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ORIGINAL PAPER

Implications of exposing mungbean (*Vigna radiata* L.) plant to higher CO_2 concentration on seed quality

Amrit Lamichaney[1] · **Kalpana Tewari**[1] · **Pardip Kumar Katiyar**[1] · **Ashok Kumar Parihar**[1] · **Aditya Pratap**[1] · **Farindra Singh**[1]

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Abstract

Understanding the crop response to elevated carbon dioxide (e[CO_2]) condition is important and has attracted considerable interest owing to the variability and crop-specific response. In mungbean, reports are available regarding the effect of e[CO_2] on its growth, physiology and yield. However, no information are available on the germination and vigour status of seeds produced at e[CO_2]. Therefore, in the present investigation, mungbean (Virat) was grown in the open top chamber during summer season of 2018 and 2019 to study the implications of e[CO_2] (600 ppm) on quality of the harvested seeds (germination and vigour). The exposure of mungbean plant to e[CO_2] had no major impact on seed quality as the percent viability (normal seedling + hard seeds) was not reduced. However, in one season (2018), the seed germination (normal seedling) was slightly reduced from 72 to 68%, attributed majorly to an increase in the hard seeds (from 13 to 19%), a predominant form of seed dormancy in mungbean. The changes in seed germination were apparent only in first year of the experiment. Accelerated ageing test (AAT) and storage studies revealed no differences in the vigour of seeds produced at ambient and e[CO_2] environments. Also, the seeds from e[CO_2] had low protein and sugar but recorded higher starch content than the seeds from ambient [CO_2].

Keywords Carbon dioxide · Climate change · Germination · Hardseededness · Mungbean

Introduction

The atmospheric carbon dioxide concentration [CO_2] is increasing continuously due to men led industrialization and is expected to reach between 730 and 1010 ppm by the end of the twentieth century (Solomon et al. 2007). It is estimated that by 2050, the world will be populated by over 9 billion people, and to achieve food security, food grain production has to be increased tremendously (Godfray et al. 2010). A physiological process of crop plants majorly decides the productivity, which is affected by the combined effect of global climate change variables including an increase in [CO_2] (Tausz-Posch et al. 2013). The previous studies on the influence of e[CO_2] are mostly dedicated towards crop growth and development, dry matter accumulation, yield and quality of the produce in terms of nutrition, post-harvest milling properties etc. The implications of e[CO_2] on growth, dry matter, photosynthesis and yield have been well documented in a number of legume and non-legume crops (Das et al. 2002; Vanaja et al. 2007; Mishra and Agrawal 2014; Sakai et al. 2019; Lv et al. 2020). In mungbean, e[CO_2] is reported to enhance plant growth, photosynthesis and grain yield, while modification in seed nutrient composition was apparent with seed having low protein and more sugars and starch (Das et al. 2000; Mishra and Agrawal 2014; Srivastava et al. 2001). Elevated [CO_2] has been linked to altering seed biochemical constituents, like organic compounds and mineral elements (Loladze 2014; Lamichaney et al. 2021a; Lamichaney and Maity 2021). Such e[CO_2]-mediated modifications in plant growth, physiology and seed nutrient composition are expected to alter the planting value (seed germination and vigour) of a seed, of which no report is available in mungbean. In general, the influence of exposing the crop to e[CO_2] on the quality of harvested seed is variable, with reports claiming increased, no change and decreased seed germination and vigour (Edwards et al. 2001; Saha et al. 2015; Hampton et al. 2016; Lamichaney et al. 2019, 2021a;

✉ Amrit Lamichaney
amritiarisst@gmail.com

1 ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh 208024, India

Thinh et al. 2017; Lamichaney and Maity 2021). Due to such variable response, understanding the effect of e[CO_2] on the mungbean plant and its seed quality has become extremely important.

Mungbean (*Vigna radiata* L.) is a short-duration crop and an important tropical food legume of the Indian subcontinent which is predominantly grown in a hot and humid climate, with a plant experiencing temperatures ranging between 30 to 48 °C throughout its growing season. It is suggested that crop response to CO_2 will be higher in hot climates than in mild and cold climates (Mooney et al. 1991). Therefore, an experiment was conducted with a hypothesis that e[CO_2]-mediated alteration on mungbean crop affects the planting value (germination and vigour) of the seed produced.

