Available online: www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2017, 9(2):233-241. DOI: 10.15835/nsb9210002

Original Article

Yield and Yield Attributes Responses of Soybean (*Glycine max* L. Merrill) to Elevated CO_2 and Arbuscular Mycorrhizal Fungi Inoculation in the Humid Transitory Rainforest

Nurudeen ADEYEMI, Olalekan SAKARIYAWO*, Muftau ATAYESE

*Federal University of Agriculture, Abeokuta (FUNAAB), Department of Plant Physiology and Crop Production, College of Plant Science and Crop Production, P.M.B. 2240, Alabata, Ogun State, Nigeria; adetanwa@yahoo.co.uk (*corresponding author)*

Abstract

Variations in yield components and grain yield of arbuscular mycorrhizal fungi (AMF) inoculated soybean varieties (*Glycine max* L. Merrill) grown in CO_2 enriched environment in the humid rainforest were tested. A screen house trial was established with soybean varieties ('TGx 1448-2E', 'TGx 1440-1E' and 'TGx 1740-2F'), AMF inoculation (with and without) and CO_2 enrichment (350±50 ppm and 550±50 ppm) in open top chamber, arranged in completely randomised design, replicated three times. A field trial was also conducted; the treatments were arranged in a split-split plot configuration fitted into randomised complete block design. In the main plot the variant was CO_2 enrichment, the sub-plot consisted of AMF inoculation (with and without), while the sub-sub plot consisted of soybean varieties, replicated three times. Both trials had significantly higher grain yield at elevated CO_2 than ambient. This could be attributed to improved yield attributes, more spore count and root colonisation. In both trials, inoculated soybean had significantly higher dry pod weight than un-inoculated, which could suggest the increased grain yield observed on the field. AMF inoculated soybean varieties outperformed un-inoculated in both CO_2 enriched and ambient concentrations. AMF inoculated soybean variety 'TGx 1740-2F' is most preferable in CO_2 enriched environment, while variety 'TGx 1448-2E' had the most stable grain yield in all growth environments.

Keywords: carbon dioxide enrichment, open top chamber, yield components

Introduction

Soybean [*Glycine max* (L.) Merrill] is a leguminous vegetable of the pea family that grows in tropical, subtropical, and temperate climates. It serves the dual purpose of being grown both as an oil seed and a pulse (Thakare *et al*., 2006). It has been a subject of research in the agronomical literature, physiologically and biochemically (Ferguson and Gresshoff, 2009). Its cultivation is increasing in most agroecological zones of Nigeria owing to its nutritional value and economic importance (Okpara *et al*., 2007). This production tempo observed in recent past could be constrained by the problem of climate change. Climate change is attributable to the natural climate cycle and human activities (Ziervogel *et al*., 2006). The most prominent cause of climate change is the increase in the level of carbon dioxide (CO_2) concentration in the atmosphere. It is predicted to double before the end of this century (Stocker *et al*., 2013). The atmospheric concentrations of carbon dioxide have been steadily rising with an average annual increase rate of about 2 ppm and projected to rise to 500-1,000 ppm by the year 2100 from the current atmospheric average of approximately 385 ppm (Keeling and Piper, 2009; Kiehl, 2011). Nigeria accounts for roughly one-sixth of the world-wide gas flaring which in turn, spews some 400 million tons of CO_2 into the atmosphere, more than any other country in Africa (IPCC, 2007). This could have serious implication on the productivity and performance of most agricultural crops, especially in the most vulnerable part of the world (Stocker *et al*., 2013). Specifically it was projected that crop yield in Africa may fall by 10-20% by 2050 or even up to 50% due to climate change (Jones and Thornton, 2009).

There are certain categories of crops that could benefit from the elevated atmospheric CO_2 concentration. The C_3 crops are limited by intracellular CO_2 concentration unlike the C_4 and CAM that possess the mechanism to increase their internal CO_2 concentration. With climate change, C_3 could theoretically benefit from this. However, the benefit accruable is dependent on the type of C_3 crop, species, developmental stage and other environmental factors. Legumes had been reported to benefit more from elevated

Received: 01 Dec 2016. Received in revised form: 20 Jun 2017. Accepted: 20 Jun 2017. Published online: 30 Jun 2017.

CO_2 than non-legumes (Poorter, 1993). Extensive reportsare available in the literature on the mechanisms that underpins this process (Aranjuelo *et al.*, 2014). The increase in growth from elevated CO_2 is likely to require more phosphorus, which is taken up from the available phosphorus pool in soil (Gentile *et al.*, 2012; Jin *et al.*, 2013). Several studies have reported that both the magnitude and the direction of the growth response of plants to elevated CO2 depend on P availability (BassiriRad *et al.*, 1997; Jin *et al.*, 2013). However, only a small proportion of total soil P (generally < 1%) is in the form of labile phosphate ions which are available to plants (Richardson *et al.*, 2009). This challenge could be ameliorated with the presence of arbuscular mycorrhizal fungi (AMF) that forms a symbiotic association with most cultivated crops (Facelli *et al.*, 2010; Shen *et al.*, 2011; Brown *et al.*, 2013) .

