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Soybean seed protein concentration is limited by nitrogen supply in tropical and subtropical environments in Brazil

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Abstract

Soybean production contributes to ca. 60% of global plant-based protein used for food and feed. Brazil is the largest soybean producer and exporter, with 60% from tropical and 40% from subtropical environments. Nitrogen (N) can play an essential role in the storage of

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proteins in seeds; thus, it could be a key factor in increasing the quantity and quality of seeds in high-yielding soybean crops. Unlike in temperate environments, there is a gap of knowledge on whether soybean grown under tropical and subtropical climates are limited by N-fertilization to sustain the seed yield increase without detriments in seed protein concentration. This study aimed to evaluate the effect of N-fertilization on soybean seed yield, protein, and oil concentrations in tropical and subtropical environments in Brazil, thus contributing to agricultural intensification procedures and food security studies. Two levels of N-fertilization (0 and 1000 ka/ha) were tested across 11 tropical or subtropical environments. The range of latitudes explored here was from 12º S to 29º S, representing the major soybean-producing regions in Brazil either under rainfed or irrigated conditions. We found that seed yield responses to N-fertilization were significant (in some environments under rainfed with an average increase of 7%) or not significant (in irrigated). Seed protein increases due to improved N-fertilization (on average 4% for irrigated and 12% for rainfed conditions) were much higher than previous reports from temperate environments. Regardless of N supply and water deficit, there was a trend of seed protein and oil concentration increasing toward lower latitudes.

Key words: rainfed, irrigated, seed quality, water deficit, latitude

Introduction

Soybean [*Glycine max* (L.) Merr.] production provides a base for global food security because it is the most important source of vegetable protein, widely used in food and feed products (Beta and Isaak, 2016; Smárason *et al*., 2019; Wajid *et al*., 2020; Parisi *et al*., 2020). Brazil is the largest soybean producer in the world, with over 41.5 million ha (based on the 2021/2022 harvest) in tropical or subtropical environments, it accounts for 36% of global soybean production (USDA, 2022). It is estimated that the world's population will rise from 7.7 to 9.8 billion between 2017 and 2050 (United Nations, 2017); this will require a considerable increase

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in the quantity and quality (protein and oil concentration) of food production (United Nations, 2017). In this sense, with limited new land on which to expand and an emphasis on sustainable systems, increases in soybean production should come primarily from increased production per unit area, requiring sustainable agricultural intensification (Cassman and Grassini, 2020; Marin *et al*., 2022).

Seed protein and oil concentration play a significant role in determining the quality of soybean food products (Clarke and Wiseman, 2000; Friedman and Brandon, 2001; Li *et al*., 2021). Usually, the soybean quality is measured by the amount of oil extracted and final crude protein concentrations in the soybean meal (Wen *et al*., 2021; Sing and Koksel *et al*., 2021). Some soybean importers currently offer premiums for soybean containing higher amounts of protein (Pathan *et al*., 2013; William *et al*., 2020; Lakkakula *et al*., 2020), while some countries exclude or apply penalties to soybeans from regions when quality requirements are not met. China, for instance, requires minimums of 33.5% of protein and 18% of oil concentration (Hertsgaard *et al*., 2019). Therefore, advances in our understanding of the effects of agricultural practices on soybean seed quality under tropical and subtropical environments can contribute to food security and agribusiness.

Soybean seed yield is linearly related to the total plant N uptake (Salvaggioti *et al*., 2008; Tamagno *et al*., 2017). The soybean crop obtains N from the symbiotic fixation of atmospheric N_2 (SNF), soil N mineralization, and N from irrigation or water table; not usually relying upon N-fertilizer application (Randall, *et al*., 2008; Herridge *et al*., 2008; Reis *et al*., 2021). Soybeans derive between 25 and 75% of their total nitrogen from SNF process (Deibert *et al*., 1979, Salvagiotti *et al*., 2008, Collino *et al*., 2015). However, there has been a concern recently about whether SNF would be sufficient to meet the increased N needs for high-yielding soybean crops (Cafaro La Menza *et al*., 2017, 2019, 2020; Basal and Szabó, 2020). These studies were conducted under temperate environments and reported that N fertilization could contribute to

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increasing soybean yield. Temperate soils are generally viewed as more favourable to crop production than tropical because of higher nutrient levels (Rosenzweig and Liverman, 1992; Caubet *et al*, 2020). In contrast, soils in humid tropics tend to be highly leached of nutrients due to high temperatures, intense rainfall, and erosion that accelerate the decay of soil organic matter, resulting in a decreased C:N ratio (Rosenzweig and Liverman, 1992; Alemayehu *et al*., 2018). The N fertilization on tropical soybean has been little explored in scientific papers.

The consistent increase in soybean yield and decrease in seed protein concentration often raise a debate on the need for extra N supply. In temperate environments, with maturity group (MG) ranging from I–IV, N-fertilization on soybean has been widely explored. For instance, Ray *et al*. (2006) and Salvagiotti *et al*. (2009) obtained seed yield increase between 130 to 438 kg/ha, while Cafaro La Menza *et al*. (2017, 2019) obtained seed yield increase around 600 kg/ha, and an average seed protein concentration increase of 0.9 g/100 g with N-fertilizer and under irrigated conditions. Further, Córdova *et al*. (2020) obtained soybean yield increase ranging 300 to 1000 kg/ha, while in a recently published study, Pannecoucque *et al*. (2022) obtained an average increase in seed yield of 209 kg/ha and a decrease of 2.0 g/100 g of protein concentration, using seed inoculation and 35 kg N/ha at R1. Finally, Wesley *et al*. (1999) and Brar and Lawley (2020) reported nonsignificant N effects on seed yield and protein concentration. In tropical and subtropical environments (with MG ranging from V–VIII), efforts have been made by a few researchers to understand the soybean responses to N-fertilization. Cordeiro and Echer (2019) obtained 439 kg/ha increase in soybean yield (there is no information about protein concentration) when combined N-fertilization with more efficient N-fixing bacteria rates under unfavourable environments, while Hungria *et al*. (2009) and Zilli *et al*. (2021) applied 200 kg N/ha and did not obtain any seed yield increase (there is no information about protein concentration).

