This decrease in K^+ concentration relates to the second process affected by salt stress: the effectiveness of K^+ in enzymatic reactions. K^+ regulates metabolic processes in the cytoplasm, e.g. enzymatic reactions, protein synthesis and ribosome functions (S. Shabala and Cuin 2008). K^+ is thus responsible for the anabolic processes which synthesize cell polymers and thereby build and strengthen the plant. However, due to the high similarity between K^+ and Na^+ in hydrated form, Na^+ can occupy K^+ binding sites and thereby block its functioning (Demidchik 2014; Pandey and Mahiwal 2020b). This competition between Na^+ and K^+ is best illustrated by the K^+:Na^+ ratio in cells. When this ratio gets too low, Na^+ reduces the functioning of a cell by blocking K^+ from binding sites (S. Shabala and Cuin 2008).

It becomes apparent that K^+ is of great importance in the functioning of plants, but its availability is threatened by high Na^+ soil-concentrations caused by salinization. This high Na^+ concentration in soil leads to Na^+ influx into the cell and consequently to a shift in the K^+:Na^+ ratio to a sub-optimal level (and thus blocking K^+ from binding sites). Na^+ influx also results in a K^+ efflux which further upsets the ratio and deprives the plant of access to K^+. Lastly, high concentrations of Na^+ in soil, reduce the K^+ uptake from soil since the uptake sites are taken by Na^+ (Shabala and Cuin 2008). This also complicates the reuptake of K^+ which left the cell though the salt induced K^+ efflux (Britto et al. 2010). When the available K^+ becomes too low, it leads to a nutrient imbalance which reduces plant growth and ultimately its yield (S. Shabala and Cuin 2008). In extreme cases, a K^+ deficiency can even lead to cytosolic proteases and endonucleases which lead to a programmed cell death (Demidchik 2014).

What is the relation between K^+ efflux and increased Na^+ concentrations?

Since K^+ efflux is caused by salt stress and has detrimental effects on plants, measuring this efflux could prove to be an efficient indicator of salt tolerance. However, to use K^+ efflux as an indicator of salt stress, the mechanism which leads to this efflux should first be addressed. There are two main hypotheses regarding this mechanism which will now be discussed. The first hypothesis assumes that K^+ efflux is caused by membrane depolarization. The second hypothesis considers the K^+ efflux as a result of cell structure integrity loss.

The first hypothesis focusses on the depolarization caused by Na^+ which leads to an efflux of K^+. The high concentration of Na^+ in soil, compared to the low concentrations of Na^+ in root cells, leads to an influx of Na^+ into root cells. This influx of positively charged ions leads to depolarization of the negatively charged cell membrane below its resting potential. To restore this depolarization, K^+ outward rectifier channels are activated which results in a K^+ efflux (Assaha et al. 2017). This presumed mechanism is substantiated by the finding that the addition of Calcium (Ca^{2+}) leads to a lower K^+ efflux. With access to Ca^{2+}, the cell has the ability to repolarize its membrane potential with the efflux of the positively charged ion Ca^{2+} instead of K^+. This finding seems to endorse the hypothesis that K^+ efflux is a consequence of cell membrane regulation and that K^+ efflux is a direct result of uptake of toxic levels of Na^+.

