The influence of K supply and soil water on cations (Ca^{+2}, Na^{+} and Mg^{+2}) concentrations in different genotypes of barley tissue

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Introduction:

Quantitatively, K^+ is usually a major cation in plant tissues. When K^+ supplies are adequate, K^+ comprises about 75 % of the total cation content of barley (Leigh and Johnston, 1983). When K^+ is in short supply, it is replaced in tissues by other cations such as Ca^{2+}, and Mg^{2+} (and sometimes Na^+) which contribute to the maintenance of the plant's osmotic potential (Flowers & Lauchli, 1983; Leigh & Johnston, 1983b; Jensen and Tophoj, 1985). As mentioned above, K^+ concentration per unit of tissue water is maintained in cereal leaves at about 200 mmol kg^{-1}, but declines when K^+ supply is limited. Of the available evidence, the study by Jensen and Tophaj (1985) is especially informative. They observed that at lowest K level, the total concentration of cations in plant tissue was reduced by about 20 % when compared with the total concentration of cations at the higher K application. K^+ accounted for more than half of the total cations (about 56 %) whilst Ca^{2+} was 25 % of the total cations. However, Na^+ and Mg^{2+} made minor contribution to cation concentration at all K levels. At adequate supplies of K fertilizer, the overall concentration of K^+ and total cations per unit tissue water were relatively constant. However, in barley, Leigh and Johnston (1983 b) found that in crops which had a low K content, the deficit was balanced by increased levels of calcium and sodium, therefore the total cation concentration in leaf cells was similar in crops at both low and high K levels.

Leigh & Wyn Jones (1984) reported that K^+ in cytoplasm has an important role in metabolic process. However, when K^+ concentration declines in tissue water K^+ in cytoplasm is not replaceable by other cations (Na^+, Ca^{2+} and Mg^{2+}), while other inorganic cations could replace vacuolar K^+. Leigh and Johnston (1983a) grew barley in K deficient soils and noted that growth decreased even when K^+ was replaced by calcium and sodium. Their crop contained 50-70 mmol kg^{-1} K^+ which is the highest that may be expected to have direct effects on potassium concentration in cytoplasm (Leigh & Wyn Jones, 1984). Clearly, there may be effects on growth related to the replacement of vacuolar K^+ by either Ca^{2+} or Na^+ but which are poorly understood.

Loué (1978) reported a reciprocal or antagonistic relationship in plants between K^+ and Ca^{2+}, Mg^{2+} or Na^+. For example, an excess of K^+ in nutrient media may result in Mg^{2+} deficiency symptoms (i.e., an antagonistic relationship) in cereals, potatoes and corn (Fecenko, 1982). Garcia *et al*. (1999) reported that plants grown in solution enriched with K had Ca^{2+} concentrations in their tissue less than in plants grown in low K^+ solution.

Application of potassium increases potassium concentrations in plant tissues and has a fairly consistent impact on lowering Ca^{2+} and Mg^{2+} concentration in tissue in most plant species (Marschner, 1995). The interaction between K^+ and Na^+ depends on the quantity of each nutrient present in nutrient media and on the plant species (Marschner, 1995). In general, an increase in one of these cations in soil solution causes a decrease in the net absorption of other cations, but the total concentrations of cations in plant tissue often remain almost stable (Mengel and Kirkby, 1987).

As yet there have been no detailed studies of the responses in terms of the potassium Supply and interactions with water supplies on concentrations of Na^+, Mg^{2+}, and Ca^{2+} in tissue water.

For this reason the experimental was amid to study the effect of different K levels on accumulation of concentrations of Na^+, Mg^{2+}, and Ca^{2+} in tissue water.

Hypotheses

1. There are no differences in term of Na^+, Mg^{2+}, and Ca^{2+} tissue between barley genotypes when they are treated grown under with the same conditions of K fertilizer and water supplies.
2. Ca^{2+}, Mg^{2+} and Na^+ concentrations in tissue water are affected by K application

Materials and methods

The experiment was done in a greenhouse and conducted in pots sized 14.5 cm diameter 15 cm deep and arranged in a fully factorial randomized complete block design with five replicates of each treatment, as described below. Each pot contained one kg of moist soil. The soil was a silty clay loam collected from an arable field

K was applied as potassium sulphate (46 % K_2O) at two levels, K0 (control) and supra optimum level K1 (equivalent to 150 kg ha^{-1}). Other macronutrients (N and P) were applied equally to each pot in all treatments. Phosphorus fertilizer was applied during soil preparation (equivalent to 100 kg P ha^{-1}) as super phosphate (16 % P_2O_5). P (and, in the K1 treatment, K) fertilizers were mixed several times with soil before adding to the pots. N fertilizers were applied in four equal doses (equivalent in total to 150 kg N ha^{-1}) as ammonium sulphate (20 % N): (1) applied during soil preparation; (2) supplied after transplanting seedlings; (3) added one month after planting; (4) applied after two months' growth. The quantities of nitrogen and phosphorus fertilizers (150 and 100 kg ha^{-1} respectively) are supposed to be optimal for barley requirement, and to avoid deficiency of these nutrients.