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the majority of terrestrial plants. Their association with crops have been implicated in the improvement in nutrient status of crops especially P and N. Other effects of their symbiotic relation with crops include enhanced water uptake in drought susceptible agroecologies, disease resistance and increased plant productivity (Smith and Read, 2008). Given their widespread importance, AMF may be a major factor in mediating plant and ecosystem responses to climate change (Drigo *et al.*, 2008; Compant *et al.*, 2010). It had been reported in the literature that elevated CO2 would benefit more the C_3 plants than the C_4 (Poorter, 1993). Soybean, being a C_3 plant, could be expected to have an enhanced performance when other growth factors are not limiting. Increased CO_2 in the atmosphere would increase the photosynthetic capacity of soybean. However, this could only be attained in the presence of nitrogen and phosphorus. Nitrogen is a limiting nutrient in this agroecology due to the loss accruable from increased intensity of precipitation and the mobile nature of this macronutrient. Soybean being a legume is capable of fixing atmospheric gaseous nitrogen in exchange for the carbon from it (Kaschuk *et al.*, 2009). Biologically fixed nitrogen would ensure availability of N towards protein synthesis, especially light harvesting complex that could increase photosynthetic capacity of soybean (Hikosaka and Terashima, 1995). Phosphorus is not readily available in the transitory rainforest. It had been directly linked with the transportation of sucrose into to the cytosol in exchange with inorganic P (Kaschuk *et al.*, 2009). This process could be limiting to the regeneration of ribulose biphosphate and consequently affect photosynthetic capacity of soybean (Rychter and Rao, 2005). AMF in association with soybean could ameliorate this effect. To what extent this symbiotic association with soybean in this agroecology under elevated CO_2 would stimulate the performance of soybean cultivars is still a subject of speculation in the literature. This investigation therefore tested the hypothesis that elevated CO2 in the presence of AMF would increase the performance of soybean cultivars and these cultivars would show differential responses under this condition.

The current investigation could increase our understanding of the effects of CO_2 elevation on AMF activities and the resulting soybean responses may be a crucial factor in a sustainable management of soybean in the transitory agroecology of Nigeria.

Materials and Methods

Two experiments were conducted both in pots and an the field. The pot experiment was conducted in the screen house of the College of Plant Science and Crop Production, FUNNAB. Carbon dioxide enrichment was facilitated in an open top chamber placed in the screen house. Three medium maturing varieties of soybean were obtained from International Institute of Tropical Agriculture (IITA), Ibadan.

Pot experiment

Experimental treatments and design

The pot experiment was a 2×2×3 factorial experiment laid out in completely randomised design, with three replicates. The treatments consisted of CO_2 enrichment [ambient (350±50 ppm) and elevated (500±50 ppm)] and AMF inoculation (inoculated and un-inoculated) on soybean varieties ('TGx 1448-2E', 'TGx 1440-1E' and 'TGx 1740-2F'). Commercially available AMF inoculum (empathy mycorrhizal root growTM fungi) was used in this trial. This inoculum was a mixture of AMF species.

Cultural operations

A total of one hundred and eight plastic pots were perforated at the bottom to allow for the drainage of excess water without depleting the soil quantity. Three planting plastic pots were used for each treatment. Each pot dimension was 0.25 m wide (diameter) at the surface, 0.36 m deep with a capacity of 10 litres (1,000 cm3). Soil (0-300 mm depth) was obtained from the Directorate of University Farms, FUNAAB. The soil sample was sieved using 2.0 mm mesh sieve. Each pot was filled with 7 kg of soil. Three seeds were planted at a depth of 20-30 mm in the soil and later thinned to one plant per pot, two weeks after sowing (WAS). AMF inoculum (2 g) was sprinkled into the base of the planting hole at the time of planting, covered with a small amount of soil and the seeds were sown.

A large open top chamber (OTC) with an internal height of 2.5 m and growth area of 12 m2 was used to enclose CO_2. OTC was constructed using polyvinyl chloride pipes covered with transparent polyvinyl chloride (PVC) nylon sheet. The pots were transferred and arranged randomly inside the OTC between 3-9 weeks after sowing.

Carbon dioxide was produced using a modified method of Saitoh *et al.* (2004). Into a beaker was placed 40 g of baker yeast, which was dissolved with lukewarm water for several minutes to dissolve air into the solution. The solution was transferred into the 5 litre bottle (CO_2 generator bottle). Into a large beaker was placed 250 g of sugar that was gently stirred to dissolve and poured into the CO_2 generator bottle, then filled with water up to 4 litres. The CO_2 generator bottle was perforated at the top to allow influx and efflux of gases (CO_2 and O_2) between the bottle and the atmosphere. Twelve CO2 generator bottles were placed in the OTC to elevate the CO_2 concentration (500±50 ppm). The maximum and minimum CO_2 concentration in the OTC was measured thrice in week throughout the enrichment period using a portable CO_2 meter [NDIR Gas Analyzer (Bentech GM8883), China].

The average was used to determine the CO_2 concentration at elevated level. The control pots were exposed to ambient atmospheric conditions. Transparent walls of the open top chamber were kept clean regularly by cleaning with water in order to minimize any differences in the light levels within and outside the chambers. Weed control was done manually as at when due and water was supplied into the pot thrice in a week.

Sampling and data collection

A composite soil sample was collected from a depth of 0–0.3 m from the field meant for the field trial to determine their physical and chemical properties. The textural class of the site was determined using the USDA textural triangle. Soil particle size distribution was determined using the hydrometer method (Bouyoucos, 1962). The active pH was determined in 1:1 soil: water using a pH meter (glass electrode) (McLean, 1982). The organic content of the samples was determined using Walkey-Black method, modify by Allison (1965). Total nitrogen was determined using modified micro-Kjeldahl digestion technique (Jackson, 1962). Available phosphorus was determined using Bray-1 (Bray and Kurtz, 1945) and determined colometrically using the method of Murphy and Riley (1962). Exchangeable bases were extracted with one normal ammonium acetate buffered at pH of 7. Sodium and Potassium in the extract were determined by flame photometry, while Ca+ and Mg+ were determined using Atomic Absorption Spectrophotometer (AAS). Total acidity (H++ Al3+) was determined using KCl as the extracting medium. Cation Exchange Capacity was determined by the summation of Total Exchangeable Bases (TEB) and Total Acidity (TA).

