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The response of some physiological traits of chickpea (*Cicer arietinum* L.) to biochar and phosphorus fertilizer application

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ABSTRACT

This study assessed the response of photosynthesis, chlorophyll content (CC), stomatal conductance (SC) and intercepted radiation (IR) of chickpea to biochar (0, 5 10 and 20 t ha^{-1}) and phosphorus (P) fertilizer (0 and 90 kg ha^{-1}) rates in Thohoyandou, South Africa in 2013/2014 summer and winter seasons. Photosynthesis, CC, SC and IR were determined at vegetative and reproductive stages. Biochar increased SC and CC by 22 - 49% and 57 – 126%, respectively. P increased CC by up to 9% in the winter sowing. IR increased with P and biochar application in both sowings. Biochar increased plant height only at 70 days after emergence and P increased plant height at all plant growth stages. Biochar and phosphorus did not affect photosynthesis in either season. Therefore the use of biochar and inorganic phosphorus fertilizer may be beneficial in chickpea cropping systems characterised by poor soils and dry winter seasons.

Key words: Chlorophyll content, Growth, Intercepted radiation, Stomatal conductance.

INTRODUCTION

In the continuous cropping systems of the semi-arid areas of North East South Africa, cultivation of crops is carried out throughout the year with minimal addition of external inputs, if any, leading to incessant depletion of soil fertility. To avert further soil fertility depletion in these areas and sustain crop productivity, management practices should be geared towards improving and maintaining soil fertility. The use of soil amendments (e.g. biochar), organic and inorganic fertilizers, and suitable crop genotypes may ameliorate the soil fertility problem and lead to improved and sustainable crop productivity. Biochar is defined as charcoal obtained when organic materials are burned under low pressure and high temperature condition through pyrolysis process under low or absence of oxygen (Lehmann 2007). Moreover, incorporation of drought tolerant legumes such as chickpea (*Cicer arietinum* L.) may increase productivity and minimize crop failure risks of current cropping systems in dry environments and thus play a significant role in achieving sustainable food security. Chickpea may contribute to agricultural sustainability through nitrogen (N) fixation and as a rotation crop.

Symbiotic N fixation can produce greater than 100 kg N ha^{-1} (Beck 1992), and provide up to 85% of the N required by a chickpea crop (Walley *et al.* 2005; Chemning'wa and Vessey 2006). According to Sinclair and Vadez (2002), phosphorus (P) is required for N fixation by legumes and thus improving P management is crucial in enhancing legume production. Thus, P may influence chickpea productivity indirectly by improving the soil N status.

The effect of P fertilizer on yield and yield components of chickpea is well documented (Madzivhandila *et al.* 2012; Turuko *et al.* 2014). However, there is a dearth of information in literature on the interactive effect of biochar and phosphorus on chickpea productivity especially in dry environments. Most documented studies on the combined effect of P and biochar on chickpea productivity were conducted in pots (Budania and Yadav 2014) and it is likely that the field response may vary. Therefore this study aimed at assessing the response of photosynthesis, chlorophyll content, stomatal conductance and intercepted radiation of chickpea to biochar and phosphorus in one representative location of dry environments in NE South Africa.

MATERIALS AND METHODS

Experimental site: Two field experiments were conducted in Thohoyandou (22°58.08´S and 30°26.4´E, and 595m above sea level), NE South Africa. The area receives an annual rainfall of ± 500 mm that falls predominantly in summer. The average maximum and minimum temperatures are 31 and 18°C, respectively (Tadross *et al.* 2006). The site is characterized by deep, well-drained clay soils (Soil Classification Working Group 1991). Soil analysis prior to

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fertiliser application indicated that initial soil P in the top 20 cm was 10.1 mg kg^{-1} (Lusiba *et al.* 2016).