The N limitation for soybean in temperate environments is driven by the yield level (larger

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limitation in high than lower yield levels) and indigenous soil N supply of the production environment (Cafaro La Menza *et al*., 2017, 2019). Still, it is unclear if these drivers also apply to tropical and subtropical environments. Seed protein concentration reports in soybean studies with N fertilization under tropical and subtropical environments are scarce, and there is comparatively less research (and without a consistent protocol) on soybean seed yield responses to N supply in tropical and subtropical than in temperate environments. Our scientific question is how N supply could improve soybean yields and protein concentration in tropical and subtropical conditions. Therefore, this research adopted consistent N-fertilization treatments applied to soybean grown in tropical and subtropical environments under irrigation or rainfed condition in Brazil to investigate whether N supply can significantly increase seed yield and/or seed protein or oil concentration (latitude range: 12º S to 29º S). We sought to test the hypothesis that soybean seed yield and/or protein concentration are limited by N supply under tropical and subtropical conditions. Our goal was to evaluate the effects of N supply on soybean yield, and seed quality under rainfed and irrigated conditions and across 17 degrees of latitude range of tropical and subtropical environments in Brazil.

Materials and methods

Field experiments

We conducted 11 field experiments under rainfed or irrigated conditions with a two-way treatment (environment × N-fertilization) structure in a randomized complete block design with four replicates. The experimental plot consisted of 12 rows, 9.0 m long, with a spacing of 0.5 m between rows and 0.04 m for planting depth. The environments were defined as the combination of locations, seasons, sowing dates, and cultivars; with different crop management:

(i) Sorriso (12°42′S, 55°48′W, 375 a.m.s.l), during two rainfed cropping seasons [2018/2019

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(SO-1) and 2019/2020 (SO-2)], with climate classification of Aw (tropical savanna climate), conducted under conventional tillage on Dystrophic Red Yellow Ferrosol, where the crop before planting was an 8-year degraded pasture area for SO-1, and maize for SO-2;

(ii) Piracicaba (22°42′S, 47°30′W, 546 a.m.s.l), during three rainfed and three irrigated cropping seasons [2017/2018 (PI-1), 2018/2019 (PI-2), and 2019/2020 (PI-3)] with a climate classification of Cwa (high-altitude tropical) (Koeppen, 1948), conducted under conventional tillage on a Eutric Rhodic Ferralic Nitisol, the previous crop was maize (*Zea mays* L.) for P1-1 and PI-3, and wheat (*Triticum aestivum* L.) for PI-2, area with a 5-year history of soybean yield > 4.5 Mg/ha;

(iii) Cruz Alta (28°38′S, 53°36′W, 476 a.m.s.l), during one rainfed cropping season [2018/2019 (CA-1)], with a climate classification of Cfa (humid subtropical, with well-defined summer and winter seasons) (Koeppen, 1948), conducted under no-tillage practices on Dystrophic Red Acrisol, the previous crop was wheat, area with a 28-year history of soybean yield > 4.5 Mg/ha;

(iv) Tupanciretã (28°48′S, 53°48′W, 466 a.m.s.l) during one rainfed and one irrigated cropping season [2019/2020 (TU-1)], with a climate classification of Cfa, conducted under no-tillage on Dystrophic Red, the previous crop was maize, area with a 26-year history of soybean yield > 4.5 Mg/ha (Table 1 and Fig.1).

The irrigation amounts applied in the experiments were determined by the potential evapotranspiration determined by the Priestley-Taylor (1972) method; computed using the daily weather data measured with well-calibrated sensors from the on-site weather station (see Table 1 and Supplementary Materials - Figs. S1 to S5). The Priestley-Taylor method was applied under minimum advection conditions and using empirical parameter α=1.26 (Pereira and Villa Nova, 1992, Figueiredo Moura da Silva *et al*., 2019, 2021, 2022). The water amount required was applied with sprinkler irrigation by centre pivot sprinklers Senninger Model i-Wob-UP3 at Piracicaba, and centre pivot sprinklers Plona KS 1500 at Tupanciretã.

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N-fertilizer treatments were defined as (i) no N-fertilizer (0N), where the soybean only relied on indigenous soil N sources (inorganic soil N, mineralized N, and SNF); and (ii) with N-fertilizer (1000N). The total amount of 1000 kg N/ha fertilizer was calculated using a soybean yield target of 8945 kg/ha; this value was based on the maximum soybean yield documented in Brazil, until the season 2016/2017, in contest areas in Brazil (CESB, 2017 and later reported by Battisti *et al*, 2018). The N fertilizer source was urea (46-0-0) applied on the surface. It is noteworthy that our objective was not to replicate the management of soybean contest areas, we only sought a maximum yield seed reference to compute supplementing N fertilizer on soybeans.

We assumed that the crop accumulates 79 kg N/ha in its aboveground biomass per each additional 1000 kg of seed yield (Salvagiotti *et al*., 2008; Tamagno *et al*., 2017), with an extra 40% of N applied to compensate for N losses through ammonia volatilization (Cantarella *et al*., 2018), and another 1.5% N applied to compensate N losses by leaching and runoff (Libardi and Reichardt, 1987; Oliveira *et al*., 2007). The urea amount applied was split into five applications during the cropping season following crop N demand reported by Thies *et al*. (1995) and Bender *et al*. (2015): (i) 10% in second-node (V2); (ii) 10% in fourth-node (V4); (iii) 20% in full bloom stage (R2); (iv) 30% in beginning pod stage (R3); (v) 30% in beginning seed stage (R5). For both treatments, the seeds were inoculated with *Bradyrhizobium elkanii* (strains SEMIA 587 and SEMIA 5019) at a concentration of $5x10^9$ CFU/ml (colony forming units).

Macronutrient fertilization was computed based on surface and subsurface soil analysis. Soil nutrient initial levels among all experiments were as follows: 0.9 to 3.1% of organic matter (Walkey-Black method), 0.7 to 27.0 mg P/dm^3 (Melich-1 method), 0.2 to 5.4 K mmol$_c$/dm^3 (Melich-1 method), 4.0 to 19 Mg mmol$_c$/dm^3 (extracted by calcium acetate), 10.6 to 44.2 Ca mmol$_c$/dm^3 (extracted by calcium acetate), 3.0 to 15.0 mg S/kg (Turbidimetry method) (see

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Supplemental Material Table S1). The soil fertilization sought to reach: P > 26 mg/m^3, K > 2.5 mmol$_c$/dm^3, Mg > 13 mmol/dm^3, Ca > 26 mmol$_c$/dm^3, S > 15 mmol/dm^3. The soybean micronutrients were supplied via foliar fertilization based on the crop cycle and extraction and export of micronutrients (see Supplemental Material Table S2).

