



## Enhancement of salinity tolerance in wheat through soil applied calcium carbide

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### Abstract

Calcium carbide ( $\text{CaC}_2$ ) has been reported to increase growth and yield of crops under normal soil conditions. This study assessed its capacity to enhance salinity tolerance in wheat (*Triticum aestivum* L.; cv- 1076) under saline conditions. Three levels of salinity: 0, 7 and 12  $\text{dS m}^{-1}$  were created using NaCl. Nitrogen, phosphorus and potassium were applied as ammonium sulphate and  $\text{KH}_2\text{PO}_4$  at 50 and 25  $\text{mg kg}^{-1}$  soil, respectively. The encapsulated calcium carbide (ECC) at 45  $\text{mg kg}^{-1}$  soil produced 1291.8  $\mu\text{mols}$  of acetylene ( $\text{C}_2\text{H}_2$ ) and 257.5  $\mu\text{mols}$  of its product ethylene ( $\text{C}_2\text{H}_4$ ) over a period of 80 days. The results of the pot study indicated that ECC increased the weight of spike, weight of grains per spike, length of spike, total water concentration, root/shoot ratio and relative leaf water content up to 17, 23, 22, 35, 33 and 3%, respectively, over the control. Contrary to this, salinity (at 12  $\text{dS m}^{-1}$ ) decreased all these parameters up to 68, 60, 26, 30, 28 and 8%, respectively, compared to the control. These results indicate that ECC enhances salinity tolerance in wheat by improving uptake of nutrients through enhanced root growth, increased hydraulic conductivity and hormonal action of ethylene released by ECC. Total water concentration was positively correlated (0.73) with grains spike<sup>-1</sup> at  $P \leq 0.05$ .

**Key words:** Ethylene, calcium carbide, acetylene, salinity

### Introduction

Soil salinity is one of the main problems for world agriculture (Ahloowalia *et al.*, 2004) since salts in the root zone affect plants at all growth stages. Several growth inhibiting effects of salinity, e.g. decreased germination rate (Bernardo *et al.*, 2000a), decreased leaf cell expansion and leaf growth (Cramer *et al.*, 2001), reduced leaf area, dry matter accumulation, diminished rates of net  $\text{CO}_2$  assimilation and relative growth have been described (Bernardo *et al.*, 2000b). The plant responses to salinity are complex and depend on duration of salinity, type of salt, development stage of plant at exposure, time of the day, and many other factors (Cramer *et al.*, 2001).

In recent years,  $\text{CaC}_2$  has emerged as a good source of  $\text{C}_2\text{H}_2$  and some studies have reported significant increases in growth and yield of wheat, cotton, maize, okra and rice (Ahmad *et al.*, 2004; Yaseen *et al.*, 2006; Kashif *et al.*, 2008).  $\text{C}_2\text{H}_2$  released from  $\text{CaC}_2$  has been reported as a potent source of plant hormone  $\text{C}_2\text{H}_4$ , derived from microbial reduction of  $\text{C}_2\text{H}_2$  (Bibik *et al.*, 1995; Ahmad *et al.*, 2008; Kashif *et al.*, 2008. Keerthisinghe *et al.* (1996) and Randall *et al.* (2001) reported improvement in yield of wheat, cotton, rice and maize crops in response to soil application of coated  $\text{CaC}_2$  (CCC), which released  $\text{C}_2\text{H}_2$  slowly. The  $\text{CaC}_2$  applied at 150  $\text{kg ha}^{-1}$  increased Satsuma (*Citrus unshiu* L.) yield over two years by an average of 49.1 % and subsequently raised the returns (Muromtsev *et al.*, 1991).

Increase in the average weight of fruit, flesh, ascorbic acid and sugar contents were also reported. Chhonkar *et al.* (2002) reported highest dry matter yield (grain + straw) after application of CCC in rice. Ahmad *et al.* (2004) reported that the number of tillers and spike length were significantly increased by  $\text{CaC}_2$  application at 60  $\text{kg ha}^{-1}$ , when applied two weeks after germination while the number of spikelets and grain yield reached a maximum when the same level of  $\text{CaC}_2$  was applied but 8 weeks after germination.