Experimental design: Experiment I was sown on 4 December 2013 and Experiment II was sown on 9 May 2014 (summer and winter sowings, respectively). Both experiments consisted of a factorial combination of four biochar (0, 5, 10 and 20 t ha^{-1}) and two phosphorus (0 and 90 kg P ha^{-1}) rates arranged in randomized complete block design with 3 replications in 3m x 3m plots. Seeds were sown manually in rows that were 30 cm apart to give a plant density of 33 plants m^{-2}. Biochar was applied a week before planting according to the treatments and incorporated into the soil to a depth of 20 cm. Phosphorus was band applied at planting as single superphosphate (8.5% P) according to the treatments.

The plots were uniformly watered (close to field capacity) immediately after sowing to promote germination, emergence and crop establishment. Supplementary irrigation (136.7 mm) was only provided in winter sowing based on soil moisture content that was determined using neutron probe. The experimental plots were kept weed free throughout the season.

Measurements

Weather data: Daily weather data for the experiments were obtained from an automatic weather station that was about 100 m from the experimental site. The following weather variables were recorded each day during the experiment: rainfall (mm); maximum and minimum air temperatures (ºC); solar radiation (MJ m^{-2} d^{-1}); reference evapotranspiration (mm); and relative humidity (%) (Table 1).

Crop growth: Crop growth was assessed by determining plant height and canopy cover. Plant height, determined in winter sowing only, was measured from the base of the plant to the apical bud of the plant using a 5 m measuring tape. Canopy cover was determined by measuring the amount of intercepted radiation by crops using the AccuPAR, model LP-80 ceptometer (Decagon Devices Ltd., Pullman, USA). The measurements were taken between 1100 and 1300 h on clear, cloudless days at 40 DAE (Experiment I) and 25, 35, 45, 55, and 70 DAE (Experiment II). The ceptometer was used to measure photosynthetically active radiation (PAR) above (Pa) and below (Pb) the canopy. The proportion of PAR intercepted by the canopy (a) was obtained as:

$\alpha = 1 - (Pb/Pa)$ (1)

Physiological parameters: Photosynthesis and stomatal conductance were measured at 40 DAE in Experiment I and 25, 35, 45, 55, and 70 DAE in Experiment II using the portable photosynthetic system (LICOR 6400, Lincoln, NE, USA) and steady state Leaf Porometer (Decagon Devices manufacturers, model version 6), respectively. On each occasion, measurements were taken between 11h00 and 13h00 on three leaves per plot. The target leaves were tagged earlier for age identification. Chlorophyll content was measured only in winter sowing at 25, 35, 45, 55, and 70 DAE from three previously selected and tagged leaves in each plot using chlorophyll content meter (CCM-200 PLUS, Opti-Sciences, Tyngsboro, Massachusetts).

Data analysis: A two-way analysis of variance in a randomised complete block design, with the aid of Genstat 16th edition, was used to assess the effect of biochar and phosphorus on plant height, intercepted radiation, chlorophyll content, photosynthesis and stomatal conductance. Significant differences between the treatments were determined at 5% level using the standard error of difference (SED) of the means.

RESULTS AND DISCUSSION

Crop growth: Biochar affected plant height at 70 DAE; plant height was greater with application of 20 t ha^{-1} of biochar compared with control at 70 DAE (Fig. 1a) in winter sowings. The increase in plant height with biochar application only at 70 DAE was probably because biochar was still mineralizing in the soil earlier in the season (Major

Table 1: Weather data at Thohoyandou during summer 2013/2014 and winter 2014 cropping seasons

Year/ Month	Mean Temp (ºC)	Solar Radiation (MJ m^{-2} d^{-1})	Total Rainfall (mm)	Total Epan (mm)	Relative Humidity (%)
Summer sowing (2013-2014)					
December	22.3	15.6	309.6	4.1	74.7
January	23.6	18.2	431.3	112.6	74.7
February	22.9	16.9	302	94.8	77.8
March	23.6	15.4	190.5	54.1	77.6
Mean/Total	23.1	16.6	1233.4	265.5	76.2
Winter sowing (2014)					
May	18.1	15.5	1	70.5	69
June	16.8	15.7	0	86.1	61.5
July	16.1	13.8	1	78.5	61
August	18.7	17.8	0.8	107.4	52.1
September	20.9	22.3	0.5	80.6	48.6
Mean/Total	18.1	17	3.3	423.1	58.4

