EFFECT OF COATED CALCIUM CARBIDE ON GROWTH, YIELD AND SOME MORPHO-
PHYSIOLOGICAL CHARACTERISTICS OF CUCUMBER (Cucumis sativus L.)

By

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M.Sc. (Hons.) Agriculture

Registration # 2003-ag-1619

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

SOIL SCIENCE

INSTITUTE OF SOIL & ENVIRONMENTAL SCIENCES

FACULTY OF AGRICULTURE

UNIVERSITY OF AGRICULTURE, FAISALABAD

PAKISTAN

2014
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We, Supervisory Committee, certify that the contents and form of thesis submitted by Mr. Muhammad Shakar (Regd. No. 2003-ag-1619) have been found satisfactory and recommend that it be processed for evaluation by the External Examiner(s) for the award of degree.

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ACKNOWLEDGEMENT

All admirations and thanks are for stupendous Allah, the Omnipotent, the Sublime, Only Creator of the universe and the source of knowledge and ingenuity Who benedict me with health, thoughts, talented teachers, helping friends and opportunity to complete this study. I offer my meekly thanks to Holy prophet (peace be upon him) the nimbus, the beacon, whose moral and spiritual teachings illuminate my heart, mind, and thrived my thoughts towards achieving high ideas of life.

I feel highly privileged to express my heartiest gratitude to my honorable supervisor Dr. Muhammad Yaseen, Professor, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, for his keen interest, full help, valuable suggestions, timely advice and sympathetic attitude throughout the study.

With deep sense of honor, I wish to extend my sincere gratitude to Dr. Muhammad Arshad (T.I.), Professor, Institute of Soil and Environmental Sciences for his inspiring help and sympathetic guidance throughout the research period. Special thanks are extended to Dr. Rashid Ahmad, Professor, Department of Agronomy, for his part as a member of supervisory committee.

My gratitude will remain incomplete if I do not mention the contribution and corporation of my sincere friend, Furqan Ijaz in completing my research work. No acknowledgement could ever adequately express my obligations to my affectionate father, Mushtaq Ahmed and Sweet Loving mother, Fahmida Mushtaq, whose hands always raised in prayers for me and without their moral and financial support, the present distinction would merely be a dream.

I express my heartiest and sincere sense of gratitude to my grandmother for her prayers and moral help during the write up of thesis and special love for my Brothers (Muhammad Baqir, Muhammad Zakir, Muhammad Asim, and Muhammad Measum) and cute nephews and nieces (Abdullah Hassanat, Muhammad Zakria, Irtaza Asim, Mishal Fatima, Hateem Ali, Fatima Measum) for long wait during my studies.

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**ABSTRACT**

Ethylene is involved in regulation of various aspects of plant growth and development and also acts as a defense mechanism under various stress conditions. A lot of work on foliar-applied source of ethylene has been evaluated as a mean to improve growth and yield of various crops in literature. Little research has been published on the use of soil-applied ethylene source for vegetable production. The objective of this project was to evaluate the effect of calcium carbide based formulation, a soil-applied source of ethylene, on growth, yield and some morpho-physiological characteristics of cucumber. Laboratory, pot and field studies were conducted in a sequence to investigate the effect of calcium carbide (CaC$_2$) on seed germination, growth, yield, nitrogen and carbon metabolism and fruit quality parameters. Preliminary studies (experiment 1 to 3) were conducted under controlled conditions to evaluate the effect of CaC$_2$ on seed germination, growth and some metabolic changes in cucumber seeds under normal and stress conditions. Results showed that application of 30 mg plate$^{-1}$ CaC$_2$ among different rates was found the best rate to promote seed germination and growth of plants. Increase in germination percentage, growth parameters and nitrogen assimilation was noted in response to 20 and 30 mg CaC$_2$ plate$^{-1}$ while higher rate of CaC$_2$ than 30 mg plate$^{-1}$ was, however, found to inhibit physiological functions. Seed germination was inhibited under salt stress, and this inhibitory effect was significantly mitigated by the addition of CaC$_2$ in the incubation medium along with increased ethylene evolution. Application of CaC$_2$ also increased the contents of soluble sugars, soluble proteins, free amino acids and activity of $\alpha$-amylase and antioxidant enzymes in response to CaC$_2$ under salt stress. The 4$^{th}$ experiment was conducted in pot where different rates and coating materials on CaC$_2$ were optimized under soil conditions for growth and yield attributes of cucumber. All rates of CaC$_2$ significantly improved growth and yield parameters but effect of coating materials was less significant. However, maximum fruit yield was obtained in the treatment of 300 mg paint coated CaC$_2$ pot$^{-1}$. In the light of the results of experiment 4, another pot experiment was conducted to investigate the genotypic response of two cucumber cultivars (Hybrid vs. Local) to soil applied paint coated CaC$_2$ which significantly improved morphological, phenological and yield characteristics of both cultivars but hybrid cultivar (Bolan-F1) surpassed local cultivar (Desi) regarding yield characteristics. Finally a field experiment of was conducted to investigate effect of paint coated CaC$_2$ on photosynthesis, nitrogen use efficiency and fruit quality of cucumber under deficient and adequate levels of nitrogen. Application of paint coated CaC$_2$ at each level of nitrogen increased photosynthesis via increase in nitrogen assimilation and stomatal conductance. Plants treated with 300 mg CaC$_2$ with sufficient nitrogen exhibited maximal photosynthesis and growth characteristics. These plants also showed maximum nitrogen uptake, nitrogen use efficiency and mineral contents along with improved fruit quality characteristics. Overall results suggest that application of 300 mg paint$^{-1}$ paint coated CaC$_2$ enhanced fruit yield by 39.6% compared to control by improving yield contributing factors. These results suggest that application of CaC$_2$ at right rate with appropriate coating material could significantly improve the quality and fruit yield in cucumber by making economic use of soil applied nitrogen.
Cucumber (*cucumis sativus* L.) locally known as “Kheera” is summer vegetable of *Cucurbitacae* family and is grown as one of the major salad crops in Pakistan. It is very highly esteemed for its freshness and vitamin contents. It was grown on 3.50 thousand hectares with total production of 50.5 thousand tons with an average yield of 14.4 tons per hectare in Pakistan during 2012-13 (FAOSTAT, 2013). Cucumber is being widely adopted vegetable especially among small land holding farmers owing to its higher yield potential and profit.

The fruits are highly nutritive containing high amount of water content and very low calories. The fruit is used as a vegetable or salad. Fruits consist about 80 percent of edible portion which contains 95 % water, 0.7 % protein, 0.1 % fat, 3.4 % sugars, 0.4 % fiber and 0.4 % ash. It is rich in minerals, thiamine, niacin and vitamin C. (0.38 g, 0.3 mg, 0.2 mg and 78 mg, respectively per 100 g of edible fruit). Cucumbers are also considered as a rich source of health-supportive phytochemicals which have anti-oxidant and anti-inflammatory properties for human health. Some of the important antioxidant phytonutrients in cucumbers are vitamin C, kaempferol, quercetin, apigenin and luleolin, which protect cells from the damage of reactive oxygen species (ROS) (Aykroyd, 1963; Milder *et al*., 2005; Nema *et al*., 2011).

Besides using conventional cultural practices, the yield of cucumber in field is quite low compared to the yields obtained at experimental stations indicating almost 45 % yield gap due to the suboptimal use of inputs. So, the conventional approaches, focusing mostly on intensive fertilization, could not only fulfill the demands of ever increasing population but also causing greater damage to environment. Therefore, along with traditional practices the use of novel approaches is highly desirable which can overcome the yield gap with reduced environmental damage. The use of plant growth regulators integrated with conventional approaches is thought to be such approach which can enhance crop yield at given level of nutrient by increasing nutrient use efficiency.

The use of plant growth regulator was started with discovery of acetylene and
ethylene gases which were primarily used to stimulate flowering in pineapple. So far, several growth regulating substances have been identified as plant growth regulators. Among them, the most commercially used are: α-naphthalene acetic acid (NAA), β-naphthalene acetic acid (BNA), indolebutiric acid (IBA), succinic acid, salicylic acid, ethylene, acetylene and calcium carbide (CaC\textsubscript{2}). However, only a few used calcium carbide as a source of ethylene and acetylene. It is believed that this plant growth regulators acts by promoting an increase of ethylene content inside the plant, more precisely in the meristematic zone (Burg and Burg, 1967; Yaseen et al., 2006; Khan et al., 2006), where the absorption of the products is faster.

In relation to other hormones, it is thought that ethylene links itself to a receptor molecule, resulting in an active complex that elicits a series of reactions including modifications in the expression of genes, leading to a wide diversity of physiological effects.

Involvement of ethylene as a potent hormone has been reported in controlling various morho-physiological processes including seed germination, root and shoot growth, flower and fruit development, senescence of various organs and also many responses under biotic and abiotic stresses (Lurssen, 1991; Archambault et al., 2005). Ethylene regulate seed germination by breaking dormancy in both dormant and non-dormant seeds (Kucera et al., 2005) because of direct relationship between ethylene production and seed germination of various plants (Calvo et al., 2004). In cucumber, it has been reported that ethylene enhanced seed germination by loosening the perisperm (Ramakrishna and Amriphale, 2005). Ethylene can also mitigate the secondary dormancy induced by salinity stress in various plants (Kepczynski and Karssen, 1985; Li et al., 2005; Chang et al., 2010; Wang et al., 2011). Recently, ethylene has also been shown to promote germination under salinity stress by scavenging ROS in germinating seeds of Arabidopsis (Lin et al., 2012). Ethylene effectively reduces plant height of different vegetable and cereal crops (Danhouse et al., 1982; Simmons et al., 1988 and Thappa et al., 2011) and in contrast to this ethylene can also stimulate elongation of certain plant organs under flooded conditions (Kende et al., 1998; Voesenek et al., 2003). Moreover, a lot of studies have also elucidated the role of non enzymatically released ethylene compounds (ethephone/ethrel) in enhancing female gene expression which subsequently leads to higher fruit number in cucumber (Rudich et al., 1969; Karchi and Govers, 1972; Yamasaki et al., 2003).
Ethylene plays important role in regulation of photosynthesis, nitrogen assimilation and plant stress responses (Khan, 2006). Ethylene affects photosynthesis indirectly by increasing leaf area and chlorophyll contents which in return capture more light enhancing photosynthesis (Khan et al., 2000). The role of ethylene as a growth promoter or inhibitor is determined by concentration of ethylene within plant system as well as sensitivity of plant to the ethylene (Pierik et al., 2006). Ethylene at low concentrations can enhance photosynthesis (Khan, 2004; khan et al., 2008) and leaf growth (Hussain et al., 1999; Khan, 2005). On the other hand, ethylene at high concentrations has been reported to reduce photosynthesis (Kays and Pallas, 1980; Khan, 2005) and leaf area (Tholen et al., 2004; Khan, 2005).

Two major control points in nitrogen metabolism are nitrate reductase (NR) activity and Glutamine synthetase (GS)/ Glutamate synthase (GOGAT) systems in the assimilation of N. These two and many other control points are coordinated by metabolic crosstalk and other signals mediated by plant growth hormones (Foyer et al., 2003). Improvement in nitrogen use efficiency by the exogenous application of ethephon in mustard (Brassica juncea L.) has been reported by Khan et al. (2008) and Iqbal et al. (2011). Results from several experiments have shown an increase in nitrate reductase activity in leaves using ethrel as source of ethylene on Brassica juncea (Mir et al., 2008a; Ashraf et al., 2010) Improvement in the chlorophyll content by application of ethrel in leaves of Brassica napus under different nitrogen fertilizers has also been reported but detrimental effects were also observed in cases where higher concentration of ethrel was used or in absence of nitrogen (Grewal and Kolar, 1990; Grewal et al., 1993). Lone et al. (2008) suggested that higher ethylene evolution after defoliation at 40 days after sowing was significantly correlated with enhanced activities of nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS) in leaves of mustard (Brassica juncea L.). Gómez-Maldonado et al. (2004b) proposed that hormonal control, particularly by gibberellic acid, is part of the regulation of cytosolic GS in the early stages of pine development. Moreover, the enhancement of cytosolic glutamine synthetase and mRNA contents in laticiferous cells in Hevea brasiliensis after the application of ethylene was reported by Pujade-Renaud et al. (1994).

Ethylene is involved in many abiotic stress responses and has long been recognized as a stress hormone (Pierik et al., 2006). Generally, seed germination is subject to tight
hormonal control (Bogatek and Gniazdowska, 2012). It has been found that ethylene can alleviate germination inhibition induced by salinity in seeds of various crops (Lin et al., 2012; Chang et al., 2012; Khan et al., 2009) and ethylene promotes germination under salinity by modulating ROS production (Lin et al., 2012). Little is known about how ethylene induced biochemical changes can regulate seed germination under salinity.

So far, most of studies have elucidated the role of non-enzymatically produced ethylene (ethephone/ethrel) on different morpho-physiological characteristics in plants which is usually restricted to foliar application. However, its application in soil environment at commercial scale came by development of a new CaC₂ based ethylene producer “Retprol” which was used as a soil amendment for crop production. The effect of this formulation upon tomato and cucumber plants was investigated and yield increase up to 70% was reported (Muromstev et al., 1988). Calcium carbide (CaC₂) upon its reaction with soil moisture produces acetylene (C₂H₂) gas which as a nitrification and denitrification inhibitor can prolong the availability of NH₄⁺ and NO₃⁻ in soil which is otherwise very low due to various losses of these ions (Walter et al., 1979; Freney et al., 1993 and 2000; Thompson, 1996; Bolman and Conrad, 1997; Hayden and Ross, 2005). Acetylene in addition to its role as controlling agent of NH₄⁺ and NO₃⁻ ions in soil, it is also reducible to ethylene by soil indigenous microbes (Muromstev et al., 1995; Yaseen et al., 2005 and 2006; Kashif et al., 2008) whose importance as a plant hormone involved in various developmental processes is elaborated in detail above.

Plant growth hormones can control the accumulation and transport of nutrients in plants and in turn, nutrient availability can regulate the concentrations of specific plant hormones (Kuiper, 1988; Kuiper et al., 1989). Among various nutrients, nitrogen availability and its endogenous distribution have a pivotal role in the regulation of different morpho-physiological characteristics of plant development that is generally endorsed to hormonal elements. In view of the importance of ethylene in plant growth and metabolism and nutrient uptake, it is assumed that cucumber (Cucumis sativus L.) may serve as model crop in the study of the influence of ethylene on plant metabolism. It was also hypothesized that ethylene play its central role in the regulation of growth and development of plant and also controls N-mediated changes in photosynthesis and nitrogen metabolism under varying levels
of nitrogen. In previous studies, it has been shown that the application of CaC$_2$ can enhance growth, yield and photosynthetic activity in various vegetables under recommended level of nitrogen (Yaseen et al., 2012; Siddiq et al., 2012; Ahmed et al., 2014). Moreover, it was also hypothesized in the above studies that CaC$_2$ treated plant would maximize uptake of available nitrogen in soil resulting in higher photosynthesis. Therefore, in-depth study regarding the interactive effect of nitrogen availability and CaC$_2$ on the photosynthesis and nitrogen assimilation enzymes still needs to be worked out. Moreover, a mechanism based study of CaC$_2$ on ROS metabolism could provide a sound theoretical basis for ethylene metabolism function in inducing cucumber tolerance to NaCl stress. Alternatively, the outcome of the present study will help in developing the use of CaC$_2$ as novel approach towards induction of salt tolerance in crops particularly cucumber during seed germination.

Therefore, by keeping above mentioned facts in view, the present project has been designed to achieve the following objectives:

- To study the effect of CaC$_2$ on seed germination, biochemical changes, root and shoot growth of cucumber under normal and stress conditions
- To evaluate different coating materials on CaC$_2$ for growth and yield attributes of cucumber
- To study ethylene emission pattern from CaC$_2$ amended soil and endogenous ethylene production from plant body after application of CaC$_2$
- To study effect of coated CaC$_2$ on morpho-phenological attributes, photosynthesis and nitrogen assimilation in cucumber under varying levels of nitrogen
- To evaluate the effect of CaC$_2$ on yield and quality of cucumber fruit
Vegetable production is considered as one of the major components of cropping pattern in Pakistan. About 0.62 M ha area is occupied by vegetables which is just 3.1% of total area under cultivation (Khokhar, 2014). Vegetable production system is considered the most intensive agriculture system accompanied with intensive nitrogen fertilization. Higher usage of nitrogen fertilizer under irrigated systems not only leads to higher losses of nitrogen but also accelerates global warming due to higher emission of nitrous oxide into atmosphere (Min et al., 2011). In near future, efficient and novel approaches are needed to be explored which not only maximize the nitrogen use efficiency but also improve morpho-physiological characteristics of plant and allowing cost-efficient production of adequate nutrition for the growing population.

Although more input of nitrogen increases growth and yield of the crops, but its excessive use causes environmental degradation phenomenon like eutrophication of the resources. Therefore, an approach is to be explored which minimizes the use of nitrogen without decreasing the growth and yield of crops. In this context, use of plant hormones may prove its potential as it has been found to enhance growth and productivity of the crop plants. In soils, N₂O is produced during the microbial processes of nitrification in aerobic conditions and denitrification in anaerobic conditions. Nitrification inhibitors, which slow down conversion of NH₄⁺-N into NO₃⁻-N, are reported to increase nitrogen use efficiency and crop yield (Prasad and Power, 1995) and also considerably reduce N₂O emission from soil (Di et al., 2007). To overcome the ecological problems and to increase crop yields, use of CaC₂ as explained in the introduction chapter may prove its potential in view of its dual action i.e. potent nitrification inhibitor (Aulakh et al., 2001; Yaseen et al., 2006; kashif et al., 2008) as well as plant growth regulator ethylene (Arshad and Frankenberger, 2002; Khalid et al., 2006b).

Calcium carbide reacts with water to release calcium ions and acetylene (C₂H₂) gas. Actylene acts as an effective inhibitor of nitrification and denitrification (Thompson, 1996;
Aulakh et al., 2001; Randall et al., 2001; Arshad and Frankenberger, 2002). Whereas its impact on calcium build up in soil is negligible due to application of calcium carbide in milligrams or in meager amount. In previous studies, the effect of acetylene released from CaC$_2$ (as one of the tools to enhance nitrogen use efficiency) have been studied in detail in cereals (Randall et al., 2001; Keerthisinghe et al., 1993; Mahmood, 2009) as well as in vegetable crops (Kashif et al., 2012, Siddiq et al., 2012, Yaseen et al., 2012). Yaseen et al. (2006) investigated the role of CaC$_2$ as potent source of acetylene i.e. a well-known nitrification and also as a potential source of biologically produced ethylene in soil.

Ethylene as a gaseous phytohormone whether synthesized endogenously in the plant body, in response to environmental stresses (Lurssen, 1991) or supplied exogenously at extremely low concentrations markedly affect plant growth and development (Arshad and Frankenberger, 2002; Woodrow and Grodzinski, 1989). Physiologically this phytohormone is attributed to break seed dormancy, improved germination, regulation of swelling and elongation, adventitious root formation, modification in root growth pattern, promotion of root hair, epinasty, hook closure, reduction in plant height, inhibition of leaf expansion, regulation of flower induction, fruit ripening, senescence and abscission. This hormone induces improvement in yield of many crops in response to the soil application of coated CaC$_2$. CaC$_2$ is coated with non-reactive substance because it is highly reactive to soil moisture and gases get dispersed within short time. The basic approach of coating like hydrophobic resins or wax is to release small/adequate amount of acetylene and ethylene gases over prolonged periods. Like other plant hormones, the physiological effects of ethylene depends on numerous variables such as the type of tissue, stage of development, physiological age of species and prevailing environmental conditions (Dithey, 1969; Pratt and Goeschi, 1969).

The direction of response to applied ethylene depends on its applied concentration. Externally applied large concentrations of ethylene generally inhibit cell elongation (Abeles et al. 1992). One of the most well-known elongation inhibiting effects of ethylene is the ‘triple response’ of seedlings grown in darkness, and was first discovered in pea seedlings exposed to ethylene (Neljubow, 1901). It was also shown that low concentration of ethylene stimulated root growth of tomato, white mustard and rice, whereas higher concentrations
inhibited growth (Koning and Jackson, 1979). Similarly, high ethylene concentration reduces leaf area (Tholen et al., 2004; Khan, 2005) and photosynthesis (Kays and Pallas, 1980; Khan, 2004, 2005). Ethylene gas causes premature senescence of many tissues, yellowing of vegetables, abscission of flowers and fruits and stimulates flower drop. The application of ethylene at higher rate has been reported to suppress flowering, fruit set and fruit yield. In cucumber, it was observed that application of high concentration of ethephon or more application times can weaken plant growth, reduce female flower rate, shorten fruit length, and decrease early and total fruit yield (Li et al., 2011). Moreover, application of coated CaC₂ at higher rates has been reported to reduce plant growth and fruit yield in cucumber, tomato and sweet pepper (Shakir et al., 2012; Siddiq et al., 2012; Ahmed et al., 2014).

The literature reviewed in this section is related to the effect of ethylene and its different sources (Etaphone/Ethrel, Retprol and CaC₂ etc.) on plant growth including morpho-physiological attributes of various crops in general while in cucumber particularly.

2.1 History of different ethylene sources and their chemical structure

Ethylene gas has played a role in agriculture long before it was recognized as a plant hormone. Some of the first documented historical techniques used to promote fruit ripening are ancient Egyptians cutting sycamore figs and the Chinese burning incense to ripen pears (Wright, 1976; Chaves and Mello-Farias, 2006). Ancient agricultural practices standing with several astute observations in the mid to late 19th century led to the identification of ethylene as a modifier of plant growth and development. The chronology of events has been conveyed in great detail in the works of Abeles et al. (1992) and Chaves and Mello-Farias (2006). In brief, the use of gas generated from coal (i.e., illuminating gas) for lighting purposes was popular throughout the 19th century. It was noticed that trees and plants growing near buried gas lines and gas lights were often stunted and injured (Crocker and Knight, 1908). These observations and experiments were validated by the seminal work of Neljubow (1901), who showed that 1 part ethylene per 1,000,000 in air (1 µmol mol⁻¹ or 1 part per million, ppm) was able to generate the same response in etiolated pea seedlings as exposure to illumination-gas tainted air. From that point onward, various efforts have been made to develop the compounds, which could release ethylene in plants or in their rhizosphere. The most important breakthrough was the development of ethephon (liquid) in 1960s, which releases
C₂H₄ chemically when absorbed by the plant tissues (Abeles et al., 1992; Arshad and Frankenberger, 2002). Control of localized ethylene applications was extremely difficult until the release of an experimental chemical called 2- chloroethylphosphonic acid (ethephon, trade name Ethrel) by Amchem Products Inc (Amchem Inc., Ambler, PA, U.S.A.) in 1969. Introduction of this liquid-form chemical provided a simplified method of exogenous ethylene applications.

The 2-chloroethylphosphonic acid (Ethephon, Ethrel, CEPA, CEPHA) has the structural formula as:

\[ \text{Cl} - \text{CH}_2 - \text{CH}_2 - \text{PO}_3\text{H}_2 \]

Ethrel is a formulation of 24% (240 g L⁻¹) ethephon solution- a compound which breaks down in plant tissues to release ethylene (Abeles, 1973), a Cl⁻ ion, and H₂PO₄⁻. Alkaline pH and high temperature promote the degradation of this chemical in the plant (Warner and Leopold, 1969). Ethrel is actively taken up in the transpiration stream and translocated to the leaves, flowers and hips (Nickell, 1979). The release of ethylene from Ethrel-treated tissues produces plant responses similar to endogenous ethylene (Cooke and Randall, 1968; Warner and Leopold, 1969). The presence of ethylene from this source likely stimulates the plant to produce additional endogenous ethylene due to its autocatalytic nature. However, use of ethylene is restricted only to foliar application. Recently after a long joint effort of a group of co-workers at the All-Union Scientific Research Institute of Agricultural Microbiology of the Lenin resulted in a new CaC₂ based C₂H₄ producing formulation under the trade name of “Retprol”. It is used as soil amendment which breaks down slowly into acetylene and calcium upon interaction with soil water. The acetylene released is reducible to ethylene by soil indigenous microorganisms as shown in an equation below:

\[ \text{CaC}_2 + \text{H}_2\text{O} \rightarrow \text{Ca}^{2+} + \text{C}_2\text{H}_2 \rightarrow \text{C}_2\text{H}_4 \]
2.2 Role of ethylene during seed germination

Ethylene’s role in seed germination is still not completely known and remains a subject of controversy. Different thoughts have been put forward to explain the mechanism of action of ethylene during seed germination. According to some scientists ethylene is produced in response to seed germination while other scientists thought that ethylene is an essential requirement for complete germination of (Petruzzelli et al., 2000; Matilla, 2000; Rinaldi, 2000). Early studies suggested that ethylene is involved in breaking primary dormancy (Ketring and Morgan, 1971; van Staden et al., 1973), while others indicated that a rise in ethylene production is merely a consequence of breaking dormancy (Satoh et al., 1984; Kebczynski and Karssen, 1985). More recently it has been suggested that ethylene reduces ABA sensitivity, and therefore reduces ABA-induced seed dormancy and increases germination in Arabidopsis (Ghassemain et al., 2000). Many questions cannot be answered because the interactions between ethylene and other important hormones, such as abscisic acid, are not completely understood.

Additional studies have led to hypotheses on how ethylene may regulate germination. One suggested mechanism developed from studies in cocklebur is that ethylene induces expression of \( \beta \)-cyanoalanine synthase (CAS), an enzyme required for cyanide metabolism during seed germination (Hasegawa et al., 1995). These studies indicate that ethylene stimulates the action of the mitochondrial CAS, which down regulates the cyanide level and at the same time causes an increase in the amino acid contents before seed germination occurs (Hasegawa et al., 1995). This supports the idea that ethylene plays a role in a more conducive environment for germination by lowering toxic cyanide levels and allowing for essential amino acids needed in the germination process (Hasegawa et al., 1995; Maruyama et al., 1997).

Another hypothesis is that ethylene promotes germination by stimulation of hydrolytic enzymes that break down the endosperm to provide an available nutrient supply for radicle emergence and subsequent germination. Ethylene was also shown to increase germination by inducing the activity of \( \beta \)-1, 3-glucanase enzyme in tobacco and pea (Petruzelli et al., 1995; Leubner-Metzger et al., 1998). The promoter region of this gene was
mapped and was found to contain ethylene-response elements, which lead to the idea that the ethylene response element binding proteins are transcription factors necessary for ethylene dependent β-1, 3-glucanase induction. The gene was also found to be positively regulated by ethylene and negatively regulated by ABA (Leubner-Metzger et al., 1998). This shows yet another incidence of interacting roles of these two hormones during seed germination. Bleecker et al. (1988) observed in Arabidopsis etr1-1 mutants that seed germination was significantly lower than wild-type seeds and that application of GA3 overcame some of the germination deficiencies.

Ethylene can break seed dormancy in dormant peanut seeds by antagonizing the inhibitory effects of ABA on seed germination (Ketring and Morgan, 1969). However, similar inhibition of ABA like chemicals by the application of ethylene was not involved to release seed dormancy in lettuce seeds (Rao et al., 1975), since that seed germination induced by the application of ethrel was diminished in the presence of ABA. Recently, in various studies by using ethylene mutants of Arabidopsis sp. it was shown that ethylene could enhance seed germination by reducing sensitivity to endogenous ABA (Beaudoin et al., 2002). Role of ethylene in the regulation of biosynthesis of ABA was further verified by using other ethylene mutants which showed faulty response to ethylene (Ghassemian et al., 2000).

Effect of acetylene and ethylene released gases from solid CaC$_2$ on seed germination and growth characteristics of okra seedlings were investigated by Kashif et al. (2012). Solid calcium carbide when reacts with moisture immediately releases acetylene gas along with small concentration of ethylene. Seed germination and plant growth characteristics of okra were studied after application of solid CaC$_2$ in sealed petri dishes under controlled conditions. Results of that experiment revealed that that application of calcium carbide appreciably enhanced germination and growth rate of root and shoot. It was also observed that CaC$_2$ treated seeds increased callus formation on the roots of germinating seeds which were converted into secondary roots within short span of time. Enhanced seed germination in CaC$_2$ treated seeds was associated with increases in ethylene evolution from germinating seeds.
i) Effect of ethylene on seed germination under salt stress

Various environmental stresses i.e. temperature, salinity, water and light can influence seed germination by affecting hormonal balance in the seed, (Ali-Rachedi et al., 2004; Alboresi et al., 2005). Salinity can cause adverse effects on seed germination through various physiological and biochemical changes in seed i.e. (a) inhibiting the germination regulating substances including gibberellins, ethylene and NO3 (b) increasing ABA activity, (c) causing ion toxicity, and (d) changing water relations and cell membrane permeability in the seed (Ungar, 1996; Khan and Ungar, 2002; Song et al., 2005; Zhang et al., 2010; Lee and Luan, 2012). Plant growth and development is inhibited at higher level of salt stress due to more production of ethylene in plants. Synthesis of ethylene in plants depends upon 1- aminocyclopropane-1 carboxylic acid (ACC) which is a recognized as a precursor of ethylene formation in plant body. Ethylene production is controlled by a critical enzyme ACC oxidase which converts ACC into ethylene, a rate limiting step in ethylene formation. Change in ethylene production from seed has also been reported under salt stress conditions (Jalili et al., 2009; Wang et al., 2011).

Various morpho-physiological and biochemical changes have been reported in plants to combat damage caused by salinity, including synthesis of plant hormone, alternation in growth pattern and ROS scavenging antioxidants (Sharma et al., 2012). Role of ethylene has also been reported to enhance seed germination under salt stress (Khan and Huang, 1988; Chang et al., 2010). Results of some experiments have shown the alleviative effect of ethylene on salt induced germination inhibition. Synergistic interaction of ethylene with glutamate was reported in enhancing seed germination under salt stress (Chang et al. 2010). Similarly, polyamines and brassinosteroids have also been reported to regulate seed germination under salt stress by interacting synergistically with ethylene (Zapata et al., 2003; Wang et al., 2011).

Salinity can reduce seed germination by limiting the water absorption, generating osmotic stress, and also by creating ion toxicity due to higher ion uptake (Dodd and Donovan, 1999; Almansouri et al., 2001). Osmotic and ionic effects caused by salinity can inhibit seed germination either by reducing water uptake (Dodd and Donovan, 1999; Duan et
Salinity has been reported to be involved in inhibiting α-amylase activity in mung bean cotyledons (Promila and Kumar, 2000), barley (Tipirdamaz et al., 1995) and wheat (Almansouri et al., 2001; Siddiqui et al., 2006).

Białecka and Kępczyński (2009) compared the effect of different rates of gibberellin (GA$_3$) and ethephon on seeds of *Amaranthus caudatus* seeds by measuring germination and starch hydrolyzing enzymes (α- and β-amylase activity) during germination under salt stress. Results indicated that both ethephon and gibberellin were effective in reducing inhibition caused by salinity stress but the effect of ethephon was found prominent compared to GA$_3$. Seed germination was only delayed at lower levels of NaCl (25 and 50 mM) but the degree of inhibition was more severe at higher rates of NaCl. Application of ethephon as well as GA$_3$ increased the activity of α-amylase significantly but activity of β-amylase was not affected during initial 14 h of incubation.

In case where dormancy of seed is induced due to the presence of salinity, ethephon is reported to promote germination. No seed germination of Chenopodiaceae (*Allenrofeea occidentalis*) was observed under 800 mM NaCl salinity level. Application of different chemical i.e. ethephon (10 μM), nitrogenous compounds (20 μM nitrate and 10 μM thiourea) and fusicoccin (5 μM),) were able to alleviate the inhibition of seed germination induced by salinity (Gul and Weber, 1998). Moreover, it has been observed that uninhibited non-dormant seeds produce more ethylene, ACC and ACC oxidase activity than dormant or non-dormant inhibited seeds (Kepczynski and Kepczynska, 1997). Gul and Weber (1998) investigated the effect of dormancy breaking compounds on seed germination under salt stress and reported that ethephon dramatically alleviated salt induced inhibition of seed germination of *Allenrofeea occidentalis*. Esashi and Leopold (1969) verified that ethylene production starts soon after imbibition is capable to stimulate seed germination. Application of ethephon relieved seed dormancy to some extent induced by salt stress in *Zygophyllum simplex* (Khan and Ungar, 1997). Similarly, application of ethephon has also been reported to alleviate germination inhibition in *A. occidentalis* completely induced by salinity (Gul and Weber, 1998). The application of ethylene and ethephon can enhance seed germination in non-dormant seeds by stimulation of germination while in dormant seeds by breaking
dormancy. In short, application of ethylene can reverse inhibition in seed germination induced by stress conditions or dormancy in various plant species (Kepczynski and Kepczynska, 1997). These above mentioned findings clearly shows that ethylene is involved during seed germination under salinity stress. Ethylene dependent mechanism during seed germination was further verified in non-dormant chickpea (Gallardo et al., 1991) and *Amaranthus caudatus* (Kepczynski and Karssen, 1985) seeds in which endogenous level of ethylene were reduced in response to various stresses i.e. salinity and high temperature.

### 2.3 Effect of ethylene on plant morphological characteristics

Phytohormones are organic chemicals in the plant body which can regulate numerous growth processes at very low concentration affecting different morphological characteristics by improving carbon metabolism (Mansfield, 1987; Reid and Howel, 1995). A lot of work has been published regarding the role of ethylene in growth stimulation i.e. increase in shoot length in rice by loosening of cell walls and enhanced formation of lysigenous aerenchyma in maize are prominent examples (Voesenek et al., 2006). Under normal conditions, negligible difference has been observed between wild-type genotypes and ethylene-insensitive genotypes regarding total biomass and growth rate of plant. However, under stress conditions a severe reduction in plant growth rate has been observed in ethylene-insensitive plants particularly during competition for light under dense canopies (Tholen, 2004).

Neljubov (1901) explained that the classical triple response in plants depends upon the sensitivity of plant to ethylene. Triple response is characterized by the horizontal expansion of the hypocotyl, reduced growth in root and hypocotyl along with typical hook formation (Guzman and Ecker, 1990; Abeles et al., 1992). Ethylene hinders the transport of auxin in shoot. Ethylene can also decrease ability of auxin to increase shoot elongation (Morgan and Gausman, 1966). Application of ethephon, an ethylene-generating chemical, has been reported to inhibit leaf and shoot elongation by enhancing lateral growth (Hayashi et al., 2001; Ouzounidou et al., 2008). Effect of applied ethylene and shoot growth responses were also studied by Fiorani et al. (2002) in four Poa (meadow-grass) species. Among these four species *Poa alpine* and *Poa compressa* were categorized into slow growing species having less relative growth rate while *Poa annua* and *Poa trivialis* were categorized into fast growing species having higher relative growth rate. Slow growing species emitted 30 to 50 %
less ethylene in contrast to the fast-growing species. Moreover, Leaf growth rate of slow growing species was promoted in response to the application of low concentration of ethylene (0.02 to 0.03 μL L⁻¹) while it was severely inhibited by increasing concentration of ethylene up to 1μL L⁻¹. However, inhibition in leaf growth rate of the fast growing species was found very less in response to application of higher ethylene. Exogenous application of ethylene has been reported to induce cell division in aquatic plants (Metzer, 1984), potato tubers (Ilker et al., 1977) and pine (Barker, 1979). In addition to cell division, it has also been reported to cause cell elongation in fig fruits (Maxie and Crane, 1968), rice (Ku et al., 1970; Smith and Robertson, 1971) and aquatic plants (Metzer, 1984). Concentration dependent response of leaf expansion to the applied ethylene has been reported in literature. It has been shown that low level of ethylene can increase leaf expansion while higher level of ethylene is proved to be inhibitory. Enlargement of leaves of *Helianthus annus* was observed in response to low concentration ethephon, while leaf expansion was reduced by the application of higher concentration of ethylene (Lee and Reid, 1997), and in mustard (Lone, 2001; Mir, 2002 and Mir et al., 2009a). Ethylene can also affect leaf expansion via inhibiting cell elongation instead of cell division (Kieber et al., 1993; Rodriguez et al., 1993).

In plants the development of root system is an elaborated and highly regulated process that is responsive to environment and is central to the overall well-being of plants and to ensure heavy crop yields (Gales, 1983). Root development is governed by cell division and cell enlargement in apical meristematic zone of root (Scheres et al., 2002). It has been shown that ethylene can severely hinder the enlargement of these cells as displayed by their reduced length (Le et al., 2001). Inhibition in root growth is caused by early cell differentiation or reduced activity in root meristem and in extreme case root completely fails to grow (Blilou et al., 2005). The development of roots is at least as sensitive to ethylene as other plant parts, and root tissues produce the gas in amounts that often reflects conditions at the root soil interface. Ethylene may, therefore, be particularly important in altering the root system. Direct exposure of ethylene gas to roots has been observed to have either a stimulatory or inhibitory influence depending on the concentration applied.

Ethylene has a significant effect on root growth of *Arabidopsis* by promoting radial expansion and inhibiting elongation. Similar responses have been obtained by using ACC as
a source of ethylene. Stimulation of root hairs has been reported by applying both ACC and ethylene (Masucci and Schiefelbein, 1996; Pitts et al., 1998; Tanimoto et al., 1995). Initiation of root hairs and reduced cell enlargement was observed within minutes after the application of ACC and ethylene (Le et al., 2001). By increasing exposure time of ethylene, it was observed that ethylene can regulate overall root shape by enhancing root girth and reducing root elongation.

Contrary to the above findings that ethylene application decreases growth and elongation it has also been reported that low concentration of ethylene can stimulate root elongation (Konings and Jackson, 1979), stem elongation (Suge et al., 1997; Pierik et al., 2003) and leaf expansion (Lee and Reid 1997; Fiorani et al., 2002). A biphasic model was proposed to describe this dual contrasting response of plant growth by application of ethylene (Konings and Jackson, 1979; Lee and Reid 1997). This model integrates both growth inhibition and growth stimulation caused by higher and low levels of ethylene, respectively into one biphasic ethylene response model. Smith and Robertson (1971) reported that exogenously applied ethylene gas at concentration less than 1 ppm inhibited extension of roots in barley while in rice and rye crops root extension was enhanced. However, concentration of 10 ppm reduced root extension in both rice and rye, the reduction was up to 25 % in rice and up to 40 % in rye. For two consecutive years, ethylene was applied as a soil treatment at the rate of 1.59 and 3.14 kg ha⁻¹ and it was observed that ethylene significantly increased the yield of cotton and sorghum by 25 and 13 % (Freytag et al., 1972). Effect of ethylene application on barley was studied by Crossett and Campbell (1975). They found that both root and shoot weights were reduced slightly by ethylene treatment but seminal root extension was inhibited greatly whereas lateral, splitting root growth was stimulated.

