The Pharma Innovation Journal 2022; 11(1): 129-134

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(1): 129-134
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www.thepharmajournal.com
Received: 05-11-2021
Accepted: 20-12-2021

Effect of optimum/high nitrogen supply and elevated CO_2 on yield and nitrogen accumulation in bread wheat

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Abstract

Wheat crop grown under elevated CO_2 (EC) often have a lowered grain nitrogen (N) and protein concentration and altered grain quality. The impact of CO_2 x N interactions on the grain yield and N accumulation is scarce in wheat. The present study describes the interactive effect of EC and optimal or high N dosage on grain yield and N accumulation. Bread wheat genotype BT- Schomburgk was evaluated under two CO_2 levels (Ambient CO_2 (AC) of 400±10 ppm and EC of 700±10 ppm) and two N levels: recommended N, 120Kg ha^{-1} (ON) and high N (150Kg ha^{-1} (HN) at ambient CO_2 (AC, 400±10 µl/l) or elevated CO_2 (EC, 700±10 µl/l) conditions. There was a significant decline in the rate of photosynthesis at the anthesis stage in EC, Indicating the onset of photosynthetic acclimation. We found a significant increase in wheat grain yield parameters by HN in EC conditions. There was a general decline in total grain N uptake in EC in ON, while grain N did not show a decrease in EC in HN conditions. The present study results indicate the potential of reproductive stage high N application in improving yield, grain N accumulation, and post-anthesis N uptake in wheat.

Keywords: Nitrogen, elevated CO_2, wheat, grain protein, grain yield, nitrogen, grain nitrogen

Introduction

Anthropogenic activities have rapidly increased the atmospheric CO_2 ([CO_2]) levels from the preindustrial era, from 280 parts per million in 1750 to more than 400 parts per million today (National Oceanic and Atmospheric Administration 2020). CO_2 emissions will continue to rise if they are not adequately controlled, reaching 800 parts per million by the end of the century. Elevated [CO_2] (EC) can both improve net photosynthesis and reduce transpiration rates since CO_2 is required for plant photosynthesis (Taub, Seemann and Coleman, 2000; Matt *et al.*, 2001) [38, 30]. As a result, EC significantly impacts plant mineral element absorption, usage, and allocation, resulting in dramatic changes in plant mineral element concentrations (Taub and Wang, 2008a; Li *et al.*, 2018; Jin, Armstrong and Tang, 2019) [39, 26, 22]. According to a meta-analysis, the concentrations of three critical nutritional elements in plants, namely nitrogen (N), phosphorus (P), and potassium (K), were all reduced by 7 to 15%, 17.5 per cent, and around 10%, respectively (Cotrufo, Ineson and Scott, 1998a; Loladze, 2014; Loladze *et al.*, 2019) [11, 27, 28]. The most common explanation for the drop in mineral concentrations under EC is dilution due to the accumulation of non-structural carbohydrates (Gifford, Lambers and Morison, 1985; STITT and KRAPP, 1999; Taub and Wang, 2008a) [16, 36, 39].

In the presence of EC, stomatal conductance is significantly reduced, leading to lower transpiration and mass flow, limiting the mineral elements absorbed by roots and partitioned to shoots and grains (R Pettersson, 1994; Mcgrath and Lobell, 2013) [34, 31]. N concentration ([N]) in plants under EC is affected by the predicted decrease in N need due to enhanced N usage efficiency and hindered nitrate absorption (Gifford, Lambers and Morison, 1985; Bloom *et al.*, 2002; Myers *et al.*, 2014) [16, 8, 32]. Plants exposed to EC have considerably different demands for water and mineral nutrients than those exposed to AC due to reduced transpiration (Mcgrath and Lobell, 2013; Houshmandfar *et al.*, 2016) [31, 20] and a shift in metabolic activity (Taub and Wang, 2008b) [40]. For example, it has been well documented that under EC, both N requirements and transpiration are lowered (Cotrufo, Ineson and Scott, 1998b; Taub and Wang, 2008b) [12, 40]. Previous hydroponic or controlled environment attempts used small containers with limited nutrient solutions, resulting in fluctuations in nutrient concentration. A deeper understanding of the fluctuations in N demand and utilisation in soil culture is critical.

