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GROWTH, ASSIMILATE PARTITIONING AND GRAIN YIELD RESPONSE OF SOYBEAN (*Glycine max* L. Merrrill) VARIETIES TO CARBON DIOXIDE ENRICHMENT AND ARBUSCULAR MYCORRHIZAL FUNGI IN THE HUMID RAINFOREST

Sakariyawo*, O.S., Adeyemi, O.N., Atayese, M.O., and Aderibigbe, S.G.

Department of Plant Physiology & Crop Production, College of Plant Science & Crop Production, Federal University of Agriculture, Abeokuta, P.M.B. 2240, Alabata, Ogun State, Nigeria

*Corresponding author's email: adetanwa@yahoo.co.uk

ABSTRACT

This investigation tested variation in the growth components, assimilate partitioning and grain yield of soybean (Glycine max L. Merrrill) varieties established in CO_2 enriched atmosphere when inoculated with mixtures of Arbuscular mycorrhizal fungi (AMF) species in the humid rainforest of Nigeria. A pot and a field experiment were established in Abeokuta (7°15'N, 3°28'E; 75 m asl), Nigeria in 2015. The pot experiment had CO_2 concentration, AMF inoculation and soybean varieties as treatments, in completely randomised design, repeated three times. On the field the treatments were in a split split-plot arrangement fitted into randomised complete block design. The main plot had CO_2 concentration [ambient (≈385 ppm) and elevated (≈550 ppm)], AMF inoculation in the sub plot (+ AMF and – AMF), while the sub sub-plot were soybean varieties (TGx 1448-2E, TGx 1440-1E and TGx 1740-2F), replicated three times. In both experiments assimilatory surface increased in CO_2 enriched atmosphere, with increased relative growth rate. The increased relative growth rate on the field was with increased leaf relative growth rate, reduced leaf area ratio and increased net assimilatory rate. Both trials had higher grain yield at elevated CO_2 than the ambient. Growth response to AMF inoculation was with reduced specific leaf area and increased leaf weight ratio. Soybean variety TGx 1448 -2E was more adaptive to variation in AMF inoculation and CO_2 enrichment. Soybean TGx 1740-2F was more responsive to combined effect of AMF inoculation and CO_2 enrichment, but less suitable in the absence of both in this ecology.

Key words: Assimilatory surface, carbon dioxide enrichment, inoculation, soybean

INTRODUCTION

Soybean is an important oil seed crop in most parts of the world. In Nigeria, its cultivation is on the rise in most agroecological systems (Obalum *et al*., 2011). Its agronomic importance is premised on its ability to biologically fix atmospheric nitrogen; hence it forms an important component of the cropping system apart from its nutritional value (Harold *et al*., 1990).

Global attention in recent past has shifted to the implications of climate change, specifically for crop growth (Poorter, 1993) and agricultural productivity (Cure and Acock, 1986). Elevation of atmospheric carbon dioxide concentration had been reported to be part of this global climate change (Leakey *et al*., 2009). Though the carbon foot print of most developing and underdeveloped regions is low compared to the developed world, there is need to establish a proactive measure against the negative impact of climate change in these regions. The need is underlined by the fact that these regions are characterized by low input agriculture, low soil fertility (Uzoh *et al*., 2015), rainfed and subsistence in nature. This farming system is unsustainable in the long run due partly to changes in their demographic profiles (Idris *et al*., 2013). It had been reported in the literature that there is interspecific variation on growth in CO_2 enriched atmosphere (Poorter, 1993). Crops with C3 carbon assimilation were observed to benefit more under elevated CO_2 than C4, especially when other growth factors are non-limiting (Poorter, 1993). However, the C4 plants could benefit in CO_2 enriched atmosphere under drought condition (Leakey *et al*., 2009). Among the C3 it was reported that leguminous crop could benefit more in CO_2 enriched atmosphere than non-leguminous crops (Poorter, 1993). The underlying mechanism for this was reported by (Aranjuelo *et al*., 2014). Other variations observed among the C3 under elevated CO_2 was between dicotyledonous and monocotyledonous crops, with the former indicating better performance than the later (Poorter, 1993).

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Soybean crop exhibits different growth habits; determinate and indeterminate. There have been reports in the literature on the effect of varietal differences on growth of soybean with different growth habits under elevated atmospheric CO_2 (Dornhoff and Shibles, 1970). It is still unknown the effect of CO_2 enriched atmosphere on the growth components of soybean varieties in the humid tropics.

The effect of symbiotic association of crops with arbuscular mycorrhizal fungi is well reported in the literature (Augé, 2004; Kaschuk *et al*., 2009; Smith and Read, 2008; Wu and Zou, 2010). It was reported that in combination with N-fixing symbionts there could be increased metabolic sink towards improved canopy photosynthesis (Kaschuk *et al*., 2009). Meghvansi et al., (2008) reported that the response of soybean cultivar to inoculation with three arbuscular mycorrhizal in combination with *Bradyrhizobium japonicum* varies with the specie of arbuscular mycorrhizal fungi involved. There is no documented evidence on the effect of Arbuscular Mycorrhizal Fungi (AMF) mixture on the performance of soybean in the humid tropical region to the best of our knowledge. Furthermore few studies have been conducted on the combined effect of symbionts on growth components of soybean under elevated CO_2 condition.

This investigation tested the hypothesis that there would be variation in the growth components of soybean varieties established in CO_2 enriched atmosphere under the combined effects of symbionts in the humid rainforest. Understanding of this process would have an impact on the sustainable production of soybean in this agroecology. The objective of this trial was to evaluate variation on the growth, growth components, assimilate partitioning and grain yield of soybean varieties under elevated atmospheric CO_2 with mixtures of arbuscular mycorrhizal fungi.

MATERIALS AND METHODS

Pot and field experiments were conducted at Abeokuta in the year 2015.

Pot experiment

This trial was conducted in the screen house of the College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta.

Experimental Treatments and Design

This experiment consisted of CO_2 enrichment [ambient (≈385 ppm) and elevated (≈550 ppm)], with soybean varieties (TGx 1448-2E, TGx 1440-1E and TGx 1740-2F) inoculated with AMF (inoculated, uninoculated). The inoculum (Empathy Mycorrhizal Rootgrow^{TM} Fungi) was sourced commercially. It was a mixture of different AMF species. The experiment was in completely randomised design, replicated three times.

Cultural operations

Three plastic pots were used for each treatment. A total of one hundred and eight plastic pots were used. Each pot was 25 cm wide at the surface and 36 cm deep with 10 litres capacity. The pots were perforated at the bottom to allow for easy drainage of excess water without depleting the soil quantity. The sampled soil was thoroughly mixed and sieved using 2.0 mm mesh sieve. Each pot was filled with 7 kg of sieved soil that was maintained at 100% field capacity. The pots were left for 72 h before planting. Soybean seeds were obtained from the International Institute of tropical Agriculture (IITA), Ibadan.

Carbon dioxide enrichment was established in an open top chamber (OTC) that was placed in the screen house. The OTC was constructed using polyvinyl chloride (PVC) pipes. These were covered with transparent PVC nylon sheet to ease the penetration of radiant energy into the chamber. The OTC was 2.5 m in height with an area of 12 m^2. To enrich the OTC with the required concentration of carbon dioxide 12 CO_2 generator bottles were placed in the chamber. Carbon dioxide production in the chamber was implemented according to the protocol developed by Saitoh *et al.* (2004).