Materials and methods

Site characteristics

The experiment was carried out at the ICAR-Indian Institute of Pulses Research (ICAR-IIPR), India during the *summer* season of 2018 and 2019. ICAR-IIPR is situated in Kanpur city of state Uttar Pradesh (26°27′ N latitude, 80°14′ E longitude and 152 m above mean sea level) and experiences a sub-tropical humid climate with cool winter while summer is dry and hot. The soil is sandy loam with electrical conductivity of 0.342 dS m^{-1}, pH 7.98, soil organic carbon 4.3 g kg^{-1}, potassium 113 mg kg^{-1}, phosphorus 7.1 mg kg^{-1} and available nitrogen 102 mg kg^{-1}.

Treatment Detail

Two different CO_2 concentrations ambient (400 ± 30 ppm) and e[CO_2] (600 ± 30 ppm)] were maintained for 8–10 h a day, from plant emergence to full maturity in open top chambers (OTCs) of size 4 m × 4 m × 4 m. The structure of the OTC was made up of polycarbonate sheets with a minimum of 80% light transmittance which was mounted in the field fabricated in an aluminium frame. The OTCs were provided with a frustum (0.5 m) at a height of 3.5 m with an open top. The desired concentration of CO_2 in OTCs was maintained using the software SCADA of Genesis Technology, India. The [CO_2] and temperature prevailing in the OTCs at every minute interval were stored in the computer in the WINLOG program as recorded by the data loggers (TC–800). Each [CO_2] treatment was replicated twice.

Agronomic Management

The soil in each OTC, prior to sowing, was prepared and leveled manually. The seeds of mungbean (*cv.* Virat) were sown on 8 April 2018 (first year) and 10 April 2019 (second year) in rows separated by 30 cm. At the time of field preparation, nitrogen, phosphorus and potassium at the rate of 20, 40 and 60 kg ha^{-1}, respectively, were applied. The crop inside OTCs was irrigated four to five times throughout the growing period to avoid any kind of stress. Likewise, one manual weeding was done 25 days after sowing to keep the OTCs free of weeds.

Seed Quality Attributes

At maturity, when the pods have turned dark brown to black in colour, the seeds from the middle four rows were manually harvested for taking observations on seed quality attributes. The harvested bulked seeds (middle four rows) were sundried, and using the oven method (130 °C for 1 h), the moisture content was monitored till it reaches 11–12%. The observation on seed length, width and thickness was recorded in 30 seeds using *Vernier* digital caliper. For seed germination assay, 100 seeds were placed between two moist germination papers, folded and covered by single butter paper to avoid moisture loss and placed in a germinator maintained at 25 °C for 7 days in the dark. After 7 days, observations on the normal, abnormal seedling and hard seeds were recorded (ISTA 2015). Germination assay was conducted in four replications for each OTC. Furthermore, ten randomly selected normal seedlings from each replication were used for measuring the shoot and root lengths and the seedlings were left to dry in a hot air oven maintained at 80 °C for 48 h to estimate the dry weight of the seedling. The seedling vigour indices were ascertained as per the formula of Abdul-Baki and Anderson (1973).

$$\text{Vigour Index} - \text{I} = \text{Germination}(\%)\text{x Mean seedling length(cm)}$$

$$\text{Vigour Index} - \text{II} = \text{Germination}(\%)\text{x Mean seedling dry weight(g)}$$

The impact of e[CO_2] on seed vigour was estimated through seed storage studies and accelerated ageing test (AAT). For storage studies, the seeds (first year of the experiment) from a[CO_2] and e[CO_2] were placed in air-tight plastic containers and stored at ambient laboratory conditions for a year. The germination test as described previously was conducted at periodic intervals of 4 months. For AAT, the seeds were kept in a muslin cloth bag and were kept in a desiccator partly filled with water such that the seed packets do not come in contact with water. The desiccator is then sealed and placed in an oven at a temperature of 41.3 ± 1 °C for 3 days. Following this, the seeds were taken out and were set for germination as described previously.

For starch and total soluble sugar (TSS) assay, the seeds (0.1 g) were homogenized in 80% ethanol and centrifuged for collecting the supernatant. Furthermore, 10 ml of distilled water was added to 1 ml of aliquot and used for TSS and starch estimation following the phenol–sulfuric acid method (Dubois et al. 1956) and anthrone method (Loewus 1952), respectively. The Kjeldahl method was used for estimating the total seed protein content (Kjeldahl 1883).