The second hypothesis focusses on the loss of structural integrity of the cell membrane caused by Na^+ influx (Demidchik 2014). The influx of Na^+ is supposed to increase the leakiness of the cell membrane which leads to the observed K^+ efflux. Normally, the cell membrane structure is stabilized by Ca^{2+}. There is however, a strong competition for binding sites between Ca^{2+} and Na^+, and although Ca^{2+} has a stabilizing effect, Na^+ has a destabilizing effect. So, an increased concentration of Na^+ leads to a replacement of Ca^{2+} for Na^+ to the binding sites, destabilizes the cell membrane and increases its leakiness. This leads to the observed K^+ efflux. This would mean that the observed K^+ efflux is not a specific reaction to saline conditions. There is an efflux of multiple ions as a reaction to the loss in structural integrity of the cell membrane. This is in line with the finding that introduction of Ammonium (NH_4^+) and K^+ itself also lead to K^+ efflux. Both NH_4^+ and K^+ have a degrading effect on membrane stability and not on cell depolarization (Britto et al. 2010). Then, this hypothesis also provides another explanation for the finding that addition of Ca^{2+} decreases K^+ efflux. The addition of Ca^{2+} shifts the ratio between Ca^{2+} and Na^+ and thereby increases its capacity for competition with Na^+ for binding sites in the cell membrane. So, K^+ efflux is not ameliorated by Ca^{2+} due to its role as replacement depolarizer, but to its role as membrane stabilizer. This also fits with the finding that Zinc (Zn^{2+}) and Lanthanum (La^{3+}) decrease K^+ efflux since they are both membrane stabilizers (Britto et al. 2010).

Although the second hypothesis sounds convincing, it seems more likely that the efflux is caused by the regulation of the membrane potential. This hypothesis fits better with the observation of immediate K^+ efflux and the long duration of this efflux. It seems unlikely that the cell integrity is compromised for the extent of time the K^+ efflux is measured. Also, experiments which considered hydrogen (H^+) efflux, found that its efflux appears to be coupled to salt uptake through an outward proton transport process (Pitman 1970). This would be in the form of an antiporter which exchanges extracellular Na+ for intracellular H^+ (Krulwich 1983). The found curve for H^+ efflux (high efflux shortly after introduction and slowly steading toward normal level (Chen et al. 2005)) indicates an antiporter system and is in line with the depolarization hypothesis but not with the cell structure degradation hypothesis. This leads to the general acceptance that K^+ efflux is caused by the membrane depolarization of root cells in plants due to the uptake of Na^+, and leads to a shift in the $K^+:Na^+$ ratio to a sub-optimal level.

How is the relation between K^+ efflux and salt tolerant traits established?

That K^+ efflux is an effect of increased Na^+ uptake due to saline conditions is clear. The efflux of K^+ is considered harmful[1] since it shifts the K^+:Na^+ ratio to a level at which K^+ cannot (properly) function. The magnitude of efflux could therefore be considered as an indicator of salt tolerance in plants. If the efflux is large, the plant experiences high salt stress and is thus not considered salt tolerant. If the efflux is low, the plant experiences less salt stress and is thus considered salt tolerant.

K^+ efflux can be measured using Non-invasive Measurements Techniques (NMT) such as the Micro-electrode Ion Flux Estimation (MIFE). The MIFE technique monitors the ion dynamics around intact roots and is thus a non-intrusive way of measuring (S. N. Shabala, Newman, and Morris 1997). It works with an ion-specific electrode to measure the efflux of this ion, in this case that would thus be a K^+ specific electrode. While measuring, the electrode moves between two positions so that the net ion fluxes can be calculated using the difference in electrochemical potential and cylindrical diffusion geometry. This ion flux is measured after the attribution of salt so that the immediate K^+ efflux can be determined. If the magnitude of this net ion flux correlates to salt tolerance, it can be used as an indicator of salt stress in plants. However, how is this relation measured? How is 'salt tolerance' defined?

There is not an absolute way to determine salt tolerance in plants. Often plants are compared to one another for their performance at specific traits. However, traits associated with salt stress regulation are abundant and can be expressed at different times and are evolved several times and in different ways. For example, *Arabidopsis thaliana* alone has already 77 genes that can potentially encode K^+ permeable channels (Demidchik 2014). So, when focussing on different traits, different levels of salt tolerance can be found. Therefore, it is important to consider the most accurate traits for determining salt tolerance, and then examine whether these traits connect to K^+ efflux.

Traits which are often ranked to indicate salt tolerance are: grain weight, yield rate, growth rate, plant survival, germination rate, leaf or root elongation rate, leaf injury and reduction of CO_2 assimilation, loss of chlorophyll and damage to the photosynthetic apparatus, Na^+ exclusion, K^+:Na^+ ratio and Cl^- exclusion (Cuin et al. 2009; Chen et al. 2005). From this list it becomes clear that there are primary responses such as cellular osmotic and ionic stress responses, but also secondary stress response on whole-plant level (Chen et al. 2005). The primary responses lead to the secondary responses and their combination gives an outline of what salt tolerance includes. K^+ efflux can be considered a primary response.