The maximum and minimum CO_2 concentration in the OTC was measured was at 11:00 am and 4:00 pm throughout the enrichment period (4 to 9 WAP) using a portable CO_2 meter [NDIR Gas Analyzer (Bentech GM8883), China]. The mean of the maximum and minimum CO_2 concentration was used to determine the CO_2level in the OTC. Transparent walls of the OTC were kept clean regularly in order to minimise any differences in the light levels between inside and outside the chamber. The control pots were grown in ambient atmospheric conditions. Two grammes of AMF granules were sprinkled into the base of the planting hole at planting. This was later covered with soil with soybean seeds. 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 planting (WAP). The initial spore count in 2 g of AMF granules was determined. The pots were transferred into the chamber at 4 WAP. Water was supplied thrice in a week to irrigate the plants. Weeding was conducted manually as at when due.

Sampling and data collection

A composite soil sample was collected before planting to determine soil physical and chemical properties. The soil for the trials was collected from the same experimental field. The soil was collected from a depth of 0-30 cm depth. Non destructive growth variables (number of trifoliate leaves per plant and trifoliate leaf area) were determined at 3, 6 and 9 WAP in one set of pot. Total leaf area of the

crop was calculated using the equation derived by Wiersma and Bailey (1975):

$$A = 0.411 + 2.008\ LW;$$

where A is trifoliate leaf area, and L and W are the maximum length and maximum width of the terminal leaflet of a trifoliate leaf, respectively.

Destructive sampling (mycorrhizal colonisation, AMF sporulation, dry matter accumulation and partitioning) was conducted at 6, 9 WAP and harvest maturity in the remaining two sets of pots. Grain yield per plant was determined at harvest maturity. Harvest maturity was evaluated visually when 95% of the pods had turned to brown, suggesting a reduction in the pod moisture content. Growth variables were determined using classical approach with two means values obtained at two harvest intervals of 21 days. The primary data (leaf area and dry weight) for the determination of growth components were transformed using log to base 10.

Relative growth rate (RGR), leaf relative growth rate (LRGR), leaf area ratio (LAR), specific leaf area (SLA), specific leaf weight (SLW) and net assimilatory rates (NAR) were determined according to the methods proposed by Hunt (2012). Fractional distribution of the assimilate to different organs was also determined for the leaf and stem. The partitioned plant parts were oven dried at 70˚C to a constant weight to determine leaf and stem weight fractions, which were the proportions of these organs to the total dry weight. Chlorophyll content was determined using SPAD chlorophyll meter.

Mycorrhizal Analysis

Root samples were collected from the pots for the determination of percentage root colonisation. Collected root samples were prepared using the method as described Phillips and Hayman (1970). Prepared root samples were rinsed off staining solution with clean tap water, cut into 1-cm pieces. They were preserved with 40% glycerol solution for further viewing under compound microscope to determine percentage root colonisation (PCR):

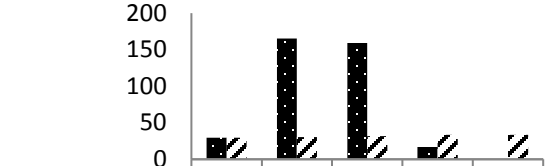
$$PCR = \frac{Number\ of\ infected\ roots}{Total\ number\ of\ roots} \times 100$$

From the rhizosphere 20 g of well mixed soil samples were collected from the pot for the determination of the soil spore count before and after inoculation. Extraction of AMF spores from the soil sample was conducted using the modified wet sieving method of Giovannetti and Mosse (1980). Extracted spores were identified using digital compound microscope and counted under dissecting slides. The initial spore count in the 20 g of the soil was between 3-5 spores.

Field experiment

Characterisation of location and experimental site

This was carried out at the Federal University of Agriculture Abeokuta (FUNAAB), Ogun State, Nigeria. The geographical location lies in the South-Western Nigeria (7˚15'N, 3˚28'E; 75 m asl). The particle size analysis showed that 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 %. The textural class of the site was determined using the USDA textural triangle. The collected soil sample was air-dried after collection. 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) as described by (McLean, 1982). The organic carbon content of the samples was determined using Walkey-Black method as modified by Allison *et al*. (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 1 N ammonium acetate buffered at pH of 7. Sodium and K^+ in the extract were determined by flame photometry, Ca^{2+} and Mg^{2+} were determined using Atomic Absorption Spectrophotometer (AAS). Total acidity (H^++Al^{3+}) was determined using KCl as the extracting medium. Cation exchange capacity was determined by the summation of total exchangeable bases and exchangeable acidity. 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).



	AUG	SEPT	OCT	NOV	DEC
RAINFALL (MM)	29.4	165.1	159.1	16.6	0
MAX TEMPERATURE ˚C	29.5	30.4	31.6	33.5	33.5

Figure 1: Rainfall pattern of the experimental location during 2015 cropping season

Experimental Treatments and Design

The field experiment had similar treatments with the pot trial. The treatments were arranged in split split-plot with CO_2 concentration [ambient (≈385 ppm) and elevated (≈550 ppm)] in the main plot, AMF inoculation in the subplot (+ AMF and – AMF), while the sub-sub-plot consisted of soybean variety (TGx 1448-2E, TGx 1440-1E and TGx 1740-2F). AMF inoculation had similar source as that in the pot trial. This arrangement was fitted into randomised complete blocked design, replicated three times.

Cultural operations

The initial spore count of the soil used was determined prior to field establishment. The field was ploughed twice and disc-harrowed once two weeks later for proper field establishment. The gross plot size measured 2 × 2 m (4 m^2) and net plot size was 1.5 × 1.5 m (2.25 m^2). The space between plots was 0.5 m, while between replicates was 1 m. Three seeds of soybean were planted per hole at a depth of 2-5 cm and at a spacing of 50 × 10 cm on 10th of August 2015. It was later thinned to one plant per stand two weeks after planting (WAP). This translated to five rows per plot, with each row consisting of 21 plants. The plant density was 105 plants per plot. Treated plots were inoculated with AMF inoculums during planting. The planting hole was sprinkled with 25 g of commercially sourced inoculums, which was covered with soil. On CO_2 enriched plots soybean seeds were established in OTC that measured 2 m height and area of 31.5 m^2. They were installed on the plots at 4WAP. They were made of the same materials as those constructed for the screen house. Generation of CO_2, its monitoring and enrichment period was as described for the screen house trial. Weeding was done manually at 3, 6 and 9 WAP.

Sampling and data collection

Five plants from the net plot were randomly chosen for the determination of growth variables. Destructive sampling was conducted from the rows outside the net plot aside from the two rows bordering the gross plot. The growth variables were the same as those determined in the screen house except Leaf Area Index on the 3, 6 and 9 WAP. Leaf area index (LAI) was determined as the ratio of leaf area to unit land area occupied by the plant. Growth analysis followed the classical approach as described for the screen house trial. Mycorrhizal colonisation, spore count, dry matter accumulation and factional distribution of the assimilates followed similar protocol as in the screen house. Grain yield per hectare was determined at harvest maturity as earlier described and was extrapolated from the net plot.