Esashi and Leopold (1969) stated that  $\text{C}_2\text{H}_4$  produced by seeds has a regulatory role in determining germination. Salt induced dormancy in *Zygophyllum simplex* is partially alleviated by ethephon (Khan and Ungar, 1998). Ethylene released from ethephon may act by stimulating the germination of non-dormant seeds or by breaking dormancy in seeds that exhibit embryo dormancy. In many species, the inhibition of seed germination due to dormancy or stress conditions can be completely or partially reversed by  $\text{C}_2\text{H}_4$  or Ethephon (Kepczynski and Kepczynska, 1997), which indicates that seeds have a  $\text{C}_2\text{H}_4$  response mechanism. Ethylene may be loosely associated with a site required for phytochrome action (Suzuki and Taylorson, 1981). Trujillo *et al.* (2008) reported that SodERF3, a novel sugarcane ethylene responsive factor (ERF), enhanced salt and drought tolerance when overexpressed in tobacco plants. Ethylene is vital in regulation of responses to environmental stresses, such as flooding and drought, and to attack by

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pathogens. However, ethylene also often initiates leaf death in response to adverse conditions, scarifying less essential parts of a plant to protect the growing tip that is responsible for producing flowers which form the reproductive organs of plants.

It appears that any environmental turbation can increase rates of  $C_2H_4$  biosynthesis, e.g. drought (El Beltagy and Hall, 1974), and salinity (El Beltagy *et al.*, 1979). The effects can be transitory or relatively long lived; they may vary considerably with species and are complicated by the fact that  $C_2H_4$  biosynthesis shows a diurnal rhythm (El Beltagy *et al.*, 1976). This growth regulator can also control its own biosynthesis via autocatalysis or auto inhibition.

Considering the roles of ethylene enumerated above, this study aimed to evaluate the effect of  $CaC_2$  (as a precursor of ethylene) on enhancement of salinity tolerance in wheat.

## Material and Methods

Soil was collected from the upper layer (0-30 cm) of experimental fields at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The soil was air-dried, ground, sieved (2 mm sieve) and analyzed for physico-chemical properties following the procedures and methods described by USDA (1954). The soil had pH, 7.8; sand, 19%; silt, 40%; clay, 41%; WHC (water holding capacity), 32.2%; organic carbon, 0.44% and total N, 0.05%. Calcium carbide (27% a.i., Ningxia National Chemical Group Co. Ltd., China) was purchased from the local market and was ground to powder. Powdered calcium carbide was encapsulated in a matrix comprising of polyethylene, wheat straw and soil and the formulation thus obtained was extruded and the noodles cut into 3-5 mm pieces (Ahmad *et al.*, 2008).

### Production of acetylene and ethylene

The acetylene and ethylene produced in soil amended with ECC was determined by gas chromatography. A 200 g sample of the processed soil was placed in a 500 mL Erlenmeyer flask at 60% WHC. A 45 mg weight of the formulation  $kg^{-1}$  soil was added at the center of the flasks; the flasks were capped with rubber corks and incubated at 30 °C for 80 days. There were three replications for each treatment. The control comprised of sterilized soil without ECC.

Release of  $C_2H_2$  and  $C_2H_4$  gases was studied over a period of 80 days by gas chromatograph (Carlo-Erba FVS-2300) following the protocol described by Arshad *et al.* (2004). The Gas chromatogram was run isothermally and capillary column packed with Porapak N was used under the following conditions: carrier gas,  $N_2$  (13 mL  $min^{-1}$ );  $H_2$

flow rate, 33 mL  $min^{-1}$ ; air flow rate, 360 mL  $min^{-1}$ ; sample volume, 1 mL; column temperature, 70 °C; detector temperature, 200 °C. Standards were run and  $C_2H_2$  and  $C_2H_4$  concentrations were determined by comparison. Ethylene identification was based on the retention time compared with  $C_2H_4$  standard (purity, 99.9%).

### Pot experiments

Same soil as described above was used in this study. There were 27 pots each containing 6 kg soil. Three levels of salinity were created by using NaCl (0, 7 and 12 dS  $m^{-1}$ ). Six seeds  $pot^{-1}$  were sown and later thinned to 3 seedlings  $pot^{-1}$  after germination. The plants were fertilized with NPK uniformly. Nitrogen was applied in the form of ammonium sulphate at the rate of 50 mg  $kg^{-1}$  soil in two split applications (at sowing and with 1<sup>st</sup> irrigation). Phosphorus and potassium were applied at sowing in the form of  $KH_2PO_4$  at the rate of 25 mg  $kg^{-1}$  soil. The ECC formulation was drenched at the depth of 5 inches in the pots at the rate of 0, 15 and 30 mg  $kg^{-1}$  soil on germination of seeds. Triplicate pots were used for each treatment following a factorial completely randomized design. The plants were grown to maturity and leaf samples were collected at flag leaf stage of growth to measure relative leaf water content (RWC) and total water concentration (g  $g^{-1}$  dry weight) at 15% moisture level of soil.

