EFFECT OF CARBON DIOXIDE AND CYTOKINE FOLIAR SPRAY ON YIELD AND GRAIN QUALITY OF BREAD WHEAT *(Triticum aestivum L.*)

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APPROVAL SHEET

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SCHOOL OF GRADUATE STUDIES

As thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance by Getachew Biru, entitled **EFFECT OF CARBON DIOXIDE AND CYTOKINE FOLIAR SPRAY ON YIELD AND GRAIN QUALITY OF BREAD WHEAT *(Triticum aestivum L.)*** I recommend that it be submitted as fulfilling thesis requirement.

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DEDICATION

I dedicate this Thesis manuscript to my late mother Olike Terfa and my late father Biru Tsegaye for their dedicated partnership for the success of my life.

STATEMENT OF THE AUTHOR

First I declare that this thesis is my work and that all sources of the materials used for this thesis have been duly acknowledged. This thesis has been submitted to in partial fulfillment of the requirements for M.Sc. degree at Jimma University, College of Agriculture and Veterinary Medicine and to be deposited at the University Library to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHOCAL SKITCH

Getachew Biru Tsegaye was born on July 24, 1988 at the vicinity of Gidam Dabsho, Horro Guduru Wollega zone. He attended his education at Hareto Primary and Secondary School from 1994 to 2001 and Hareto Secondary High School from 2002 to 2003, respectively. After completing secondary high school, he joined Alage ATVT College of Agriculture in 2004 academic year and certified with Diploma in Plant science in June 2006. After he completed his study (in June, 2006) he worked as supervisor from 2007 to 2009 in Jimma Genet woreda .Again after three years he joined Jimma University College of Agriculture and Veterinary Medicine for up grading in academic year of 2009/10 and he graduated from Jimma University (JUCAVM) in June, 2011, in BSc. Degree in Horticulture. And then he worked from 2012 to 2013 in agricultural office as expert in Jimma Genet distinct and final join Jimma University College of Agriculture and Veterinary Medicine for MSc. degree in Agronomy (2014).

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Abbreviation and acronyms

CSA	Central Statistical Agency
CK	Cytokine
FAOSTAT	Food and Agriculture Organization of the United Nation
GDP	Growth and Development Programme
IPCC	Intergovernmental Panel on Climate Change
DAP	Di-amonium phosphate
N	Nitrogen
P	Phosphorus
WUE	Water Use Efficiency

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EFFECT OF CARBON DIOXIDE AND CYTOKINE FOLIAR SPRAY ON YIELD AND GRAIN QUALITY OF BREAD WHEAT *(Triticum aestivum L.*)

Abstract

Wheat (Triticum aestivum L.) is one of the important cereal grain crops cultivated worldwide. Wheat is grown on more land area than any other crop in the world. World trade for wheat is greater than for any other cereal. Ethiopia is the second largest wheat producer in sub-Saharan Africa, after South Africa and also among the most important crops in Ethiopia, ranking fourth in total cereals production. The demand for wheat is highly increased with the increasing population number and urban expansion. However currently the production of wheat and productivity become difficult due to climate change. Therefore the objective of these studies were to increase the productivity and the production of wheat under variable climate change by applying CO_2 and Cytokine foliar spray at different growth stages on grain yield and grain quality of bread wheat (Digalu), studies were conducted both under field and control environment at Mana distinct and Jimma University College of Agriculture and Veterinary Medicine in (2014 and 2015) respectively. Carbon dioxide and cytokine were applied at rate of 680mmole and 100mmole respectively at different stage (tillering stage, heading stage and seed filling stage) and Randomized complete block design (RCBD) with three replications were used. The results showed that in both case (in control environment and open field),the number of tiller, days to 50% heading, days to 90% physiological maturity, plant height, spike length, number of seed per plant, biomass, grain yield and Harvest Index (HI) were highly significantly ($p<0.01$) affected by the effects of Carbon dioxide, whereas, number of tiller, grain nitrogen content, straw nitrogen content, nitrogen uptake efficiency, nitrogen utilization efficiency, nitrogen use efficiency, grain protein content and grain thousand weight were highly significantly ($p<0.01$) affected by cytokine. Furthermore, the combination of carbon dioxide and cytokine at different stages (Tillering, flowering & seed filling) highly significantly affected, days to heading, days to maturity height of plant, thousand kernels weight, number of effective tillers, biomass yield, number of kernels per spike, harvest index, protein content, nitrogen uptake efficiency, nitrogen utilization efficiency, nitrogen use efficiency and grain yield. The application of both carbon dioxide and cytokine at tillering stages on wheat in green house and under field conditions resulted in highest tiller number (7) and (5.5) respectively, the maximum biomass yield (15.68 ton ha^{-1}& 14.8o ton ha^{-1}) were both in green house and under field condition due to the foliar application of both carbon dioxide & cytokine at flowering stages, protein content (16.75% & 16.76%) and grain yields were (13.44 ton ha^{-1}& 11.88 ton ha^{-1}) at flowering stages respectively. From this study, the use of both carbon dioxide and cytokine at wheat flowering stage (Digalu) was identified for good crop stand, high grain yield and seed quality. Therefore to ensure further research will be important for fundamental understanding of the effect of carbon dioxide and cytokine foliar spray on yield and grain quality on bread wheat in different location.

Key words: *CO_2, Cytokine, efficiency, grain yield, Nitrogen uptake, Nitrogen utilization, Nitrogen use efficiency, seed quality.*

1. Introduction

Wheat *(Triticum aestivum L.*) is one of the important cereal grain crops which is cultivated worldwide (*Belderok et al., 2000;* Shewry and Peter, 2009). According to FAO (2015) reports, wheat is grown on larger land area than any other crop in the world and its trade is greater than any other cereal crops. Ethiopia is the second largest wheat producer in sub-Saharan Africa after South Africa and the crop is among the most important crops in Ethiopia, ranking fourth in total cereals production (16 per cent) next to maize, sorghum and teff (CSA, 2009). Wheat, sorghum, and maize supply over 50% of average daily caloric intake in Ethiopia. Cereal production accounts for roughly 60% of rural employment and 80% of total cultivated land in Ethiopia. Households spend an average of 40% of their total food budget on cereals (Adugna, 2007). Although most of the wheat grown in Ethiopia is bread wheat, there is some durum wheat which is often grown mixed with bread wheat. Wheat is used not only for making bread, biscuit and pastry products, but also used for the production of starch and gluten (Hanson *et al*., 1982, Ziobro *et al*., 2016).

It is grown as a staple food in the highlands at altitudes ranging from 1500 to 3000 masl, between 6-16 ^{0} N latitude and 35- 42 ^{0} E longitude. The most suitable agro- ecological zones, however, fall between 1900 and 2700 masl (Bekele *et al*., 2000, Getachew *et al*., 2014). In all African countries, wheat consumption has been steadily increasing during the past 20 years as a result of growing population, changing food preferences and a strong urbanization trend which has led to a growing 'food gap' in all regions, largely met by imports (FAO, 2015; Abu, 2013). Nearly all wheat in country is produced under rain-fed conditions predominantly by small farmers. A few government's owned large-scale (state) farms and commercial farms also produce wheat. Despite the recent expansion, Ethiopia falls short of being self-sufficient in wheat production, and is currently a net importer of wheat grain (FAOSTAT, 2011).

However, wheat yield in Ethiopia is also lagging behind other major producers in Africa, with an average yield of 2.11 tonnes per ha in 2012, which is about 41 percent below Kenya and 77 percent below South African averages (FAO,2014).The apparent low productivity can be attributed to several factors, including, the production technologies which are predominantly characterized by low agricultural inputs (fertilizer, improved seeds, pesticides) using traditional farming techniques (Arndt *et al*., 2011).Not only this but also climate variability, particularly rainfall variability and associated droughts have been causes for food insecurity in Ethiopia (Seleshi and Zanke, 2004; Rosell, 2011).

Climate change is expected to pose more challenges and to further reduce the performance of the economy (Arndt *et al*., 2011). However according to World Bank (2006) report, the correlation between rainfall variability and the overall performance of the country´s GDP: years of poor rainfall were associated with low, whereas years with high rainfall were associated with high country‘s total and agricultural GDP. For this cause, some studies assessed impacts of climate change on Ethiopian economy (Block *et al.*, 2008; Deressa and Hassan, 2009; Mideksa, 2010; Arndt *et al*., 2011; Robinson *et al*., 2012) and determinants of farmers choice for adaptation practices (Deressa *et al*., 2009). A quantitative understanding of current climate variability, its impacts and how farmers respond to this, is an essential step for adapting agricultural systems to future climate change. Moreover, anticipating impacts of future climate change and evaluating potential adaptation options for various climate change scenarios is highly relevant for Ethiopia‘s agricultural production and improving food security (Bewket *et al*., 2015).

Therefore to reduce the current and the future impact of climatic variability and improve food security uses of new technology that increasing water use efficiency, water and plant nutrients absorption from the soil such as carbon dioxide fertilization and Cytokine hormones are very important, because of the impact of climate change scenarios was more severe in the tropical latitudes than in the mid- or high-latitudes. For example, averaged over all three global climate model (GCM) scenarios, wheat production changes predicted in Brazil, India, China and Canada were -47, -43, -11 and -20%, respectively, without CO_2 effects, and -28, -13, +8 and +16% with CO_2 fertilization effects (calculated from data of Rosenzweig and Parry, 1993; Rosenzweig and Iglesias, 1994).

On average, they found photosynthetic enhancements of 33 and 25%, respectively, for C_3 and C_4 plants, along with biomass enhancements of 44 and 33%, respectively, for a doubling of the air's CO_2 concentration (Prior *et al*., 2003).In general, elevated CO_2 increases plant growth (both above-and below ground) and improves plant water relations (reduces transpiration and increases water use efficiency (WUE) (Gifford, 1988). Although elevated CO_2increased overall yields, the proportion of protein in grains is reduced because under these conditions the plant did not increase its nitrogen uptake at the same rate as its growth rate (Pleijel & Uddling, 2012).Cytokine hormone, on the other hand, has been shown to slow down plant aging and prevent protein breakdown, activate protein synthesis and assemble nutrients from nearby tissues(Campbell *et al*., 2008).Cytokines are a group of compounds that stimulate water uptake, increase cell division, promote organ development and lead to regeneration and proliferation of shoots (Leham *et al*., 1983).It is therefore imperative to evaluate the effects of CO_2 and cytokines hormone on yield and protein content of wheat as to design optimum scenario for climate mitigation.

This study was, therefore, initiated with the objectives of

- Evaluating effects of CO_2 and Cytokine hormone foliar spray on wheat crop growth, yield and protein contents in control environment and under field condition.
- Identifying the phenological stage of wheat growth at which the applications are effe ctive both in control environment and under field condition.