Statistical analysis

Analysis of Variance (ANOVA) using a fixed model was conducted to determine significant differences among the means at 5% probability level. A mixed model ANOVA was conducted for the field trial. Carbon dioxide, AMF and soybean variety were the fixed factors. Discrete data were transformed using square root transformation prior to analysis. Significant means were separated using Least Significant Difference (LSD). The statistical package used was Genstat 12th Edition.

RESULTS

Pot Experiment

Soybean crop exposed to elevated CO_2 had significantly more number of trifoliate leaves and leaf area at 6 and 9 WAP than those exposed to ambient CO_2 concentration except at 3 WAP, where there were no significant differences on the number of trifoliate leaves in soybean at different atmospheric CO_2 concentration (Table 1). At all periods of investigation chlorophyll content was similar in both enriched and ambient concentration of CO_2 in the atmosphere. There were no significant differences between inoculated and uninoculated soybean on the number of trifoliate leaves except at 3 WAP. Inoculated soybean had more number of trifoliate leaves than uninoculated. Similar pattern was observed on the leaf area at 3 and 6 WAP. Inoculation had no significant effect on the chlorophyll content at all periods of investigation. There were no significant varietal differences on the aforementioned growth variables during the period of investigation (Table 1).

Soybean crop exposed to CO_2 enriched atmosphere had significantly higher aboveground dry weight than those exposed to ambient CO_2 concentration at 9 WAP and at harvest maturity (Table 2). Inoculated soybean crop had significantly higher aboveground dry weight than uninoculated ones at all periods of investigation except 6 WAP. Soybean varieties had similar aboveground dry weight at all periods of investigation (Table 2). The CO_2 enrichment had no significant effect on leaf weight ratio and stem weight ratio at all periods of investigation (Table 3). At 9 WAP more assimilates were partitioned into leaf in inoculated soybean than uninoculated. Contrarily, at 9 WAP more assimilates were partitioned into the stem in uninoculated soybean than inoculated. There were no significant varietal differences on leaf mass and stem mass ratio at all periods of investigation (Table 3). Soybean crop grown in enriched atmosphere had similar specific leaf area with those grown at ambient CO_2 concentration (Table 4). Similar pattern was observed on specific leaf weight and leaf area ratio at all periods of investigation (Table 4). Uninoculated soybean crop had significantly

Table 1: Effect of CO_2 enrichment and AMF inoculation on number of trifoliolate leaves, trifoliolate leaf area and chlorophyll content of soybean cultivars at 3, 6 and 9 WAS (screen house experiment)

	Number of trifoliolate leaves			Leaf area (cm^2)			Chlorophyll content		
Treatments	3 WAP	6 WAP	9 WAP	3 WAP	6 WAP	9 WAP	3 WAP	6WAP	9 WAP
CO_2 Level (C)									
Ambient	2.55	10.06	18.11	30.91	65.4	101.1	37.3	34.2	32.2
Elevated	2.61	15.67	27.11	27.14	91.8	155.1	37.6	35.1	33.5
LSD	NS	2.41**	2.52**	NS	6.8**	17.5**	NS	NS	NS
AMF (A)									
Inoculated	3.0	13.28	23.56	33.28	82.8	133.1	37.8	35.0	33.1
Un-inoculated	2.17	12.44	21.67	24.78	74.4	123.1	37.11	34.2	32.7
LSD	0.36**	NS	NS	3.79**	6.8*	NS	NS	NS	NS
Varieties (V)									
TGx 1448-2E	2.50	11.67	21.92	26.93	79.9	130.4	36.6	35.9	34.2
TGx 1440-1E	2.58	14.08	23.75	30.32	76.2	129.4	39.7	34.5	33.5
TGx 1740-2F	2.67	12.83	22.17	29.83	79.7	124.4	36.2	33.4	31.2
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS
C × A	NS	NS	NS	NS	NS	NS	NS	NS	NS
C× V	NS	NS	NS	NS	11.8*	NS	NS	NS	NS
A × V	NS	NS	NS	NS	NS	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS	16.65*	NS	NS	NS	NS

*Significant at 5% probability level; **Significant at 1% probability level NS: Not significant, WAP: weeks after planting

Table 2: Effects of CO_2 enrichment and AMF inoculation on aboveground dry weight of soybean cultivars at 6 and 9 WAP and harvest maturity (screen house experiment)

	Aboveground dry weight (g $plant^{-1}$)		
Treatments	6 WAP	9 WAP	Harvest maturity
CO_2 level (C)			
Ambient	1.78	5.25	20.34
Elevated	2.22	8.74	31.27
LSD	NS	1.87**	3.43*
AMF (A)	1.99	7.97	30.15
Inoculated			
Un-inoculated	2.01	6.03	21.46
LSD	NS	1.87*	3.43*
Varieties (V)			
TGx 1448-2E	1.86	6.77	23.10
TGx 1440-1E	2.06	7.14	27.28
TGx 1740-2F	2.08	7.08	27.03
LSD	NS	NS	NS
C × A	NS	NS	4.73**
C× V	NS	NS	NS
A × V	NS	NS	NS
C × A × V	NS	NS	8.19*

*Significant at 5% probability level; **Significant at 1% probability level, NS: not significant, WAP: weeks after planting

Table 3: Effects of CO_2 enrichment and AMF inoculation on leaf weight ratio and stem weight ratio of soybean cultivars at 6 and 9 WAS (screen house experiment)

Treatments	Leaf weight ratio		Stem weight ratio	
	6 WAP	9 WAP	6 WAP	9 WAP
CO_2 level (C)				
Ambient	0.78	0.77	0.22	0.23
Elevated	0.77	0.76	0.23	0.24
LSD	NS	NS	NS	NS
AMF (A)				
Inoculated	0.78	0.80	0.22	0.20
Un-inoculated	0.77	0.73	0.23	0.27
LSD	NS	0.065*	NS	0.065*
Varieties (V)				
TGx 1448-2E	0.77	0.77	0.23	0.23
TGx 1440-1E	0.78	0.79	0.22	0.21
TGx 1740-2F	0.78	0.73	0.22	0.27
LSD	NS	NS	NS	NS
C × A	NS	NS	NS	NS
C × V	NS	NS	NS	NS
A × V	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS

*Significant at 5% probability level; **Significant at 1% probability level, NS: Not significant, WAP: weeks after planting

Table 4: Effects of CO_2 enrichment and AMF inoculation on specific leaf area, specific leaf weight and leaf area ratio of soybean cultivars at 6 and 9 WAS (pot experiment)

Treatments	Specific leaf area (cm^2 g^{-1})		Specific leaf weight (g cm^{-2})		Leaf area ratio	
	6 WAP	9 WAP	6 WAP	9 WAP	6 WAP	9 WAP
CO_2 level (C)						
Ambient	53.9	30.6	0.023	0.042	42.0	22.8
Elevated	57.0	28.1	0.019	0.044	44.2	19.5
LSD	NS	NS	NS	NS	NS	NS
AMF (A)						
Inoculated	58.5	23.4	0.019	0.047	45.9	18.5
Un-inoculated	52.4	35.4	0.022	0.039	40.2	23.7
LSD	NS	11.6*	NS	NS	NS	NS
Varieties (V)						
TGx 1448-2E	63.2	30.7	0.018	0.039	48.8	23.1
TGx 1440-1E	50.0	26.2	0.023	0.046	38.9	19.9
TGx 1740-2F	53.1	31.2	0.021	0.044	41.5	20.3
LSD	NS	NS	NS	NS	NS	NS
C × A	NS	NS	NS	NS	NS	NS
C × V	NS	NS	NS	NS	NS	NS
A × V	NS	NS	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS	NS	NS

