

# Ethylene and ethane production in response to salinity stress

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**Abstract.** Ethylene and ethane production in mung bean hypocotyl sections were evaluated as possible indicators of stress due to contact with four salts that are common in natural sites. Ethylene production decreased with increasing concentrations of applied NaCl and KCl. When  $\text{CaCl}_2$  was applied, the ethylene evolution was greater. However, when  $\text{MgCl}_2$  was applied, ethylene evolution remained high then decreased and at higher salt concentrations again showed an increase. NaCl (up to  $0.1 \text{ kmol m}^{-3}$ ) and KCl (up to  $0.5 \text{ kmol m}^{-3}$ ) caused a concentration-dependent increase in ethane production. The ethane production with  $\text{CaCl}_2$  was the lowest among the salts tested and only a minute increase was noticed with the increase of concentration from  $0.01$  to  $1 \text{ kmol m}^{-3}$ . Ethane production showed a distinct maximum at  $0.2 \text{ kmol m}^{-3}$   $\text{MgCl}_2$ . The introduction of  $0.01 \text{ kmol m}^{-3}$   $\text{CaCl}_2$ , as well as anaerobic conditions obtained by purging vials with  $\text{N}_2$ , eliminated that high ethane production. Respiratory activity of the mung bean hypocotyl sections in  $\text{MgCl}_2$  concentrations from  $0$  to  $0.5 \text{ kmol m}^{-3}$  was correlated with ethane but not with ethylene production. The ethane/ethylene ratio showed three patterns for the four salts tested.

**Key-words:**  $\text{CaCl}_2$ ; ethane; ethylene; KCl;  $\text{MgCl}_2$ ; NaCl; salinity stress; *Vigna radiata*.

## Introduction

Stress caused by temperature, water deficit, mechanical stimuli, exposure to chemicals, or infection by pathogens generally stimulates production of ethylene in plant tissues (Abeles, 1973; Yang & Hoffman, 1984). As the severity of stress increases, there is a decrease in the production of ethylene (Apelbaum & Yang, 1981; Field, 1981; Yu, Adams & Yang, 1980).

Ethane evolution has been associated with disruption of cell membranes caused by stress due to freezing temperatures (Elstner & Konze, 1976), chemicals (Bressan *et al.*, 1979; Garcia & Einset, 1983; Peiser & Yang, 1979) or mechanical damage (Curtis, 1969; Lieberman & Mapson, 1962). This has

been observed consistently enough to propose the emission of ethane as an index of sensitivity of some plant tissues to certain air pollutants (Bressan *et al.*, 1979; Kimmerer & Kozlowski, 1982), NaCl and a herbicide (Garcia & Einset, 1983; Simon, Decoteau & Craker, 1983).

It has been proposed that ethylene and ethane production be used as an indicator of moderate and severe stress, respectively, in tissue culture (Garcia & Einset, 1983), leaves (Kimmerer & Kozlowski, 1982) and in whole plants (Peiser & Yang, 1979; Simon *et al.*, 1983; Tingey, 1980).

The cations that most frequently contribute to salinity effects in natural sites are  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . In the present study they were applied in chloride form to mung bean hypocotyl sections in an attempt to use ethylene and ethane production as indicators of the intensity of salt stress.