2. Literature Review

2.1 Impact of climatic change on wheat production

Wheat is a cool season crop and increasing temperature shortens its growth period by accelerating Phenological development, resulting in reduced yield (Asseng *et al.*, 2011). As Liu *et al.* (2008) reported in SSA, average annual temperature in 1990 was 20.3°C in wheat harvest areas, which already exceeded the optimum wheat growing temperature of 15 20°C. The exact level of the effects of climate change differ by location, but some studies suggest that a 1°C increase in temperature above norm reduces wheat yield by 10% (Brown 2009). Without considering the temperature effect on photosynthesis and grain- set, Hatfield *et al.* (2008) reported 7% yield reduction per 1°C increase in air temperature between 18 and 21°C and 4% reduction per 1°C increase above 21°C.

Although by plotting the multiple simulation studies from low- latitude areas, Easterling *et al.* (2007) reported a linear relationship between an increase in temperature and the reduction in wheat yield with about 7% decrease for a 2°C rise in temperature and 34% yield decrease for a 4°C rise in temperature for example as Ethiopia ((Mc Sweeney *et al.*, 2010). Many simulation studies have projected a greater impact on wheat yield compared to other crops in East Africa (Liu *et al.*, 2008; Fischer 2009; Nelson *et al.*, 2009; Ringler *et al.*, 2010).

Fischer (2009), used CM3 under the A2 storyline, projected 63% and 44% yield reduction for rain fed wheat in eastern and southern Africa, respectively, by the 2050s even under carbon fertilization and adapted crop cultivars (A2 storyline mean that Economic development is regionally divided; global population continually grows; consistent fossil fuel use; relatively higher increase in GHG emissions) (Mc Sweeney *et al.*, 2010). Using the CSIRO climate model under the same storyline, the author projected 30%, 48%, and 72% yield reduction for rain fed wheat in eastern Africa by 2020s, 2050s, and 2080s, respectively, even considering carbon fertilization. Using the National Centre for Atmospheric Research (NCAR) and CSIRO models and without considering carbon fertilization, Nelson *et al.* (2009) projected 34% to 36% reduction in wheat under the A2 scenario by 2050 in SSA.

2.2. Adaptation of climate change for wheat production

Carbon dioxide is an important greenhouse gas and burning of carbon-based fuels since the industrial revolution has rapidly increased its concentration in the atmosphere, leading to global warming. It is also a major source of ocean acidification since it dissolves in water to form carbonic acid (National Research Council, 2010).Carbon dioxide is regarded as the driving factor of climate change, however its direct effect on plant is positive (Warrick, 1988). CO_2 enriches atmosphere positively and affects the plants in two ways. First, it increases the photosynthesis process in plants. This effect is termed as carbon dioxide fertilization effect. This effect is more prominent in C_3 plants because higher level of CO_2 increases rate of fixed carbon and also suppresses photorespiration. Second, increased level of CO_2 in atmosphere decreases the transpiration by partially closing of stomata and hence declines the water loss by plants.

Both aspects enhance the water use efficiency of plants causing increased growth (Ghannoum *et al*., 2000; Sage & Kubien, 2003). In additional to improved plant water relations, elevated CO2 can also affect water movement through the landscape. Water in filtration can be increased and sediment loss through runoff can be decreased in high CO2 environments (Prior *et al*., 2010).

The crops which exhibit positive responses to enhanced CO_2 are characterized as C_3 crops including wheat, rice, soybean, cotton, oats, barley and alfalfa whereas the plants which show low response to enhanced CO_2 are called C_4 crops including maize, sugarcane, sorghum, millet and other crops (Pervez *et al*., 2010).C_3 plants typically respond better to atmospheric CO_2 enrichment than do C_4 plants in terms of increasing their rates of photosynthesis and biomass production (Bernacchi *et al*., 2003; Long *et al.*, 2004). Hence, it has periodically been suggested that in a world of rising atmospheric CO_2 concentration, C_3 plants may out-compete C_4 plants and displace them, thereby decreasing the biodiversity of certain ecosystems. It will inevitably be overwhelmed by all the adverse effects on plant reproduction and growth as the world becomes warmer caused by the carbon dioxide increase, along with the increase in the other greenhouse gases (GHGs) (Stern, 2006).CO_2 fertilization may be slowing down the expected accumulation of carbon dioxide in the atmosphere by increasing carbon accumulation in terrestrial vegetation and soil (Harrison, 2004).

According to Reilly and Hohmann (1993) Studies in the early 1990s found that climate change would have limited agricultural impacts globally, but with varying effects across regions. Therefore as they suggested the adaptation and carbon dioxide (CO_2) fertilization effects were the two largest sources of variation in the results. They also suggested that, the mean biophysical yield effect with no incremental CO_2 fertilization is a 17% reduction globally by 2050 relative to a scenario with unchanging climate. They further suggested that endogenous economic responses reduce yield loss to 11%, increase area of major crops by 11%, and reduce consumption by 3%.

Table 1. Mean climate change impacts on SSA crop yields, with and without CO_2 fertilization effects across 5 GCM models (percent change in 2046-2055 relative to 1996-2005) on current (2000) cropland.

Full CO_2 fertilization effects				No CO_2 fertilization effects			
A1b	A2	B1	Mean	A1b	A2	B1	Mean
8.4	7.8	6.8	7.5	-8.2	-8.5	-5.9	-7.6

Source: Muller *et al*., 2010.

As the author reported by the 2046-2055 periods, average crop yields are projected to increase by 7.5% with CO_2 fertilization effects, and decline by an average of 7.6% without CO_2 fertilization. However, the study strongly caveats these results, particularly with regard to production benefits from CO_2.

2.3. Agro-ecology and soil suitability for bread wheat production

Wheat in Ethiopia is produced from 1600-3200 masl. However, Wheat is best grown from 1800-2800 masl with an average temperature of 15-25 ^{0}C (Bekele *et al*., 2000). On average rainfall of 400-1200 mm is required. The distribution of rainfall throughout the crop growth period is very important. Wheat can be produced on different soil types such as black clay soils, red and brown soils. Sandy soils and soils with problems of water logging are unsuitable for wheat production. Suitable soil pH for wheat ranges from 5.5-8.0 (Ministry of Agriculture of Ethiopia, 2002).

2.4. Uses and importance of bread wheat in Ethiopia

Agriculture is the main source of livelihood for about 85% of Ethiopia‘s population, contributes 50% of the GDP and generates more than 80% of the foreign exchange earnings (Deressa and Hassan, 2009).Wheat is among the most important crops in Ethiopia, ranking fourth in total cereals production (16 per cent) next to maize, sorghum and teff (CSA, 2009). Wheat, sorghum, and maize supply over 50% of average daily caloric intake. Cereal production accounts for roughly 60% of rural employment and 80% of total cultivated land. Households spend an average of 40% of their total food budget on cereals (Adugna, 2007).

Although most of the wheat grown in Ethiopia is bread wheat, there is some durum wheat which is often grown mixed with bread wheat. Wheat is used not only for making bread, biscuit and pastry products, but also for the production of starch and gluten. The raised bread is possible because the wheat kernel contains gluten, an elastic form of protein that traps minute bubbles of carbon dioxide when fermentation occurs in leavened dough, causing the dough to rise (Hanson *et al*., 1982).Also wheat and barley are the main staple foods in highland temperate areas, and selling wheat is generally not a primary economic activity for producers (Waddington *et al*, 2009). Wheat accounts for nearly 20% of daily caloric intake in Ethiopia, second to maize (Rashid, 2010). The Ethiopian Commodity Exchange reports that household consumption accounts for about 60% of wheat produced, 20% is sold and the remainder is used for seed, in-kind payments for labor, and animal feed (*http://www.ecx.com.et/commodities.aspx)*

2.5. Effect of carbon dioxide and cytokine on wheat production

2.5.1 Effect of carbon dioxide on wheat production

Carbon dioxide is regarded as the driving factor of climate change, however its direct effect on plant is positive (Warrick, 1988). CO_2 enriches atmosphere positively and affects the plants in two ways. First, it increases the photosynthesis process in plants. This effect is termed as carbon dioxide fertilization effect. This effect is more prominent in C_3 plants because higher level of CO_2 increases rate of fixed carbon and also suppresses photorespiration. Second, increased level of CO_2 in atmosphere decreases the transpiration by partially closing of stomata and hence

declines the water loss by plants. Both aspects enhance the water use efficiency of plants causing increased growth (Ghannoum *et al*., 2000; Sage & Kubien, 2003).

Carbon dioxide is not only a major greenhouse gas, but also is essential for plant growth (Kramer, 1981; Dahlman *et al*., 1985; Warrick, 1988; Kimball, 2011).Carbon dioxide links the atmosphere to the biosphere and is an essential substrate for photosynthesis. Elevated CO_2 stimulates photosynthesis leading to increased carbon (C) uptake and assimilation, thereby increasing plant growth (Kimball, 2011).However, as a result of differences in CO_2 use during photosynthesis, plants with a C_3 photosynthetic pathway often exhibit greater growth response relative to those with a C_4 pathway (Amthor, 1995; Amthor and Loom 1996; Rogers *et al*., 1997).

Plants with a C_4 photosynthetic pathway show a smaller response to elevated CO_2 than plants with a C_3 pathway. Previous studies (e.g. Wand *et al*. (1999)) indicated that rising in the air's CO_2 content will lead to C_3 plants replacing C_4 plants in the vast majority of earth's ecosystems. For a doubling of the air's CO_2 concentration, Prior *et al*. (2003)found an average photosynthetic enhancements of 33 and 25%, respectively, for C_3 and C_4 plants, along with biomass enhancements of 44 and 33%, respectively asCO_2 usually increases the growth of C_3 plants, in which a doubling of the CO_2 level has also been shown to increase vegetative growth of C_3 plants with an average of 41% (data on 156 species, Poorter, 1993) and 47% (data on 250 species, Poorter *et al*., 1996). The growth response varied considerably, both between and within species. For example, in wheat the increase in vegetative growth varied between 7 and 97% (Poorter, 1993).

An elevated CO_2 increase plant growth (both above-and below ground) and improves plant water relations (reduces transpiration and increases WUE) (Gifford, 1988). Carbon dioxide increase grain yield and biomass of wheat by increasing the number of grain sunder stood (Pleijel and Uddling, 2012). Rising carbon dioxide enhances vegetable and grain crops grow more quickly and become more drought-resistant and produce potentially higher yields (*http://southwestfarmpress.com/news/climate-weeds-0409/).*

Some modelling results indicated that climate change without carbon dioxide (CO_2) fertilization could reduce the rice and wheat yields by up to 37% in the next 20–80 years(Ainsworth *et al*.,

2008). The net effect on yields for C_3 crops has been measured as an average increase of 14% for 580 mmole for C_4 species such as maize and sorghum, very few experiments have been conducted but the observed effect is much smaller and often statistically insignificant (Leakey, 2009).Some other studies suggested that CO_2 enrichment to 550 mmole under field conditions consistently increases biomass and yields in the range of 5–15% (Lin Erda, *et al.*, 2005).However, the long-term response to CO_2 remains uncertain and will depend on environmental constraints.

