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Enhancing sustainability in kohlrabi production by balancing nitrogen use without compromising inner and outer quality aspects

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Summary

Sustainability of vegetable production is a declared goal for a more sustainable agriculture in the future. Nitrogen is a crucial macronutrient for achieving high yields and quality such as intense greenness in vegetables, particularly in leafy varieties. However, excessive nitrogen contributes to greenness but can lead to groundwater pollution through nitrate leaching. This study evaluates a strategy to enhance the sustainability of kohlrabi production (*Brassica oleracea*) by reducing N application while improving N use efficiency. It further investigates how product quality such as glucosinolate concentration is affected under these conditions. By applying 80% and 50% of the nitrogen fertilizers which contain an urease inhibitor or an additional nitrification inhibitor, higher sustainability through the mitigation of nitrogen losses is expected. The results show that a 20% reduction in N application is feasible with minimal yield loss and no significant effects on crop vitality or leaf greenness. The type of fertilizer had little impact on yield when 80% of N was supplied. While a 20% N reduction influences glucosinolate profiles, the effect on taste and quality, remains unclear. Overall, a 20% reduction in nitrogen fertilization can be accomplished without yield loss or significant changes in outer quality, although it may slightly alter glucosinolate profiles and taste perception.

Key words: Kohlrabi, nitrogen, nitrate, ammonium, nitrification inhibitor, quality, glucosinolates

Introduction

Plants require macro- and micronutrients for vegetative and reproductive growth. Nitrogen (N) in form of nitrate, ammonium, urea, or amino acids and oligopeptides are considered most important nutrients which are mainly responsible for high yield and optimum quality in vegetable production (YOLDAS et al., 2008; BLUMENTHAL et al., 2008). However, nitrate derived from agricultural production is considered the main reason for groundwater pollution. Within the sustainability aspect of the European Union, especially in regions where leafy vegetables are grown (SONG et al., 2009) nitrate fertilization levels possibly leading to leaching is under discussion. In order to obtain an equilibrium between sufficient crop yield, environmental protection, and product quality aspects, a strategy is needed which combines reduced N input with increased N use efficiency and maintaining high inner and outer product quality. A reduction of the N fertilization is the most direct method to lower environmental nitrate levels, but yield and quality losses might be expected. For example, in vegetable production, the greenness of leafy vegetables is relevant for consumer acceptance. The chlorophyll concentration in leaves, observed as the greenness, is directly correlated with N supply and to a certain extent increases with nitrate, urea, or ammonium fertilization (JAVED et al., 2022). Cruciferous vegetables such as kohlrabi, broccoli or cabbage are well-known for their content in glucosinolates which are considered beneficial and health promoting substances in human nutrition as they may have anti-inflammatory, antioxidant and chemo-protective effects (CONOLLY et al., 2021; VERKERK et al., 2009). Their content has been also shown to depend on the N supply (FALLOVO et al., 2011).

In addition to an overall reduction in N supply, the application of inhibitors to reduce N leaching and ammonia volatilization, and to improve N use efficiency might be an additional suitable strategy (DAWAR et al., 2010; DURUY et al., 2017). While urease inhibitors slow down the transformation of urea into ammonia and thus minimize the risk of ammonia volatilization, nitrification inhibitors slow down microbial transformation of ammonia into nitrate and thus minimize nitrate leaching and nitrous oxide production (SOARES et al., 2012; VANGELI et al., 2022). A field study using the urea fertilizer concluded that the use of nitrification inhibitor was able to keep the nitrate concentration in the soil solution constant (CUI et al., 2011; KIRSCHKE et al., 2019). However, only few studies have explored the effect of inhibitors in combination with urea fertilizers in vegetable production (CUI et al., 2011). Very little is known about whether addition of urease and/or nitrification inhibitors can contribute to an additional reduction in N input in vegetable production and which effects occur on product quality aspects (ABALOS et al., 2014).