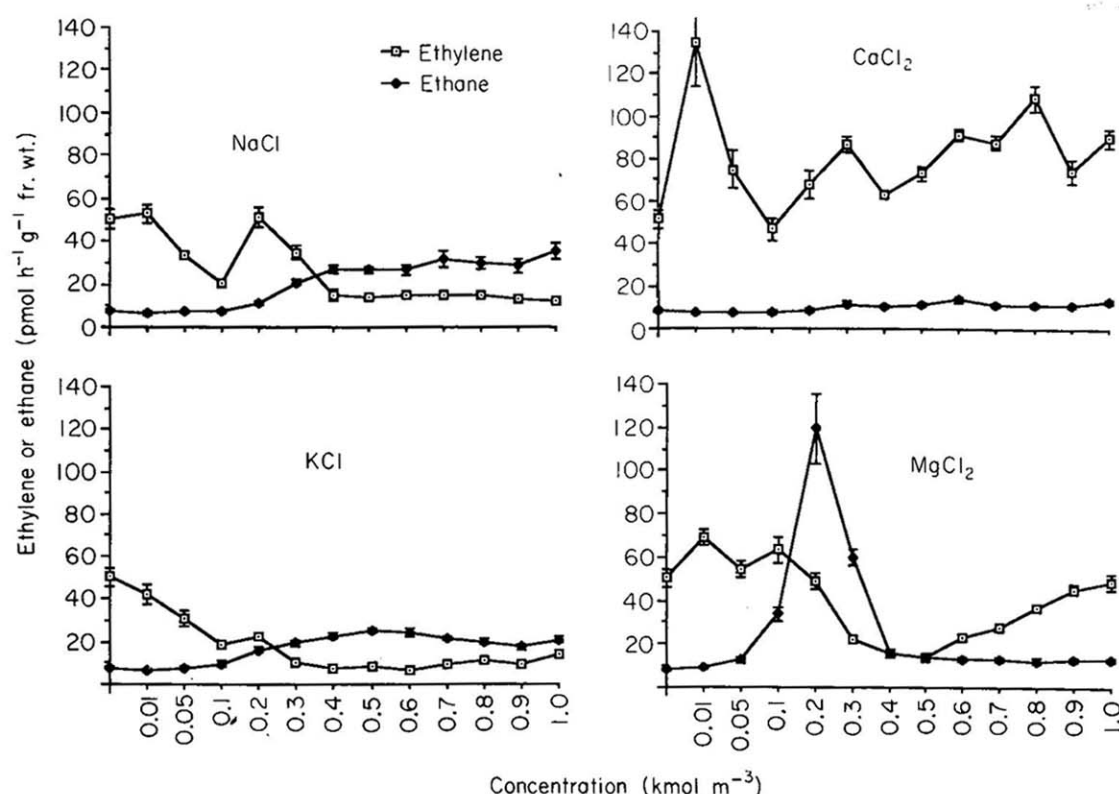
## Materials and methods

The system designed by Sakai & Imaseki (1971) to study ethylene metabolism was used with modification (no buffer was used). Mung bean (*Vigna radiata* (L.) Wilczek) seeds were placed into moist perlite and grown in the dark at  $30^\circ\text{C}$  for 3 days. When hypocotyl length was approximately 6–7 cm, 1 cm long segments were cut. Six randomly chosen segments (actual weight determined for each sample) were placed in each of a series of glass vials ( $7 \text{ cm}^3$ ) containing  $0.2 \text{ cm}^3$  of different salt solutions ( $0$ – $1 \text{ kmol m}^{-3}$ ), sealed with a rubber serum stopper and shaken gently (80 r.p.m.) in darkness at  $30^\circ\text{C}$ . Depending on the experiment, ethylene and ethane production were analysed several times between 4 h and 24 h following beginning of the treatment. A gas chromatograph (Packard 824) equipped with an alumina column (2 m) was operated isothermally at  $60^\circ\text{C}$  using a flame ionization detector. The concentration of ethylene and ethane were determined by electronic integration of GC peaks. Each experiment was repeated at least twice with at least eight replications for each treatment.

Calcium chloride ( $0.01 \text{ kmol m}^{-3}$ ) was added to a similar series of vials containing a concentration range of  $\text{MgCl}_2$  to determine whether  $\text{CaCl}_2$  would overcome the stimulation effect of  $\text{MgCl}_2$  on the ethane production. To determine the pattern of ethane and  $\text{CO}_2$  production in rapidly killed tissue,

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**Figure 1.** Effect of NaCl, KCl, CaCl<sub>2</sub> and MgCl<sub>2</sub> on ethylene and ethane production by mung bean hypocotyl sections after 24 h incubation in darkness at 30°C. Each point represents the average of eight replicates in two experiments. The bars represent standard errors.

