Differences in response between nitrate- and ammonium-fed maize to salinity stress and its amelioration by potassium

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The effects of salinity (80mM NaCl) on the growth in hydroponics of ammonium- and nitrate-fed maize and the ameliorative role of potassium on salinity toxicity in these plants was assessed from measurements of growth rates and gas exchange characteristics. Ammonium-fed plants were more sensitive to salinity stress than their nitrate-fed counterparts. The photosynthetic and transpiration rates of ammonium-fed plants were significantly reduced by salinity whereas those of nitrate-fed plants were not significantly affected. Salinity caused 59 and 60% reductions in the shoot and root growth of ammonium-fed plants, respectively, whereas shoot and root growth of nitrate-fed plants was reduced by only 27 and 34%, respectively. In both nitrate- and ammonium-fed plants, raising the potassium concentration from 0.2 to 5mM in the presence of salinity produced a significant increase in maize growth.

Die uitwerking van saliniteit (80mM NaCl) op die groei in waterkultuur van mielies wat met ammonium en nitraat gevoed is en die verbeterende rol van kalium op toksiese saliniteit by hierdie plante is met behulp van metings van groeitempo's en gaswisselingskenmerke bepaal. Plante wat met ammonium gevoed was, was meer gevoelig vir saliniteitstremming as dié wat met nitraat gevoed was. Die fotosintese- en transpirasietempo's van plante wat met ammonium gevoed was, is betekenisvol deur saliniteit verminder, terwyl dié van plante wat met nitraat gevoed was, nie noemenswaardig beïnvloed is nie. Saliniteit het verminderings van onderskeidelik 59 en 60% veroorsaak in die groei van stingels en wortels van plante wat met ammonium gevoed was, terwyl stingel- en wortelgroei van plante wat met nitraat gevoed was, slegs met onderskeidelik 27 en 34% gereduseer is. By plante wat met nitraat gevoed was sowel as by dié wat met ammonium gevoed was, het 'n verhoging van die kaliumkonsentrasie vanaf 0.2 na 5mM in die teenwoordigheid van saliniteit tot 'n betekenisvolle toename in die groei van mielies gelei.

Keywords: Salinity toxicity, nitrate nutrition, ammonium nutrition, potassium nutrition, photosynthesis, maize.

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Introduction

Soil salinity has become an increasingly serious and costly problem for agriculture. Widespread efforts to improve salt tolerance in traditional crops have been encouraged because of the reduction in yields brought about by secondary salinization of freshwater supply by irrigation water, and the growing need in many developing countries for agricultural output from semi-arid and maritime regions fed by aquifers (Blits & Gallagher 1990).

Growth reduction caused by NaCl could be due to either osmotic or ionic effects, or a combination of both (Lewis *et al.* 1989). The ionic effects include interference with nitrogen uptake, dislocation of nitrogen assimilation and protein assembly, interference with transport of essential ions within the plant and lowering of net photosynthetic rates (Greenway & Munns 1980). The osmotic effects are associated with lack of cell wall extension and cellular expansion leading to cessation of growth (Greenway & Munns 1980; Hayward & Spurr 1943). It is, however, uncertain which of these major effects dominates the growth response of salinized plants. Rawson (1986), for example, reported a salinity-induced decrease in photosynthetic rates in wheat and barley exposed to salinity levels of up to 150mM NaCl, while the water use efficiency was only marginally affected, indicating a purely ionic effect of salinity. Plaut *et al.* (1990) also reported a decrease in carbon dioxide assimilation in cowpea (*Vigna angulata*) exposed to salinity levels of up to 173mM NaCl. In contrast, Terry and Waldron (1986) observed no effect on the rate of photosynthesis in sugarbeet exposed to salinity levels of up to 250mM NaCl, and Lewis *et al.* (1989) discerned no effect on the rate of photosynthesis in nitrate-fed maize plants that were exposed to salinities of up to 80mM NaCl.