In the present study we explored (*i*) the possibility to reduce N input in kohlrabi (*Brassica oleracea* var. Gongylodes) production by applying 20% less N. Moreover, we tested a nitrate/ammonium based (CAN) and two urea-based N fertilizers with urease inhibitor (PIAGRAN® pro) or with combined urease and nitrification inhibitor (ALZON® neo-N) (*ii*) to test effects on yield and quality of kohlrabi. The impact of different N supply levels on yield, greenness and internal quality i.e. glucosinolate levels of kohlrabi was evaluated.

Materials and methods

Experimental setup and harvest

Young kohlrabi plants (Rijk Zwaan Zaadteelt en Zaadhandel B.V, Bode F1 variety Gongylodes) were grown in 7.5 L plastic pots in a glasshouse under controlled light (14/10 h day/night) and temperature (22°C / 18°C) at the University of Hohenheim (Germany; 48° 42′ 43″ N, 9°12′ 50,6″ O). The substrate-sand mixture was made from a seedling substrate and sand at a ratio of 2:1 (v/v). The initial N content was 0.33 g N per pot. One plant per pot was grown. At planting, plants were fertilized based on an estimated yield of 390 d t ha^{-1} corresponding to a demand of 200 kg N ha^{-1}, 50 kg P ha^{-1}, 220 kg K ha^{-1}, 40 kg S ha^{-1}, 60 kg Mg ha^{-1} and 95 kg Ca ha^{-1}. Assuming a planting density of 16 plants per m^2, the nutrient demand

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for each plant was calculated. After subtracting the amounts of nutrients provided by the seedling substrate (for details see supplementary material S1), the nutrients supplemented for each pot at the time of planting are presented in Tab. 1. Seedlings were applied with solid nitrogen fertilizer, followed by compensatory applications of potassium dihydrogen phosphate and calcium sulfate two days later.

Tab. 1: The amount of nutrients per pot

	N	P_2O_4	K_2O	S	MgO	CaO
Nutrients before fertilization (g per pot)	0.33	0.37	0.93	0.37	0.37	-
To fertilize at 100% N* (g per pot)	0.92	0.37	0.93	0.37	0.37	0.59
To fertilize at 80% N* (g per pot)	0.67	0.37	0.93	0.37	0.37	0.59
To fertilize at 50% N* (g per pot)	0.30	0.37	0.93	0.37	0.37	0.59

* calculated on the basis of usual filed application amount in kohlrabi cultivation, see material and method section

Nitrogen was supplied in the form of three different fertilizer types: (*i*) CAN (calcium ammonium nitrate) containing 26% N as ammonium nitrate (EuroChem Antwerpen NV, Belgium); (*ii*) PIAGRAN® pro containing 46% N as urea and the UI 2-NPT (N-(2-nitrophenyl) phosphoric triamide) (SKW Stickstoffwerke Piesteritz GmbH); (*iii*) ALZON® neo-N containing 46% N as urea, the UI 2-NPT and the NI MPA (N-([3(5)-methyl-1H-pyrazol-1-yl] methyl) acetamide) (SKW Stickstoffwerke Piesteritz GmbH). Three different levels of N were applied, corresponding to 100%, 80% and 50% of the N demand, respectively. This experimental setup resulted in 9 different treatments (3 fertilizer types: CAN, PIAGRAN, ALZON and 3 N levels: N100, N80, N50). Each treatment included six biological replicates, totaling 54 pots. The pots were randomly arranged in a column-row layout.
Plants were grown for a total of 49-57 days after planting (DAP). Full maturity of the kohlrabi was defined as the day when the bulbs reached a diameter of 95 mm. This occurred on DAP 49 (CAN N100, CAN N80, ALZON N100, ALZON N80), on DAP 55 (PIAGRAN N100 and PIAGRAN N80) and on DAP 57 (CAN N50, ALZON N50, PIAGRAN N50), respectively. At harvest, fresh weight of bulbs and leaves was determined separately. One half of each bulb was cut into cubes, frozen at -80 °C and freeze dried before measurement of glucosinolates.