N is an essential nutrient frequently low in nature, preventing plant growth (De Groot *et al.*, 2003) [17]. Due to N deficiency, plants cultivated under EC may have rapid photosynthetic adaptation, premature senescence, and quality degradation (Agüera *et al.*, 2006; De la Mata *et al.*, 2013) [3, 25]. In a study by (Dier *et al.*, 2019) [13], EC boosted post-anthesis N uptake in wheat. In wheat, the total N intake at maturity is higher than at anthesis. As a result, post-anthesis N absorption could be a significant source of N remobilised to wheat grain in the presence of EC. Under EC, wheat produced with low N at pre-anthesis and high N at post-anthesis showed a significant increase in post-anthesis N uptake with no change in grain N content, according to (Fernando *et al.*, 2017) [15]. The current work proposes raising N supply to keep up with carbon (C) inputs to offset the adverse effects of EC on plants (Pettersson and McDonald, 1994; Aranjuelo *et al.*, 2015; Halpern *et al.*, 2019) [35, 6, 18]. We anticipated that variations in N consumption in EC-grown plants would differ significantly from those in AC, potentially affecting grain production and N accumulation.

Methodology

Plant growth

The study employed bread wheat genotype BT- Schomburgk (BTS) seeds maintained at the Division of Plant Physiology, ICAR-IARI, New Delhi. Based on previous experiments, the variety showed high nitrogen responsiveness and nitrogen use efficiency (NUE) (Mahmoud *et al.*, 2020; Padhan *et al.*, 2020; Adavi and Sathee, 2021) [29, 33, 2]. Experiments were conducted during the *rabi* 2019-20, 2020-21 cropping seasons, average of the 2 seasons are presented. The plants were grown in pots in the open-top chambers (OTC) of the Division of Plant Physiology, ICAR-IARI, New Delhi. Plants were raised in pots (12 inches in diameter) with a 2:1:1 potting combination of soil, sand, and compost. In all of the pots, the typical package of procedures included a basal dose of single superphosphate (1250 mg/pot) and muriate of potash (333 mg/pot).

N treatments CO_2 exposures

The pots containing uniformly germinated seedlings were housed inside OTCs at two different CO_2 levels at 25 days after sowing (DAS): ambient CO_2 (AC: 400 10 ppm) or elevated CO_2 (EC: 700 10 ppm). CO_2 in the chambers was measured at regular intervals using an infrared gas analyser (IRGA) from a portable photosynthetic system (LI- 6400 XT, LiCOR, Lincoln Nebraska, USA). To keep the soil's field capacity, pots were irrigated regularly. Plants were given two different N dosages at each CO_2 level. The N dosages (in mg/pot of urea) were treated at three crop stages: basal dose, booting stage, and 50% anthesis stage. optimal N (120Kg ha^{-1}) (ON; 450 mg: 225 mg: 225 mg), and high N (HN: (150 Kg ha-1) (450 mg: 337.5 mg: 337.5 mg) were the different treatments.

Gas exchange parameters

At the anthesis stage of the crop, the LI-6400XT Portable Photosynthesis System (Model: LI-COR, Lincoln, Nebraska, USA) was used to record photosynthetic rate (PN, mol CO_2 m^{-2} s^{-1}), stomatal conductance (Gs, mmol H_2O $m^{-2}s^{-1}$), and transpiration rate (Tr, mmolH_2O m^{-2} s^{-1}).

Evaluation of yield parameters

The plant height was measured and expressed in centimetres. The total number of tillers and effective tillers per plant were counted at harvest. The plant samples were dried in a hot air oven at 60°C, and the dry weight was measured. Ear length, ear weight, the number of spikelets per ear and the number of grains per ear was determined. Hundred individual grains were counted, and the weight was recorded subsequently.

Calculation of total nitrogen accumulation

The total nitrogen content of shoot and grains was calculated using the micro-Kjeldahl method. In the Kjeldahl digestion tubes containing concentrated H_2SO_4 (10 ml) and 3–3.5 g of catalyst mixture (K_2SO_4:$CuSO_4$ in 10:1 ratio), 500 mg of grains were added. The sample was pre-digested for 10 minutes at 2000°C before digested at 380 °C temperature. After cooling the sample to room temperature, 35 mL of double-distilled water was added to dilute it. In the distillation apparatus (KELPLUS, CLASSIC-DX VATS (P)), the diluted digested material was steam distilled for 8 minutes with a strong alkali of 40% NaOH. The emitted ammonia was measured by titrating against 0.1N HCl with a 4 per cent boric acid-containing indicator (methyl red) (Kjeldahl, 1883) [24].

Statistical analysis

GraphPad Prism version 8 (La Jolla, California, USA) was used for the two-way analysis of variance (ANOVA) with CO_2 and N treatments as treatment effects to compute adjusted P values and level of significance.