All plants were fully irrigated for first two weeks to allow successful seedling establishment. Thereafter, two droughtiness regimes were established by varying irrigation frequency. Irrigated plants were grown in soil maintained at a minimum water content of 40 % (as read by using a Theta probe; Delta-T Instruments, Cambridge, UK), which is close to field capacity for this soil. This moisture content was achieved by irrigating every 7-9 days. Droughted plants watered less frequently such that soil moisture content was maintained around 20-25 %, the interval between irrigations

depending on moisture content. Moisture was recorded in all pots every two days using the Theta probe.

Two spring barley (*Hordeum vulgare* L.) genotypes were selected: Westminster, characterized by long and with potentially greater drought tolerance, and Scout, with shorter roots and potentially more drought-susceptible

Seeds were placed on wet filter paper in Petri dishes at room temperature (20-25 °C) to germinate. After one day the seeds which started to germinate were transferred to the pots. Six seeds per pot were planted at 1 cm depth. Seedlings were thinned to three per pot after six days. The experiment contained a total of 200 pots.

The experiment had five harvests, with three weeks between each harvest, the first occurring 35 day after planting. Harvested plants were fractionated into green leaves, stems and spikes and each component weighed.

Each tissue subsample (160 in total) in each harvest was digested in 4.5 ml of digest reagent comprising sulphuric acid (112 ml), lithium sulphate (3.6 g) and hydrogen peroxide (93.3 ml). Samples were placed on a digestion block for 2 hours at 360 °C until colourless solutions were obtained. Each digested sample was transferred to a 50 ml centrifuge tube and made up to volume (50 ml) with distilled deionised water. Na^+, Ca^{2+} and Mg^{2+} concentrations were determined by an Atomic Absorption Spectrophotometer (Perkin Elmer A Analyst 100 AAS). Na^+, Ca^{2+} and Mg^{2+} were measured using wavelengths of 766.5, 589.0, 422.7 and 285.2 nm respectively.

Data were analysed using analysis of variance (general linear models) in MINITAB. To test for significant differences between means, Tukey tests were used.

Results

The effect of low and high K fertiliser, droughtiness on other cations in barley plants

In general, the concentrations of Ca^{2+}, Mg^{2+} and Na^+ in barley tissue water at all harvests increased with the age of plant until the fourth harvest (hence the significant effect of time in GLM). However, they decreased at the fifth harvest (maturity period). Also, drought conditions tended to increase Ca^{2+}, Mg^{2+} and Na^+ concentrations in tissue water compared with in frequently watered plants. Also, concentrations of these cations were inversely related to increasing rate of K application. This could be attributed to decrease of cations content when K applied.

Calcium concentration in whole plant per unit tissue water

Droughtiness, K supply, time and genotype had highly significant influences on Ca^{2+} concentration per unit tissue water. Ca^{2+} concentration was decreased slightly when the rate of K fertilizer was increased. In both genotypes the concentration of Ca^{2+} was lower in plants at 150 kg K ha^{-1}, compared to those given no K fertilizer at all harvests regardless of irrigation treatment. For example, the highest mean value of Ca^{2+} concentration in Scout and Westminster occurred at the fourth harvest (2.06 and 2.65 mg

g^{-1} respectively). These concentrations were measured in droughted plants that did not receive K fertilizer. The concentration of Ca^{2+} per unit tissue ranged from 0.36 mg g^{-1} at the onset of growth to 2.77 mg g^{-1} at the fourth harvest. The concentration of Ca^{2+} declined at the fifth harvest under all experimental conditions.

In addition to K fertilizer, frequency of watering also had direct effects on Ca^{2+} concentration in barley tissue. Ca^{2+} concentration increased when plants were subjected to water stress. Plants in droughted treatments maintained higher Ca^{2+} concentration in their tissue than did fully watered plants. As mentioned above, Ca^{2+} concentration varied significantly with genotype Westminster continued to keep Ca^{2+} concentration higher than the Scout regardless of K supply or water frequency.

Despite the significant influence of droughtiness on Ca^{2+} concentration ($P < 0.001$), significantly increase in Ca^{2+} concentration occurred at the third and fourth harvests in drought treatments compared to fully watered treatments in both genotypes. Increases in the rate of K application from 0 to 150 kg ha^{-1} in fully watered and droughted treatments did not increase Ca^{2+} significantly in both genotypes during the first and second harvest.

- Magnesium concentration per unit tissue water

The concentration of magnesium in barley tissue water was influenced significantly by genotype, droughtiness, K level and time ($P < 0.001$). Genotypes, droughtiness and K level also had significant interactions with time on Mg^{2+} concentration ($P < 0.001$).