Non-destructive measurement of leaf greenness (SPAD)

Leaf greenness was determined non-destructively using a SPAD-502Plus device (Conioca-Minolta). An average SPAD value was taken from 2 older and 1 younger leaf of each plant once per week between DAP 10-46.

Nitrate concentration of kohlrabi bulbs

Nitrate concentrations in kohlrabi bulbs was photometrically quantified on the basis of a salicylic acid-sulphuric acid method (Zhang et al., 2021). Briefly, freeze-dried material (20 mg) was extracted in 1 mL ddH_2O and heated at 90 °C for 30 min at 850 rpm. After cooling, samples were centrifuged at 11000 × *g* for 10 min. A volume of 80 µL salicylic acid-sulfuric acid (5 vol/100 vol) was added to 20 µL of the supernatant, mixed well and allowed to stand at room temperature for 20 min, before adding 1.9 mL 2 M NaOH. The absorbance of all samples was measured at 410 nm. Each sample was determined in three technical replicates.

Glucosinolate determination

The aliphatic glucosinolates sinigrin and glucoiberin, and the indolic glucosinolates glucobrassicin, 4-methoxyglucobrassicin, and 1-methoxyglucobrassicin were determined from freeze-dried plant material using the near-infrared spectroscopic (NIRS) method (Hartwig, 2004). The method is based on the absorption bands of molecular vibrations excited by electromagnetic radiation in the near-infrared range (NIR-concept GmbH, 2020). Here, the XDS Rapid Content™ analyzer (Fa. Foss Denmark) was used (wavelengths 400-2000 nm, intervals of 2 nm). The calibration curve was derived from a previous glucosinolate determination for broccoli according to Sahamishirazi et al. (2017).

Statistics

Data were statistically analyzed using SPSS statistics. One-way and two-way analysis of variance ANOVA with LSD were then used to show significant differences and figures were made by R 3.6 (Posit Inc) and Origin pro 2023 (Origin lab Inc).

Results

Leaf greenness

The SPAD (greenness) values were measured weekly, and mean values taken over a period of 6 weeks are shown in Fig. 1. SPAD values varied between 42 and 54 (Fig. 1), and neither fertilizer type nor N supply level had a significant effect on mean SPAD values. Therefore, no significant interaction exists between leaf greenness and either the level of fertilization or the type of fertilizer used (S2).

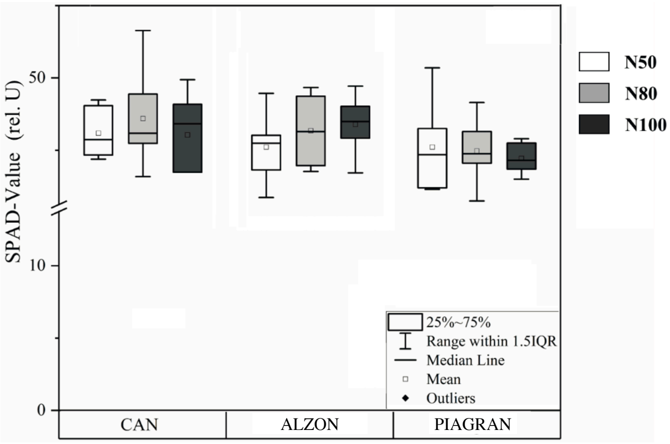

Fig. 1: Mean SPAD (greenness) values of kohlrabi leaves measured weekly during a period of 6 weeks. Box plots with N50 (white), N80 (grey), N100 (black) and three fertilizer types as indicated. No significant differences between all bars.