*Significant at 5% probability level; **Significant at 1% probability level, NS: Not significant, WAP: weeks after planting

Table 5: Effects of CO_2 enrichment and AMF inoculation on relative leaf growth rate, relative growth rate, net assimilatory ratio and grain yield of soybean cultivars (pot experiment)

Treatments	Leaf relative growth rate ($g\ g^{-1}\ day^{-1}$)	Relative growth rate ($g\ g^{-1}\ day^{-1}$)	Net assimi-latory ratio	Grain yield ($g\ plant^{-1}$)
	R2	R2	R2	Harvest maturity
CO_2 level (C)				
Ambient	0.048	0.049	0.0021	7.27
Elevated	0.062	0.064	0.0026	10.68
LSD	NS	0.012*	NS	1.03**
AMF (A)				
Inoculated	0.065	0.064	0.0026	9.31
Un-inoculated	0.046	0.050	0.0021	8.64
LSD	0.014*	0.012*	NS	NS
Varieties (V)				
TGx 1448-2E	0.058	0.058	0.0021	9.90
TGx 1440-1E	0.058	0.058	0.0025	8.31
TGx 1740-2F	0.050	0.054	0.0024	8.71
LSD	NS	NS	NS	1.26*
C × A	NS	NS	NS	NS
C × V	NS	NS	NS	NS
A × V	0.024*	0.021*	NS	NS
C × A × V	0.034*	NS	NS	2.52*

*Significant at 5% probability level; **Significance at 1% probability level, NS: Not significant, WAP: weeks after planting, R2: 6-9 WAP

Table 6: Effects of CO_2 elevation and AMF inoculation on percent mycorrhizal colonization and AMF sporulation of soybean cultivars at 6 and 9 WAP (screen house experiment)

Treatments	Percent mycorrhizal colonization (%)		Spore count	
	6 WAP	9 WAP	6 WAP	9 WAP
CO_2 LEVEL (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
Uninoculated	30.7	43.9	36.4	51.4
LSD	4.27**	2.81**	4.63**	5.81**
Varieties (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

*Significant at 5% probability level; **Significant at 1% probability level, NS: Not significant, WAS: Weeks after planting

higher specific leaf area than inoculated at 9 WAP except at 6 WAP, when there were no significant differences between both. There were no significant varietal differences on specific leaf area, specific leaf weight and leaf area ratio at all periods of investigation (Table 4). The relative growth rate of soybean grown in CO_2 enriched atmosphere was significantly higher than ambient at R2 (Table 5). Leaf relative growth rate of inoculated soybean was significantly higher than uninoculated at R2. Similar pattern was observed on the relative growth rate.

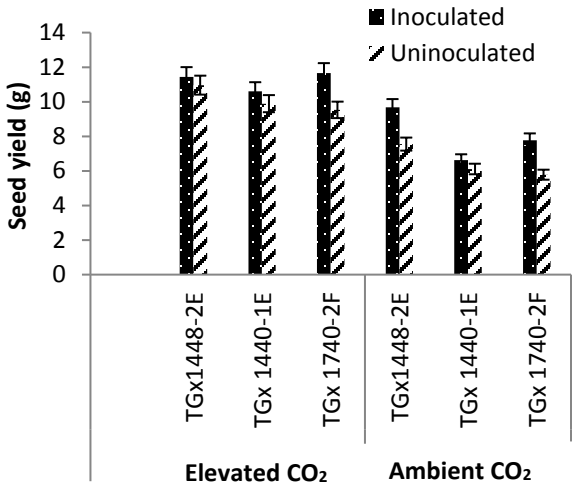


Figure 2: Interaction of CO_2 enrichment × AMF inoculation × variety on seed yield, pot experiment. Bars in each column indicate standard error of mean (±SE).

There were no significant differences among the varieties of soybean on leaf relative growth rate, relative growth rate and net assimilatory ratio at R2 (Table 5). Grain yield per plant was significantly higher in soybean grown in CO_2 enriched atmosphere than ambient (Table 5). Significant varietal differences were observed on grain yield per plant. Grain yield per plant decrease was in the order TGx 1448-2E > TGx 1740-2F > TGx 1440-1E (Table 5). Significant CO_2 × AMF× Variety was observed on grain yield per plant (Fig. 2). Seed yield per plant of inoculated soybean was significantly higher than uninoculated at both CO_2 enriched and ambient growth environments. Under CO_2 enriched growth environment seed yield of inoculated soybean varieties was in a decreasing order of TGx 1740-2F > TGx 1448-2E > TGx 1440-1E. Under CO_2 enriched growth environment seed yield per plant of uninoculated soybean varieties was in a decreasing order of TGx 1448-2E > TGx 1440-1E >TGx 1740-2F. Soybean sown at elevated CO_2 concentration had significantly higher percentage mycorrhizal colonisation than ambient at all periods of investigation (Table 6). Similar pattern was observed on spore count at 9 WAP except at 6 WAP, where no significant differences were observed on spore count between soybean sown at elevated CO_2 and those grown under ambient environment. At all period of investigation percentage AMF colonisation was significantly higher in inoculated soybean than un-inoculated. Similar pattern was observed on spore count at all sampling period. There were no significant varietal differences on percentage AMF colonisation and spore count at all investigation periods (Table 6).

Table 7: Effects of CO_2 enrichment and AMF inoculation on number of trifoliolate leaves and chlorophyll content of soybean cultivars at 3, 6 and 9 WAP (field experiment)

Treatments	Number of trifoliolate Leaves			Chlorophyll contemt		
	3 WAP	6 WAP	9 WAP	3 WAP	6 WAP	9 WAP
CO_2 level (C)						
Ambient	2.23	14.04	22.15	44.79	41.98	36.43
Elevated	2.46	16.11	27.61	43.96	41.77	36.34
LSD	NS	0.28*	0.38*	NS	NS	NS
AMF (A)						
Inoculated	2.34	16.44	25.29	45.32	41,92	36.89
Uninoculated	2.35	13.71	24.47	43.43	41.83	35.88
LSD	NS	0.28*	NS	NS	NS	NS
VARIETIES (V)						
TGx 1448-2E	2.08	13.30	25.24	44.44	42.86	36.52
TGx 1440-1E	2.53	15.79	25.32	45.37	41.88	36.89
TGx 1740-2F	2.43	16.12	24.08	43.32	40.89	35.73
LSD	NS	NS	NS	NS	NS	NS
C × A	NS	0.39*	NS	NS	NS	NS
C× V	NS	NS	NS	NS	NS	NS
A × V	NS	NS	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS	NS	NS

*Significant at 5% probability level; **Significance at 1% probability level, NS: Not significant, WAP: weeks after planting

Table 8: Effects of CO_2 enrichment and AMF inoculation on leaf area and leaf area index of soybean cultivars at 3, 6 and 9 WAP in 2015 (field experiment)