Epan: Reference evapotranspiration

et al. 2010). In contrast, P fertilizer increased plant height at all plant growth stages in the winter sowing (Fig. 1b). The increase in plant height with P application could be attributed to the effect of P on cell division and elongation. P is one of the minerals required in large quantities in roots and shoots where metabolic activities and cell division are high hence sufficient supply of P fertilizer may lead to increased crop growth. Initial soil analysis prior to sowing indicated very low levels of P (Lusiba *et al*. 2016) hence the crop likely benefitted from the added P fertilizer. In beans, Turuko *et al*. (2014) reported a similar increase in plant height with application of fertilizer P (up to 20 kg ha^{-1}) and attributed the negative response at higher rates of P to the negative effect of high P levels on the availability of other plant nutrients. .

The proportion of intercepted radiation increased with biochar and P fertilizer rates at all measurement dates in the winter sowing (Fig. 2a & b). However, the interaction between biochar and P fertilizer affected the proportion of IR in the summer sowing (Fig. 3). Application of P decreased the proportion of IR by 48% in plots where biochar were not added. In contrast, P application increased the proportion of IR by 58% at 20 t ha^{-1} of biochar (Fig. 3). The positive response of radiation capture to biochar in the winter sowing could be attributed partly to larger canopy size (Burke *et al*. 2014; Varela *et al*. 2013) and greater chlorophyll content with biochar application. The proportion of IR is affected by both the leaf extinction coefficient (chlorophyll content-dependant) and the canopy size. Plant height and shoot biomass (Lusiba, 2016) was greater (suggesting a greater canopy size) with biochar application

Although fertilizer P did not affect chlorophyll content in both sowings, the proportion of IR increased with phosphorus application in the winter sowing. Therefore it is likely that the increase in the proportion of IR in this study was due to a larger leaf canopy with P application. Availability of P mineral to plants enhances physiological and metabolic processes hence leading to improved crop growth as a result of rapid cell division. In support of this, P increased both plant height and shoot biomass suggesting a larger crop canopy. Our findings are comparable to results from previous studies. For example, Ogola *et al*. (2013) attributed an increase in WUE of chickpea with P application to better partitioning of evapotranspiration into transpiration and direct evaporation from the soil. They postulated that the better partitioning of ET with P application was probably

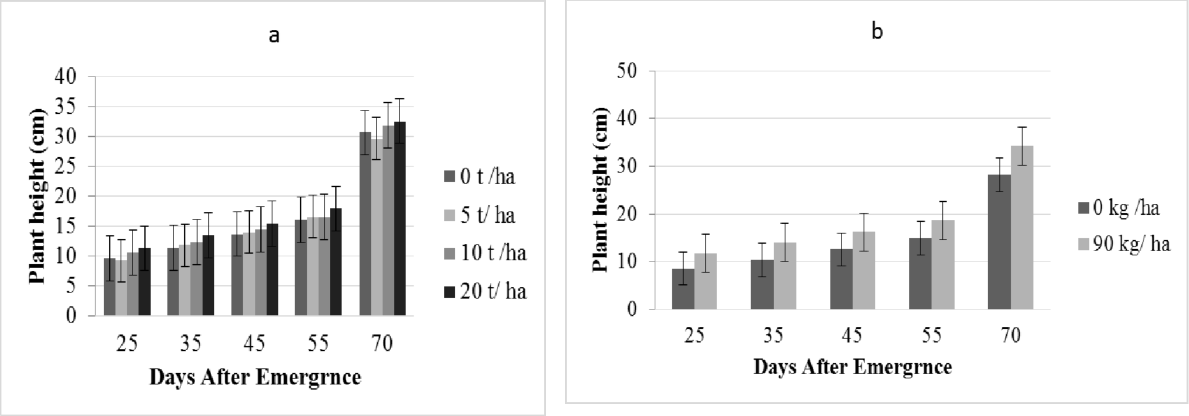

Fig 1: The effect of biochar (a) and P fertilizer (b) on plant height in the winter sowing.