For relative leaf water content, fresh weight was recorded and the samples were placed in Petri plates containing water and were left over night at 20 °C. After 24 h the turgid weight was recorded. Samples were oven dried to a constant weight at  $65 \pm 2$  °C and the oven dry weight was taken. Relative leaf water content was calculated following Jiang and Huang (2001):

$$RWC = (Fresh\ weight - Oven\ dry\ weight) / (Turgid\ weight - Oven\ dry\ weight)$$

Total water concentration was calculated according to Jackson (1962)

$$(Fresh\ weight - Oven\ dry\ weight) / Oven\ dry\ weight$$

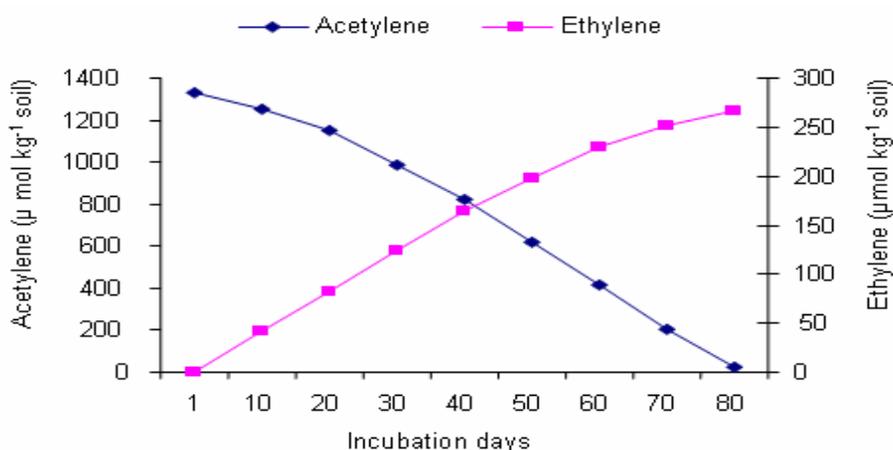
All data were subjected to ANOVA and significantly different means separated using Duncan's Multiple Range Test at 5% level of probability (Steel and Torrie, 1980)

## Results

Gas chromatography analysis (Figure 1) revealed that on the day of incubation 1330.4  $\mu mol$  of  $C_2H_2$  was released which decreased gradually over a period of time to 20.6  $\mu mol$  on the 80<sup>th</sup> day. No production of  $C_2H_4$  was recorded on the day of incubation, but on the 5<sup>th</sup> day of incubation 20.5  $\mu mol$ s of  $C_2H_4$  was observed. A linear correlation was observed between time of incubation and  $C_2H_2$  produced.

The relative leaf water content was decreased significantly ( $P \leq 0.05$ ) by salinity (Figure 2). A decrease of 3 and 8.32% compared with control was recorded at 7 and 12 dS  $m^{-1}$ , respectively. Calcium carbide formulation increased RWC by 1.64 and 2.8% over control, respectively; however, this increase was non-significant at  $p \leq 0.05$ . Maximum RWC (82.7%) was observed in the treatment with 30 mg of  $CaC_2$  in the absence of salinity and minimum (73.2%) in the treatment having highest salinity (12 dS  $m^{-1}$ ) in the absence of ECC. Salinity decreased the total water concentration by 18.52 (7 dS  $m^{-1}$ ) and 30.04% (12 dS  $m^{-1}$ ) over control, respectively (Figure 2). However, ECC significantly ( $p \leq 0.05$ ) increased total water concentration and an increase of 20 and 35% over control

Maximum weight of grains spike $^{-1}$  (2.54 g) was recorded in the treatment having 15 mg ECC in the absence of salinity and minimum (0.78 g) was observed in the treatment where highest salinity (12 dS  $m^{-1}$ ) was applied in the absence of ECC. Weight of spike was increased by ECC up to 22% (ECC at 15 mg  $kg^{-1}$  soil) over control and was decreased by salinity up to 26 % (12 dS  $m^{-1}$ ) over control (figure 2). Maximum weight of spike (12.89 g) was recorded in the treatment where no salinity was applied along with 30 mg of ECC  $kg^{-1}$  soil and minimum (7.34 g) was observed in the treatment having maximum salinity in the absence of ECC. Root/ shoot ratio was increased by ECC up to 17% and salinity (12 dS  $m^{-1}$ ) decreased it up to 31% over control (Figure 2). The effect of two levels of ECC was at par with