Various attempts to improve the growth of plants in salinized media have been made by adjusting the chemical composition of the nutritional source of these plants. Helal and Mengel (1979), for example, reported an improvement in growth and nitrogen utilization in young salinized barley plants by increasing the potassium concentration of the saline nutrient medium. Lewis *et al.* (1989) found nitrate-fed maize plants to be more tolerant of salinity than their ammonium-fed counterparts, indicating some protective effect of nitrate against salinity toxicity. In this paper we report on further experimentation designed to investigate chemical methods for the alleviation of salinity stress in plants. This included the growing of salt-stressed maize plants under different nitrogen feeding regimes in order to assess the optimal nitrogen source for growth under these conditions, and the use of different concentrations of potassium in the feeding medium of these plants to observe their effect on salinity tolerance. The effect of these treatments on the growth of salinized maize was quantified by the measurement of growth rate, root:shoot ratio, photosynthetic rate and transpiration rate in the experimental plants.

Materials and Methods

Growth conditions

Maize plants (*Zea mays* L. var PNR 394) were germinated and grown in a controlled environment chamber (Furcold, Cape Town) under conditions suitable for plants possessing the C_4 pathway. Day irradiance in the chamber was maintained at between 1300 and 1500 μmol m^{-2} s^{-2} over a 14-h photoperiod. Light was supplied by a mixture of 28 × 400-W Wotan metal halide, 14 × 400-W Wotan sodium vapour and 14 × 150-W Osram incandescent lamps. Day temperature was maintained at 35°C and night temperature at 25°C. Relative humidity was maintained at 50% during the light period and 65% during the dark period.

Hydroponic growth

Seedlings of maize were germinated in aerated water and grown in vermiculite. After 5 days, seedlings were transplanted into 20-l troughs containing a well-aerated Long Ashton nutrient medium modified to contain 4mM nitrate or ammonium with 1.25mM $FeSO_4$ as an iron source (Hewitt 1966). In all the experiments, eight plants were grown in each of the 20-l plastic containers with holes drilled in their lids to accommodate the plants. Experimental plants were allowed to grow a day in hydroponics before they were exposed to the salinity treatment (80mM NaCl, final concentration); the controls continued growth at 0mM NaCl. On the first day, NaCl was added at 40mM to prevent osmotic shock and then increased to 80mM the following day. A concentration of 80mM was chosen for the present study because this level was previously found by us to be satisfactory for the production of stress symptoms without killing the plant (results not shown).

Nutrient solutions were renewed a week after the commencement of feeding and every fourth day thereafter. Because of its tendency to oxidize, additional $FeSO_4$ (0.9mM) was added to the growth media every third day to ensure that there was enough supply for the plants. The pH of each nutrient medium was monitored every second day and adjusted to 5.5 with 5mM NAOH or H_2SO_4. After growth in hydroponics for 16 days, plants were harvested, divided into root and shoot sections and their fresh weights immediately determined. Each root and shoot was then oven-dried at 80°C for 48 h and the dry weight of each was determined.

Gas exchange analysis

Net photosynthetic rate (*A*) and transpirational water loss (*E*) were determined using Parkinson's leaf chamber linked to an ADC LCA2 infra-red gas analyser (Analytical Development Corporation, Hoddesdon, England). Determinations were made on 10 (experiment 1) or 5 (experiments 2 and 3) randomly chosen plants from each treatment, and in each plant the youngest fully-matured leaf was considered. Gas exchange determinations were made half-way through the light period under the optimum growth conditions described above.

Experimental design

Experiment 1. Differences between nitrate- and ammonium-fed maize plants in their response to salinity

This experiment was conducted to establish whether nitrate-fed and ammonium-fed maize plants respond differently to salinity. Sixteen troughs were used in this experiment. Of these 16 troughs, 8 contained nitrate-fed plants and the other 8 contained ammonium-fed plants. For both nitrate- and ammonium-fed plants, 4 troughs served as controls and the other 4 received 80mM NaCl. Plants were grown in hydroponics for 14 days.

Experiments 2 and 3. Effect of potassium on the responses of nitrate-fed (experiment 2) and ammonium-fed plants (experiment 3) to salinity

These experiments were conducted to investigate the effects of external K^+ concentrations on the growth of nitrate-fed and ammonium-fed plants under saline (80mM NaCl) conditions. In both these experiments, 16 troughs were used. Half of these 16 troughs served as controls and the remaining half received 80mM NaCl. Both the experimental and the control troughs were further divided into different potassium treatments (0.2, 1.0, 2.5 and 5.0mM K^+) with two troughs per single potassium treatment. Plants were grown in hydroponics for 16 days.