Low concentrations of ethylene, usually well below 1 μL L⁻¹ have been found to promote root elongation in many species including broad bean, rice, tomato, maize and peanut when roots of intact seedlings of these species are grown under well aerated conditions. Ethephon also found to involve in increasing seedling root length in watermelon (Citrillus lanatus). Research suggests that a small amount of ethylene enhances root extension in a range of species in relation to the amount of endogenous ethylene present (Mattoo and Suttle, 1991).
Use of CaC\(_2\) based formulation for improving growth and yield of various crops has been documented. Kashif\textit{ et al.} (2012) reported significant root and shoot increase in okra seedlings (\textit{Lycopersicon esculentum} Mill.) with application of acetylene and ethylene releasing compound calcium carbide. Similarly, increase in weight of root and shoot in response to soil applied coated CaC\(_2\) has been observed in cucumber (shakir\textit{ et al.}, 2012), tomato (Siddiq\textit{ et al.}, 2012) and sweet pepper (Ahmed\textit{ et al.}, 2014).

\textbf{i) Role of ethylene in flower induction}

The conversion from vegetative to reproductive growth plays a decisive role in plant development. Plants recognize environmental conditions such as temperature, light intensity and photoperiod, and change the developmental processes from vegetative to reproductive growth (Murfet, 1977; Halevy, 1985). Consequently, plants have developed certain mechanisms with the passage of time to regulate flower induction. Plants have evolved unique ability to combine various environmental signals (Internal and external) to form an integrated response. Basically, flower induction in plants is the net result of integrated effect of internal competence to flower and environmental signals conducive for flowering (Mouradov\textit{ et al.}, 2002).

It includes flowering-time genes, floral meristem identity genes and organ identity genes (Schultz and Haughn, 1993; Weigel and Meyerowitz, 1993; Weigel, 1995; Weigel and Nilsson, 1995; Mandel and Yanofsky, 1995; Nilsson\textit{ et al.}, 1998). The genetic and molecular interactions among genes involved in the transition from vegetative to reproductive growth have been elucidated (Yanofsky, 1995; Pineiro and Coupland, 1998; Mouradov\textit{ et al.}, 2002). It has been shown that the gibberellin (GA) pathway acts additively to regulate flowering time (Blazquez\textit{ et al.}, 1997, 1998), and that phytochrome also affects the floral transition (Bagnall\textit{ et al.}, 1995; Koornneef\textit{ et al.}, 1995, Peeters and Koornneef, 1996; Halliday\textit{ et al.}, 1997). Experiments applying ethylene and chemical inhibitors of its biosynthesis and function have confirmed that ethylene is involved in floral promotion in mango, pineapple, and \textit{Plumbago indica} and in floral inhibition in short-day plants such as Japanese morning glory, cocklebur, \textit{chrysanthemum}, \textit{tobacco} and \textit{Chenopodium} (Abeles\textit{ et al.}, 1992). Role of ethylene seems quite complicated in \textit{Arabidopsis}. However, late flowering was attributed to ethylene insensitivity which signifies the role of ethylene in flower initiation.
ii) Ethylene as a regulator of sex expression

It is well established that ethylene is the key hormonal regulator of sexual expression in cucurbits, controlling not only the sexual fate of individual floral buds, but also pistillate flower initiation i.e. the time at which the first female flower appears. A lot of studies have revealed that ethylene is involved in determining sex expression in numerous monoecious species of the *Cucurbitaceae* (Rudich, 1990). Role of ethylene in promoting pistillate bud production and inhibiting male buds was verified in these studies by using ethephon (ethylene liberating compound), aminoethoxyvinyl glycine (inhibitor of ethylene biosynthesis ethylene) and silver nitrate (an ethylene action inhibitor) in cucurbits (McMurray and Miller 1968; Robinson *et al*. 1969).

Sex determination in cucumber is affected by phytohormones and genotype (Takahashi *et al*. 1983). The genetic control of sex expression in cucumber is determined by the *F* and *M* loci which interact to form three phenotypes: monoecious (*M-ff*), gynoecious (*F-M-*) and andromonoecious (*mmff*) (Kubicki 1969a, 1969b; Pierce and Wehner 1990). Ethylene has also been reported to influence sex expression in the cucumber (Mac-Murray and Miller 1968; Saito and Takahashi 1987; Yin and Quinn1995). Induction of female flower has been reported by application of 2-chloroethylphosphonic acid (ethephon) (MacMurray and Miller, 1968; Iwahori *et al*., 1970). Lines of cucumber and melon differing in sex expression also differ in ethylene sensitivity and production and it was found that levels of ethylene evolution from monoecious or andromonoecious lines were less compared to gynoecious lines of cucumber (Owens *et al*., 1980; Yamasaki *et al*., 2001). It has also been reported that the andromonoecious sex phenotype of melon and cucumber is the result of a reduction in ethylene production conferred by a mutation in the *CmACS7* and *CsACS2* genes (Boualem *et al*., 2008, 2009; Li *et al*., 2009).

The role of ethylene perception to regulate pistillate flower development was investigated in melon by using transgenic plants for the mutant ethylene receptor *etr1-1* of *Arabidopsis*. It was found that ethylene perception is necessary for development and maturity of pistillate flowers (Papadopoulou *et al*., 2005; Little *et al*., 2007). Ethylene production and sensitivity is essential in the regulation of sexual expression in cucurbits, controlling both the conversion from male to female phases of development in monoecious and andromonoecious genotypes and the number of male and female flowers per plant. Yamasaki *et al*. (2003)
investigated the role of ethylene in sex determination at different growth stages of flower buds in cucumber by using ethephon (an ethylene-generating compound) or aminoethoxyvinyl glycerine (an inhibitor of ethylene biosynthesis). Sex expression in both monoecious and gynoecious lines was affected by these treatments only at the floral stage when stamen primordia were differentiated. Moreover, in situ hybridization technique was performed to understand the relationships and accumulation pattern of mRNA of the ethylene-receptor-related genes (CS-ETR1, CS-ETR2, and CS-ERS) and the ACC synthase gene (CS-ACS2) involved in producing female flowers in the cucumber. Results revealed that mRNAs accumulation of ethylene producing genes was detected in the pistil primordia of gynoecious cucumbers whereas its position was just below the pistil primordia and at the adaxial side of the petals in monoecious cucumbers. However, location of mRNAs of ethylene receptor genes were found to overlapped in both monoecious and gynoecious cucumbers. Yu-mei, (2009) investigated the effect of ethylene-concentration and its suitable application methods on some morphological and floral attributes of cucumber. The result showed that ethylene application can obviously inhibit the vine growth, shorten the internode length, increase the number of female flower and reduce the number of male flower. The treatments had no obvious effect on the node of 1st female flower. Among six treatments, the treatment of 100 mg/L of ethylene showered in 1st leaf period can increase obviously the number of female flower and reduce the number of male flower only.

iii) **Conclusions on effect of ethylene on morphological characteristics**

In this review, we examined the effect of ethylene on morphological traits in terms of growth, flower initiation and sex expression. As explained above, ethylene treatment will generally lower the growth of a plant, especially if high concentrations are used. Furthermore, an increasing body of literature reveals a stimulating effect of low ethylene concentrations on both leaf expansion and root growth. Role of ethylene in flower induction and female expression has been revealed by using molecular and genetic approaches. Specifically, in cucumber it has been found that ethylene perception is necessary for regulation of female sex expression. Previous studies have shown that exogenously applied soil and foliar sources of ethylene can induce female flowering in cucumber. In short, exogenous application of various sources of ethylene at optimum rate can manipulate morphology in desired direction in vegetable crops.
2.3 Effect of ethylene on physio-biochemical characteristics

i) Effect of ethylene on photosynthesis

Contradictory results have been reported in the literature relating ethylene-induced stomatal opening and photosynthesis (Taylor and Gunderson, 1986; Gunderson and Taylor, 1989; Kamaluddin and Zwiazek, 2002). Ethylene can influence the process of photosynthesis independently of the senescence pathway. For instance, a lot of studies have shown that ethylene can improve photosynthetic rate of *Brassica juncea* depending on concentration of ethylene applied (Subrahmanyam and Rathore, 1992; Khan, 2004b), most likely via an increase in stomatal conductance (Khan, 2004b). Ethylene has been reported to cause stomatal opening in *Vicia faba* via inducing auxin production (Merritt *et al*., 2001) while close stomata in *Arabidopsis thaliana* by stimulating abscisic acid production (Tanaka *et al*., 2005). Similarly, stomata closure has been reported by various scientists in response to the application of ethylene (Pallas and Kays, 1982; Madhavan *et al*., 1983; Desikan *et al*., 2006). Ethylene can regulate photosynthetic rate and stomatal conductance depending upon plant species and concentration of ethylene applied (Khan, 2004a).

The effect of exogenous application of ethephon on photosynthesis has been well documented including both stimulatory (Subrahmanyam and Rathore, 1992; Grewal *et al*., 1993; Khan *et al*., 2008; Iqbal *et al*., 2011) as well as inhibitory (Kays and Pallas, 1980; Rajala and Peltonen-Sainio, 2001) effect depending upon ethylene concentration and sensitivity of plant species (Pierik *et al*., 2006). Several explanations have been suggested regarding the effect of ethylene on photosynthesis by the application of ethylene generating compounds. Ethylene-induced increase in photosynthesis has been attributed to increase in leaf area via harvesting more solar radiation by leaves (Woodrow and Grodzinski, 1989) or by enhancing chlorophyll contents per unit leaf area (Grewal *et al*., 1993).

Besides improving stomatal conductance, ethylene has also been reported to regulate photosynthetic rate via affecting sugar sensitivity in plants. Glucose is also known as feedback regulator of photosynthesis by down-regulation of Calvin cycle related enzymes (Paul and Pellny, 2003). León and Sheen (2003) investigated interactive effect of ethylene, abscisic acid and sugar sensing on photosynthesis and suggested that ethylene could regulate
gene expression of photosynthesis. Zhou et al. (1998) reported that ethylene-insensitive mutants were more sensitive to endogenous glucose contents. Moreover, reduced photosynthetic rate in ethylene-insensitive plants was attributed to increased sensitivity to endogenous concentration of glucose. Similar response was observed by Grbić and Bleecker (1995) in which chlorophyll contents and Rubisco activity of ethylene-insensitive etr1−1 mutants was found less than wild-type Arabidopsis leaves. Recently, involvement of ethylene perception has been reported in the regulation of photosynthesis. It was found that the absence of functional ethylene receptors caused a reduction in photosynthetic rate and Rubisco expression in both tobacco and Arabidopsis. However, the two species vary significantly regarding the effect on stomatal conductance. The reduced rate of photosynthesis associated with decrease in Rubisco contents was related to an increased sensitivity to endogenous glucose contents (Tholen et al., 2007).

In short, it is implausible to consider that low level of endogenous ethylene inhibits photosynthetic activity. Therefore, use of exogenous ethylene at low concentration could maintain plant growth when plant leaf during active photosynthesis has accumulated sugars contents. Similarly, effect of soil applied CaC₂ on growth, yield and photosynthesis of various vegetable crops has also been documented. It has been shown that at optimum rate CaC₂ can be used as potential source of ethylene to improve photosynthesis in potato, tomato and sweet pepper (Abbasi et al. 2009; Siddiq et al. 2012 and Ahmed et al. 2014).

**ii) Effect of ethylene on nutrient availability**

Among various environmental factors, nutrient availability is one of major factor affecting different growth and morpho-physiological characteristics of plants. Although, availability of essential nutrients is limited in most of soils but plants have certain mechanisms to maintain persistent level of essential nutrients for their survival. Plants in response to nutrient deficient conditions can stimulate different morpho-physiological responses directed to enhance nutrient acquisition that, in many circumstances, result in metabolic and morphological changes in whole plant (Lopez-Bucio et al., 2002). Biosynthesis of plant hormones can influence nutritional homeostasis due to close interdependence between hormonal and nutritional signals. (Krouk et al., 2011). Interplays between mineral nutrients and different hormones has been reported in literature: sulfur(S)
and cytokinins (Maruyama-Nakashita et al., 2004), phosphorus (P) and cytokinins (Franco-Zorrilla et al., 2005), P and auxin (Nacry et al., 2005), potassium (K) and auxin (Vicente-Aguillo et al., 2004), K and jasmonic acid (Armengaud, 2004), iron (Fe) and cytokinins (Seguela, 2008), K and ethylene (Jung et al., 2009), N and ethylene (Tari, and Szen, 1995; Lynch and Brown, 1997; Iqbal et al., 2011), calcium (Ca) and ethylene (Lau and Yang, 1976) and many more.

Role of ethylene has been documented in mineral toxicity and deficiency in the literature. Ethylene has been reported to cope with abiotic stress i.e. nutrient stress by regulating root responses (Lynch and Brown, 1997). Ethylene can regulate lateral root spread, root elongation and also induce root hair emergence via determining cell fate of roots. Role of ethylene has been revealed in numerous stress conditions such as nutrient stress by using ethylene sensitivity mutants. (Zhang et al., 2003).

### iii) Mechanism of action of ethylene under nutrient stress

Ethylene evolution from plant tissue enhanced under various stress conditions (Abeles, 1992). However, ethylene is synthesized through the same pathway under stress such as under optimal conditions. In response to stress different reactive oxygen species are produced causing oxidative damage to the plant cells. Sudden oxidative burst causes activation of mitogen-activated protein kinases cascade (MAPK), and as a result production of ethylene is enhanced by phosphorylation of ACC synthase enzyme which carries on conversion of SAM to ACC (Liu and Zhang, 2004). To understand the mechanism of action of ethylene during nutrient stress conditions a diagrammatic view is show in Figure 1.1.

It is shown that oxidative stress is produced in response to nutrient stress which causes the activation of MAPK cascade and consequently ethylene production is enhanced in plant system. Ethylene can improve stress tolerance by acting individually or integrating with other hormones. Ethylene can maintain cellular homeostasis by inducing gene expression which acts as a defense mechanism against stress.
Figure 1.1 Diagrammatic illustration of interaction between ethylene, nutrient and plant response adapted from Iqbal et al. (2011)
Ethylene has been reported to increase nutrient uptake under nutrient deficient conditions via increasing auxin sensitivity or adventitious root development (Visser et al., 1996), root hair initiation (Dolan, 2001). Similarly, effect of ethylene and auxin to promote root hair formation was observed by Pitts et al. (1998) and Rahman et al. (2002). Ethylene can also enhance root hair development by activating genes responsible for cell expansion (Cho and Cosgrove, 2002) which further increases nutrient uptake. Ethylene has also been reported to increase nutrient uptake and transport by activating nutrient transporters under nutrient deficient conditions leading to nutrient tolerance. Effect of ethylene on plant responses under nutrient stress conditions explained in a simplified flow chart (Figure 1.1).

iv) Effect of ethylene on nitrogen metabolism

In natural environments, nitrogen is commonly considered as deficient nutrient and therefore, in order to overcome this deficiency plants have developed certain mechanisms to absorb nitrogen from their environment efficiently and then assimilate it into amino acids. Plants absorbs nitrogen in forms of ammonium ($\text{NH}_4^+$) and nitrate ($\text{NO}_3^-$) but later one is the most common form of nitrogen (N) available to higher plants. After absorption of $\text{NO}_3^-$ by the plant it is reduced in the roots as well as in leaves and can also be accumulated in vacuoles. Reduction of nitrate to ammonium takes place in two successive steps. In the first step nitrate is converted into nitrite by nitrate reductase (NR) and probably considered rate-limiting step that plays a critical role in the regulation of N assimilation. Moreover, different environmental factors i.e. source of nitrogen, light, temperature, $\text{pH}$ and carbon dioxide have been found to affect the activity of NR enzyme (Long et al., 1992).

Activity of nitrate reductase is dependent on $\text{NO}_3^-$ availability from a putative metabolic pool (Aslam et al., 1976; Ferrari et al., 1973). However, most of the tissue $\text{NO}_3^-$ may be in a storage pool in vacuoles, and not readily available for enzyme induction (Granstedt and Huffaker, 1982; Martionoia et al., 1981). The movement of $\text{NO}_3^-$ from the storage pool to the metabolic pool probably determines NR activity. Therefore, plant growth regulators such as ethylene which increase membrane permeability would probably enhance NR activity by promoting availability of storage $\text{NO}_3^-$ for enzyme induction and activity. Plant hormones have been shown to affect the regulation of ion transport (Van-Steveninck,
Glutamine synthetase (GS) found in cytosol is known for its key role in converting ammonium ion into glutamine. Results from various experiments have shown that cytosolic isoform of GS is particularly important for flowering plants and it can assimilate ammonium from both primary nitrogen assimilation and recycling. Genetic studies on maize crop revealed that GS activity was increased during seed germination which caused early germination. During seed germination various cytosolic GS genes have been detected displaying certain cellular expression (Canton et al., 1999; Glevarec et al., 2004; Rodriguez et al., 2006). Gomez-Maldonado et al. (2004a) suggested that gibberellic acid is involved in the regulation of cytosolic GS activity particularly during initials growth stages of pine development.

Palmer (1985) reported that nitrate reductase activity was stimulated in roots and stems, but suppressed in leaves of potato plants grown in nutrient culture by 30 mg L\(^{-1}\) ethephon applied to the culture solution. In stems, nitrate reductase activity was stimulated after 5 h and by 24 h it was more than two fold that of the control. The magnitude of stimulation by ethephon was less in roots compared to stems. Ethephon treatment enhanced ethylene production by roots, stems and leaves but the level of production was not significantly different in these organs. The stimulation of nitrate reductase activity was prevented by cydoheximide and cordycepin suggesting the involvement of new protein synthesis. However, ethephon enhanced trichloroacetic acid (TCA) precipitable protein and amino nitrogen levels in both roots and stems while that in leaves was not significantly affected.

Ge et al. (2008) investigated the effect of ethephon on nitrogen metabolism and photosynthesis characters of peanut under field conditions. Applying different concentrations of ethephon could increase chlorophyll contents and photosynthetic rate of functional leaves.
of peanut, which was in favor of synthesis and accumulation of photosynthesis outcome, and development of grain yield and quality. The transports of nitrogen from functional leaves to grain were increased. The experiment showed that the decreasing magnitudes of total N and protein N in the functional leaves of treatment were higher as compared with control. The activities of the nitrate reductase, glutamine synthetase, glutamine transferase and protease of functional leaves were obviously increased, and the contents of total N, protein N of grain were increased with applying different concentrations of ethephon. From the experiment we could conclude that 150 mg/L was the optimum spraying concentration of ethephon.

Recently, Iqbal et al. (2011) reported that application of exogenous ethephon increased photosynthetic characteristics, nitrogen use efficiency and growth in mustard under both deficient and adequate levels of nitrogen. It was also observed that ethylene production increased at much higher rate under deficient level of nitrogen which caused decrease in photosynthesis and nitrogen use efficiency in mustard. This higher production rate of endogenous ethylene in plants grown under nitrogen deficient conditions was considered as stress ethylene which showed inhibitory effect on plant growth and photosynthetic characteristics. It was concluded that the application exogenous ethephon can improve nitrogen use efficiency and photosynthesis via increasing ethylene perception in plants grown under deficient and adequate levels of nitrogen.

v) Conclusions on effect of ethylene on physio-biochemical characteristics

Above literature highlights the pivotal role played by the exogenous ethylene along with basal nutrients in the form of nitrogen on various physio-biochemical characteristics like: photosynthesis, nitrate reductase activity, nutrient accumulation, nitrogen efficiency like nitrogen uptake efficiency, nitrogen use efficiency and physiological use efficiency. Ethylene trigger the nitrogen metabolism by transporting nitrogen from functional leaves to reproductive parts and also enhance activity of nitrogen assimilation enzymes. Ethylene can improve photosynthesis either increasing leaf area or by enhancing chlorophyll contents per unit leaf area. Further, under nutrient stress conditions, exogenous ethylene can enhance stress tolerance individually or integrating with other hormones. Ethylene can maintain cellular homeostasis by inducing gene expression increase nutrient uptake by increasing auxin sensitivity or enhancing root hairs and adventitious development. A complete
understanding of the ethylene and nutrients interaction would provide new strategies for improving crop vigor and development under changing environment.

2.4 Effect of ethylene on attributes related to fruit quality

The chemical composition and content of nutrients found in vegetables make an important contribution to human health (Vicente et al., 2009). Because the nutritive value of the cucumber is lower than those other vegetables, the quality of cucumber fruits is usually classified based on the shape, uniformity, skin color and defects. In Pakistan, the cucumber fruits are harvested at immature stage based on fruit length (about 20 cm) and fruit weight (about 100 g) as a standard. Recently, because consumers are becoming more interested in their health, and there is growing interest in minerals, amino acids and antioxidants contained in cucumber fruits.

In fresh vegetables, the main components affecting the taste are amino acid and sugar. In fruits, amino acid and sugar synthesis is performed by the use of photosynthate synthesized by leaves and absorbed inorganic nitrogen from roots. Recently, sweetness is key factor for the taste quality and preference of cucumber. Sugar concentration in cucumber fruits is low just after anthesis that increases with enlargement of the fruits especially and accumulated rapidly just before harvest (Davies and Kempton, 1976). Strength of sweetness is different depending on the kind of sugar because the sweetness degree is high in fructose, sucrose, and glucose in this sequence. The main soluble sugars in cucumber fruits are glucose and fructose, at nearly equal amounts, with extremally small amount of sucrose and others (Nakamachi et al., 2002; Davies and Kempton, 1976; Horie, 2011). Just before harvest stage, the fruits rapidly enlarged, therefore, a large amount of photosynthate supply during this period is required for increasing the sugar concentration in fruits. On the other hand, sugar content is known to decrease when photosynthate supply is not enough under unfavorable conditions (Horrie, 2011). As role of ethylene hormones is known regarding efficient nitrogen utility and photosynthesis and it is likely that improved translocation of the photosynthate and nitrogenous metabolite can produce high quality cucumber fruits.
2.5 Effect of different sources of ethylene on yield attributes

On the basis of mode of application, ethylene sources are broadly categorized into two following types

i) Foliar-applied sources

ii) Soil-applied sources

In foliar application mode, different sources of ethylene i.e. ethephon/ethrel are directly sprayed on foliage surfaces which liberate ethylene after getting entry into plant tissue. In soil applied sources, different precursors of ethylene i.e. methionine, ethanol and CaC₂ etc. are applied into rhizosphere from which soil microorganisms can derive ethylene (C₂H₄) which at physiologically active concentration in soil environment can influence different morpho-physiological and yield characteristics of plant.

i) Effect of foliar-applied ethephon/ethrel on yield attributes

The manipulation of the plant frame and inducing flowering at early stages is the prime focus of research regarding productivity enhancement and early development of vegetables using various plant growth regulators. Brown and Early (1973) studied the effect of ethrel (an acetylene + ethylene producer) application on wheat and oat. Their result showed that ethrel application @ 2.24 kg ha⁻¹ effectively reduced lodging in both crops while application @ 0.56 kg ha⁻¹ increased yield significantly. The yield increase was 15.8 % in wheat and 7.8 % in oat. Soil trenched or foliarly applied ethephon can affect the growth of various plants.

The influence of the three plant growth regulators, maleic hydrazide, ethephon and naphthalene acetic acid, on the morphological, floral and yield traits of cucumber was investigated by Thappa et al. (2011). Two of the each plant growth regulators, maleic hydrazide and ethephon, were applied at two different concentrations of 100 and 200 ppm and the third was applied at 50 and 100 ppm at the two-, four- and six-leaf and full-bloom stages using variety “Cucumber Long Green”. The results revealed that combined application of 100 ppm maleic hydrazide and 100 ppm ethephon induced early development, maximized the sex ratio with regard to yield and was comparatively helpful in reducing plant expansion. This treatment also produced the best economic results for the production of cucumber.
Hong-yan (2007) reported that application of ethrel to the plant increased the number of cucumbers' pistillate flowers, the overgrowing of cucumber was controlled and the yield of it was improved, furthermore, this kind of treatment affects the physical activity and quality of cucumber. By treating with suitable concentration of ethrel (50 or 150μl·L⁻¹ separately), either the differentiation of pistillate flowers was promoted, or the physical activity of leaves was maintained well, which won't cause premature senescence, consequently laid a foundation for the high yield and high quality of cucumber.

Bin-bin et al. (2009) investigated the effect of ethrel in different rates, different dates and different times on the yield and the main agronomic characters of Cucumber "Jing Chun No.4". The results showed that the treatment with the concentrations of 100 μL L⁻¹ ethephon at different concentrations in one-leaf and two-leaf period by one time could efficiently increase female flower node ratio and yield, especially the prophase yield, and had no effect of aberration. The internode length and plant height were shortened; node ratio without flower, the female node, the female flower ratio and the number of affected node was reduced after the cucumber were sprayed with ethrel.

Li et al. (2011) studied effect of different concentrations of ethephon and applying times on cucumber Lüdao No.3. The results showed that using 80 to 100 mg/kg ethephon two times on cucumber Lüdao No.3 at the stage of two-leaf and three-leaf seedling could raise its early yield and total yield, and could improve growth and fruit quality which indicates that this method can be applied in Lüdao No.3 greenhouse in spring crop. High concentration of ethephon or more application times would weaken plant growth, reduce female flower rate, shorten fruit length, and decrease early and total yield.

ii) Effect of soil-applied calcium carbide on yield attributes

A number of studies have revealed that slow release of ethylene from different ethylene releasing compounds in the soil environment, can increase yield of different vegetables like potato cucumber, tomato, , and hempseed. The influences of ethylene released from calcium carbide (CaC₂) were observed by Bibik et al. (1995) and results reported that CaC₂ could increase tuber development, potato yield, and tuber shelf life of potato. It is expected that a appropriate formulation of CaC₂ could enhance plant growth due to dual role, i.e., inhibiting nitrification losses of nitrogen through action of acetylene and
also by releasing ethylene as plant growth regulator in rhizosphere (Yaseen et al., 2006). The effects of soil-applied ethylene released from CaC$_2$ on morpho-physiological and yield characteristics of crops are reviewed below:

The hormonal effect of ethylene liberated by ECC under soil moisture conditions enhances the activity of nutrient use by the plant. The results of field trial conducted by Saleem et al. (2002) to evaluate influence of encapsulated calcium carbide on growth, yield and chemical composition of okra showed that application of CaC$_2$ @ 90 kg ha$^{-1}$ was most effective in yield and yield contributing factors. Increase in horizontal expansion of plant, yield of green pods, number of green pods per plant, fresh and dry weights of shoot and root and internodal length was recorded by applying this rate of CaC$_2$. While plant height decreased with increase in CaC$_2$ application rate. The chemical analysis of green pods and plant root revealed that increased P and K contents with increased application of CaC$_2$. Phosphorus contents in shoots were decreased while that of K increased with increase in CaC$_2$ application from 0-90 kg ha$^{-1}$.

Kashif et al. (2008) conducted experiments under laboratory and field conditions to study the effect of CaC$_2$ on growth and yield of okra (Hibiscus seulentus L.). On the basis of their results obtained, it was shown that calcium carbide can effectively be used as a potent source of acetylene (nitrification inhibitor) and biologically produced ethylene in soil. Application of calcium carbide @ 60 kg ha$^{-1}$ along with half of the recommended N fertilizer (60 kg N ha$^{-1}$) increased pod yield about 37 % compared to control and fertilizer alone treatments.

Shakir et al. (2012) studied the effect of different concentrations of paint coated calcium carbide (0, 100, 200, 300 and 600 mg pot$^{-1}$) on growth and yield parameters of cucumber cv. Bolan-F1. Calcium carbide was applied along with and without recommended rates of NPK fertilizer. Results revealed a significant increase in total number of flowers, female flower percentage, number of fruit, fruit weight, fruit length, fruit diameter and ultimately yield as compared to control. Moreover, calcium carbide applied at all rates significantly decreased the number of days to flower initiation, flower drop percentage and plant height compared to recommended fertilizer alone and control.
Kashif et al. (2012) also observed that calcium carbide can effectively improve the germination of okra seeds with a callus formation on roots during the germination stage which gave an early secondary root formation as well as a well-developed root system at initial stages thereby increasing plant ability to absorb nutrients from a wider root zone, which ultimately give early flowering and fruiting even increasing green pod yield of okra up to 27% over control which was attributed towards calcium carbide application.

Siddiq et al. (2012) evaluated responses of tomato (Lycopersicon esculentum Mill.) cultivars to different rates (0, 100, 200 and 300 mg pot\(^{-1}\)) of polyethylene coated CaC\(_2\) applied after two weeks of transplanting into pots. Results revealed a significant reduction in plant height, number of days to flowering with increased number of fruits per plant from CaC\(_2\) treated plants. These parameters ultimately contributed to increase tomato fruit yield. Data regarding fruit yield showed that the mean maximum yield was actually 39 % more over control. Statistically, effect of CaC\(_2\) application at the rate of 300 mg pot\(^{-1}\) showed most significant results among all doses of CaC\(_2\).

Similarly, hormonal properties of calcium carbide were studied regarding its impact on physiological nutrient use efficiency and vegetative growth of sweet pepper (Ahmed et al. 2014). Application of 20 mg PCC kg\(^{-1}\) soil with soil applied recommended dose of NPK fertilizers significantly improved the net photosynthetic rate by 32%, stomatal conductance by 11%, transpiration rate by 14%, carboxylation efficiency by 47%, physiological water use efficiency by 13%, physiological nitrogen use efficiency by 29% over the control treatment. This improvement in physiological attributes resulted in increase in leaf area by 20%, leaf area index by 78%, total plant dry weight by 35%, flower and fruits by 29% and fruit yield by 24% compared to the treatment of alone recommended dose of NPK fertilizers.

### iii) Conclusions on effect of different sources of ethylene on yield attributes

Exogenous application of foliar as well as soil-applied sources of ethylene can enhance yield production of different vegetable crops by stimulating nitrogen and carbon metabolism, flowering, fruit setting and yield contributing factors. For cucumber, yield and its contributing traits such as days to fruit maturity, number of female flowers, number of fruits per vine, yield per plant and yield per hectare were influenced by the application of
optimum concentration of ethylene either soil or foliar-applied source of ethylene. For cucumber production, increased female sex ratio, earliness in fruiting and higher fruit yield are an important concern. Due to correlation between fruit yield and sex expression, the importance of modification of sex expression is considered a great interest for plant scientists. Therefore, any treatment that would increase the formation of pistillate flowers would therefore, be beneficial in producing high yield.

2.6 General conclusion

The literature described in detail above on the effect of calcium carbide on different aspects of plant growth clearly indicates the role of calcium carbide in influencing different morpho-physiological attributes of plants. Improvement in photosynthesis and nitrogen use efficiency has been well documented in various vegetables under the supply of recommended N levels in the presence of calcium carbide as a potent source of acetylene and ethylene (Yaseen et al., 2012; Siddiq et al., 2012; Ahmed et al., 2014), but the interactive effect of CaC$_2$ as source of ethylene and N availability on the photosynthesis and nitrogen assimilation enzymes has not been worked out in detail using cucumber as a test crop. Moreover, the effect of ethylene on seed germination and ROS metabolism under normal and salt stress conditions especially in cucumber (Cucumis sativus L.), a salt sensitive crop, is need to be studied whether exogenous application of CaC$_2$ as a cheaper source of ethylene could mitigate germination inhibition of seeds under salinity stress. In short, present study is focusing to explore different dimensions of the use of CaC$_2$ as a growth promoter which is not previously studied in detail. The outcome of the present study will surely add some new findings in the current literature.
A series of experiments was conducted to study the effect of CaC\textsubscript{2} on morphological and physiological parameters of cucumber under normal and stress conditions. These experiments were conducted at the Soil Fertility and Plant Nutrition laboratory, wire house and fields of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Details of the experiments are given below under different experiments:

A) Laboratory experiments under controlled conditions

Experiment 1: Seed germination response of cucumber to different rates of calcium carbide

Experiment 2: Effect of different rates of CaC\textsubscript{2} on biochemical changes, chlorophyll contents and nitrogen metabolism in cucumber

Experiment 3: Effect of CaC\textsubscript{2} on germination of cucumber seeds to alleviate salinity stress

B) Pot experiments under wire house conditions

Experiment 4: Evaluation of different rates and coating materials on calcium carbide for morphological, floral and yield attributes of cucumber

Experiment 5: Optimizing different rates of paint coated CaC\textsubscript{2} for ethylene production, some morpho-phenological and yield attributes of two cucumber cultivars differing in yield potential

C) Field experiment at Institute of Soil and Environmental Sciences

Experiment 6: Effect of paint coated CaC\textsubscript{2} on photosynthesis, nitrogen use efficiency and fruit quality of cucumber under deficient and adequate levels of nitrogen

A) Laboratory experiments under controlled conditions

Experiment 1 Seed germination response of cucumber to different rates of calcium carbide

The experiment was conducted with the objective of selecting the best level of CaC\textsubscript{2} on the basis of germination and growth response of cucumber to different rates of CaC\textsubscript{2}. The experiment was carried out by following procedures as given below
1.1 Site of experiment

The experiment was carried out under controlled growth conditions in an incubator (Sanyo MIR 253) at 25 ± 1 °C with 14 h photoperiod in the laboratory of Soil Fertility and, Plant Nutrition, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad.

1.2 Cucumber cultivar

Seeds of cucumber cultivar Bolan F-1 were sown in sterilized disposable petri plates. Prior to using the seeds in this experiment, seeds were sterilized by washing with 70% ethanol (to remove any fungus/dust present on seed coat), then washed several times with distilled water under aseptic conditions in a laminar flow cabinet. These surface sterilized seeds were used in this experiment.

1.3 Experimental set up

Plastic petri plates with covers were used for germination of seeds. Holes were made in lids of petri plates; rubber septa were plugged in holes and sealed with silicon gel. Round shaped sheets of filter papers (Whatman 42, Schleicher and Schuell) were placed in petri plates. Analytical grade calcium carbide (27 % a.i. CaC₂, Ningxia National Chemical Group Co. Ltd., China) was used. De-ionized water was injected in petri plates through rubber septum using disposable syringe (10 ml) for watering.

1.4 Experimental design

The experiment was laid out according to Completely Randomized Design (CRD) with four replications as described by Steel et al. (1997). All seed germination related indexes were analyzed at different rates of CaC₂ (as mentioned in section 1.6) by subordination function. The calculation formulae are as given below:

\[ X(\mu) = \frac{(X - X_{\text{min}})}{(X_{\text{max}} - X_{\text{min}})} \]  \hspace{1cm} (1)

\[ \text{Or } X(\mu) = 1 - \frac{(X - X_{\text{min}})}{(X_{\text{max}} - X_{\text{min}})} \]  \hspace{1cm} (2)

1.5 Experimental conditions

Petri plates were arranged randomly according to the experimental design in incubator (Sanyo MIR 253) at 25 ± 1 °C day/night temperature with 14 h photoperiod using cool white fluorescent light.
1.6 Treatment plan

- **T<sub>1</sub>**  Control (without CaC<sub>2</sub>)
- **T<sub>2</sub>**  CaC<sub>2</sub> @ 10 mg plate<sup>-1</sup>
- **T<sub>3</sub>**  CaC<sub>2</sub> @ 20 mg plate<sup>-1</sup>
- **T<sub>4</sub>**  CaC<sub>2</sub> @ 30 mg plate<sup>-1</sup>
- **T<sub>5</sub>**  CaC<sub>2</sub> @ 40 mg plate<sup>-1</sup>

In control plates, calcium sulphate was added to adjust amount of calcium added through CaC<sub>2</sub> in calcium carbide treated petri plates.

1.7 Methodology

Effect of calcium carbide on seed germination of cucumber seed was studied under controlled growth conditions of incubator and laboratory. Required amount of CaC<sub>2</sub> was spread over round shaped filter paper placed at the bottom of each petri plate. Five sterilized seeds of cucumber cultivar Bolan-F1 were sown on another round shaped filter paper placed above the CaC<sub>2</sub>. The seeds were covered with a third round shaped filter paper to control floating and displacement of seeds from their original positions after application of deionized water. Deionized water was injected into petri plates through rubber septa. After putting the lid back, petri plates were sealed with the help of parafilm and arranged in incubator according to the conditions mentioned in section 1.5. Each treatment was repeated four times.

1.8 Parameters studied

The following parameters were studied during growth period:

**i- Ethylene analysis**

Ethylene (C<sub>2</sub>H<sub>4</sub>) concentrations were determined by using method as described by Khalid *et al.* (2006b) from gas samples collected from petri plates using Gas Chromatograph (Shimadzu GC 2010) fitted with flame ionization detector (FID) and a capillary column (Porapak Q 80-100) operating isothermally under the following conditions: Carrier gas, N<sub>2</sub> (13 ml min<sup>-1</sup>); H<sub>2</sub> flow rate, 30 ml min<sup>-1</sup>; Air flow rate, 300 ml min<sup>-1</sup>; Sample volume 1 ml; Column temperature 70 °C. Ethylene concentrations were determined by comparison with reference standards of C<sub>2</sub>H<sub>4</sub>. 
ii- Germination potential

Root length of 0.2 cm was taken as the germination mark of seeds. The germination potential (GP) of cucumber seeds were calculated after culturing for 2 days by using following formula:

\[ GP(\%) = \frac{\text{Number of germinated seeds after 2 d}}{\text{Total seed number}} \times 100 \]  

(3)

iii- Germination rate

The germination rate (GR) of cucumber seeds were calculated after culturing for 4 d by using following formula

\[ GR(\%) = \frac{\text{Number of germinated seeds after 4 d}}{\text{Total seed number}} \times 100 \]  

(4)

iv- Germination index

The germination index (Gi) was calculated by using the formula:

\[ Gi = \sum \left( \frac{Gt}{Dt} \right) \]  

(5)

v) Growing vigor index

Growing vigour index (GVI) was calculated by multiplying average fresh weight of seedlings with germination index:

\[ GVI = Gi \times \text{average fresh weight of seedlings} \]  

(6)

1.9- Morphological characteristics

The length and width of tap root and hypocotyledonary axis were determined after culturing for 7 days with the help of mm scale and vernier caliper, respectively. At the same time, the fresh weight and number of lateral roots were determined.
Experiment 2 Effect of different rates of CaC\textsubscript{2} on biochemical changes, chlorophyll contents and nitrogen metabolism in cucumber

This experiment was conducted in two parts, in part (1) effect of different rates of CaC\textsubscript{2} with and without addition of KNO\textsubscript{3} on germination and seedling growth parameters were studied. In part (2) response of isolated cotyledons to different rates of CaC\textsubscript{2} with and without addition of KNO\textsubscript{3} on nitrogen metabolism indicators was studied.