10%, w/v, trichloroacetic acid (TCA) was applied directly to the hypocotyls in the vials at the start of the experiment. In all experiments, blank runs were used to correct the data for actual ethylene, ethane and CO<sub>2</sub> production.

## Results

### Effect of salts on ethylene production

The ethylene production of mung bean hypocotyls (Fig. 1) decreased with increasing concentrations of NaCl and KCl. While MgCl<sub>2</sub> at concentrations up to 0.2 kmol m<sup>-3</sup> caused an ethylene increase, ethylene production dropped below the control level in the range of 0.3–0.5 kmol m<sup>-3</sup> and reached the control level at 1.0 kmol m<sup>-3</sup>. The ethylene production with CaCl<sub>2</sub> was stimulated over almost the entire concentration range with a significant maximum at 0.01 kmol m<sup>-3</sup>. There was no decrease in ethylene production even at 1 kmol m<sup>-3</sup> CaCl<sub>2</sub>.

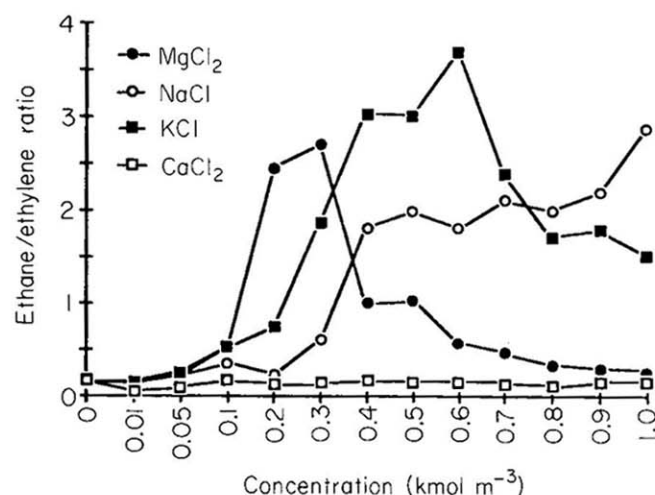
### Effect of salts on ethane production

Ethane production (Fig. 1) increased with increasing concentrations of NaCl. The KCl treatment increased ethane production up to 0.5 kmol m<sup>-3</sup> with no further increase at higher concentrations. The ethane production due to CaCl<sub>2</sub> treatment was the lowest among the salts tested and only a slight increase was noticed as the concentration increased from 0.01 to 1 kmol m<sup>-3</sup>. In contrast, with MgCl<sub>2</sub>, a conspicuous 12-fold increase of ethane was