To get the most complete overview of the level of salt tolerance of a plant, it is best to take a combination of both responses and look at multiple traits. However, when focussing on the performance of a specific trait under saline conditions, only this specific trait is of importance. For example, when selecting the most salt tolerant food crops for further breeding, the crop yield is the trait to focus on. Since this paper is to evaluate the potential of K^+ efflux as an indicator of salt tolerance, all traits (for which research is done) will be considered to get the most substantiated answer. Based on the possible correlation with other salt tolerant indicating

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[1] However, there is also the hypothesis that K^+ efflux is a mechanism to cope with the toxic concentrations of Na^+. This suggestion regards the decrease in cytosolic K^+ content as a mechanism to inhibit energy-consuming reactions and thus save energy for adaptation and reparations needs caused by the increased Na^+ concentration. This reduction of energy-consuming processes leads to a reduction in plant growth, however, this reduction is a positive effect instead of negative one (Demidchik 2014).

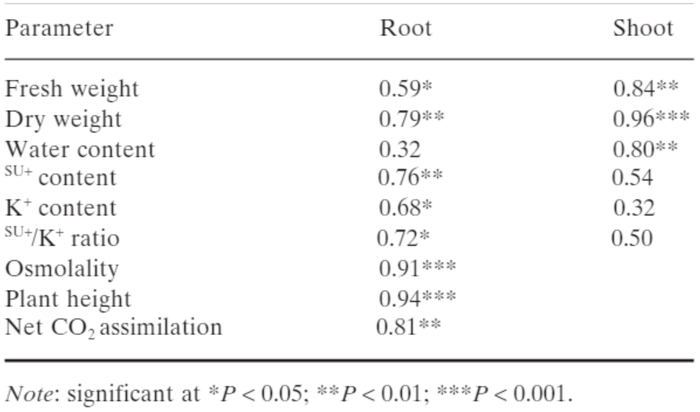
traits, it will be determined whether K^+ efflux can be considered an indicator of salt tolerance as well. If so, it will enrich the list with possible screening traits and offer a relative fast and cheap way to determine salt tolerance.

What is the relation between K^+ efflux and salt tolerance in earlier experiments?

So far, K^+ efflux has been measured in multiple experiments to determine its relation to salt tolerance. Some plants of interest were barley, wheat, grapevine rootstock and irises (Chen et al. 2005; Cuin et al. 2009; Fu et al. 2019; Li and Zhang 2014). The studies used slightly different methods, used different traits to investigate salt tolerance and found different results. These results will be addressed below.

Barley

In a study addressing salt tolerance in barley, "a very strong negative correlation between the magnitude of K^+ efflux from the root and salt tolerance of particular cultivar" was found (Chen et al. 2005). The study used seven, 3-day old barley cultivars and measured the K^+ efflux in the mature root zone after introduction of various concentrations NaCl. The K^+ efflux was found to correlate with shoot dry weight (r2 = 0.96), plant height (r2 = 0.94) and osmolality of flagleaf (r2 = 0.91), see Figure 1. There was a clear difference in K^+ efflux between the salt tolerant cultivars (20-25 nmol m-2 s-1) and the salt sensitive cultivars (150-180 nmol m-2 s-1) (Chen et al. 2005). The distinction between salt tolerant and salt sensitive plants was based on biomass indicators and shoot water content since they proved significantly different between the cultivars under saline conditions. It is, therefore, not surprising that these are also the traits which most strongly correlated with K^+ efflux.