2.1 Site of experiment

It was conducted under controlled conditions in an incubator (Sanyo MIR 253) at 25 ±1 °C with 14 h photoperiod in the laboratory of Soil Fertility and Plant Nutrition, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad.

2.2 Cucumber cultivar

Seeds of cucumber cultivar Bolan-F1 were washed and sterilized as described in section 1.2 with 70\% ethanol to remove any fungus and dust present on seed coat, then washed several times with distilled water. Sterilized seeds were then used in experiment.

2.3 Experimental materials

Plastic petri plates with covers were used for germination of seeds. Holes were made in lids of petri plates; rubber septa were plugged in holes and sealed with silicon gel. Round shaped filter papers (Whatman 42, Schleicher and Schuell) were placed in petri plates as described in section 1.3. Analytical grade calcium carbide (27 \% a.i. CaC\textsubscript{2}, Ningxia National Chemical Group Co. Ltd., China) was used. De-ionized water was injected in petri plates through rubber septum by using disposable syringe (10 ml) for moistening the seeds.

2.4 Experimental design

The experiment was laid out according to 2-Factor Completely Randomized Design (CRD) with four replications according to the procedures described by Steel et al. (1997).

2.5 Treatment plan

Levels of CaC\textsubscript{2}: 4 (0, 20, 30 and 40 mg CaC\textsubscript{2})

Levels of NO\textsubscript{3}: 2 (0, 20 mM)

Calcium sulphate was added in control petri dishes to equalize amount of calcium added through CaC\textsubscript{2} in calcium carbide treated petri plates.
2.6 Methodology

2.6 Methodology (Part 1):

Sterilized seeds of cucumber cultivar Bolan-F1 as used in the experiment were sown on round shaped filter paper sheet placed above the CaC₂ powder in bottom of sterilized disposable petri plates as described in section 1.7 of experiment 1. These plates were randomly placed win an incubator (Sanyo MIR 253) at 25 ± 1 °C with 14 h photoperiod. In Petri plates CaC₂ was applied at different rates (0, 20, 30 and 40 mg plate⁻¹) with and without KNO₃ (20 mM). After adding CaC₂, petri plates were sealed with the help of parafilm and solution of 20 mM KNO₃ was injected into petri plates through rubber septa. Germination was recorded after 48 h when the radical became visible. The growth analysis was started by observing 7-day-old seedlings raised in the petri plates. Growth analysis was performed on the basis of 7th day observations to determine the length and weight of root and shoot of cucumber.

2.6 Methodology (Part 2):

Same sterilized seeds of cucumber cultivar Bolan-F1 were sown in sterilize disposable petri plates as described in sections 1.7 and 2.6 to study germination in an incubator (Sanyo MIR 253) at 25 ± 1 °C for 48 h. Then cotyledons were excised and left under constant illumination at 25 ± 1 °C for 72 hours for growth and greening. Uniform sized cotyledons were selected and transferred to petri plates where CaC₂ was applied at different rates (0, 20, 30 and 40 mg plate⁻¹) with and without NO₃⁻. After adding CaC₂, petri plates were sealed with the help of parafilm and solution of 20 mM KNO₃ was injected into petri plates through rubber septa. Treated cotyledons were illuminated with fluorescent light, at a light intensity of 100 u W m⁻² S⁻² for 48 h, and then subjected to biochemical analyses. Controls were incubated either in distilled water or in 20 mM KNO₃.

2.7 Biochemical Parameters studied

The following biochemical parameters were studied after incubation:

i- Extraction procedure

Fully expanded cotyledons were taken after 48 hour of incubation in light to determine the concentration of total soluble carbohydrates, free amino acids and proteins in each replication. Fresh weight was recorded and samples were stored at -20°C until processing. Sample processing and extraction of free amino acids and soluble carbohydrates
were optimized for cucumber (Hwang et al., 1997). With a pre-chilled mortar and pestle, frozen cotyledon samples were ground to a powder state. A solution of 80% ethanol was added to the ground tissue in a ratio of 3:1 (v/w), ground for a few more seconds and 1 mL sample was transferred to eppendorf tubes. The sludge was centrifuged at 10,000 g for 20 min at room temperature. The clear supernatant was collected and stored at -20°C until use for quantification of total free amino acids and carbohydrate concentrations. The pellet from the ethanol extraction was homogenized in 1 mL of 1 M of NaOH and incubated for 2 hours at 37°C. The homogenate was centrifuged at 10,000 g for 10 min at room temperature. The supernatant was transferred to a new eppendorf tube. The pellet was reextracted under the same conditions with an additional 0.5 mL of 1 M of NaOH and the supernatant mixed with the previous extraction. The resulting greenish supernatant (about 1.5 mL) was quickly stored at -20°C until quantification of total protein.

ii- Total soluble sugars

The anthrone method was used to quantify total carbohydrates following manufacturer’s specifications (Sigma Chemical Corp, St Louis, MO). An anthrone solution was prepared right before analysis by dissolving 0.1 g of anthrone in concentrated sulfuric acid. A total of 1.5 mL of anthrone and sulfuric acid solution was transferred to glass test tubes. Twenty-five mL of standard solution (with known concentrations of glucose) or cotyledon samples were added in duplicates to the anthrone solution. The mixture was heated in a boiling water bath for 10 min. Tubes were left to cool down at room temperature before reading the absorbance at 600 nm with a spectrophotometer. Total carbohydrate content was expressed per gram of fresh weight tissue.

iii- Total free amino acids

Total free amino acids were quantified using ninhydrin reagent as described in Yemm and Cooking (1955). A solution of 1:2 of ninhydrin reagent Ni632 (Sigma Chemicals, St. Louis, MO) and distilled water was prepared and 1 mL put into glass test tubes. A standard curve was prepared with leucine in 0.05% glacial acetic acid ranging from 0.1 to 1.5 mg mL⁻¹. Twenty-five mL of standards or cucumber samples were added to the ninhydrin solution in duplicates and mixed by vortexing. Immediately, the tubes were placed in a boiling water bath for 10 min. After the heat treatment, tubes were left to cool down at room temperature and 2 mL of 95% ethanol was added to each tube. The contents of tubes were
mixed by vortexing and absorbance was read at 570 nm on spectrophotometer. Total free amino acids were expressed per gram of fresh weight tissue.

**iv- Total Soluble proteins**

Bradford’s method (Bradford, 1976) was used to quantify total protein content. The concentrated dye was prepared by diluting the Bradford reagent fivefold in dH$_2$O (1 part Bradford: 4 parts dH$_2$O). After filtering the diluted reagent through Whatman 540 paper it was stored at 4°C.

**v- Estimation of nitrate reductase activity**

Nitrate reductase activity (NR) in vivo was assayed by following Jawarski (1971) method. Cotyledons were cut into small round discs. Approximately 200 mgs of leaf discs were suspended in a screw cap vial (25 ml) containing 5 ml of a medium consisting of 0.1 M phosphate buffer (pH 7.5), 0.02 M KNO$_3$, 5% propanol and two drops of chloramphenicol (0.5 mgs/ml). The vial was sealed and kept in the dark at 25°C for 30 minutes. The reaction was stopped by adding 0.1 ml of zinc acetate (1M) and 1.9 ml of ethanol (70%). The contents were centrifuged at 3000 g for 10 minutes and supernatant was collected. Eleven ml of sulphanilamide (1%) and 1 ml of 0.02% N-Naphthyl ethylene diamine dihydrochloride (N-NEDD) were added to supernatant and incubated at room temperature for 20 minutes and absorbance was recorded at 540 nm. Nitrate reductase activity was determined from a standard curve of KNO$_2$ and expressed as n moles NO$_2$ formed per gram fresh weight per hour.

**vi- Estimation of Glutamine synthetase activity**

Glutamine synthetase (GS) activity in cotyledon was measured using the ‘transferase’ assay (Lea and Blackwell, 1993). Approximately 0.5 g of cotyledon was ground in a mortar and pestle, then homogenized in 5 mL GS extraction buffer containing 50 mm Tris-HCl, 1 mm EDTA, 2 mm dithiothreitol (Sigma), 10 mm MgSO$_4$ (Sigma), 5 mm glutamate (Sigma), 10% v/v ethanediol [Ethylene glycol (synonym)] (Sigma) and 0.1% insoluble polyvinylpyrrolidone (PVP) (Sigma); buffer pH was set to 7.8 using 1 M NaOH. The homogenized extract was centrifuged at 17, 000 g for 45 min at 4 °C. GS activity was measured in a buffer consisting of 100 mm Tris-HCl at pH 7.8, 5 mm NH$_2$OH (Sigma), 50 mm MgSO$_4$ (Sigma), 50 mm glutamate (Sigma) and 20 mm ATP (Sigma). 0.375 mL of assay buffer was pre-incubated at 30°C, followed by addition of 0.3 mL supernatant. The reaction
was allowed to proceed for 30 min, and terminated by the addition of 1 mL FeCl₃ reagent (Sigma) [2.5% w/v FeCl₃, 5% w/v trichloro acetic acid (Sigma) in 1.5 M HCl. Controls were performed under identical conditions, except that ATP was absent. The resulting precipitate was centrifuged at 10,000 g for 5 min, and the absorbance of the supernatant was measured at 540 nm, and compared with a standard curve of glutamyl hydroxymate (Sigma).

**vii- Estimation of chlorophyll contents**

Chlorophyll a, b and carotenoids were determined using the method of Dere *et al.* (1998). The weighed samples, 100% acetone (50 ml for each gram), were homogenized with the B-Brawn type homogenizer at 1000 rpm for one minute. The homogenate was filtered through two layer cheese cloths, and was centrifuged at 1000 g for ten minutes. The supernatant was separated and the absorbance was read at 400-700 nm on T80 UV/VIS spectrophotometer, PG instruments limited. It was recorded that chlorophyll a showed the maximum absorbance at 662 nm, chlorophyll b at 646 nm and total carotenoids at 470 nm and the amount of these pigments in ug g⁻¹ fresh weight was calculated by using equations given below:

\[
\text{Chl a} = (11.75 \times A_{662} - 2.350A_{645}) \times \text{volume of supernatant} \times \text{dilution factor/sample mass}
\]

\[
\text{Chl b} = (18.61 \times A_{645} - 3.960 \times A_{662}) \times \text{volume of supernatant} \times \text{dilution factor/sample mass}
\]

\[
\text{Car} = [(1000\times A_{470} - 2.270\times \text{Chl a} - 81.4\times \text{Chl b})/227] \times \text{volume of supernatant} \times \text{dilution factor/sample mass}
\]
Experiment 3 Effect of CaC$_2$ on germination of cucumber seeds to alleviate salinity stress

This experiment was performed in four parts under controlled conditions to investigate germination response of cucumber seeds to CaC$_2$ under salinity stress. Details of each part are given in section 3.4.

3.1 Site of experiment

This experiment was conducted under controlled conditions in an incubator (Sanyo MIR 253) at 25 ±1 °C with 14 h photoperiod in the laboratory of Soil Fertility and Plant Nutrition, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad.

3.2 Cucumber cultivar

Seeds of cucumber cultivar Bolan F1 were washed and sterilized as described in section 1.2 of the experiment 1.

3.3 Experimental materials

Sterilized disposable plastic petri plates with covers were used for germination of seeds. Holes were made in lids of petri plates; rubber septa were plugged in holes and sealed with silicon gel. Round shaped filter papers sheet (Whatman 42, Schleicher and Schuell) were placed in petri plates. Analytical grade calcium carbide (27 % a.i. CaC$_2$, Ningxia National Chemical Group Co. Ltd., China) was used. De-ionized water was injected in petri plates through rubber septum by using disposable syringe, 10 ml capacity.

3.4 Experimental design

The experiment was laid out according to Completely Randomized Design (CRD) with four replications as described by Steel et al. (1997).

3.5 Treatment plan (Part 1)

In this part of the experiment, germination response of cucumber seeds to different concentrations of NaCl during 6 d of incubation was studied. Treatments applied were as follows:
$T_1 = 0 \text{ mM NaCl}$

$T_2 = 50 \text{ mM NaCl}$

$T_3 = 100 \text{ mM NaCl}$

$T_4 = 150 \text{ mM NaCl}$

$T_5 = 200 \text{ mM NaCl}$

**i- Methodology**

Sterilized seeds of cucumber cultivar Bolan-F1 were placed randomly in sterilized disposable petri plates (9.0 cm diameter) containing whatman 42 filter paper soaked with 0.5mM CaCl$_2$ solution containing either 0 (control) or different concentrations of NaCl (0, 50, 100, 150 and 200mM) at 25 ±1 °C with 14 h photoperiod in the incubator (Sanyo MIR 253) for 6 d of incubation. There were 20 seeds in each Petri plate and the seeds were soaked with 5 mL treatment solution.

**ii- Germination Percentage**

Germination (%) was recorded at 24 h interval up to 144 h. Seeds were considered to be germinated at the emergence of the radicle and scored.

**3.5 Treatment Plan Part (2)**

In 2$^{nd}$ part of this experiment, effect of different levels of CaC$_2$ was evaluated on seed germination and ethylene evolution under 150 mM NaCl stress. We selected 150 mM concentration of NaCl for further studies because maximum reduction in germination after 48 h of incubation was observed at this concentration. The treatments followed were given below:

$T_1 = \text{ Control}$

$T_2 = \text{ NaCl (150 mM)}$

$T_3 = T_2 + \text{ CaC}_2 (10 \text{ mg plate}^{-1})$

$T_4 = T_2 + \text{ CaC}_2 (20 \text{ mg plate}^{-1})$

$T_5 = T_2 + \text{ CaC}_2 (30 \text{ mg plate}^{-1})$

$T_6 = T_2 + \text{ CaC}_2 (40 \text{ mg plate}^{-1})$

**i- Methodology**

Sterilized seeds of cucumber cultivar Bolan-F1 were placed randomly in sterilized disposable plastic petri plates (9.0 cm diameter) containing filter paper moistened with 5 ml distilled water or the same volume 150 mM NaCl made in distilled water. The effect of CaC$_2$
on seed germination in the presence and absence of 150 mM NaCl was investigated by treating seeds with the required weights of powdered calcium carbide on filter paper sheet placed at the bottom and covered with another piece of filter paper according to the procedures as described in section 1.7. Twenty seeds of cucumber cultivar Bolan-F1 were spread on the covering filter paper in each petri plate. After putting the lid back, petri plates were sealed with the help of parafilm and arranged in an incubator (Sanyo MIR 253) at 25 ±1 °C for 48 h under dark conditions. Ethylene gas samples were taken after 48 h of incubation as described in section 1.8. Each treatment was repeated four times.

ii- Seed Germination

Germination (%) was recorded after 48 h of incubation. Seeds were considered to be germinated at the emergence of the radicle and scored.

iii- Ethylene evolution

Ethylene evolution was determined after 48 hours of incubation by using same method as described in section 1.8.

3.5 Treatment Plan Part (3)

In the 3rd part of this experiment, effect of 30 mg CaC\textsubscript{2} under 150 mM NaCl salinity was investigated in the presence and absence of ethylene action inhibitor CoCl\textsubscript{2} and perception inhibitor AgNO\textsubscript{3}. We selected 30 mg CaC\textsubscript{2} as the best dose of CaC\textsubscript{2} from experiment 1 and 2 with the following treatment plan as given below:

- T\textsubscript{1} = Control
- T\textsubscript{2} = NaCl (150 mM)
- T\textsubscript{3} = CaC\textsubscript{2} (30 mg plate\textsuperscript{-1}) + NaCl (150 mM)
- T\textsubscript{4} = T\textsubscript{3} + CoCl\textsubscript{2} (100 uM)
- T\textsubscript{5} = T\textsubscript{3} + AgNO\textsubscript{3} (50 uM)

i- Methodology

Sterilized seeds of cucumber cultivar Bolan-F1 were placed randomly in sterilized disposable petri plates (9.0 cm diameter) containing filter paper moistened with 5 ml distilled water or the same volume of 150 mM NaCl made in distilled water. The effect of CaC\textsubscript{2} on seed germination under salinity stress was investigated by treating seeds with 30 mg of powdered calcium carbide on filter paper sheet placed at the bottom and covered with another piece of filter paper (as described in section 1.7) in the presence and absence of
CoCl₂ (100 uM) and AgNO₃ (50 uM). Twenty seeds of cucumber cultivar Bolan-F1 were spread on the covering filter paper in each petri plate. Rest of procedure was same as described in section 1.7. After putting the lid back, petri plates were sealed with the help of parafilm and arranged randomly in an incubator (Sanyo MIR 253) at 25 ± 1 °C for 48 h in dark.

**ii- Seed Germination**

Germination % was recorded after 48 h of incubation. Seeds were considered to be germinated at the emergence of radicle and scored.

**3.5 Treatment Plan Part (4)**

In the 4th part of this experiment, the effect of 30 mg CaC₂ was investigated during different intervals of incubation on some physio-biochemical attributes of germinating cucumber seeds with the following treatments:

- **T₁** = Control (NaCl = 150 mM NaCl)
- **T₂** = CaC₂ (30 mg plate⁻¹) + NaCl (150 mM NaCl)

**i- Methodology**

Sterilized seeds of cucumber cultivar Bolan-F1 were placed randomly in sterilized disposable petri plates (9.0 cm diameter). Just before germination, two treatments were set by putting 30 mg CaC₂ of powdered calcium carbide on filter paper sheet placed at the bottom and other without CaC₂ as control where CaSO₄ was used just to balance the calcium amount added through CaC₂ in both treatments. Then it was covered with another piece of filter paper where seeds were spread on the covering filter paper in each petri plate. Another filter paper was used to cover the seeds and to save seeds from floating after the application of salt solution. The procedure followed was same as described in section 1.7. Salt solution (150 mM NaCl) was injected into petri plates through rubber septa. After putting the lid back, petri plates were sealed with the help of parafilm and arranged in incubator (Sanyo MIR 253) at 25 ± 1°C with 14 h of photoperiod. Seeds were sampled at 1, 2, 3, 4 and 5 d after application of treatments for biochemical and physiological measurements and another batch was sampled at 2 d after treatments for the determination of different reactive oxygen species and antioxidant enzymes.
ii- Biochemical parameters studied

Total soluble sugars, free amino acids and soluble proteins were determined by following the same procedure as described in section 2.7 and other biochemical parameters were studied by following procedures.

iii- α-Amylase activity

The α-Amylase enzyme were extracted and estimated according to the procedure of Kishorekumar et al. (2007) and Tárrago and Nicolás (1976). One gram of germinating seeds was ground and homogenized in a pre-chilled mortar and pestle with 10 ml ice-cold distilled water at 4 °C. The extract was centrifuged at 15,000 g for 30min at 4 °C. The supernatant was collected for estimating α-Amylase activity. Activity of α- Amylase was assayed after inactivating β-Amylase activity under higher temperature. Five ml of enzyme extract and 3ml of 3mM CaCl₂ were mixed and incubated at 70 °C for 5min. The reaction mixture (2ml) contained 0.1mM citrate buffer (pH 5.0), 2% soluble starch solution and 0.7 ml of hot enzyme extract. The reaction was incubated at 30 °C for 5min and stopped by adding 2 ml colour reagent (the colour reagent was obtained by dissolving 1 g 3, 5-dinitrosalicylic acid in 20 ml of 2M NaOH and 30 g potassium sodium tartrate in 100 ml distilled water). The mixture was heated at 50 °C for 5min, and the final volume of the solution was made up to 10 ml with de-ionized water. Activity of α-Amylase was then determined spectrophotometrically at 540 nm. Soluble protein content in seeds was determined according to Bradford (1976) using bovine serum albumin (BSA) as standard. α- Amylase activity was expressed as units mg⁻¹ protein, and one unit is equivalent to release of one milligram maltose from starch per minute by the enzyme.

iv- Hydrogen peroxide measurement (H₂O₂)

Hydrogen peroxide was estimated by formation of titanium-hydro peroxide complex (Mukherjee and Choudhari, 1983). Seeds (0.5 g) were ground in 10 ml cooled acetone in a chilled mortar and pestle kept in ice bucket. The homogenate was filtered through Whatman No.1 filter paper followed by addition of 4 ml of titanium reagent and 5 ml of ammonium hydroxide solution to precipitate the titanium-hydro peroxide complex. The reaction mixture was centrifuged at 10,000 g for 10 min. The precipitate was dissolved in 10
ml of 2 M concentrated sulphuric acid and re-centrifuged. The supernatant was read at 415 nm against blank and H$_2$O$_2$ expressed as mmol H$_2$O$_2$ g$^{-1}$f.wt.

v- Enzyme extraction and antioxidant enzyme assay

Germinating seeds (one gram) were homogenized in potassium phosphate buffer (1:5 w/v) (pH 7.0) containing 0.2 mmol L$^{-1}$ ascorbate and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000 g for 30 min at 0 $^\circ$C. The supernatant was collected and used for enzyme assays. Total soluble protein was measured using BSA as a standard for all the enzyme activities.

vi- Catalase activity

Catalase activity (CAT) was assayed by measuring the disappearance of H$_2$O$_2$ ($\epsilon$ = 39.4 mM$^{-1}$cm$^{-1}$) (Aebi, 1984). The 3 ml reaction mixture contained sodium phosphate buffer (50 mM, pH 7.0) and 10 mM H$_2$O$_2$. Absorbance was measured at 240 nm in UV Visible spectrophotometer. The specific activity was expressed as nmol H$_2$O$_2$ reduced min$^{-1}$mg$^{-1}$ protein.

vii- Peroxidase activity

Peroxidase activity (POX) was measured by monitoring the formation of tetraguaiacol ($\epsilon$ = 26.6 mM$^{-1}$cm$^{-1}$) from guaiacol (Rao et al., 1996). The POX reaction mixture (3 ml) contained 0.5 mM phosphate buffer (pH 6.1), 16 mM guaiacol, 2 mM H$_2$O$_2$ and 20 ml of enzyme extract. Changes in absorbance of the reaction mixture at 470 nm were followed every 30 s. The specific activity was expressed as nmol tetraguaiacol formed min$^{-1}$mg$^{-1}$ protein.

viii- Ascorbate peroxidase activity

Ascorbate peroxidase activity (APX) was determined by monitoring the oxidized rate of ascorbic acid. Every 1 ml of reaction mixture contained 50 mM Na-potassium phosphate (pH 7.0), 0.5 mM H$_2$O$_2$, 0.5 mM ascorbate, 0.1 mM EDTA and 25 ml of enzyme extract. The reaction was started by addition of H$_2$O$_2$, and oxidation rate of ascorbate was estimated by recording decrease of absorbance at 290 nm ($\epsilon$ = 2.8 mM$^{-1}$cm$^{-1}$) for 1 min. The APX activity was expressed as mmol ascorbate oxidized g$^{-1}$ fresh weight min$^{-1}$ (Costa et al., 2002).

ix- Superoxide dismutase activity

Superoxide dismutase activity (SOD) was assayed using photochemical method as described by Steward and Bewley (1980). Every 1 ml of the reaction mixture contained 0-
200 µ ml enzyme extract, 1.3 mM riboflavin, 13 mM methionine, 63 mM nitroblue tetrazolium (NBT), 50 mM Na-phosphate buffer (pH 7.8), and 0.1 mM EDTA. The test tubes containing the reaction mixture were shaken and placed 30 cm below a light blank consisting of two 15-w fluorescent tubes. The reaction was allowed to run for 10 min, and then stopped by covering the tubes with a piece of black cloth. The reduction in NBT was followed by reading the absorbance at 560 nm. The non-irradiated reaction mixtures were used as the blank. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition to the initial reaction rate (the rate of reaction in the absence of the enzyme).

**x- Lipid peroxidation**

Lipid peroxidation was measured by the thiobarbituric acid (TBA) test that determines malondialdehyde (MDA) as an end product of lipid peroxidation (Costa *et al.*, 2002). Seeds were hand-homogenized in a mortar with pestle in 4 ml of 5% (v/v) trichloroacetic acid (TCA), followed by centrifuging at 10,000 g for 20 min, the supernatants obtained were used for MDA determination. To 1 ml of the supernatant, 1 ml of 20% TCA containing 0.5% (w/v) TBA was added. The mixtures were heated at 95°C for 30 min, and then quickly cooled in an ice-water-bath to room temperature. The mixtures were then centrifuged at 10,000 g for 10 min, and the absorbance of the supernatants was read at 532 nm. Non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex was calculated from the extinction coefficient 155 mM⁻¹cm⁻¹.
B) Pot experiments under wire house conditions

Experiment 4 Evaluation of different rates and coating materials on calcium carbide for morphological, floral and yield attributes of cucumber

4.1 Site of experiment

Evaluation of different coating materials for growth and yield of cucumber was studied in a pot experiment. Pot experiment was conducted in the wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad under natural conditions during the period 2010-2011. The wire house has a glass roof with no control over temperature, humidity and light as the sides are open having only a wire net to control birds. During the experimental period, day and night temperatures in the wire house were 26±5 °C and 20±5 °C, respectively and average relative humidity was 50±7%. Three coating materials, wax coated, paint coated and gelatin encapsulated were compared for their performance regarding growth and yield parameters.

4.2- Cucumber cultivar used

Two seedlings of cucumber cultivar Bolan-F1 (Hybrid) from fifteen days old nursery were transplanted into glazed pots and finally one plant was maintained in each pot.

4.3- Experimental setup

Glazed pots having 30 cm width and 35 cm length with the capacity of 12 kg soil filling per pot lined with polyethylene bags were used for the experiment. Soil (air-dried, ground and sieved) taken from upper soil layer (0-30 cm depth) from vegetable research area, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, was filled in pots at the rate of 10 kg per pot. Pots were kept under natural day light.

4.4- Soil physical and chemical analyses

The soil samples collected from surface soil layer of 0-30 cm depth from vegetable research area of Institute of soil and Environmental science, and were analyzed for physico-chemical properties (Table 1.1). Physical and chemical properties of soil were determined by using the methods described by Page et al. (1982) or methods otherwise used are mentioned.

i- Mechanical analysis

Fifty grams of air dried soil sample was taken in 500 ml beaker. Then 40 ml of 1 % sodium hexametaphosphate solution and 150 ml of distilled water were added. These soil samples were kept for overnight soaking. Next day soil suspension was stirred with a
mechanical stirrer for ten minutes and transferred to 1 L plastic cylinder. With the help of metal plunger the suspension was shaken vigorously. Initial reading was recorded after 40 seconds of the shaking and final reading was noted after 2 hours on Bouyoucos Hydrometer (Moodie et al., 1959). Soil textural class was established by using textural class triangle of United States Department of Agriculture.

ii- Saturation percentage

Saturated soil paste was transferred to a tared china dish and oven dried to a constant weight at 105° C. Saturation percentage was calculated using the formula (Method 10-2.3, U.S. Salinity Lab. 1954)

\[
SP = \frac{\text{Mass of wet soil} - \text{Mass of dry soil}}{\text{Mass of oven dry soil}} \times 100
\]

iii- Soil pH (pHw)

Weigh 5 g of air dried soil in 50 ml plastic beaker, add 5 ml of distilled water with automatic pipette, mix thoroughly for 5 seconds and allowed to stand for 10 minutes. pH was recorded by pH meter (3510 JENWAY) with glass electrode after standardizing the meter with buffer of 7.0 and 4.0 pH as standards (McLean, 1982).

iv- Organic matter

Organic matter was determined by Walkely Black method. Two gram soil sample was mixed with 10 mL 1 N potassium dichromate solution and 20 mL concentrated sulphuric acid. Add 150 mL of distilled water and 10 ml of 85% phosphoric acid, 0.2 g of sodium fluoride and 30 drops of diphenylamine indicator (0.5 g diphenylamine + dissolve it 20 ml distilled water + 100 ml of conc. sulphric acid). The solution is back titrated with 0.5 N ferrous sulphate solution to a brilliant green end point. A blank was also run (Jackson, 1962).

v- Electrical conductivity of saturated soil extract (ECe)

Saturated soil extract was taken by using vacuum pump (Method 3a, U.S. Salinity Staff, 1954) and its electrical conductivity was measured using digital conductivity meter model 4510 JENWAY (Method 4b, U.S. Salinity Staff, 1954).
Table 1.1 Physico-chemical characteristics of soil used for pot and field trials

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>%</td>
<td>58</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>%</td>
<td>23</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>%</td>
<td>19</td>
</tr>
<tr>
<td>Textural class</td>
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<tr>
<td>Saturation percentage</td>
<td>%</td>
<td>34</td>
</tr>
<tr>
<td>Organic matter</td>
<td>%</td>
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</tr>
<tr>
<td>ECe</td>
<td>dS m⁻¹</td>
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<tr>
<td>pH</td>
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<td>8.0</td>
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<tr>
<td>K</td>
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<tr>
<td>Na</td>
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<tr>
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<td>meq. L⁻¹</td>
<td>6.20</td>
</tr>
<tr>
<td>CO₃</td>
<td>meq. L⁻¹</td>
<td>Absent</td>
</tr>
<tr>
<td>HCO₃</td>
<td>meq. L⁻¹</td>
<td>6.4</td>
</tr>
<tr>
<td>Cl</td>
<td>meq. L⁻¹</td>
<td>7.2</td>
</tr>
<tr>
<td>SO₄</td>
<td>meq. L⁻¹</td>
<td>1.4</td>
</tr>
<tr>
<td>Cation exchange capacity</td>
<td>cmol. kg⁻¹</td>
<td>4.15</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>%</td>
<td>0.04</td>
</tr>
<tr>
<td>Available phosphorus (P)</td>
<td>mg kg⁻¹ soil</td>
<td>5.06</td>
</tr>
<tr>
<td>Available Potassium (K)</td>
<td>mg kg⁻¹ soil</td>
<td>160</td>
</tr>
</tbody>
</table>
vi- Cation exchange capacity (CEC)

Five grams soil sample was taken in centrifuge tube and washed three times successively by adding 33 mL of saturating solution (0.4 N NaOAC-0.1 N NaCl, 60% ethanol) discarding the supernatant each time. Then add 33 mL of extracting solution (0.5 N magnesium nitrate) and washed three times successively as above. While giving washing with extracting solution supernatant liquid was preserved in 100 mL flask and its volume was made up to the mark. Sodium and chloride concentrations of this liquid were determined by using Sherwood 410 flame photometer and cation exchange capacity was calculated (Rhoades, 1982a).

vii- Calcium plus magnesium

Soluble calcium plus magnesium were determined by titration of saturated soil extract against 0.01 N EDTA solution using eriochrome black-T as an indicator in the presence of buffer to a blue green end point (Method 7, U.S. Salinity Staff, 1954).

viii- Sodium and potassium

Soluble sodium and potassium in the saturated soil extract were determined by using flame photometer (Sherwood 410) using NaCl and KCl as standard solutions (Method 13-4, U.S. Salinity Lab. 1954).

ix- Carbonates and bicarbonates

Carbonates and bicarbonates in the saturation extract were determined by titration with standard 0.1 N H₂SO₄ (Method 12, U.S. Salinity Staff, 1954).

x- Chlorides

Chlorides in the saturation extracts were determined by titration with standard (0.05 N) silver nitrate using potassium chromate as an indicator (Method 13, U.S. Salinity Staff, 1954)

xi- Sulphate

Sulphate was determined by difference i.e. TSS - (CO₃²⁻ + HCO₃⁻ + Cl⁻).

xii- Total nitrogen

Nitrogen was determined by Gunning and Hibbard’s method of sulphuric acid digestion and distillation of ammonium into 4 % boric acid by macro distillation apparatus (Jackson, 1962).
Available phosphorus

Five g soil was extracted with 0.5 M NaHCO$_3$ solution (pH 8.5). Then 5 mL aliquot of clear filtrate was taken in 25 mL volumetric flasks and added to it 5 mL of colour developing reagent (ascorbic acid, ammonium molybdate, antimony potassium tartarate and sulphuric acid). Volume was made up to the mark with distilled water and reading was recorded by T80UV/VIS spectrophotometer, PG Instruments Ltd. (Olsen and Sommers, 1982).

Available potassium

Five g soil was saturated with 50 ml of 1 M ammonium acetate solution (pH 7.0). Extraction was made with same solution and extractable or available K was determined by Sherwood 410 flame photometer (Carson, 1980).

4.5 Experimental design

The experiment was laid out according to Completely Randomized Design (CRD) with four replications as described by Steel et al. (1997).

4.6 Treatment plan

Five coating materials (wax, paraffin, paint, polyethylene and gelatin capsules) were used for coating of powdered CaC$_2$. Calcium carbide was coated with different coating materials by adopting method as described by Mahmood (2009) as shown in figure 1.2 and following treatment plan was adopted:

- T$_1$: Paraffin coating without CaC$_2$ (control)
- T$_2$: Paraffin coated CaC$_2$ @ 100 mg pot$^{-1}$
- T$_3$: Paraffin coated CaC$_2$ @ 200 mg pot$^{-1}$
- T$_4$: Paraffin coated CaC$_2$ @ 300 mg pot$^{-1}$
- T$_5$: Wax coating without CaC$_2$ (control)
- T$_6$: Wax coated CaC$_2$ @ 100 mg pot$^{-1}$
- T$_7$: Wax coated CaC$_2$ @ 200 mg pot$^{-1}$
- T$_8$: Wax coated CaC$_2$ @ 300 mg pot$^{-1}$
- T$_9$: Paint coating without CaC$_2$ (control)
- T$_{10}$: Paint coated CaC$_2$ @ 100 mg pot$^{-1}$
- T$_{11}$: Paint coated CaC$_2$ @ 200 mg pot$^{-1}$
Calcium sulphate was added in control pots to equalize the amount of calcium coming through the application of calcium carbide. In this experiment each coating material without CaC\textsubscript{2} was also included as control treatment to test its influence on morphological and yield parameters of cucumber plants.

4.7- Fertilizers

Nitrogen, phosphorus and potassium were applied at recommended rate of 100- 75-60 kg ha\textsuperscript{-1} in the form of urea, single super phosphate and murate of potash, respectively. All P and K were applied at sowing time by mixing in soil before pot filling whereas urea was applied in two splits i.e. half at seedling transplantation time by mixing in soil and other half dose at the time of flowering.

4.8 Date of nursery sowing and transplanting

Cucumber nursery plants were grown in thermo pore cups containing media of compost and sand in 1:1 ratio in controlled temperature room during last week of January 2010. Fifteen days old cucumber seedlings were then used to transplant in earthen glazed pots.

4.9 Methodology

After filling pots with soil having characteristics as described in Table 3.1, the pots were randomly placed in the wire house. Recommended doses of fertilizers (NPK) were applied as discussed in section 4.8. Two seedlings of each cucumber cultivar from fifteen days old nursery were transplanted into pots and finally one plant was maintained in each pot. The uprooted seedlings were chopped and buried in the soil of pot. Required rates of coated calcium carbide were placed 6 cm deep in soil in the center of pots three days after transplanting. Canal water treated with Topsin-M @ 0.25% was used for irrigation of plants throughout the growth period.
Figure 1.2 Coating of calcium carbide with different coating materials (Modified from Siddiq, 2012)
Recommended insecticide was sprayed two times during the growing period to protect plants from fruit borer. Data was statistically analyzed to find out the best rate of CaC₂.

4.10 Parameters studied

Data on following parameters were collected during this study.

4.10 (A) Morphological characteristics

i- Vine length

The length of the primary stem was measured from ground level (the point of emergence of the plant) to the top of the vine on each plant with the help of a centimeter scale after 60 days of sowing.

ii- Number of nodes on main vine

All the nodes appearing on the main stem were calculated.

iii- Internodal distance

The distance between the nodes of plant was measured from the middle of plant using a scale.

4.10 (B) Floral characteristics

i- Days to first female flower appearance

The number of days to the appearance of the first female flower was recorded on each plant.

ii- Days to fruit maturity

The number of days taken by a bud to reach a marketable fruit was calculated with at least three buds from tagged plant being selected at the time of bud initiation stage.

4.10 (C) Yield characteristics

i- Number of fruits per plant

The number of fruit per plant was recorded as the average of the cumulative number of fruits in all pickings of each cultivar at a marketable stage.

ii- Fruit yield per plant (g)

Fruits harvested from each plant were weighted separately at each picking and the cumulative yield per vine was calculated.
Experiment 5 Optimizing different rates of coated CaC₂ on some morpho-phenological and yield attributes of two cucumber cultivars differing in yield potential

5.1 Site of experiment

Response of cucumber cultivars to different rates of calcium carbide was studied in a pot experiment in wire house of experimental area, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. Two cucumber cultivars, one hybrid and other local cultivar were compared for their performance in treated and non-treated plots.

5.2- Cucumber cultivar used

Two seedlings of each cucumber cultivar (Bolan-F1 (Hybrid) and Desi (local)) from thirty days old nursery were transplanted into glazed pots and finally one plant was maintained in each pot.

5.3- Experimental setup

Experimental set up followed was the same as described in section 4.3.

5.4- Soil physical and chemical analyses

The soil used for pot filling was collected from same site having same physical and chemical characteristics as described in Table 3.1.

5.5- Experimental design

The experiment was laid out according to Completely Randomized Design (CRD) with four replications as described by Steel et al. (1997).

5.6- Treatment plan

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>T₁</td>
<td>Control (without CaC₂)</td>
</tr>
<tr>
<td>T₂</td>
<td>Paint coated CaC₂ @ 100 mg pot⁻¹</td>
</tr>
<tr>
<td>T₃</td>
<td>Paint coated CaC₂ @ 200 mg pot⁻¹</td>
</tr>
<tr>
<td>T₄</td>
<td>Paint coated CaC₂ @ 300 mg pot⁻¹</td>
</tr>
<tr>
<td>T₅</td>
<td>Paint coated CaC₂ @ 400 mg pot⁻¹</td>
</tr>
</tbody>
</table>

In control treatments calcium sulphate was added to adjust the amount of calcium added from CaC₂.