Mg^{2+} concentration was unaffected by any treatment in both genotypes at the first and second harvests. The highest Mg^{2+} concentration was recorded in drought treatments at the third harvest in Scout genotype (3.07 mg g^{-1}). Droughtiness was stronger influence than genotype and K level on Mg^{2+} concentration. In both genotypes, drought treatments maintained higher Mg^{2+} concentrations in their tissue more than plants grown in the well watered. In addition, K level caused Mg^{2+} concentration in plant tissue to decrease. The lowest mean values of Mg^{2+} during all harvests occurred in irrigated and fertilised treatments. Mg^{2+} concentration increased throughout the experiment in all treatments irrespective of K supply, water frequency and genotype until the fourth harvest then declined at the fifth harvest. Mg^{2+} concentration in both genotypes at the fifth harvest did not differ significantly with K supply or watering frequency

-Sodium concentration per unit tissue water

Genotype, droughtiness, time and K levels all had significant effects on Na^{+} concentration in barley tissue water ($P < 0.03$). The concentration of sodium responded to K applied. For example, Na^{+} concentration decreased by increasing the rate of K application irrespective of the water treatment or genotype. Also, Na^{+} concentration was increased with barley age until the fourth harvest then declined at the fifth harvest. Also, Na^{+} concentration per unit tissue water was respond to, droughtiness where plants that were grown under drought maintained Na^{+} at higher concentration in their tissue than plants which received water frequently in both genotypes.

Genotypes differed significantly in terms of Na^+ concentration. However, these variations were dependent on irrigation treatment more than on K supply. For example, the highest mean value of Na^+ concentration was recorded in Scout under drought condition during the fourth harvest (3.69 mg g^{-1}), while it was 2.15 mg g^{-1} in Westminster. Statistically, the effect of water stress on Na^+ concentration occurred at the third harvest in Scout genotype, while in the Westminster their effect occurred at the fourth harvest. Additionally, droughtiness had a stronger influence than K application on Na^+ concentration in tissue water.

Discussion

The results of this experiment showed that watering frequency caused significant influence on Ca^{2+} concentration in plant tissue water. Droughted plants had more Ca^{2+} ions in whole plant per unit tissue water than plants which received periodical water. These results are in agreement with Egilla *et al*. (2001) who said that Ca^{2+} in Hibiscus tissue water increased about 50 % in drought treatments more than in well watered plants. Low K^+ concentration can be accompanied by enhanced Ca^{2+} concentration. Similar observations have been reported by Leigh and Johnston (1983a) for spring barley which did not receive K fertilizer: Ca^{2+} concentration was increased in response to lower K^+ concentration because plants need to maintain balance for anion uptake "to act as osmotica."

Droughtiness had also effects on Na^+ concentration. Plants subjected to water stress maintained Na^+ in their tissue at greater concentrations than in fully watered plants. However, this effect is not always seen in experiments.

The use of potassium fertilizer led to reductions in tissue Ca^{2+} concentrations in both genotypes. This could be explained by enhanced amounts of monovalent ions (i.e., K^+) in soil solution (antagonistic relationship between K^+ and Ca^{2+}) (Jonathan and Läuchli, 1985; Rageb, 1979). Dibb and Thompson (1985) found that reductions in Ca^{2+} concentration were attributed to a dilution effect. Similar findings were recorded by Jensen and Tophaj (1985), who observed that the application of potassium fertilizer led to a decrease (20 %) in the concentration of Ca^{2+} in the barley shoot per unit tissue water.

Mg^{2+} concentration in plant tissue decreased when the rate of K fertilizer increased that resulted to increase K^+ concentration. This could be attributed to dilution in Mg^{2+} concentrations in soil solution. For this reason fertilized treatments maintained Mg^{2+} in their tissue more than plants which had received 150 kg ha^{-1} irrespective of droughtiness. Tang (1998) stated that Mg^{2+} concentration in lupin and clover shoot was decreased from 6.03 to 5.33 g kg^{-1} and from 6.81 to 3.50 kg g^{-1} respectively when K fertilizer increased from nil K to 240 mg kg^{-1}. In general, The results reported in this experiment agree with those of other authors who observed that K applications are associated with decreases in Mg^{2+} concentration in cotton leaf tissue water (Pervez *et al*., 2006) and in barley (Leigh and Johnston, 1983a).

The concentration of sodium in barley shoots decreased with increasing rate of K fertilizer from 0 to 150 kg ha^{-1}. This could be attributed to competition between Na^+ and K^+ ions in the soil solution.

At the end of the study, there was antagonism and / or reciprocal interaction between applying K and cations (Ca^{2+}, Mg^{2+} and Na^+) concentration, especially under water stress condition where cation concentration decreased when the rate of K fertilizer increasing. All cations (Ca^{2+}, Mg^{2+} and Na^+) per unit tissue water had increased in concentration throughout most of the experiment with time. All cations which were measured in plant tissue water had highly significant effect on barley genotypes.

Conclusions

Ca^{2+}, Mg^{2+} and Na^+ concentrations in plant tissue water were depended on genotype, droughtiness and K fertilizer. However, the reciprocal or antagonistic influence between K and cations rely on the quantity available for each element in soil and plant species (Marschner, 1971). All cations increased with plant age and declined by the end of the experiment when barley had started to produce reproductive structures by the end of the experiment.

All experiment parameters had significant effect on cations concentration. However, genotypes differed significantly amongst themselves to response on cations concentrations (Ca^{2+}, Mg^{2+} and Na^+) were not due to K supply and / or droughtiness because their interactions with genotype were not significant.

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