Water deficit was computed as the difference between water input (precipitation and/or irrigation) and computed potential evapotranspiration. Phenological stages, according to Fehr and Caviness (1977), were tracked every five days in all experiments and recorded dates of emergence (VE), beginning flowering (R1), beginning pod (R3), beginning seed (R5), and physiological maturity (R7) (Fig. 2), when 50% of the plants were at each stage (Fehr and Caviness, 1977). Field measurements for each experiment included: (i) seed yield (kg/ha), (ii) seed protein concentration (Kjeldahl method); and (iii) seed oil concentration (Goldfish method). The seed yield, seed protein, and oil concentration values were expressed at 0.13 kg H_2O/kg seed. Seed yield, seed protein, and oil concentration samples were collected (hand-harvested) on three centre rows (9 m^2) of the experimental plot for each treatment and replication of each season.

Statistical analysis

Bartlett test (Milliken and Johnson, 2009) was applied to investigate the homogeneity of variances, and no significant differences were observed ($P > 0.05$) for all experiments. Once the homogeneity of within-environment variances was obtained, we conducted the combined analysis of variance (AOV) to determine the effect of environments and N treatments on seed yield, seed protein, and oil concentration; and the means were compared by the Tukey test considering the significance level of 5% (agricolae package, Team R Core, https:/www.r-project.org). The N-fertilizer and environment were considered a fixed effect, and blocks were considered a random effect. Linear regression was used to estimate the relation: (i) between

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seed protein or oil concentration and water deficit; and (ii) between seed protein or oil concentration and latitude.

Results

The higher N supply in the 1000N treatment significantly increased the seed yield by 277 kg/ha (7 %) and the seed protein concentration by 4.3 g/100 g (12%) in tropical and subtropical environments in Brazil under rainfed conditions (Table 2, Fig. 2, and Fig. 3). However, the magnitude of the response varied according to the environment from -300 kg/ha (-7 %) to 596 kg/ha (+20 %) for seed yield and from 2.2 g/100 g (6 %) to 6.2 g/100 g (16 %) for protein. In the case of irrigated experiments, the increased N supply in the 1000N treatment did not affect the seed yield but increased by 1.6 g/100 g (4 %) seed protein concentration (Table 2, Fig. 2, and Fig. 3). Furthermore, the magnitude of responses in seed oil concentration due to N supply also varied across irrigated and rainfed conditions from -1.2 g/100 g (-8 %) to 1.8 g/100 g (+11%). While seed yield responses to 1000N were significant (in some environments under rainfed) or not significant (in irrigated), the seed protein concentration consistently increased across both water availability conditions.

We observed that 1000N treatment only promoted a significant seed yield increase at 476 kg/ha for PI-1, 596 kg/ha for PI-2, 627 kg/ha for SO-1, and 452 kg/ha for CA-1 (Fig. 2). The increase in N supply through the 1000N treatment provided statistically significant gains in seed protein concentration under irrigated and rainfed conditions ($P < 0.001$; Table 2; Fig. 3). The average seed protein concentration was 41.0 g/100 g for 1000N and 39.4 g/100 g for 0N treatment under irrigated condition and 40.5 g/100 g for 1000N and 36.2 g/100 g for 0N under rainfed conditions. The average seed protein concentration increased in the 1000N compared to the 0N treatment on 1.6 g/100 g in irrigated experiments and 4.3 g/100 g in rainfed experiments. However, the magnitude of the treatment response varied from 2.2 to 6.5 g/100 g

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in rainfed experiments.

For experiments conducted under irrigated environments, the seed oil concentration ranged from 14.1 to 18.7 g/100 g (Fig. 4). The N treatments provided no statistically significant results for seed oil concentration under irrigated conditions. Under rainfed conditions, seed oil concentrations ranged from 14.8 to 20.0 g/100 g (Fig. 4). The response to N treatments on seed oil concentration was unclear. The average seed oil concentration obtained in rainfed environments was 17.8 % with 0N and 17.7 % with 1000N.

There was a positive linear relationship between the seed protein concentration difference ((1000N - 0N) / 0N × 100) and the water deficit, with $R^2 = 0.84$ (Fig. 5). The relative response in seed protein concentration due to N supply increased by 4 % for every 100 mm of water deficit. For seed oil concentration, the water deficit did not explain the variability.

We also found that a large portion of the variability in seed protein and oil concentration across environments under rainfed or irrigated conditions was related to the latitude in which the experiments were conducted (Fig. 6A and Fig. 6B). It is important to note that the relationship was established only using only 4 observations representing the sites. The higher seed protein and oil concentration values occurred in lower latitudes (12°S). The soybean harvested across Southern Brazil experiments showed consistently lower seed protein and oil concentrations than Northern Brazil. The magnitude of the decrease in seed protein concentration with latitude was slightly higher than in oil (-0.34x vs -0.20x).

Discussion

Unlike in temperate environments, soybean seed yield failed to respond to increases in N supply in most tropical and subtropical environments evaluated in this study. Salvagiotti *et al* (2009) and Cafaro La Menza *et al*. (2017, 2019, 2020) documented the existence of N limitation in highly productive environments in trials in Argentina and the USA, with N-fertilization

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under irrigated conditions. Their findings revealed the potentiality of soybean seed yield increases with ample N supply in temperate environments with a yield level of >4.5 Mg/ha (based on the yield history of the area). In our areas with yield level >4.5 Mg/ha, the seed yield responded to the 1000N treatment only in PI-3 (rainfed conditions) and Cruz Alta. In these sites, we sowed cultivars with maturity groups between 6.2 to 6.5, which are much longer maturity groups than the ones used in previous studies in temperate zones (maturity groups between 0.0 to 3.8). For PI-1, PI-2, and TU-1, the indigenous soil N sources were sufficient to reach the same seed yield level as soybeans with N-fertilizer. Still, the yield target of 8,945 kg/ha was not reached in any experiment. It means that the sowing date may have been a limiting factor in reaching higher yield; because for the Southern Hemisphere, sowing between late September and mid-October may be more ideal for the soybean crop to reach its peak of development (between R5 and R6) in December (greatest solar energy availability) (Zanon *et al*., 2016; Tagliapietra *et al*., 2021). Therefore, soybean could have accumulated more carbohydrates and acquire N via fixation or fertilization.

In our experiments, the SNF could have been indirectly affected in the few cases where increased seed protein also accompanied seed yield response to N fertilization, for instance in SO-1. This environment was limited for SNF for two reasons: firstly, although the seeds were inoculated with *Bradyrhizobium elkanii*, the soil probably had a poor inoculum of N-fixing bacteria because, before planting, it was a degraded pasture area with no prior soybean crop history (Cordeiro and Echer, 2019), Secondly, a low initial soil phosphorus content (see Supplementary Material Table - S1) may reduce root nodulation (Hungria *et al*., 2006; Pavanelli and Araújo, 2009). Although the soil was fertilized with phosphorus, it cannot be certain that the element was available in the early stages of inoculation. Consistent with our results, Cordeiro and Echer (2019) obtained an increase of 439 kg/ha (22%) in the soybean yield using N-fertilizer (50 kg/ha) in the first year of sowing in areas following degraded

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pasture.