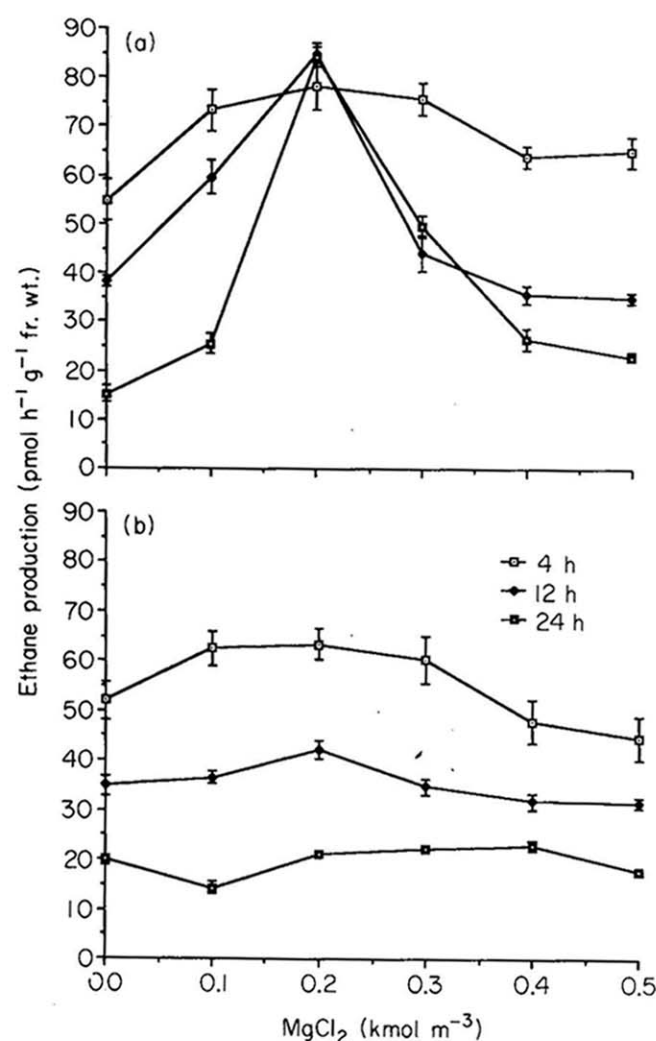
observed at 0.2 kmol m<sup>-3</sup>. Duncan multiple range test indicated that the ascending (0.1 kmol m<sup>-3</sup>) and descending (0.3 kmol m<sup>-3</sup>) values of the maximum production of ethane were significantly different at the 0.01% level when compared to the base line.

### Ethane/ethylene ratio

The ethane/ethylene ratio (Fig. 2) showed three patterns. (1) It was low and stable as the concentration of CaCl<sub>2</sub> increased. (2) It increased as the concentration of NaCl increased. (3) It produced an irregular bell-shaped curve for MgCl<sub>2</sub> and KCl.



**Figure 2.** Changes in ethane/ethylene ratios as affected by varied NaCl, KCl, CaCl<sub>2</sub> and MgCl<sub>2</sub> concentration applied to mung bean hypocotyl sections. Each point represents the mean of two experiments presented in Fig. 1.



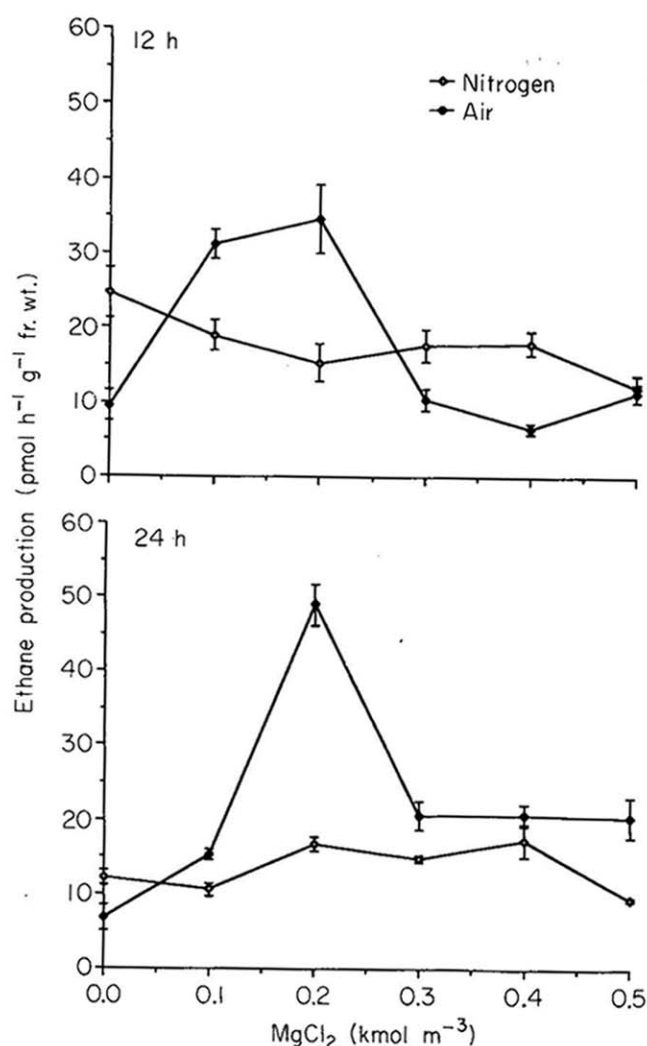
**Figure 3.** Interaction of MgCl<sub>2</sub> and CaCl<sub>2</sub> in affecting ethane production (cumulative data) in mung bean hypocotyl sections after 4, 12 and 24 h incubation in the darkness at 30 °C. (a) MgCl<sub>2</sub>; (b) MgCl<sub>2</sub> + CaCl<sub>2</sub> (0.01 kmol m<sup>-3</sup>). Each point represents the average of 32 replicates in four experiments. The bars represent standard errors.