5.7-Procedure for paint coating of calcium carbide

Calcium carbide was coated with paint by adopting same method as described by Mahmood (2009).
5.8 Fertilizers

Fertilizer application practices adopted were the same as described in section 4.8.

5.9 Date of nursery sowing and transplanting cucumber in field

Cucumber nursery plants were prepared by using the same method as described in section 4.9. Fifteen days old cucumber seedlings were then used to transplant in earthen glazed pots during 1st week of February 2011.

5.10 Methodology

Methodology adopted was the same as described in section 4.10.

5.11 Parameters studied

Prior to initiating the pot study, C2H4 production in soil amended with five different levels of paint coated CaC2 was monitored over a period of 56 d of incubation under ambient conditions (24 ± 3 °C). Pre-harvest parameters were taken at 60 days after sowing from each treatment to measure plant growth characteristics i.e. vine length (cm), days to 1st female flower initiation and number of fruits per plant as described in section 4.9 while other parameters were determined by methods given below.

i-Ethylene production in soil

For this purpose, 50 g sandy clay loam soil (Typic Haplocambids) was taken in 125 mL Erlenmeyer flasks fitted with rubber supa-seal. The content of the flask were maintained at 60% water contents (WHC). Paint coated CaC2 was added at the rate of 10, 20, 30 and 40 mg kg⁻¹ soil. Equivalent amount of calcium present in CaC2 was also added in control flasks by using CaSO4. This experiment was carried out according to completely randomized design and each treatment was repeated four times. C2H4 gas was collected by withdrawing 1 cm³ gas samples from the head space of the flask above the soil with a gas tight glass hypodermic syringe. Concentrations of C2H4 were determined by gas chromatography (Shimadzu-4600) as described by Khalid et al. (2006b); fitted with a flame ionization detector (FID), and a capillary column (Porapak Q 80-100) operating isothermally under the following conditions: sample volume, 1mL; column temperature, 70°C; detector temperature, 200°C; carrier gas used, N2 (13 mL min⁻¹); flow rate of H2, 33 mL min⁻¹; flow rate of air, 330 mL min-1. The C2H4 concentrations were calculated by comparing with reference standards which were made by diluting 99.5% C2H4 obtained from Matheson (Secancus, NJ, USA).
ii-Ethylene production from leaves

After 40 days of seed sowing, ethylene was analyzed using the second developing leaf from the top of the plant. Leaf was excised 2 mm above the stem surface from each selected plant at 10 a.m. using stainless steel blade. It was placed immediately in 150 mL glass tubes, each containing 5 mL of H2O for immersing the leaf base in water to prevent drought stress. Glass tubes were then sealed using rubber supa-seal and kept under a 1,000-lux fluorescent light for 8 h. One-mL gas samples were then withdrawn from the glass tube head space and concentrations of C2H4 were determined by gas chromatography (Shimadzu-4600) as described by Khalid et al. (2006b). Data were then recorded as nL of C2H4 g⁻¹ FW h⁻¹.

iii- Days to fruit setting

The number of days to the conversion of the female flower to initial fruit setting was recorded on each plant.

iv- Days to fruit maturity

The number of days taken by initial fruit to reach a marketable fruit size was counted on each plant.

v- Number of female flowers per plant

The total number of female flowers per vine was calculated.

vi- Fruit setting percentage

Fruit setting percentage per plant was calculated by using following formula

\[
\text{Fruit setting} \,(\%) = \frac{\text{Total number of fruits per plant}}{\text{Total female flowers per plant}} \times 100
\]

vii-Fruit yield per plant (g)

Fruits harvested from each plant were weighted separately at each picking and the cumulative yield per vine was calculated.

viii- Early yield per plant (g)

Fruits harvested from each plant were weighted separately for first five pickings and the cumulative yield per vine was calculated.
C) Field experiment at Institute of Soil and Environmental Sciences

6 Effect of coated CaC\textsubscript{2} on photosynthesis, nitrogen use efficiency and fruit quality of cucumber under nitrogen deficient and adequate levels

6.1 Site of experiment

Response of cucumber cultivar Bolan-F1 (Hybrid) to different levels of paint coated calcium carbide under nitrogen deficient and adequate level was conducted on research area of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad (Figure 1.3) under agro-climatic zone of Northern irrigated plain. The region has semi-arid to arid subtropical climate with a hot dry summer, a warm and humid rainy season and cold winter months. The mean maximum and minimum temperature in summer are 39 \degree C (102 \degree F) and 27 \degree C (81 \degree F) respectively. In winter it peaks at around 21 \degree C (70 \degree F) and 6 \degree C (43 \degree F) respectively. The summer season starts from April and continues till October. May, June and July are the hottest months. The winter season starts from November and continues till March. December, January and February are the coldest months. The average yearly rainfall lies only at about 300 mm (12 in) and is highly seasonal with approximately half of the yearly rainfall takes place in July and August.

6.2 Soil physical and chemical analysis

Physical and chemical properties of soil collected from surface soil layer of 0-30 cm depth from vegetable research area of Institute of soil and Environmental science, were same as described in table 3.1.

6.3 Experimental design

The experiment was laid out according to 2-Factor Randomized complete Block Design (RCBD) with four replications as described by Steel \textit{et al.} (1997). There were two factors (CaC\textsubscript{2} and Nitrogen) and each has three levels making total 9 treatments as given below.
Figure 1.3 The location of wire house and the Research Area, Institute of Soil and Environmental Sciences (Google Earth 2014)
6.4- Treatment plan

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<table>
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<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Control (without CaC&lt;sub&gt;2&lt;/sub&gt;)</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Paint coated CaC&lt;sub&gt;2&lt;/sub&gt; @ 200 mg pot&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Paint coated CaC&lt;sub&gt;2&lt;/sub&gt; @ 300 mg pot&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Nitrogen @ 50 kg ha&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Nitrogen @ 50 kg ha&lt;sup&gt;-1&lt;/sup&gt; + Paint coated CaC&lt;sub&gt;2&lt;/sub&gt; @ 200 mg pot&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Nitrogen @ 50 kg ha&lt;sup&gt;-1&lt;/sup&gt; + Paint coated CaC&lt;sub&gt;2&lt;/sub&gt; @ 200 mg pot&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Nitrogen @ 100 kg ha&lt;sup&gt;-1&lt;/sup&gt;</td>
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<tr>
<td>T&lt;sub&gt;8&lt;/sub&gt;</td>
<td>Nitrogen @ 100 kg ha&lt;sup&gt;-1&lt;/sup&gt; + Paint coated CaC&lt;sub&gt;2&lt;/sub&gt; @ 200 mg pot&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;9&lt;/sub&gt;</td>
<td>Nitrogen @ 100 kg ha&lt;sup&gt;-1&lt;/sup&gt; + Paint coated CaC&lt;sub&gt;2&lt;/sub&gt; @ 300 mg pot&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

In all control treatments calcium sulphate was added to adjust the amount of calcium added from CaC<sub>2</sub>.

6.5- Methodology

The nursery was prepared on 2 January 2012 by using same procedure as described in section 4.9 under controlled conditions. After complete germination of the seeds at 15 d after sowing, transplanting was carried out into two rows 100 cm apart, keeping six plants per treatment tagged to document various observations. Each treatment had four replicates in four individual plots of 4 × 2 m<sup>2</sup> wide (36 plots). Each plot contained 6 plants. N Fertilizer was applied @ 0, 50 and 100 kg ha<sup>-1</sup> using urea (46%-N) as a source of nitrogen. Paint coated calcium carbide @ 0, 200 and 300 mg plant<sup>-1</sup> was placed 6 cm deep in soil three days after transplanting. Nitrogen as urea while phosphorus (60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) as single super phosphate and potassium (60 kg K<sub>2</sub>O ha<sup>-1</sup>) as sulfate of potash. Half of N and whole phosphorus and potassium were applied as the basal dose whiles other half nitrogen at time of flowering. The plants were irrigated when required depending on the soil moisture regime. Plants were protected from insect pests and diseases using appropriate plant protection measures from the initial stages of crop growth until crop senescence.

6.6 (A)-Pre-harvest parameters

Among the pre-harvest parameters vine length, leaf area, No. of primary branches, photosynthetic rate and nitrate reductase activity in leaves were determined after 8 weeks of sowing.
i- Growth measurement

Leaf area was measured with a portable Living Leaf Area Meter (YMJ-A/ YMJ-B). Plant dry mass was determined by drying plants in an oven at 80°C until a constant weight was reached.

ii- Length of main stem

The length of the primary stem was measured from ground level (the point of emergence of the plant) to the top of the vine on each tagged plant with the help of a meter scale and the average length was calculated.

iii- Number of primary branches per vine

The total number of branches arising from the main stem was counted from each tagged plant and the average was calculated.

iv- Days to 1st female flowering

Days to 1st female flowering were determined as described in previous section 3.3

v- Days to fruit setting

Days to fruit setting were determined as described in previous section 3.4

vi- Photosynthetic activity

Photosynthesis rate, carboxylation efficiency and plant water use efficiency were determined. Fully expanded youngest leaves were used for photosynthesis measurements. Infrared gas analyzer (IRGA, model ADC, Bioscientific Ltd., England) was used for measurement at 1100-1200 h when photosynthetic active radiations above the canopy were present. The inside temperature of the leaf cuvette was set at 30 ± 2 °C. The light responses curves were carried out at ambient CO₂ concentrations (300-350 μmol mol⁻¹). Carboxylation efficiency was calculated as the ratio of photosynthesis to intercellular CO₂ concentration (Farquhar and Sharkey, 1982). Water use efficiency (WUE) was calculated as the ratio of photosynthesis to gs to avoid effects of small differences in vapour pressure between measurements (Von Cammerer and Farquhar, 1981).

vii- Nitrate reductase activity

Nitrate reductase activity in fresh leaf samples was determined by using same method as described in section 2.7
6.6 (B) Post-harvest observations recorded

i- Fruit yield and quality:
   Cucumber fruits at marketable stage were harvested twice weekly. At harvest time the, number of fruits/plant, mean weight of fruit, fruit length, fruit diameter and yield (kg/plant) in each treatment were recorded.

ii- Stem dry weight
   Stems were detached from soil surface and their oven dried weights were recorded using an electronic digital balance.

iii- Leaves dry weight
   After detaching leaves from branches were oven dried and their weights were recorded using an electronic digital balance

iv- Fruit dry weight
   After each picking fruits were oven dried their weights were recorded using an electronic digital balance.

v- Root dry weight
   After detaching main stem of plant, underground root portion with earthen boll was washed thoroughly with tap water so that they were free from soil. These were air dried in laboratory for some time and then over dried up to constant weight by using a digital electronic balance, to record their dried weights.

vi- Total nitrogen uptake by plant
   Nitrogen uptake by each plant part (leave, stem, fruit and root) was calculated by multiplying dry weight of each plant part (leave, stem, fruit and root) with their respective nitrogen concentration and total was taken as the sum of all plant parts of cucumber.

6.7- Chemical contents
   Chemical analysis of cucumber leaves was carried out after harvest to determine mineral contents (N, P, K, Ca and Mg). The plant materials were dried in an oven at 70 °C until a constant mass was reached and then they were grounded for chemical analysis. Total nitrogen was determined using the micro-Kjeldahl method (Plank, 1992). Phosphorus was determined colorimetrically using a spectrophotometer, according to the ammonium molybdate method (Murphy and Riley, 1962).
Moreover, K was determined by using flame photometer while Ca and Mg contents were determined by the atomic absorption spectrometry (Plank, 1992).

6.8- Fruit compositional analysis

Upon reaching the marketable size five individual cucumber fruits from each treatment were picked, washed with tap water to remove dust and dried with soft tissues. These fruits were used for determination of chemical composition of fruits. After the determination of physical parameter fruits were homogenized using a blender cup and homogenizer, Bosh easy mix (model CNHR6 Germany) followed by centrifugation with 8060 x g, for 20 minutes. The resulting supernatant was filtered using cheese cloth and then used later for compositional analysis of the following parameters.

i- Total soluble solids

Total soluble solids (TSS) were measured as stated by Korsten (2008). One to two drops of the supernatant as prepared above was placed on the prism of the digital refractometer (Model ATAGO, Japan) and TSS was reported as °Brix (noted in percentage).

ii- pH

The value of pH supernatant (as prepared in section 3.3.10.3) was measured with digital prob pH meter (3510 JENWAY) using buffer of 7.0 and 4.0 pH as standards.

iii- Titratable acidity (TA)

Titratable acidity was measured by titration according to the AOAC (1999). Each cucumber sample supernatant (6 g) was weighed out and diluted in 40 mL of distilled water. Two to five drops of phenolphthalein was added in this juice. A 10 mL aliquot was taken in a titration flask and, then titrated against 0.1N NaOH till permanent light pink color appeared. The samples were titrated with 0.1 N NaOH to endpoint of pH 8.2. Titratable acidity was expressed as percentage of malic acid by using following formula

\[
\text{TA} (\%) = \frac{\text{(mL NaOH used) (Normality of NaOH) (Equivalent wt. of malic acid)}}{\text{(Wt. of sample) (Vol. of aliquot taken)}}
\]

iv- Ascorbic acid

The method used for ascorbic acid determination was described by the AOAC (1999). Ascorbic acid content was expressed as mg per 100 g fresh weight.
5 ml cucumber juice was taken in a volumetric flask of 100 ml and volume was made by adding 0.4 % oxalic acid solution. Out of this filtered aliquot 10 ml was taken, added some distilled water for making end point clear and titrated against standardized (0.04 %) 2,6-dichlorophenolindophenol sodium salt hydrate dye, to light pink end point which should persist for at least 15 seconds and the vitamin C was calculated as ascorbic acid by using the following formula:

\[
\text{Ascorbic acid (mg 100g}^{-1}) = \frac{1 \times R_1 \times V \times 100}{R \times W \times V_1}
\]

R= ml of dye used to titrate against 2.5 ml of reference solution (1 ml standard ascorbic acid + 1.5 ml 0.4% oxalic acid)
R$_1$= ml of dye used to titrate against V$_1$ of aliquot
V= volume of aliquot made by 0.4% oxalic acid
V$_1$= ml of aliquot taken for titration
W= ml of juice taken

v- Total soluble sugars, proteins and free amino acids

Fresh fruit samples (0.5 g) were crushed with cold phosphate buffer (50 mM KH$_2$PO$_4$, pH 7.0) and centrifuged at 12,000 g for 15 min. The resulting supernatant was used for the determination of total free amino acids, total soluble sugars and soluble proteins by the same methods as given in section 3.3.

6.9- Nitrogen use efficiency

The effect of nitrogen on cucumber growth and yield was determined by using recovery efficiency, physiological nitrogen use efficiency (PNUE) and the agronomic efficiency (NUE-AE) indices (Dobermann, 2007; IFA, 2007). Following formulae were used to calculate both the efficiencies.

\[
\begin{align*}
\text{RE} &= 100 \times \Delta TN/FN, \\
\text{AE} &= \Delta GY/FN, \\
\text{PE} &= \Delta GY/\Delta TN, \\
\text{NUE} &= \text{AE}/\text{PE},
\end{align*}
\]
where $\Delta T N$ was total aboveground plant N accumulation in the plot that received N fertilizer minus total aboveground plant N accumulation in the zero-N control, $F N$ was the amount of N fertilizer applied, $\Delta G Y$ was fruit yield in the plot that received N fertilizer minus fruit yield in the zero-N control.

### 6.10 Statistical analysis

All agronomic data obtained from experiments were analyzed statistically according to the analysis of variance (ANOVA) procedure as detailed by Steel *et al.* (1997) utilizing Statistix 8.1 software. Significant differences ($p<0.05$) among means were identified using Fisher’s least significant difference (LSD) tests at 5 % probability level.
The research work presented in this manuscript was conducted at Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. The results of a series of experiments conducted under laboratory, pot and field conditions to evaluate the effect of calcium carbide on seed germination, morpho-physiological and biochemical changes, fruit yield and quality of cucumber are presented below separately according to each experiment.

A) **Laboratory experiments under controlled conditions**

Experiment 1: Seed germination response of cucumber to different rates of calcium carbide

Experiment 2: Effect of different rates of CaC$_2$ on biochemical changes, chlorophyll contents and nitrogen metabolism in cucumber

Experiment 3: Effect of CaC$_2$ on germination of cucumber seeds to alleviate salinity stress

B) **Pot experiments under wire house conditions**

Experiment 4: Evaluation of different rates and coating materials on calcium carbide for morphological, floral and yield attributes of cucumber

Experiment 5: Optimizing different rates of paint coated CaC$_2$ for ethylene production, some morpho-phenological and yield attributes of two cucumber cultivars differing in yield potential

C) **Field experiment at Institute of Soil and Environmental Sciences**

Experiment 6: Effect of paint coated CaC$_2$ on photosynthesis, nitrogen use efficiency and fruit quality of cucumber under deficient and adequate levels of nitrogen
A) Laboratory experiments under controlled conditions

Experiment 1: Seed germination response of cucumber to different rates of calcium carbide

1.1 Introduction

Seed germination is considered as one of major factor that determines the productivity of the crops. Seed germination and seedling growth is influenced by various environmental and hormonal factors (Kucera et al., 2005). Plant hormones can extend or release dormancy, stimulate root growth, enhance or inhibit growth, increase fruit number or size, or affect the growth and/or yield of various crops (Anna et al., 1979). Among various plant hormones, gibberellic acid and ethylene are well known regarding their role in breaking seed dormancy caused by ABA (Matilla and Matilla-Vazquez, 2008; Linkies et al., 2009). Ethylene, a gaseous plant hormone, has been reported to be involved in regulation of various morphological and physiological processes, including root hair formation, seed dormancy, fruit ripening and numerous abiotic stresses (Matilla, 2000; Nath et al., 2006). Ethylene interacts with other plant hormones for its signaling to produce a physiological response (Druege, 2006). Seed germination has been correlated with ethylene emission in several species, although the mechanism of action of ethylene in seed germination is still debatable. Thus, some scientists argued that ethylene is produced in response to germination process, while others oppose that ethylene is mandatory to complete the process of germination (Matilla, 2000; Petruzzelli et al., 2000). Although, role of ethylene during seed germination is a well-known fact but its mechanism of action still needs to be studied.

In most of the cases ethylene can stimulate the germination of seeds which may be inhibited due to embryo or coat dormancy, adverse environmental conditions or under the influence of inhibitors (Rao et al., 1975; Esashi, 1991). These results show that ethylene based mechanism is involved during seed germination, and it was also observed that critical concentration of ethylene is required for seed germination.

Application of ethylene releasing compounds could be much easier than application of ethylene in gaseous form. Calcium carbide (CaC$_2$) is now renowned source of acetylene and ethylene affecting different morpho-physiological characteristics ranging from seed germination to senescence (Abeles et al., 1992; Randall et al., 2001; Kashif et al., 2012).
This aim study was carried out to select optimum rate of CaC$_2$ that improves germination and morphological characteristics of cucumber by providing the basis for further studies on cucumber germination under different stress condition.

1.2 Materials and Methods
Methodology of the experiment is described in the experiment 1 of chapter 3.

1.3 Results

i) Germination characteristics of cucumber seeds after application of CaC$_2$

The differences in seed germination rate with the application of different rates from 0 to 40 mg plate$^{-1}$ of CaC$_2$ were significant and maximum germination rates (94.8% and 93.3%) were recorded in the plates receiving 20 and 30 mg CaC$_2$ plate$^{-1}$, respectively. The increase in germination rate was about 39% on an average with respect to control. In comparison to the control, germination rate in the treatment of 40 mg plate$^{-1}$ was decreased by 41.8%. Similar trend was observed in germination potential and germination index which were consistently increased up to 30 mg plate$^{-1}$ CaC$_2$ and then decreased with the further increase in the rate of CaC$_2$ i.e. 40 mg plate$^{-1}$. Overall, germination potential and germination index were increased by 131.8 and 60.0% in the treatment of 30 mg plate$^{-1}$ of CaC$_2$ while these were decreased by 100 and 52.0% in the treatment receiving 40 mg plate$^{-1}$ of CaC$_2$, respectively, compared to the control. Growing vigor index increased significantly with increasing the rate of CaC$_2$ up to 30 mg plate$^{-1}$. The increase recorded was 42.7, 91.8 and 146.6% at 10, 20 and 30 mg CaC$_2$ plate$^{-1}$, respectively, while at 40 mg plate$^{-1}$ it was decreased 62.5% compared to control (Table 1.2).

ii- Ethylene evolution during germination

Data concerning ethylene release from germinating seeds of cucumber per petri plate under the application of CaC$_2$ during incubation is presented in Fig.1.4. The gas chromatography analysis of the samples collected from petri plates, treated with different rates of CaC$_2$ at different intervals of incubation, showed significant differences in ethylene evolution pattern.
Table 1.2 Effect of different rates of CaC₂ on germination characteristics of cucumber

<table>
<thead>
<tr>
<th>Calcium carbide (mg plate⁻¹)</th>
<th>Germination rate (%)</th>
<th>Germination potential (%)</th>
<th>Germination index</th>
<th>Seedling vigour index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>67.54 c</td>
<td>23.83 d</td>
<td>1.36 d</td>
<td>1.62 d</td>
</tr>
<tr>
<td>10</td>
<td>83.73 b</td>
<td>34.33 c</td>
<td>1.73 c</td>
<td>2.31 c</td>
</tr>
<tr>
<td>20</td>
<td>93.35 a</td>
<td>43.56 b</td>
<td>1.99 b</td>
<td>3.10 b</td>
</tr>
<tr>
<td>30</td>
<td>97.87 a</td>
<td>55.23 a</td>
<td>2.18 a</td>
<td>3.99 a</td>
</tr>
<tr>
<td>40</td>
<td>39.23 d</td>
<td>00.00 e</td>
<td>0.65 e</td>
<td>0.60 e</td>
</tr>
<tr>
<td>Mean</td>
<td>76.34</td>
<td>31.39</td>
<td>1.59</td>
<td>2.33</td>
</tr>
</tbody>
</table>

Means sharing same letter in each column are statistically non-significant at P> 0.05

*CC = CaC₂ (mg plate⁻¹)
Values are means of 4 measurements and bars represent ± SE (n = 4)

Figure 1.4 Effect of different rates of CaC₂ on ethylene evolution from germinating seeds of cucumber during 5 days of incubation period
Ethylene released from germinating seeds was increased with increasing rates of CaC₂ except the treatment where the highest level of CaC₂ (40 mg plate⁻¹) was applied which significantly reduced the ethylene release. After 48 h of incubation, maximum peak of ethylene evolution (8.87 nl g⁻¹ dry seed weight) was observed in the treatment where CaC₂ was applied @ 20 mg plate⁻¹, followed by 30 mg plate⁻¹ (8.57 nl g⁻¹ dry seed weight) while the lowest peak (2.83 nl g⁻¹ dry seed weight) was observed at the highest level of CaC₂ (40 mg plate⁻¹). The same trend among treatments was observed after 72 h of incubation but emission peaks at all levels of CaC₂ were higher than 48 h of incubation. After 5 days of incubation, maximum ethylene emission (14.02 nl g⁻¹ dry seed weight) was recorded in the treatment where CaC₂ was applied @ 30 mg plate⁻¹ which was 46% higher than that of control. The application of the highest level of CaC₂ (40 mg plate⁻¹) inhibited the emission of ethylene throughout incubation by 32, 22 and 35% compared to control at 48, 72 and 96 h of incubation, respectively.

iii- Morphological characteristics of cucumber after application of CaC₂

The length of root, number of 1st lateral root, the length and width of hypocotyledonary axis and seedlings fresh weight are important indicators for determining the growth status of plants. So the above indices of plants under different CaC₂ rates were determined and results are shown in Table 1.3. The length of taproot and hypocotyledonary axis of cucumber increased at low levels and then decreased with increasing rate of CaC₂. When CaC₂ application rate reached 30 mg plate⁻¹, the lengths of root and hypocotyledonary axis of cucumber were reached to maximum, which were 148.5 and 77.7% of the control, respectively. But at 40 mg plate⁻¹ of CaC₂, the lengths of root and hypocotyledonary axis of cucumber were decreased by 26.4 and 12.6% of the control, respectively. The width of hypocotyledonary axis was significantly increased with the increasing rate of CaC₂. Maximum hypocotyledonary width (0.21 cm) was found in the treatment of 40 mg plate⁻¹ of CaC₂. Here the increase was almost 31% more than control. The number of 1st lateral root and fresh weight of cucumber seedlings were significantly increased with increasing rate of CaC₂ application. But at the 40 mg plate⁻¹ of CaC₂, the number of 1st later root and fresh weight of seedlings were decreased by 28.5 and 21.8%, respectively, compared to the control.
iv- Analysis of the subordination function

Analysis of the subordination function is a comprehensive method to evaluate different rates CaC\textsubscript{2} for seed germination and growth indices. The correlation of subordination function values of germination and growth indices of cucumber were investigated and found that the correlation coefficients of all studied indices except the width of hypocotyledonary axis were found highly significant (Table 1.5). According to the analysis of subordination function, 30 mg plate\textsuperscript{-1} of CaC\textsubscript{2} was found the best rate which enhanced the germination and seedling growth of cucumber (Table 1.4).

1.4 Discussion

The results of this experiment revealed that germination rate, germination index, germination potential and growing vigor index of cucumber was significantly improved at lower rates of CaC\textsubscript{2} (10 to 30 mg CaC\textsubscript{2} plate\textsuperscript{-1}) compared to the highest rate of CaC\textsubscript{2} (40 mg plate\textsuperscript{-1}) suppressed all germination characteristics as shown in Table 1.1. In addition to germination, CaC\textsubscript{2} was also found effective in enhancing the root length, hypocotyledonary axis, number of 1st lateral roots and fresh weight of seedling of cucumber at lower rates of CaC\textsubscript{2} i.e. 10 to 30 mg plate\textsuperscript{-1} of CaC\textsubscript{2}. However, the highest rate of CaC\textsubscript{2} i.e. 40 mg plate\textsuperscript{-1} of CaC\textsubscript{2} showed reverses results. Further, it was demonstrated that the CaC\textsubscript{2} induced improvement in seed germination indices was related to its enhancement of ethylene production during imbibition (Fig. 1.1). Similar results in cucumber had been reported by Lijin (2007) by using ethephon as ethylene source where significant increase in germination rate, germination potential, germination index and activity index than those of control was reported. The germination rate and germination potential of cucumber seeds treated with 4000 and 5000 mg/L of ethephon had extremely significant differences compared to control.

Similarly, improved seed germination in both dormant and non-dormant seeds have been reported in response to the application of ethylene (Kepczynski and Kepczynska, 1997; Matilla, 2000; Kucera et al., 2005). In this experiment, enhanced seed germination by the application of CaC\textsubscript{2} was associated with increased ethylene evolution and similar results have been reported by Machabee and Saini (1991), and Calvo et al. (2004), signifying that ethylene is involved in regulation of seed germination.
Table 1.3 Effect of different rates of CaC\textsubscript{2} on some plant morphological characteristics of cucumber

<table>
<thead>
<tr>
<th>Calcium Carbide (mg plate\textsuperscript{-1})</th>
<th>No. of lateral roots</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Shoot width (cm)</th>
<th>Seedling weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.7 d</td>
<td>5.46 d</td>
<td>4.45 d</td>
<td>0.16 d</td>
<td>1.19 d</td>
</tr>
<tr>
<td>10</td>
<td>18.34 c</td>
<td>8.21 c</td>
<td>5.66 c</td>
<td>0.17 c</td>
<td>1.33 c</td>
</tr>
<tr>
<td>20</td>
<td>21.45 b</td>
<td>10.13 b</td>
<td>6.58 b</td>
<td>0.19 b</td>
<td>1.56 b</td>
</tr>
<tr>
<td>30</td>
<td>24.21 a</td>
<td>13.57 a</td>
<td>7.91 a</td>
<td>0.19 b</td>
<td>1.83 a</td>
</tr>
<tr>
<td>40</td>
<td>11.23 e</td>
<td>4.02 e</td>
<td>3.89 e</td>
<td>0.21 a</td>
<td>0.93 e</td>
</tr>
<tr>
<td>Means</td>
<td>18.19</td>
<td>8.28</td>
<td>5.70</td>
<td>0.18</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Means sharing same letter in each column are statistically non-significant at P> 0.05

Table 1.4 The Subordination function values for germination and morphological indices of cucumber at different rates of CaC\textsubscript{2}

<table>
<thead>
<tr>
<th>Calcium Carbide (mg plate\textsuperscript{-1})</th>
<th>X1</th>
<th>X2</th>
<th>X3</th>
<th>X4</th>
<th>X5</th>
<th>X6</th>
<th>X7</th>
<th>X8</th>
<th>X9</th>
<th>The Subordination function</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.48</td>
<td>0.43</td>
<td>0.46</td>
<td>0.30</td>
<td>0.34</td>
<td>0.15</td>
<td>0.13</td>
<td>0.00</td>
<td>0.28</td>
<td>2.60</td>
</tr>
<tr>
<td>10</td>
<td>0.75</td>
<td>0.62</td>
<td>0.71</td>
<td>0.50</td>
<td>0.54</td>
<td>0.43</td>
<td>0.44</td>
<td>0.20</td>
<td>0.44</td>
<td>4.66</td>
</tr>
<tr>
<td>20</td>
<td>0.92</td>
<td>0.78</td>
<td>0.87</td>
<td>0.73</td>
<td>0.78</td>
<td>0.64</td>
<td>0.66</td>
<td>0.60</td>
<td>0.70</td>
<td>6.72</td>
</tr>
<tr>
<td>30</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.60</td>
<td>1.00</td>
<td>8.60</td>
</tr>
<tr>
<td>40</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Note: X1-X9= Germination rate, Germination potential, Germination index, Seedling vigor index No. of lateral roots, Root length, Shoot length, Shoot width, and Fresh seedling weight, respectively.
### Table 1.5 Correlation coefficients among germination and growth indices

<table>
<thead>
<tr>
<th>Indexes</th>
<th>X1</th>
<th>X2</th>
<th>X3</th>
<th>X4</th>
<th>X5</th>
<th>X6</th>
<th>X7</th>
<th>X8</th>
<th>X9</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X2</td>
<td>0.986**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X3</td>
<td>0.998**</td>
<td>0.994**</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>X4</td>
<td>0.957*</td>
<td>0.987**</td>
<td>0.97**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X5</td>
<td>0.937*</td>
<td>0.994*</td>
<td>0.983**</td>
<td>0.998**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X6</td>
<td>0.908*</td>
<td>0.951*</td>
<td>0.925*</td>
<td>0.986**</td>
<td>0.975**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X7</td>
<td>0.911*</td>
<td>0.950*</td>
<td>0.927*</td>
<td>0.986**</td>
<td>0.976**</td>
<td>0.999**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X8</td>
<td>-0.338</td>
<td>-0.281</td>
<td>-0.321</td>
<td>-0.127</td>
<td>-0.172</td>
<td>0.003</td>
<td>0.016</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>X9</td>
<td>0.937*</td>
<td>0.977**</td>
<td>0.955</td>
<td>0.997**</td>
<td>0.992*</td>
<td>0.988*</td>
<td>0.987**</td>
<td>-0.09</td>
<td>1</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01.

Note: X1-X9= Germination rate, Germination potential, Germination index, Seedling vigor index No. of lateral roots, Root length, shoot Length, Shoot width, fresh seedling weight
However, the mechanistic details of ethylene to stimulate seed germination are not fully clear. Ethylene produced after onset of imbibition has been reported to interfere with ABA-induced seed dormancy to enhance seed germination (Beaudoin et al., 2000). Weakening of perisperm has been reported to regulate seed germination in muskmelon (Welbaum et al., 1990) and in cucumber (Ramakrishna and Amrithale, 2005). This mechanism was further verified recently by Linkies et al. (2009) in which he reported that improved seed germination in Arabidopsis thaliana and Lepidium sativum was due to weakening of endosperm cap and endosperm rupture by antagonizing inhibitory effect of ABA on both processes. A similar mechanism may also account for improved germination in response to CaC\textsubscript{2} application in this experiment.

Different peaks of ethylene evolution were observed after the beginning of imbibition in cucumber seeds while ethylene emission was increased with passage of time. Different patterns of ethylene evolution have been reported in various plant species. Similar results regarding ethylene evolution were reported by Takayanagi and Harrington (1971) in rapeseed in which ethylene evolution was strongly coincided with the rupture of the seed coat, radicle emergence and cotyledon enlargement. Meheriuk and Spencer (1964) also detected ethylene evolution in oat seeds before radicle emergence which was increased with passage of incubation time. In lettuce, Small et al. (1993) detected a higher peak of ethylene evolution after the emergence of radical while in some cases main peak of ethylene was detected at the time of radical emergence (Fu and Yang, 1983; Saini et al., 1986).

Application of different rates of calcium carbide affected seed germination and seedling growth in a different pattern but application of 30 mg CaC\textsubscript{2} plate\textsuperscript{-1} was found the best and appropriate rate of application regarding seed germination and seedling growth indices in cucumber (Tables 1.1, 1.2 and 1.3). This might be due to release of appropriate amount of ethylene in proximity of seeds that stimulated seed germination in petri plates treated with CaC\textsubscript{2}. Dose response seedling growth and germination was observed in the treatments where CaC\textsubscript{2} was applied at different rates. Similar trend has been reported by Kashif et al. (2012) where results showed a significant relationship between critical level of CaC\textsubscript{2} and seed germination. Calcium carbide gave a different pattern of ethylene production from germinating seed as compared to control and the best results were found at 30 mg plate\textsuperscript{-1}.
regarding root and shoot growth and seed germination in okra seeds. Similar results were observed by Abbasi et al. (2009) where the application of CaC$_2$ significantly reduced days to sprouting of potato tubers. Results are also in agreement with findings of Siddiq et al. (2011) where application of CaC$_2$ at increasing rates significantly improved seed germination of different genotypes of tomato. It was also observed that addition of maximum dose of CaC$_2$ enhanced growth rate of root and shoot maximally which was proved non-toxic at this rate of application.

The results of this experiment provide information that application of calcium carbide at right rate could be useful for early germination and seedling emergence as is obvious from figures 1.5 and 1.6. Moreover, this information could be particularly useful for vegetable growers where they need early and fast growth of vegetables to fetch more benefit from the market. These results suggested that application of CaC$_2$ can be used to stimulate early root growth. However, to explore the role of ethylene under nitrogen nutrient stress conditions are required to further elaborate its mode of action. As a lot of information is available regarding the effect of CaC$_2$/ethylene in the absence or presence of ammonium form of nitrogen fertilizer on plant growth in the literature and work conducted by other students of this laboratory. Therefore, these results should also be verified in the presence and absence of exogenous nitrate nitrogen source to study physiological response of cucumber cultivar under the influence of ethylene released from CaC$_2$. The details are given in the next experiment.
Figure 1.5 Effect of 20 and 30 mg CaC₂ plate⁻¹ on seedling growth in cucumber after 7 days of incubation
Figure 1.6 Effect of CaC\textsubscript{2} at different rates on seedling growth in cucumber after 7 days of incubation
Experiment 2: Effect of different rates of CaC$_2$ on biochemical changes, chlorophyll contents and nitrogen metabolism in cucumber

2.1 Introduction

Application of exogenous plant hormones can stimulate different morpho-physiological and metabolic changes in plant tissues causing modification in plant structure. Plant hormone’s role in germination, photosynthesis, nitrogen assimilation enzymes and respiration is well documented (Goswami and Srivastava, 1989; Chanda et al., 1998, Khan, 2006). Moreover, role of plant hormones has been investigated at cellular and subcellular level and it is proposed that they can influence the nutrient uptake, (i) by changing cell membrane permeability through direct interaction or indirectly by modifying enzymatic reactions associated with membrane permeability, and (ii) by changing the energy metabolism by affecting ATP synthesis (Luttge and Higinbotham, 1979).

Ethylene is considered as an important hormone regarding its role in nutrient signaling and uptake. Evidences have been accumulated regarding the role of ethylene in root morphology especially under nutrient stress (Lynch and Brown, 1997). It can affect root morphology by regulating root length, lateral root spread and root hair production. Deficiency of nutrients can also affect these processes in similar fashion. Therefore, the use of mutants of ethylene biosynthesis has been convenient and useful technique to understand the involvement of ethylene under different stresses including nutrient stress (Zhang et al., 2003). Different kinds of interaction between nitrogen and ethylene have reported in literature. Lynch and Brown (1997) reported that response of ethylene depends upon nitrogen status of. Ethylene production in wheat plants was increased under nitrogen deficient conditions (Tari and Szen, 1995). Exogenous application of ethephon, as foliar-applied source of ethylene, has been found to enhance nitrogen use efficiency in barley and wheat crops (Van Sanford et al., 1989; Bulman and Smith, 1993).

The role of ethylene in nitrogen metabolism under stress conditions is still very limited. Therefore, ethylene based regulation of N-metabolism due to environmental constraints requires more detailed study to understand germination and seedling growth under N-deficiency. Recently, calcium carbide (CaC$_2$), a potent source of soil-applied source of
ethylene, has received attention being after being determined that it can induce germination and early seedling growth in okra and tomato plants (Kashif et al., 2008; Siddiq et al., 2009). By studying the effect of ethylene on different nitrogen metabolism indicators may provide a method for the diagnosis of nitrogen nutrient disorder in plants. Therefore, effects of exogenous CaC\(_2\) on growth, development and nitrogen metabolism in cucumber must be determined to understand physiological responses to N-nutrition.

The objectives of this study were to investigate the role of CaC\(_2\) in regulation of nitrogen metabolism related enzymes and growth parameters like NR (nitrate reductase) activity, GS (glutamine synthetase) activity, chlorophyll synthesis, soluble carbohydrate content, total soluble proteins and free amino acids, percent seed germination and seedling development of cucumber (Cucumis sativus L.).

### 2.2 Materials and Methods

Methodology of the experiment is described in the experiment 2 of chapter 3.