2.5.2 Effect of cytokine on plant growth

The Cytokine hormone is one of the factors that determine the cell division, growth and development of wheat. Seed growth as a major economical factor is composed of several growth stages including cell differentiation and division and photosynthates storage (Koch, 2004). Transition from one growth stage in the seeds after anthesis doesn't occur directly but occurs gradually. For example in wheat and barley the mythosis activity of endosperm stops gradually due to cell expansion and photosynthates storage (Olsen *et al*., 1992).

2.5.3 Importance of cytokine for bread wheat (crops) production.

Cytokines have been shown to slow aging of plant organs by preventing protein breakdown, activating protein synthesis, and assembling nutrients from nearby tissues (Campbell *et al*., 2008). Cytokines are a group of compounds that stimulate water uptake, increase cell division, promote organ development, and lead to regeneration and proliferation of shoots (Leham *et al*., 1983). Previous studies e.g. Xie *et al*. (2004), indicated that greatest biological yield was obtained from foliar application of cytokine hormones at pollination and grain filling compared to the check. For example, foliar applications at pollination and both at pollination & grain filling produced grain yield by 5.6 and 6.1 t/ha, respectively, which showed an increase in 50 and 60.2% in grain yield compared to the check, respectively. The probable reason would be that cytokine increase cell divisions causing the number of endospermic cells to increase. These had positive correlations with the increase in grain yield and harvest index (Morris *et al*., 2005).Despite their established role in plant/crop development, information as to how endogenous cytokines are reduced under stressful conditions is meagre.

3. Materials and Methods

3.1 Description of the area

These experiments were conducted both in Green house (pot experiment) and in a field, at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) and Mana distinct Specifically Somodo water shade, respectively. The distinct is located 352 km South West of Addis Ababa, Ethiopia. At 7º40' N, $37^0$26' E and altitude of 2000 masl. The mean annual rainfall is 1639 mm. The average annual maximum and minimum temperature of the area are 26.6^0C and 13.9^0C, respectively (JARC, 2013/14 Unpublished report). The farmers grow bread wheat on brown soil found in the area. Though the area has both Dega and woinadega agro-ecologies this study was carried out on the farmer's field in the woinadega parts of the area.

3.3.1. Experimental treatments and design

In both greenhouse and field, the experiments were arranged in RCBD with ten treatments

T_0: No spray (Check),

T_1:CO_2 foliar spray at tillering,

T_2:CO_2foliar spray at flowering,

T_3:CO_2foliar spray at grain filling,

T_4: Both $CO_2$and Cytokine foliar Sprays at tillering

T_5: Both CO_2 and Cytokine foliar spray at flowering,

T_6: Both CO_2 and Cytokine foliar spray at grain filling,

T_7: Cytokine foliar spray at tillering,

T_8: Cytokine foliar spray at flowering

T_9: Cytokine foliar sprays at grain filling each with three replications .The experiment was conducted in controlled environment (pot experiment). The pots filled with soil that mixed with different type of soil, sand soil, clay soil, loam soil, forest soil and compost. The area of the pots is 0.0157m^2 that has 20cm and fourteen seeds are planted per pot. After the germination of the seeds, seven vigorous seedlings per pot are selected through thinning that was irrigated with the interval of one day.

The field experiment was well tilled and planted in row. The length and width of each experimental plot was 2 m x 2 m ($4m^2$). Each of the experimental plots had 10 rows, and the spacing between rows was 0.2m. The spacing between plots and replications were 0.4 m and 1.5 m, respectively. Both of the experiments were applied with the conc. of 680 mmole $litre^{-1}$ (0.68 millilitre per litre of water) of CO_2 (Warrick, 1988; Ainsworth and Long, 2005) and 100 mmole $litre^{-1}$ (100mg per litre of water) (Shahin Babakhaani *et al.*, 2013) of cytokine through foliarly in each pot and plot at different growth stages until very wet. Carbon dioxide applied three times in a week for two consecutive weeks. The nitrogen (protein) contents of wheat grains, straw nitrogen and available nitrogen in the soil (before sowing wheat crops) were analysed in Animal nutrition laboratory of Jimma University College of Agriculture and Veterinary Medicine. All treatments received NP fertilizer at 1:1 ratio (DAP, 100kg/ha and Urea, 100kg/ha). DAP was applied at sowing time while urea was applied in two splits at sowing and at tillering. The field experimental seeds were planted on July 29, 2014 and that of green house was in January 12, 2015.

3.3.2. Data collection

Composite soil sample were taken from the upper 30cm soil depth at different six randomly spotted plots and were mixed properly to characterize the N content (%) of the trial site before nitrogen application or planting. In addition, to soil nitrogen content data on grain yield, agronomic performance, disease reaction, grain quality, and nitrogen use efficiency were collected from each plot as follow:

1. Days to heading (day): this parameter of the plant was determined by counting the number of days from sowing to the time when 50% of the plants had produced spikes
2. Days to maturity (day): days to maturity were determined by counting the number of days from emergence to the period when 75% of the plants had reached the physiological maturity based on visual observations
3. Number of Tiller/plant (No): tillers were counted on five randomly selected plants from central rows of each plot.
4. Plant Height (cm): the height of the plant from ground level to the top of the spike were recorded from five randomly selected plants from the middle four rows of each treatment

5. Spike Length (cm): the main spikes from the five sample plants were measured in cm and averaged to represent the mean spike length in cm.
6. Number of spikelets per spike (NSKPS): The number of spikelets in main tillers of each of the five randomly taken plants was registered for each treatment.
7. Number of Seed per Spike (NSPS): number of seeds per spike from the five randomly selected plants from the central rows of each plot was counted.
8. Thousand Seeds Weight (TSW): Grain weight of thousand seeds sample at random from total grain harvest of the experimental plot was weighted on analytical balance expressed in gram.
9. Biomass yield (TDW) (ton/ha): the total above ground biomass produce was recorded for each plot.
10. Grain yield (GDW) (ton/ha): Grain yield in g/plot at 12.5 % moisture content was taken from the central eight rows.
11. Harvest Index (%): the proportion of grain yield to biomass was determined. HI = GDW/TDW
12. Grain Protein content (GP) (%): The grain protein content of the grain wheat was taken randomly from ten sample to determine (% protein = % N X5.75) (Bremner and Mulvaney, 1982).
13. Total nitrogen content of the straw was the amount of nitrogen in the straw. The straw nitrogen was determined by Kjeldahl method of nitrogen analysis. This method is used as standard and universal method for analysis consists of digestion, distillation, and titration processes (Bremner and Mulvaney, 1982).
14. Total plant Nitrogen (TN) was amount of N in the plant (grain N + Vegetative N)
15. Total N Supplied (NS) was the amount N applied and available N in the soil sample.
16. Biomass nitrogen is the amount of N in the above ground part of the plant.
17. Cytokine Use Efficiency for Protein (CUEP): the ability to produce grain protein at the expense of applied Cytokine was determined. CUEP = TGN/TN = (TGN/TDW) (TDW/TN) (TN/NS).TGN = Total grain nitrogen, TN = Total nitrogen, TDW = Total dry weight.

18. Nitrogen uptake efficiency (NUPE): the ability of the plant by using carbon dioxide and cytokine to utilize the available N in the soil was determined. NUPE = TN/NS, TN = Total nitrogen, NS = Nitrogen supplied.
19. Biomass Production Efficiency (BPE): the ability of the plant to produce biomass at the expense of applies CO_2 and Cytokine was determined. BPE = TDW/NS, TDW = Total dry weight, NS = Nitrogen supplied.
20. CO_2 and Cytokine utilization efficiency is defined as a crop's ability to convert the absorbed nutrient into grain yield. Utilization efficiency = TGrN/TN, TGrN = Total grain nitrogen, TN = Total nitrogen used plant.
21. CO2 and Cytokine-use efficiency (GW) = uptake efficiency x Utilization efficiency (Weih *et al*., 2011). GW=TN/NS * GrN/TN, GW = GrN/NS, GrN = Grain nitrogen, NS = Nirtogen supplied

3.4. Data Analysis

The collected data were subjected to analysis of variance (ANOVA) using GLM function of SAS 2008 version9.2 software. For statistically significant means, mean separations were done using Duncan multiple range test was used to identify significantly different treatments. Correlations between the different variables were determined by calculating simple correlation coefficient between each of the variable using Pearson correlation coefficient.

4. RESULT AND DISCUSSION

4.1 Effect of Carbon dioxide and Cytokine hormone foliar spray on Phenological and Growth of Bread wheat

4.1.1 Number of tillers

Statistical analysis of the data for both experiments in controlled environment and under field condition revealed that effect of carbon dioxide and cytokine on the number of tillers had showed highly significant differences ($P < 0.01$, Appendix Table 1 &2).The result of the control environment experiment showed that, the treatments that received carbon dioxide and both carbon dioxide and cytokine at tillering stages produced the highest tiller numbers (6 and 7) respectively (Table 2). The lowest number of tillers (3) was recorded in treatments that received cytokine at both flowering and seed filling stages and check. For field grown wheat crops, the treatments that received carbon dioxide and both carbon dioxide and cytokine at tillering produced the highest tiller numbers(5.5 and 5.6)(Table 3),while the lowest number of tillers (three in both cases) were recorded in treatments that received cytokine at flowering and seed filling stages and check. Therefore the result indicated that the application of carbon dioxide and cytokine and carbon dioxide & cytokine at tillering stages increases the number of tillering, prolong the stage of vegetative period, and promote vegetative growth.

These studies confirmed with previous reports (Baker and Allen, 1993), who indicated that Carbon dioxide enrichment stimulated greater amount of branching or tillers by increasing photosynthesis, reducing photorespiration and transpiration. The increased in tiller number with CO_2 fertilization also increases tiller production and survival (Combe, 1981; Amthor, 2001).

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4.1.2. Days to heading.

In both experiments the analysed result showed, carbon dioxide and cytokine application at tillering significantly affected days to heading ($P < 0.01\%$, Appendix Table 1 & 2). Among the treatments applied in the greenhouse, those received carbon dioxide and both carbon dioxide and cytokine at tillering took long days to flowering (67.5 days and 68.37days) respectively. While the application of the carbon dioxide, cytokine and both carbon dioxide and cytokine at flowering or seed filling had no effect on the number days to flowering, they were similar to check (63 days) (table 2).