During the cropping season at PI-2, there were water deficit periods between R3 and R6 [critical period for N accumulation and seed number determination (Monzon *et al*., 2021; Rotundo *et al*., 2012; Egli, 2017)], the accumulated rainfall was only 54.3 mm, with 97.8% of rainfall concentration on 77 and 84 DAP (Fig. 2; Supplemental Material Fig.S1-b). Water stress affects the survival and synthesis of leghemoglobin and nodule function (Sprent, 1971; Patterson and Hudak, 1996; Santachiara *et al*., 2019). Similarly, several studies have shown that N uptake and assimilation from the soil are less sensitive to temporary water deficits than SNF (Purcell and King, 1996; Ray *et al*., 2006; Purcell, 2014). However, it is still unclear how much yield loss can be consistently captured with an economically feasible N-fertilizer amount. Also unknown, is which type, timing, and placement of the N fertilizer would maximize yield response and minimize N losses in rainfed environments that usually experience diverse drought conditions (amount and timing of water deficit).

N-fertilization on soybean could impact seed composition despite not necessarily affecting seed yields (Assefa *et al*., 2019). We found an average potential increase of 1.6 g/100 g (4%) in soybean seed protein concentration due to N-fertilizer application under experiments conducted under irrigated conditions. This potential increase was *ca.* 60% higher than the average of 0.9 g/100 g documented by Cafaro La Menza *et al*. (2017, 2019, 2020) with N-fertilization under irrigated conditions in temperate environments. Yet, in our experiments conducted under rainfed conditions, we found a larger potential response to N fertilizer in terms of seed protein concentration (4.3 g/100 g). This potential response was 2-fold higher than the 2.0 g/100 g documented by Bosaz *et al*. (2021) under rainfed conditions in a temperate environment.

Different from results obtained for seed protein concentration, our findings showed unclear relation between N supply and seed oil concentration: 1) no response in all experiments under

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irrigated conditions and PI-2 under rainfed conditions, 2) negative response in SO-1, SO-2, PI-1, and TU-1 under rainfed conditions, and 3) positive response in PI-3 and CA-1 under rainfed conditions. In a meta-analysis study, Rotundo and Westgate (2009) reported that water stress decreased seed oil concentration dramatically for soybean grown in the USA. However, their results were not consistent with ours in subtropical and tropical environments in which the water deficit did not consistently affect seed oil concentration. The seed oil and protein concentration can be affected by genotype, environment, management, and season in a complex and still unclear way (Rotundo and Westgate, 2009; Rotundo *et al*, 2016; Assefa *et al.*, 2019; Bosaz *et al.*, 2021; Grassini *et al*., 2021). Unlike protein, the interaction between N supply, latitudes, and water deficit for seed oil did not follow a clear pattern in tropical and subtropical environments.

Our findings highlighted that N supply is a critical factor in tropical and subtropical soybean systems to increase the seed protein concentration and that the protein gap due to N supply is much larger in tropical and subtropical than in temperate environments. Indeed, more research efforts are needed to diagnose nutrient limitations in tropical and subtropical environments and test N-fertilization timing, rates, placement, and fertilizer types for an economical and environmentally sound response (Rotundo *et al*., 2022). At the cropping system level, increasing indigenous soil N supply with cover crops or different crop rotations and developing novel bacteria strains with improved SNF performance and survival in the soil will be needed to close the N gap for seed yield and protein in soybean.

Yet, our findings also revealed that the soybean harvested in lower latitudes showed consistently higher seed protein and oil concentrations than in higher latitudes within tropical and subtropical environments. It should be noted that we used only 4 points to establish this relationship, so this trend needs to be confirmed with more data in tropical conditions. In a meta-analysis, Assefa *et al*. (2019) reported a reduction in seed oil and increases in soybean

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protein concentration in higher latitudes for soybean sowed in the USA. In the same way, Rotundo *et al.* (2016) documented higher protein concentrations in lower latitudes in the northern hemisphere. Thakur and Hurburgh (2007) compared the quality of samples from different locations (in USA and Argentina) and documented that Brazilian soybean had the highest seed oil and protein concentration. The reason for this higher protein concentration may be related to genetic breeding (flowering-time control and latitudinal adaptation, see Lu *et al*., 2017) inherent to cultivars recommended for low-latitude regions. Yet, this has not enough been elucidated by science, more studies are required to advance our understanding of interactions between soybean protein and latitude.

Although the purpose of this study was not to test N-fertilization on soybean to make recommendations, it is important to clarify that N-fertilization applications of that magnitude can generate substantial volatilization losses that can exceed 40% (Cantarella *et al*., 2018). These results alerted us to a considerable chance of increasing environmental losses under subtropical and tropical conditions by high rates of the volatilization of N-NH_3 associated with urea application. Hence, this heavy fertilization practice (1000N) is not consistent with the sustainable intensification principles and is not cost-effective or environmentally appropriate — farmers should not consider the application of 1000 kg N/ha on soybean in tropical environments. Thus, improvements in N-management on soybean might increase protein production in Brazil and contribute to the global plant-based protein production for a growing population. However, our findings indicated that improvements in N-management on soybean might increase protein production in Brazil and contribute to the global plant-based protein production for a growing population.

Conclusions

Eleven soybean experiments were conducted under tropical and subtropical environments in

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Brazil with rainfed or irrigated conditions combined with N-fertilization treatment (0 or 1000 kg N/ha). The N-fertilization under subtropical and tropical conditions resulted in significant (in some environments under rainfed) or no soybean seed yield effect (under irrigation) and inconsistent seed oil concentration responses. However, our findings showed that the ample N supply in the 1000N treatment consistently increased seed protein concentration in the tropical and subtropical environments evaluated, and its magnitude was larger than what was previously reported for temperate environments. Finally, in lower latitude soybean regions of Brazil, the seed protein and oil concentration were higher than in higher latitudes.

This paper expands upon the work of the first author's PhD thesis.

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Authorship

E. H. Figueiredo Moura da Silva conceived and designed the study, conducted data gathering in Piracicaba-SP, performed statistical analyses, wrote and edited the article, N. Cafaro La Menza, wrote and revised the article, G. G. Munareto conducted data gathering in Cruz Alta-RS and Tupanciretã-RS, A. J. Zanon revised the article, K. Santos Carvalho conducted data gathering in Sorriso-MT, F. R. Marin conceived and designed the study, revised the article.