Parameter	Root	Shoot
Fresh weight	0.59*	0.84**
Dry weight	0.79**	0.96***
Water content	0.32	0.80**
$^{SU+}$ content	0.76**	0.54
K^+ content	0.68*	0.32
$^{SU+}/K^+$ ratio	0.72*	0.50
Osmolality	0.91***	
Plant height	0.94***	
Net CO_2 assimilation	0.81**	

Note: significant at $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Figure 1: *Linear correlation (r^2) between net K^+ efflux and plant physiological characters (Chen et al. 2005)*

The study also investigated the optimal measuring conditions for using K^+ efflux as salt tolerance indicator. It found that a NaCl concentration between 40-160 mM is suitable for screening, and that 80mM NaCl is optimal. This concentration gives a clear response in efflux with a clear distinction between salt tolerant and salt sensitive cultivars. Furthermore, the strongest correlation between K^+ efflux and salt tolerance was found with a measuring time of 40min (Chen et al. 2005).

Wheat

In a substantial study into salt tolerance in wheat, it was found that K^+ efflux did not predict salinity tolerance (Cuin et al. 2009). It used twenty-five, 6-day old bread and durum wheat cultivars. The ranking of salinity tolerance was based on relative grain yield under saline conditions compared to control plants. As can be seen in Figure 2, there is no relation between

K^+ efflux and relative grain yield. However, the K^+ efflux measurement in this study used a measurement time of only 5 min and a NaCl concentration of 150 mM. Since the optimal measuring conditions in barley were a measurement time of 40 min and a NaCl concentration of 80 mM NaCl, it might be of use to repeat the measurement with these parameters (Chen et al. 2005).

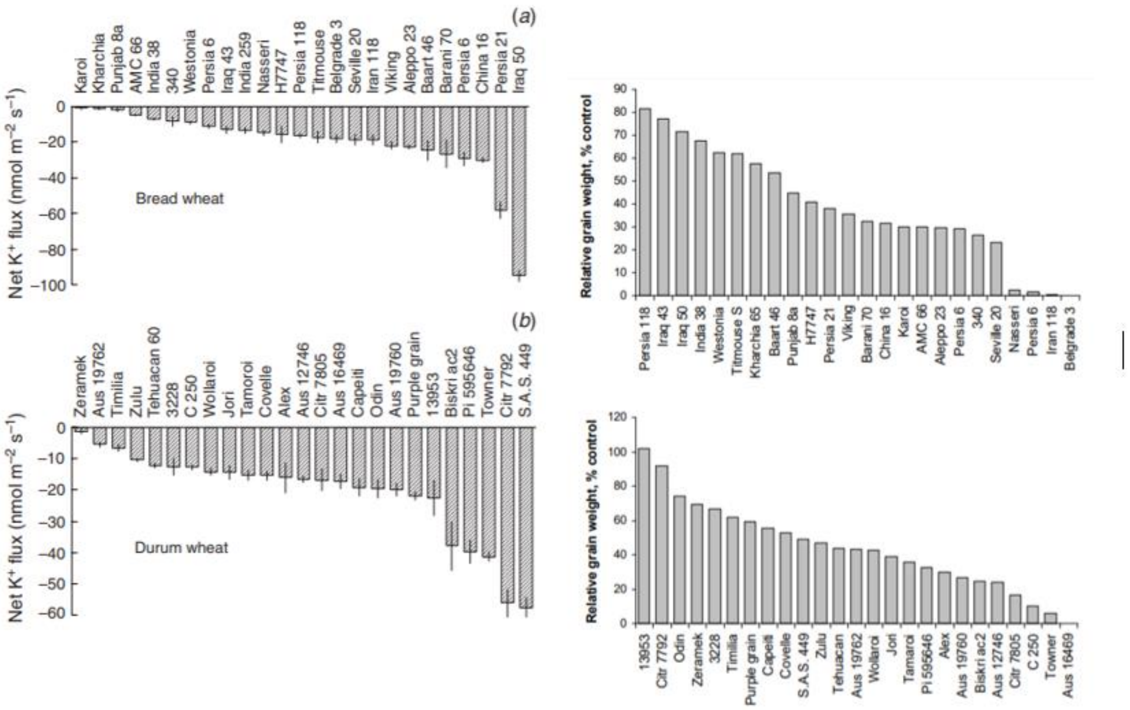


Figure 2: *On the left is the K^+ efflux in the mature rootzone of 6-day-old (a) bread and (b) durum wheat cultivars. On the right is the relative grain yield (%) in (a) bread and (b) durum wheat cultivars. There is no correlation between the K^+ efflux and the relative grain yield* (Cuin et al. 2009).