At 95% harvest maturity, data were collected on the number of pods per plant, number of seeds per pod, dry pod weight, 100 seed weight, biomass yield and seed yield per plant, shelling percentage and harvest index.

Mycorrhizal analysis

Rhizosphere soil and roots samples were collected from each experimental pot at 6, 9 WAS and 95% harvest maturity for the determination of both AMF sporulation and mycorrhizal colonisation. Spore population of the soil was determined by collecting 20 g of the soil sample from the well mixed soil using modified wet sieving method of Giovannetti and Mosse (1980). Extracted spores were identified using digital compound microscope and counted under dissecting slides. Roots samples collected were prepared using the method of Phillips and Hayman (1970) to determine percentage AMF colonisation. Prepared root samples were rinsed off staining solution with clean tap water. They were preserved with 40% glycerol solution for further viewing under compound microscope to determine percentage root colonisation. Percentage root colonisation was determined as*:*

Percentage root colonization = number of root infected/total number of roots × 100

The initial spore count in the 20 g of the soil was between 3-5 spores.

Field experiment

Description of experimental location and site

A field experiment was conducted at FUNAAB (latitude 7°15'N, longitude 3°28'E and altitude of 76 m a.s.l.), Nigeria.

Experimental treatments and design

The field experiment treatment structure was a 2×2×3 factorial combination laid out in randomised complete block design in split-split plot arrangement, with three replicates. The main plot consisted of CO_2 enrichment [ambient (350±50 ppm) and elevated (500±50 ppm)]. The sub-plot consisted AMF inoculation (inoculated and un-inoculated) and the sub sub-plot was made of soybean varieties ('TGx 1448-2E', 'TGx 1440-1E' and 'TGx 1740-2F').

Cultural operations

The field was ploughed twice and disc harrowed once. Each gross plot measured 2×2m (4 m2) and net plot size was 1.5×1.5 (2.25 m2). The distance between plots was 0.5 m and 1 m between replicates. Planting was conducted on the 10th of August 2015. During planting, 25 g of AMF granules was placed at the base of the planting hole with three seeds of soybean, which was covered with soil. The planting depth was 20-30 mm at spacing of 50×10 cm. This gave five (5) rows per plot including the first row and each row would contain 21 plants. This translated to 105 plants per plot. The number of plants per stand was later retained as one after thinning at 2 WAP. Weeding was carried out manually at 3, 6 and 9 WAS. Harvesting was conducted manually at 95% harvest maturity (when the pods turned brownish in colour).

CO_2 enrichment

Three large OTC with an internal chamber height of 2 m and growth area of 7×4.5 m each was utilised to contain CO_2. Materials for the construction of OTC on the field were the same as that used in the screen house. The OTCs were installed on the plots at 3 WAP. Carbon dioxide enrichment on the plot commenced from 4 till 9 WAP using yeast that produced CO_2. Four CO_2 generator bottles were placed in each plot. The maximum and minimum CO_2 concentration in the OTCs was measured as described for the screen house trial. The control plot was exposed to ambient atmospheric conditions.

Sampling and data collection

Five plants from the net plot were randomly chosen for the determination of grain yield and its components. Grain yield and its components were determined according to the protocol described for the pot experiment.

Mycorrhizal analysis

Rhizosphere soil samples were collected from each experimental plot at 3, 6 and 9 WAS. Root samples and their rhizosphere soil samples were taken to determine root colonisation and spore count respectively. Sample preparation, determination of mycorrhizal colonisation and spore count followed the protocol earlier described for the pot experiment.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA). The pot trial had a fixed model ANOVA with CO2 enrichment, AMF inoculation and soybean variety as the fixed factors, while replicate was the random factor. The field trial had a mixed model ANOVA. Differences among treatment means were separated using Least Significant Differences (LSD) at 5% probability level. Discrete data were transformed using square root transformation before subjecting them to ANOVA. The statistical package used was Genstat 12th Edition.

Results

Characterisation of experimental site

The textural class of the soil was sandy with a pH that was slightly acidic (6.5). It has total nitrogen of 0.8 mg kg-1 and available P value of 5.23 mg kg-1 with soil organic matter of 1.12% (Table 1).

Table 1. Pre-planting soil physical and chemical properties of the experimental site

Soil Property	Value
Texture	Sand
Sand (%)	87.9
Silt (%)	7.49
Clay (%)	4.61
pH (H_2O)	6.5
Organic Matter (%)	1.12
Nitrogen (mg kg^{-1})	0.80
Available Phosphorus (mg kg^{-1})	5.23
Potassium (cmol kg^{-1})	0.44
Calcium (cmol kg^{-1})	2.54
Magnesium (cmol kg^{-1})	0.73
Sodium (cmol kg^{-1})	0.29
Total Exchangeable Acidity (cmol kg^{-1})	0.110
Exchangeable Cation Exchange Capacity (c mol kg^{-1})	3.920

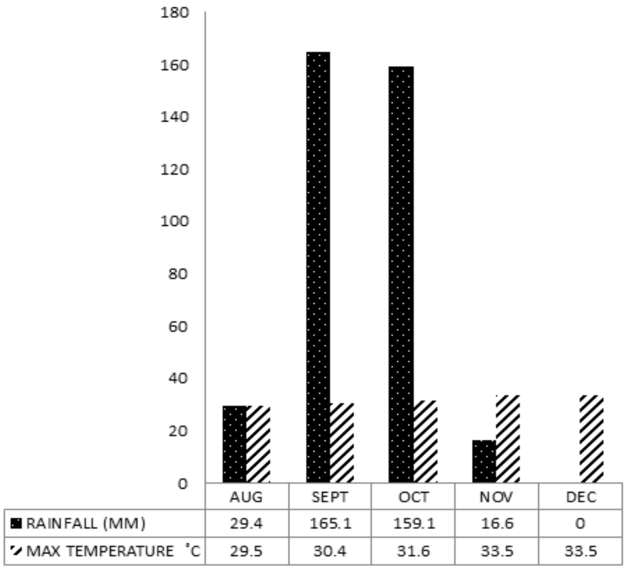

Fig. 1. Rainfall pattern of the experimental location during 2015 cropping season

During the cropping season in the year 2015 the rainfall pattern was in the range of 165.1 mm (September) - no precipitation (December). The maximum temperature during the cropping season was observed in November and December (33.5 °C), while the minimum was recorded in August (29.5 °C) (Fig. 1).