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Conflicts of Interest

The authors declare there are no conflicts of interest.

Ethical Approval

Not applicable.

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Table 1. Description of field experiments conducted in Piracicaba, Sorriso, Cruz Alta, and Tupanciretã.

Sites	Environment	Cultivar (MG[1])	Season	Sowing date	Growing season[2] (days)	Sowing density (plant/m^2)	Rainfall (mm)	Irrigation (mm)
Sorriso	SO-1	TMG7063 (7.0)	2018/19	Nov 20	98	30	903	—
	SO-2	NS701 (7.0)	2019/20	Nov 01	100	30	893	—
Piracicaba	PI-1	TMG7062 (7.0)	2017/18	Dec 26	96	28	632	50
	PI-2	TMG7062	2018/19	Nov 09	100	28	692	260
	PI-3	TMG7062	2019/20	Nov 27	103	28	830	150
Cruz Alta	CA-1	65i65RSF (6.5)	2018/19	Nov 28	123	35	923	—
Tupanciretã	TU-1	65i65RSF (6.5)	2019/20	Dec 03	118	35	352	270

[1]MG – Maturity group, [2]Time between VE (emergence) and R7 (beginning of maturity), using phenological stages proposed by Fehr and Caviness (1977).

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Table 2. Analysis of variance for effects of environments, nitrogen (N)-fertilization treatment, and interaction between environments × N-fertilization in randomized complete block design structure in experiments with rainfed conditions.

Source of Variation		Seed yield	Seed protein	Seed oil
		Irrigated experiments		
Design structure	df	F	F	F
Blocks	3	0.1	0.2	1.6
Treatment structure				
Environment	3	28.9***	33.7***	231.1***
N	1	2.0	15.0***	12.9**
Environment × N	3	0.2	0.9	7.9**
Error structure		MS	MS	MS
Block × treatments	21	304464	1.6	0.1
		Rainfed experiments		
Design structure	df	F	F	F
Blocks	3	2.0	0.7	2.7
Treatment structure				
Environment	6	117.8***	41.4***	124.0***
N	1	15.2***	177.3***	1.58
Environment × N	6	3.6**	4.4**	16.42***
Error structure		MS	MS	MS
Block × treatments	39	69005	1.6	0.1

df = degrees of freedom; F = F-statistic; MS= mean square. Statistical significance was indicated by: * =p-value

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<0.05; ** = p-value <0.01; ***= p-value<0.001.

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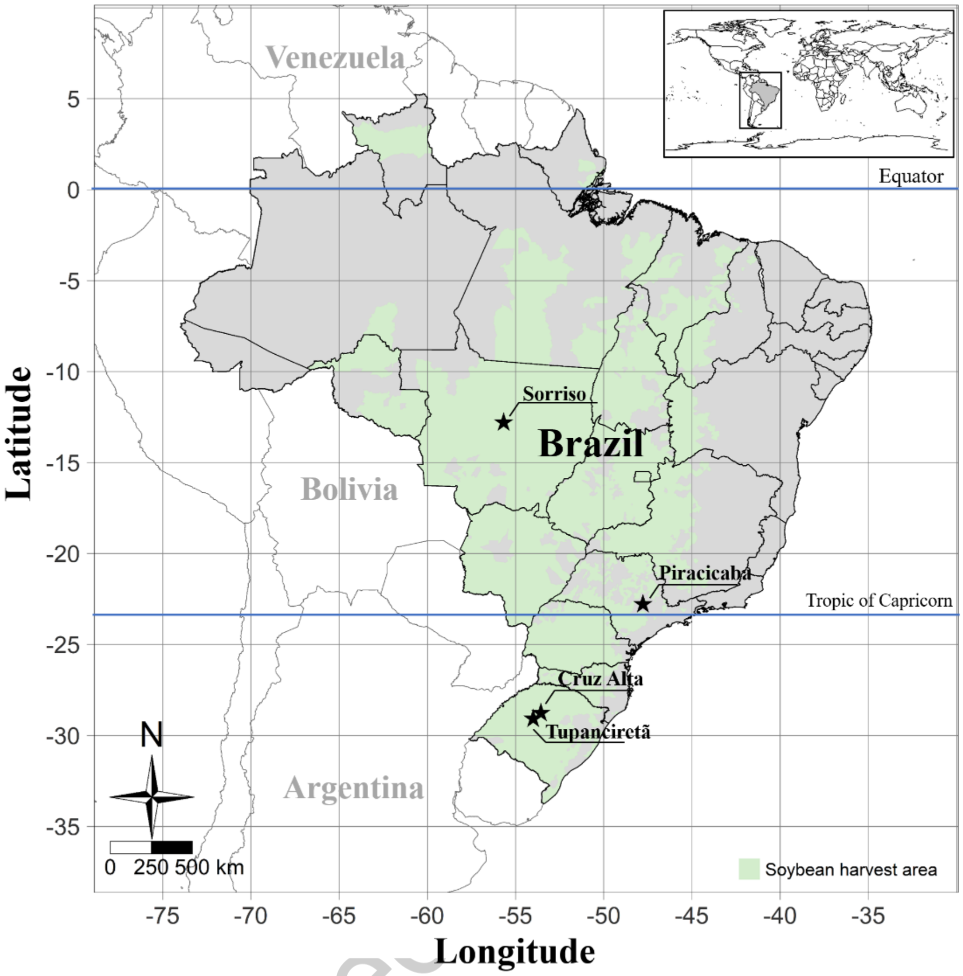

Fig. 1. Soybean harvest area distribution in Brazil. Data source: Brazilian Institute of Geography and Statistics (IBGE, 2021). Black stars indicate field experiments.