Results and Discussion

Differences in the response of nitrate- and ammonium-fed maize plants to salinity (experiment 1)

The respective growth responses of nitrate- and ammonium-fed maize plants to salinity stress can be observed in Figure 1. The dry-weight data recorded in Figure 1 show that under non-saline conditions, nitrate-fed maize plants did not differ significantly in size from ammonium-fed plants ($p > 0.05$, t-test). These results are contrary to those of other researchers (Murphy 1984; Lewis *et al.* 1989) who found that under non-saline conditions, ammonium-fed maize plants produced greater dry mass than nitrate-fed plants. The dry weight shoot:root ratio of non-salt-stressed ammonium-fed plants was significantly larger than that of non-salt-stressed nitrate-fed plants ($p < 0.05$, t-test), indicating that ammonium nutrition exerted a deleterious effect on the growth of maize roots (Figure 1). This effect has also been observed in other plant species such as barley (Lewis & Chadwick 1983) and wheat (Lewis *et al.* 1987) and has been attributed to decreasing root-medium pH due to H^+ excretion by roots in exchange for NH_4^+ absorption. In this study, the root-inhibiting effect of ammonium nutrition was still evident in spite of strict pH control preventing the development of an acidic root environment.

Under saline conditions the dry weights of shoots and roots of nitrate-fed maize plants were significantly larger than those of ammonium-fed plants, a feature contrasting with the results reported above for maize plants grown under non-saline conditions (Figure 1). While all the nitrate-fed plants still showed healthy growth at the 80mM salinity level, many of the ammonium-fed plants showed leaf necrophily (wilting and dying-back of tips), indicating that ammonium-fed maize plants are more sensitive to salinity stress than their nitrate-fed counterparts. Even though salinized nitrate-fed plants grew significantly better than ammonium-fed plants, the growth of plants under both treatments was nevertheless significantly reduced compared to growth under non-saline conditions ($p < 0.05$, t-test) (Figure 1). Salinity caused 27 and 59% growth reduction in the shoot of nitrate- and ammonium-fed plants, respectively, while root growth of nitrate- and ammonium-fed plants was reduced by 34 and 60%, respectively. Other workers have found the salinity effects on the growth of other plant species such as wheat (Lewis *et al.* 1989) and barley (Delane *et al.* 1982), to be more pronounced in shoots than roots. Shoot:root ratio results in our study (Figure 1) show, however, that in both nitrate- and ammonium-fed maize plants the salinity effect on the growth of maize was nearly similar in both shoots and roots.

The reasons for the improved growth of nitrate-fed plants compared with ammonium-fed plants under saline conditions is not known, but could possibly be ascribed to one or a combination of the following factors:

(i) In maize, nitrate assimilation takes place primarily in the shoot and ammonium assimilation in the root (Murphy 1984). As the root is in close contact with the saline nutrient medium, it is possible that nitrogen assimilation in ammonium-fed plants is impaired by ionic effects which would be absent in the leaf.

(ii) In nitrate-fed plants a malate-nitrate shuttle is operative between shoot and root (Lips *et al.* 1970). The absence of this shuttle in ammonium-fed plants could bring about ion translocatory problems which are exacerbated by the uptake of NaCl.

(iii) The assimilation of the bulk of nutrient nitrogen in the roots of ammonium-fed plants necessitates the diversion of large quantities of carbon to the root to provide the carbon skeletons required for nitrogen assimilation. It is possible that this carbon metabolism is inhibited by the presence of high concentrations of sodium and chloride ions in the root.

From Figure 2 it can be seen that both photosynthetic and transpiration rates of nitrate-fed maize plants showed no significant response to salinity ($p > 0.05$, t-test), whereas those of ammonium-fed plants were significantly reduced by salinity ($p < 0.05$, t-test). This indicates that at least one