Pot experiment

Effect of CO_2 enrichment and AMF inoculation on yield variables of soybean varieties

The number of pods per plant was significantly ($P<0.05$) higher in CO_2 enriched soybean than those grown in ambient CO_2 (Table 2). Similar trend was observed in total dry biomass, all other yield attributes and grain yield per plant except number of seeds per pod and harvest index. Significant varietal differences were observed on number of seeds per pod, dry pod weight, harvest index and seed yield per plant. Soybean variety 'TGx 1448-2E' had significantly the highest number of seeds per pod. This pattern was also observed on harvest index and grain yield per plant. The highest dry pod weight (16.59 g plant^{-1}) was observed in soybean variety 'TGx 1440-1E'. However, a converse pattern was observed on the number of seed per pod, harvest index and seed yield per plant.

Significant interaction of CO_2 enrichment × AMF × variety was observed on the seed yield per plant (Fig. 2). Inoculated soybean with AMF at elevated CO_2 had seed yield increase in the following decreasing order 'TGx 1740-2F' > 'TGx 1448-2E' > 'TGx 1440-1E'. However, in the absence of CO_2 enrichment in the growth environment, but when inoculated with AMF, soybean variety 'TGx 1448-2E' had the highest seed yield per plant than other soybean varieties. This soybean variety equally displayed the highest seed yield per plant in CO_2 enriched environment, but without being inoculated with AMF and in the environment in the absence of both CO_2 enrichment and inoculation with AMF. The soybean variety with the least seed yield per plant in the last two mentioned growth environments was variety 'TGx 1740-2F'.

Percentage of AMF colonization was significantly higher in CO_2 enriched soybean than ambient at 6 and 9 WAP (Table 3). Similar pattern was observed on the spore count at 9 WAP. At all period of investigation, inoculated soybean

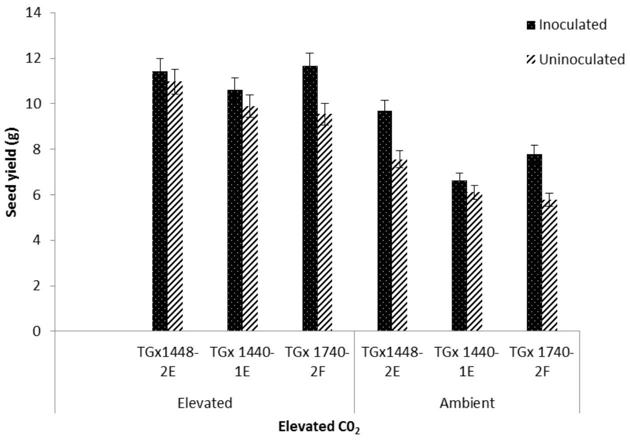

Fig. 2. Interaction of CO_2 enrichment × AMF inoculation × variety on seed yield, screen house. Bars in each column indicate standard error of mean (±SE)

Table 2. Effect of CO_2 enrichment and AMF inoculation on number of pods, number of seeds per pod, dry pod weight, 100-seed weight, dry biomass, harvest index and grain yield per plant of soybean varieties at harvest maturity (pot experiment)

Treatments	Number of pods per plant	Number of seeds per pod	Dry Pod weight (g plant^{-1})	100-seed Weight (g)	dry biomass (g plant^{-1})	Harvest index (%)	Seed yield per plant (g)
CO_2 (C)							
Ambient	35.11	2.56	12.12	8.03	20.34	37.6	7.27
Elevated	46.67	2.57	17.76	9.34	31.27	36.3	10.68
LSD	3.59**	NS	1.31**	0.66**	3.43**	NS	1.03**
AMF (A)							
Inoculated	41.61	2.53	17.18	8.71	30.15	41.6	9.31
Un-inoculated	38.17	2.59	12.70	8.65	21.46	32.4	8.64
LSD	NS	NS	1.31**	NS	3.43**	5.18**	NS
Variety (V)							
'TGx 1448-2E'	40.08	2.73	12.98	8.99	23.10	45.4	9.90
'TGx 1440-1E'	40.58	2.36	16.59	8.59	27.28	32.9	8.31
'TGx 1740-2F'	39.00	2.60	15.25	8.47	27.03	32.7	8.71
LSD	NS	0.14**	1.60**	NS	NS	6.35**	1.26*
C × A	NS	NS	1.85**	NS	4.73**	NS	NS
C× V	NS	NS	NS	NS	NS	NS	NS
A × V	NS	NS	2.26*	NS	NS	NS	NS
C × A × V	NS	NS	3.19**	NS	8.19*	NS	2.52*

* indicates significance at $P < 0.05$ probability level; ** indicates significance at $P < 0.01$ probability level; NS: Not significant

Table 3. Effect of CO_2 enrichment and AMF inoculation on percentage mycorrhizal colonization and AMF sporulation of soybean variety at 6 and 9 WAP (Pot experiment)