### 2.3 Results

This experiment was conducted in two parts. The part 1 includes studying the effect of different rates of CaC\(_2\) with and without addition of KNO\(_3\) on germination and seedling growth parameters were studied. Moreover, part 2 includes studying the response of isolated cotyledons to different rates of CaC\(_2\) with and without addition of KNO\(_3\) for some biochemical changes.

#### 2.3 (Part 1) Growth analyses

Calcium carbide induced several morphological changes depending on the rate applied, as the highest rate of CaC\(_2\) was found to have inhibitory action on the growth of cucumber plants. Data elucidate that seed germination after 48 h was observed as the highest in the treatment of 30 mg CaC\(_2\) plate\(^{-1}\) with or without NO\(_3^-\). It was also noted that effect of CaC\(_2\) was more significant in the presence of NO\(_3^-\) than in the absence of NO\(_3^-\) (Table 2.1). However, 40 mg plate\(^{-1}\) CaC\(_2\) rate caused reduction in germination by 28.8% compared to control (no CaC\(_2\)). Observations were gathered for 7 days to determine the influence of CaC\(_2\) on seedlings growth.
Table 2.1 (Part 1) Effect of different rates of CaC$_2$ on growth characteristics of cucumber seedlings with and without 20 mM KNO$_3$ after 7 d of incubation period

<table>
<thead>
<tr>
<th>CaC$_2$ (mg plate$^{-1}$)</th>
<th>KNO$_3$ (mM)</th>
<th>Germination (%)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Root weight (mg)</th>
<th>Shoot weight (g)</th>
<th>Seedling weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>63.8 f</td>
<td>5.03 f</td>
<td>6.43 f</td>
<td>22.23 f</td>
<td>0.37 f</td>
<td>0.58 f</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>71.56 e</td>
<td>5.42 e</td>
<td>7.21 e</td>
<td>28.35 e</td>
<td>0.474 e</td>
<td>0.75 e</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>73.2 d</td>
<td>5.98 d</td>
<td>8.12 d</td>
<td>31.36 d</td>
<td>0.523 d</td>
<td>0.83 d</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>81.2 c</td>
<td>6.68 c</td>
<td>9.23 c</td>
<td>35.45 c</td>
<td>0.585 c</td>
<td>0.93 c</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>84.23 b</td>
<td>7.56 b</td>
<td>12.2 b</td>
<td>40.21 b</td>
<td>0.837 b</td>
<td>1.33 b</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>91.67 a</td>
<td>9.56 a</td>
<td>14.2 a</td>
<td>45.08 a</td>
<td>0.924 a</td>
<td>1.46 a</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>45.45 h</td>
<td>3.81 g</td>
<td>4.44 g</td>
<td>16.45 g</td>
<td>0.263 g</td>
<td>0.41 g</td>
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<td></td>
<td>20</td>
<td>59.37 g</td>
<td>3.87 g</td>
<td>4.41 g</td>
<td>16.10 g</td>
<td>0.251 g</td>
<td>0.39 g</td>
</tr>
</tbody>
</table>

Means sharing same letter in each column are statistically non-significant at $P > 0.05$
Results indicated that 20 µM of NO$_3^-$ in conjugation with 30 mg plate$^{-1}$ of CaC$_2$ caused increase in root and shoot lengths, while higher dose of CaC$_2$ than this was found to have inhibitory effect both with or without NO$_3^-$ (Table 2.1). Growth determining parameters revealed that effect of CaC$_2$ in cucumber have been mediated by the specific rate of CaC$_2$ rather than the supply of external NO$_3^-$. Overall, 30 mg plate$^{-1}$ of CaC$_2$ exhibited the highest fresh seedling weight (1.46 g per plant) among 7 days old seedlings, while the highest level of CaC$_2$ reduced fresh seedling weight even in the presence of NO$_3^-$ (Table 2.1). Similarly, weight and length of root and shoot of cucumber seedlings were significantly increased (P<0.05) by the application of lower rates of CaC$_2$ (20 to 30 mg CaC$_2$ plate$^{-1}$) while these were decreased in the treatment of 40 mg plate$^{-1}$ of CaC$_2$ under the presence as well as absence of external nitrate application. Maximum increase in weight and length of root and shoot of cucumber seedlings was found in the treatment of 30 mg plate$^{-1}$ of CaC$_2$ plus external nitrate (20 mM KNO$_3$) as is shown in Table 2.1.

2.3 (Part 2) Biochemical analyses

i) Nitrate reductase activity

To see the effect of CaC$_2$ on the possible correlation between NO$_3^-$ assimilation and NR activity, CaC$_2$ treated cucumber cotyledons were studied for nitrate reductase (NR) activity in the absence as well as presence of NO$_3^-$ nutrition (Figure 2.1). In absence of NO$_3^-$ application, CaC$_2$ increased NR activity by 35% compared to control (411.5 nmol NO$_2^-$ h$^{-1}$g$^{-1}$FW) in the treatment of 30 mg CaC$_2$ plate$^{-1}$ and then significantly reduced in the treatment of 40 mg plate$^{-1}$ of CaC$_2$. While, the presence of NO$_3^-$ further increased NR activity and it was maximum in the treatment of 30 mg plate$^{-1}$ of CaC$_2$. However, 10 % reduction in NR activity was recorded in the treatment of 40 mg plate$^{-1}$ of CaC$_2$ compared to control. It was estimated that 30 mg plate$^{-1}$ of CaC$_2$ in the presence of NO$_3^-$ increased NR activity by 81 %, however, higher rate of CaC$_2$ (40 mg plate$^{-1}$) reduced it by 10.3% compared to control.

ii) Glutamine synthetase activity

To determine the interactive effect of the nitrogen nutrition as NO$_3^-$ and CaC$_2$ on nitrogen assimilation in cucumber cotyledons, Glutamine synthetase (GS) activity was investigated in absence as well as presence of NO$_3^-$ nutrition (Figure 2.3).
Figure 2.1 Effect of CaC\textsubscript{2} on nitrate reductase activity (NR) in cotyledon of cucumber seedlings with and without 20 mM KNO\textsubscript{3} after 48 h of incubation period.

Figure 2.2 Effect of CaC\textsubscript{2} on glutamine synthetase (GS) activity in cotyledon of cucumber seedlings with and without 20 mM KNO\textsubscript{3} after 48 h of incubation period.

Means sharing same letter in each bar are statistically non-significant at P> 0.05

FW= fresh weight, NR= Nitrate reductase, GS= Glutamine synthetase
In absence of exogenous NO$_3^-$, application of CaC$_2$ increased GS activity by 32.3% (10767.4 nmol glutamyl hydroxamate h$^{-1}$g$^{-1}$FW) up to 30 mg CaC$_2$ plate$^{-1}$ and then significantly reduced at 40 mg CaC$_2$ plate$^{-1}$ (Table 2.3). While, in the presence of NO$_3^-$, GS activity was maximum at 30 mg plate$^{-1}$ of CaC$_2$, however it was reduced at 40 mg plate$^{-1}$ level of CaC$_2$ compared to control. Overall results indicated that up to 30 mg plate$^{-1}$ of CaC$_2$ in the presence of NO$_3^-$, GS activity was increased by 56.1% compared to control. After that higher rate of CaC$_2$ (40 mg plate$^{-1}$) reduced it by 10.3% of control.

iii) Photosynthetic pigments

Photosynthetic pigments were recorded separately as chlorophyll a, b and carotenoids. Low rates of CaC$_2$ (20 to 30 mg CaC$_2$ plate$^{-1}$) gradually increased chlorophyll a, b and carotenoids contents, however 30 mg plate$^{-1}$ of CaC$_2$ showed maximum increase which decreased significantly thereafter at highest level of CaC$_2$ both in the absence as well as presence of NO$_3^-$ (Figure 2.3, 2.4 and 2.5). Application of 30 mg CaC$_2$ plate$^{-1}$ increased chlorophyll a, b and carotenoids contents in cotyledonary tissues by 114.4, 78.7 and 91%, respectively in the presence of NO$_3^-$ while contents of chlorophyll a, b and carotenoids were increased by 73.6, 55.2 and 63.1%, respectively in the absence of NO$_3^-$, in comparison to control. All photosynthetic pigments were reduced significantly at 40 mg plate$^{-1}$ CaC$_2$ treatment both in presence and absence of NO$_3^-$ (20 mM KNO$_3$).

iv) Total soluble sugars

The content of soluble sugar increased gradually at lower rates of CaC$_2$ until optimal contents reached at 30 mg plate$^{-1}$ of CaC$_2$ compared to the control as well as alone NO$_3^-$ treatment and decreased at the highest rate of CaC$_2$ (40 mg plate$^{-1}$) (Figure 2.6). The effect of CaC$_2$ was more significant in presence of external NO$_3^-$1. The application of 30 mg plate$^{-1}$ CaC$_2$ with 20 mM NO$_3^-$ increased the contents of sugars up to maximum by 64.8% compared to nitrate alone treatment, however the least being in plants treated with 40 mg plate$^{-1}$ CaC$_2$ without NO$_3^-$1. In the absence of NO$_3^-$ similar trend was observed by getting maximum increase (51.9%) in sugar contents at 30 mg plate$^{-1}$ compared to the control, while 40 mg plate$^{-1}$ CaC$_2$ reduced sugar contents.
Figure 2.3 Effect of CaC\textsubscript{2} on chlorophyll a contents in cotyledon of cucumber with and without 20 mM KNO\textsubscript{3} after 48 h of incubation period

Means sharing same letter in each bar are statistically non-significant at $P>0.05$

**FW= fresh weight**
Figure 2.5 Effect of CaC\textsubscript{2} on carotenoids contents in cotyledon of cucumber with and without 20 mM KNO\textsubscript{3} after 48 h of incubation period

Means sharing same letter (s) in each bar are statistically non-significant at P> 0.05

FW= fresh weight

Figure 2.6 Effect of CaC\textsubscript{2} on total soluble sugars in cotyledon of with and without 20 mM KNO\textsubscript{3} after 48 h of incubation period

Means sharing same letter (s) in each bar are statistically non-significant at P> 0.05

FW= fresh weight
v) Soluble proteins

The content of soluble proteins in cucumber cotyledons after application of different rates of CaC$_2$ was analyzed on fresh weight basis. Gradual increase in soluble proteins contents were observed up to 30 mg plate$^{-1}$ of CaC$_2$ in comparison to control, while the soluble protein contents declined sharply at the higher rate of CaC$_2$ (40 mg plate$^{-1}$) (Figure 2.7). Concentration based CaC$_2$ response was more prominent in the presence of exogenous NO$_3^-$ in comparison to without NO$_3^-$. In the presence of nitrate, the highest level of soluble proteins was observed at 30 mg CaC$_2$ plate$^{-1}$ (7.2 mg g$^{-1}$ FW) which was 64% higher than that of control. Similar trend was observed in the presence of exogenous nitrate by getting maximum increase (41.3%) in soluble proteins contents at 30 mg plate$^{-1}$ compared to the aqueous control. Application of 40 mg plate$^{-1}$ CaC$_2$ reduced soluble proteins contents both in presence as well as absence of exogenous NO$_3^-$. 

vi) Free amino acids

Calcium carbide induced changes in the level of free amino acids were analyzed on fresh weight basis in the cotyledons of cucumber. Free amino acid contents increased significantly in the treatments of low rates of CaC$_2$ (20 to 30 mg CaC$_2$ plate$^{-1}$) compared to control, while free amino acid level declined sharply at the higher rate of CaC$_2$ (40 mg plate$^{-1}$) (Figure 2.8). Application of 30 mg plate$^{-1}$ of CaC$_2$ along with NO$_3^-$ increased the contents of free amino acids up to the maximum by 84.8% compared to control. Concentration based CaC$_2$ response was more prominent in the presence of NO$_3^-$ compared to without NO$_3^-$. In the presence of nitrate, the highest level of free amino acids was observed at 30 mg CaC$_2$ plate$^{-1}$(1.22 mg g$^{-1}$FW) whereas; in the absence of exogenous nitrate it was 1.03 mg g$^{-1}$ FW (Figure 2.8). Application of 40 mg plate$^{-1}$ of CaC$_2$ reduced free amino acid level in presence as well as absence of NO$_3^-$. 
Figure 2.7 Effect of CaC$_2$ on total soluble proteins in the cotyledon of cucumber seedlings with and without 20 mM KNO$_3$ after 48 h of incubation period

Means sharing same letter (s) in each bar are statistically non-significant at P> 0.0

Figure 2.8 Effect of CaC$_2$ on free amino acids in cotyledon of cucumber seedlings in the presence and absence of 20 mM KNO$_3$ after 48 h of incubation period

Means sharing same letter (s) in each bar are statistically non-significant at P> 0.0

FW= fresh weight
2.4 Discussion

Calcium carbide induced physic-biochemical effects are found concentration dependent. Higher doses of CaC\textsubscript{2} were found to cause inhibitory action in cucumber plants. Percentage of seed germination was found significantly promoted at lower rates (20 to 30 mg plate\textsuperscript{-1}) of CaC\textsubscript{2} and sharply reduced thereafter at higher rate (40 mg plate\textsuperscript{-1}) both in the absence as well as presence of nitrate application. The interactive effect of CaC\textsubscript{2} and nitrate was more prominent than individual effects. Since suitable environmental conditions are required for seed germination, therefore, role of exogenously applied ethylene in seed germination is a well-known fact (Khan and Prusinski, 1989; Huang and Khan, 1992; Nascimento, 1998; Nascimento \textit{et al}., 1999a, 1999b), but the mechanism of action is not completely known. Enhancement of seed germination by application of CaC\textsubscript{2} can be attributed to ethylene induced activation of degradative enzymes as has been reported in other species (Cervantes \textit{et al}., 1994; Petruzzelli \textit{et al}., 2000). It has been suggested that ethylene can influence seed germination i) by interaction with growth inhibitors e.g. abscisic acid; ii) by interaction with growth promoters required to maximize a given physiological response; iii) by affecting enzyme synthesis and secretion (Ketring, 1977).

In order to check the influence of nitrate on seed germination under controlled conditions, a moderate level of KNO\textsubscript{3} (about 20 mM) was used and significant improvement in seed germination was observed. Similarly, the role of nitrate to break seed dormancy and improve seed germination has been reported in various experiments under controlled conditions (Steinbauer and Grigsby, 1957; Popay and Roberts, 1970; Hendricks and Taylorson, 1974; Slade and Causton, 1979; Schimpf and Palmblad, 1980; Singh and Amritphale, 1992; Gul and Weber, 1998; Henig-Sever \textit{et al}., 2000). Despite the fact the mechanism of action is not completely understood still to date, positive effect of nitrate on seed germination has been reported in several species. Different environmental factors have been found to interact with nitrate to produce stimulating effect (Roberts and Benjamin, 1979; Saini \textit{et al}., 1987; Karssen \textit{et al}., 1988).

Nitrate as a signal molecule can regulate various aspects of plant growth and metabolism in spite of its role as nutrient (Scheible \textit{et al}., 1997a, b; Wang \textit{et al}., 2003). Role of nitrate as a signal molecule was shown in \textit{Sisymbrium officinale} where it enhanced seed germination by
stimulating GA synthesis in close interaction with light (Hilhorst and Karssen, 1988, 1989). Later on, nitrate was shown to enhance seed germination in Arabidopsis Landsberg erecta ecotype and in the Cape Verde Island ecotype by decreasing light requirement of seeds (Batak et al., 2002) and interacting with ABA levels in imbibed seeds (Ali-Rachedi et al., 2004), respectively.

The enhanced seed germination in the present study may be due to conversion of nitrate into nitrite which has been reported to stimulate germination of some seeds (Hendricks and Taylorson, 1974; Roberts and Benjamin, 1979). The promotion of germination due to the conversion to nitrite within the seed by nitrate treatments has been suggested by Hendricks and Taylorson (1974). Synergistic interaction of ethylene and nitrite may have been due to the increased seed sensitivity to nitrite converted from nitrate (Egley, 1984). Similarly, enhanced seed germination was observed in Arabidopsis due to signaling role of nitrate. Results in previous literature also showed that dual affinity nitrate transporter NRT1.1 (CHL1) was involved in passing on the nitrate signal into seeds (Alboresi et al., 2005). Higher level of CaC$_2$ provided toxic level of ethylene in the vicinity of seeds which might inhibited nitrate uptake system and caused retardation in growth and development of cucumber. Data regarding growth analysis indicated that CaC$_2$ behaved as a growth stimulator at lower concentrations while at higher concentration growth inhibition was observed. Similar responses of CaC$_2$ regarding seed germination and root and shoot growth parameters have been reported by Kashif et al. (2012) and Siddiq et al. (2011) in okra and tomato, respectively.

The results of 2nd part of experiment demonstrated a differential effect of CaC$_2$ induced ethylene on different nitrogen metabolism parameters of cotyledons of cucumber. Results regarding all nitrogen metabolism parameters showed a positive correlation with all rates of CaC$_2$ except at the highest rate where inhibitory response was observed. Nitrogen is an integral part of various biochemicals i.e. amino acids, nucleic acids, lipids, proteins, chlorophyll, and a lot of other nitrogen containing metabolites. Amino acids are synthesized after assimilation of inorganic nitrogen and are generally correlated with nitrogen status of leaves (Khamis et al., 1990; Scheible et al., 1997a). Inorganic nitrogen especially nitrate contents has been reported to influence carbohydrate metabolism in dicots (Scheible et al.,
Similarly, instant increase in amino acid contents has been observed when excised leaves are exposed to ammonia or nitrate nutrition (Foyer et al., 1994a). Interim increase in amino acids is generally due to increased supply of substrate or enzyme activation while long-term changes are due to modified expression of enzymes such as nitrate reductase (NR) (Scheible et al., 1997b). Two major control points in nitrogen metabolism are nitrate reductase (NR) and glutamine synthetase (GS) activity which are involved in the assimilation of inorganic nitrogen. These and many other control points, are coordinated by metabolic crosstalk and other signals mediated by plant growth hormones (Foyer et al., 2003).

It is assumed that in response to CaC₂ treatment, ethylene induced response is produced which has been shown to persist in plant under laboratory studies in petri dishes (Kashif et al., 2012). Cotyledons of CaC₂ treated plants had significantly higher NR and GS activity with and without external nitrate (NO₃⁻) during the 48 h of incubation which reached maximum at 30 mg plate⁻¹ of CaC₂ (Figure 2.1 and 2.2). It is highly likely that this was a direct effect of hormone on enzyme stimulation or ethylene that probably altered cellular metabolism or processes which resulted in increased NR activity. This stimulation of NR activity in cotyledon was accompanied with synthesis of soluble proteins, suggesting a dependence on new protein synthesis (Figure 2.7). Moreover, NO₃⁻ induced NR activity dependent on de novo protein synthesis has been reported in literature (Ferrari and Varner, 1969; Smith and Thompson, 1971).

Although CaC₂ stimulation of NR activity is dependent on protein synthesis, other factors may contribute to overall enhancement of activity. If CaC₂ generated ethylene increased membrane permeability in cotyledon tissues as is the case with other systems (Hanson and Kende, 1975; Kende and Baumgartner, 1974), then this would allow increased movement of NO₃⁻ from the storage pool to the metabolic pool. The increased protein and amino acid accumulation in cotyledon in response to CaC₂ (Figure 2.7 and 2.8) is consistent with published reports (Marei and Romani, 1971; Hensen, 1967; Hulme et al., 1971). This may involve elevated biosynthesis in view of reports indicating increased ribosome production and higher levels of rough endoplasmic reticulum in response to ethylene (Lieberman et al., 1983; Sargent and Osborne, 1975). If it is assumed that ethylene altered the amino nitrogen
fraction in favor of amino acids which enhanced NR activity (Radin, 1977, Oaks, 1977), then this could account for the observed results as increase in amino acid contents is shown in results (Figure 2.8).

Increase in glutamine synthetase (GS) activity might be directly stimulated by ethylene as it has also been reported to enhance glutamine synthetase activity and mRNA level in *Hevea brasiliensis* (Pujade-Renaud et al., 1994). Indirectly, ethylene can enhance GS activity by increased membrane permeability in cotyledon tissues and allowing increased movement of NO$_3^-$ from the storage pool to the metabolic pool as involvement of the higher cytosolic GS activity in the assimilation of ammonium originating from nitrate reduction nitrate has been suggested (Peat and Tobin, 1996; Tobin and Yamaya, 2001). Ethylene stimulation of respiration, resulting in increased ATP synthesis (Solomos and Lades, 1975), may also be important in the observed enhancement of cotyledon NR activity and GS activity.

Moreover, increase in NR contents in this study coincides with the several published results in which increase in nitrate reductase activity in leaves of *Brassica juncea* was reported (Mir et al., 2008a; Ashraf et al., 2010). Likewise, improvement in the chlorophyll content by application of ethrel in leaves of *Brassica napus* was reported but detrimental effects were also observed in cases where higher concentration of ethrel was used (Grewal et al., 1993; Grewal and Kolar, 1990).

The present results allow to draw conclusion that CaC$_2$ induced stimulation of NR, GS, and photosynthetic pigments are associated with increased levels of both soluble proteins and free amino acids under optimal rate of CaC$_2$ in the presence or absence of NO$_3^-$ but results were more prominent under nitrate nutrition i.e., in the presence of nitrate. In addition to nutritional stress, ethylene has also been reported to be involved in salinity stress conditions. Therefore, further studies are needed to verify the involvement of CaC$_2$ in improving germination under salinity stress by investigating its effect on reactive oxygen species and antioxidant enzymes during germination. Details of experiment are given in the next experiment.
Experiment 3: Effect of CaC\(_2\) on germination of cucumber seeds to alleviate salinity stress

3.1 Introduction

Soil salinity is the most important limiting factors to crop production, particularly in arid regions of the world and also being considered as a serious hurdle towards the reclamation of new salt affected soils. Salinity has affected about 20% the total world’s cultivated land and nearly half of the irrigated land (Zhu, 2001). Sodium chloride is the most abundant salt as far as composition of salt affected soils is concerned. Increase in salt affected area is reducing the production of many crops including most of vegetables. Among various processes of plant life cycle, seed germination is the most critical phase vulnerable to salinity stress (Cesur and Tabor, 2011) which ultimately leads to reduction in germination rate and early growth (Singh et al., 2012). Being the first step in plant life cycle it is very important to study the physiological mechanisms of poor seed germination under the influence of salinity and also to develop appropriate techniques to ameliorate the adverse effects of salinity regarding seed germination and plant establishment on salt affected soils. A large number of studies have reported the effects of salinity on physiological processes. Salinity inhibits the seed germination process through imbalance of plant growth hormones, inadequate imbibition, interference with metabolism, ionic toxicity, and destruction of enzymes (Ungar, 1995), finally leading to the inactivation of antioxidant enzymes and production of reactive oxygen species (Tanou et al., 2009). Production of reactive oxygen species (ROS) during seed germination is being considered an important mechanism to regulate seed germination under both normal and stress conditions. Certain concentration of ROS is essential for releasing dormancy and improving seed germination in many plant species (Bailly et al., 2008; Leymarie et al., 2012). However, elevated levels of ROS are produced in response to salinity, causing oxidative stress (Miller et al., 2010). As a result plants have evolved different protective mechanisms including enzymatic and non-enzymatic ROS-scavenging antioxidants to counteract the damage caused by ROS (Yin et al., 2008). Therefore, increasing the antioxidant enzyme activity in plant systems is essential for improving salt tolerance in plants.
Generally, seed germination is dependent on strict hormonal regulation (Bogatek and Gniazdzowska, 2012). Ethylene can improve germination inhibition caused by salt stress in many seeds (Khan et al., 2009, Chang et al., 2010; Lin et al., 2012) but not alone rather interacting with other factors factor controlling this process. Ethylene has been reported to enhance seed germination under salt stress by regulating ROS production during seed germination (Lin et al., 2012).

Cucumber (Cucumis sativus L.) is considered as a salt sensitive crop, especially during germination stage. Therefore, cucumber was selected as plant material to investigate if application of CaC₂ could reduce germination inhibition caused by salinity. Simultaneously, the effects of CaC₂ on ROS production and antioxidant enzymes activities were studied in salt treated cucumber seeds by using CaC₂. Therefore, the investigation of ROS metabolism in response to exogenous application of CaC₂ could be a useful study to explore defense mechanism in cucumber under salt stress. Moreover, the outcome of this study would help in developing CaC₂ as cheaper technology to induce salt tolerance in cucumber.

3.2 Materials and Methods
Methodology of the experiment is described in experiment 3 of chapter 3.

3.3 Results

This experiment was conducted in four consecutive parts. The part 1 includes studying germination response of cucumber seeds to different concentrations of NaCl during 6 d of incubation. In 2nd part of this experiment, effect of different levels of CaC₂ was evaluated on seed germination and ethylene evolution under 150 mM NaCl stress. In the 3rd part of this experiment, effect of 30 mg CaC₂ under 150 mM NaCl salinity was investigated on seed germination in the presence and absence of inhibitor of ethylene synthesis (CoCl₂) and perception (AgNO₃). In the 4th part of this experiment, the effect of 30 mg CaC₂ was investigated during different intervals of incubation on some physio-biochemical attributes of germinating cucumber seeds.
3.3 (Part 1) Seed germination in cucumber as affected by salinity

Salinity (NaCl) significantly (P<0.05) delayed or inhibited seed germination rate of hybrid cultivar Bolan-F1, compared to the control (without NaCl) (Figure 3.1). As the salinity level increased germination percentage delayed. After 24 hours of incubation maximum germination (46.3%) was observed in the control, followed by 39.5% at 50 mM NaCl level and there was no germination occurred at higher salinity level (Figure 3.1). As the salinity level increased germination percentage delayed. After 2 days of incubation minimum germination (15.6 %) was observed at 150 mM NaCl compared to control (67.3 %). At 100 mM NaCl salinity, about 61% germination was observed 3 days after transfer to light compared to almost 93% in the control while at 150 and 200 mM NaCl levels, seed germination rate was severely inhibited (Figure 3.1). After 6 days of incubation seed germination at 200 mM NaCl was increased to 64.3%, followed by 86.7% at 150 mM NaCl.

3.3 (Part 2) Ethylene emission and seed germination after application of CaC\textsubscript{2} under salinity stress

Effect of CaC\textsubscript{2} on ethylene production and seed germination was investigated under salinity stress. Ethylene production rate was markedly reduced from germinating seeds in response to NaCl stress (Figure 3.2). On the other hand, addition of CaC\textsubscript{2} to the petri plates significantly alleviated inhibition in ethylene production caused by NaCl stress from germinating seeds (Figure 3.2). After 48 h of incubation, ethylene emission was reduced by 54% in 150 mM NaCl stress compared to the control but ethylene suppression was reduced by increasing concentration of CaC\textsubscript{2} and maximum ethylene evolution (9.78 nl g\textsuperscript{-1} FW h\textsuperscript{-1}) was observed at 30 mg plate\textsuperscript{-1} of CaC\textsubscript{2}. Seed germination was observed in the same treatment after 48 h of incubation. Minimum seed germination (21.3%) was observed in 150 mM NaCl stress where no CaC\textsubscript{2} was applied. Seed germination was significantly enhanced by increasing rates of CaC\textsubscript{2} and maximum germination (64.6%) was observed at 30 mg plate\textsuperscript{-1} of CaC\textsubscript{2} which was increased by 207% compared to the control without CaC\textsubscript{2}. 

Figure 3.1 Germination responses of cucumber seeds to salinity stress at different intervals of incubation. [Values are means of 4 measurements [Bars represent ± SE (n = 4)]

Figure 3.2 Effect of different rates of CaC\textsubscript{2} on ethylene evolution and seed germination under 150 mM NaCl stress after 48 h of incubation [wt. = weight]

Means sharing same letter at each bar and at each point on line graph are statistically non-significant at P> 0.05
3.3 (Part 3) Germination responses along with ethylene inhibitors after application of CaC\textsubscript{2} under salinity stress

To further verify the involvement of ethylene for enhanced seed germination after application of CaC\textsubscript{2} in the absence and presence of NaCl, we investigated the effect of CaC\textsubscript{2} on seed germination by using ethylene perception (AgNO\textsubscript{3}) and ethylene action inhibitor (CoCl\textsubscript{2}). There was significant reduction in seed germination (77\%) under salinity stress (150 mM NaCl) compared to the control (without NaCl) (Figure 3.3). However, in the treatment of 30 mg plate\textsuperscript{-1} of CaC\textsubscript{2}, a marked increase in seed germination (208\%) was observed compared to the control (150 mM NaCl without CaC\textsubscript{2}). Maximum decrease (35\%) in seed germination was observed in the treatment where AgNO\textsubscript{3} was applied, followed by 27\% decrease in the treatment where CoCl\textsubscript{2} was applied with respect to treatment where only CaC\textsubscript{2} (30 mg plate\textsuperscript{-1}) was applied under salinity stress (Figure 3.3).

3.3 (Part 4) Effect of CaC\textsubscript{2} on some biochemical changes in cucumber seeds under salt stress

i) Effect of CaC\textsubscript{2} on α-Amylase activity under salinity stress

In the 4\textsuperscript{th} part of experiment, the impact of CaC\textsubscript{2} on α-amylase activity was investigated under salinity stress during 5 days of incubation (Figure 3.4). In the beginning, α-amylase activity was low and increased thereafter till day 3 of treatment and then decreased at day 4 and 5 of the treatment. Maximum amylase activity (1.62 mg maltose mg\textsuperscript{-1} protein min\textsuperscript{-1}) was observed after 3 days of treatment. Compared to the control, exogenous CaC\textsubscript{2} (30 mg CaC\textsubscript{2} plate\textsuperscript{-1}) significantly enhanced α -amylase activity in cucumber (P < 0.05) during 5 days of incubation (Figure 3.4).

ii) Effect of CaC\textsubscript{2} on H\textsubscript{2}O\textsubscript{2} contents under salinity stress

Contents of H\textsubscript{2}O\textsubscript{2} in cucumber seed increased during germination under salt stress (Figure 3.5). Exogenous application of CaC\textsubscript{2} significantly alleviated the salt induced accumulation of H\textsubscript{2}O\textsubscript{2} in the germinating cucumber seeds (P < 0.05). Compared to the control, H\textsubscript{2}O\textsubscript{2} content in CaC\textsubscript{2} treated seeds at days 1, 2, 3, 4 and 5 after treatment decreased to 42.3, 40.2, 31.1, 25.9 and 23.1\%, respectively.
Figure 3.3 Germination response of cucumber seeds to CaC$_2$ along with ethylene synthesis and action inhibitors under salinity stress after 48 hours of incubation.

Figure 3.4 Effect of CaC$_2$ on total $\alpha$-amylase activity in cucumber seeds during different intervals of incubation under salinity (150 mM NaCl) stress.

Means sharing same letter at each bar (Fig. 3.3) and each day (Fig. 3.4) are statistically non-significant at $P > 0.05$. 

NaCl = 150 mM, CC = CaC$_2$ @ 30 mg plate$^{-1}$  CoCl$_2$ = 100 uM and AgNO$_3$ = 50 uM
iii) **Effect of CaC$_2$ on total soluble sugars under salinity stress**

Exogenous treatment of CaC$_2$ significantly increased the salt induced inhibition of sugar contents (P < 0.05) (Figure 3.6). Initially, there was no significant difference in sugar contents after application of CaC$_2$ at 24 h of incubation. Compared to the control, sugar content in CaC$_2$ treated seeds at day 2, 3, 4 and 5 after treatment increased to 40.9, 50.6, 25.5 and 16%, respectively.

iv) **Effect of CaC$_2$ on free amino acids under salinity stress**

Exogenous application of CaC$_2$ significantly increased the salt induced inhibition of amino acid contents (P < 0.05) (Figure 3.8). Free amino acid contents in CaC$_2$ treated seeds were increased by 54.2, 28.6, 41.7, 21.8 and 24.8% at day 1, 2, 3, 4 and 5, respectively.

v) **Effect of CaC$_2$ on antioxidative enzymes, malondialdehyde and protein contents under salinity stress**

Exogenous CaC$_2$ application significantly alleviated the salt induced accumulation of malondialdehyde (MDA) in the germinating cucumber seeds (P < 0.05). Exogenous CaC$_2$ treatment significantly increased superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities by 94.8, 83.2 and 71.3%, respectively during germination under salt stress (P < 0.05; Table 3.1). The inducible effect of exogenous CaC$_2$ on the activity of ascorbate peroxidase (APX) in cucumber was not significant. Application of exogenous CaC$_2$ also significantly increased the total soluble protein contents by 40.4% compared to control under salinity stress (Table 3.1).

3.4 Discussion

In the present study, significant inhibition in seed germination of cucumber was observed under the influence of various concentrations of NaCl and the suppression of seed germination caused by NaCl stress was improved by the addition of CaC$_2$. Moreover, it was confirmed that germination inhibition caused by NaCl stress was related to its reduction in ethylene evolution during imbibition.
Figure 3.5 Effect of CaC$_2$ on concentration of H$_2$O$_2$ in germinating seeds of cucumber during 5 days of incubation under salinity (150 mM NaCl) stress

Figure 3.6 Effect of CaC$_2$ on total soluble sugars in cucumber seeds during 5 days of germination under salinity (150 mM NaCl) stress

Means sharing same letter at each day are statistically non-significant at P > 0.05

FW = Fresh weight
Figure 3.7 Effect of CaC\textsubscript{2} on free amino acids in cucumber seeds during 5 days of germination under salinity (150 mM NaCl) stress

Table 3.1 Effect of CaC\textsubscript{2} on superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX), malondialdehyde (MDA) and soluble proteins in germinating cucumber seeds at 48 h of incubation under salinity (150 mM NaCl) stress.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>*SOD</th>
<th>CAT</th>
<th>POD</th>
<th>APX</th>
<th>MDA</th>
<th>Soluble Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>242.34 b</td>
<td>421.23 b</td>
<td>137.56 b</td>
<td>225.4 a</td>
<td>4.12 a</td>
<td>27.12 b</td>
</tr>
<tr>
<td>CaC\textsubscript{2} (30 mg plate\textsuperscript{-1})</td>
<td>472.1 a</td>
<td>721.4 a</td>
<td>252.56 a</td>
<td>212.4 a</td>
<td>2.12 b</td>
<td>35.32 a</td>
</tr>
</tbody>
</table>

Means sharing same letter at each day (Fig. 3.1) and each column (Table 3.1) are statistically non-significant at P > 0.05

*(SOD= units g\textsuperscript{-1} FW, CAT= nmol H\textsubscript{2}O\textsubscript{2} reduced min\textsuperscript{-1} mg\textsuperscript{-1} protein, POD= nmol tetraguaiacol formed min\textsuperscript{-1} mg protein\textsuperscript{-1}, APX= µmole ascorbate g\textsuperscript{-1} FW min\textsuperscript{-1}, MDA= µmol g\textsuperscript{-1} FW, Soluble proteins= mg g\textsuperscript{-1} FW)

FW = Fresh weight
Results of present experiment are suggesting that the mitigating effect of CaC$_2$ to enhance seed germination under salinity stress might be due to activation of ethylene biosynthesis in imbibed seeds. This hypothesis was further supported when application of antagonist of ethylene synthesis significantly diminished the mitigating effect of CaC$_2$ on germination inhibition caused by salt stress. Application of CaC$_2$ at optimum rate sustains critical level of ethylene required for germination process which may be suppressed in response to salinity stress.

Role of ethylene in releasing seed dormancy is a widely accepted fact (Matilla and Matilla-Vazquez, 2008), and dormancy release in most of crops is associated with an increase in ethylene production (Gniazdowska et al., 2010; Bogatek and Gniazdowska, 2012). Involvement of ethylene in mitigating germination inhibition induced by salinity has been reported in literature which may also account for our findings (Kepczynski and Karssen, 1985; Li et al., 2005). Our results regarding reduced ethylene evolution from cucumber seeds under NaCl stress were consistent with findings of Wang et al. (2011) and Chang et al. (2010). Chang et al. (2010) reported that ethephon and 1-aminocyclopropane-1-carboxylic acid (ACC) as a source of ethylene can alleviate the salt induced inhibition in germinating cucumber seeds and also demonstrated that essential level of ethylene concentration is mandatory to continue germination process under salt stress. Similar to our results, Khan and Huang (1988) also reported that ethylene evolution was reduced in response to NaCl stress in lettuce seeds.

The growth potential of germinating seeds can be affected by breakdown of reserve materials particularly insoluble proteins into soluble proteins and amino acids which are fundamental units for cell formation. In this experiment, results showed that CaC$_2$ as a potent source of ethylene significantly enhanced contents of free amino acids and soluble proteins in germinating seeds of cucumber that ultimately increase the tolerance to salt stress by improving nitrogen metabolism. Moreover, accumulation of nitrogen containing compounds (amino acids, amides, quaternary ammonium compounds and proteins) is correlated with salinity tolerance in plants (Mansour, 2000). Similarly, free amino acids and the soluble sugar contents which are produced by breakdown of starch are source of solutes for lowering osmotic potential in growing embryos. Salinity has been reported to cause decrease in
chlorophyll pigments, soluble proteins, carbohydrates contents as well as amylase contents in Vigna sinensis plants (Alsokari, 2011). Gibberellins have been reported to promote the activity of protease enzymes to mobilize proteins into amino acids (Propagation and Culture, 2008). As, ethylene increases sensitivity to gibberellins during germination that may account for increased contents of free amino acids in cucumber seeds by application of CaC₂. Results are also in coincidence with reported findings by Ahmad et al. (2012) in which significant increase in soluble proteins were observed in wheat leaves by the application of encapsulated CaC₂ both under normal and salt stress conditions. Moreover, salinity-induced proteins by the application of CaC₂ were found correlated with its salt tolerance in wheat.

The increase of α-amylase activity can be associated with an adaptive mechanism to combat salinity stress. Salinity has been reported to cause an increase in α-amylase activity and total soluble sugars by decreasing starch contents in Gossypium vitifolium and Oryza sativa (Dubey and Singh, 1999; Ashraf et al., 2002). In this experiment, we observed that activity of α-Amylase was enhanced significantly during 5 d of incubation which is in agreement with findings of Białecka and Kępczyński (2009) in which increased α-amylase activity in Amaranthus caudatus seeds was reported during the first 14 h of incubation after application of ethephon and GA₃ under salt stress condition.