Results and Discussion

Atmospheric CO_2 is steadily increasing and is expected to reach 720 ppm by 2100 (Stocker *et al.*, 2013) [37]. The beneficial effects of EC on photosynthesis and yield of field-grown crops have been confirmed under various free-air atmospheric CO_2 enrichment (FACE) experiments (Ainsworth and Long, 2005) [4]. Though EC is generally expected to increase yield, sometimes there is no substantial yield gain at all (Högy and Fangmeier, 2008) [19]. This is majorly due to acclimation of photosynthesis rates at higher [CO_2], negatively affecting grain production. In the present study, also we found significant variation in the rate of photosynthesis in response to EC and N levels. After long term exposure to EC, there was a substantial decline in the rate of photosynthesis; further, the HN treatment was inhibitory to photosynthesis in both AC and EC **(**Fig 1). Elevated CO_2 (EC) increases the growth of C3 species, albeit the longer-term adaptive phenomena, photosynthetic acclimation (Ainsworth and Rogers, 2007) [5]. The acclimation of photosynthesis under EC usually occurs due to a reduction in stomatal conductance (gs) and a decrease in RuBP regeneration (Zhang *et al.*, 2009) [42].

The number of tillers per plant and productive tillers per plant differed significantly. W. r. to AC, the number of tillers per plant showed a significant increase in EC. The number of ears also showed a similar trend **(**Fig 2). Length and weight of ears were also significantly different in CO_2 and N treatments. In ON, ear length was higher in EC, while in HN, there was a significant increase of ear length in EC. EC and HN significantly increased ear weight (Fig 3). In general EC grown plants showed a higher ear weight.

Only an average yield increment of 10% was observed in wheat under FACE experiments (Kimball, 2016) [23]. We found a significant increase in wheat grain yield in EC. The effect of HN was significant and positive in AC conditions (Fig 3). There was a significant increase of 100-grain weight in EC compared to AC. The 100 seed weight was higher in HN treatment in AC. In EC, the 100-grain weight was more elevated than AC in both ON and HN conditions. Grain number per ear was significantly different in N and CO_2 treatments. HN treatments significantly increased the grain number per ear in AC and EC (Fig 4). The grain number per ear was substantially higher in EC conditions at both N levels. Inconsistent effects of EC on grain yield parameters in wheat is described. High 100grian weight was observed by Högy and Fangmeier (2008) [19] and Fernando *et al*. (2014a; 2014b) [14], while Kimball *et al*. (2001) and Högy *et al*. (2009) reported no change or even decreasing 100-grain weight.

The most pressing attribute of plants grown in EC is the reduced nitrogen (N) content and grain protein concentration (Fernando *et al*., 2014) [14]. The reduced shoot and grain N levels in EC are ascribed to the dilution effect by non-structural carbohydrates, reduction in nitrate and ammonia assimilation, and altered N signalling (Adavi and Sathee, 2020; Padhan *et al*., 2020) [1, 33]. Recent reports suggest that the incremental application of nitrate N is either inhibitory (Jauregui *et al*., 2020; Padhan *et al*., 2020) [21, 33] or promotive to plant growth and N accumulation of EC grown plants (Asensio *et al*., 2015; Bloom *et al*., 2010) [7, 9]. There was a general decline in total grain N uptake in EC in ON. In the HN condition, the grain N was highest in EC (Fig 5). Shoot N uptake was higher in EC grown plants at both the N doses. The reduction in tissue N concentration is due to high NUE without limiting plant growth under EC (Zerihun, Gutschick and Bassirirad, 2000) [41]. When expressed per plant basis, the N content is high in low and high N treatments under EC compared to AC. Studies by Coleman *et al*. (1993) [10] also showed that plants responded to EC with reduced tissue N concentration relative to AC grown plants when comparisons were made at a common time. Even though N decreases on a weight basis in EC, the organic N content per plant often increases (Hocking and Meyer, 1991). Dier *et al*. (2019) [13] indicated that EC increased post-anthesis N uptake in wheat. Acquisition of N at maturity was also enhanced compared to that under anthesis. This indicated that post-anthesis N uptake had an essential role in the supply of N to grains with increased demand under EC. The present study results also show the potential of reproductive stage high N application in improving yield, grain N accumulation, and post-anthesis N uptake in wheat.