However, a smaller study regarding four different wheat cultivars, did find that K^+ efflux is a significant indicator of salt tolerance when considering the relative plant yield, see Figure 3. The measurements in this study were made on 6-day old cultivars at 10 mm from the root tip (so the mature zone), it used a 80 mM NaCl concentration and started measuring after 60s for a duration of 60 minutes (Cuin et al. 2008).

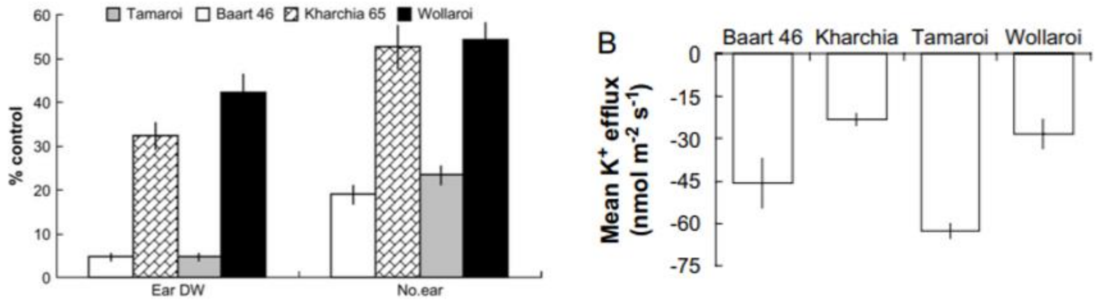


Figure 3: *On the left is the relative plant yield (%) of plants grown on 150mM NaCl. On the right is the K^+ efflux of 6-day-old seedlings in the mature zone (10 mm from the root tip)* (Cuin et al. 2008).

So, it is not yet conclusive whether K^+ efflux indicates salt tolerance in wheat due to the contrasting findings. However, the second study does seem promising and its methods are more in line with the optimal conditions found in barley.

Grapevine Rootstock

An experiment concerning grapevine rootstock looked at the salt tolerance of two F1 hybrids and its male parent. The experiment found that the hybrids were more salt tolerant then their parent, this was based on their capacity to more efficiently exclude Na^+ from their roots, a lower Na^+ accumulation in the whole plant (especially leaves), and the capacity to maintain higher levels of K^+. The F1 hybrids also displayed a smaller K^+ efflux in roots after introduction of NaCl, see Figure 4. This K^+ efflux was measured for 30min after introduction of 100mM NaCl. This indicated that the salt tolerance in grapevine rootstock correlates to the K^+ efflux in roots (Fu et al. 2019).

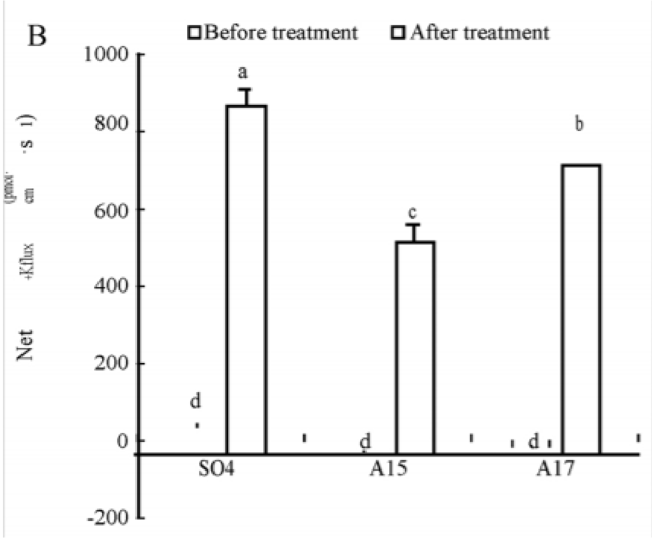


Figure 4: *The average K^+ efflux in F1 hybrids A15 and A17 and their male parent SO4. Different letters on the error bars indicate significant difference in K^+ efflux* (Fu et al. 2019)