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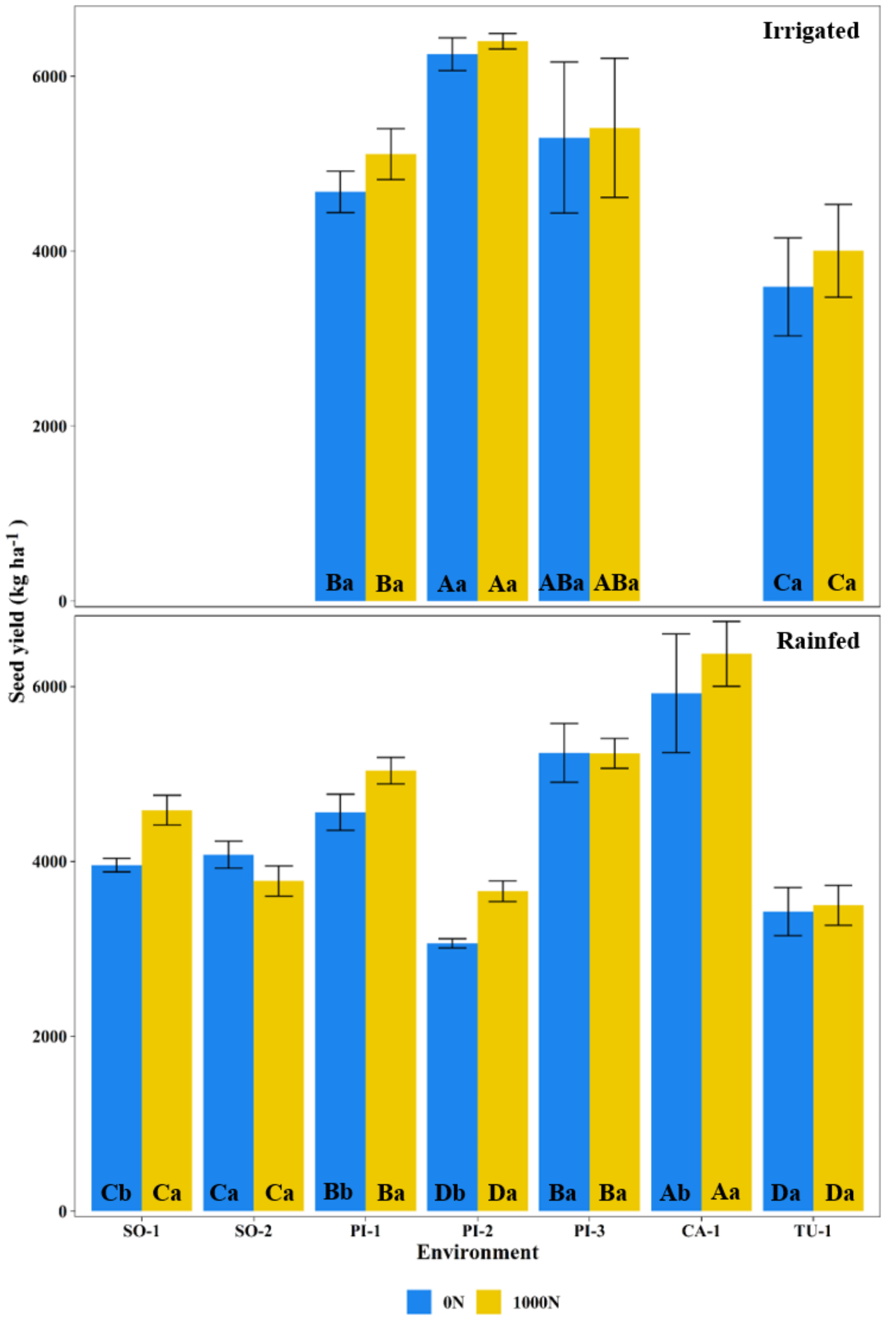

Fig. 2. Seed yield under irrigated or rainfed conditions, with zero (0N) or 1000 kg N/ha (1000N). For experiments conducted in Piracicaba (PI) under three seasons, Sorriso (SO) under two seasons, Cruz Alta (CA) under one season, and Tupanciretã (TU) under one season. The experiments were shown from the locations of low latitude (left) to high latitude (right). Means followed by different letters, uppercase among environments and lowercase between N-treatment for each environment, differ by Tukey test ($p < 0.05$).

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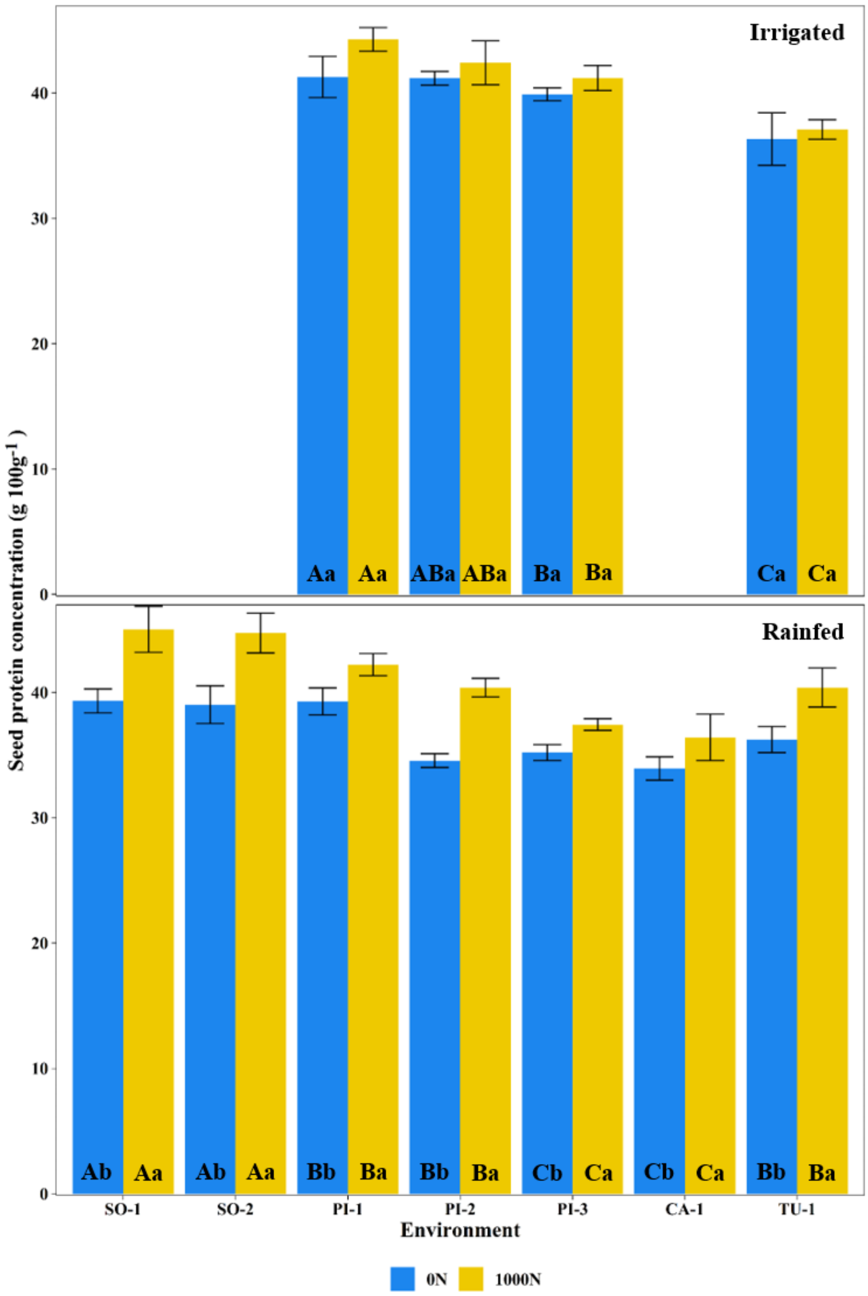

Fig. 3. Seed protein concentration under irrigated or rainfed conditions, with zero (0N) or 1000 kg N/ha (1000N). For experiments conducted in Piracicaba (PI) under three seasons, Sorriso (SO) under two seasons, Cruz Alta (CA) under one season, and Tupanciretã (TU) under one season. The experiments were shown from the locations of low latitude (left) to high latitude (right). Means followed by different letters, uppercase among environments and lowercase between N-treatment for each environment, differ by Tukey test ($p < 0.05$).