Biomass of kohlrabi bulbs

Bulb fresh weights increased with nitrogen application amount showing that the fertilization management of the pot experiment was effective and has a biomass increase effect from N50-N80-N100 (S2). The biomass was highest at the highest N supply level for all fertilizer types (Fig. 2). Bulb fresh weights were marginally different between N100 (365 ± 4 g) and N80 (356 ± 26 g). N100 fresh weights were significantly higher than at N50 (312 ± 2 g) across all fertilizer types. No significant differences were found between 3 fertilizer types at each N level (Fig. 2), bulb fresh weights were highest in CAN-N100 (375 g ± 7 g) and smallest in PIAGRAN-N50 (303 g ± 4 g).

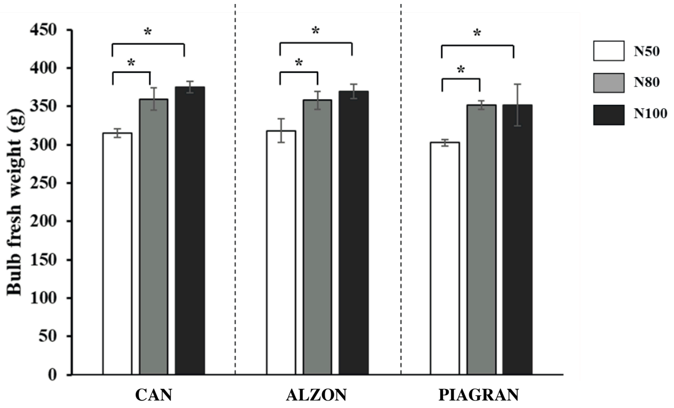

Fig. 2: Fresh weights of kohlrabi bulbs fertilized with three fertilizer types at three N supply levels (N50 white, N80 grey; N100 black). Bars represent means, error bars represent standard errors (n=6). One-way ANOVA with LSD was performed among different N levels within the same fertilizer type, and among different fertilizer types at the same N level. “*”, $p \leq 0.05$. No significant differences were found between fertilizer types at the same N level.

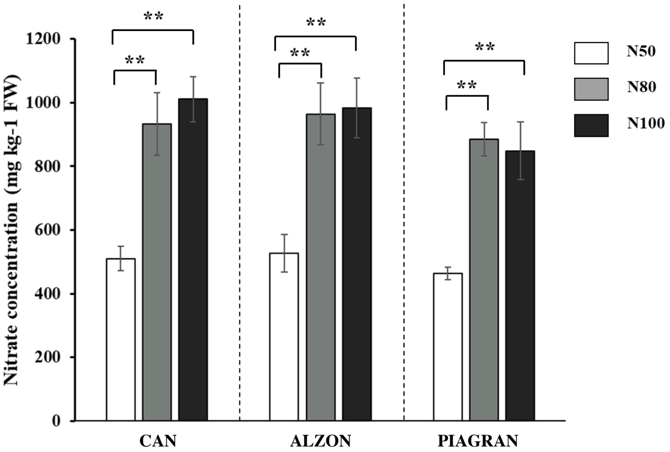

Fig. 3: Nitrate concentrations of kohlrabi bulbs (N50 white, N80 grey; N100 black). Bars represent means, error bars represent standard errors (n=6). One-way ANOVA with LSD was performed among different N levels within the same fertilizer type, and among different fertilizer types at the same N level. “**”, $p \leq 0.01$. No significant differences were found between fertilizer types at the same N level.

Nitrate concentrations of kohlrabi bulbs

The highest nitrate concentration of bulbs was 1011 mg kg^{-1} FW-1 in CAN-N100 (Fig. 3). This concentration is high but still below the limit of 5 g nitrate per kg FW. Kohlrabi is a vegetable prone to a higher risk of nitrate accumulation in tissues (Lošák et al., 2015). Mean nitrate concentrations in the bulbs across all fertilizer types were nearly identical in N100 (0.94 ± 0.2 g/kg FW) and N80 (0.93 ± 0.2 g/kg FW) treatments, and significantly higher than in N50 (0.55 ± 0.1 g/kg FW) (Fig. 3). At each N level, nitrate concentrations in the bulbs were consistently lowest in the PIAGRAN fertilizer type, although these differences were not statistically significant.