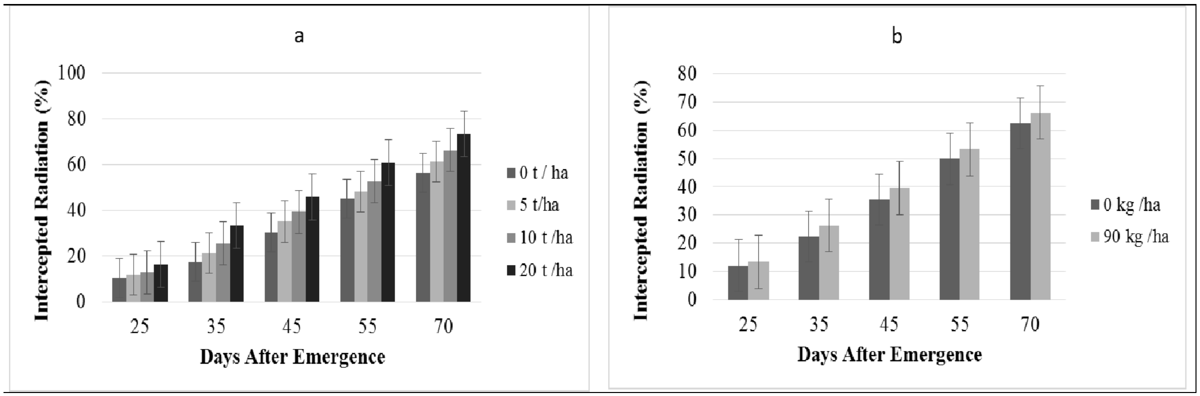

Fig 2: The effect of biochar (a) and P fertilizer (b) on the proportion of intercepted radiation in the winter sowing

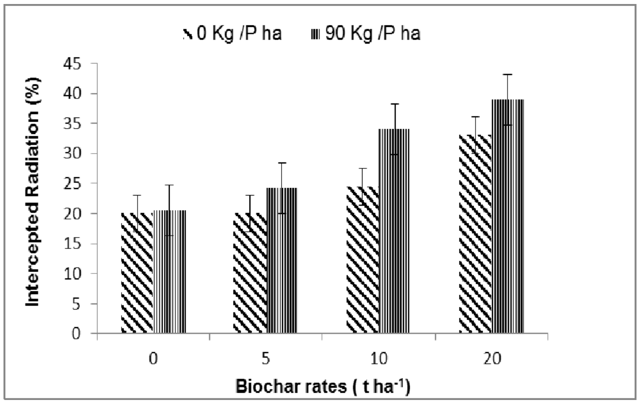

Fig 3: Interactive effect of biochar and P fertilizer on the proportion of intercepted radiation at 40 DAE in summer 2013/14

due to a larger crop canopy (proxied with larger shoot biomass at harvest maturity) with P application.

Photosynthesis, stomatal conductance and chlorophyll content: Biochar and phosphorus did not affect photosynthesis in both seasons (Table 2) but the effect of biochar on stomatal conductance was significant. This may imply that stomatal factors were not limiting to photosynthesis (Table 3). Alternatively, it could be due to unfavourable leaf nitrogen status and/or soil type (Xu *et al.* 2007). Although nitrogen fertilizer was applied at planting, the unusually high rainfall (146% greater than the long term average) received in the summer sowing likely led to huge N losses from the soil through surface runoff, leaching and denitrification. Also, no nodulation was observed in both sowings showing that biological nitrogen fixation did not contribute to soil N budget in this study. However, we did not determine the leaf nitrogen status in the current study. The non-response of photosynthesis, attributed to dilution of leaf chlorophyll content with biochar application, to biochar has similarly been observed in rice (Varela *et al.* 2013) and cotton (Burke *et al.* 2014). In contrast to our study, Burke *et al.* (2014) and Varela *et al.* (2013) did not measure photosynthesis directly but rather used the leaf chlorophyll content as a proxy.