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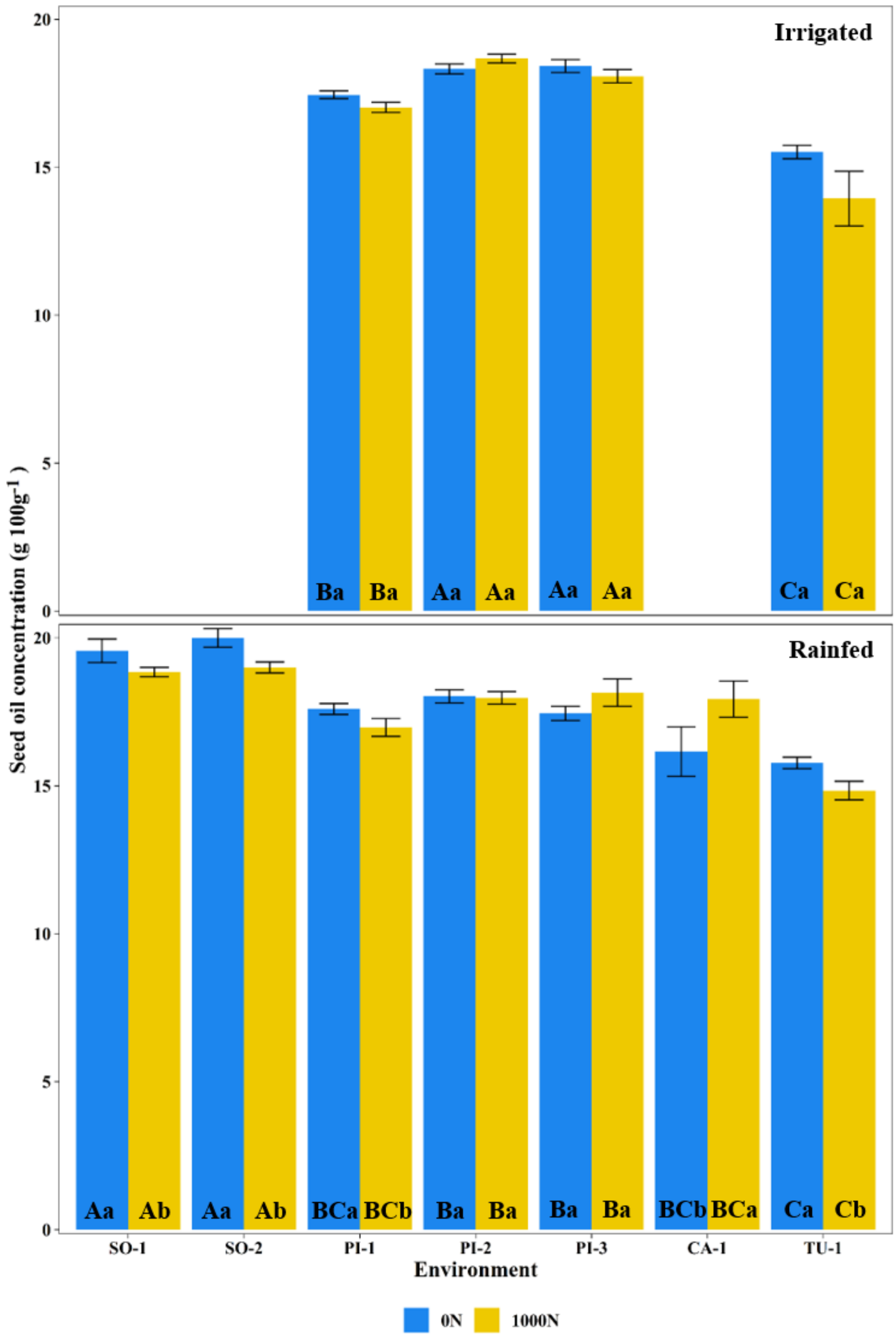

Fig. 4. Seed oil concentration under irrigated or rainfed conditions, with zero (0N) or 1000 kg N/ha (1000N). For experiments conducted in Piracicaba (PI) under three seasons, Sorriso (SO) under two seasons, Cruz Alta (CA) under one season, and Tupanciretã (TU) under one season. The experiments were shown from the locations of low latitude (left) to high latitude (right). Means followed by different letters, uppercase among environments and lowercase between N-treatment for each environment, differ by Tukey test ($p < 0.05$).

