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Enhancement of salinity tolerance in wheat through soil applied calcium carbide

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Abstract

Calcium carbide (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 dS m^{-1} were created using NaCl. Nitrogen, phosphorus and potassium were applied as ammonium sulphate and KH_2PO_4 at 50 and 25 mg kg^{-1} soil, respectively. The encapsulated calcium carbide (ECC) at 45 mg kg^{-1} soil produced 1291.8 µmols of acetylene (C_2H_2) and 257.5 µmols of its product ethylene (C_2H_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 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^{-1} 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 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, CaC_2 has emerged as a good source of C_2H_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). C_2H_2 released from CaC_2 has been reported as a potent source of plant hormone C_2H_4, derived from microbial reduction of C_2H_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 CaC_2 (CCC), which released C_2H_2 slowly. The CaC_2 applied at 150 kg ha^{-1} increased Satsuma (*Citrus unsbiu* 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 CaC_2 application at 60 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 CaC_2 was applied but 8 weeks after germination.

Esashi and Leopold (1969) stated that C_2H_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 C_2H_4 or Ethephon (Kepczynski and Kepczynska, 1997), which indicates that seeds have a C_2H_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 flaks 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 isothermically 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 1st 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 ± 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 μmol of C_2H_2 was released which decreased gradually over a period of time to 20.6 μmol on the 80th day. No production of C_2H_4 was recorded on the day of incubation, but on the 5th day of incubation 20.5 μmols 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 12dS 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 (12dS 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 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 (12dS m^{-1}).

Length of spike was decreased (up to 68%) in comparison with control by salinity (12dS 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). 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 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).

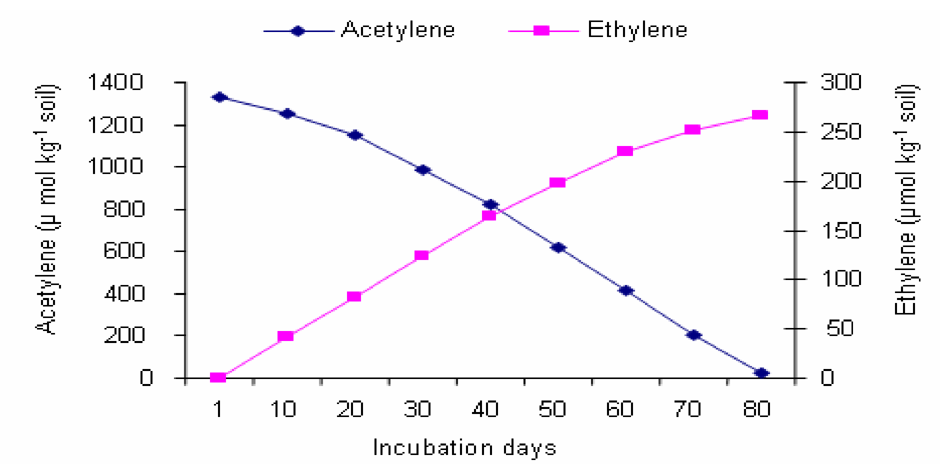

Figure 1. Production of acetylene and ethylene from soil amended with encapsulated calcium carbide

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).

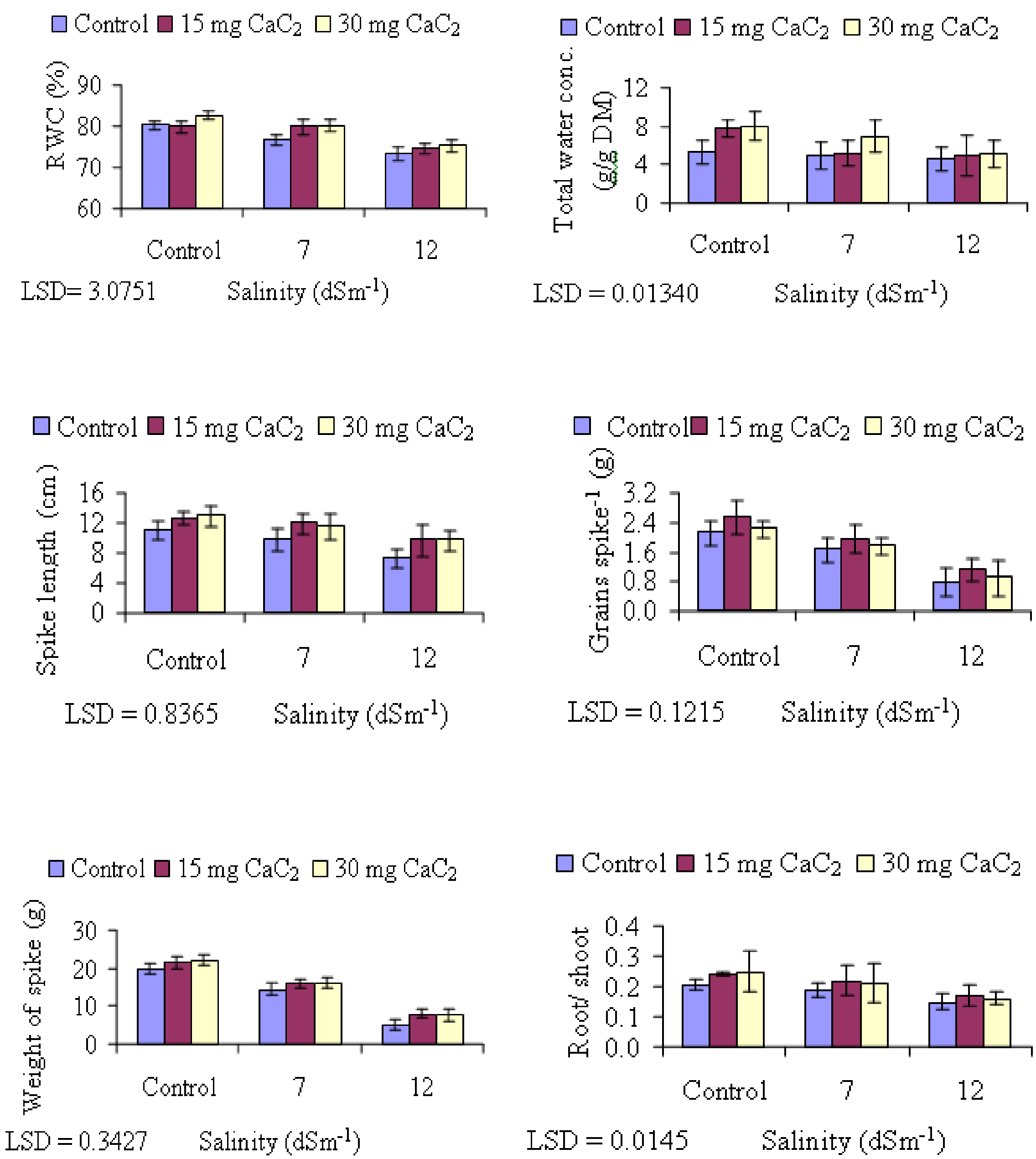

Figure 2: Enhancement of salinity tolerance in wheat through soil applied calcium carbide

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_2 enhanced the weight of spike and weight of grains per spike, it implies that this effect of CaC_2 is most likely because of the hormonal action of C_2H_4, 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_2 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_4-form over extended period of time resulting in more availability of both NO_3 and NH_4 to the plant. Nitrification inhibitors have indeed been reported to improve crop yields by decreasing losses of N through denitrification and NO_3 leaching (Kashif *et al*., 2007). In addition, CaC_2 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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