Statistical Analysis

The *t* test was used to calculate the significance of differences between the treatments. All the percentage data were arcsine transformed before statistical analysis for normalization of data.

Results

The [CO_2] concentration inside the elevated chamber ranged between 582 and 625 ppm, while, in the ambient chamber, the value ranged between 376 and 415 ppm. Likewise, the average air temperature inside e[CO_2] chamber was 1.2–1.3 °C higher than the average air temperature of the ambient [CO_2] chamber (Fig. 1).

Exposure of mungbean plants to e[CO_2] had no effect on physical seed quality such as seed length, seed width and 100 seed weight except for seed thickness (+3 to 6%) (Table 1). With respect to physiological quality, in the first year, e[CO_2] resulted in a small but significant decrease in germination and an increase in the percentage of hard seeds. This response did not occur in the second year. The

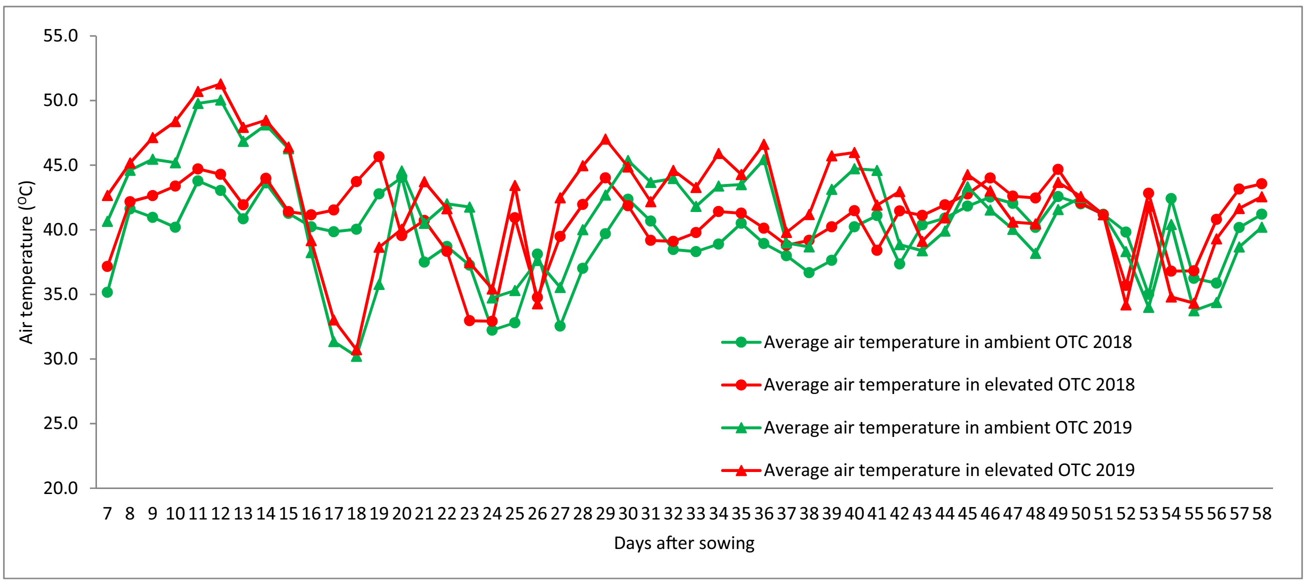

Fig. 1 Variation in the levels of air temperature (°C) inside ambient and elevated [CO_2] open top chamber (OTC)

Table 1 Effect of ambient and elevated [CO_2] on physical seed quality of mungbean

`	Treatment	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	100-seed weight (g)
2018	Ambient	4.39 ± 0.04	3.40 ± 0.02	3.31 ± 0.03	4.35 ± 0.17
	Elevated	4.44 ± 0.05	3.49 ± 0.03	3.51 ± 0.04	4.37 ± 0.02
	P value	ns	0.02	0.000019	ns
2019	Ambient	4.82 ± 0.62	3.51 ± 0.03	3.41 ± 0.03	4.42 ± 0.04
	Elevated	4.84 ± 0.65	3.50 ± 0.02	3.53 ± 0.03	4.51 ± 0.03
	P value	ns	ns	0.001	ns
Year		0.003	0.03	0.03	ns
Treatment		ns	ns	0.0001	ns
Year × treatment		ns	ns	ns	ns

ns not significant

percentage of abnormal seedlings, seedling dry weight and vigour indices remain unaffected by e[CO_2] exposure of the mother plant (Table 2). The seed vigour, as estimated by accelerated ageing test and seed storage studies (data not shown), revealed no considerable difference in the germination and related parameters of seeds produced from two different [CO_2] environments. The seeds produced at e[CO_2] condition had low total soluble sugar (− 17%) and protein (− 8%) but higher total starch (17%) as compared to seeds from a[CO_2] (Fig. 2).