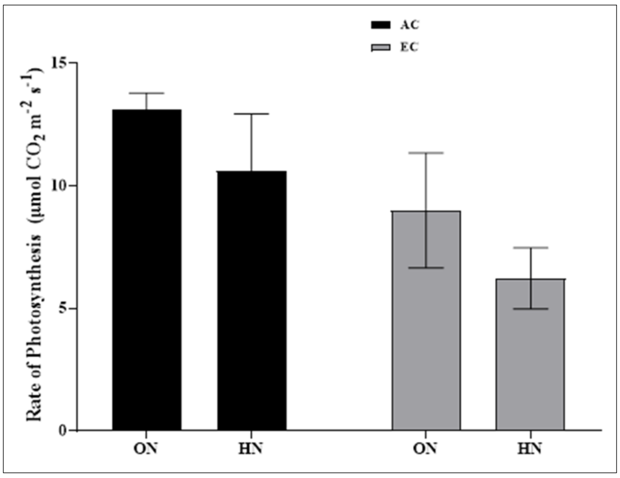

Fig 1: Effect of elevated $[CO_2]$ and nitrogen supply on rate of photosynthesis at 50% anthesis in flag leaves of wheat genotype BT-SCHOMBURGK with recommended N, 120Kg ha^{-1} (ON) and high N 150Kg ha^{-1} (HN) at ambient CO_2 (AC, 400±10 µl/l) or elevated CO_2 (EC, 700±10 µl/l) conditions

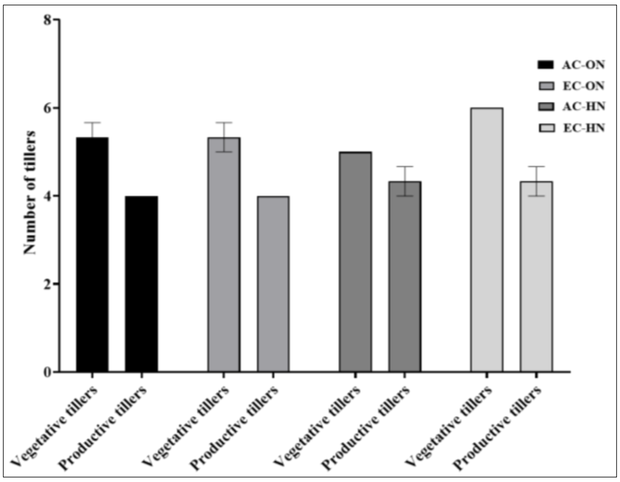

Fig 2: Effect of elevated $[CO_2]$ and nitrogen supply on vegetative and productive tillers of wheat genotype BT-SCHOMBURGK with recommended N, 120Kg ha^{-1} (ON) and high N 150Kg ha^{-1} (HN) at ambient CO_2 (AC, 400±10 µl/l) or elevated CO_2 (EC, 700±10 µl/l) conditions

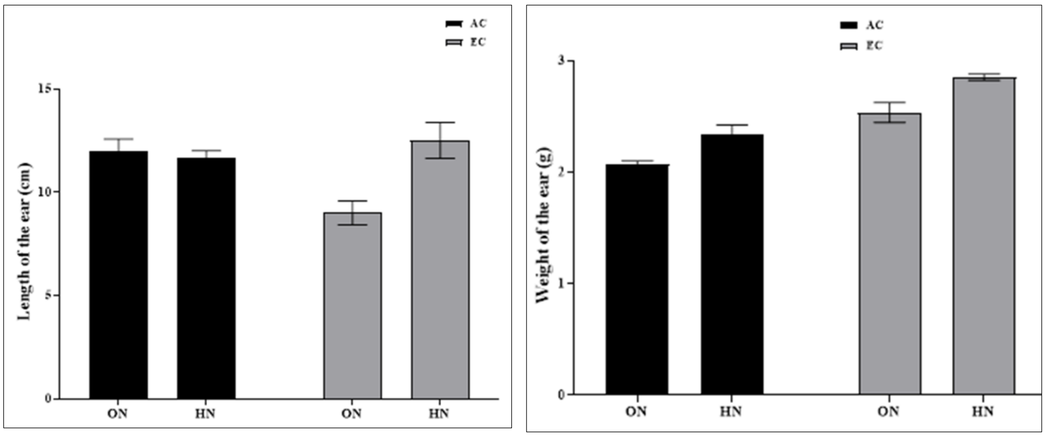

Fig 3: Effect of elevated $[CO_2]$ and nitrogen supply on length of ears and weight of ear in wheat genotype BT-SCHOMBURGK with recommended N, 120Kg ha^{-1} (ON) and high N 150Kg ha^{-1} (HN) at ambient CO_2 (AC, 400±10 µl/l) or elevated CO_2 (EC, 700±10 µl/l) conditions