	Mycorrhizal Colonization (%)		Spore Count	
Treatments	6 WAP	9 WAP	6 WAP	9 WAP
CO_2 (C)				
Ambient	36.9	50.6	41.5	53.4
Elevated	41.9	62.2	45.4	63.1
LSD	4.27*	2.81**	NS	5.81**
AMF (A)				
Inoculated	48.0	68.9	50.5	65.0
Un-inoculated	30.7	43.9	36.4	51.4
LSD	4.27**	2.81**	4.63**	5.81**
Variety(V)				
TGx 1448-2E	41.1	56.7	43.7	57.0
TGx 1440-1E	37.8	56.9	42.1	59.9
TGx 1740-2F	39.2	55.6	44.7	57.8
LSD	NS	NS	NS	NS
C × A	6.04*	3.97*	NS	NS
C× V	NS	NS	NS	NS
A × V	NS	4.87*	NS	NS
C × A × V	NS	NS	NS	NS

* indicates significance at $P < 0.05$ probability level; ** indicates significance at $P < 0.01$ probability level; NS: Not significant; WAP: Weeks after planting

had significantly higher percentage of AMF colonization than the control. The same pattern was observed on the number of spores at 9 WAP.

Field experiment

Effect of elevated CO_2 and AMF inoculation on total dry mass, yield components, grain yield variables of soybean

The response pattern of grain yield and its attributes to CO_2 enrichment observed in the screen house was validated on the field (Table 4). Dry pod weight response pattern when soybean was inoculated with AMF in the screen house was validated on the field. Furthermore, on the field inoculated soybean had significantly higher number of pods per plant and seed yield per hectare than un-inoculated. This pattern was observed on dry biomass. Significant varietal differences were observed on the number of seeds per pod and harvest index. Soybean variety 'TGx 1448-2E' had significantly higher number of seed per pod and harvest index than others. The least significant number of seeds per

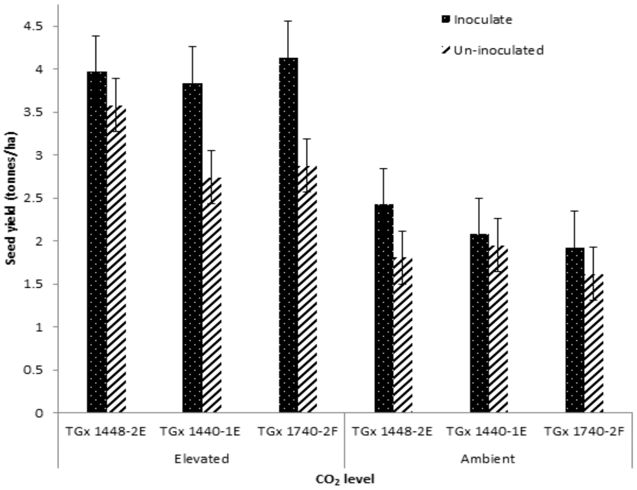

Fig. 3. Interaction of CO_2 elevation × AMF inoculation × variety on seed yield. Bars in each column indicate standard error of mean (±SE). (Field experiment)

Table 4. Effect of CO_2 enrichment and AMF inoculation on number of pods, number of seeds per pod, dry pod weight, 100-seed weight, dry, total dry biomass yield per plant, harvest index and grain yield ha^{-1} of soybean varieties at 90 % harvest maturity (Field experiment)

Treatments	Number of pods per plant	Number of seeds per pod	Dry pod weight (g $plant^{-1}$)	100-seed Weight (g)	Dry biomass (g $plant^{-1}$)	Harvest index (%)	Grain yield (t ha^{-1})
CO_2 (C) (df 1)							
Ambient	42.9	2.48	12.4	9.22	26.8	36.8	1.97
Elevated	62.7	2.52	21.4	11.10	40.3	41.0	3.52
LSD	7.3**	NS	2.42**	0.81**	5.28**	NS	0.44**
AMF (A) (df 1)							
Inoculated	56.9	2.51	19.4	10.31	38.4	36.7	3.01
Un-inoculated	48.7	2.49	14.5	10.01	28.7	41.1	2.48
LSD	7.3*	NS	2.42**	NS	5.28**	NS	0.44*
Variety (V) (df 2)							
'TGx 1448-2E'	51.0	2.74	17.4	10.33	33.8	42.2	2.95
'TGx 1440-1E'	56.3	2.28	17.4	10.10	34.7	40.0	2.65
'TGx 1740-2F'	51.1	2.49	15.9	10.05	32.1	34.4	2.64
LSD	NS	0.11**	NS	NS	NS	6.37*	NS
C × A (df 1)	NS	NS	NS	NS	NS	NS	0.62*
C × V(df 2)	NS	NS	NS	NS	NS	NS	0.77*
A × V (df 2)	NS	NS	NS	NS	NS	NS	NS
C × A × V (df 2)	NS	NS	NS	NS	NS	NS	1.08*

* indicates significance at P< 0.05 probability level; ** indicates significance at P< 0.01 probability level; NS: Not significant; WAS: Weeks after planting; df: degree of freedom

Table 5. Effect of CO_2 elevation and AMF inoculation on percentage mycorrhizal colonisation and AMF sporulation of soybean varieties at 3, 6 and 9 WAP (Field experiment)

Treatments	Colonization (%)			Spore count		
	3 WAP	6 WAP	9 WAP	3 WAP	6 WAP	9 WAP
CO_2 (C)(df 1)						
Ambient	21.7	44.2	60.7	11.2	39.8	45.7
Elevated	17.8	50.2	74.7	9.1	41.2	50.4
LSD	NS	3.85**	3.48**	NS	NS	4.0*
AMF (A)(df 1)						
Inoculated	28.9	57.6	82.7	13.6	55.6	63.1
Un-inoculated	10.6	36.9	52.7	6.7	25.4	33.1
LSD	4.8*	3.85**	3.48**	2.25**	5.27**	4.0**
Variety (V)(df 2)						
'TGx 1448-2E'	20.8	49.3	68.0	11.1	41.6	48.0
'TGx 1440-1E'	18.3	45.3	68.3	8.7	37.8	49.0
'TGx 1740-2F'	20.0	47.0	66.7	10.7	42.2	47.3
LSD	NS	NS	NS	NS	NS	NS
C × A(df 1)	NS	NS	4.92	NS	NS	5.7*
C× V(df 2)	NS	NS	NS	NS	NS	NS
A × V(df 2)	NS	NS	NS	NS	NS	6.9*
C × A × V(df 2)	NS	NS	NS	NS	NS	NS

* indicates significance at P < 0.05 probability level; ** indicates significance at P < 0.01 probability level; NS: Not significant ; WAP: Weeks after planting, df: degree of freedom

pod was observed in soybean variety 'TGx 1440-1E'. Soybean variety 'TGx 1740-2F' had significantly the least harvest index compared to others (Table 4).