Stomatal conductance was 24% greater at 20 t ha^{-1} biochar compared with the control in summer sowing (Table 3). Similarly, stomatal conductance was greater (by 22%, 49%, 40%, 29%, 29% at 25, 35, 45, 55 and 70 DAE, respectively) at 20 t ha^{-1} biochar compared with the control in winter sowing (Table 3). The increase in stomatal conductance with biochar application was greater in the winter (drier) season compared to the summer (wetter) season probably due to the effect of biochar on soil moisture retention and plant available water (Glaser *et al.* 2002). Similarly, stomatal conductance in tomatoes decreased in no biochar soils that easily dried out (Akhtar *et al.* 2015), and in cowpea stomatal conductance was greater in soils having greater moisture content (Ulyett *et al.* 2014). These effects of biochar on stomatal conductance were attributed to an increase in soil water holding capacity with biochar application; stomatal closure is usually the first line of defence against water stress.

Phosphorus did not affect stomatal conductance in the summer sowing but P increased (by 8.0 and 9.0% at 55 and 70 DAE, respectively) stomatal conductance in the winter sowing (Table 3). Since stomatal responses to phosphorus are driven by the effects of P on plant growth and anatomy (Sarker *et al.* 2010), it is likely that the P effects on plant growth and anatomy are more pronounced at the later growth stages. Xu *et al.* (2007) similarly observed that stomatal conductance increased with increase in P rates. The

Table 2: Effect of biochar and P fertilizer on photosynthesis rate (μmol CO2 m^{-2} s^{-1}) during summer 2013/4 and winter 2014

Season/ Year	Summer 2013/14	Winter 2014				
Treatments	40 DAE	25 DAE	35 DAE	45 DAE	55 DAE	70 DAE
Biochar (t ha^{-1})						
0	32.48	41.33	41.2	42.4	43.55	43.42
5	30.9	43.08	44.07	43.85	44.1	45.4
10	33.58	41.43	41.53	43.27	46.38	48.47
20	32.18	38.48	39.57	41.83	44.67	48.12
SED	0.922	2.146	2.077	1.827	2.258	2.611
P fertilizer (kg ha^{-1})						
0	32.83	41	41.31	42.32	44.75	46.16
90	31.74	41.17	41.88	43.36	44.6	46.54
SED	0.652	1.518	1.469	1.292	1.597	1.846
F-test probability						
Biochar (B)	ns	ns	ns	ns	ns	ns
P fertilizer	ns	ns	ns	ns	ns	ns
Biochar* P fertilizer	ns	ns	ns	ns	ns	ns
CV (%)	11.6	9	8.6	7.5	3.5	0.4

** Highly significant (P<0.001), significant * (P<0.05), and ns (not significant).
Means in the same column followed by the same letter are not significantly different, and standard error of difference of the means (SED).
Days after emergence (DAE).

Table 3: Effect of biochar and P fertilizer on stomatal conductance (mmol H_2O m^{-2} s^{-1}) during summer 2013 and winter 2014

Treatments	**Summer 2013**	**Winter 2014**				
Biochar (t ha^{-1})	**40 DAE**	**25 DAE**	**35 DAE**	**45 DAE**	**55 DAE**	**70 DAE**
0	27.35^{c}	31.3^{b}	27.8^{c}	29.48^{c}	30.82^{d}	30.57^{d}
5	25.15^{d}	29.5^{c}	28.7^{c}	30.27^{c}	33.93^{c}	34.08^{c}
10	30.10^{b}	29.6^{c}	31.0^{b}	33.00^{b}	35.63^{b}	36.37^{b}
20	33.98^{a}	38.3^{a}	41.4^{a}	41.22^{a}	39.70^{a}	39.57^{a}
SED	1.872	2.99	3.01	1.798	1.309	1.601
P fertilizer (Kg ha^{-1})						
0	29.58^{a}	32.4^{a}	32.0^{a}	32.99^{a}	33.61^{b}	33.68^{b}
90	28.71^{a}	32.0^{a}	32.4^{a}	33.99^{a}	36.43^{a}	36.61^{a}
SED	2.12	2.11	2.13	1.271	0.926	1.132
F- probability						
Biochar (B)	**	*	*	*	**	**
P fertilizer	ns	ns	ns	ns	*	*
Biochar*P fertilizer	ns	ns	ns	ns	ns	ns
CV (%)	0.4	1.4	7.8	5.3	2.6	5.6

** Highly significant ($P<0.001$), significant * ($P<0.05$), and ns (not significant).
Means in the same column followed by the same letter are not significantly different, and standard error of difference of the means (SED).
Days after emergence (DAE).

interaction between biochar and phosphorus did not affect stomatal conductance in both sowings (Table 3).