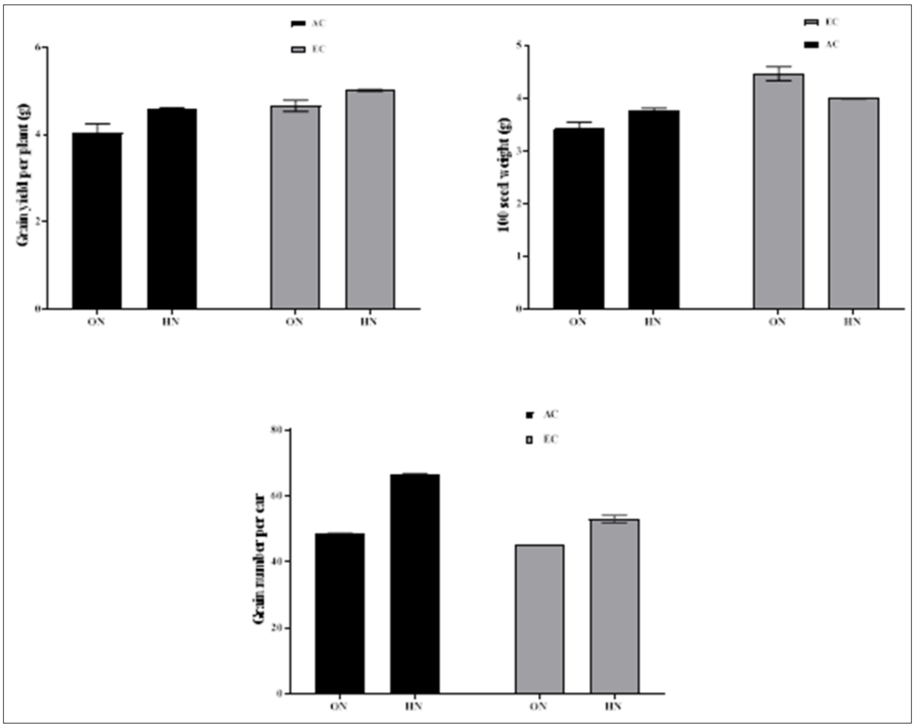

Fig 4: Effect of elevated [CO_2] and nitrogen supply on grain yield, 100grain weight and grain number per year in wheat genotype BT-SCHOMBURGK with recommended N, 120Kg ha^{-1} (ON) and high N 150Kg ha^{-1} (HN) at ambient CO_2 (AC, 400±10 µl/l) or elevated CO_2 (EC, 700±10 µl/l) conditions

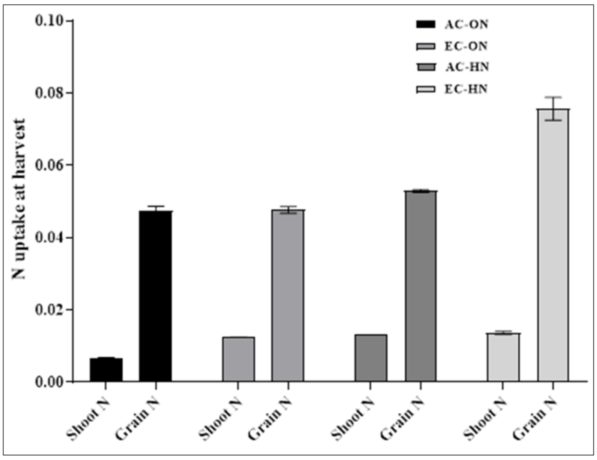

Fig 5: Effect of elevated [CO_2] and nitrogen supply on shoot and grain nitrogen uptake in wheat genotype BT-SCHOMBURGK with recommended N, 120Kg ha^{-1} (ON) and high N 150Kg ha^{-1} (HN) at ambient CO_2 (AC, 400±10 µl/l) or elevated CO_2 (EC, 700±10 µl/l) conditions

Author contributions

SA conducted the experiments with the help of DS. LS designed the study and wrote the manuscript. LS, VC, SKJ, RR and MP provided the necessary facilities and finalised the study.

Acknowledgements

The authors are thankful to the ICAR-Indian Agricultural Research Institute for funding and necessary facilities. SA acknowledges ICAR for the junior research fellowship support received during the study.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

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