Similarly, for field grown wheat plants the experiment that received carbon dioxide and both Carbon dioxide and cytokine at tillering stages took long days to flowering (74 days and 76 days) respectively, when we compared to treatments that received carbon dioxide and cytokine at flowering and seed filling stages and check one (70 days) (table 3).This is probably because of both carbon dioxide and cytokine help the plants to stay longer in the vegetative stage by stimulating cell division, cell elongation, preventing protein break down, activating protein synthesis, increasing photosynthesis, and nutrient absorbed, reducing photorespiration and transpiration.

These result agreed with that of Chaudhuri *et al*., (1990) who reported that increasing levels of carbon dioxide delay advancement to the next growth stage. And also cytokines have been reported that, slow aging of plant organs by preventing protein breakdown, activating protein synthesis, and assembling nutrients from nearby tissues that helped crops in staying in the same stage for long period of time (Campbell *et al*., 2008).

4.1.3 Days to maturity

The data analysed indicated that both control environment and field experiments revealed significant effect of Carbon dioxide and Cytokine application (P <0.01) on days to maturity of wheat crop (Appendix Table 1& 2). The maximum days to reach physiological maturity (122 days) (table 2) were recorded from the application of both carbon dioxide and cytokine at tillering stage, While the minimum (114 days) was recorded from the check.Similarly the treatment that conducted under field was also indicated that the maximum days to reach physiological maturity (135 days) (table 3) was recorded from the interaction of both carbon dioxide and cytokine at tillering stage , While the minimum (122 days) was recorded from the check one.

Therefore these result showed that the application of both CO2 and cytokine help the crops to increases days of maturity because it delays both stages by slow aging of crop organs, activating protein synthesis and helped crops in staying in the same stage for long period of time. These results agreed with the (Chaudhuri *et al*., 1990) who reported that increasing levels of carbon dioxide delay advancement to the next growth stage. And also cytokines have been reported that, slow aging of plant organs by preventing protein breakdown, activating protein synthesis, and assembling nutrients from nearby tissues that helped crops in staying in the same stage for long period of time (Campbell *et al*., 2008).

Table 2: Mean values for different phenology and vegetative traits in bread wheat (Digalu variety) tested under green house.

TRT	Mean				
	NT (No)	DH (day)	DM (day)	PH (cm)	SPN (No)
Control	3.12^{f}	63.00^{d}	114.67^{d}	90.00^{c}	20.00^{d}
$(CO_2)T$	6.23^{b}	67.50^{b}	118.88^{bc}	92.00^{a}	21.63^{c}
$(CO_2)F$	4.08^{e}	63.00^{d}	118.04^{c}	90.67^{bc}	23.83^{b}
$(CO_2)SF$	4.01^{e}	63.00^{d}	118.00^{c}	90.67^{bc}	21.05^{d}
(CK)T	4.42^{d}	65.13^{c}	118.03^{c}	91.00^{b}	20.27^{f}
(CK)F	3.12^{f}	63.00^{d}	117.27^{c}	90.67^{bc}	21.07^{d}
(CK)SF	3.07^{f}	63.00^{d}	117.23^{c}	90.00^{c}	20.63^{e}
$(CO_2$ X CK)T	7.12^{a}	68.37^{a}	122.36^{a}	92.40^{a}	21.43^{cd}
$(CO_2$ X CK)F	4.77^{c}	63.00^{d}	120.00^{b}	90.87^{b}	24.14^{a}
$(CO_2$ X CK)SF	4.07^{e}	63.00^{d}	120.00^{b}	90.73^{b}	21.22^{de}
Standard Error	0.73	1.41	0.68	0.47	0.74
CV (%)	3.28	0.38	0.85	0.42	0.69

CV = Coefficient of Variation; Values following by the same letter within the Column or row are not significantly different at 0.05 probability level, TRT=Treatment' NT= number of tiller, DH= Days of Heading, DM= Days of Maturity, PH= Plant height, SPN= Number of spikelet, T= Tillering, F= Flowering, SF= Seed Filling, CK= Cytokine, CO_2= Carbon dioxide.

Table 3: Mean values for different phenology and vegetative traits in bread wheat (Digalu variety) tested at field, 2015.

TRT	Mean				
	NT(No)	DH (day)	DM (day)	PH(cm)	SPN (No)
Control	3. 00^{d}	70.00^{d}	122.16^{d}	91.12^{d}	19.65^{f}
(CO_2)T	5.50^{a}	74.17^{b}	131.67^{ab}	95.57^{a}	20.03^{ef}
(CO_2)F	4.10^{bc}	70.24^{d}	130.55^{b}	94.46^{b}	22.30^{b}
(CO_2)SF	3.80^{c}	70.00^{d}	130.52^{b}	94.55^{b}	20.69^{def}
(CK)T	4.17^{bc}	73.43^{c}	129.89^{b}	92.80^{c}	19.66^{f}
(CK)F	3.17^{d}	70.25^{d}	128.48^{c}	94.05^{b}	21.28^{bd}
(CK)SF	3.05^{d}	70.05^{d}	128.47^{c}	93.73^{c}	20.36^{def}
(CO_2 X CK)T	5.83^{a}	75.66^{a}	134.58^{a}	96.32^{a}	20.87^{de}
(CO_2 X CK)F	4.48^{b}	70.40^{d}	131.51^{ab}	94.73^{b}	23.90^{a}
(CO_2 X CK)SF	3.90^{c}	70.02^{d}	131.57^{ab}	94.58^{b}	21.70^{b}
Standard Error	0.5	1.5	1.8	0.5	0.8
CV (%)	6.11	0.47	0.56	0.47	2.96

CV = Coefficient of Variation; different at 0.05 probability level, TRT=Treatment' NT= number of tiller, DH= Days of Heading, DM= Days of Maturity, PH= Plant height, SPN= Number of spikelet, T= Tillering, F= Flowering, SF= Seed Filling, CK= Cytokine, CO_2= Carbon dioxide

4.2 Effect of Carbon dioxide and Cytokine hormone foliar spray on yield and yield components traits of Bread wheat

4.2.1 Thousand grain weight

The result of analysis both the experiments in control environment and under field condition revealed effect of carbon dioxide and cytokine application on thousand seed weight had showed highly significant differences ($P < 0.01$) (Appendix Table 3 & 4).The result of the experiment in control environment showed that, the treatments that received both carbon dioxide and cytokine and also cytokine at flowering seed filling produced the highest thousand grain weight, 50.07g, 51g and 52g, respectively (Table 4). While the lowest grain weight 43g were recorded in check and treatment that received carbon dioxide at tillering stage.

In field conditions, treatments that received both carbon dioxide and cytokine and cytokine at flowering and seed filling produced the highest thousand grain weight of 53.35g, 53.53g and 53.42g, respectively (Table 5). The highest in grain thousand weights might be due to the application of cytokine and carbon dioxide at seed filling stage that help to activate the cell division of seed that finally caused the increment of seed size. While the lowest grain thousand weight 45.80g, 45.86g and 45.88g were recorded in check and treatments that received carbon dioxide at flowering stage and carbon dioxide at tillering stage respectively. These results were in line with Amthor (2001) report who reported that Carbon dioxide enrichment caused an increase in seed number per plant when applied during the flowering period and in seed size when applied during seed filling. Xie *et al*. (2004) also reported that cytokine increases 1000-kernel weight through retarding ageing and increasing the seed's active growth period.

4.2.2 Grain yield (ton ha^{-1})

Statistical analysis of the data in both experiments in control environment and under field condition revealed significant effect of Carbon dioxide and Cytokine foliar spray application ($p < 0.01$) (Appendix Table 3 & 4).In greenhouse, the highest grain yield 13.44 ton ha^{-1} was produced by the application of both carbon dioxide and cytokine at flowering stage (Table 4). Furthermore, the treatments that received carbon dioxide at flowering and both carbon dioxide and Cytokine at seed filling stage, produced the next higher yields (11.96 ton ha^{-1} & 11.73 ton ha^{-1}) respectively. On the other hand, the lowest mean yield of 6.6 ton ha^{-1} and 7.2 ton ha^{-1} were recorded in check and the treatments that received cytokine at tillering stage, respectively.

Similarly, the result of the field experiment showed that, wheat crops that received carbon dioxide and both the carbon dioxide and cytokine at flowering stage produced the highest grain yield (11.59 ton ha^{-1}&11.89 ton ha^{-1}). On the other hand, the lowest mean yield of 6.2 ton ha^{-1} and 6.8 ton ha^{-1} were recorded in check and the treatments that received cytokine at tillering stage (Table 5). The reason of increased grain yield may be due to enhanced nutrients and water absorption, increment of photosynthesis, high assimilation allocated to grain seed in the needed time(at flowering and seed filling period), photo respiration and transpiration reduced, increase cell division, promote grain seed development by the application of carbon dioxide and cytokine.

The result agreed with the Amthor (2001) report, the grain yield increased when carbon dioxide enrichment was added during flowering and seed filling periods. No effect of carbon dioxide enrichment on grain yield was observed when the additional carbon dioxide was given during the vegetative period. Carbon dioxide enrichment caused an increase in seed number per plant when applied during the flowering period and in seed size when applied during seed filling. Similarly besides increasing the number of grain seeds per plant Morris *et al*. (2005) reported that an increasing cytokine concentration in seeds during cell division caused the number of endospermic cells to increase which had positive correlation with the increase in grain yield.

4.2.3 Biomass yield (ton ha^{-1})

Analysed data indicated that both the experiments in control environment and under field condition showed high significant effect of Carbon dioxide and Cytokine applied at different stages ($p < 0.01$) on biological yield (Appendix Table 3 & 4).The result of the experiment that conducted in controlled environment showed that, the maximum biomass yield 15.50 ton ha^{-1}and15.30 ton ha^{-1}were produced by the treatments that received carbon dioxide and both with carbon dioxide and cytokine at tillering stage (Table 4). On the other hand, the minimum biomass yield was obtained from the treatment that received cytokine at tillering stage (10.83tonha^{-1}).

Similarly, the result of the treatment that conducted under field condition showed that, maximum biomass yield 14.8 ton ha^{-1} was obtained from the treatment combination of both carbon dioxide and cytokine applied at flowering stage (Table 5). On the other hand, the minimum biomass yields were obtained from a check and a treatment that received cytokine at tillering stage (11.30 ton ha^{-1} and 11.10 ton ha^{-1}) respectively.

The result showed that biological yield was increased due to high number of tillers, number of grain per spike and plant height in the treatment of high plant population and low yield and plant height than in the treatment of lower plant population (Table 4 & 5).