Irises

Another experiment focused on the salt tolerance in two populations (Xj and Bj) of Chinese irises (*Iris lacteal*). It measured K^+ efflux at 12-day-old cultivars after addition of 140mM NaCl. The efflux was measured from the apex along the root axis up to 10.8 mm in intervals of 0.6 mm. It was determined that Xj was better adapted to saline conditions than Bj based on the relative growth rate of the plants and its higher K^+:Na^+ ratio in the shoots. In line with this finding it was found that the K^+ efflux in the mature root zone was lower for Xj than it was for Bj, see Figure 5 (Li and Zhang 2014). Thus, K^+ efflux seems to function as an indicator of salt tolerance in Chinese irises.

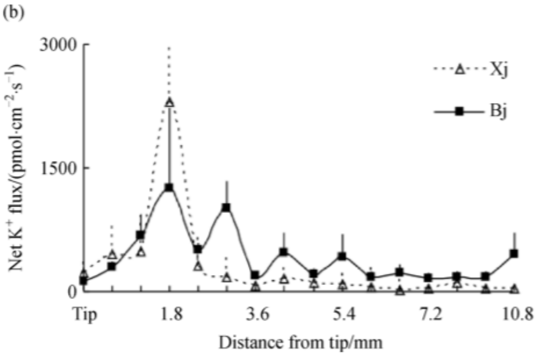


Figure 5: *K^+ efflux for two Chinese iris populations (*Iris lacteal*) after addition of 140mM NaCl. Measurement took place in intervals of 0.6 mm starting at the root tip up to 10.8 mm* (Li and Zhang 2014)

How best to apply K^+ efflux as an indicator of salt tolerance?

As became clear from these experiments, K^+ efflux in roots can often be used as an indicator for salt tolerance. Strong correlations between K^+ efflux and the salt tolerance was found for the indicators: shoot dry weight (barley), plant height (barley), osmolality of flagleaf (barley), relative plant yield (wheat), exclusion of Na^+ (grapevine rootstock), low Na^+ accumulation in the whole plant (grapevine rootstock), maintenance of high K^+ levels (grapevine rootstock), relative growth rate of the whole plant (irises) and higher K^+:Na^+ ratio in shoots (irises). Although these are all different indicators, it is clear that there is a relation between the magnitude of K^+ efflux and the primary responses such as cellular osmotic and ionic stress and secondary stress responses on whole plant level. So, it seems like K^+ efflux is an accurate indicator of salt stress in general and can thus help rank plants in accordance to salt tolerance.

The only uncertainty remains with regard to wheat. The accuracy of K^+ efflux as an indicator of salt tolerance proved uncertain due to contractionary findings. The uncertainty can be caused because K^+ efflux as an indicator simply does not work in wheat, but it can also be caused by the different methods used in the experiments. To ban such uncertainties in the future and make the use of K^+ efflux as an indicator of salt tolerance as reliable and efficient as possible, it is proposed that a protocol is used in further research. This would improve the reliability of the results and provide the possibility to compare results of different studies. A proposal for a protocol is as follows:

1. Determine the place of the mature rootzone with a scan along the root axis. The mature rootzone is identified as the most robust place to measure since the fluxes in this zone show less variability along the axes than the fluxes in the elongation zone (Chen et al. 2005). A small inaccuracy in electrode placement in the elongation zone might lead to a considerably different magnitude of K^+ efflux. This risk is lower in the mature rootzone due to the lower variability of ion fluxes along the root. Therefore, it is advised to determine the mature rootzone and measure the ion fluxes there.