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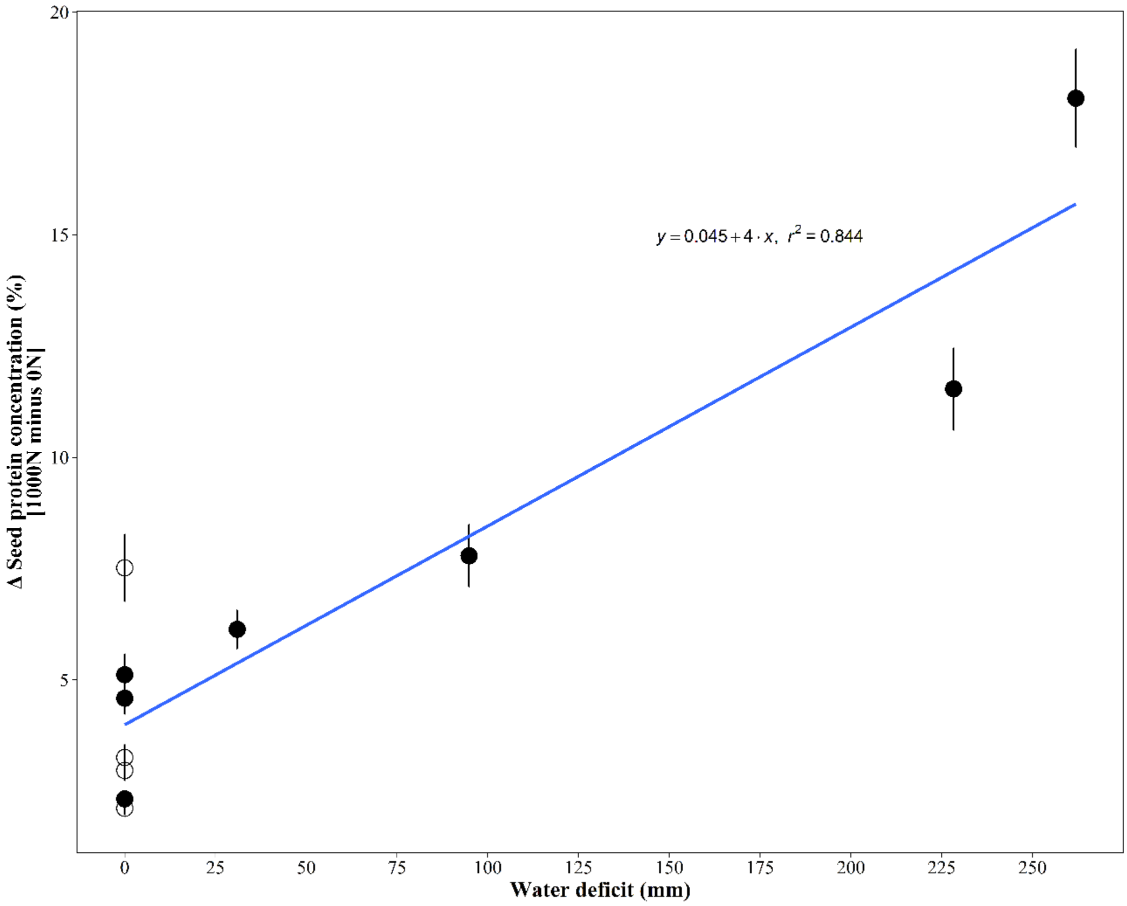

Fig. 5. Variation (Δ) of seed protein concentration (%) between 1000N and 0N treatments (1000N minus 0N) with water deficit across all rainfed conditions (close circles) in this study (total of seven environments). The Δ of seed protein concentration in irrigated environments is also shown (open circles). Water deficit was calculated as the difference between water input (precipitation and/or irrigation) and potential evapotranspiration using Priestley-Taylor method. A linear regression adjusted to the rainfed environments indicates the increase in Δ of seed protein concentration due to N supply with increasing water deficit.

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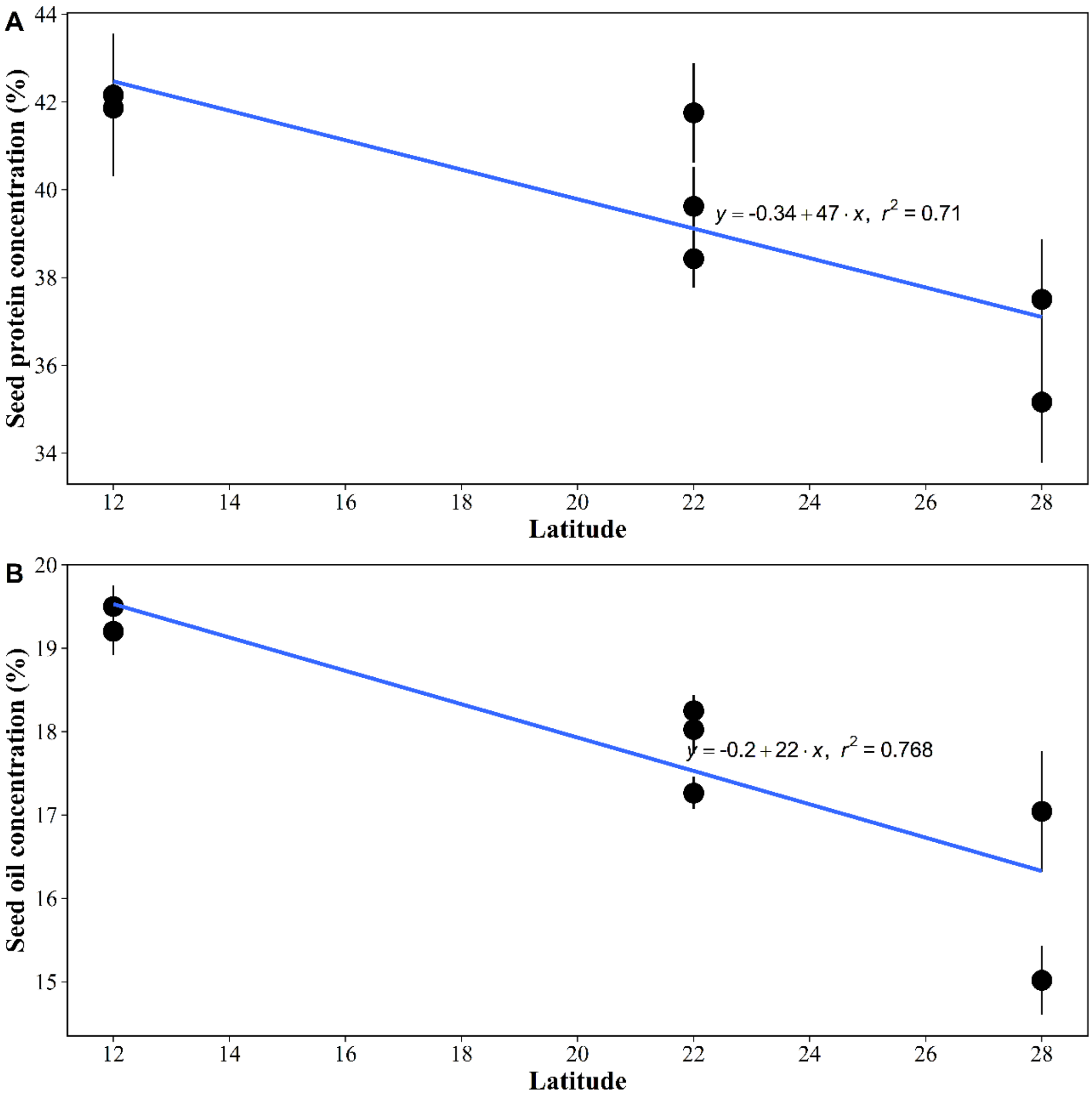

Fig. 6. Average of seed protein (A) and oil concentration (B) (g/100 g) for experiments conducted under rainfed or irrigated conditions across different latitudes. Each average includes the four replicates of the 0N and 1000N treatments. Linear regression was adjusted to each relationship between seed protein or oil to latitude.

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