Treatments	Leaf area (cm^2)			Leaf area index		
	3 WAP	6 WAP	9 WAP	3 WAP	6 WAP	9 WAP
CO_2 Level (C)						
Ambient	28.7	108.6	151.9	0.13	3.05	6.80
Elevated	31.0	160.8	189.7	0.15	5.33	10.71
LSD	NS	1.92**	17.21**	NS	1.003*	1.71**
AMF (A)						
Inoculated	29.9	140.3	182.4	0.14	4.65	9.50
Un-inoculated	29.8	129.2	159.2	0.14	3.73	8.01
LSD	NS	NS	17.21*	NS	NS	NS
Varieties (V)						
TGx 1448-2E	30.0	139.1	182.4	0.13	3.92	9.37
TGx 1440-1E	31.6	135.8	187.0	0.16	4.40	9.82
TGx 1740-2F	28.0	129.1	143.1	0.14	4.26	7.08
LSD	NS	NS	21.08**	NS	NS	2.09*
C × A	NS	NS	NS	NS	NS	NS
$C_\times$ V	NS	NS	NS	NS	NS	NS
A × V	NS	NS	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS	NS	NS

*Significant at 5% probability level; **Significant at 1% probability level, NS: Not significant, WAP: weeks after planting, R1: 3-6 WAP, R2: 6-9 WAP

Table 9: Effects of CO_2 enrichment and AMF inoculation on leaf weight ratio and stem weight ratio of soybean cultivars at 3, 6 and 9 WAP in 2015 (field experiment)

Treatments	Leaf weight ratio			Stem weight ratio		
	3 WAP	6 WAP	9 WAP	3 WAP	6 WAP	9 WAP
CO_2 level (C)						
Ambient	0.64	0.73	0.72	0.36	0.27	0.28
Elevated	0.63	0.72	0.70	0.37	0.28	0.30
LSD	NS	NS	NS	NS	NS	NS
AMF (A)						
Inoculated	0.65	0.73	0.75	0.35	0.27	0.25
Un-inoculated	0.63	0.72	0.67	0.38	0.28	0.33
LSD	NS	NS	0.06*	NS	NS	0.06*
Varieties (V)						
TGx 1448-2E	0.63	0.72	0.72	0.37	0.28	0.28
TGx 1440-1E	0.65	0.73	0.74	0.35	0.27	0.26
TGx 1740-2F	0.62	0.73	0.68	0.38	0.27	0.32
LSD	NS	NS	NS	NS	NS	NS
C × A	NS	NS	NS	NS	NS	NS
$C_\times$ V	NS	NS	NS	NS	NS	NS
A × V	NS	NS	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS	NS	NS

*Significant at 5% probability level; **Significant at 1% probability level, NS: Not significant, WAP: weeks after planting

Field experiment

The response of the number of trifoliate leaves and leaf area of soybean to enriched CO_2 at 6 and 9 WAP followed similar pattern as was observed in the screen house trial (Tables 7 and 8). Inoculated soybean had significantly higher number of trifoliate leaves at 6 WAP than uninoculated. At 9 WAP similar pattern was observed on leaf area (Table 8). Significant varietal differences were observed on leaf area at 9 WAP. Increase in leaf area among the varieties was in the order TGx 1440-1E > TGx 1448-2E > TGx 1740-2F. Similar pattern was observed on leaf area index at 9 WAP (Table 8). At 9 WAP leaf weight ratio was more in inoculated than uninoculated (Table 9). Contrarily, stem weight ratio was more in un-inoculated soybean than inoculated at 9 WAP. Above ground dry biomass was significantly higher in soybean exposed to elevated CO_2 than ambient atmospheric condition at all period of investigation except at 3 WAP (Table 10). Uninoculated soybean had significantly more above ground dry biomass than inoculated at 9 WAP; however at harvest maturity a reverse pattern was observed. There were no significant varietal differences on above ground dry biomass at all periods of investigation (Table 10). Enrichment of the growth environment had no significant effect on leaf area ratio at all periods of investigation except on the 9 WAP. It was observed that soybean sown at elevated CO_2 had significantly higher leaf area ratio than those sown in the ambient CO_2 (Table 11). At all periods of investigations, leaf relative growth rate, relative growth rate and net assimilatory rate were significantly higher in soybean grown in CO_2 enriched environment that those in ambient CO_2 concentration except leaf relative growth rate at R2 (Table 12). There were no significant differences on leaf relative growth rate of soybean grown at elevated and ambient CO_2 concentration at R2 (Table 12). Grain yield was significantly higher in soybean grown at elevated CO_2 than those at ambient CO_2 concentration. At R2 leaf relative growth rate and relative growth rate was significantly higher in inoculated soybean than uninoculated. Inoculated soybean had significantly higher grain yield than uninoculated. There were no significant differences with respect to the aforestated leaf morphological characters and grain yield among the soybean varieties. Significant CO_2 × AMF × Variety was observed on the grain yield per hectare (Fig. 3). Inoculated soybean had significantly higher grain yield per hectare for all soybean varieties at both elevated and ambient CO_2 concentration. The order of decrease in grain yield for soybean varieties inoculated at elevated CO_2 was TGx 1740-2F > TGx 1448-2E > TGx 1440-1E. The order of decrease in grain yield of uninoculated soybean grown at elevated

Table 10: Effects of CO_2 enrichment and AMF inoculation on aboveground dry biomass of Soybean cultivars at 3, 6, 9 WAP and harvest maturity in 2015 (field experiment)

Treatments	Aboveground dry biomass (g plant^{-1})			
	3 WAP	6 WAP	9 WAP	Harvest maturity
CO_2 level (C)				
Ambient	0.25	2.39	7.03	26.8
Elevated	0.24	2.99	11.77	40.3
LSD	NS	0.56*	2.27**	5.28*
AMF (A)				
Inoculated	0.23	2.67	8.18	38.4
Un-inoculated	0.25	2.71	10.62	28.7
LSD	NS	NS	2.27*	5.28**
Varieties (V)				
TGx 1448-2E	0.25	2.51	9.06	33.8
TGx 1440-1E	0.24	2.76	9.53	34.7
TGx 1740-2F	0.24	2.80	9.61	32.1
LSD	NS	NS	NS	NS
C × A	NS	NS	NS	NS
C× V	NS	NS	NS	NS
A × V	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS

Significant at 5 % probability level; ** Significance at1 % probability level, NS: Not significant, WAP: Weeks after planting

Table 11: Effects of CO_2 enrichment and AMF inoculation on specific leaf area, specific leaf weight and leaf area ratio of soybean cultivars at 3, 6 and 9 WAP in 2015 (field experiment)

	Specific leaf area (cm^2 g^{-1})			Specific leaf weight (g cm^{-2})			Leaf area ratio		
Treatments	3 WAS	6 WAS	9 WAS	3 WAS	6 WAS	9 WAS	3 WAS	6 WAS	9 WAS
CO_2 Level (C)									
Ambient	189.9	80.4	26.4	0.006	0.017	0.035	121.5	49.7	25.8
Elevated	213.6	68.3	37.5	0.005	0.014	0.045	132.1	57.7	17.3
LSD	NS	NS	NS	NS	NS	NS	NS	NS	6.1**
AMF (A)									
Inoculated	208.0	78.9	26.8	0.005	0.015	0.043	132.6	57.7	20.1
Un-inoculated	195.5	69.8	37.1	0.006	0.016	0.037	121.0	49.7	23.0
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS
Varieties (V)									
TGx 1448-2E	200.9	86.5	35.9	0.005	0.014	0.035	125.2	62.3	25.0
TGx 1440-1E	212.5	70.2	30.9	0.005	0.015	0.039	137.7	50.8	21.7
TGx 1740-2F	191.9	66.4	29.1	0.006	0.017	0.046	117.6	48.0	17.9
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS
C × A	NS	NS	NS	NS	NS	NS	NS	NS	NS
C × V	NS	NS	NS	NS	NS	NS	NS	NS	NS
A × V	NS	NS	NS	NS	NS	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Significant at 5% probability level; **Significant at 1% probability level, NS: Not significant, WAP: weeks after planting