These results were in agreement with (Li *et al*., 2000; Amthor, 2001; Obrist and Arnone, 2003) who reported that increasing carbon dioxide concentration in wheat plants should display increasingly greater rates of photosynthesis and biomass production, which should lead to ever greater grain yields in this important cereal crop, even under conditions of low soil moisture or

poor soil fertility. Because elevated CO_2 stimulated rates of photosynthesis in wheat biomass and grain yield.

4.2.4. Harvest index

Harvest index is the partitioning of dry matter by plant among biomass and economic yield. Statistical analysis of the data for both experiments, in control environment and under field condition revealed significant effect of Carbon dioxide and Cytokine applied at different stage ($p < 0.01$) on harvest index (Appendix Table 3 & 4). Highest harvest index was observed in the treatment that received carbon dioxide and both carbon dioxide & cytokine at flowering stage (87.28%) and (91.86%), respectively. While lowest harvest index was observed from the check (57.31%) (Table 4).

Similarly, the result of the treatment that conducted under field condition showed that the highest harvest index was observed in a plots that received carbon dioxide and combination of carbon dioxide and cytokine at flowering stages (78.4%) and (80.13%) respectively, while the lowest harvest index was observed from the check (55%) (Table 5).

These result showed that harvest indexes were low when applied carbon dioxide, cytokine and both carbon dioxide and cytokine at tillering stage that increase number of tillers, all photo assimilates are allocated to vegetative that stimulate vegetative growth. These increasing plant density caused increases competition for resource, finally produced low grain yield and maximum biomass yield. While the maximum harvest indexes obtained from carbon dioxide and both interaction of carbon dioxide and cytokine applied at flowering (pollination) and or seed filling period that helps to increase, nutrient absorbed, photosynthesis, photo assimilated allocation to sink organ, stimulate cell division and elongation of seeds, reduce photorespiration and transpiration, moisture competition minimum, that result number of tiller inter mediate, long spike length, large grain seed size, large number of grain seeds per plant, high grain yield per unit areas and finally maximum harvest index.

The obtained results were in agreement with the findings of (Amthor, 2001) the foliar application of cytokine increased the source ability through the size of the source by increasing the rate of cell division in growing seeds. As a result, a greater portion of the photosynthates was allocated to the seeds and the harvest index increased (Morris *et al*., 2005).

Table 4: Mean values for different grain yield & other yield components in bread wheat (Digalu variety) tested in control environment.

TRT	Mean					
	SPL(cm)	NS(no)	TWS(g)	BMY(tonha^{-1})	GY(tonha^{-1})	HI (%)
Control	9.00^{i}	80.00^{g}	43.05^{f}	11.03^{e}	6.61^{g}	57.31^{f}
(CO_2)T	9.65^{e}	86.05^{b}	43.06^{f}	15.50^{a}	8.98^{e}	66.62^{d}
(CO_2)F	12.20^{b}	88.10^{a}	45.40^{e}	13.37^{c}	11.96^{b}	87.28^{a}
(CO_2)SF	9.73^{e}	86.00^{c}	45.31^{e}	14.01^{bc}	10.25^{c}	73.28^{bc}
(CK)T	9.00^{g}	82.03^{f}	49.70^{b}	10.83^{e}	7.20^{g}	57.96^{e}
(CK)F	9.57^{e}	84.00^{d}	52.00^{a}	12.10^{d}	9.10^{e}	75.04^{bc}
(CK)SF	9.22^{f}	83.05^{e}	51.03^{a}	12.10^{d}	8.41^{f}	69.60^{cd}
(CO_2 X CK)T	10.58^{d}	86.08^{c}	47.01^{d}	15.69^{a}	10.00^{d}	65.49^{d}
(CO_2 X CK)F	12.58^{a}	88.27^{a}	50.07^{b}	14.65^{ab}	13.44^{a}	91.86^{a}
(CO_2 X CK)SF	10.91^{c}	86.16^{b}	48.37^{c}	14.65^{ab}	11.73^{b}	78.80^{b}
Standard Error	0.62	1.30	0.79	469.45	793.77	6.77
CV (%)	2.68	0.46	0.54	4.50	1.02	4.92

CV = Coefficient of Variation, TRT=Treatment, SPL = Spike length, NS = Number of seed per plant, TWS = Thousand seed weight, BMY= Biomass yield, GY = Grain yield, HI = Harvest Index, F= Flowering, SF= Seed Filling, CK= Cytokine, CO_2= Carbon dioxide.

Table 5: Mean values for different grain yield & other yield components of wheat (Digalu variety) under field condition.

TRT	Mean					
	SPL	NS per pt	TSW	BMY	GY	HI
Control	8.35^{c}	78^{f}	45.80^{e}	11.30^{h}	6.24^{i}	55.23^{j}
(CO_2)T	8.58^{d}	87.25^{b}	45.86^{e}	12.00^{g}	7.83^{f}	65.27^{h}
(CO_2)F	10.22^{b}	88.22^{a}	45.88^{e}	14.68^{b}	11.65^{b}	78.40^{b}
(CO_2)SF	9.62^{bc}	86.28^{cd}	46.56^{e}	13.88^{d}	10.08^{d}	73.10^{d}
(CK)T	8.54^{d}	81.20^{e}	48.85^{c}	11.41^{h}	6.80^{h}	61.14^{i}
(CK)F	9.62^{bc}	87.25^{b}	53.93^{a}	12.32^{f}	8.88^{e}	72.14^{e}
(CK)SF	9.08^{cd}	85.92^{d}	53.84^{a}	12.30^{f}	8.35^{g}	68.17^{f}
(CO_2 X CK)T	8.88^{cd}	86.42^{cd}	48.01^{d}	12.60^{e}	8.53^{f}	67.48^{g}
(CO_2 X CK)F	11.05^{a}	88.27^{a}	53.35^{ab}	14.81^{a}	11.89^{a}	80.13^{a}
(CO_2 X CK)SF	9.67^{bc}	86.50^{c}	51.57^{b}	14.31^{c}	10.95^{c}	75.57^{c}
Standard Error	0.5	0.7	1.35	642.6	934.6	3.7
CV (%)	4.6	0.36	0.89	2.46	1.3	0.5

CV = Coefficient of Variation; TRT = Treatment, SPL = Spike length, NS = Number of seed per plant, TWS = Thousand seed weight, BMY= Biomass yield, GY = Grain yield, HI = Harvest Index, F= Flowering, SF= Seed Filling, CK= Cytokine, CO_2= Carbon dioxide.

4.3. Effect of Carbon dioxide and cytokine on grain seed quality of wheat.

4.3.1 Grain protein content

Statistical analysis of the data for both experiments in controlled environment and under field condition revealed significant effect of carbon dioxide and cytokine applied at different stages ($P < 0.01$) on the grain wheat protein content (Appendix Table 5& 6).The result of the experiment in control environment showed that, the treatments that received cytokine at flowering and seed filling stages contain the maximum grain proteins (16.75% and 14.87%) respectively (Table 6). The minimum grain protein content was recorded in treatment that received carbon dioxide at tillering stage (9.25%).

The experiment in the field plots showed that treatments received cytokine at flowering and seed filling contain the maximum grain proteins (16.76% and 14.88%) respectively (Table 7). And also the minimum grain protein content was recorded in treatment that applied carbon dioxide at tillering stage (9.26%).

These findings were in agreement with (Campbell *et al*., 2008) who reportedly cytokines have been shown to slow aging of plant organs by preventing protein breakdown, activating protein synthesis, and assembling nutrients from nearby tissues. And also agreed with (Gornall *et al*., 2010; Sinclair *et al*., 2000) who reported an elevated CO_2 is detrimental to wheat flour quality through reductions in protein content.

4.3.2 Straw Nitrogen content

The analysed data for both experiments in controlled environment and under field condition revealed significant effect of carbon dioxide and cytokine applied at different stages ($P < 0.01$) on straw wheat nitrogen content (Appendix Table 5& 6).The result of the treatments that conducted in controlled environment showed that, the maximum straw content of nitrogen (0.31g/pot) in both cases were recorded in treatments that received carbon dioxide and both carbon dioxide and cytokine at tillering stages (Table 6). On the other hand the minimum straw content of nitrogen was recorded in treatment that received carbon dioxide at flowering stage (0.13g/pot).

Similarly in field condition, the treatments that received both carbon dioxide and cytokine at tillering stage contained the maximum straw nitrogen contents (70.56g/plot) (Table 7), while the

minimum straw nitrogen content was recorded in treatment that received carbon dioxide at flowering stage (32.79g/plot).

These results indicated that application of carbon dioxide, cytokine and combination of both carbon dioxide and cytokine at tillering stage, it initiate crops to bear great tiller and increases nitrogen content of straws of wheat by increasing nitrogen absorbed, photosynthesis and water use efficiency, reduce photorespiration and transpiration that promote vegetative growth, increase, the number of tillers, stem diameter, plant height and leaf area.

4.3.3 Total Nitrogen in the Plants.

The analysed data for both the experiments in control environment and under field condition showed significant effect of Carbon dioxide and Cytokine applied at different stages ($p < 0.01$) on total nitrogen in a plant (Appendix Table 5 & 6).The result of the experiment in controlled environment showed that, the maximum total nitrogen in a crops (0.57 g/pot) was recorded in treatments that received both carbon dioxide and cytokine at tillering stage (table 6), While the minimum total nitrogen in plants (0.46g/pot) was recorded in treatment that received carbon dioxide at flowering stage.

Similarly the experiment under field condition, showed that the treatments that received cytokine at flowering stage and both carbon dioxide and cytokine at tillering stages gave the maximum total nitrogen in crops (159.64 g/plot and 155.64 g/plot) respectively (Table 7). And also the minimum straw nitrogen content was recorded in treatment that received carbon dioxide at flowering stage (105.23g/plot).

The total nitrogen in the plants is total nitrogen that in all plants body parts that found above ground. The result showed that the most total nitrogen in the plant stored were in the straw than grain seed, except the grain seed that treated with the cytokine at flowering and seed filling stages. The application of carbon dioxide, cytokine and both combination of carbon dioxide & cytokine at tillering stages are increases the absorption nitrogen, nutrients, water, photosynthesis, number of tillering, promote vegetative growth than grain yield.

These result agreed with (Baker and Allen, 1993; Campbell, 2011) who reported that Carbon dioxide enrichment stimulated greater amount of branching or tillering by increasing photosynthesis, reducing photorespiration and transpiration. However the application of Carbon dioxide fertilizer during tillering period indicated that early growth and tillering were relatively unimportant. And also agree with (Campbell *et al*., 2008) who reported that the Cytokines have been shown to slow aging of plant organs by preventing protein breakdown, activating protein synthesis, and assembling nutrients from nearby tissues.