Significant interaction of CO_2 × AMF × variety was observed on seed yield ha^{-1} (Fig. 3). Seed yield ha^{-1} of CO_2 enriched soybean was significantly higher than those grown in ambient CO_2 concentration. In both elevated and ambient CO_2 concentrations soybean inoculated with AMF had significantly higher seed yield ha-1 than un-inoculated. In CO_2 enriched environment soybean inoculated with AMF had seed yield ha-1 response pattern as observed in the screen house. Soybean variety 'TGx 1448-2E' had the highest seed yield ha-1 when growth in ambient CO_2 concentration, but inoculated with AMF, and in the environment where soybean was not inoculated with AMF, while the growth environment was enriched with CO_2. Soybean variety 'TGx 1740-2F' had the least seed yield ha^{-1} in the environments when inoculated with AMF but without CO_2 enrichments and without AMF inoculation but with CO_2 enrichments in the growth environment.

Effect of CO_2 enrichment and AMF inoculation on root colonisation and spore count (field experiment)

Percent root colonisation

It was observed that elevated CO2 significantly (P<0.05) increased the percentage root colonisation of the soybean varieties at 6 and 9 WAS (Table 5). However, spore count displayed similar response with CO2 enrichment only at 9 WAP.

Percentage AMF colonisation and spore count displayed similar response pattern to inoculation as observed in the screen house at all period of investigation. On the field the soybean variety had similar AMF colonisation and spore count.

Discussion

The yield potential of a grain legume crop depends upon its yield components. Dahmardeh *et al.* (2010) posited that the plant biomass is also an important factor that could affect yield and yield components of grain legumes. This could be through the provision of assimilates prior to the formulation of crop reproductive structures. These assimilates could serve as a buffer in case the sink was compromised at the reproductive growth stage through remobilisation. The improved seed yield in both trials under elevated CO_2 could be attributed to increased yield components. This was corroborated by the findings of Kimball *et al.* (2002) who reported that an increase in atmospheric concentration of CO_2 could enhance the growth and yield of C3 species. Ziska (2000) reported that elevated CO_2 increased photosynthetic rates in soybean varieties by an average of 75%, which lead to CO_2-induced increases in seed yield that averaged 40%. The increased number and weight of the reproductive structures observed in this trial with increasing CO_2 could have been as a result of increased concentration of intracellular CO_2. Increased cellular CO_2 concentration could increase carboxylation rate and reduce photorespiration that is predominant in C_3 crops. This response pattern is capable of increasing photosynthetic efficiency of soybean in the short run. However, under field condition there was stability in the grain yield increase under CO_2 enrichment. It had earlier been reported that the proportion of nitrogen partitioned to the leaf at elevated CO_2 would be reduced, with increase leaf senescence and consequently reduced photosynthetic efficiency of the canopy (Makino and Mae, 1999). The increased grain yield in the long run under field condition enriched with CO_2 could have suggested that the biological N-fixing ability of soybean could have been responsible for this seed yield response pattern in the long run contrary to earlier theoretical prediction. Increased sporulation and colonisation of soybean roots at elevated CO_2 could have suggested that the carbon economy of soybean crop supported the growth of AMF structures. The presence of these structures could act as a metabolic sink towards the stimulation of carbon assimilation apart from P uptake. Increased carbon assimilation, though not validated in this study could have suggested the availability of assimilates as observed in significantly higher yield components and grain yield of soybean inoculated with AMF than the un-inoculated.

Availability of assimilates to the fungi could stimulate uptake of P and other nutrients to soybean. This is possible in this context since the available P in the soil is below the critical P level for most tropical soils (Olatunji *et al.*, 2007). Availability of P would stimulate several key plant functions, including energy transfer (Cakmak *et al.*, 1999), canopy photosynthesis, transformation of sugars and starches (de Groot *et al.*, 2003) and nutrient movement within the plant. This is achievable since exchange of inorganic P with sucrose would aid the transportation of sucrose from the chloroplast into the cytosol. Other explanation for the stimulating effect of P on canopy photosynthetic rate could be on photophosphorylation that would make available NADPH and ATP as electron donor for the dark reaction (Bashir *et al.*, 2011). The reduced available P in this soil is another stimulus for the increased sporulation and colonization of soybean roots. Available literature had indicated that AMF infection is most predominant in marginal soils, leading to the formation of root exudates by the host for increased colonisation and infection (Smith and Read, 2008). Bass and Kuiper (1989) further reported that under AMF inoculation there could be an increase in the cytokinins content in shoots and leaves to mediate other physiological processes like stomatal behaviour and other gas exchange properties of soybean crop. Cytokinin had been implicated in increasing stomatal density and transpiration that could positively affect carbon assimilatory process and increase soybean performance (Farber *et al.*, 2016). The earlier enumerated mechanisms could have underpinned the increased seed yield ha-1 observed on the field though not experienced in the screen house. Available literature had indicated that the number of pod per plant is the precursor towards the increased sink strength observed in soybean (Borrás *et al.*, 2004). Soybean compared with wheat and maize is capable of increased response to assimilate availability (Borrás *et al.*, 2004). This pronounced sink strength on the field could be as a result of different environmental factors on the field than the pot experiment. The radiant energy on the field during the grain set and filling could exacerbate the positive influence of AMF on assimilate availability and partitioning. Other explanation on the comparatively reduced performance of soybean in the pot to that on the field with AMF inoculation could be the restriction the pot had on root volume. Reduced root volume is capable of reducing sink strength and ultimately compromise photosynthetic capacity of soybean through feedback inhibition of photosynthesis from its produce. Other reports indicated a high remobilization of assimilate in soybean compared to maize (Borrás *et al.*, 2004). This could have explained the increased HI with AMF.