Table 12: Effects of CO_2 enrichment and AMF Inoculation on relative leaf growth rate, relative growth rate and net assimilatory ratio of soybean cultivars (field experiment)

Treatments	Leaf relative growth rate ($g\ g^{-1}\ day^{-1}$)		Relative growth rate ($g\ g^{-1}\ day^{-1}$)		Net assimilatory ratio		Grain yield ($t\ ha^{-1}$)
	R1	R2	R1	R2	R1	R2	
CO_2 Level (C)							
Ambient	0.112	0.049	0.106	0.049	0.152	0.068	1.97
Elevated	0.126	0.062	0.119	0.065	0.174	0.092	3.52
LSD	0.012*	NS	0.01*	0.012*	0.016**	0.017**	0.44**
AMF (A)							
Inoculated	0.121	0.065	0.114	0.064	0.163	0.086	3.01
Un-inoculated	0.118	0.046	0.111	0.051	0.162	0.074	2.48
LSD	NS	0.014*	NS	0.012*	NS	NS	0.44**
Varieties (V)							
TGx 1448-2E	0.114	0.058	0.108	0.058	0.156	0.080	2.95
TGx 1440-1E	0.122	0.058	0.116	0.058	0.165	0.078	2.65
TGx 1740-2F	0.122	0.050	0.114	0.055	0.166	0.081	2.64
LSD	NS	NS	NS	NS	NS	NS	NS
C × A	NS	NS	NS	NS	NS	NS	0.62*
C × V	NS	NS	NS	NS	NS	NS	0.77*
A × V	NS	NS	NS	NS	NS	NS	NS
C × A × V	NS	NS	NS	NS	NS	NS	1.08**

*Significant at 5% probability level; **Significant at 1% probability level, NS: Not significant, WAP: weeks after planting R1: 3-6 WAP, R2: 6-9 WAP

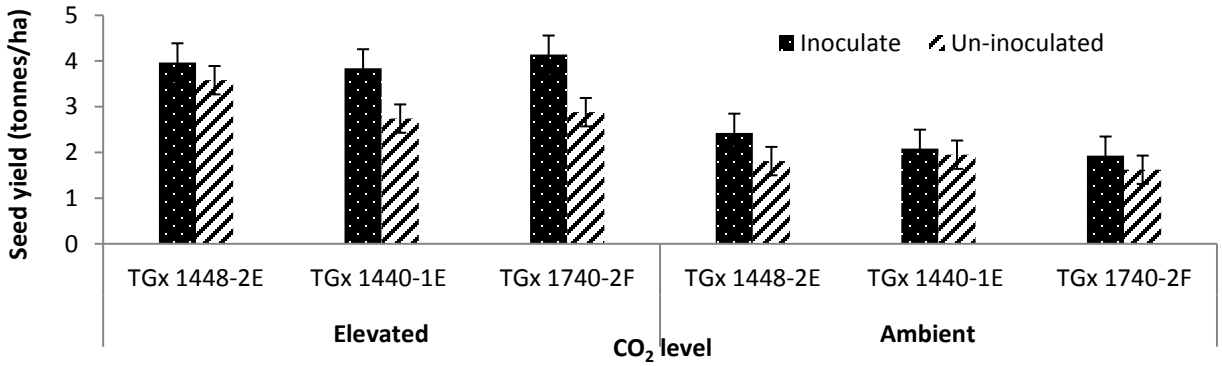


Figure 3: Interaction of CO_2 elevation × AMF inoculation × variety on seed yield $t\ ha^{-1}$, field experiment. Bars in each column indicate standard error of mean (±SE)

CO_2 was TGx 1448-2E > TGx 1740-2F > TGx 1440-1E. At ambient CO_2 concentration grain yield ha^{-1} of inoculated soybean varieties decreased in the order TGx 1448-2E > TGx 1440-1E > TGx 1740-2F. At ambient CO_2 concentration grain yield ha^{-1} of uninoculated soybean varieties decreased in the order TGx 1440-1E > TGx 1448-2E > TGx 1740-2F. Mycorrhizal colonisation was significantly higher in soybean grown at elevated CO_2 than at ambient CO_2 concentration at all periods of investigation except at 3 WAP, when there was no significant effect on this variable (Table 13). There was no significant effect of CO_2 enrichment on the soil spore count at all periods of investigation except at 9 WAP. At 9 WAP soybean crop grown at elevated CO_2 concentration had significantly higher soil spore count than those grown at ambient CO_2 concentration. Percentage AMF colonisation and soil spore count were significantly higher in inoculated soybean than uninoculated at all periods of investigation.

DISCUSSION

Increased number of trifoliate leaves and leaf area at 6 and 9 WAP in both trials under elevated CO_2 in the growth environment could have suggested increased canopy closure with increased light capture to facilitate carbon assimilation. On the field at the canopy level increased assimilatory surface in CO_2 enriched environment was facilitated by significantly higher leaf area index than soybean grown at ambient CO_2 concentration. Ainsworth *et al*. (2002) reported similar response where she indicated that there was 39 % and 13 % increase in photosynthetic rate and leaf area of soybean respectively under elevated CO_2 condition. The high carbon assimilatory process under elevated CO_2 in the atmosphere could have supported increased relative growth rate especially at the earlier period of soybean growth. Rogers *et al*. (1984) had reported that there was a significant depression in the stimulating effect of elevated CO_2 on growth parameters with time. In this investigation,

Table 13: Effects of CO_2 elevation and AMF inoculation on percent mycorrhizal colonization and AMF sporulation of soybean cultivars at 3, 6 and 9 WAP (field experiment)

Treatments	Percent mycorrhizal colonization (%)			Spore count		
	3 WAP	6 WAP	9 WAP	3 WAP	6 WAP	9 WAP
CO_2 Level (C)						
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)						
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**
Varieties (V)						
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	NS	NS	4.92	NS	NS	5.7*
C× V	NS	NS	NS	NS	NS	NS
A × V	NS	NS	NS	NS	NS	6.9*
C × A × V	NS	NS	NS	NS	NS	NS