Table 6: Mean values of grain seed Nitrogen (protein) & nitrogen contents of others yield components in wheat treated at different stages in control environment.

TRT	MEAN				
	GrP (%)	TNStr (g/g)	TNP (g /g)	NS (g/g/)	BN(g /g)
Check	12.00^{d}	0.24^{e}	0.47^{e}	120.3^{a}	0.47^{e}
(CO_2)T	9.25^{j}	0.31^{a}	0.51^{b}	120.3^{a}	0.51^{b}
(CO_2)F	9.37^{i}	0.13^{h}	0.46^{f}	120.3^{a}	0.46^{f}
(CO_2)SF	9.75^{h}	0.22^{e}	0.47^{e}	120.3^{a}	0.47^{e}
(CK)T	13.37^{c}	0.277^{b}	0.49^{c}	120.3^{a}	0.49^{c}
(CK)F	16.75^{a}	0.26^{d}	0.51^{b}	120.3^{a}	0.51^{b}
(CK)SF	14.87^{b}	0.275^{c}	0.49^{c}	120.3^{a}	0.49^{c}
(CO_2 X CK)T	10.25^{g}	0.31^{a}	0.57^{a}	120.3^{a}	0.57^{a}
(CO_2 X CK)F	10.31^{f}	0.18^{g}	0.48^{d}	120.3^{a}	0.48^{d}
(CO_2 X CK)SF	10.5^{e}	0.184^{f}	0.49^{c}	120.3^{a}	0.49^{c}
Standard Error	0.74	24.17	13.12	0.00	13.12
CV (%)	0.06	0.005	0.0023	0.0006	0.0023

CV = Coefficient of Variation, TRT = Treatment, GrP = Grain protein, TNStr = Total nitrogen in straw, TNP = Total nitrogen in plant, NS = Nitrogen supply, BN = Biomass yield nitrogen.

Table 7: Mean values of grain seed nitrogen (protein) & nitrogen contents of others yield components in wheat treated at different stages in field.

	Mean Square				
TRT	GrP (%)	TNStr (g/g)	TNP (g/g)	NS (g/g)	BN (g/g)
Check	12.00^{d}	0.49^{e}	120.11^{g}	28840.1^{a}	120.11^{g}
(CO_2)T	9.26^{j}	56.01^{b}	143.00^{c}	28840.1^{a}	143.00^{c}
(CO_2)F	9.38^{i}	32.79^{j}	105.23^{i}	28840.1^{a}	105.23^{h}
(CO_2)SF	9.76^{h}	48.10^{f}	111.13^{h}	28840.1^{a}	111.13^{g}
(CK)T	13.38^{c}	52.33^{c}	137.34^{d}	28840.1^{a}	137.34^{d}
(CK)F	16.76^{a}	43.10^{h}	159.64^{a}	28840.1^{a}	159.64^{a}
(CK)SF	14.88^{b}	49.53^{d}	142.94^{c}	28840.1^{a}	142.94^{c}
$(CO_2$ X CK)T	10.26^{g}	70.56^{a}	155.64^{b}	28840.1^{a}	155.64^{b}
$(CO_2$ X CK)F	10.32^{f}	40.79^{i}	121.97^{f}	28840.1^{a}	121.97^{f}
$(CO_2$ X CK)SF	10.51^{e}	46.94^{g}	122.68^{e}	28840.1^{a}	122.68^{e}
Standard Error	0.57	16.87	12.63	0.00	0.02
CV (%)	0.12	1.03	0.21	0.0004	0.21

CV = Coefficient of Variation; TRT = Treatment, GrP = Grain protein, TNStr = Total nitrogen in straw, TNP = Total nitrogen in plant, NS = Nitrogen supply, BN = Biomass yield nitrogen.

4.3.5. Nitrogen uptake efficiency (NUPE).

Statistical analysis of the data for both experiments, in control environment and under field condition revealed a highly significant effect of carbon dioxide and cytokine applied at different stages ($p < 0.01$) on the nitrogen uptake efficiency in wheat crops (Appendix Table 7 & 8). The result of the experiment in controlled environment showed that, the maximum nitrogen uptake efficiency (0.49% and 0.52%) had used by the treatments that treated with cytokine at flowering and both carbon dioxide and cytokine at tillering stages (Table 8) respectively. On the other hand, the minimum nitrogen grain use efficiencies were obtained from the treatment that received carbon dioxide at flowering and seed filling stages (0.44% & 0.44%) respectively.

Similarly the result of the treatment that conducted under field showed that, the maximum nitrogen use efficiencies were 0.54% and 0.53% used by the treatments applied cytokine and

both combination of carbon dioxide & cytokine at flowering stages (Table 9) respectively. On the other hand, the minimum nitrogen uptake efficiency was obtained from the treatment that applied carbon dioxide at flowering stage (0.42%).

As the result indicated nitrogen up take efficiency high at early stage of growth (tillering stage), that enhance the wheat to increase the number of tillers. These mean that the amount of nitrogen in the straw is greater than the amount nitrogen in the grain wheat except the treatments that received cytokine at flowering and seed filling. Therefore the application of carbon dioxide, cytokine, and both combination of carbon dioxide and cytokine at early stage helps the plant to maximum nitrogen uptake efficiency. However the importance of both carbon dioxide and cytokine application at this stage is less for wheat crop productivity. It may be important for vegetable crops, whose vegetative parts are consumed. Then to increase photosynthetic efficiency, enhancing sink capacity, and improving N uptake of wheat would be applied both carbon dioxide and cytokine at flowering stage is potentially help to increase grain yield. Therefore nitrogen uptake efficiency is increased by the application of cytokine hormone at flowering for wheat crops.

These finding agree with (Baker and Allen, 1993; Campbell, 2011) who reportedly that Carbon dioxide enrichment stimulated greater amount of branching or tillering by increasing photosynthesis, reducing photorespiration and transpiration. However the application of Carbon dioxide fertilizer during tillering period indicated that early growth and tillering were relatively unimportant.

4.3.6. Straw Nitrogen use efficiency

The analysed data for both experiments in controlled environment and under field condition had showed significant effect of carbon dioxide and cytokine applied at different stages ($P < 0.01$) on straw nitrogen use efficiency (Appendix Table).The result of the experiment in control environment showed that, the treatments that received cytokine and both carbon dioxide and cytokine at tillering stages were contains the maximum straw nitrogen use efficiency (0.24% and 0.27%)(Table 8) respectively. The minimum straw nitrogen use efficiencies were recorded in treatments that received carbon dioxide and both carbon dioxide & cytokine at flowering stages (0.16% and 0.165%) respectively.

Similarly the experiment under field condition showed that, the treatments that received carbon dioxide and both carbon dioxide & cytokine at tillering stages were contained the maximum straw nitrogen use efficiency (0.255%)(Table 9). And also the minimum straw nitrogen contents were recorded in treatment that applied carbon dioxide at flowering stage (0.145%).Similarly as above discussed the nitrogen use efficiency of straws are high at the tillering stages, when carbon dioxide, cytokine and both the interaction of carbon dioxide and cytokine applied at the tillering stage of wheat growth that promote vegetative growth, increases the number of tillering per plant and prolong the stage of vegetative that help the plant to store high nitrogen in its straw. However the importance of both carbon dioxide and cytokine application at this stage is less for wheat crop productivity. It may important for vegetable crops, those consumed their vegetative parts.

The result agree with (Baker and Allen, 1993; Campbell, 2011) who reportedly that Carbon dioxide enrichment stimulated greater amount of branching or tillering by increasing photosynthesis, reducing photorespiration and transpiration. However the application of Carbon dioxide fertilizer during tillering period indicated that early growth and tillering were relatively unimportant.

4.3.7 Effect of Carbon dioxide and Cytokine on wheat grain Nitrogen utilization efficiency.

The analysed data for both experiments in controlled environment and under field condition had showed significant effect of carbon dioxide and cytokine applied at different stages ($P < 0.01$) on the grain wheat nitrogen utilization efficiency (Appendix Table 7 & 8).The result of the control environment experiment showed that, the treatments that received carbon dioxide and both carbon dioxide and cytokine at flowering stages used maximum grain wheat nitrogen utilization efficiency (80% and 76%)(Table 8) respectively. The minimum grain wheat nitrogen utilization efficiencies were recorded in treatments that received carbon dioxide and both carbon dioxide & cytokine at tillering stages (50% and 55%) respectively.

Similarly under field condition the treatments that received carbon dioxide and both carbon dioxide and cytokine at flowering stages used maximum grain wheat nitrogen utilization efficiency (74% and 78%) (Table 9) respectively, While the minimum grain wheat nitrogen utilization efficiency were recorded in treatment that received carbon dioxide and both carbon dioxide &cytokine at tillering stages (45% and 53%) respectively.

These results showed that the most important time of application of carbon dioxide and both the interaction of carbon dioxide & cytokine for maximum grain seed nitrogen utilization efficiency is at time crop flowering period. These indicated that most photo assimilates directly allocated to sink which increases number of grain seed per plant, size of grain seed, length of spike, quality and finally grain yield per unit area. Cytokine increases 1000-kernel weight through retarding ageing and increasing the seed's active growth period and positively influencing source tissues and increasing photosynthetic capacity (Xie *et al*., 2004).

4.3.8 Effect of carbon dioxide and cytokine on wheat grain nitrogen use efficiency.

The analysed data for both the experiments in control environment and under field condition showed significant effect of Carbon dioxide and Cytokine applied at different stages ($P < 0.01$) on grain nitrogen use efficiency (Appendix Table 7 & 8).The result of the experiment in control environment showed that, the treatments that received both carbon dioxide and cytokine at flowering stage contained the maximum grain seed nitrogen use efficiency (38.4%)(Table 8). The minimum grain seed nitrogen use efficiency recorded in treatment that received cytokine at tillering stages (24.00%).

Similarly under field condition the experiment showed that, the treatment that received both carbon dioxide & cytokine at flowering stage contained the maximum grain seed nitrogen use efficiency (41.60%) (Table 9), while the minimum grain seed nitrogen use efficiency recorded in treatment that received cytokine at tillering stage (22.75%).The term use efficiency is the result nutrient uptake multiplied by utilization efficiency. Then the application of both carbon dioxide and cytokine at flowering stage help the crop to increase nutrient uptake, photosynthesis, reduce photorespiration, use the nitrogen efficiently by allocating most of the photo assimilates to the grain seed that resulted high yield and quality.