**Figure 1. Production of acetylene and ethylene from soil amended with encapsulated calcium carbide**

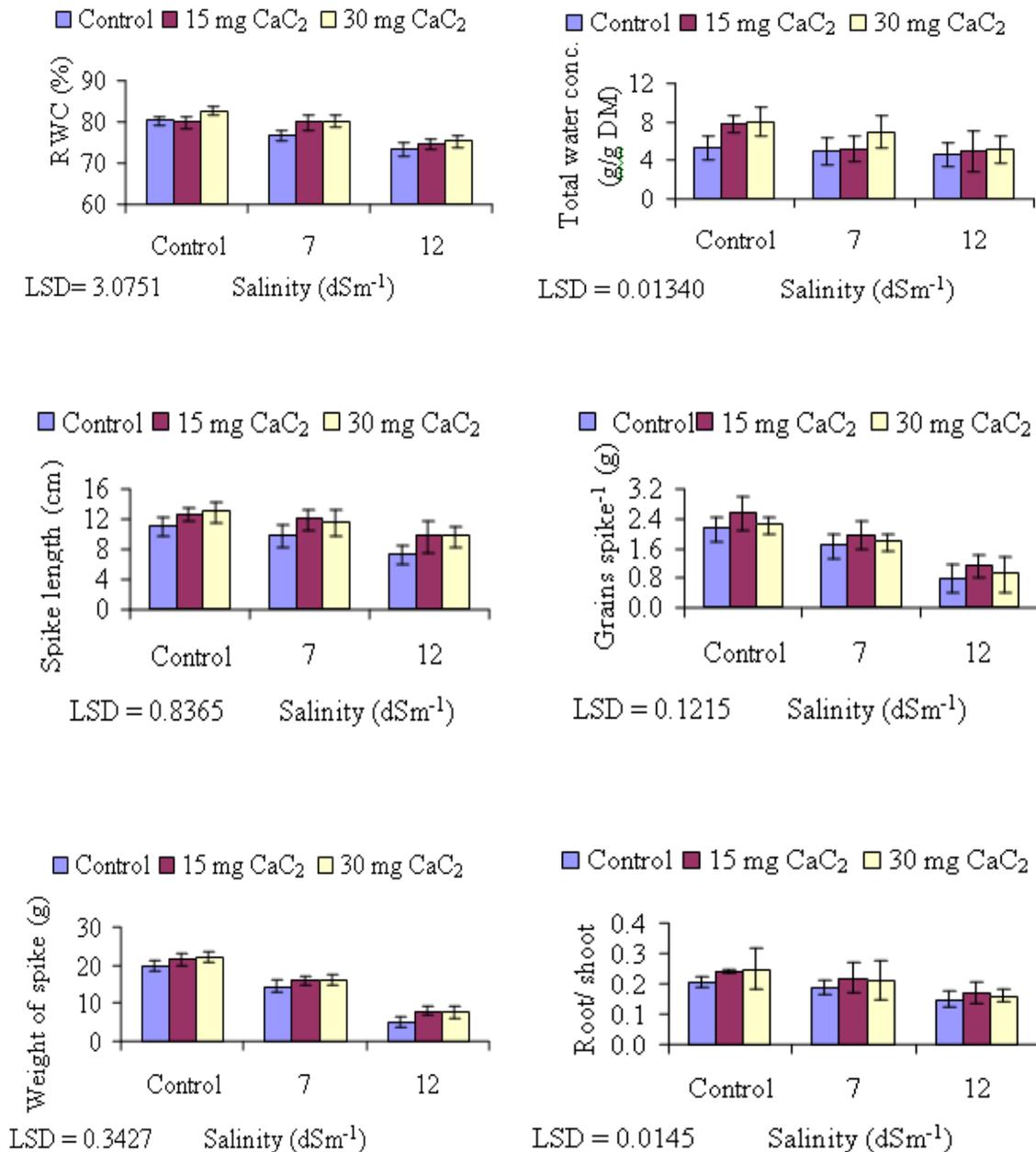
was recorded where formulation was applied at the rate of 15 and 30 mg  $kg^{-1}$  soil, respectively. Maximum total water concentration (8.0 g  $g^{-1}$  DM) was recorded in the treatment where no salinity was applied along with 30 mg ECC and minimum (4.5 g  $g^{-1}$  DM) was observed in the absence of ECC along with highest salinity (12 dS  $m^{-1}$ ).