Role of ethylene has been reported to be involved in the regulation of various stress related responses. Ethylene has also been regarded as a stress hormone and plays its essential role by taking part in the expression of oxidative harm (Abeles et al., 1992; Kendrick and Chang, 2008). Increased ethylene evolution in response to various stresses has been associated with an enhanced plant injury, suggesting that ethylene is harmful to plants. Nevertheless, it has been reported that ethylene improved stress tolerance in tobacco plants under various stresses via stimulation of ERF (ethylene response factor) in signal transduction pathway which finally reduced accumulation of reactive oxygen species (Wu et al., 2008). Salinity has been reported as one of the major causes of oxidative damage to plant tissues (Jalali-e-Emam et al., 2011). However, plants can escape the damaging effects of reactive oxygen species (ROS) by developing a strong defense system including antioxidant enzymes like superoxide dismutase (SOD) catalase (CAT) and peroxidase (POD) (Joseph and Jini, 2011).
It was observed that H$_2$O$_2$ and MDA contents increased in response to salinity stress, which ultimately caused inhibition in seed germination of cucumber (Fig. 3.5). Interestingly, the application of CaC$_2$ significantly reduced the H$_2$O$_2$ and MDA contents in germinating seeds under salinity stress, demonstrating that ethylene-induced decrease in H$_2$O$_2$ and MDA contents may be underlying mechanism of salt tolerance in cucumber. These results were in agreement with reported results by Lin et al. (2012) that ethylene promoted germination under salinity by reducing the endogenous contents of H$_2$O$_2$ in germinating seeds of Arabidopsis. These findings demonstrated that ethylene was involved in regulating germination as an initiator of the process rather than result, and that ethylene promoted germination by regulating the H$_2$O$_2$ contents in germinating seeds under salinity.

However several salt stresses resulted in an inhibition of the antioxidative enzyme catalase and peroxidase. The effects of C$_3$H$_4$ on different enzymes have been reviewed by Abeles et al. (1992) and both positive and negative effects were reported.

In our work, CaC$_2$ increased the activity of superoxide dismutase (SOD), peroxidase (POD) and catalases (CAT) significantly, while the ascorbate peroxidase (APX) activity remained unaffected under the salinity stress (Table 3.1). An increase in non-enzymatic antioxidants has been reported in regenerated laves of peach by the application of ethephon and methionine (Molassiotis et al., 2005). Similarly, an increase in the both polyphenol oxidase (PPO) and CAT activity has been reported in spinach leaves by application of ethephon under salinity stress. However, a decrease in the activity of the POD enzyme was observed in contrast to our results that might be due to difference in concentration and plant tissue (Ozturk and Demir, 2003). In another study, application of ethephon significantly enhanced in vitro activities of peroxidase (POD) and catalase (CAT) in spinach leaves (Ozturk et al. 2008). In accordance with our results, De and De (2003) also reported that different concentrations of ethephon had significant correlation with ethylene-induced activities of catalase and peroxidase in the seedlings of Tigonella foenum-graecum. Moreover, decrease in reactive oxygen species and production of antioxidant enzymes by application of CaC$_2$ in this study might be due to co-production of nitric oxide (NO) along with ethylene under salinity stress. Interrelationship of NO and ethylene was explained by Lin et al. (2013) and
showed a very close association between NO production and ethylene biosynthesis in improving seed germination under salt stress by reducing reactive oxygen species (ROS).

In conclusion, our results showed that enhanced ethylene production after application of CaC$_2$ was underlying process which inhibited H$_2$O$_2$ and MDA contents caused by salt stress, which are the main reactive oxygen species to cause oxidative damage to plant during seed germination. Moreover, exogenous application of CaC$_2$ enhanced the activity of SOD, CAT and amylase enzymes and contents of soluble proteins, amino acids and soluble sugars that could be the possible mechanisms through which CaC$_2$ enhanced seed germination in response to salt stress.

Results of laboratory experiments need to further verify under the field conditions. It is highly likely that the calcium carbide formation should be developed to convert it into an application form. For this purpose, two pot experiments are conducted to select on the coating material and right rate of CaC$_2$ for soil medium.
B) Pot experiment under wire house conditions

Experiment 4: Evaluation of different rates and coatings on calcium carbide for morphological, floral and yield attributes of cucumber

4.1 Introduction

Exogenous ethylene is acknowledged to intensify germination of dormant and non-dormant seeds if they are exposed to precise and controlled level of concentration as too much ethylene defers or hinders germination. Ethylene is also involved in shoot, root growth and differentiation (Clark et al., 1999; Nicolas et al., 2001) and adventitious root formation (Pan et al., 2002). In the past, the effect of liquid CaC\textsubscript{2} based formulation upon tomato and cucumber plants with yield increases up to 70\% had been reported (Muromtsev et al., 1993, 1995). Recently, calcium carbide (CaC\textsubscript{2}) is a well-known precursor of ethylene and due to its fast reaction with water and rapid release of gases; it has to apply to soil in some suitable formulation which leads to consistent slow release of gases for a longer period of time. The use of ethylene based formulation is an innovative approach to develop a soil amendment to improve/enhance vegetative growth and yield. Keeping in view the above facts, there is need to find out some coating material on CaC\textsubscript{2} that can not only control/slow down the releases of gases in a specific time span but also prolong and maintain its pressure in the soil.

The experiment reported here was planned to study the response of cucumber to acetylene and ethylene gases released from calcium carbide based formulations for different morpho-physiological characteristics. The objective of this experiment was to find out the best rate and coating material of CaC\textsubscript{2} in terms of its effect on different morpho-physiological characteristics of cucumber while growing in soil environment under wire-house conditions. The coatings on CaC\textsubscript{2} grains are applied to ensure consistent slow release of gases. Results of previous experiments (1, 2 and 3) have revealed that calcium carbide application at optimum concentration improved different morpho-physiological characteristics of cucumber under controlled conditions. Therefore, the influence of CaC\textsubscript{2} application in soil medium on different morpho-physiological attributes was needed to be investigated. This pot experiment was conducted in wire-house where same hybrid cucumber cv “Bolan-F1” was grown as used in previous laboratory experiments.
4.2 Materials and Methods

Methodology of the experiment is described in experiment 4 in chapter 3.

4.3 Results:

4.3 (A) Morphological characteristics

i) Vine Length
Data regarding the effect of various rates and calcium carbide based coating materials on vine length (cm) of cucumber is presented in Table 4.1. Reduction in vine length was noted by the application of CaC\textsubscript{2} compared to control, which ultimately resulted in the decrease in inter-nodal distance but also improved fruit number as well as fruit yield. However, there were a lot of variations observed regarding reduction in vine length both due to variable rates and coating materials on CaC\textsubscript{2}. Application of all rates of CaC\textsubscript{2} showed significant reduction in vine length. Mean minimum vine length (139.1 cm) was observed in plants treated with paint coated CaC\textsubscript{2} @ 300 mg pot\textsuperscript{-1} and mean maximum vine length (165.4 cm) was found in the control plants. Overall vine length was reduced where CaC\textsubscript{2} was applied in either formulation along with NPK fertilizer compared to that of control plants receiving NPK fertilizers alone. According to comparison of means, mostly CaC\textsubscript{2} application @ 300 mg pot\textsuperscript{-1} was more effective in reducing vine length than 100 and 200 mg pot\textsuperscript{-1} CaC\textsubscript{2}. Later two rates of CaC\textsubscript{2} were statistically at par in reduction of plant height. Calcium carbide coating materials did not differ significantly from each other in their influence on vine length.

ii) Number of primary branches
An increase in number of primary branches in cucumber was noted where calcium carbide plus recommended NPK was applied compared to control plants where only recommended NPK fertilizer was applied. Number of primary branches were significantly increased at all rates of CaC\textsubscript{2} but application of high rate of CaC\textsubscript{2} i.e. 300 mg pot\textsuperscript{-1} caused significant increase in primary branches among all rates of CaC\textsubscript{2}. 
Table 4.1 Effect of different rates and coating materials on CaC₂ on vine length (cm) of cucumber

<table>
<thead>
<tr>
<th>CaC₂ Rate (mg pot⁻¹)</th>
<th>Coating materials on CaC₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paraffin</td>
</tr>
<tr>
<td>0</td>
<td>*165.40 a</td>
</tr>
<tr>
<td>100</td>
<td>145.50 b</td>
</tr>
<tr>
<td>200</td>
<td>124.50 d</td>
</tr>
<tr>
<td>300</td>
<td>108.30 f</td>
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<tr>
<td>Mean</td>
<td>135.93 A</td>
</tr>
</tbody>
</table>

*Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test

Table 4.2 Effect of different rates and coating materials on CaC₂ on number of primary branches of cucumber

<table>
<thead>
<tr>
<th>CaC₂ Rate (mg pot⁻¹)</th>
<th>Coating materials on CaC₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paraffin</td>
</tr>
<tr>
<td>0</td>
<td>*4.32 g</td>
</tr>
<tr>
<td>100</td>
<td>4.71 f</td>
</tr>
<tr>
<td>200</td>
<td>4.8 e</td>
</tr>
<tr>
<td>300</td>
<td>5.01 d</td>
</tr>
<tr>
<td>Mean</td>
<td>4.71 A</td>
</tr>
</tbody>
</table>

*Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test
Maximum number of primary branches (5.6) was noted in treatment where CaC₂ applied at 300 mg pot⁻¹ coated with paint whereas the minimum number of primary branches (4.3) was recorded in untreated plants (control) (Table 4.2). An estimated increase of 30.6 % in number of primary branches per plant in treated plants was recorded. Differences regarding number of primary branches by different coating materials for number of primary branches were not statistically significant.

**iii) Number of nodes on main vine**

Comparative effect of different rates and coatings on calcium carbide with recommended doses of NPK on number of nodes on main vine is presented in Table 4.3. Data clearly indicate that application of different rates of CaC₂ affected number of nodes on main vine significantly. Maximum number of nodes on main vine (32.6) was recorded in treatment where paint coated CaC₂ @ 300 mg pot⁻¹ was applied while it was minimum (24.0) in control plants grown in pots. Overall, Statistical means of treatments showed significant increase in number of nodes on main vine from 6.9 to 24.3 % over control by the application of CaC₂ from 0 to 300 mg pot⁻¹, respectively. However, effect of different coating materials on CaC₂ on number of nodes on main vine was not significant.

**iv) Inter-nodal distance**

Data regarding the effect of rate and type of coating on calcium carbide on inter-nodal distance in cucumber plants is presented in Table 4.4. It is observed from the data that inter-nodal distance greatly responded to application rate of CaC₂. Minimum inter-nodal distance (6.1 cm) was observed by the application of 300 mg pot⁻¹ paint coated CaC₂. But maximum inter-nodal distance (7.7 cm) was observed in control plants. Overall, application of CaC₂ @ 100 to 300 mg pot⁻¹ along with recommended NPK fertilizer showed significant influence and decreased the inter-nodal distance by 8.8, 16.0 and 20.0 %, respectively as compared to control among rates of CaC₂ applied. However, the effect of different coating materials on CaC₂ regarding inter-nodal distance in cucumber was not significant.

**4.3 (B) Floral characteristics**

**i) Days to 1st female flower initiation**

Comparative effect of rate and type of coating on CaC₂ on days to 1st female flower
Table 4.3 Effect of different rates and coating materials on CaC$_2$ on number of nodes on main vine of cucumber

<table>
<thead>
<tr>
<th>CaC$_2$ Rate (mg pot$^{-1}$)</th>
<th>Coating materials on CaC$_2$</th>
<th>Paraffin</th>
<th>Wax</th>
<th>Paint</th>
<th>Gelatin</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>24.09 e</td>
<td>24.05 e</td>
<td>24.03 e</td>
<td>24.05 e</td>
<td>24.06 D</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>25.60 de</td>
<td>25.10 de</td>
<td>26.40 cd</td>
<td>25.80 de</td>
<td>25.73 C</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>26.50 cd</td>
<td>26.50 cd</td>
<td>28.50 bc</td>
<td>26.70 cd</td>
<td>27.05 B</td>
</tr>
<tr>
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<td></td>
<td>28.40 bc</td>
<td>29.12 b</td>
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<td>29.50 b</td>
<td>29.91 A</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>26.15 A</td>
<td>26.19 A</td>
<td>27.88 A</td>
<td>26.51 A</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4 Effect of different rates and coating materials on CaC$_2$ on inter-nodal distance (cm) of main vine of cucumber

<table>
<thead>
<tr>
<th>CaC$_2$ Rate (mg pot$^{-1}$)</th>
<th>Coating materials on CaC$_2$</th>
<th>Paraffin</th>
<th>Wax</th>
<th>Paint</th>
<th>Gelatin</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>*7.69 a</td>
<td>7.71 a</td>
<td>7.67 a</td>
<td>7.68 a</td>
<td>7.69 A</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>7.06 b</td>
<td>7.05 b</td>
<td>6.98 b</td>
<td>7.96 b</td>
<td>7.26 B</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>6.46 c</td>
<td>6.47 c</td>
<td>6.41 c</td>
<td>6.49 c</td>
<td>6.46 C</td>
</tr>
<tr>
<td>300</td>
<td></td>
<td>6.14 d</td>
<td>6.19 d</td>
<td>6.08 d</td>
<td>6.18 d</td>
<td>6.15 D</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>6.84 A</td>
<td>6.86 A</td>
<td>6.79 A</td>
<td>7.08 A</td>
<td></td>
</tr>
</tbody>
</table>

*Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test
Table 4.5 Effect of different rates and coating materials on CaC₂ on days to first female flower initiation in cucumber

<table>
<thead>
<tr>
<th>CaC₂ Rate (mg pot⁻¹)</th>
<th>Coating materials on CaC₂</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paraffin</td>
<td>Wax</td>
</tr>
<tr>
<td>0</td>
<td>*52.2 a</td>
<td>52.3 a</td>
</tr>
<tr>
<td>100</td>
<td>50.2 b</td>
<td>50.4 b</td>
</tr>
<tr>
<td>200</td>
<td>47.8 de</td>
<td>48.2 cd</td>
</tr>
<tr>
<td>300</td>
<td>45.7 fg</td>
<td>45.7 fg</td>
</tr>
<tr>
<td>Mean</td>
<td>48.98 A</td>
<td>49.15 A</td>
</tr>
</tbody>
</table>

*Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test

Table 4.6 Effect of different rates and coating materials on CaC₂ on days to fruit maturity in cucumber

<table>
<thead>
<tr>
<th>CaC₂ Rate (mg pot⁻¹)</th>
<th>Coating materials on CaC₂</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paraffin</td>
<td>Wax</td>
</tr>
<tr>
<td>0</td>
<td>*13.5 a</td>
<td>13.49 a</td>
</tr>
<tr>
<td>100</td>
<td>11.7 b</td>
<td>11.73 b</td>
</tr>
<tr>
<td>200</td>
<td>10.4 cd</td>
<td>10.6 cd</td>
</tr>
<tr>
<td>300</td>
<td>10.01 d</td>
<td>10.1 d</td>
</tr>
<tr>
<td>Mean</td>
<td>11.40 A</td>
<td>11.48 A</td>
</tr>
</tbody>
</table>

*Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test
 initiation in cucumber plants is presented in Table 4.5. Days to 1st female flower initiation was significantly reduced by all rates of CaC₂ but minimum days to 1st female flower initiation (43.7) was noted in treatment where paint coated CaC₂ @ 300 mg pot⁻¹ was applied. Maximum days to 1st female flower initiation (52.2) were noted in control. Overall, an estimated decrease of 4.3, 9.19 and 13.50 % in days to 1st female flower initiation was recorded where CaC₂ applied @ 100, 200 and 300 mg pot⁻¹, respectively compared to control. However, the effect of different coating materials on CaC₂ regarding days to 1st female flower initiation in cucumber was statistically non-significant.

ii) Days to fruit maturity
A reduction in days to fruit maturity in cucumber was noted where calcium carbide was applied compared to control plants. Results indicated that application of all rates of CaC₂ i.e. 100 to 300 mg pot⁻¹ caused significant early maturity in cucumber. Maximum numbers of days to fruit maturity (13.5) were noted in control plants whereas the minimum number of days to fruit maturity (9.2) was recorded in treatment where CaC₂ was applied at 300 mg pot⁻¹ coated with paint (Table 4.6). An estimated reduction of 27.1 % in time period for fruit maturity in treated plants was recorded. However, differences among various coating materials on CaC₂ regarding the days taken to fruit maturity were statistically non-significant.

4.3 (C) Yields characteristics

i) Number of fruits per plant
Number of fruit per plant was also significantly enhanced by both rate of application of calcium carbide and type of coating materials on calcium carbide (Table 4.7). Maximum number of fruits per plant (17.4) was observed in the treatment where paint coated CaC₂ @ 300 mg pot⁻¹ was applied. Minimum number of fruits per plant (12.0) was recorded in control treatment where no CaC₂ was applied. Overall, an estimated increase of 18.7, 26.1 and 33.9 % in number of fruits was recorded where CaC₂ applied @ 100, 200 and 300 mg pot⁻¹, respectively compared to control. Comparison among different coating materials averaged across different rates of CaC₂ revealed that maximum fruit number (15.3) was obtained in paint coated CaC₂ followed by gelatin capsulated CaC₂ (14.3) whereas no significant difference was observed between paraffin and wax coated CaC₂.
ii) Fruit yield per plant

The most important character affecting the yield of a cultivar is fruit weight per plant because average yield per unit area depends upon this character. The ultimate goal of the study was maximization of fruit yield by the application of different formulation of \( \text{CaC}_2 \). Increase in fruit yield is directly correlated with different plant growth parameters like inter-nodanal distance, number of nodes, fruit number and fruit weight. Data on fruit yield per plant reveal significant differences \((p < 0.05)\) among rates of \( \text{CaC}_2 \), coating materials and their interactions. Data in Table 4.8 show that different doses and coating materials on \( \text{CaC}_2 \) along with recommended doses of fertilizer showed significant effect on fruit yield compared to fertilizer alone i.e. control. It is obvious from the data that calcium carbide application rate significantly affected fruit yield of cucumber plant. Maximum fruit yield (2.2 kg plant\(^{-1}\)) by cucumber was observed where 300 mg pot\(^{-1}\) paint coated calcium carbide was applied while minimum fruit yield (1.5 kg plant\(^{-1}\)) was observed in control plants (without \( \text{CaC}_2 \)). Overall, an estimated increase of 19.2, 26.6 and 34.6 \% in fruit yield per plant was observed where \( \text{CaC}_2 \) applied @ 100, 200 and 300 mg pot\(^{-1}\), respectively over the control plants. Among coating materials, paint coating was found the best to enhance the fruit yield of cucumber plant (1.9 kg palnt\(^{-1}\)) followed by gelatin coating (1.7 kg plant\(^{-1}\)) whereas no significant difference was observed between paraffin and wax coating on \( \text{CaC}_2 \).

4.4 Discussion

Differential response of cucumber in terms of morphological, floral and yield parameters due to the application of coated calcium carbide is owing to the specific acetylene flux and then ethylene production from various rates and coatings of calcium carbide. Ethylene released from calcium carbide reduced plant height, number of days to flowering, days to fruit maturity while improved number of nodes on main vine and number of fruits. These parameters ultimately contributed increase cucumber yield. Reduced plant height, increase in number of primary branches and number of nodes on main vine in response to calcium carbide application is due to classical triple response of plant to ethylene (Frankenberger and Fitzpatrick, 1984) and plant polar auxin transport (Arora et al., 1982).
Table 4.7 Effect of different rates and coating materials on CaC$_2$ on number of fruits per plant in cucumber

<table>
<thead>
<tr>
<th>CaC$_2$ Rate (mg pot$^{-1}$)</th>
<th>Coating materials on CaC$_2$</th>
<th>Paraffin</th>
<th>Wax</th>
<th>Paint</th>
<th>Gelatin</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>*11.8 h</td>
<td>12.1 h</td>
<td>12.2 h</td>
<td>11.92 h</td>
<td>12.01 D</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>13.77 g</td>
<td>13.81 g</td>
<td>15.4 cd</td>
<td>14.03 fg</td>
<td>14.25 C</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>14.5 e</td>
<td>14.4 ef</td>
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<td>15.25 d</td>
<td>15.14 B</td>
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<td>15.4 cd</td>
<td>15.7 cd</td>
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<td>15.86 c</td>
<td>16.09 A</td>
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<tr>
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<td>13.87 B</td>
<td>14.00 B</td>
<td>15.35 A</td>
<td>14.27 AB</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.8 Effect of different rates and coating materials on CaC$_2$ on fruit yield per plant (kg) in cucumber

<table>
<thead>
<tr>
<th>CaC$_2$ Rate (mg pot$^{-1}$)</th>
<th>Coating materials on CaC$_2$</th>
<th>Paraffin</th>
<th>Wax</th>
<th>Paint</th>
<th>Gelatin</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
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<td>1.51 f</td>
<td>1.45 f</td>
<td>1.49 f</td>
<td>1.48 D</td>
</tr>
<tr>
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<td></td>
<td>1.69 e</td>
<td>1.70 e</td>
<td>1.93 bc</td>
<td>1.74 e</td>
<td>1.76 C</td>
</tr>
<tr>
<td>200</td>
<td></td>
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<td>1.77 de</td>
<td>2.05 ab</td>
<td>1.89 cd</td>
<td>1.87 B</td>
</tr>
<tr>
<td>300</td>
<td></td>
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<td>1.93 bc</td>
<td>2.18 a</td>
<td>1.97 bc</td>
<td>1.99 A</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.71 B</td>
<td>1.73 B</td>
<td>1.90 A</td>
<td>1.77 AB</td>
<td></td>
</tr>
</tbody>
</table>

*Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test*
Similarly, the results regarding increased number of branches in eggplant and pepper has been reported by Miller et al. (1969) which induced lateral bud development and damaged the terminal growing bud by the application of ethrel, are in complete agreement with the present findings. Inhibition of both cell division and cell elongation has been found with the application of growth retardants, resulting in production of shorter shoots and leaves in melon (Rajala and Peltonen-Saino, 2001). Similar reports have been published by Ouzounidou et al. (2008) in melon (Cucumis melo L.).

The results of the present study indicated that all rates of CaC₂ irrespective of coating materials had a significant effect on floral characters, which agreed with other research workers who found that foliar application of ethylene (Sulochanamma, 2001; Bhat et al., 2004; Thappa et al., 2011) as well as soil applied ethylene (Shakir et al., 2012; Yaseen et al., 2012) not only enhanced early flowering but also shifted sex expression towards femaleness. Yu-mei (2009) also reported that exogenous ethylene application can obviously inhibit the vine growth, shorten the internode length, increase the number of female flower and reduce the number of male flower with no obvious effect on the node of 1st female flower in cucumber. Such effects could be attributed to the fact that a lower concentration of CaC₂ slightly inhibited vegetative growth, increased lateral development, reduced respiration (thus increasing carbohydrate levels) and enhanced the development of early pistillate flowers. Such synergistic effects not only increased early fruit setting, but also accelerated the fruit development processes (Abdel-Rahman and Thompson, 1969). Moreover, exogenous ethylene application has been reported to enhance carbohydrate content in cells (Almodares et al., 2013; Yang, 1986) which in turn promoted pistillate flower production in the vine. It was also noticed that application of CaC₂ reduced inter-nodal distance, thus increasing the source-sink relationship and helped in the early accumulation of photosynthates necessary for the flowering and fruiting processes of the plant.

Yield of cucumber also depends upon the parameters which directly or indirectly contribute to it. Increase in fruit yield in this study may be due to better early flowering, fruiting and increase in number of fruits per plant. Guanping (1983) found that ethrel application could lower the site of the first female flower on the plant, promote the female flower to occur, increase the number of fruits, at the same time, increase the leaf area, decrease the amount of
male flowers, and finally, speed up the fruit setting and increase the early yield of cucumber. Results of present study also agreed with the findings of Binbin (2007) who showed that the treatment with the concentrations of 100 μL/L ethephon at different concentrations in one-leaf and two-leaf period by one time could efficiently increase female flower node ratio and yield, especially the prophase yield in cucumber and had no effect of aberration. This all happened most probably due to addition of calcium carbide which provided persistent release of microbial produced ethylene in the soil environment as a plant growth regulator. The treatments of paint coated CaC$_2$ @ 300 mg pot$^{-1}$ gave the best results in improving number of fruits and yield per plant as this rate provided the optimum level of ethylene necessary for plant growth and development. The other CaC$_2$ treatments also followed this treatment in improving the growth and yield of cucumber and thus produced significantly more fruit yield compared to control.

As the application of CaC$_2$ consistently supplied ethylene in the proximity of plant that stimulated the developmental processes of cucumber and thus resulting in more flowers, then fruits which in turn produced maximum total fruit yield per plant. In contrast with the finding that ethylene treatment inhibits elongation growth, there are reports that low concentrations (i.e., below 0.1 μL L$^{-1}$) can stimulate leaf expansion (Lee and Reid, 1997; Fiorina et al., 2002), stem elongation (Emery et al. 1994; Sage et al., 1997; Pieria et al., 2003), hypocotyl elongation (Smaller et al. 1997), root elongation (Kennings and Jackson, 1979) and photosynthetic rate and nitrogen uptake (Khan et al., 2008, Iqbal et al., 2011). To explain these differential responses to ethylene, a biphasic model was suggested (Kennings and Jackson 1979; Lee and Reid 1997), with low Levels promoting and high levels inhibiting cell expansion. However, the exact range of ethylene concentrations that are needed to stimulate or inhibit growth in plants are the integrative result of environmental conditions, internal signals (e.g. hormones) and species-specific characteristics tentatively related to selection pressure in their habitat of origin. The observed enhanced effects of different rates and coating materials on CaC$_2$ on flowering and yield in this study are also supported by the findings of Yaseen et al. (2006), Kashif et al. (2008), Abbasi et al. (2009), Siddiq et al. (2012) and Ahmed et al. (2014). Results of this experiment were further verified by selecting the best rate and coating material on CaC$_2$ for different genotypes of cucumber in the next experiment.
Experiment 5: Optimizing different rates of paint coated CaC\textsubscript{2} for ethylene production, some morpho-phenological and yield attributes of two cucumber cultivars differing in yield potential

5.1 Introduction

Ethylene is a plant hormone produced in all plant organs including roots, stem, leaves, tuber, flowers, and seeds (Lieberman et al., 1996). Ethylene is difficult to use for agricultural production in the form of gas. The results of several studies have proved that an exogenous source of ethylene could be used for enhancing agricultural production because it has different mode of action than ethylene produced internally in the plant body (Yaseen et al., 2006). Calcium carbide is one of the compounds which can be a source of ethylene when calcium carbide is introduced into the soil under the influence of soil moisture. Due to its rapid reaction with water it is mostly applied into soil in some encapsulation form so that a sustained supply of acetylene and ethylene gases may be maintained to produce physiologically active concentration of ethylene in rhizosphere as well as improved nitrogen economy by inhibiting nitrification. Evaluation of different rates and coating materials on CaC\textsubscript{2} for morphological and yield attributes of cucumber cv “Bolan-F1” under wire-house conditions was investigated in previous experiment. The purpose was to find out better coating material so that coating of this material on CaC\textsubscript{2} should ensure sustainable supply of released gases. The summarized results on all plant growth parameters elucidated that different rates and type of coating materials on CaC\textsubscript{2} significantly affected every stage of cucumber plant, however, the application rate of CaC\textsubscript{2} 300 mg pot\textsuperscript{-1} averaged over all coating materials contributed more to increase ultimate outcome i.e. fruit yield. While difference among coating materials averaged over all rates of CaC\textsubscript{2} was not significant except paint coating which significantly enhanced fruit number and yield per plant compared to other coating materials. So, on the basis of these results I selected paint coating among the best coating materials due to easily available items and simple procedure for preparation at large scale. I further tested this coating material to study genotypic response of cucumber.

For this purpose paint coated calcium carbide was applied at different rates and response of two cucumber cultivars (Local vs Hybrid) was tested in a pot trial with an aim to select a better rate with respect to different growth and yield parameters. Fertilizers containing N, P
and K were applied in all treatments including control. Here, plants in control are actually showing response to recommended levels of fertilizers while plants in all other treatments of CaC₂ are showing response to CaC₂ in the presence of recommended doses of fertilizers.

5.2 Materials and Methods

Methodology of the experiment is described in experiment 5 in chapter 3.

5.3 Results

5.3 (A) Ethylene production from soil and plant leaves

i) Ethylene production from soil amended with paint coated CaC₂

There was a significant increase in C₂H₄ production in all the treatments (Figure 5.1). Initially, no C₂H₄ was detected in the control and very low ethylene emission in the CaC₂ amended incubations. But from 7th day onwards, significant amount of C₂H₄ emission was detected, which increased with the passage of time (Fig. 5.1). In un-amended control, the release of C₂H₄ was considered as a reference for native C₂H₄ production in the soil, although its concentration was too low. Maximum C₂H₄ (223.3 nmol kg⁻¹soil) was observed in the treatment of 40 mg kg⁻¹ soil paint coated CaC₂, while minimum ethylene production (7.3 nmol kg⁻¹soil) was recorded in un-amended soil after 14 days of incubation. This substrate-dependent C₂H₄ release in the amended soil continued even after 56 d of incubation that allowed sufficient time to the plant roots for exposure to slowly released ethylene. Previous studies have shown that C₂H₄ concentrations as low as 1 n mole L⁻¹ can evoke a plant response (Arshad and Frankenberger, 1988).

ii) Ethylene production from plant leaves

Data presented in Table 5.1 show that paint coated CaC₂ treated plants released significantly higher amounts from the leaves compared to untreated or control plants. Overall, Bolan-F1 released 22.5% more ethylene than Desi cultivar. The trend of ethylene release at different rates of CaC₂ further explained and differentiated the results. Maximum ethylene production (0.71 nl g⁻¹FW h⁻¹) was observed at the highest application rate of paint coated CaC₂ (400 mg pot⁻¹) in Bolan-F1 (Hybrid) cultivar.
Figure 5.1 Ethylene release in the soil amended with paint coated CaC$_2$ at different intervals of incubation [Values are means of 4 measurements. Bars represent ± SE (n = 4)]

Table 5.1 Effect of different rates of paint coated CaC$_2$ on ethylene release (nl g$^{-1}$FW h$^{-1}$) from young leaves in two cultivars of cucumber at 40 d after sowing

<table>
<thead>
<tr>
<th>CaC$_2$ rate (mg pot$^{-1}$)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.25 h</td>
<td>0.32 E</td>
</tr>
<tr>
<td>100</td>
<td>0.31 g</td>
<td>0.36 D</td>
</tr>
<tr>
<td>200</td>
<td>0.36 f</td>
<td>0.41 C</td>
</tr>
<tr>
<td>300</td>
<td>0.46 d</td>
<td>0.50 B</td>
</tr>
<tr>
<td>400</td>
<td>0.66 b</td>
<td>0.69 A</td>
</tr>
<tr>
<td>Mean</td>
<td>0.41 B</td>
<td>0.50 A</td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test
However, the best rate of application of paint coated CaC$_2$ i.e. 300 mg pot$^{-1}$ caused 42% increase in ethylene release in same cultivar while in Desi cultivar it was increased to 84% compared to control plants receiving no CaC$_2$. It was further observed that ethylene release by the application of paint coated CaC$_2$ up to 300 mg pot$^{-1}$ was found associated with the increase in female flower initiation and then fruit yield per plant while the higher rate (400 mg pot$^{-1}$) paint coated CaC$_2$ had inhibitory effect on almost all the yield parameters.

5.3 (B) Morphological traits

i) Vine length

Data (Table 5.2) show that vine length of Desi (147.0 cm) showed significant increases when compared with Bolan-F1 (hybrid) cultivar (124.5 cm). Variation in vine length was observed in relation to different rates of calcium carbide (Table 5.2). Maximum vine length (148.4 cm) was recorded in the control (without CaC$_2$) treatment. Minimum vine length (120.8 cm) was noted in the treatment where CaC$_2$ was applied at the rate of 40 mg kg$^{-1}$ soil (400 mg CaC$_2$ pot$^{-1}$ on the basis of 10 kg soil per pot) (Figure 5.2) which was closely followed by the treatment where CaC$_2$ was applied at the rate of 300 mg pot$^{-1}$. Results revealed significant interaction effect between cultivar and CaC$_2$. Overall, maximum vine length (160.2 cm) was recorded in Desi (local) cultivar in the control treatment where no CaC$_2$ was applied. Similarly, vine length of Bolan-F1 cultivar was also found maximum in the control. Minimum vine length (112.7 and 128.9 cm) in Bolan-F1 and Desi cultivar respectively was observed in the treatment of 400 mg CaC$_2$ pot$^{-1}$. Here, the reduction in vine length was about 17.4 and 19.6% in in Bolan-F1 and Desi cultivar, respectively, compared to control treatment (Table 5.2).

5.3 (C) Floral attributes

i) Days to 1st female flower

Both cultivars differ significantly regarding days to first female flower (Table 5.3). A significant decrease (26.6%) in number of days to first female flower was recorded in Bolan-F1 cultivar if compared with Desi cultivar. In terms of days to first female flower at different rates of CaC$_2$, statistically significant differences were recorded in the both cultivars.
Table 5.2 Effect of different rates of paint coated CaC$_2$ on vine length in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC$_2$ rate (mg pot$^{-1}$)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td>Bolan-F1(Hybrid)</td>
</tr>
<tr>
<td>0</td>
<td>160.23 a</td>
<td>136.5 d</td>
</tr>
<tr>
<td>100</td>
<td>153.67 b</td>
<td>126.5 ef</td>
</tr>
<tr>
<td>200</td>
<td>147.3 c</td>
<td>124.1 fg</td>
</tr>
<tr>
<td>300</td>
<td>144.8 c</td>
<td>122.6 g</td>
</tr>
<tr>
<td>400</td>
<td>128.9 e</td>
<td>112.7 h</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>124.48 B</strong></td>
<td><strong>146.98 A</strong></td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at $P < 0.05$ according to LSD test

Table 5.3 Effect of different rates of paint coated CaC$_2$ on days to first Female flower initiation in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC$_2$ rate (mg pot$^{-1}$)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td>Bolan-F1(Hybrid)</td>
</tr>
<tr>
<td>0</td>
<td>50.5 a</td>
<td>41 e</td>
</tr>
<tr>
<td>100</td>
<td>46 b</td>
<td>39.5 f</td>
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<tr>
<td>200</td>
<td>45 c</td>
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<td>34.19 g</td>
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<tr>
<td><strong>Mean</strong></td>
<td><strong>45.8 A</strong></td>
<td><strong>33.6 B</strong></td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at $P < 0.05$ according to LSD test
Minimum (36.6 days) to first female flower was recorded in the 400 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment which was closely followed by 200 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment (38.6), while 300 and 400 mg CaC\textsubscript{2} pot\textsuperscript{-1} showed at par difference in number of days to first female flower (Table 5.3). Significant interaction between cultivar and CaC\textsubscript{2} also elucidated that maximum days to first female flower (50.5) was taken by Desi cultivar in the control treatment while minimum days to first female flower (32.2) was recorded in 400 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment closely followed by 300 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment (34.2) in Bolan-F1 cultivar.

**ii) Female flower count**

Data shown in Table 5.4 elaborate that female flower count in Blan-F1 (26.01) was statistically higher than that in Desi (15.9) cultivar. Moreover, significant variation in female flower count at different rates of CaC\textsubscript{2} was recorded. The lowest number of female flower count (17.4) was recorded in the control treatment which was closely followed by 100 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment (19.7) while the highest female flower count (24.7) was recorded in the 400 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment, here estimated increase was 42.3% compared to control (Table 5.4). Results also revealed that there was a significant interaction effect between cultivar and CaC\textsubscript{2}. The lowest female flower count (12.6) was recorded in Desi cultivar in the control treatment whereas maximum female flower count (31.0) was recorded in Bolan-F1 cultivar.