Therefore the application of both carbon dioxide and cytokine at pollination stage was very important for nitrogen use efficiency that providing high grain yield and quality. These founding agree with (Xie *et al*., 2004), who reported that Cytokine increases 1000-kernel weight through retarding ageing and increasing the seed's active growth period and positively influencing source tissues and increasing photosynthetic capacity. And also agree with (Fischer *et al*., 1998) who reported that there is mounting evidence that the yield potential of many crops is limited by their capacity to exploit sufficient Carbon dioxide during their lifecycle, limiting grain size and quantity. “Carbon dioxide fertilization” through increased CO_2 levels would be ideal for yield increase.

Table 8: The mean values of wheat crop nitrogen use efficiency, nitrogen uptake efficiency and nitrogen utilization efficiency for different treatments in control environment.

TRT	MEAN			
	NUPE (%)	BPNE (%)	NUtE (%)	NUE (%)
$(CO_2)T$	0.48^{bc}	0.25^{b}	55^{h}	26.40^{d}
$(CO_2)F$	0.44^{e}	0.16^{e}	76^{b}	33.44^{ab}
$(CO_2)SF$	0.44^{e}	0.18^{d}	69^{d}	30.36^{cd}
(CK)T	0.48^{bc}	0.24^{b}	50^{i}	24.00^{e}
(CK)F	0.49^{b}	0.19^{cd}	67^{e}	32.83^{bc}
(CK)SF	0.46^{d}	0.21^{c}	63^{f}	28.98^{cd}
$(CO_2$ X CK)T	0.52^{a}	0.27^{a}	61^{g}	31.72^{c}
$(CO_2$ X CK)F	0.47^{cd}	0.165^{e}	80^{a}	38.40^{a}
$(CO_2$ X CK)SF	0.47^{cd}	0.195^{cd}	71^{c}	33.37^{abc}
	0.01	0.03	6.11	0.02
CV (%)	1.49	3.77	1.18	5.55

CV = Coefficient of Variation, TRT = Treatment, NUPE = Nitrogen uptake efficiency, By product nitrogen efficiency, N Utilization efficiency, and NUE= N used efficiency.

Table 9: The mean values of the wheat crop nitrogen use Efficiency, N uptake efficiency and N utilization efficiency for different treatments under field condition.

TRT	MEAN			
	NUPE (%)	BPE (%)	NUtE (%)	NUE (%)
(CO_2)T	0.455^{e}	0.24^{b}	57.5^{g}	25.16^{h}
(CO_2)F	0.420^{f}	0.145^{c}	74.5^{b}	31.29^{d}
(CO_2)SF	0.440^{ef}	0.185^{b}	69^{d}	30.36^{e}
(CK)T	0.50^{b}	0.185^{b}	45.5^{i}	22.75^{i}
(CK)F	0.54^{a}	0.175^{b}	66.5^{e}	35.91^{b}
(CK)SF	0.46^{c}	0.175^{b}	63.5^{f}	29.21^{f}
$(CO_2$ X CK)T	0.50^{b}	0.255^{a}	53.5^{h}	26.75^{g}
$(CO_2$ X CK)F	0.53^{a}	0.185^{b}	78.5^{a}	41.60^{a}
$(CO_2$ X CK)SF	0.450^{e}	0.185^{b}	71.5^{c}	32.17^{c}
Standard Error	0.01	0.02	6.42	0.03
CV (%)	1.49	3.47	1.27	2.62

CV = Coefficient of Variation, TRT = Treatment, NUPE = Nitrogen uptake efficiency, BNE= By product nitrogen efficiency, NUtE= nitrogen utilization efficiency, and NUE = nitrogen used efficiency.

4.4 Correlation analysis

4.4.1. The correlation between parameter of growth rate, yield and yield components in control environment

The Correlation analysis of wheat that conducted in control environment) between growth parameters, yield and yield related traits, were given in (Table 1 & 3). The simple correlation analysis showed that number of tiller had highly significant (P <0.01) and positively correlated with days of heading (r=0.91), number of spikelet per spike (r=0.99), spike length per plant (r=0.98), thousand seed weight (r=0.98),grain yield (r=90),harvest index (r=0.99) and It negatively correlated with date of maturity (r=0.99),plant height (r=0.99),number of seed(r=0.99), and biomass yield(r=0.97). The date of heading has highly significant (P <0.01) and positively correlated with plant height (r=0.76) and negative correlated with harvest index (r=0.72).

Day to maturity was highly significantly ($p<0.01$) and positively correlation with plant height(r=0.99), number of seed per spike(r=0.97) and biomass yield(r=0.98) and it negatively correlated with number of spikelet per spike(r=0.99), spike length per plant(r=0.97), thousand seed weight(r=0.99), grain yield(r=0.88) and harvest index(r=0.99). Plant height was highly significant ($P<0.01$) and positively correlation with number of seed per spike(r=0.99) and biomass yield(r=0.98) and negatively correlated with number of spikelet per spike (r=0.99), spike length per plant (r=0.97), thousand seed weight (r=0.97), grain yield (r=0.90) and harvest index(r=0.99).

Number of spikelet per spike was highly significantly ($P <0.01$) and positively correlated with spike length per plant (r=0.98), thousand seed weight (r=0.98), grain yield(r=0.91) and harvest index (r=0.99) and negatively correlated with number of seed per spike(r=0.99) and biomass yield(r=0.97). Spike length was highly significant ($P<0.01$) and positively correlation with thousand seed weight (r=0.96), grain yield (r=0.94) and harvest index (r=0.99) and negatively correlated with number of seed per spike(r=0.97) and biomass yield(r=0.94). Number of seed per spike was highly significant ($P <0.01$) and positively correlated with biomass yield(r=0.98) and negatively correlated with thousand seed weight(r=0.99), grain yield(r=0.88) and harvest index(r=0.99).

Thousand seed weight was highly significant ($p\leq0.01$) and positively correlated with grain yield (r=0.86) and harvest index(r=0.98) and negatively correlated with biomass yield (r=99). Biomass yield was highly significant ($P <0.01$) and negatively correlated with grain yield (r=0.83) and harvest index (r=0.96). Finally grain yield was highly significantly ($P<0.01$) and positively correlated with harvest index(r=0.89).

1. The relationship of different growth, yield and yield component parameters at green house.

	NT	DH	DM	PH	NSP	SPL	NS	TSW	BIO	GYW	HI
NT	1.00	0.91**	-0.99**	-0.99**	0.99**	0.98**	-0.99**	0.98**	-0.97**	0.90**	0.99**
DH		1.00	-0.11ns	0.76**	-0.09ns	-0.16ns	0.13ns	-0.45ns	0.51ns	-0.27ns	-0.72**
DM			1.00	0.99**	-0.99**	-0.97**	0.99**	-0.99**	0.98**	-0.88**	-0.99**
PH				1.00	-0.99**	-0.97**	0.99**	-0.99**	0.98**	-0.90**	-0.99**
NSP					1.00	0.98**	-0.99**	0.98**	-0.97**	0.91**	0.99**
SPL						1.00	-0.97**	0.96**	-0.94**	0.94**	0.99**
NS							1.00	-0.99**	0.98**	-0.88**	-0.99**
TSW								1.00	-0.99**	0.86**	0.98**
BIO									1.00	-0.83**	-0.96**
GYW										1.00	0.89**
HI											1.00

* and ** = Correlation is significant and highly significant at p<0.01 and p<0.05 levels, respectively.; DH =Day of heading ,DM =Day of maturity , NT =Number of tiller , PH =Plant height , NSP =Number of spike let per plant, SPL =Spike length , NS =Number of seed per spike , TSW =Thousand seed weight , BIO =Biomass yield , GY =Grain yield , HI = Harvest index(field).

4.4.2. The relationship between parameter of growth rate, yield and yield components under field

The Correlation analysis of Bread wheat conducted under field between growth parameters, yield and yield related traits, were given in (Table 2 &4). The simple correlation analysis showed that the number of tiller was highly significantly (P<0.01) and positively correlated with day to heading (r=0.82) and significant at (P<0.5) and negatively correlated with thousand seed weight (r=0.50).Days of heading has highly significant (P <0.01) and negatively correlated with, date of heading (r=0.69 and spike length per plant (r=0.58). And significant (P <0.5) and negatively correlated with, grain yield (r=0.47) and harvest index(r=0.53). Day to maturity has highly significantly (p<0.01) and positively correlated with plant height(r=0.62), number of spikelet per spike(r=0.67) spike length per plant (r=0.70), grain yield(r=0.69) and harvest index(r=0.76). Plant height was highly significant and positively correlated with number of spike let per spike (r=0.79), spike length(r=0.69), grain yield (r=0.79) and harvest index(r=0.85).

Number of spikelet per spike was highly significantly (P <0.01) and positively correlated with spike length (r=0.82), grain yield(r=0.83) and harvest index (r=0.83). And also significantly (P<0.5) and positively correlated with number of seed(r=0.65). Spike length was highly significant (P<0.01) and positively correlation with grain yield (r=0.76) and harvest index

(r=0.82). And also significantly (P<0.5) positively correlated with number of seed(r=0.49). Number of seed per spike was highly significant (P <0.01) and positively correlated with grain yield(r=0.69) and harvest index (r=0.62).And also significantly (P<0.5) positively correlated with biomass yield (r=0.56).

Thousand seed weight was significantly ($p \leq 0.5$) and positively correlated with biomass yield (r=0.56). Biomass yield was significant (P <0.5) and positively correlated with grain yield (r=0.55). Finally grain yield was highly significantly (P<0.01) and positively correlated with harvest index(r=0.96).

2. The relationship of different growth, yield and yield component parameters at field.

Para	NT	DH	DM	PH	NSP	SPL	NS	TSW	BIO	GY	HI
NT	1.00	0.82**	-0.39^{ns}	0.09^{ns}	-0.03^{ns}	-0.25^{ns}	0.19^{ns}	-0.50*	-0.11^{ns}	-0.10^{ns}	-0.09^{ns}
DH		1.00	-0.69**	0.42ns	-0.41ns	-0.58**	-0.06ns	-0.43ns	-0.06ns	-0.47*	-0.53*
DM			1.00	0.62**	0.67**	0.70**	0.17ns	0.44ns	0.16ns	0.69**	0.76**
PH				1.00	0.79**	0.67**	0.63*	0.08ns	0.19ns	0.79**	0.85**
NSP					1.00	0.82**	0.65*	0.38ns	0.37ns	0.83**	0.83**
SPL						1.00	0.49*	0.37ns	0.17ns	0.76**	0.82**
NS							1.00	0.19ns	0.46*	0.69**	0.62**
TSW								1.00	0.56*	0.43ns	0.32ns
BIO									1.00	0.55*	0.32ns
GYW										1.00	0.96**
HI											1.00

* and ** = Correlation is significant and highly significant at p<0.01 and p<0.05 levels, respectively.; DH =Days of heading ,DM =days of maturity , NT =number of tiller , PH =plant height , NSP =number of spikelet per spike, SPL =spike length , NS = number of seed per spike, TSW =thousand seed weight , BIO =biomass yield , GY =grain yield , HI = harvest index

5. SUMMARY AND CONCLUSIONS

A study on carbon dioxide and cytokine application at different stages on growth, yield and grain quality of Digalu (HAR 3116) were conducted at Jimma, Ethiopia in (2014 to 2015). The experiments were arrangement in RCBD for ten treatments with three replications. The study focused on the effect of carbon dioxide and cytokine foliar spray at different stage in both control environment and field condition that thousand seed weight, grain yield, harvest index, grain seed protein, nitrogen utilization efficiency and nitrogen use efficiency were significantly affected by the effect of CO_2, cytokine and both combination of carbon dioxide and cytokine at different stages while nitrogen supply was not significant due to both the available nitrogen in the soil and the amount nitrogen applied (urea) were the same for all treatments.