Glucosinolate concentrations of kohlrabi bulbs

Five different glucosinolates were detected in kohlrabi bulbs, namely the indolic glucosinolates: glucobrassicin, 1-methoxyglucobrassicin (neoglucobrassicin NGB), and 4-methoxyglucobrassicin (4-ME), and the aliphatic glucosinolates: sinigrin and glucoiberin (Fig. 4, 5). The most abundant glucosinolates were 4-ME and sinigrin, with concentration ranges of 194 ± 2 mg/kg DM and 135 ± 1 mg/kg DM, respectively, across all fertilizer treatments. However, no significant differences of sinigrin and 4-ME were observed in the concentrations in response to varying nitrogen levels (data not shown). With the exception of 4-ME, all indolic glucosinolates were significantly affected by the N supply level (Fig. 4, S2). Glucoiberin and NGB were significantly influenced by fertilizer type, (Fig. 4, Fig. 5, S2); there was a significant difference between the fertilizers ALZON and PIAGRAN when 20% nitrogen was reduced (Fig. 4B, Fig. 5). A significant interaction between fertilizer type and N level was observed for NGB (Fig. 4B, S2). Glucobrassicin increased with increasing N supply for all three fertilizer types (Fig. 4A), while NGB decreased with N supply level with the exception of ALZON at N100 (Fig. 4B). Glucoiberin was overall decreasing with increasing N supply levels with all fertilizer types, but to different extents (Fig. 5).

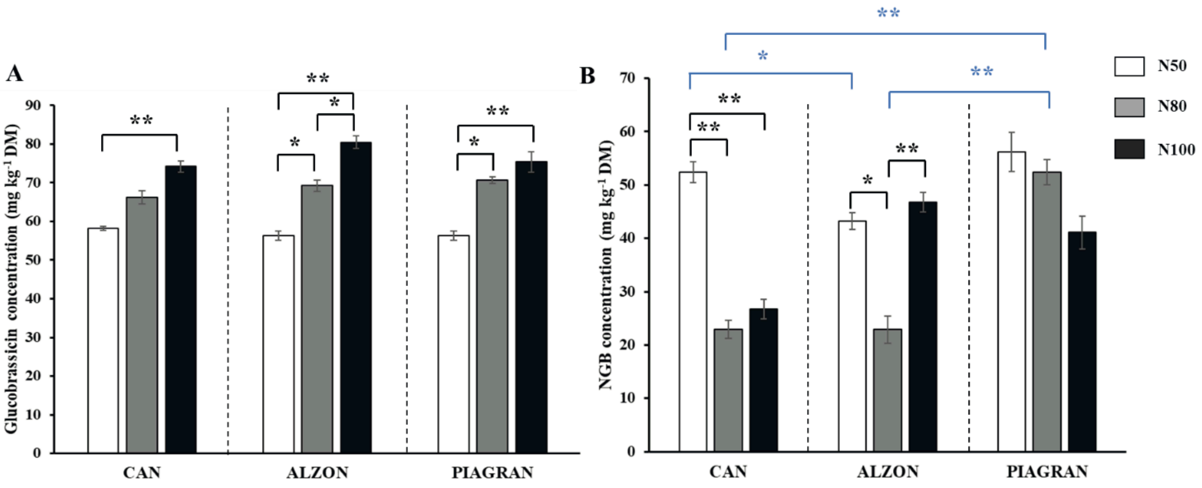

Fig. 4: Concentrations of the indolic glucosinolates: (A) glucobrassicin, (B) 1methoxyglucobrassicin (NGB) and in kohlrabi bulbs fertilized with three fertilizer types at three N supply levels (N50 white, N80 grey; N100 black). Bars represent means, error bars represent standard errors (n=6). One-way ANOVA with LSD was performed among different N levels within the same fertilizer type (black star label), and among different fertilizer types at the same N level (blue star label). “*”, $p \leq 0.05$; “**”, $p \leq 0.01$ (no significant differences in all treatments of 4-ME.)