#### *MgCl<sub>2</sub>-induced ethane production in relation to time, CaCl<sub>2</sub>, and aeration*

Ethane production of tissue exposed to MgCl<sub>2</sub> between 0 h and 4 h (Fig. 3a) was high and showed little change with increased MgCl<sub>2</sub> concentration. Between 4 h and 12 h and 12 h and 24 h, ethane evolution at 0.2 kmol m<sup>-3</sup> continued at a very high rate when compared to other concentrations (Fig. 3a). The rate of ethane production at 0.2 kmol m<sup>-3</sup> MgCl<sub>2</sub> concentration, after 24 h, remained as high as the 4 h period. When CaCl<sub>2</sub> (0.01 kmol m<sup>-3</sup>) was added to vial containing MgCl<sub>2</sub> solutions, the stimulation of ethane production was removed (Fig. 3b). Purging vials with nitrogen also resulted in removing of the increase in ethane production that consistently occurred at 0.2 kmol m<sup>-3</sup> MgCl<sub>2</sub> (Fig. 4) at both the 12 h and 24 h period.

#### *CO<sub>2</sub> production from treated tissues*

The CO<sub>2</sub> concentration in the vials was determined to give an indication of the metabolic state of the

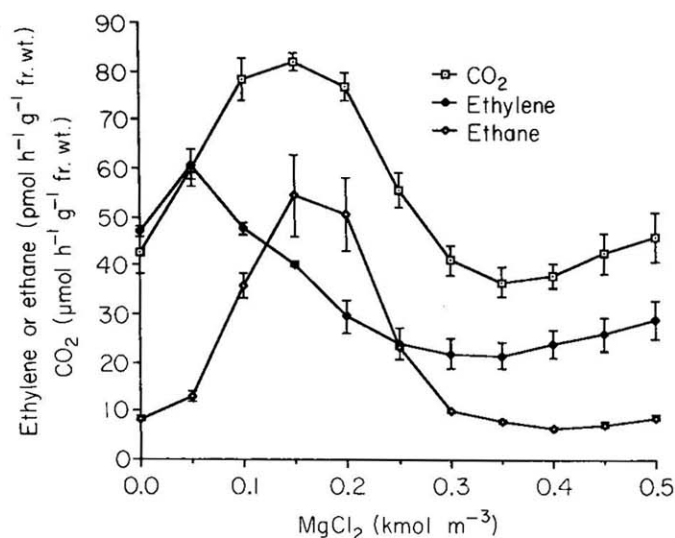


**Figure 4.** Ethane production (cumulative data) by mung bean hypocotyl sections during 12 and 24 h incubation in darkness, at 30 °C, in vials with air and with nitrogen. Each point represents the average of 24 replicates in three experiments. The bars represent standard errors.

tissue. The results shown in Fig. 5 illustrate an increase in CO<sub>2</sub> accumulation and ethane production in the concentration range of 0.1 and 0.2 kmol m<sup>-3</sup> MgCl<sub>2</sub>. On the other hand, ethylene production does not follow the pattern of CO<sub>2</sub> and ethane production, since the highest production occurred early at 0.05 kmol m<sup>-3</sup> MgCl<sub>2</sub>. Treatment with 10% TCA inhibited both ethane (Fig. 6) and CO<sub>2</sub> (Fig. 7) production. At 0.2 kmol m<sup>-3</sup> MgCl<sub>2</sub> the ethane and CO<sub>2</sub> evolution were both high.