The maximum thousand seed weight (52 & 53.93g) observed both in green house and field respectively, due to the application of cytokine at flowering stages. The maximum grain yield relatively to the others (13.44 ton ha^{-1}& 11.89 ton ha^{-1}) were observed in both green house and field respectively due the application of both carbon dioxide and cytokine at flowering stages. The highest grain protein contents relatively the others (16.75% & 16.76%) were observed in both green house and field respectively due to the application of cytokine at flowering stages.

The highest nitrogen utilization efficiency (80% & 78%) were observed both in green house and field respectively, due to the application of both CO_2 and cytokine at flowering stages. And finally the highest nitrogen use efficiency (38. & 41.6) were observed as compared with other different treatments, both in green house and field respectively, due to the application of both carbon dioxide and cytokine at flowering stages.

Results of this experiment indicated highest significant differences in grain yield per hectare and grain protein content (%) both in green house and field. Numerically, that have both the highest grain yield per hectare and optimum seed quality(protein) were recorded comparatively to the other treatments both in green house and field, due to the foliar application of both carbon dioxide and cytokine at flowering stages. Also relatively to the other treatments the foliar application of both carbon dioxide and cytokine at seed filling stages both in green house and field were comparative good both in grain yield per hectare and seed quality. The foliar application of carbon dioxide at flowering stages in both, in green house and field were high

grain yields but low seed quality (protein).However, this tentative generalization, based on one season at one location, required confirmation with further studies to give a valid recommendation. From the forgoing results, grain yield and quality (protein) can be substantially improved by the use of foliar application of both carbon dioxide and cytokine at flowering stage in Jimma and similar areas for further study is good for bread wheat (Digalu).

From the present findings, it is possible to suggest the followings as high priority research areas:

- Research on effect of carbon dioxide and cytokine foliar application of bread wheat productivity and grain quality should be continued. Because the population number, Urban expansion and wheat demand radically increase in a country, however the productivity of wheat in Ethiopia is very low grain yield, due to the climate change such as rain fall shortage, variability, and lack of uniform distribution that cause the shortage of moisture in the soil.
- Breeder will be done on increasing nitrogen uptake, assimilatory and responsive genes would ensure optimal nitrogen allocation toward expanding source and sink tissues under elevated CO_2 levels as well as improving grain yield and quality.
- Breeder will be work on increasing cytokine hormone in plant tissue that help wheat crops to increase nitrogen uptake and protein synthesis in grain seed.

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7. APPENDIX

Appendix Table 1.Analysis of variance table showing mean square values of growth parameters received carbon dioxide, cytokine and their combination at different stages in control environment

Source	Df	Mean Square				
		NT	DH	DM	PH	SPN
Model	8	5.432***	14.101***	8.316***	1.643***	5.566***
Error	18	0.022	0.061	1.013	0.148	0.224
Corrected total	26					
F Value			232.15	8.21	11.06	247.97
Pr> F		< 0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV (%)		3.28	0.38	0.85	0.42	0.69

DF = degree of freedom, %CV = % coefficient of variation, *** highly significant (P < 0.001), ** highly significant (p<0.01), *Significant (P < 0.05), Ns = non-significant difference, NT = Number of tiller, DH = Days of heading, DM = Days of maturity, PH = plant height, SPN = number of spikelet per spike**.**

Appendix Table 2. Analysis of variance table showing mean square values of yield component received carbon dioxide, cytokine and their combination at different stages under field condition.

Source	Df	Mean square					
		SPL	NS	TSW	BMY	GRY	HI
Model	8	4.728***	30.83***	26.950***	7892289.22***	10966563.14***	346.361***
Error	18	0.077	0.148	0.067	375644.42	10518.04	3.264
Correct total	26						
F Value		61.75	208.11	398.98	21.01	1042.64	26.11
Pr>F		<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV (%)		2.68	0.46	0.54	4.50	1.02	4.92

DF = degree of freedom, %CV = % coefficient of variation, ******* highly significant (P < 0.001), ** highly significant (p<0.01), *****Significant (P < 0.05), Ns = non-significant difference, SPL = Spike length, NS = Number of seed per plant, TSW = thousand seed weight, BMY = Biomass yield, GRY = Grain yield, HI = Harvest index.

Appendix Table 3.Analysis of variance table showing mean square values of growth parameters that received carbon dioxide, cytokine and their combination at different under field condition

Source	Df	Mean Square				
		NT	DH	DM	PH	SPN
Model	8	2.657***	15.440***	29.994***	3.019***	5.085***
Error	18	0.066	0.113	0.533	0.195	0.396
Corrected total F	26					
Value		39.98	136.32	56.26	15.46	12.94
Pr>F		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV (%)		6.11	0.47	0.56	0.48	2.96

.DF = degree of freedom, %CV = % coefficient of variation, *** highly significant (P < 0.001), ** highly significant (p<0.01), *Significant (P < 0.05), Ns = non-significant difference, NT = Number of tiller, DH = Days of heading, DM = Days of maturity, PH = plant height, SPN = number of spikelet per spike.

Appendix Table 4. Analysis of variance table showing mean square values of yield component that received carbon dioxide, cytokine and their combination at different stages under field condition

Source	Df	Mean square					
		SPL	NS	TSW	BMY	GRY	HI
Model	8	1.911***	3.152***	35.562***	5309783***	9548926***	123.65***
Error	18	0.190	0.098	0.195	37.22	6.41	0.12
Correct total	26						
F Value		10.06	32.18	181.88	142651	1490295	1052.87
Pr> F		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV (%)		4.59	0.36	0.89	2.5	1.3	0.5

DF = degree of freedom, %CV = % coefficient of variation, ******* highly significant (P < 0.001), ** highly significant (p<0.01), *****Significant (P < 0.05), Ns = non-significant difference, SPL = Spike length, NS = Number of seed per plant, TSW = thousand seed weight, BMY = Biomass yield, GRY = Grain yield, HI = Harvest index.

Appendix Table 5.Analysis of variance table showing mean square values of grain quality and nitrogen contents of other yield component received carbon dioxide, cytokine and their combination at different stages in control environment.

			Mean	square	
Source	Df	GrP	TNStr	TNP	NB
Model	8	14.741***	2759.624***	1379.71***	1379.71***
Error	9	0.0002	0.045	2.000	2.000
Correct total	17				
F Value		73703.4	61325	689.85	689.85
Pr> F		0.0001	0.0001	0.0001	0.0001
CV (%)		0.12	0.15	0.46	0.46

DF = degree of freedom, %CV = % coefficient of variation, *** highly significant (P < 0.001), ** highly significant (p<0.01), *Significant (P < 0.05), Ns = non-significant difference, Trt = Treatment, GrP = Grain protein, TNStr = Total nitrogen in straw, TNP = Total nitrogen in plant, NS = Nitrogen supply, BN = Biomass yield nitrogen.

Appendix Table 6.Analysis of variance table showing mean square values of grain seed and other yield component of nitrogen use efficiency received carbon dioxide, cytokine and their combination at different stages in control environment.

			mean	square		
Source	Df	GrNUE	NUPE	BPNE	NUtE	NUE
Model	8	8.206***	1.25***	3.551***	0.0186***	3.1014**
Error	9	0.000001	0.000001	6.111	0.00006	2.555
Corrected total	17					
F Value		16.88	25.00	0.95	304.98	12.14
Pr> F		<0.0001	<0.0001	<0.0001	0.0001	<0.0001
CV (%)		2.28	1.48	3.77	1.18	5.55

DF = degree of freedom, %CV = % coefficient of variation, *** highly significant (P < 0.001), ** highly significant (p<0.01), *Significant (P < 0.05), Ns = non-significant difference, Trt = Treatment, GrNUE = grain nitrogen use efficiency, NUPE = Nitrogen uptake efficiency, BPNE = By product nitrogen efficiency, NUtE = Nitrogen Utilization efficiency, and NUE = Nitrogen used efficiency.

Appendix Table 7.Analysis of variance table showing mean square values of grain quality and nitrogen contents of other yield component of different treatment at different stages under field condition.

		Mean square			
Source	Df	GrP	TNStr	TNP	NB
Model	8	14.741***	1401.050***	5240.848***	5240.848***
Error	9	0.0002	1.597	0.500	0.5
Correct total	17				
F Value		73703.4	877.22	10481.7	10474.8
Pr> F		0.0001	0.0001	0.0001	0.0001
CV (%)		0.12	1.03	0.21	0.21

DF = degree of freedom, %CV = % coefficient of variation, *** highly significant (P < 0.001), ** highly significant (p<0.01), *Significant (P < 0.05), Ns = non-significant difference, TRT = Treatment, GrP = Grain protein, TNStr = Total nitrogen in straw, TNP = Total nitrogen in plant, NS = Nitrogen supply, BN = Biomass yield nitrogen.

Appendix Table 8.Analysis of variance table showing mean square values of grain seed protein and other yield component of nitrogen use efficiency of different treatments at different stages in control environment.

Source		mean square				
	Df	GrNUE	NUPE	BPEN	NUtE	NUE
Model	8	4.922***	3.039***	2.314***	0.0225***	4.403***
Error	9	5	5	4.444	0.0001	6.25
Corrected total	17					
F Value		10.94	60.78	52.06	337.96	70.46
Pr> F		0.0001	0.0001	0.0001	0.0001	0.0001
CV (%)		2.49	1.49	3.47	1.27	2.62

DF = degree of freedom, %CV = % coefficient of variation, *** highly significant (P < 0.001), ** highly significant (p<0.01), *Significant (P < 0.05), Ns = non-significant difference, TRT = Treatment, GrNUE = grain nitrogen use efficiency, NUPE = Nitrogen uptake efficiency, By product nitrogen efficiency, NUtE = Nitrogen utilization efficiency, and NUE = Nitrogen used efficiency.