The outstanding performance of soybean variety 'TGx 1448-2E' compared to other soybean varieties in both trials could be premised on its comparatively high harvest index and number of seeds per pod. The high harvest index observed on the field could be as a result of the influence of radiant energy on seed set and filling. Similar observation was made by (Borrás *et al.*, 2004). In the pot experiment the significantly higher dry pod weight observed in that variety than others could have suggested that this yield attribute contributed to its performance, though it was not validated on the field. The conservation of pod dry weight in the screen house and significant varietal variation of number of seeds per pod on the field could have suggested the presence of a stress factor on the growth and development of soybean varieties. These varieties were established in the late cropping season when the amount of rainfall was comparatively lesser than the earlier cropping season. It had been reported that there is a kind of compensatory relationship between number and weight of reproductive structures under stressful condition (Squire, 1990), with the later conversed than the former. Probably soybean variety 'TGx 1448-2E' possesses mechanism to ensure more stable yield performance under stressful conditions needed further investigation. The response pattern of AMF colonisation in both trials could be confounded by the specie composition and spore count in the soil before the trial.

The increased seed yield in both trials when soybean varieties were inoculated at different CO_2 concentrations in the atmosphere could have suggested that the presence of AMF together with other N-fixing symbionts in the soil could have acted as metabolic sink towards the stimulation of carbon assimilation and the availability of nutrient, especially P to support physiological processes in soybean. This pattern was more pronounced at elevated CO_2 in the growth environment. In both trials the superior performance of soybean variety 'TGx 1740-2F' under this condition could have indicated the genetic basis for the modification of physiological processes that lead to increased performance for this variety. Similar argument could be provided for the converse seed yield pattern observed in the absence of both growth conditions. The underlying mechanism requires further exploration in the future. The seed yield stability and superior of soybean variety 'TGx 1448-2E' than others in both trials at different combinations of CO_2 and AMF inoculation except at growth condition with elevated CO_2 concentration and when inoculated with AMF, where it occupied intermediate performance could have suggested the genetic contribution of this variety to its performance.

Conclusions

It could be concluded that at elevated CO_2 soybean varieties responded with improved sink strength as observed in the yield component positive responses. This response could be premised on the metabolic sink created by combined effects of CO_2 elevation, AMF colonization and the presence of N-fixing bacteria in symbiotic association with soybean. The evidences reported hereby supported the hypothesis of the positive effect of AMF on yield components which was validated on the field. The superior performance of soybean variety 'TGx 1448-2E' in most conditions could be premised on number of seed per pod through increased assimilate partitioning to the reproductive structure; however, when inoculated with AMF and at elevated atmospheric CO2 concentration soybean variety 'TGx 1740-2F' is most preferable.

References

Allison L (1965). Organic carbon. In: Black CA (Ed), Methods of soil analysis. Part 2. Madison: American Society of Agronomy pp 1307-1378.

Aranjuelo I, Cabrerizo PM, Aparicio-Tejo PM, Arrese-Igor C (2014). Unravelling the mechanisms that improve photosynthetic performance of N_2-fixing pea plants exposed to elevated [CO 2]. Environmental and Experimental Botany 99:167-174.

Bashir K, Ali S, Umair A (2011). Effect of different phosphorus levels on xylem sap components and their correlation with growth variables of mash bean. Sarhad Journal of Agriculture 27:595-601.

BassiriRad H, Griffin KL, Reynolds JF, Strain BR (1997). Changes in root NH_4^+ and NO_3^- absorption rates of loblolly and ponderosa pine in response to CO_2 enrichment. Plant and Soil 190(1):1-9.

Bass R, Kuiper D (1989). Effects of vesicular arbuscular mycorrhizal infection and phosphate on *Plantago major* spp. pleiosperma in relation to internal cytokinin concentration. Physiologia Plantarum 76: 211-215.

Borrás L, Slafer GA, Otegui ME (2004). Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Research 86(2):131-146.

Bouyoucos G (1962). Hydrometer method improved for making particle size analysis of soil. Agronomy Journal 54: 464-465.

Bray R, Kurtz L (1945). Determination of total, organic and available forms of phosphorus in soil. Soil Science 59:39-45.

Brown L K, George TS, Barrett GE, Hubbard SF, White PJ (2013). Interactions between root hair length and arbuscular mycorrhizal colonisation in phosphorus deficient barley (*Hordeum vulgare*). Plant and Soil 372(1-2):195-205.

Cakmak I, Engels C, Rengel Z (1999). Role of mineral nutrients in photosynthesis and yield formation. In: Mineral nutrition of crops. Fundamental mechanisms and implications. New York: The Haworth Press Inc pp 141–168.

Compant S, Van Der Heijden MG, Sessitsch A (2010). Climate change effects on beneficial plant–microorganism interactions. FEMS Microbiology Ecology 73(2):197-214.