*Significant at 5% probability level; **Significant at 1% probability level, NS: Not significant, WAP: weeks after planting

this was supported by the significant increase in the speed of canopy development as evidence in significantly higher leaf relative growth rate on the field at R1 and R2 than soybean cultivated under ambient CO_2 condition. This evidence was supported by the observation made by Cure *et al.* (1987). This response pattern was equally corroborated by Poorter (1993), where he indicated that among the C3 crops the faster their growth under elevated CO_2 the higher the stimulating effect of CO_2. On the field a converse pattern was observed on leaf area ratio with time under elevated CO_2. Available literature had indicated that with increase in CO_2 concentration in the atmosphere this could lead to the accumulation of starch (Makino and Mae, 1999) and increase in support tissue (Konings *et al*., 1989). Accumulation of starch in the chloroplast could facilitate feedback inhibition of carbon assimilation (Clough *et al*., 1981). The increase in the support tissue could lead to the mutual shading in the canopy and a down regulation of photosynthetic process (Konings *et al*., 1989). Increased above ground dry matter accumulation at 9 WAP in the screen house under elevated CO_2 condition could have suggested a favourable carbon budgeting. Bunce and Caufield, (1991) reported a reduced respiratory rate per biomass in CO_2 enriched environment in three herbaceous perennial species. This observation could have implied that more of carbon is incorporated into the formation of growth process than maintenance respiration. Finn and Brun (1982) and Cure *et al*. (1987) indicated that at elevated CO_2 increased dry weight observed could be due to the accumulation of non structural carbohydrate. Increased relative growth rate in both trials in CO_2 enriched environment especially at the early period of soybean development could have explained the significantly higher grain yield per plant observed in the screen house. This response pattern was equally validated on the field. However, on the field it could be suggested that the increased relative growth rate at R1 and R2 was as a result of increased net assimilatory rate in both periods with a significant depression in leaf area ratio at 9 WAP. Poorter (1993) suggested that photosynthetic rate and growth among different species under elevated CO_2 could also be stimulated by the source:sink balance. In this investigation the presence of nitrogen fixing and AMF symbionts could have acted as metabolic sink. This hypothesis is supported by the significant increase in percentage AMF colonisation and spore count under elevated CO_2 than soybean cultivated in ambient CO_2 condition. In both trials increased spore count and percentage AMF colonisation could have acted as metabolic sink through the use of assimilates provided by the host soybean and the stimulation of photosynthetic rate through the supply of phosphorus and nitrogen from the symbionts. Supply of nitrogen is very important for the synthesis of light harvesting complex and the enzymes responsible for the normal functioning of photosynthesis. Cave *et al*. (1981) had earlier reported that leaves of *Trifolium subterraneum* experienced reduced chlorophyll a to b ratio per dry weight in CO_2 enriched atmosphere. They further posited that under this condition accumulation of starch granules in the chloroplast together with the earlier mentioned observation could result in chlorosis. However, soybean as a legume can fix N biologically. This could have explained the non-significant effect of elevated CO_2 on chlorophyll content observed in these trials. Phosphorus is a critical growth limiting factor in this agroecology. The availability of which could improve the performance of soybean through its effect on the supply of reducing agents to drive the dark reaction of photosynthesis.

Similar interpretation could be adduced to the AMF effect on the assimilatory surface (leaf area and number of trifoliate leaves) of soybean under elevated CO_2 in both trials. However, the increased assimilatory surface observed in AMF inoculated soybean could be ascribed to further changes in soybean morphology and their earlier development. This was evident in the increased leaf weight ratio, a reduction in the specific leaf area and rapid canopy development especially in the screen house trial. The field trial was established during the August break of the cropping season. The reduced rainfall at the time of sowing might have affected leaf expansion in the field. Water availability affects cellular elongation and growth (Boyer, 1988). The reduced specific leaf area might have increased chloroplast surface area and CO_2 concentration in the intracellular spaces (Siebenkäs *et al*., 2015). Siebenkäs *et al*. (2015) also reported that increased intracellular CO_2 could lead to a reduced Rubisco:CO_2 ratio. Considering these response patterns of soybean to AMF inoculation, there might have been increased carboxylation and reduced oxygenation resulting in an increased photosynthetic process. This supposition could be inferred from the increased relative growth rate and dry matter accumulation observed in soybean inoculated with AMF in both trials. Inoculation of soybean with AMF would explain the higher spore count and AMF colonisation observed. Together with the presence of N-fixing bacteria in soybean they could have acted as metabolic sink to stimulate photosynthesis, growth and grain yield in both trials.

Varietal differences on grain yield had soybean variety TGx 1448-2E with the highest performance in both trials though not significant on the field. The underlying growth mechanism indicated that it occupied intermediate position in leaf area index and the least significant leaf area at 9 WAP (field), probably a mechanism to forestall mutual shading in the canopy. This soybean variety showed a more adaptive performance as evidenced in both CO_2 enriched environment without AMF inoculation and in ambient CO_2 concentration when inoculated with AMF. It occupied an intermediate position when both factors were present or absent. However, soybean variety TGx 1740-2F is more responsive to the combined effect of elevated CO_2 and inoculation with AMF with a converse pattern when grown in ambient CO_2 concentration in the absence of AMF inoculation. Meghvansi *et al*. (2008) reported genetic variability in AMF colonisation capacity of barley, grapevine, pepper and tomato. Similar conclusion was arrived at by Taiwo and Adegbite (2001) on selected soybean varieties in Nigeria. This suggests synergistic effect of the symbionts. However, Meghvansi *et al*. (2008) observed a selected synergistic relationship between the symbionts. Harris *et al*. (1985), who used one species of AMF in ambient CO_2 concentration, posited that the combined effects of the symbionts in soybean reduced source:sink ratio with an increase in metabolic sink through increase in nodule activity in soybean. Other mechanisms behind the combined effects of the symbionts on the relative growth rate of soybean include increase in gross photosynthetic rate, increased leaf P concentration, increased specific leaf area with reduced leaf weight and increased starch mobilisation (Harris *et al*., 1985). Aranjuelo *et al*. (2014) reported that elevated CO_2 regulated activity of the pathways for carbohydrate metabolism in N-fixing pea plants. Specifically, there was an increase in starch and sucrose level in CO_2 enriched environment. However, to ameliorate feedback inhibition of starch there was also increase in the activities of the protein responsible for the degradation of starch. Taken together the combined effects of CO_2 enriched atmosphere with aforestated symbionts had not been fully explored in the literature. We hypothesize that for the N-fixing legumes elevated CO_2 in the atmosphere would increase the intracellular CO_2 concentration, increase carboxylation and reduce oxygenation process. This would result in increased photosynthetic rate. Presence of metabolic sink through AMF colonisation and N-fixing bacteria would supply P and N respectively to the legume. This metabolic stimulation would drive transportable form (sucrose) into the structures of the symbionts and lead to the degradation of starch to support this process at the same time ameliorate the feedback inhibition of starch. Our findings indicated that there could be varietal variation in this mechanism. The factors underlying this would require further studies.

CONCLUSION

Increased assimilatory surface observed in both trials at elevated CO_2 could have increased the capacity of soybean varieties to intercept more light and increase gross photosynthetic rate. This could have explained increased growth rate in both trials, albeit with variation in the components of growth in both screen house and field trials. Presence of symbionts could have altered source:sink balance in preference for increased metabolic sink and stimulation of growth through photosynthesis. Growth stimulation under inoculation with AMF followed similar pattern with CO_2 enrichment. The reduced specific leaf area under AMF inoculation could have altered chloroplast morphology and gas exchange properties positively for increased photosynthesis and growth. Soybean variety TGx 1448-2E is more adaptive to variation in AMF inoculation and CO_2 enrichment in the atmosphere. Soybean TGx 1740-2F is more responsive to combined effect of AMF inoculation and N-fixing bacteria, while it less suitable in the absence of either of the symbionts or their combined absence.