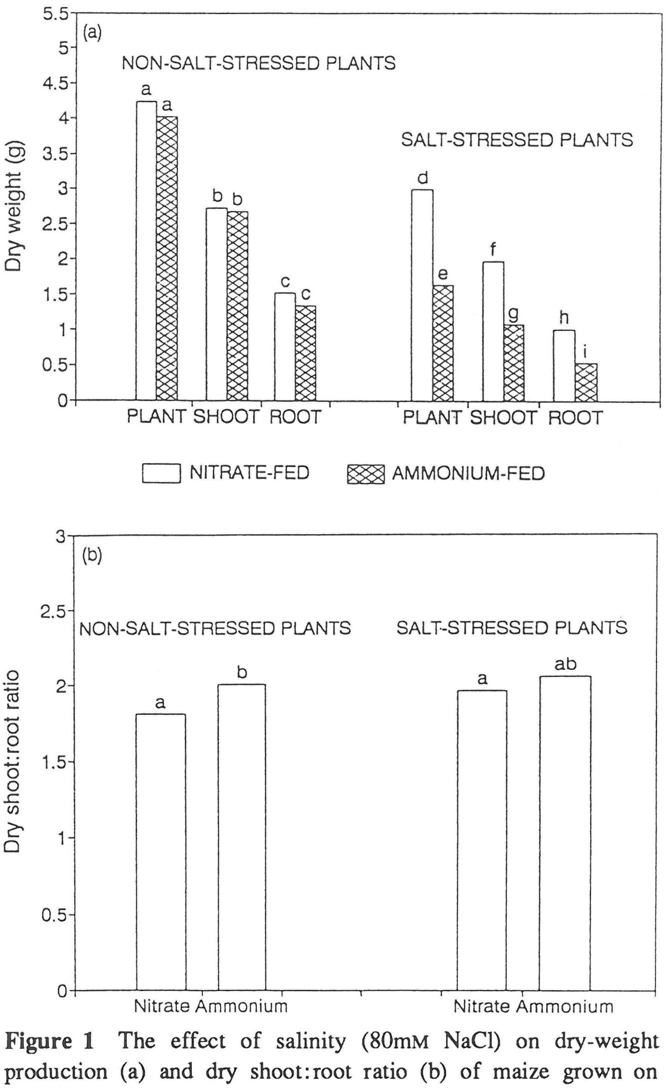

Figure 1 The effect of salinity (80mM NaCl) on dry-weight production (a) and dry shoot:root ratio (b) of maize grown on 4mM ammonium or 4mM nitrate. Means are of 32 replicates. Similar letters above bars indicate non-significant differences between treatments ($p > 0.05$, Student's t-test), different letters indicate significant differences ($p < 0.05$).