Chlorophyll content was greater at 10 and 20 t ha^{-1} of biochar compared with control at all measurement dates in the winter sowing (Table 4). This response could partly be attributed to the effect of biochar on plant nutrient status (Lusiba *et al*., 2016). Biochar acts as an absorber, reducing N leaching, and increasing leaf N content and N use efficiency (Steiner *et al*. 2008; Agegnehu *et al*. 2015; Partey *et al*. 2015). In support of this, chlorophyll content increased with crop growth stage, from 1.85 (25 DAE) to 2.81 mmol cm^{-2} s^{-1} (70 DAE) (Table 3). Also, the biochar used in the current study contained appreciable levels of some essential plant nutrients such as carbon, calcium, magnesium and potassium (Lusiba *et al*. 2016). Similar findings have been reported in cotton (Burke *et al*. 2014), rice and wheat (Akhtar *et al*. 2014), and peanut (Agegnehu *et al*. 2015). For example, Burke *et al*. (2014) reported that although chlorophyll content decreased (due to dilution effect) with biochar application, the contrast was the case for dry matter yield which they associated with increased plant nutrient availability. Furthermore, Agegnehu *et al*., 2015 attributed the increase in chlorophyll content with biochar application to an increased leaf nitrogen content and improved N, P and C uptake.

Phosphorus did not affect chlorophyll content in the winter sowing (Table 4). Earlier, Hossain *et al*. (2010) attributed the non-significant effect of fertilizer P

Table 4: Effect of biochar and P fertilizer on chlorophyll content (mmol cm^{-2} s^{-1}) during winter 2014.

Treatments	**Winter 2014**				
Biochar (t ha^{-1})	**25 DAE**	**35 DAE**	**45 DAE**	**55 DAE**	**70 DAE**
0	1.45^{b}	1.34^{b}	1.47^{c}	2.03^{b}	2.08^{b}
5	1.33^{b}	1.55^{b}	1.73^{c}	1.92^{b}	2.15^{b}
10	2.35^{a}	2.37^{a}	2.58^{b}	3.08^{a}	3.52^{a}
20	2.27^{a}	2.72^{a}	3.32^{a}	3.53^{a}	3.49^{a}
SED	0.284	0.556	0.341	0.724	0.900
P fertilizer (Kg ha^{-1})					
0	1.78^{a}	1.89^{a}	2.24^{a}	2.67^{a}	2.75^{a}
90	1.92^{a}	2.09^{a}	2.31^{a}	2.62^{a}	2.87^{a}
SED	0.201	0.393	0.241	0.512	0.636
F- probability					
Biochar (B)	*	**	**	**	*
P fertilizer	ns	ns	ns	ns	ns
Biochar *P fertilizer	ns	ns	ns	ns	ns
CV (%)	13.6	13.5	11.4	16.1	5.9

** Highly significant ($P<0.001$), significant * ($P<0.05$), and ns (not significant).
Means in the same column followed by the same letter are not significantly different, and standard error of difference of the means (SED).
Days after emergence (DAE).

application on chlorophyll content to plant age. However, this cannot explain our findings since our measurements were taken at different growth stages both at the vegetative and reproductive phases. Therefore, further investigations on the effect of fertilizer P application on chlorophyll content of inoculated chickpea need to be carried out. It is apparent from our findings that application of pinewood biochar and fertilizer P may improve the productivity of chickpea at the site of the current study (and other sites having similar environmental conditions) by affecting certain physiological traits. Nonetheless, we recommend further studies, involving more chickpea genotypes and sites, before definite conclusions may be drawn

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