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REFERENCES

Ainsworth, E.A., Davey, P.A., Bernacchi, C.J., Dermody, O.C., Heaton, E.A., Moore, D.J. and Long, S.P. (2002). A meta-analysis of elevated [CO_2] effects on soybean (*Glycine max*) physiology, growth and yield. *Glob. Chang. Biol.*, 8 (8), 695–709

Allison, L., Bollen, W.B., and Moodie, C.D. (1965). Total carbon. *Methods of Soil Analysis. Part 2. Chemical and Micro-biological Properties*, (methodsofsoilanb), 1346–1366

Aranjuelo, I., Cabrerizo, P.M., Aparicio-Tejo, P.M. and Arrese-Igor, C. (2014). Unravelling the mechanisms that improve photosynthetic performance of N 2-fixing pea plants exposed to elevated [CO_2]. *Environ. Exp. Bot.*, 99, 167–174

Augé, R.M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Can. J. Soil Sci.*, *84*(4), 373–381

Bouyoucos, G.J. (1962). Hydrometer method improved for making particle size analyses of soils. *Agron. J.*, 54 (5), 464–465

Boyer, J.S. (1988). Cell enlargement and growth-induced water potentials. *Physio. Plant*, 73 (2), 311–316

Bray, R. and Kurtz, L. (1945). Determination of total, organic and available forms of phosphorus in soil. *Soil Sci.*, 59, 39–45

Bunce, J.A. and Caufield, F. (1991). Reduced respiratory carbon dioxide efflux during growth at elevated carbon dioxide in three herbaceous perennial species. *Ann. Bot.*, 325–330

Cave, G., Tolley, L.C. and Strain, B.R. (1981). Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in Trifolium subterraneum leaves. *Physio. Plant*, 51 (2), 171–174

Clough, J.M., Peet, M.M. and Kramer, P.J. (1981). Effects of high atmospheric CO_2 and sink size on rates of photosynthesis of a soybean cultivar. *Plant Physiol.*, 67 (5), 1007–1010

Cure, J.D. and Acock, B. (1986). Crop responses to carbon dioxide doubling: a literature survey. *Agric. For. Meteorol.*, 38 (1), 127–145

Cure, J.D., Rufty Jr, T.W. and Israel, D.W. (1987). Assimilate utilization in the leaf canopy and whole-plant growth of soybean during acclimation to elevated CO_2. *Bot. Gaz.*, 67–72

Dornhoff, G.M. and Shibles, R.M. (1970). Varietal differences in net photosynthesis of soybean leaves. *Crop Sci.*, 10 (1), 42–45

Finn, G.A. and Brun, W.A. (1982). Effect of atmospheric CO_2 enrichment on growth, nonstructural carbohydrate content, and root nodule activity in soybean. *Plant Physiol.*, 69 (2), 327–331

Giovannetti, M. and Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.*, 84 (3), 489–500

Harold, H., Keyser and Fudi, L. (1990). Potantial for increasing biological nitrogen fixation in soybean. In J. Ladha, T. George, and C. Bohloot (Eds.), *Biological Nitrogen Fixation for Sustainable Agriculture* (pp. 119–135). Netherlands: Springer

Harris, D., Pacovsky, R.S. and Paul, E.A. (1985). Carbon economy of soybean–Rhizobium–Glomus associations. *New Phytol.*, *101*(3), 427–440

Hunt, R. (2012). *Basic Growth Analysis: Plant Growth Analysis for Beginners*. Springer Science and Business Media

Idris, A., Rasaki, K., Hodefe, O.J. and Hakeem, B. (2013). Consumption pattern of Ofada rice among civil servants in Abeokuta Metropolis of Ogun State, Nigeria. *J. Biol. Agric. Healthcare*, 3 (6), 106–112

Jackson, M. (1962). *Chemical Soil Analysis*. New Delhi: Prentice Hall of India Pvt, Ltd.

Kaschuk, G., Kuyper, T.W., Leffelaar, P.A., Hungria, M. and Giller, K.E. (2009). Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biol. Biochem.*, 41 (6), 1233–1244

Konings, H., Koot, E. and Tijman-de Wolf, A. (1989). Growth characteristics, nutrient allocation and photosynthesis of Carex species from floating fens. *Oecologia*, 80 (1), 111–121

Leakey, A.D., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P. and Ort, D.R. (2009). Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J. Exp. Bot.*, 60 (10), 2859–2876

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

McLean, E. (1982). Soil pH and lime requirements. In: A. Page, R. Miller, and R. Keneey (Eds.), *Methods of Soil Analysis* (2nd Ed., pp. 199–223). Agronomy Society of America

Meghvansi, M.K., Prasad, K., Harwani, D. and Mahna, S.K. (2008). Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and Bradyrhizobium japonicum in the alluvial soil. *Eur. J. Soil Biol.*, 44 (3), 316–323

Murphy, J. and Riley, J.P. (1962). A Modified Single Solution Method for Determination of Phosphate in Natural Waters. *Anal. Chim. Acta*, 27, 31–36

Obalum, S.E., Igwe, C.A., Obi, M.E. and Wakatsuki, T. (2011). Water use and grain yield response of rainfed soybean to tillage-mulch practices in southeastern Nigeria. *Sci. Agricola*, 68 (5), 554–561

Phillips, J.M.and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *T. Brit. Mycol. Soc.*, 55 (1), 158–I61

Poorter, H. (1993). Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. *Vegetation*, 104 (1), 77–97

Rogers, H.H., Cure, J.D., Thomas, J.F. and Smith, J.M. (1984). Influence of elevated CO_2 on growth of soybean plants. *Crop Sci.*, 24(2), 361–366

Saitoh, Y., Hattori, J., Chinone, S., Nihei, N., Tsuda, Y., Kurahashi, H. and Kobayashi, M. (2004). Yeast-generated CO_2 as a convenient source of carbon dioxide for adult mosquito sampling. *J. Am. Mosq. Control Asso.*, 20 (3), 261–264

Siebenkäs, A., Schumacher, J. and Roscher, C. (2015). Phenotypic plasticity to light and nutrient availability alters functional trait ranking across eight perennial grassland species. *AoB Plants*, *7*, plv029

Smith, S. and Read, D. (2008). *Mycorrhizal Symbiosis*, (3rd edition). Amsterdam; Boston: Academic Press

Taiwo, L. and Adegbite, A. (2001). Effect of arbuscular mycorrhiza and Brydyrhizobium inoculation on growth, N2 fixation and yield of promiscuously nodulating soybean (*Glycine max*). *J. Agric. Res.*, 2, 110–118

Uzoh, I.M., Obalum, S.E. and Ene, J. (2015). Mineralization rate constants, half-lives and effects of two organic amendments on maize yield and carbon-nitrogen status of loamy Ultisol in Southeastern Nigeria. *Agro-Sci.*, 14 (3), 35–40.

Wiersma, J.V. and Bailey, T.B. (1975). Estimation of leaflet, trifoliolate, and total leaf areas of soybeans. *Agron. J.*, 67 (1), 26

Wu, Q.S. and Zou, Y.N. (2010). Beneficial roles of arbuscular mycorrhizas in citrus seedlings at temperature stress. *Sci. Hortic.*, 125 (3), 289–293