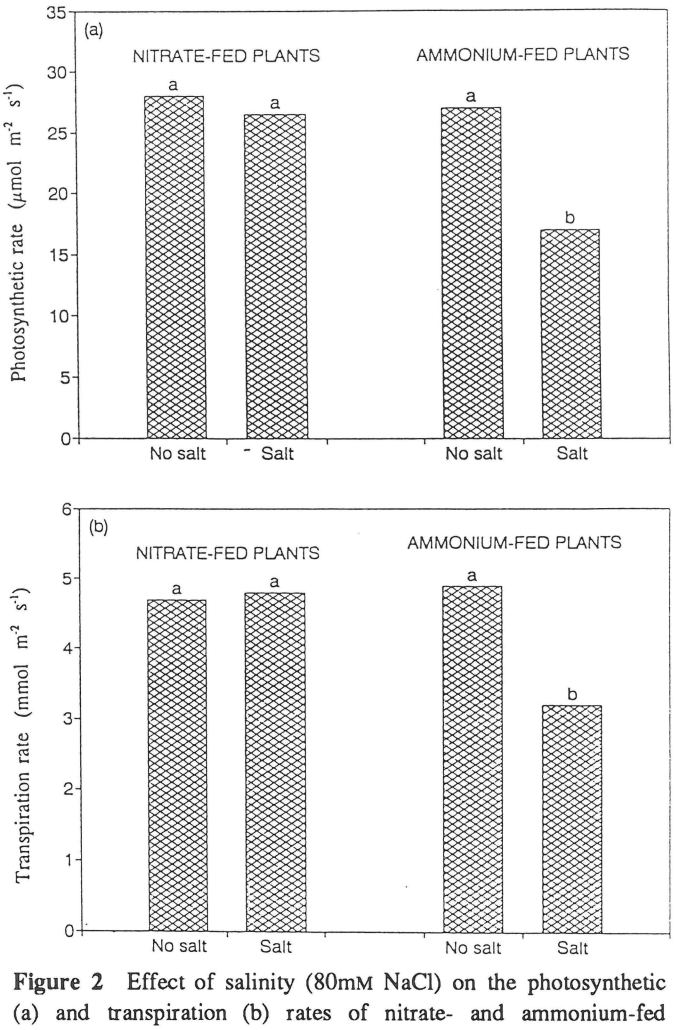

Figure 2 Effect of salinity (80mM NaCl) on the photosynthetic (a) and transpiration (b) rates of nitrate- and ammonium-fed maize. Means are of 10 replicates. Similar letters above bars indicate non-significant differences between treatments ($p > 0.05$, Student's t-test), different letters indicate significant differences ($p < 0.05$).

effect of salinity in ammonium-fed plants was its influence on stomatal conductance, and that nitrate feeding was able to ameliorate this effect.

Effect of potassium on the responses of nitrate-fed and ammonium-fed plants to salinity (experiments 2 and 3)

Experimental results of this study showed an ameliorative effect of potassium on the growth of salt-stressed maize plants fed either nitrate or ammonium (Figure 3). These results are in agreement with those of Helal and Mengel (1979) who found that additions of potassium (5 and 10mM) to a saline (80mM NaCl) nutrient medium improved the growth of barley plants. However, it should be noted that in this study the growth-enhancing effect of the higher potassium concentration was also apparent in the ammonium-fed control plants; increasing the potassium concentration from 2.5 to 5.0mM resulted in a significant increase in the growth of ammonium-fed plants but not in that of nitrate-fed plants, indicating a higher optimal concentration of potassium for ammonium-fed maize than used in conventional feeding solutions. When studying the effects of potassium concentration (0.1 to 5.0mM) on various physiological characteristics of ammonium- and nitrate-fed wheat, Lips *et al.* (1990) found biomass production of ammonium-fed plants to be more dependent on the concentration of K^+ in the nutrient medium than nitrate-fed plants. The proposed K^+ shuttle in plants, which operates in nitrate-fed plants and not in ammonium-fed plants (Lips *et al.* 1970), could explain the larger requirement of potassium for growth in ammonium-fed plants.

Because of the significant depression in photosynthetic and transpiration rates noted in ammonium-fed (but not nitrate-fed) maize (Figure 2), the photosynthetic and transpiration rates of salt-stressed ammonium-fed plants were determined at different concentrations of potassium to investigate possible salinity-ameliorating effects of this ion on these two processes. These results are presented in Figure 4. From them it can be concluded that varying the potassium supply to these plants did not have any significant effect on the photosynthetic or transpiration rates of ammonium-fed maize ($p > 0.05$, one-way ANOVA).

Conclusions

The most significant findings of this research study are as follows:

(i) In hydroponically grown maize, ammonium-fed plants showed a significantly greater sensitivity to salinity than