Dahmardeh M, Ramroodi M, Valizadeh J (2010). Effect of plant density and cultivars on growth, yield and yield components of faba bean (*Vicia faba* L.). African Journal of Biotechnology 9(50):8643-8647.

de Groot C C, van den Boogaard R, Marcelis LF, Harbinson J, Lambers H (2003). Contrasting effects of N and P deprivation on the regulation of photosynthesis in tomato plants in relation to feedback limitation. Journal of Experimental Botany 54(389):1957-1967.

Drigo B, Kowalchuk GA, van Veen JA (2008). Climate change goes underground: effects of elevated atmospheric CO2 on microbial community structure and activities in the rhizosphere. Biology and Fertility of Soils 44(5):667-679.

Facelli E, Smith SE, Facelli JM, Christophersen HM, Andrew Smith F (2010). Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. New Phytologist 185(4):1050-1061.

Farber M, Attia Z, Weiss D (2016). Cytokinin activity increases stomatal density and transpiration rate in tomato. Journal of Experimental Botany pp 398.

Ferguson BJ, Gresshoff PM (2009). Soybean as a model legume. Grain Legumes 53 (7).

Gentile R, Dodd M, Lieffering M, Brock SC, Theobald PW, Newton PC (2012). Effects of long-term exposure to enriched CO2 on the nutrient-supplying capacity of a grassland soil. Biology and Fertility of Soils 48(3):357-362.

Giovannetti M, Mosse B (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84(3): 489-500.

Hikosaka K, Terashima I (1995). A model of the acclimation of photosynthesis in the leaves of C3 plants to sun and shade with respect to nitrogen use. Plant, Cell & Environment 18(6):605-618.

IPCC (2007). Climate change 2007: The physical basis. Contribution of the working group I to the fourth assessment report of the intergovernment panelon climate change. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Everyt K, Miller H (Eds). United Kingdom: Cambridge University Press.

Jackson M (1962). Soil chemical analysis. New Delhi: Prentice Hall of India Pvt, Ltd.

Jin J, Tang C, Armstrong R, Butterly C, Sale P (2013). Elevated CO_2 temporally enhances phosphorus immobilization in the rhizosphere of wheat and chickpea. Plant and Soil 368(1-2):315-328.

Jones PG, Thornton PK (2009). Croppers to livestock keepers: livelihood transitions to 2050 in Africa due to climate change. Environmental Science & Policy 12(4):427-437.

Kaschuk G, Kuyper TW, Leffelaar PA, Hungria M, Giller KE (2009). Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? Soil Biology and Biochemistry 41(6):1233-1244.

Keeling R, Piper S (2009). Atmospheric CO_2 records from sites in the SIO air sampling network (pp. 16–26). In: Boden T, Kaiser D, Sepanki R, Stoss F (Eds). Trends: A compedium of data on global change. Oak Bridge: Carbon dioxide information analysis centre, Oak ridge National Laboratory, U.S. Department of Energy.

Kiehl J (2011). Lessons from Earth's past. Science 331(6014):158-159.

Kimball BA, Kobayashi K, Bindi M (2002). Responses of agricultural crops to free-air CO_2 enrichment. Advances in Agronomy 77:293-368.

Makino A, Mae T (1999). Photosynthesis and plant growth at elevated levels of CO_2. Plant and Cell Physiology 40(10):999-1006.

McLean E O (1982). Soil pH and lime requirement. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, (methodsofsoilan2): 199-224.

Murphy J, Riley JP (1962). A modified single solution method for determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36.

Okpara DA, Muoneke CO, Ihediwa N (2007). Influence of liming on the performance of high-yielding soybean varieties in southeastern Nigeria. Agro-Science 6(2):16-24.

Olatunji US, Ayuba A, Oboh V (2007). Growth and yield of Okra and tomato as affected by pig dung and other organic manures. Proceedings of the 30th Annual Conference of the Soil Science Society of Nigeria. Makurdi.

Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55(1):158-IN18.

Poorter H (1993). Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Plant Ecology 104(1):77-97.

Richardson AE, Hocking PJ, Simpson R J, George TS (2009). Plant mechanisms to optimise access to soil phosphorus. Crop and Pasture Science 60(2):124-143.

Rychter A, Rao I (2005). Role of phosphorus in photosynthetic carbon metabolism. Handbook of photosynthesis. Tucson: Taylor and Francis Group, LLC pp123-148.

Saitoh Y, Hattori J, Chinone S, Nihei N, Tsuda Y, Kurahashi H, Kobayashi M (2004). Yeast-generated CO_2 as a convenient source of carbon dioxide for adult mosquito sampling. Journal of the American Mosquito Control Association 20(3):261-264.

Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang F(2011). Phosphorus dynamics: from soil to plant. Plant Physiology 156(3):997-1005.

Smith S, Read D (2008). Mycorrhizal symbiosis (3rd ed). Amsterdam; Boston: Academic Press.

Squire G (1990). The physiology of tropical crop production. Wallingford, UK: CABI.

Stocker T, Qin D, Plattner G, Plattner M, Tignor M, Allen S, Boschung J (2013). Summary for the policy makers in climate change 2013: The physical basis. Contribution of the working group I to the fifth assessment report of the intergovernmental panel on climate change. New York: Cambridge University Press.

Thakare K, Chore C, Deolate R, Kamble P, Suyata B, Shradha R (2006). Influence of nutrient and hornimes on biochemnical, yield and yield contributing parameters of soybean. Journal of Soils and Crops 16(1):210-216.

Ziervogel G, Nyong A, Osman B, Conde C, Cortes S, Dowing T (2006). Climate variability and change: Implications for household food security. (Assessments of impacts and adaptations to climate change (AIACC) No. 20). Washinton DC, USA: The AIACC Project Office pp 678-691.

Ziska LH (2000). The impact of elevated CO_2 on yield loss from a C3 and C4 weed in field-grown soybean. Global Change Biology 6(8):899-905.