**iii) Fruit setting**

Fruit setting (%) showed a significant variation both due to cultivar and CaC\textsubscript{2} rates. A significant increase (11.3%) in fruit setting was recorded in Bolan-F1 cultivar if compared with Desi cultivar (Table 5.5). At different rates of CaC\textsubscript{2} significant difference in fruit setting (%) was recorded. Minimum fruit setting (50.7%) was recorded from treatment where CaC\textsubscript{2} was applied at the highest rate i.e. 400 mg CaC\textsubscript{2} plot\textsuperscript{-1}. It was closely followed by control treatment (38.6%). Maximum fruit setting (78.0%) was recorded in the 300 mg CaC\textsubscript{2} pot\textsuperscript{-1} which was 19.3% more compared to control. The treatment of 200 mg CaC\textsubscript{2} pot\textsuperscript{-1} (71.3%) closely followed the treatment of 300 mg CaC\textsubscript{2} pot\textsuperscript{-1} (Table 5.5). The interaction between cultivar and CaC\textsubscript{2} was found significant where maximum fruit setting (82.0 %) was recorded in Bolan-F1 cultivar in the 300 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment while minimum fruit setting (45.9%) was recorded in Desi cultivar in the 400 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment. This higher rate of CaC\textsubscript{2} inhibited fruit setting and reduction recorded was 25.8% as compared to control (Table 5.5).
Table 5.4 Effect of different rates of paint coated CaC₂ on female flower count in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC₂ rate (mg pot⁻¹)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td>Bolan-F1( Hybrid)</td>
</tr>
<tr>
<td>0</td>
<td>12.6 i</td>
<td>22.1 d</td>
</tr>
<tr>
<td>100</td>
<td>15.0 h</td>
<td>24.5 c</td>
</tr>
<tr>
<td>200</td>
<td>16.1 g</td>
<td>25.1 c</td>
</tr>
<tr>
<td>300</td>
<td>17.3 f</td>
<td>27.4 b</td>
</tr>
<tr>
<td>400</td>
<td>18.5 e</td>
<td>30.9 a</td>
</tr>
<tr>
<td>Mean</td>
<td>15.9 B</td>
<td>26.0 A</td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test

Table 5.5 Effect of different rates of paint coated CaC₂ on fruit setting (%) in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC₂ rate (mg pot⁻¹)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td>Bolan-F1(Hybrid)</td>
</tr>
<tr>
<td>0</td>
<td>61.9 f</td>
<td>68.9 c</td>
</tr>
<tr>
<td>100</td>
<td>64.9 e</td>
<td>69.0 c</td>
</tr>
<tr>
<td>200</td>
<td>67.7 e</td>
<td>74.9 b</td>
</tr>
<tr>
<td>300</td>
<td>74.1 d</td>
<td>82.0 a</td>
</tr>
<tr>
<td>400</td>
<td>45.9 h</td>
<td>55.5 g</td>
</tr>
<tr>
<td>Mean</td>
<td>62.9 B</td>
<td>70.1 A</td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test
Overall, Bolan-F1 cultivar showed significant higher (p< 0.05) fruit setting than Desi cultivar.

iv) Days to fruit setting

Data regarding days to fruit setting showed a significant variation in relation to different cultivars (Table 5.6). A significant decrease (11.0 %) in days to fruit setting was recorded in Bolan-F1 cultivar compared to Desi cultivar. Data on days to fruit setting at different rates of CaC₂, indicate that minimum days to fruit setting (13.4) was recorded by the application of 400 mg CaC₂ pot⁻¹ and estimated reduction was 23.7% compared to control. This treatment was closely followed by 200 mg CaC₂ pot⁻¹ treatment (15.2). Maximum days to fruit setting (17.6) were recorded in the control treatment which was closely followed by 100 mg CaC₂ pot⁻¹ treatment (16.8). Interaction between cultivar and CaC₂ regarding days to fruit setting was found significant. This interaction revealed that maximum days to fruit setting (18.7) was recorded in Desi cultivar in the control treatment while minimum days to fruit setting (12.45) was recorded in the treatment of 300 and 400 mg CaC₂ pot⁻¹ in Bolan-F1. The treatment 200 mg CaC₂ pot⁻¹ set fruits in 14.6 and 15.5 days in Bolan-F1 and Desi, respectively.

v) Days to fruit maturity

Days to fruit maturity showed a significant variation regarding cultivars. A significant decrease (13.9 %) in days to fruit maturity was recorded in Bolan-F1 cultivar when compared with Desi cultivar (Table 5.7). Different rates of paint coated CaC₂ significantly affected days to fruit maturity in both the cultivars. Minimum days to fruit maturity (9.15) was recorded in the 400 mg CaC₂ pot⁻¹ treatment which were reduced by 32.8 % compared to control. This treatment was closely followed by the 200 mg CaC₂ pot⁻¹ treatment (11.21) while treatments 400 and 300 mg CaC₂ pot⁻¹ showed more or less parallel results regarding days to fruit maturity (Table 5.7). However, response of both cultivars to both treatments was obvious. Maximum days to fruit maturity (13.6) were recorded in the control treatment which was closely followed by 100 mg CaC₂ pot⁻¹ treatment (12.3). The interaction between cultivar and CaC₂ revealed differences in days to fruit maturity regarding both rate and cultivar.
Table 5.6 Effect of different rates of paint coated CaC$_2$ on days to fruit setting in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC$_2$ rate (mg pot$^{-1}$)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td>Bolan-F1(Hybrid)</td>
</tr>
<tr>
<td>0</td>
<td>18.75 a</td>
<td>16.5 c</td>
</tr>
<tr>
<td>100</td>
<td>17.5 b</td>
<td>16.1 d</td>
</tr>
<tr>
<td>200</td>
<td>15.8 d</td>
<td>14.62 e</td>
</tr>
<tr>
<td>300</td>
<td>14.45 e</td>
<td>12.45 f</td>
</tr>
<tr>
<td>400</td>
<td>14.5 e</td>
<td>12.4 f</td>
</tr>
<tr>
<td>Mean</td>
<td>16.2 A</td>
<td>14.4 B</td>
</tr>
</tbody>
</table>

Table 5.7 Effect of different rates of paint coated CaC$_2$ on days to fruit maturity in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC$_2$ rate (mg pot$^{-1}$)</th>
<th>Cultivars</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td>Bolan-F1(Hybrid)</td>
</tr>
<tr>
<td>0</td>
<td>14.65 a</td>
<td>12.6 c</td>
</tr>
<tr>
<td>100</td>
<td>13.5 b</td>
<td>11.1 e</td>
</tr>
<tr>
<td>200</td>
<td>11.8 d</td>
<td>10.6 f</td>
</tr>
<tr>
<td>300</td>
<td>10.5 f</td>
<td>8.4 i</td>
</tr>
<tr>
<td>400</td>
<td>9.5 g</td>
<td>8.8 h</td>
</tr>
<tr>
<td>Mean</td>
<td>12.0 A</td>
<td>10.3 B</td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test.
Maximum days to fruit maturity (14.6) was recorded in Desi cultivar in the control treatment while minimum days to fruit maturity (9.9, 8.4) was recorded in 400 and 300 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatments, respectively in Bolan-F1 cultivar (Table 5.7).

### 5.4 Yield attributes

#### i) Total number of fruits per plant

Both cultivars differed significantly in total number of fruits per plant at each level of CaC\textsubscript{2}. A significant increase (93.5\%) in total number of fruits per plant was recorded in Bolan-F1 cultivar compared to Desi cultivar (Table 5.8). In terms of total number of fruits per plant in relation to different rates of CaC\textsubscript{2}, a statistically significant difference was recorded in the present trial. Minimum total number of fruits per plant (12.0) was recorded in the control treatment which was closely followed by the treatment of 100 mg CaC\textsubscript{2} pot\textsuperscript{-1} (13.93). The highest number of fruits per plant (17.2) was recorded in the 300 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment and increase noted here was 43.3 \% as compared to control. The treatment of 200 mg CaC\textsubscript{2} pot\textsuperscript{-1} produced 15.8 fruits per plant (Table 5.8). Total number of fruits per plant was also affected by the interaction between cultivar and CaC\textsubscript{2}. Maximum number of fruits per plant (22.5) was recorded in Bolan-F1 cultivar in 300 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment while minimum number of fruits per plant (7.8) was recorded in Desi cultivar in the control treatment.

#### ii) Total yield per plant

Total yield per plant showed a significant variation in relation to both CaC\textsubscript{2} rate and cultivar. A significant increase (223.3 \%) in total yield per plant was recorded in Bolan-F1 cultivar compared to Desi cultivar (Table 5.9). Total yield per plant in relation to different rates of CaC\textsubscript{2} was found statistically significant. Minimum total yield per plant (1606.7 g) was recorded in the control treatment which was closely followed by 400 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment (1891.3 g). Maximum total yield per plant (2592.7 g) was recorded in 300 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment which was increased by 61.4 \% as compared to control and it was closely followed by 200 mg CaC\textsubscript{2} pot\textsuperscript{-1} treatment (2313.6 g) (Table 5.9). This indicates that application of CaC\textsubscript{2} above 300 mg pot\textsuperscript{-1} rate severely affected fruit yield. So it is found safe to apply 200 to 300 mg CaC\textsubscript{2} pot\textsuperscript{-1}. Significant interaction was recorded between cultivar and CaC\textsubscript{2} regarding total yield per plant.
Table 5.8 Effect of different rates of paint coated CaC₂ on number of fruits per plant in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC₂ rate (mg pot⁻¹)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
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</tr>
<tr>
<td>0</td>
<td>7.83 g</td>
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<tr>
<td>100</td>
<td>9.96 f</td>
<td>13.93 c</td>
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<tr>
<td>200</td>
<td>10.72 f</td>
<td>15.76 b</td>
</tr>
<tr>
<td>300</td>
<td>11.85 e</td>
<td>17.17 a</td>
</tr>
<tr>
<td>400</td>
<td>8.5 g</td>
<td>12.85 d</td>
</tr>
<tr>
<td>Mean</td>
<td>9.8 b</td>
<td>18.9a</td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test.

Table 5.9 Effect of different rates of paint coated CaC₂ on total fruit yield per plant in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC₂ rate (mg pot⁻¹)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>698.3 e</td>
<td>1606.7 C</td>
</tr>
<tr>
<td>100</td>
<td>927.3 de</td>
<td>1917.4 B</td>
</tr>
<tr>
<td>200</td>
<td>1106.4 d</td>
<td>2313.6 A</td>
</tr>
<tr>
<td>300</td>
<td>1250.5 d</td>
<td>2592.7 A</td>
</tr>
<tr>
<td>400</td>
<td>894.2 de</td>
<td>1891.3 bc</td>
</tr>
<tr>
<td>Mean</td>
<td>975.3 B</td>
<td>3153.4 A</td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test.
Maximum total yield per plant (3934.9 g) was recorded in Bolan-F1 cultivar in 300 mg CaC$_2$ pot$^{-1}$ treatment while minimum total yield per plant (698.31 g) was recorded in Desi cultivar in the control treatment. The 400 mg CaC$_2$ pot$^{-1}$ treatment producing 894.16 g fruit yield in Desi cultivar performed more close to the control treatment. Overall, Bolan-F1 performed relatively better at all CaC$_2$ rates than Desi cultivar (Table 5.9).

iii) Early yield per plant

Early yield is related to earliness in fruit maturity. Early yield per plant also varied due to both CaC$_2$ rate and cultivar. About 298.5% increase in early yield per plant was recorded in Bolan-F1 cultivar compared to Desi cultivar (Table 5.10). Different rates of CaC$_2$ also significantly affected early yield per plant. Minimum early yield per plant (793.0 g) was recorded in the control treatment which was closely followed by 100 mg CaC$_2$ pot$^{-1}$ treatment (975.1 g). Maximum early yield per plant (1530.3 g) was recorded in the 300 mg CaC$_2$ pot$^{-1}$ treatment which was increased by 93.0% as compared to control (Table 4.9). Significant interaction was recorded between cultivar and CaC$_2$ regarding early yield per plant. This interaction pointed out maximum early yield per plant (2416.5 g) in Bolan-F1 cultivar in 300 mg CaC$_2$ pot$^{-1}$ treatment while minimum early yield per plant (290.51 g) in Desi cultivar in the control treatment. The results of control treatment were followed by the results of 400 mg CaC$_2$ pot$^{-1}$ treatment (385.3 g) in Desi cultivar (Table 5.9). However, the performance of both the cultivars could be separated easily as both performed differently at each level of CaC$_2$.

iv) Early yield/total yield ratio

Early yield/total yield ratio showed a significant variation in relation to both rate and cultivar. A significant increase (23.72%) in early yield/total yield ratio was recorded in Bolan-F1 cultivar compared to Desi cultivar (Table 5.10). Early yield/total yield ratio at different rates of CaC$_2$ further explained and differentiated the results. Minimum ratio (0.47) was recorded in 100 mg CaC$_2$ pot$^{-1}$ treatment. Maximum ratio (0.57) was recorded in 300 mg CaC$_2$ pot$^{-1}$ treatment which was increased by 21.3 % as compared to control. This ratio was 0.51 in 200 mg CaC$_2$ pot$^{-1}$ treatment (Table 5.11). These results could be further established in the light of interaction between cultivar and CaC$_2$ which was statistically significant.
Table 5.10 Effect of different rates of paint coated CaC₂ on early yield per plant in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC₂ rate (mg pot⁻¹)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>290.5 g</td>
<td>793.0 D</td>
</tr>
<tr>
<td>100</td>
<td>395.0 fg</td>
<td>975.1 C</td>
</tr>
<tr>
<td>200</td>
<td>493.2 f</td>
<td>1240.5 B</td>
</tr>
<tr>
<td>300</td>
<td>644.1 e</td>
<td>1530.3 A</td>
</tr>
<tr>
<td>400</td>
<td>385.3 fg</td>
<td>965.1 C</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>441.6 B</strong></td>
<td><strong>1760.0 A</strong></td>
</tr>
</tbody>
</table>

Table 5.11 Effect of different rates of paint coated CaC₂ on early yield/total yield ratio in two genotypes of cucumber

<table>
<thead>
<tr>
<th>CaC₂ rate (mg pot⁻¹)</th>
<th>Cultivar</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desi (local)</td>
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</tr>
<tr>
<td>0</td>
<td>0.42 g</td>
<td>0.47 D</td>
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<tr>
<td>100</td>
<td>0.43 fg</td>
<td>0.49 C</td>
</tr>
<tr>
<td>200</td>
<td>0.45 e</td>
<td>0.51 B</td>
</tr>
<tr>
<td>300</td>
<td>0.52 d</td>
<td>0.58 A</td>
</tr>
<tr>
<td>400</td>
<td>0.44 f</td>
<td>0.49 C</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>0.46 B</strong></td>
<td><strong>0.56 A</strong></td>
</tr>
</tbody>
</table>

Values sharing common letter(s) in table body (non-bold) (a) last column (b) last row do not differ significantly at P < 0.05 according to LSD test
Maximum early yield/total yield ratio (0.62) was recorded in Bolan-F1 cultivar in 300 mg CaC₂ plot⁻¹ treatment while minimum early yield/total yield ratio (0.42) was recorded in Desi cultivar in the control treatment which was closely followed by 100 mg CaC₂ plot⁻¹ treatment (0.43) in Desi cultivar (Table 5.10).

5.5 Discussion

Results indicated that paint coated CaC₂ acted as a potent source of ethylene. When CaC₂ added to soil, it reacts with soil moisture and releases C₂H₂ gas, which is then converted to C₂H₄ (a potent plant hormone) (Muromtsev et al., 1995). Thus, to exploit CaC₂ potential it was coated with paint material to make its release slow and persistent; hence roots may be exposed to this metabolite during critical stages of development. Similar findings have been reported by some other workers (Porter, 1992; Bibik et al., 1995; Yaseen et al., 2006; Kashif et al., 2008).

Ethylene performs various physiological functions in plants. It has stimulatory as well as inhibitory effect depending upon its concentration and sensitivity to plants. It is also of interest to note that the stimulated growth of both cultivars of cucumber coincided with the plant ethylene level, since plants treated with 300 mg pot⁻¹ paint coated CaC₂ which produced the highest parameters of plant growth and yield are attributed to physiological active concentration of ethylene in cucumber plants (Tables 5.2 to 5.8). These findings may suggest that the positive impact of paint coated CaC₂ on cucumber plant growth is by stimulating ethylene production because ethylene induced cell division (Barker 1979; Ilker et al. 1977; Metzer 1984; Riad, 1996) and enlargement (Ku et al. 1970; Riad, 1996) in higher plant tissues of several plant species has been reported.

The results regarding morphological attributes revealed that paint coated calcium carbide was effective in reducing the length of the main stem in a dose dependent manner. Similar reduction in plant height by application of CaC₂ has been reported in various vegetable crops (Kashif et al., 2008; Siddiq et al., 2009, 2012; Shakir, 2012; Ahmed et al., 2014). The inhibition of apical growth may have been due to ethylene released from CaC₂ which inhibits elongation and promotes radial expansion because of its more impact on cell enlargement instead of division of cells (Kieber et al., 1993) and plant polar auxin transport (Arora et al., 1982). At higher concentrations, ethephon showed more severe and lasting inhibition on
vegetative growth (Abdel-Rahman and Thompson, 1969). Similar findings were reported by Arora et al. (1994) in long melon and by Murthy et al. (2007) in gherkin. A possible reason might be that ethylene acts as an anti-gibberellin and causes cessation of the mitotic processes in the meristem of root and shoot, thereby affecting the length of the plant (Hayashi et al., 2001).

Flowering has been hastened or delayed by plant hormones depending on species (Latimer, 1991). Flower number of different crops was found to be positively responsive towards exogenous application of growth substances (Morgan et al., 1983; Friends, 1985). For better vegetative growth, ability of plant to produce and to respond ethylene, is very important (Solano and Ecker, 1998). Ethylene is a critical plant hormone for such growth and developmental processes. The results of the present study indicated that paint coated CaC\(_2\) as a potent source of ethylene in soil reduced days to flowering, days to fruit setting and maturity and increased female flowers, total yield and early yield in both cultivars differing in yield potential. Such effects could be attributed to the fact that a lower concentration of CaC\(_2\) slightly inhibited vegetative growth, increased lateral development, reduced respiration (thus increasing carbohydrate) and enhanced the development of early pistillate flowers. Such synergistic effects not only increased early fruit setting, but also accelerated the fruit development processes (Abdel-Rahman and Thompson, 1969). Yamasaki et al. (2000) reported that higher levels of endogenous ethylene production caused greater accumulation of CS-ETR2 and CS-ERS mRNA, which play a role in the development of female flowers in cucumber.

Modification of sex expression in cucurbits has remained a great interest for plant scientists because there is a correlation between fruit yield and the number of pistillate flowers per plant. Any treatment that would increase the formation of pistillate flowers would therefore, be beneficial in producing high yield. Results of this experiment also showed that hybrid cultivar produced more ethylene than local cultivar. Higher ethylene production in Bolan-F1 cultivar (Hybrid) might be due to more female flowers because female buds of cucurbitaceous plants produced greater amounts of ethylene than those produced by male buds (Rudich et al. 1972). These results are in agreement with previous findings of McMurray and Miller (1968), Robinson et al. (1969) Rudich et al. (1969), Karchi and
Govers (1972), Arora et al. (1985) and Muromtsev et al. (1995). Exogenous application of ethylene was correlated with the amount of 1-aminocyclopropane-1 carboxylic acid (ACC) in the time. ACC changed the direction of sexual differentiation in potentially male buds to female buds. Ultimately the total number of female flower was increased in cucumber plant under the present study. Similar results were also found in bottle gourd and reported by Ying et al. (1994).

Similar results were reported by application of coated/encapsulated CaC₂ in soil with recommended dose of fertilizers which not only resulted in early flowering but also brought early fruiting and maturity and enhanced fruit yield in tomato (Siddiq et al., 2012) and cucumber (Shakir et al., 2012). A probable reason suggested by Ries (1985), which endorsed the present results, was that the application of growth regulator like ethylene increased the endogenous ethylene level which triggered metabolic processes and affected the C:N ratio in plants, in turn stimulating flowering, fruit set, sex ratio and thereby yield. Early fruit maturity from CaC₂ treated plants was might be due acceleration of physiological maturity by ethylene hormone accompanied with loss of chlorophyll and breakdown of carbohydrate, protein and RNA with increasing activity of chlorophylase, protease, ribonuclease etc. (Hossain, 2004).

The results of this study revealed that lower rates of paint coated CaC₂ (100 to 300 mg pot⁻¹) in relation to both cultivars showed dose dependent growth stimulatory response while level of 400 mg pot⁻¹ proved to be inhibitory concerning most of morpho-phenological and yield attributes. Growth promotion activity is due to the fact that when CaC₂ reacts with water release large amount of acetylene (C₂H₂), that is renowned nitrification inhibitor in soil, and then this acetylene reduces to plant hormone, ethylene (C₂H₄) by soil microbes with the passage of time at physiological active concentration (Walter et al., 1979 and Yaseen et al., 2004; Yaseen et al., 2006). Comparison among different rates of paint coated calcium carbide showed that the treatment where paint coated calcium carbide was applied @ 300 mg pot⁻¹ along with recommended dose of NPK fertilizer was more effective in improving growth and yield of cucumber due to its slow and persistent release of gases for longer period. A sound improvement of physiologically active concentration of C₂H₄ from the microbial reduction of C₂H₂ might have contributed in growth promotion, which afterward
resulted in better yield of treated plant. These results are in line with the findings of Abeles, (1992), Arshad et al. (1993), Arshad and Frankenberger (2002), Yaseen et al. (2006), Siddiq et al. 2012 and Ahmed et al. 2014).

A series of experiments in laboratory and wire-house in pots revealed that calcium carbide has positive and beneficial effects on morpho-physiological and yield attributes. Therefore, it was planned to conduct experiment on similar lines in the light of results of this and previous experiments under field conditions for the confirmation and validation of results of previous experimental work and finally for preparation of set of technology for the use of farmers.
6.1 Introduction

Plant growth regulators have been found to be engaged in enhancing growth and productivity of crop plants. There are many instances which suggest that growth regulators and nutrients can interact in a variety of ways. Deficient and toxic levels of nutrients can affect the concentration of specific hormones, and in turn, hormones have the capacity to direct the translocation and accumulation of nutrients in plants (Kuiper, 1988; Kuiper et al., 1989). Among various nutrients the nitrogen availability and internal distribution plays a critical role in the regulation of various growth-related and morphogenetic aspects of plant development that are usually attributed to hormonal factors. Increased N supply stimulates plant growth and productivity as well as photosynthetic capacity of leaves through increased amounts of stromal and thylakoid proteins in leaves (Hikosaka, 2004; Teixeira Filho et al., 2011).

Among various factors influencing photosynthesis, plant hormones are important in the regulation of photosynthesis and growth processes (Brenner and Cheikh, 1995). Ethylene as a plant hormone influences many aspects of plant growth and photosynthesis (Abeles et al., 1992; Fiorani et al., 2002; Pierik et al., 2006; Acharya and Assmann, 2009). It has also been shown that ethylene modifies the rate of photosynthesis (Gunderson and Taylor, 1991; Khan, 2004). The effect of ethylene depends on ethylene production and sensitivity limits (Pierik et al., 2006). A low rate of ethylene release increases photosynthesis (Subrahmanynam and Rathore 1992; Khan 2004) and leaf growth (Lee and Reid, 1997; Hussain et al., 1999; Fiorani et al., 2002; Khan, 2005). In contrast, high ethylene concentration reduces leaf area (Tholen et al., 2004; Khan, 2005) and photosynthesis (Kays and Pallas, 1980; Khan, 2004).

In some studies, the influence of nitrogen availability on ethylene evolution, growth and photosynthesis was investigated and it was reported that application of ethephon can increase photosynthesis and growth of mustard under high N levels (Khan et al., 2000, 2008). In another study, application of ethephon at each level of nitrogen (deficient and sufficient) was
reported to increase photosynthesis via its effect on the photosynthetic machinery and stomatal conductance by increasing ethylene and decreasing glucose sensitivity (Iqbal et al., 2011).

Recently, CaC\textsubscript{2} has been shown to be an important biological precursor of ethylene in soil environment (Bibik et al., 1995; Khalid et al., 2006; Arshad and Frankenberger, 2002) which has been shown to influence the plant growth in various crops by providing ethylene at physiological active concentration (Arshad and Frankenberger, 2002; Yaseen et al., 2006, Kashif et al., 2012 and Ahmed et al., 2014). Many studies have shown positive effect of coated CaC\textsubscript{2} on growth, photosynthesis and nitrogen use efficiency in tomatoes (Siddiq et al., 2012) and sweet pepper (Ahmed et al., 2014) but limited information is available regarding interactive effect of nitrogen availability and soil applied CaC\textsubscript{2} on nitrogen metabolism, photosynthesis and fruit quality of cucumber. The present study was designed in the light of laboratory and pot experiments to evaluate the response of cucumber (\textit{Cucumis sativus} L. cv Bolan-F1) Hybrid to CaC\textsubscript{2} applied with different nitrogen fertilizer (Urea-46\% N) rates under field conditions. In this experiment the optimum rates of 200 and 300 mg pot\textsuperscript{-1} from previous pot experiments were chosen to further study interaction of CaC\textsubscript{2} with different levels of nitrogen on growth, photosynthesis and nitrogen metabolism in cucumber.

6.2 Materials and Methods
Methodology of the experiment is described in experiment 6 in chapter 3.

6.3 Results

6.4 Morphological characteristics

i) Leaf area

Soil applied paint coated CaC\textsubscript{2} significantly increased leaf area at each level of nitrogen (Figure 6.1) and its maximal effect on leaf area was noted in the treatment of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} in combination with 100 kg N ha\textsuperscript{-1}. However, this response was less in in plots treated with 50 kg N ha\textsuperscript{-1} and 300 mg CaC\textsubscript{2} plant\textsuperscript{-1}. About 47\% increase in leaf area was recorded in the treatment of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} with 100 kg N ha\textsuperscript{-1} compared to 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} with 0 kg N ha\textsuperscript{-1}. The plants receiving 50 kg N ha\textsuperscript{-1} with 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} were able to increase leaf area by 30\% compared to 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} plus 0 kg N ha\textsuperscript{-1} (Figure 6.1).
ii) Vine length

Data in Figure 6.2 show significant reduction in vine length by application of paint coated CaC₂ at each level of N. Maximum vine length (190.3 cm) was recorded in treatment where 100 kg N ha⁻¹ was applied without CaC₂. This treatment caused an increase in vine length about 26.4 % as compared to control (without N and CaC₂). The maximum reduction in vine length at each level of N was observed by the application of 300 mg CaC₂ plant⁻¹ which was 3, 8 and 7.29% at 0, 50 and 100 kg N ha⁻¹, respectively compared to their respective controls (without CaC₂).

iii) Number of primary branches

Soil applied paint coated CaC₂ also significantly increased number of primary branches at each level of nitrogen (Figure 6.2) which was recorded maximum in the application of 300 mg plant⁻¹ CaC₂ in plants received 100 kg N ha⁻¹. The plants treated with 50 kg N ha⁻¹ responded less to the application of 300 mg plant⁻¹. Number of primary branches was increased by 29.1% with the treatment of 300 mg plant⁻¹ CaC₂ and 100 kg N ha⁻¹ compared with 300 mg plant⁻¹ CaC₂ and 0 kg N ha⁻¹. The plants receiving 50 kg N ha⁻¹ with 300 mg plant⁻¹ CaC₂ could increase number of primary branches by 15.9% compared with 300 mg plant⁻¹ CaC₂ and 0 kg N ha⁻¹. The effect of 200 mg plant⁻¹ of CaC₂ plus 50 or 100 kg N ha⁻¹ was less compared to 300 mg CaC₂ plant⁻¹ alone.

iv) Total plant dry weight

Application of paint coated CaC₂ with and without N treatments significantly increased total plant dry mass of cucumber. The maximum increase occurred in the combined treatment of 300 mg plant⁻¹ CaC₂ and 100 kg N ha⁻¹. The total dry mass was increased by 71% with 300 mg plant⁻¹ CaC₂ and 100 kg N ha⁻¹ compared to the application of 300 mg plant⁻¹ CaC₂ with 0 kg N ha⁻¹ (Figure 6.3). Application of 300 mg plant⁻¹ CaC₂ on plants grown with 50 kg N ha⁻¹ could result in an increase of 33.3% in the total dry mass compared to 300 mg plant⁻¹ CaC₂ applied with 0 mg kg N ha⁻¹. A lower level of CaC₂ (200 mg plant⁻¹) either with 50 or 100 kg N ha⁻¹ proved to be less effective compared to 300 mg CaC₂ rate (Figure 6.3).
**Figure 6.1** Effect of paint coated CaC$_2$ on leaf area of cucumber at 8 weeks after sowing under nitrogen deficient and adequate levels

Means sharing same letter at each bar and line are statistically non-significant at $P>0.05$.

**Figure 6.2** Effect of paint coated CaC$_2$ on vine length and number of primary branches per plant at 8 weeks after sowing under nitrogen deficient and adequate levels

Means sharing same letter at each bar and line are statistically non-significant at $P>0.05$. **N**= Nitrogen rate (kg ha$^{-1}$) **CC**= Paint coated calcium carbide rate (mg plant$^{-1}$)
6.5 Phenological Attributes

i) Days to first female flower initiation

Results showed that CaC\textsubscript{2} significantly reduced the days to first female flower initiation at each level of nitrogen (Figure 6.13, 6.14, 6.15). However, maximum reduction was observed by application of 300 mg plant\textsuperscript{-1} of CaC\textsubscript{2} to plants received 100 kg N ha\textsuperscript{-1} (Figure 6.13). Addition of alone nitrogen also significantly reduced the days to fruit maturity but relatively of less magnitude when added with CaC\textsubscript{2}. Days to first female flower and fruit maturity decreased by 19\% with the treatment of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} and 100 kg N ha\textsuperscript{-1} compared with 300 mg plant\textsuperscript{-1} of CaC\textsubscript{2} with 0 kg N ha\textsuperscript{-1}. The effect of 200 mg plant\textsuperscript{-1} of CaC\textsubscript{2} plus 50 or 100 kg N ha\textsuperscript{-1} was found relatively less as compared to 300 mg CaC\textsubscript{2} rate (Figure 6.6).

ii) Days to fruit maturity

Days to fruit maturity was significantly affected by application of both CaC\textsubscript{2} and Nitrogen treatments. Minimum number of days to fruit maturity (12.3) was recorded in the 300 mg plant\textsuperscript{-1} of CaC\textsubscript{2} when plants treated with 100 kg N ha\textsuperscript{-1}(Figure 6.4) and it was at par with the 200 mg CaC\textsubscript{2} plant\textsuperscript{-1} rate under adequate rate of nitrogen (100 kg ha\textsuperscript{-1}). Maximum number of days to fruit maturity (17.2) was observed in control plants receiving no CaC\textsubscript{2} and nitrogen. Addition of nitrogen significantly reduced the days to fruit maturity, whereas application of CaC\textsubscript{2} reduced days to fruit maturity at each level of nitrogen. Plants receiving 300 mg CaC\textsubscript{2} plant\textsuperscript{-1} under adequate level of nitrogen (100 kg ha\textsuperscript{-1}) decreased the number of days to fruit maturity by 7.5\% compared with 300 mg plant\textsuperscript{-1} of CaC\textsubscript{2} with 0 kg N ha\textsuperscript{-1}. The effect of 200 mg plant\textsuperscript{-1} of CaC\textsubscript{2} plus 50 or 100 kg N ha\textsuperscript{-1} was comparatively less in magnitude as compared to 300 mg CaC\textsubscript{2}.

6.6 Physio-Biochemical attributes:

i) Photosynthesis

Application of CaC\textsubscript{2} significantly enhanced all photosynthetic characteristics in cucumber by interacting positively with N. Deficiency of N reduced photosynthetic characteristics and CaC\textsubscript{2} application at each level of N increased these characteristics.
Figure 6.3 Effect of paint coated CaC$_2$ on total plant dry weight of cucumber under nitrogen deficient and adequate levels

Figure 6.4 Effect of paint coated CaC$_2$ on days to first female flower initiation and fruit setting under nitrogen deficient and adequate levels

Means sharing same letters at each bar and line are statistically non-significant at P> 0.05
Figure 6.4.1 Effect of 300 mg plant$^{-1}$ paint coated CaC$_2$ on early female flowering in cucumber under adequate level of nitrogen
Figure 6.4.2 Effect of 300 mg plant⁻¹ paint coated CaC₂ on early female flowering in cucumber under deficient level of nitrogen.
Figure 6.4.3 comparison of 200 and 300 mg plant\(^{-1}\) paint coated CaC\(_2\) on female flowering in cucumber under adequate level of nitrogen
Carboxylation efficiency was calculated by using linear regression between photosynthetic rate ($P_N$) and intercellular CO$_2$ concentration [$CO_2]i$ (Figure 6.8). Application of 300 mg plant$^{-1}$ CaC$_2$ proved superior to 0 or 200 mg plant$^{-1}$ CaC$_2$. In comparison with the alone 300 mg plant$^{-1}$ CaC$_2$, plants treated with 300 mg plant$^{-1}$ CaC$_2$ plus 100 kg N ha$^{-1}$ had the strongest stimulating effect on photosynthetic rate (68.4%, Figure 6.5), intercellular CO$_2$ concentration (27.6%, Figure 6.6), stomatal conductance (39.4%, Figure 6.7), carboxylation efficiency (33.3%, Figure 6.9) and water use efficiency (21.4%, Figure 6.10). Application of 300 mg plant$^{-1}$ of CaC$_2$ to plants under 50 kg N ha$^{-1}$ caused increase in photosynthetic rate ($P_N$), stomatal conductance ($g_s$), intercellular CO$_2$ concentration [$CO_2]i$, carboxylation efficiency and water use efficiency by 33.1, 18.2, 12.0, 20.4 and 13.2%, respectively compared to application with 300 mg plant$^{-1}$ of CaC$_2$ plus 0 kg N ha$^{-1}$. A lower level of CaC$_2$ (200 mg plant$^{-1}$) either with 50 or 100 kg N ha$^{-1}$ proved to be less effective than that of 300 mg plant$^{-1}$ rate.

ii) Nitrate reductase activity

Data elucidated increase in the activity of nitrate reductase (NR) due to the application of CaC$_2$ at each level of N. Maximum increase occurred in the combined treatment of 300 mg plant$^{-1}$ CaC$_2$ and 100 kg N ha$^{-1}$. The activity of NR was enhanced by 57.3% due to the combined application of 300 mg plant$^{-1}$ CaC$_2$ with 100 kg N ha$^{-1}$ compared to the application of 300 mg plant$^{-1}$ CaC$_2$ with 0 kg N ha$^{-1}$. Moreover, application of 300 mg plant$^{-1}$ CaC$_2$ to plants grown with 50 kg N ha$^{-1}$ could result in an increase of only 29.1% in the activity of NR compared to the application of 300 mg plant$^{-1}$ CaC$_2$ to the plants treated with 0 kg N ha$^{-1}$. A lower level of CaC$_2$ (200 mg plant$^{-1}$) either with 50 or 100 kg N ha$^{-1}$ proved to be less effective compared to 300 mg CaC$_2$ rate (Figure 6.11).

iii) Mineral contents (N, P, K, Ca and Mg) in leaves

Statistical analysis of data in this study is evident that both the rate of CaC$_2$ and N significantly enhanced N, P, K, Ca and Mg contents in leaves of cucumber (Table 6.1). Increase in N, P, K, Ca and Mg contents in leaves was occurred by the combined application of 300 mg plant$^{-1}$ CaC$_2$ and 100 kg N ha$^{-1}$. 
Figure 6.5 Effect of CaC\textsubscript{2} on rate of photosynthesis in leaves at 8 weeks after sowing under nitrogen deficient and adequate levels

Figure 6.6 Effect of paint coated CaC\textsubscript{2} on intracellular CO\textsubscript{2} concentration in leaves at 8 weeks after sowing under nitrogen deficient and adequate levels

Means sharing same letters at each bar and line are statistically non-significant at P> 0.05
Fig. 6.7 Effect of paint coated CaC$_2$ on stomatal conductance in cucumber at 8 weeks after sowing under nitrogen deficient and adequate levels.

Fig. 6.8 Linear regression between photosynthetic rate and intracellular CO$_2$ drawn to calculate carboxylation efficiency of cucumber plants.
Fig. 6.9 Effect of paint coated CaC$_2$ on carboxylation efficiency of cucumber at 8 weeks after sowing under nitrogen deficient and adequate levels

Fig. 6.10 Effect of paint coated CaC$_2$ on water use efficiency of cucumber at 8 weeks after sowing under nitrogen deficient and adequate levels

Means sharing same letters at each bar are statistically non-significant at P> 0.05

*N= Nitrogen rate (kg ha$^{-1}$)  **CC= Paint coated calcium carbide rate (mg plant$^{-1}$)
The contents of N, P, K, Ca and Mg in leaves were increased by 17.2, 60.8, 12.4, 17.9, and 42.5%, respectively with 300 mg plant\(^{-1}\) CaC\(_2\) and 100 kg N ha\(^{-1}\) compared to the application of 300 mg plant\(^{-1}\) CaC\(_2\) with 0 kg N ha\(^{-1}\). The application of 300 mg plant\(^{-1}\) CaC\(_2\) to plants grown with 50 kg N ha\(^{-1}\) also resulted in an increase of 14.1, 23.1, 10.1, 5.1 and 37.0% in N, P, K, Ca and Mg contents of leaves respectively compared with 300 mg plant\(^{-1}\) CaC\(_2\) along with 0 kg N ha\(^{-1}\). A lower level of CaC\(_2\) (200 mg plant\(^{-1}\)) either with 50 or 100 kg N ha\(^{-1}\) significantly increased mineral contents in leaves but relatively lesser in magnitude than 300 mg CaC\(_2\) rate (Table 6.1).

**iv) Nitrogen use efficiency indices**

Application of paint coated CaC\(_2\) with different rates of N significantly affected recovery efficiency (RE), agronomic efficiency (AE), physiological efficiency (PE) and nutrient use efficiency (NUE) of nitrogen (Table 6.2). Decreasing trend at higher nitrogen level was observed in all nitrogen use efficiency indices except PE which was slightly increased at higher level of nitrogen and slightly decreased at lower level of nitrogen with the application of CaC\(_2\). Maximum increase in RE, AE and NUE was observed in the treatment of combined application of 300 mg plant\(^{-1}\) CaC\(_2\) with 50 kg N ha\(^{-1}\) was done. The values of RE, AE and NUE were increased by 203.8, 204.2 and 174.7%, respectively while value of PE decreased by 9.9% due to the application of 300 mg plant\(^{-1}\) CaC\(_2\) with 50 kg N ha\(^{-1}\) compared to the application of 50 kg N ha\(^{-1}\) with 0 mg plant\(^{-1}\) CaC\(_2\). The application of 300 mg plant\(^{-1}\) CaC\(_2\) on plants grown with 100 kg N ha\(^{-1}\) resulted in an increase of 139.7, 144.8 and 124.2% in the values of RE, AE and NUE, respectively and decrease of 6.7% in the value of PE if compared to 0 mg plant\(^{-1}\) CaC\(_2\) plus 100 kg N ha\(^{-1}\). A lower level of CaC\(_2\) (200 mg plant\(^{-1}\)) either with 50 or 100 kg N ha\(^{-1}\) followed similar trend but was found less effective (Table 6.2).