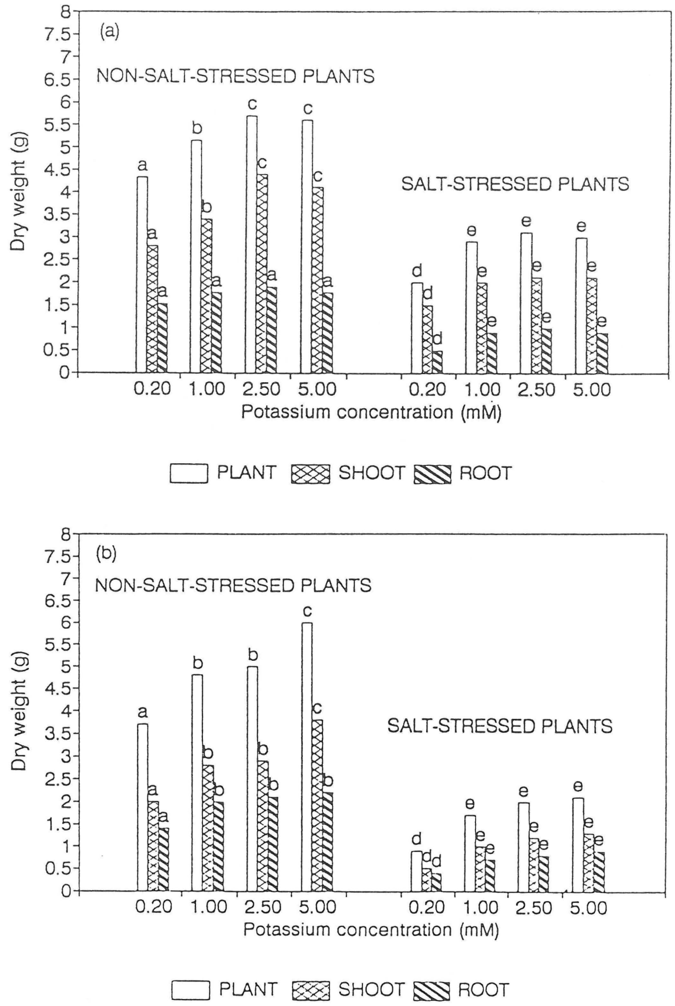

Figure 3 Effect of potassium on the growth of salinity-stressed (80mM NaCl) nitrate-fed (a) and ammonium-fed (b) maize. Means are of 16 replicates for each treatment. Similar letters above bars indicate non-significant differences among treatments ($p > 0.05$, one-way ANOVA), different letters indicate significant differences ($p < 0.05$).

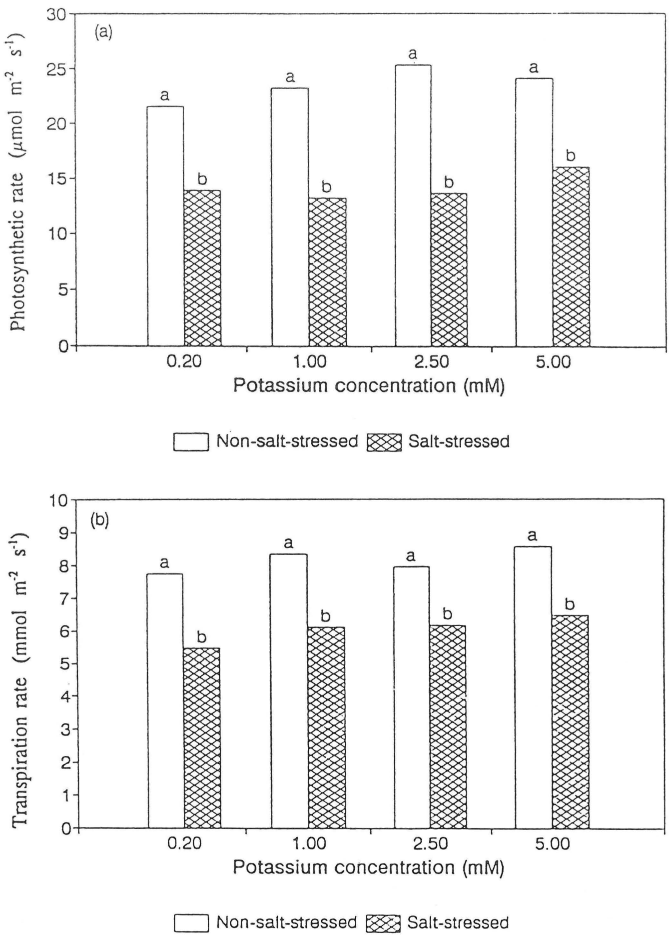

Figure 4 The effect of potassium on the photosynthetic (a) and transpiration (b) rates of salt-stressed (80mM NaCl) maize grown on ammonium. Means are of 5 replicates for each treatment. Similar letters above bars indicate non-significant differences among treatments ($p > 0.05$, one-way ANOVA), different letters indicate significant differences ($p < 0.05$).

did nitrate-fed plants. This effect is very likely to occur under field conditions.

(ii) The photosynthetic and transpiration rates of nitrate-fed maize plants were not significantly affected by salinity, whereas those of ammonium-fed plants were significantly reduced. The reasons for this effect are not immediately apparent.

(iii) Potassium plays a role in enhancing the growth of nitrate- and especially ammonium-fed maize plants under saline conditions.

These results suggest that the productivity of maize plants growing under saline conditions would be enhanced by supplying nitrate nitrogen rather than ammonium nitrogen, and that growth under both nitrogen regimes (and especially the latter) would benefit from the provision of high levels of potassium.

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