2. It would also be advisable to use a standard measuring time. As can be seen in the results from two different studies on wheat with different measurement times, the results were considerably different (Cuin et al. 2008; 2009). One study found that K^+ efflux correlates to salt tolerance but the other study did not. Partly due to the difference in measuring time, it is uncertain whether K^+ is an efficient indicator in wheat or not, or whether the measuring time was simply too short. To ban such unnecessary uncertainties, it would be advisable to use a standard measuring time. A measuring time of 40 minutes is proposed here, since this was found to give the strongest correlation for barley and there is no further research into the optimal measuring time so far (Chen et al. 2005).

3. The use of a standardized concentration of NaCl to measure K^+ efflux would be the last recommendation to unify the measuring protocol. The studies mentioned earlier all used concentrations between 80-140 mM NaCl, this is in line with the recommendation by Chen to use concentrations between 40-160 mM NaCl (Chen et al. 2005). The best correlation between K^+ efflux and salt stress was found at 80 mM NaCl for barley, it is also the concentration used in the experiments with wheat. Therefore, it seems like 80 mM NaCl is a reasonable concentration to use.

Ideally, the use of such a protocol as described above can increase validity of future research. Repetition of K^+ efflux measurements in wheat, using this protocol, can also help establish the link between K^+ efflux and salt tolerance. As can be noted from the protocol, there is no recommendation regarding the number of cultivars in experiments. This is because the number of cultivars does not seem to influence the efficiency of K^+ efflux as an indicator. The studies addressed in this paper used between 2-7 cultivars, with the exception of the wheat experiment which used 25 cultivars. Since the findings in wheat were contradictory, it could be concluded that group size affects result. However, this does not seem to be the case. It seems more probable that the lack of a correlation can be explained by the different protocol than the sample size, since the study found no correlation at all. If the sample size was too large, it would be more logical if there was no correlation but still a rough distinction between salt tolerant plants and K^+ efflux. It might be that K^+ efflux as an indicator is not capable of making fine grained distinctions between salt tolerance with the large sample size, but it seems like it should be able to make a rough distinction. However, as can be seen in figure 2, K^+ efflux does not even provide a rough distinction between salt tolerant and salt sensitive cultivars. If the deviating protocol was at fault however, the outcome is more likely to show no distinction at all since the gathered data is not complete and thus incapable of making even a rough distinction between salt tolerant and salt sensitive cultivars.

Also worth mentioning is the beforementioned existence of different salt-resistance traits which could influence the found results. These traits (e.g. storing Na^+ in the vacuole, relocating Na^+ to older leaves (Burns and Hutsby 1986; Wang 2019) could operate independent from the trait which regulates Na^+ uptake through roots and thus interfere with the results.

Nevertheless, the best thing to do would be to repeat the study with twenty-five wheat cultivars and use the proposed protocol, this could provide some clarity as to why this study did not find a correlation and how K^+ efflux can be used.

Conclusion/recommendations

This paper aims to answer the question whether NaCl induced K^+ efflux in roots is an accurate indicator of salt tolerance in plants. This measurement could be of use to identify salt tolerant cultivars quickly and easily and thereby improve the breeding of salt tolerant plants. This would be beneficial in light of the increase in worldwide food demand and the negative effects salinization has on plant growth and yield.

K^+ efflux is thought to indicate salt stress since it relates to the capacity of a plant to retain K^+ and exclude Na^+. This is important because K^+ has many functions e.g. membrane voltage regulation and regulation of metabolic processes through enzymatic reactions. K^+ is compromised in this function by its efflux and also by the influx of Na^+ to toxic levels.

Although there are still some contradicting findings, K^+ efflux seems like an accurate indicator of salt tolerance in general. This was found in studies regarding K^+ efflux in barley, wheat, grapevine rootstock and Chinese irises. To resolve some contradictory findings, it is proposed to use a standardized protocol in further studies. This protocol would consist of (1) a scan along the root axis to determine the mature rootzone which is considered the best place for measurements, (2) a standard measuring time of 40 minutes and (3) a standardized concentration of NaCl which should be preferable between 80-140 mM NaCl with a preference for 80mM NaCl.

Overall, K^+ efflux proves to be a promising indicator of salt tolerance in plants and could therefore help optimize breeding programmes for salt tolerance.

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