Discussion

Till date, very less research attention has been put forward on the impact of e[CO_2] on the planting value of the seed. Since, quality seed production is largely dependent on the prevailing weather variables during the entire growth period of the mother plant, it is expected that e[CO_2] may alter the quality of the seed. However, the limited information available reports the effect of e[CO_2] to be variable (increase, decrease or no change) on the seed germination, seedling establishment and vigour in various crops (Hampton et al. 2013; Marty and BassiriRad 2014; Saha et al. 2015; Lamichaney et al. 2019, 2021a; Lamichaney and Maity 2021; Thomas et al. 2009; Jablonski et al. 2002). In the present investigation, exposing the mungbean plant to 600 ppm [CO_2] did not affect the weight, length and width of the seed except for its thickness. In general, exposing the mungbean plant to e[CO_2] had no major impact on seed quality as the percent viability (normal seedling + hard seeds) was not reduced. However, the seed germination (normal seedling) was significantly reduced in the first year of the experiment from 72 to 68% due to exposure of the mungbean plant to e[CO_2], which was attributed to an increase in the percent of hard seeds (53%). Hardseededness is the most predominant

Table 2 Effect of ambient and elevated [CO_2] on physiological seed quality of mungbean

`	Treatment	Normal seedling (%)	Abnormal seedlings (%)	Hard seeds (%)	Seedling dry weight (g)	Vigour Index I	Vigour Index II
2018	Ambient	72.00 ± 1.00	15.33 ± 3.53	12.67 ± 1.76	0.093 ± 0.001	1251.55 ± 51.18	6.72 ± 0.19
	Elevated	68.00 ± 1.15	12.67 ± 1.76	19.33 ± 0.67	0.100 ± 0.002	1225.88 ± 47.83	6.35 ± 0.14
	P value	0.03	ns	0.01	ns	ns	ns
2019	Ambient	85.33 ± 2.67	12.00 ± 2.31	1.33 ± 0.67	0.11 ± 0.01	2109.39 ± 85.01	9.46 ± 0.62
	Elevated	83.33 ± 6.76	12.67 ± 6.77	3.33 ± 0.67	0.12 ± 0.01	2090.65 ± 296.77	10.16 ± 0.79
	P value	ns	ns	ns	ns	ns	ns
Year		0.004	ns	0.0002	0.0002	0.0006	0.0002
Treatment		ns	ns	0.003	ns	ns	ns
Year × treatment		ns	ns	ns	ns	ns	ns

ns not significant

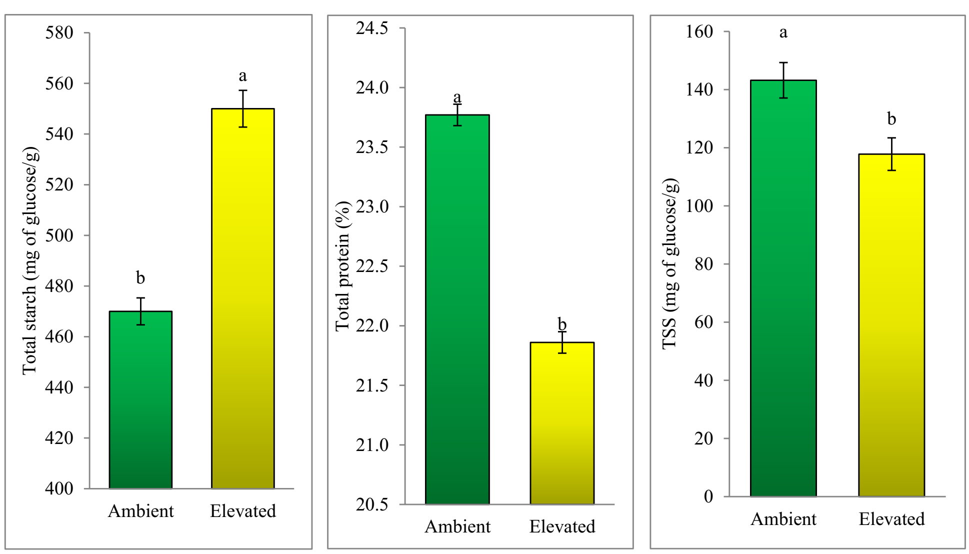

Fig. 2 Total starch, protein and total soluble sugar (TSS) of mungbean seed produced at ambient and elevated [CO_2]. Different lowercase letters represent significant differences at $P < 0.05$ following *t*-test