#### **Discussion**

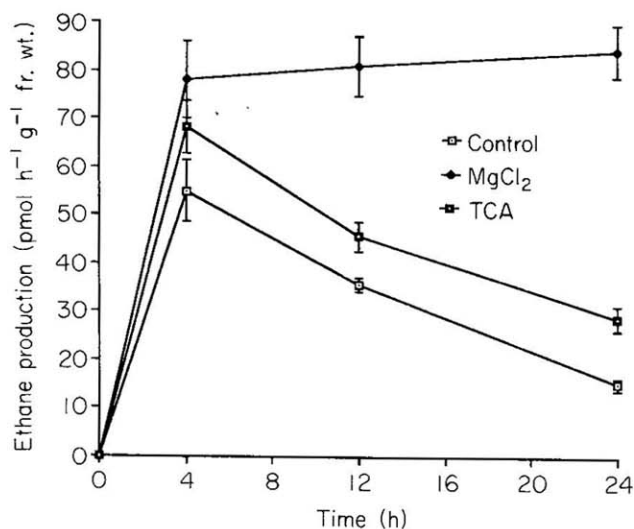
Results obtained with NaCl and KCl treatments are consistent with the concept of ethylene production decreasing and ethane increasing with increased severity of stress (Eltner & Konze, 1976; Rasmussen, Furr & Cooper, 1969). Low concentrations of CaCl<sub>2</sub> (0.01 and 0.05 kmol m<sup>-3</sup>) and of MgCl<sub>2</sub> (0.01–0.1 kmol m<sup>-3</sup>) stimulated ethylene production. These results are in agreement with the report of Lieberman & Wang (1982) that Ca<sup>2+</sup> and



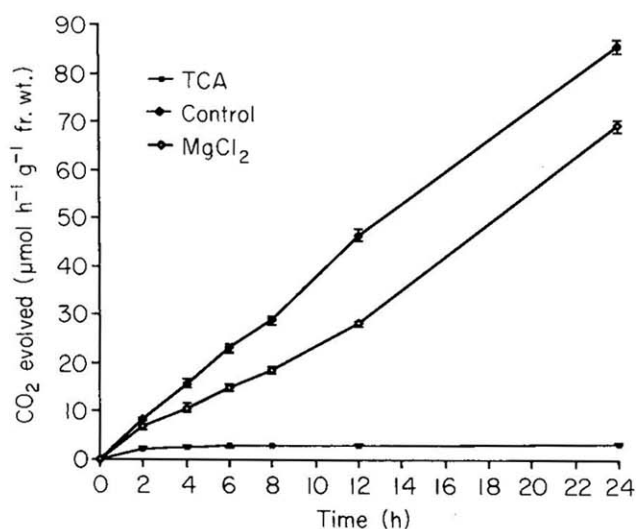
**Figure 5.** Relationship between production of ethylene, ethane and CO<sub>2</sub> from hypocotyls treated with 0.2 kmol m<sup>-3</sup> MgCl<sub>2</sub> after 24 h incubation in darkness, at 30°C. Each point represents the average of 24 replicates in three experiments. The bars represent standard errors.

Mg<sup>2+</sup> maintained the integrity of the ethylene-forming system in apple fruit slices. In mung bean hypocotyl sections, Ca<sup>2+</sup> applied in a very low concentration (μmol m<sup>-3</sup>) also sustained ethylene production (Lau & Yang, 1975). Our results support these findings.