Length of spike was decreased (up to 68%) in comparison with control by salinity (12 dS  $m^{-1}$ ) while, ECC increased it up to 17 % over control when applied at 30 mg  $kg^{-1}$  soil (Figure 2). There were no significant differences ( $p \leq 0.05$ ) between effects of different levels of ECC. Maximum length of spike (22.12 cm) was recorded in the treatment having 30 mg  $CaC_2$  in the absence of salinity and minimum (4.9 cm) was observed in the treatment with highest salinity (12 dS  $m^{-1}$ ) in the absence of  $CaC_2$ . Similarly, weight of grains spike $^{-1}$  was also significantly ( $p \leq 0.05$ ) increased by  $CaC_2$  treatment, but also the decrease due to salinity was significant (Figure 2).

each other statistically in the case of root/shoot ratio. There was a negative correlation between root/shoot ratio and weight of spike (-0.45), and weight of grains spike $^{-1}$  (-0.42).

## Discussion

Laboratory studies revealed that ECC acted as a source of  $C_2H_2$  in soil, which was partially reduced to  $C_2H_4$  over time. No ethylene was detected (data not shown) in  $CaC_2$ -amended sterilized soil, which may imply that the release of  $C_2H_2$  from  $CaC_2$  is a chemical reaction while reduction of  $C_2H_2$  to  $C_2H_4$  is a biochemical transformation. Scientists have reported involvement of microorganisms in the synthesis of  $C_2H_4$  in soil and in pure culture (Muromtsev *et al.*, 1991; Bibik *et al.*, 1995; Akhtar *et al.*, 2005; Yaseen *et al.*, 2006; Kashif *et al.*, 2008; Ahmad *et al.*, 2008). Ethylene being a potent plant growth regulator is reported to influence plant growth from germination to harvesting (Abeles *et al.*, 1992; Bibik *et al.*, 1995).



**Figure 2: Enhancement of salinity tolerance in wheat through soil applied calcium carbide**

Increase in RWC, total water concentration and yield components is attributed to the fact that under hypoxic (salinity or water logging) conditions ethylene regulates stomatal conductance, root hydraulic conductivity and increased water flow which helps the plants to mitigate the water deficit caused by oxygen deficiency (Kamaluddin and Zwiazek, 2002). It may further be attributed to the fact that ethylene induces root proliferation and increase in the soil

volume being explored by the roots for nutrient and water acquisition (Ahmad *et al.*, 2004). Since the application of encapsulated CaC<sub>2</sub> enhanced the weight of spike and weight of grains per spike, it implies that this effect of CaC<sub>2</sub> is most likely because of the hormonal action of C<sub>2</sub>H<sub>4</sub>, which evolved a physiological response (Yaseen *et al.*, 2006; Kashif *et al.*, 2008). This increase could also be attributed to improved uptake of N, P and K (data not shown) which

has been reported by Ahmad *et al.* (2004), Yaseen *et al.* (2006) and Kashif *et al.* (2008). In addition to increased availability of nutrients, CaC<sub>2</sub> may also lead to changes in the form of available nutrients, e.g. N. Since acetylene is inhibitory to nitrification, part of the N fertilizer will remain in NH<sub>4</sub>-form over extended period of time resulting in more availability of both NO<sub>3</sub> and NH<sub>4</sub> to the plant. Nitrification inhibitors have indeed been reported to improve crop yields by decreasing losses of N through denitrification and NO<sub>3</sub> leaching (Kashif *et al.*, 2007). In addition, CaC<sub>2</sub> may also serve as a source of Ca which is useful for plant growth (Sharma and Yadav, 1996). Results of organic carbon, dehydrogenase activity and presence of 26 and 58 kDa proteins, in the same study (data not shown) provide sufficient clue that ECC increased microbial activity and brought about molecular changes which could have enhanced tolerance in wheat under saline conditions (Klose and Tabatabai, 2000; Trujillo *et al.*, 2008).

In summary, the results of this study showed a positive effect of ECC on RWC, total water concentration, weight of spike, weight of grains per spike, length of spike and root/shoot ratio, in wheat under salinity stress. Induction of salinity tolerance is mainly due to increased uptake of N, P and K, regulation of stomatal conductance and root hydraulic conductivity, increased microbial activity and induction of changes at protein level.

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