form of seed dormancy occurring in legumes which is characterized by the development of a water-impermeable seed coat during the later phase of the seed developmental process (Lamichaney et al. 2018). The hard seed is reported to be temperature dependent and higher temperature tends to favour its occurrence (Hill and Rattigan 1986; Ghaleb et al. 2021). The average air temperature inside the a[CO_2] and e[CO_2] OTCs varied from 39.5 to 40.95 and 40.85 to 42.19 °C, respectively (Fig. 1). Such CO_2-mediated increase in temperature might have increased the proportion of hard seeds in freshly harvested seeds. The implication of high-temperature stress on seed quality has been reported in a number of crops (Rashid et al. 2018a; 2018b; 2020; Lamichaney et al. 2021b). In general, the proportion of hard seeds averaged around 15% in 2018 and only 2% in 2019. In both the year, the mean relative humidity (RH) prevailing in the experimental site was about 67% during the entire crop growth period (sowing to maturity), whereas, the mean RH during the reproductive period (flowering to maturity) was 67.03% and 59.84% for 2018 and 2019, respectively. Hence, the higher proportion of hard seeds recorded could be attributed to the high RH observed during the reproductive period in 2018 as compared to 2019. Our experience and also reports suggest that the occurrence of hard seeds in mungbean is more in kharif/rainy season as compared to the summer season (Debashis et al. 2018). Therefore, RH during the seed developmental stage (flowering to maturity) could be an important factor for the development of hard seeds in mungbean. However, in the present investigation, the percent occurrence of hard seeds in e[CO_2] and a[CO_2] was the same after 8 months of storage, implying that the e[CO_2]-mediated increase in percent hard seeds is temporal and would not affect the seedling establishment if used in the next cropping season. Seed vigour, defined as 'the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments' (ISTA 2015), is considered to represent precisely the quality of a seed under stressful field conditions. The vigour of the seeds harvested from a[CO_2] and e[CO_2] environments assessed by accelerated ageing test and storage studies revealed no change. Similar results on no alteration in seed vigour due to e[CO_2] are reported in chickpea (Lamichaney et al. 2021a), while Lamichaney et al. (2019) report reduced vigour of rice seeds produced under [CO_2] of > 610 ppm. Our results further revealed that the seeds produced at e[CO_2] had higher starch content with low protein and total soluble sugar. The reduction in seed protein content is very obvious and may be due to the dilution effect caused by carbohydrate enrichment (Wu et al. 2004) and reduced nitrogen pool (Fangmeier et al. 1999). The mungbean plant is capable of fixing atmospheric nitrogen, but reduced seed protein suggests its limitation. Similar reports of increased starch and reduced protein content in seeds of mungbean produced at e[CO_2] have been reported (Mishra and Agrawal 2014). The seed soluble sugars is an important substrate for successful germination and subsequent seedling growth (Naegle et al., 2005; Da Silva Ferreira et al. 2009), reduction of which might result in an increased percentage of abnormal seedlings (Lamichaney et al., 2019). Furthermore, a decrease in seed protein implies a reduction in seed nitrogen (N) content. The importance of seed N content on faster germination and subsequent seedling growth is apparent (Andalo et al. 1996; Hara and Toriyama, 1998). Also, a positive correlation was reported for seed N with seed germination and seed vigour, implying that reduction in seed protein content due to e[CO_2] might result in poor germination and vigour.

Conclusions

Exposing mungbean plants to e[CO_2] had no major impact on seed quality as the percent viability (normal seedling + hard seeds) was not reduced. Increasing [CO_2] from 400 to 600 ppm in the first year of the experiment resulted in a small but significant reduction in fresh seed germination, which was attributed to an increase in the proportion of hard seeds. Thus, further investigation is required to verify the effect of a higher concentration of CO_2 on the seed quality of mungbean. Most importantly, e[CO_2] resulted in a reduction in seed protein and soluble sugar content of the seed, which might affect the germination speed and subsequent growth of seedlings.

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Declarations

Conflict of interest The authors declare no competing interests.

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