Ethane may be produced from lipid oxidation or from membrane damage (Kimmerer & Kozlowski, 1982; Riely, Cohen & Lieberman, 1974). Among eight salts tested by Leopold & Willing (1984), NaCl was one of the most toxic in inducing lesions in cell membranes. Increased production of ethane with increasing NaCl concentrations applied here is in agreement with the results where NaCl was applied to the tobacco callus (Garcia & Einset, 1983). While



**Figure 6.** Production of ethane by mung bean hypocotyl sections treated with MgCl<sub>2</sub> (0.2 kmol m<sup>-3</sup>) trichloroacetic acid (TCA 10%) and untreated control. Each point represents the average of 24 replicates in three experiments. The bars represent standard errors.



**Figure 7.** Evolution of CO<sub>2</sub> from mung bean hypocotyl sections treated with MgCl<sub>2</sub> (0.2 kmol m<sup>-3</sup>) trichloroacetic acid (TCA 10%) and untreated control. The CO<sub>2</sub> concentration was determined by infra-red CO<sub>2</sub> analyses. Each point represents the average of 24 replicates in three experiments. The bars represent standard errors. Note that the tissue poisoned with 10% TCA produced little CO<sub>2</sub> whereas nearly normal CO<sub>2</sub> production occurs in the presence of MgCl<sub>2</sub>.

MgCl<sub>2</sub> was not as toxic as NaCl (Leopold & Willing, 1984), nevertheless in our study it promoted high production of ethane with a maximum at 0.2 kmol m<sup>-3</sup> as shown in Fig. 1. MgCl<sub>2</sub> concentrations approaching 1 kmol m<sup>-3</sup>, used in our studies are extremely high and would result in severely stressed or damaged tissue (Levitt, 1980). Since other salts such as NaCl and KCl continue to stimulate ethane production even up to 1 kmol m<sup>-3</sup> concentrations, why should concentrations of MgCl<sub>2</sub> greater than 0.2 kmol m<sup>-3</sup>, which have been shown to be less toxic to membranes, bring about a reduction in ethane production? Perhaps the site of ethane production is more sensitive to MgCl<sub>2</sub> than NaCl.

The observation that over the complete concentration range of CaCl<sub>2</sub> the ethane/ethylene ratio remains stable would suggest that CaCl<sub>2</sub> is protecting the appropriate membranes and that little membrane breakdown is occurring. Our results with the mung bean hypocotyl assay would support the concept of CaCl<sub>2</sub> acting as a protective agent.

The most interesting part of our result is the unusual increase in ethane production at 0.2 kmol m<sup>-3</sup> MgCl<sub>2</sub>. This maximum is not due to the instant killing of the tissue because we noted that when tissue was treated with TCA (10%), the ethane and CO<sub>2</sub> production was substantially reduced (Figs 6, 7). In contrast, even at high concentration of MgCl<sub>2</sub> we obtained considerable CO<sub>2</sub> evolution indicating the tissues were living (Fig. 7). However, when we purged the atmosphere of the vial with nitrogen, ethane production due to MgCl<sub>2</sub> effect was completely eliminated (Fig. 4).

Konze & Elstner (1978) purged potato tuber mitochondria with nitrogen and thus reduced ethane

production by approximately 70%. Curtis's (1969) observation that oxygen is required for ethane production is supported by our data at least for  $\text{Mg}^{2+}$  stimulated ethane production. The addition of  $\text{CaCl}_2$  ( $0.01 \text{ kmol m}^{-3}$ ) in concentrations  $20\times$  lower than most effective concentration of  $\text{MgCl}_2$  ( $0.2 \text{ kmol m}^{-3}$ ), also eliminated the ethane production. This may indicate (a) protective action of  $\text{Ca}^{2+}$  for membrane damages or (b) competition for the reaction sites between  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ .

Our results relate primarily to the cations since the chloride anion was present in each of the salts applied. The patterns of production of ethane and ethylene differed with the application of various salts and in many cases were not related to the concentration range applied. These patterns were modified when two salts were mixed. Therefore, we suggest that ethane or ethylene evolution can be used as indicators of saline stress only in situations where plants are exposed to one particular compound for which a satisfactory relationship has been determined over the concentration range in question.

### Acknowledgment

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