**iv) Total nitrogen uptake**

Combined application of CaC\(_2\) with different rates of N caused marked increase in the total nitrogen uptake. Maximum increase in total nitrogen uptake was observed in the combined application of 300 mg plant\(^{-1}\) CaC\(_2\) and 100 kg N ha\(^{-1}\). This treatment increased total nitrogen uptake by 100.5% compared to application of 300 mg plant\(^{-1}\) CaC\(_2\) with 0 kg N ha\(^{-1}\).
Fig. 6.11 Effect of paint coated CaC₂ on nitrate reductase activity in leaves at 8 weeks after sowing under nitrogen deficient and adequate levels

Table 6.1 Effect of paint coated CaC₂ on nutrient contents in leaves of cucumber at 8 weeks after sowing under nitrogen deficient and adequate levels

<table>
<thead>
<tr>
<th>N level (kg ha⁻¹)</th>
<th>CaC₂ (mg plant⁻¹)</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2.30 i</td>
<td>0.41 h</td>
<td>3.37 i</td>
<td>2.43 i</td>
<td>0.44 i</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2.62 h</td>
<td>0.48 g</td>
<td>3.77 g</td>
<td>2.63 g</td>
<td>0.48 h</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>3.13 e</td>
<td>0.62 e</td>
<td>4.30 d</td>
<td>2.88 d</td>
<td>0.54 f</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>2.88 g</td>
<td>0.52 f</td>
<td>3.67 h</td>
<td>2.50 h</td>
<td>0.52 g</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>3.22 d</td>
<td>0.64 d</td>
<td>4.27 e</td>
<td>2.82 e</td>
<td>0.64 d</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>3.60 b</td>
<td>0.76 b</td>
<td>4.73 b</td>
<td>3.03 c</td>
<td>0.74 b</td>
</tr>
<tr>
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<td>0</td>
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<td>0.67 c</td>
<td>4.23 f</td>
<td>2.70 f</td>
<td>0.60 e</td>
</tr>
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<td>0.75 b</td>
<td>4.53 c</td>
<td>3.10 b</td>
<td>0.66 c</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>3.67 c</td>
<td>1.00 a</td>
<td>4.83 a</td>
<td>3.40 a</td>
<td>0.77 a</td>
</tr>
</tbody>
</table>

Means sharing same letter at each bar (Figure 6.11) and column (Table 6.1) are statistically non-significant at P > 0.05
Application of 300 mg plant\(^{-1}\) CaC\(_2\) to plants grown with 50 kg N ha\(^{-1}\) could cause increase of only 53.1% in the total nitrogen uptake compared to 300 mg plant\(^{-1}\) CaC\(_2\) with 0 kg N ha\(^{-1}\). Application of lower rate of CaC\(_2\) (200 mg plant\(^{-1}\)) either with 50 or 100 kg N ha\(^{-1}\) was found less effective than 300 mg CaC\(_2\) regarding total nitrogen uptake in cucumber (Figure 6.12).

### 6.7 Yield components

Numbers of fruits per plant and fruit yield per plot or fruit yield per hectare were significantly increased by the combined application of CaC\(_2\) and nitrogen. The application of 300 mg plant\(^{-1}\) CaC\(_2\) plus 100 kg N ha\(^{-1}\) caused the maximal increase in number of fruits per plant and fruit yield per plot or fruit yield per hectare with an estimated increase of 59.8, 77.9 and 77.9%, respectively compared to the application of alone 300 mg plant\(^{-1}\) CaC\(_2\). The plants receiving application of 300 mg plant\(^{-1}\) CaC\(_2\) with 50 kg N ha\(^{-1}\) were able to increase 31.1, 36.4 and 36.4% in number of fruits per plant and fruit yield per plot or fruit yield per ha, respectively compared to alone 300 mg plant\(^{-1}\) CaC\(_2\) i.e. with 0 kg N ha\(^{-1}\). Application of lower rate of CaC\(_2\) (200 mg plant\(^{-1}\)) either with 50 or 100 kg N ha\(^{-1}\) also increased all yield components in similar fashion but impact was less in magnitude compared to 300 mg plant\(^{-1}\) CaC\(_2\) rate (Table 6.3).

### 6.8 Fruit quality characteristics

Both physical and chemical characteristics of fruits were measured which are described below separately.

#### 6.8 (A) Physical characteristics

All treatments significantly affected physical characteristics of cucumber fruits. The maximal increase in length, weight and diameter of fruits was observed in the treatment of 300 mg plant\(^{-1}\) CaC\(_2\) with 100 kg N ha\(^{-1}\). This treatment caused an estimated increase of 33.4, 11.4 and 33.2% in length, weight and diameter of fruits, respectively compared to the application of 300 mg plant\(^{-1}\) CaC\(_2\) with 0 kg N ha\(^{-1}\). The application of 300 mg plant\(^{-1}\) CaC\(_2\) on plants grown with 50 kg N ha\(^{-1}\) brought about an increase of 13.4, 4.1 and 18.1% in length, weight and diameter of fruits, respectively compared to 300 mg plant\(^{-1}\) CaC\(_2\) along with 0 mg kg N ha\(^{-1}\).
Table 6.2 Effect of paint coated CaC\textsubscript{2} on recovery, agronomic, physiological and nutrient use efficiency of nitrogen in cucumber under nitrogen deficient and adequate levels

<table>
<thead>
<tr>
<th>N level (kg ha\textsuperscript{-1})</th>
<th>CaC\textsubscript{2} rate (mg plant\textsuperscript{-1})</th>
<th>Recovery efficiency (RE)</th>
<th>Agronomic efficiency (AE)</th>
<th>Physiological Efficiency (PE)</th>
<th>Nutrient use efficiency (NUE)</th>
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<td>51.0 e</td>
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<td>18.0 d</td>
<td>7.90 a</td>
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<td>22.1 a</td>
<td>3.10e</td>
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<td>133.9 b</td>
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</table>

*N= Nitrogen rate (0, 50, 100 kg ha\textsuperscript{-1})  **CC= Paint coated calcium carbide rate (0, 200, 300 mg plant\textsuperscript{-1})

**Fig. 6.12 Effect of paint coated CaC\textsubscript{2} on total nitrogen uptake by cucumber under nitrogen deficient and adequate levels**

Means sharing same letters at each column are statistically non-significant at P > 0.05
Table 6.3 Effect of paint coated CaC\textsubscript{2} on physical characteristics of fruit quality and yield components of cucumber under nitrogen deficient and adequate levels

<table>
<thead>
<tr>
<th>N level (kg ha\textsuperscript{-1})</th>
<th>CaC\textsubscript{2} (mg plant\textsuperscript{-1})</th>
<th>Number of fruits per plant</th>
<th>Average fruit weight (g)</th>
<th>Average fruit length (cm)</th>
<th>Average fruit diameter (cm)</th>
<th>Fruit yield per plot (kg)</th>
<th>Fruit yield per hectare (t)</th>
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<td>9.58 f</td>
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Means sharing same letter at each row and column are statistically non-significant at P > 0.05
Application of lower rates of CaC\textsubscript{2} (200 mg plant\textsuperscript{-1}) either with 50 or 100 kg N ha\textsuperscript{-1} also improved the length, weight and diameter of fruits but less than 300 mg CaC\textsubscript{2} (Table 6.3).

6.8 (B) Chemical characteristics

i) Titratable acidity

Titratable acidity (TA) was significantly increased in cucumber fruits by the combined application of both CaC\textsubscript{2} and N. The maximum increase was observed in the treatment of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} plus 100 kg N ha\textsuperscript{-1}. Titratable acidity was increased by 23.7\% in the plant receiving 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} and 100 kg N ha\textsuperscript{-1} compared with 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} with 0 kg N ha\textsuperscript{-1}. The application of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} on plants grown with deficient rate i.e. 50 kg N ha\textsuperscript{-1} also resulted in an increase of 13.9\% in titratable acidity compared with 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} along with 0 mg kg N ha\textsuperscript{-1}. However, lower rate of CaC\textsubscript{2} (200 mg plant\textsuperscript{-1}) either with 50 or 100 kg N ha\textsuperscript{-1} increased TA compared to control but showed decreasing trend compared to 300 mg CaC\textsubscript{2} (Table 6.3).

ii) Ascorbic acid

Application of CaC\textsubscript{2} and N in combination significantly enhanced ascorbic acid contents in cucumber fruits. The maximum increase occurred with the combined application of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} and 100 kg N ha\textsuperscript{-1}. This treatment increased ascorbic acid contents by 38.2\% compared to application of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} with 0 kg N ha\textsuperscript{-1}. The application of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} with 50 kg N ha\textsuperscript{-1} also resulted in an increase of (19.0\%) ascorbic acid contents compared to 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} along with 0 kg N ha\textsuperscript{-1}. Application of lower level of CaC\textsubscript{2} (200 mg plant\textsuperscript{-1}) either with 50 or 100 kg N ha\textsuperscript{-1} increased TA compared to control but showed decreasing trend compared to 300 mg CaC\textsubscript{2} (Table 6.4).

iii) Soluble solid contents

Statistical analysis of data clearly showed that soluble solid contents (SSC) were significantly enhanced by application of CaC\textsubscript{2} in the presence of N. The maximal increase (51.8\%) in soluble solid contents was observed in the treatment of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} and 100 kg N ha\textsuperscript{-1} compared to the treatment of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} with 0 kg N ha\textsuperscript{-1}. The application of 300 mg plant\textsuperscript{-1} CaC\textsubscript{2} plus 50 kg N ha\textsuperscript{-1} also caused increase (30.2\%) in soluble solid contents
compared with 300 mg plant$^{-1}$ CaC$_2$ along with 0 kg N ha$^{-1}$. However, application of CaC$_2$ @ 200 mg plant$^{-1}$ either with 50 or 100 kg N ha$^{-1}$ showed comparatively less increase in soluble solid contents (Table 6.3).

iv) Dry matter

Dry matter of cucumber fruits was significantly enhanced by the combined application of CaC$_2$ and N. Maximum increase was observed in the treatment of 300 mg plant$^{-1}$ CaC$_2$ and 100 kg N ha$^{-1}$ with an estimated increase of 63.2% compared to 300 mg plant$^{-1}$ CaC$_2$ with 0 kg N ha$^{-1}$. The application of 300 mg plant$^{-1}$ CaC$_2$ on plants grown with 50 kg N ha$^{-1}$ also showed increase of 26.1% in dry matter compared with 300 plant$^{-1}$ CaC$_2$ along with 0 kg N ha$^{-1}$. A lower level of CaC$_2$ (200 mg plant$^{-1}$) either with 50 or 100 kg N ha$^{-1}$ proved to be less effective (Table 6.3).

v) Total soluble sugars (TSS)

Application of both CaC$_2$ and N alone and in combination increased total soluble sugars (TSS) in cucumber fruits. The maximal increase was observed in the combined application of 300 mg plant$^{-1}$ CaC$_2$ and 100 kg N ha$^{-1}$. This treatment increased total soluble sugars by 27.4% compared to the application of 300 mg plant$^{-1}$ CaC$_2$ with 0 kg N ha$^{-1}$. The application of 300 mg plant$^{-1}$ CaC$_2$ on plants grown with 50 kg N ha$^{-1}$ also caused increase in total soluble sugars by 10.1% compared to 300 mg plant$^{-1}$ CaC$_2$ along with 0 kg N ha$^{-1}$. Application of lower rate of CaC$_2$ (200 mg plant$^{-1}$) either with 50 or 100 kg N ha$^{-1}$ also increased TSS significantly but found less in magnitude compared to 300 mg CaC$_2$ (Table 6.3).

vi) Total soluble protein and free amino acid contents

It is obvious from statistical analysis of data that application of CaC$_2$ with and without nitrogen significantly enhanced total soluble protein and free amino acid contents in cucumber fruits. The maximum increase occurred with the combined application of 300 mg plant$^{-1}$ CaC$_2$ and 100 kg N ha$^{-1}$. Total soluble protein and free amino acid contents were increased by 42.6 and 115.4% respectively with 300 mg plant$^{-1}$ CaC$_2$ and 100 kg N ha$^{-1}$. 

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Table 6.4 Effect of CaC$_2$ on chemical characteristics of fruit quality in cucumber under nitrogen deficient and adequate levels

<table>
<thead>
<tr>
<th>N level (kg ha$^{-1}$)</th>
<th>CaC$_2$ (mg plant$^{-1}$)</th>
<th>Titratable acidity (mg100g$^{-1}$FW)</th>
<th>Ascorbic acid contents (mg kg$^{-1}$FW)</th>
<th>Total soluble solids (%)</th>
<th>Dry matter (g 100g$^{-1}$FW)</th>
<th>Soluble sugars (mg g$^{-1}$fw)</th>
<th>Protein contents (mg g$^{-1}$FW)</th>
<th>Free amino acids (mg g$^{-1}$FW)</th>
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Means sharing same letter at each column are statistically non-significant at P > 0.05
The application of 300 mg plant⁻¹ CaC₂ on plants grown with 50 kg N ha⁻¹ could result in an increase of 37.7 and 66.7% in total soluble protein and free amino acid contents, respectively compared with 300 mg plant⁻¹ CaC₂ along with 0 kg N ha⁻¹. Application of lower rate of CaC₂ (200 mg plant⁻¹) either with 50 or 100 kg N ha⁻¹ proved to be less effective than 300 mg CaC₂ (Table 6.4).

6.9 Discussion

Metabolism provides the power and building blocks for plant life, whereas hormones regulate the growth of individual parts and integrate these parts to produce the form that we recognize as a plant (Davies, 1987). The nutrient status of a plant influences its metabolism and growth and can affect the synthesis and distribution of growth substances (Haru et al., 1982; Green, 1983). Considering the complex interactions of plant hormones and multiplicity of plant functions they control, the impact of nutrients on hormones is an important issue (Whenham et al., 1989; Thoresteinsen and Eliasson, 1990; Cao et al., 1993). Taking this fact into consideration, the present study of the impact of coated CaC₂ as a potent source of ethylene on nitrogen metabolism, photosynthesis and fruit quality in cucumber was of significant importance because of their involvement in dry matter accumulation and yield of plants. Ethylene as a gaseous phytohormone whether synthesized endogenously in the plant body, in response to environmental stresses (Lurssen, 1991) or supplied exogenously at extremely low concentrations markedly affect plant growth and development (Arshad and Frankenberger, 2002; Woodrow and Grodzinski, 1989). In the present study, we observed that the application of CaC₂ in soil rhizosphere significantly improved the growth (leaf area, number of lateral branches), fruit yield (number of fruits, fruit yield per plot), photosynthetic characteristics, nitrogen metabolism and fruit quality in cucumber plants under the deficient as well as adequate levels of nitrogen. Of the various rates of nitrogen in combination with paint coated CaC₂ employed in this study, the combined application of 300 mg plant⁻¹ CaC₂ and 100 kg N ha⁻¹ was found the best in improving the values for most of the morphophysiological characteristics.

By increasing nitrogen level, plant height was significantly increased while coated CaC₂ application reduced the length of the main stem at each level of nitrogen due to classical triple response of plant exposure to ethylene (Neljubow, 1901). The inhibition of apical
growth may have been due to ethylene released from CaC₂ which inhibits elongation and promotes radial expansion because of its more impact on cell enlargement instead of division of cells (Kieber et al., 1993) and plant polar auxin transport (Arora et al., 1982). Early female flowering, fruit setting and fruit yield attributed to the fact that an optimum concentration of CaC₂ consistently supplied ethylene in the proximity of plant that stimulated the developmental processes of cucumber and thus resulting in enhanced lateral growth, slightly inhibited vegetative growth, and enhanced early female flowers. Such synergistic effects not only increased female flower count and fruit setting, but also accelerated the fruit development processes (Abdel-Rahman and Thompson, 1969; Thappa et al., 2011).

The improvement in morpho-physiological characteristics with increase in nitrogen fertilizer rate could be attributed to increased uptake of nitrogen and its associated role in chlorophyll synthesis and hence the process of photosynthesis and carbon dioxide assimilation (Jasso-chaverria et al., 2005) leading to enhanced growth. In addition, nitrogen stimulates vegetative growth resulting in large stems and leaves. Considering that potassium and phosphorus was applied at recommended rates, it is possible that their uptake was enhanced by nitrogen fertilizer which has been reported to mediate the uptake and utilization of potassium, phosphorus and other elements in plants (Brandy, 1984). The significant response of leaf area to different rates of nitrogen (Urea 46%) fertilizer may be an indication that nitrogen was taken up by the plant and subsequently utilized in cell multiplication, amino acid synthesis and energy formation that acts as structural compound of the chloroplast which carries out photosynthesis. Nitrogen fertilizer has been reported to be a constituent of chlorophyll (Lawlor, 2002). However, nitrogen insufficiencies have been reported to reduce the individual leaf area, leaf area index, and total leaf area resulting to reduced surface light interception for photosynthesis (Cechin and Fumis, 2004).

Experimental data in this study clearly indicated that application of CaC₂ @ 300 mg plant⁻¹ with 100 kg N ha⁻¹ had pronounced effect on all photosynthetic characteristics. The reports on the interaction of ethylene and N are available in the literature. Nitrogen influences the quantity, structure, and composition of the photosynthetic apparatus and hence plays a crucial role in determining the photosynthetic capacity of the plant in both natural and agricultural environments (Abrol et al., 1999). In crops, rubisco content increases linearly with N uptake.
and leaf N (Sage et al., 1987; Makino et al., 1997; Nakano et al., 1997). The present work suggests that CaC₂ application at each level of N influences stomatal, photosynthetic, and growth responses. Ethylene increased the stomatal conductance of plants, thereby increasing the diffusion of CO₂ and thus photosynthesis (Figures 6.7 and 6.8). Ethylene can alter the rate of photosynthesis by affecting the diffusion rate of CO₂ from the atmosphere to the intercellular cavities by influencing stomatal aperture (Pierik et al., 2006; Acharya and Assmann, 2009; Wilkinson and Davies, 2010). The enhancement in photosynthesis by CaC₂ application in this experiment was associated with an increase in stomatal conductance and intercellular CO₂, indicating that ethylene may also have increased photosynthesis by increasing the availability of CO₂.

In addition, ethylene could stimulate photosynthesis by increasing the allocation of N to the photosynthetic machinery. The higher photosynthetic rate with application of CaC₂ may be the result of higher ethylene production in the rhizosphere of the plant which increased vegetative growth by increasing availability of N (more N uptake) to the plants (Keerthisinghe et al., 1996; Seneweera et al., 2003). The higher ethylene evolution led to higher leaf area and thus more light interception and net photosynthesis. The role of ethylene in regulating leaf growth of plants (Abeles et al., 1992; Hussain et al., 1999; Khan et al., 2000; Khan, 2004a; Mir et al., 2009b), ethylene-induced leaf emergence in cereal seedlings (Ivenish and Kreicberg, 1992), and leaf expansion (Kieber et al., 1993; Rodrigues-Pousada et al., 1993) has been reported. The application of 300 mg plant⁻¹ of CaC₂ and adequate-N (100 kg N ha⁻¹) maximally increased stomatal conductance and photosynthetic responses.

Coated CaC₂ along with nitrogen fertilizer treatment enhanced the total plant dry mass, accumulation of N, P, K, Ca and Mg in plant leaves and total nitrogen uptake by the plant. This is most likely due to more prolific root growth in response to paint coated CaC₂. Enhanced root growth actively explores more volume of soil and could help to fetch more nutrients from the soil and thus contribute to increase improved quality fruits. Enhanced uptake of nutrients may also be attributed to interaction of CaC₂ dependent ethylene with other endogenous hormones in such a way that net impact resulted in enhanced uptake of nutrients (Leopold 1980; Yang and Hoffman, 1984; Stepanova and Alonso, 2005). Similarly, Ahmed et al., (2003) also reported significant increase in the uptake of N, P, and K
in cotton crop if applied after two weeks of germination. Our results were consistent with findings of Arshad et al. (1993) in which exogenously soil applied L-methionine significantly increased N, P, K, Ca and Mg contents in Albizia lebbeck L. Benth (black ciris). The results obtained in this study also highlighted improved nutrient uptake by increased root hair formation probably stimulated by ethylene (Tanimoto et al., 1996). This improved nutrient uptake may also be owing to the ability of CaC$_2$ to inhibit nitrification (Hazzrika and Sarkar, 1996), improving nitrogen economy of the soil and reducing N losses and thus assuring its availability for a longer period of time.

Application of CaC$_2$ with different rates of N significantly affected recovery efficiency (RE), agronomic efficiency (AE), physiological efficiency (PE) and nutrient use efficiency (NUE) of nitrogen. Decreasing trend at higher nitrogen level was observed in all nitrogen use efficiency indices except PE which was increased at adequate level of nitrogen and slight decrease at each level of nitrogen was observed by application of CaC$_2$. That decrease was due to more uptakes of nutrients which increased the yield potential but decreased the physiological efficiency. In contrast to this RE, and AE were significantly enhanced by application of CaC$_2$ due to more uptake of nitrogen and higher yield, respectively. Similar results have been reported by using different rates and coating materials on CaC$_2$ in improving NUE indices in sweet paper (Ahmed et al. 2014) and in tomatoes (Siddiq et al., 2012). Moreover, Mir (2002) reported impressive increase in the nitrogen uptake efficiency, nitrogen use efficiency and nitrogen utilization efficiency of mustard in response to ethrel application at basal 80 kg N ha$^{-1}$. Similarly CaC$_2$ application at 300 mg plant$^{-1}$ under adequate N resulted in the highest leaf nitrate reductase (NR) activity and leaf N concentration. Several cases of interactions between ethylene and nitrogen have been reported. Van Sanford et al. (1989) and Bulman and Smith (1993) also found positive effects of ethephon on nitrogen-use efficiency of winter wheat and barley. Significant increase the nitrate reductase activity in leaves of Brassica juncea has been reported by application of ethrel (200 µL/L) at basal 80 kg N ha$^{-1}$ (Mir 2002; Mir et al., 2008 a; Ashraf et al., 2010).

Calcium carbide dependent ethylene release resulted in significant improvement in quality of fruiting elements at each level of nitrogen. Application of CaC$_2$ along with nitrogen fertilizer (Urea-46%) enhanced nitrogen uptake which resulted in enhanced growth.
and dry-weight production in the fruits (Mattson et al., 1991; McDonald et al., 1996), thereby lowering the water content (Table 6.4). Similarly, increase in soluble proteins, organic acids and amino acids via increase in NR activity and nitrogen uptake has been reported by Ruiz and Romero (1998) while increase in SSC and soluble sugar contents was reported by Raese and Drake (1997). Ahmed et al. (2007) reported that an increase in nitrogen application resulted in maximum fruit length, fruit weight, vine length and yield of cucumber. Moreover, significant increase in total soluble solids, organoleptic quality and acidity has been reported in mango fruits by the application of nitrogen and CaC₂. The improvement in the quality of the fruit of tomatoes (an increase in the content of dry matter by 0.5-0.6 %, an increase in the total sugars by (0.55-0.73 %) was reported by Muromtsev et al. (1988). Muromtsev et al. (1991) also found that calcium carbide applied @ 200 kg ha⁻¹ not only increased yield of Satsuma (Citrus unshiu L.) but also increased the average weight of fruit, flesh, ascorbic acid and sugar contents.

Results regarding the effect of calcium carbide @ 300 mg plant⁻¹ in combination with nitrogen @ 100 kg ha⁻¹ showed maximum increase in number of fruits per plant and fruit yield per hectare of cucumber (Table 6.3). Likewise, positive interaction of nitrogen with CaC₂ was reported in okra where maximum green pod yield was observed in treatment receiving N-CaC₂ of 30-30 mg kg⁻¹ soil compared to control (Kashif et al., 2007). However, physiological disorder was observed at higher level of nitrogen in contrast to our results that might be due to the change in plant sensitivity to soil applied ethylene at highest level of nitrogen. Similarly, application of CaC₂ in combination with nitrogen was also reported in rice where maximum number of tillers per plant and paddy yield was obtained by application of CaC₂ in combination with full dose of N fertilizer by increasing agronomic, apparent and physiological efficiencies of nitrogen (Yaseen et al., 2009).

All the observed effects of calcium carbide application reported in the present study suggest that use of calcium carbide in combination with chemical fertilizer could therefore be used as a tool of non-conventional approaches for improving growth, yield and quality of cucumber and other vegetable crops and thus may serve as an innovative approach in the country towards the attainment of global food security.
Ethylene can act as a growth stimulator as well as growth inhibitor depending upon concentration, plant species, exposure time and sensitivity of plant organ. Cucumber shows a great diversity regarding floral morphology and sex expression. Ethylene, the plant growth regulator associated with several physiological processes in plants is also known to alter the sex expression in cucurbits. Any treatment that would increase the formation of pistillate flowers would therefore, be beneficial in producing high yield because there is a correlation between fruit yield and number of pistillate flowers per plant. Yield of cucumber has been improved by different conventional approaches or technologies i.e. incorporation of disease resistance into cultivars and use of improved cultural practices have also contributed to the improvement of yield in cucumber but still gap exists between potential yield and yield obtained at farm level. The use of coated CaC\textsubscript{2} would be a desirable approach in this scenario to minimize this gap for enhancing vegetable production.

At present, various compounds have been reported to provide biologically produced ethylene in soil and among them coated CaC\textsubscript{2} has emerged as a very good source of C\textsubscript{2}H\textsubscript{2} (nitrification inhibitor) which upon its reduction is converted to C\textsubscript{2}H\textsubscript{4} by micro biota in the soil. Keeping in view the role of calcium carbide in plant growth and developmental processes, it was planned to explore its potential in the soil environment of Pakistan. It could be an innovative activity to enhance crop yield by combining the non-conventional approaches with conventional approaches. Because ethylene does not act singly but instead a net balance after interaction with other hormones determines its overall effect on plant growth. A complete understanding of the ethylene and nutrients interaction would provide new strategies for improving crop vigor and development under changing environment.

A series of laboratory, wire house and field experiments were conducted to evaluate the effects of coated CaC\textsubscript{2} on morpho-physiological, and biochemical changes in cucumber. Since literature revealed that release of acetylene and ethylene from applied CaC\textsubscript{2} could give
better germination of dormant and non-dormant seeds of various crop species so different rates were compared regarding germination and morphological characteristics. The results of this experiment revealed that germination rate, germination index, germination potential and growing vigor index of cucumber was significantly improved by using low rates of CaC₂ (10 to 30 mg CaC₂ plate⁻¹) and that the highest rate of CaC₂ (40 mg plate⁻¹) suppressed all germination characteristics. In addition to germination, CaC₂ was also found effective in enhancing the root length, hypocotyledonary axis, number of 1st lateral roots and fresh weight of seedling of cucumber at low rates of CaC₂ i.e. 10 to 30 mg plate⁻¹ of CaC₂. However, the highest rate of CaC₂ i.e. 40 mg plate⁻¹ of CaC₂ showed reverses results. Further, it was demonstrated that the CaC₂ induced improvement in seed germination indices was related to its enhancement of ethylene production during imbibition. After 5 days of incubation, maximum ethylene emission (14.02 nl g⁻¹ dry seed weight) was recorded in the treatment where CaC₂ was applied @ 30 mg plate⁻¹ which was 46% higher than that of control while it was inhibited at higher rate of CaC₂. When CaC₂ rate reached 30 mg plate⁻¹, the length of root and hypocotyledonary axis of cucumber were reached the maximum, which were 148.5 and 77.7% of the control, respectively. According to the analysis of subordination function, 30 mg plate⁻¹ of CaC₂ was found the best rate which enhanced the germination and seedling growth of cucumber.

In the next experiment under laboratory controlled conditions, effect of different rates of CaC₂ in the presence as well as absence of nitrate on seedling growth parameters and nitrogen metabolism indicators was investigated in cucumber. Calcium carbide caused concentration dependent increase in all nitrogen metabolism indicators in isolated cotyledons of cucumber. Application of 30 mg plate⁻¹ of CaC₂ along with NO₃⁻ increased the contents of chlorophyll a, chlorophyll b, carotenoids, nitrate reductase (NR) activity, glutamine synthetase (GS) activity, soluble sugars, soluble proteins and free amino acids up to the maximum by 114.4, 78.7, 91, 81 56.1, 64.8, 41.3, 64 and 84.8%, respectively compared to control (without nitrate and CaC₂).

Percentage of seed germination, root and shoot lengths and root and shoot weight was found significantly promoted at lower rates (20 to 30 mg plate⁻¹) of CaC₂ and sharply reduced thereafter at higher dose (40 mg plate⁻¹) both in the absence as well as presence of
nitrate application. However, higher dose of CaC\textsubscript{2} were found to cause inhibition in all physiological and biochemical activities in cucumber cotyledon. Overall, the interactive effect of CaC\textsubscript{2} and nitrate was more prominent than individual effects.

In addition to nutritional stress, ethylene has also been reported to be involved in salinity stress condition. Therefore, another laboratory study was conducted for the verification of involvement of CaC\textsubscript{2} in improving germination under salinity stress by investigating its effect on reactive oxygen species and antioxidant enzymes during germination. This experiment was performed in 5 parts under controlled conditions to investigate the effect of CaC\textsubscript{2} on germination and different biochemical changes in cucumber seeds under different levels of NaCl stress (0 to 200 mM). In the first part seed germination response was studied under different levels of salinity. Results showed that as the salinity level increased germination percentage delayed. After 72 h of incubation, maximum germination (93.3\%) was observed in the control while in 150 and 200 mM NaCl treatments, seed germination was severely inhibited. In 2\textsuperscript{nd} part of experiment, effect of different doses of CaC\textsubscript{2} (0 to 40 mg plate\textsuperscript{-1}) on pattern of ethylene emission in relation to seed germination was studied under salinity stress (150 mM). Addition of CaC\textsubscript{2} to the incubation medium significantly alleviated the NaCl-induced suppression of ethylene evolution from the imbibed seeds. After 48 h of incubation, ethylene emission was reduced by 54\% in 150 mM NaCl stress compared to the control but ethylene suppression was reduced by increasing concentration of CaC\textsubscript{2} and maximum ethylene evolution was observed at 40 mg plate\textsuperscript{-1} of CaC\textsubscript{2}. Seed germination was significantly enhanced by increasing rates of CaC\textsubscript{2} and maximum increase in germination (207\%) was observed at 40 mg plate\textsuperscript{-1} CaC\textsubscript{2} compared to the control (150 mM NaCl) without CaC\textsubscript{2}.

To further verify the involvement of ethylene for enhanced seed germination after application of CaC\textsubscript{2} under NaCl (150 mM) stress, effect of the best rate of CaC\textsubscript{2} (300 mg plate\textsuperscript{-1}), selected from the 2\textsuperscript{nd} part, was investigated by using ethylene perception (AgNO\textsubscript{3}) and ethylene action inhibitor (CoCl\textsubscript{2}) in the 3\textsuperscript{rd} part of experiment. After application of 30 mg plate\textsuperscript{-1} of CaC\textsubscript{2} a marked increase in seed germination (208\%) was observed compared to control (150 mM NaCl without CaC\textsubscript{2}). Maximum decrease (35\%) in seed germination was observed in the treatment where AgNO\textsubscript{3} was applied followed by 27\% decrease in the
treatment where CoCl$_2$ was applied with respect to the treatment of 30 mg plate$^{-1}$ of CaC$_2$. Results verified that ethylene is involved in breaking seed dormancy under salt stress.

In the 4$^{th}$ part, effect of best rate of CaC$_2$ (300 mg plate$^{-1}$) on some biochemical changes including $\alpha$-amylase activity, H$_2$O$_2$ contents, soluble proteins and free amino acids was studied during 5 d of incubation under salinity stress. Results showed that CaC$_2$ as a potent source of ethylene significantly enhanced contents of free amino acids, soluble sugars while decreased H$_2$O$_2$ contents at each interval in germinating seeds of cucumber that ultimately increase the tolerance to salt stress by improving nitrogen metabolism in cucumber. Moreover, in another batch, effect of the best rate of CaC$_2$ on MDA contents, antioxidants enzymes and proteins contents was investigated after 48 h of incubation. Results showed that CaC$_2$ application significantly alleviated the salt induced accumulation of malondialdehyde (MDA) in the germinating cucumber seeds. Application of CaC$_2$ significantly increased the total soluble protein contents, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities by 40.4, 94.8, 71.3 and 80.3%, respectively compared to the control without affecting activities of ascorbate peroxidase (APX).

In the light of the results of laboratory experiments, a pot experiment was planned to study the response of cucumber for different morpho-physiological characteristics to acetylene and ethylene gases released from calcium carbide based formulations. The objective of this experiment was to find out the best rate and coating material of CaC$_2$ so that to release gases slowly in soil environment under wire-house conditions. Ethylene released from different rates of calcium carbide (0 to 300 mg pot$^{-1}$) significantly reduced vine length, number of days to flowering, days to fruit maturity while improved number of nodes on main vine but differences among various coating materials on CaC$_2$ were found statistically non-significant regarding morphological and floral attributes. Similarly, maximum fruit yield (2.2 kg plant$^{-1}$) by cucumber was observed in the treatment where paint coated calcium carbide was applied @ 300 mg pot$^{-1}$ while minimum fruit yield (1.5 kg plant$^{-1}$) was observed in control plants (without CaC$_2$). Overall, an estimated increase of 34 % in fruit number and yield per plant was observed where CaC$_2$ was applied @ 300 mg CaC$_2$ pot$^{-1}$, respectively over the control plants. However, among coating materials, paint coating was found the best.
to enhance the fruit number and yield of cucumber plant followed by gelatin coating whereas no significant difference was observed between paraffin and wax coating on CaC₂.

So, on the basis of results from the above pot experiment, paint coating material was selected and tested to study genotypic response of cucumber. For this purpose paint coated calcium carbide was applied at different rates (0 to 400 mg pot⁻¹) and response of two cucumber cultivars (Local vs Hybrid) was studied. The results indicate that the rate of 300 mg paint coated CaC₂ reduced number of days to flowering, days to fruit setting and maturity and increased female flowers, earliness in yield, total yield and ratio of early yield : total yield in both cultivars differing in yield potential. However, the highest rate of paint coated CaC₂ (400 mg pot⁻¹) exhibited inhibitory effect regarding fruit setting, early yield, total yield and ratio of early yield/total yield in both cultivars.

Results obtained in laboratory and wire house trials were verified by conducting a field trial. In this experiment the optimum rates of paint coated CaC₂ (200 and 300 mg pot⁻¹) from previous pot experiment were chosen to further study interaction of CaC₂ with deficient and adequate levels of nitrogen (50 and 100 kg ha⁻¹) on growth, photosynthesis, nitrogen assimilation and fruit quality attributes of cucumber. All parameters regarding morphological (leaf area, number of primary branches, total dry weight and total nitrogen uptake per plot) and yield attributes (number of fruits per plant, fruit yield per plot and fruit yield per hectare) responded positively and maximally to the treatment of 300 mg plant⁻¹ paint coated CaC₂ and adequate-N level (100 kg ha⁻¹). The values of these parameters were lower in plants grown with 0 or 50 kg N ha⁻¹, but the application of paint coated CaC₂ (200 or 300 mg plant⁻¹) with these N levels caused increase in these parameters. It was also noted that addition of paint coated CaC₂ significantly reduced vine length, days to first female flower and days to fruit maturity at each level of nitrogen.

Similarly, the plants receiving 300 mg plant⁻¹ paint coated CaC₂ plus 100 kg N ha⁻¹ had the strongest stimulating or increasing effect on nitrate reductase activity (57.3%), photosynthetic rate (68.4%), intercellular CO₂ concentration (27.6%), stomatal conductance (39.4%), carboxylation efficiency (33.3%) and water use efficiency compared to alone 300 mg plant⁻¹ of paint coated CaC₂. These increases are also depicted from enhanced recovery efficiency (RE), agronomic efficiency (AE), physiological efficiency (PE) and nutrient use
efficiency (NUE) of nitrogen due to application of CaC$_2$ with different rates of N. Decreasing trend at higher nitrogen level was observed in all nitrogen use efficiency indices except PE which was slightly increased at higher level of nitrogen and slightly decreased at lower level of nitrogen due to the application of CaC$_2$. Maximum increase in RE (203.8%), AE (204.2%) and NUE (174.7%) was observed in the treatment of 300 mg plant$^{-1}$ CaC$_2$ plus 50 kg N ha$^{-1}$ while PE was decreased by 9.9% compared to the treatment where no CaC$_2$ with same level of nitrogen was applied.

Paint coated calcium carbide application also markedly improved physical and chemical quality parameters of cucumber fruit. Parameters like length, width, weight, titrable acidity, total soluble solids, ascorbic acid, total soluble sugars, protein contents and free amino acids were found better/improved in the fruits of plants treated with CaC$_2$ treated plants compared to fruits of untreated plants at each level of nitrogen. All these parameters had maximum values in the treatment of 300 mg plant$^{-1}$ of paint coated CaC$_2$ applied with adequate nitrogen level. The enhancement in growth, yield and different morpho-physiological attributes was probably due to the ethylene induced changes, produced in soil environment by the application of coated CaC$_2$. More precisely, these may be due to improved nitrogen economy in soil through nitrification inhibition by acetylene released from CaC$_2$.

**Concluding Remarks**

Results of laboratory experiments showed that CaC$_2$ formulation can be used as innovative approach to enhance seed germination under normal and stress conditions like nitrogen and salinity stress by increasing the activity of nitrogen assimilation enzymes and antioxidant enzymes during germination. Results obtained from laboratories, pot and field experiments indicate that cucumber crop responded well to calcium carbide in the root zone. A probable reason of enhanced growth and fruit yield might be due to increased level of endogenous ethylene in plants by the application of exogenous CaC$_2$ in root zone which promoted metabolic processes and changed the C:N ratio in plants, resulting more female flowering, fruit setting, early fruit formation and improved nitrogen use efficiency. All these factors contributed directly or indirectly to increase the yield obtained. In the net shell, application of CaC$_2$ effectively improved the fruit yield of cucumber as well as its quality.
parameters. Results from all trials on morpho-physiological parameters (more number of flowering, fruit set, total biomass, total fruit yield, higher photosynthesis and nitrogen assimilation) and physical and compositional characteristics of fruits suggest that application of paint coated CaC\(_2\) at the rate of 300 mg plant\(^{-1}\) is the appropriate formulation for cucumber crop under field conditions.

**Future Directions**

This work demonstrates that use of paint coated calcium carbide at the rate of 300 mg plant\(^{-1}\) can successfully be used to increase yield and quality of cucumber when applied with recommended doses of NPK fertilizers. This study opens a gateway to explore the effectiveness of coated calcium carbide for further studies to find its effectiveness in other horticultural crops. The effect of ethylene on regulating stomatal conductance and nitrogen assimilation enzymes has been reported in this study which leads to increased photosynthesis and nitrogen uptake, but whether ethylene functions alone or in co-ordination with other phytohormones remains to be tested. Phytohormones, cytokinin, auxin, and gibberellins are known as signaling components in response to N deficiency. A single hormone can regulate an amazingly diverse array of cellular and developmental processes, while at the same time multiple hormones often influence a single process. Therefore, further study may be focused on whether ethylene regulates different morpho-physiological processes under varying N levels alone or in co-ordination with other hormones. For this, the study of the relationship of ethylene with, abscisic acid (ABA), salicylic Acid, gibberellic acid (GA) and nitric oxide (NO) in the regulation of nitrogen assimilation enzymes, antioxidants enzymes and stomatal conductance will give more insight in the net growth responses under optimal and/or stress conditions. Moreover, gene expression studies would be a step forward towards unraveling signal transduction components specifically involved in the stimulatory and inhibitory phases of ethylene responses.


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