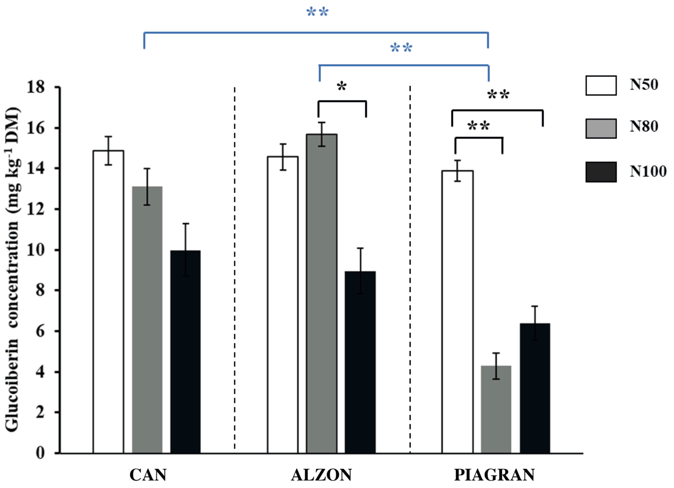

Fig. 5: Concentrations of the aliphatic glucosinolate: glucoiberin in kohlrabi bulbs fertilized with three fertilizer types at three N supply levels (N50 white, N80 grey; N100 black). Bars represent means, error bars represent standard errors (n=6). One-way ANOVA with LSD was performed among different N levels within the same fertilizer type (black star label), and among different fertilizer types at the same N level (blue star label). “*” $p \leq 0.05$; “**”, $p \leq 0.01$. (no significant differences in all treatments of sinigrin.)

Discussion

N supply can be reduced by 20% in kohlrabi without significant reduction of crop yield

Reducing the amount of N supply to agricultural fields is one of the main requirements for a more sustainable agriculture, especially in non-legume vegetable production, which is considered one of the main drivers for nitrate pollution in water bodies. Therefore it is a goal to test whether a reduction of N fertilization in relevant vegetables may lead to a yield decrease or to a quality change. Moreover, the potential to reduce environmental effects has to be evaluated under controlled conditions. Although our experimental approach was a pot trial, it clearly demonstrated that a 20% reduction in nitrogen (N) of the common practice did not result in biomass losses or lower greenness values, irrespective of the fertilizer type used. However, we are aware that pot trials should not be directly extrapolated to field conditions, and therefore, we do not intend to make practical recommendations. Nevertheless, our findings are clear: a 20% reduction in N has minimal impact on biomass yield but can already lead to changes in quality. Only when N supply was reduced to 50%, yield was significantly lowered. This indicates that at least in pot grown kohlrabi production, the suggested reduction by 20% of N supply in certain regions (so-called “red nitrogen areas”) might be feasible without major yield loss. This is also supported by the lack of response of chlorophyll concentrations (Fig. 1) to the three N supply levels. Based on studies with other representatives of the species *Brassica olerace*a (Vidigal et al., 2021; Westerveld et al., 2002), indicating that even at N50 no severe N deficiency occurred despite a biomass reduction by approximately 15% compared to N100. This also demonstrates, that reduction of 50% N was not a deficiency treatment and that the applied N amount were well-chosen.

In the present study, the fertilizer type had no statistically significant effect on the kohlrabi bulb biomass, indicating that at least in pot-grown plants, where leaching was non-existent due to an optimum watering, CAN and urea-based fertilizers with urease inhibitors are similarly suitable for kohlrabi production. Also, the presence of urease and nitrification inhibitors did not seem to be relevant for overall ‘crop yield’. Similar results were shown previously (Pfab et al., 2012; Scheer et al., 2017). However, it can be assumed that the inhibitors may be relevant under field conditions, where ammonia emission and leaching cannot be prevented and where the activity of microbiota could vary more than in pots. Depending on weather conditions, this variability could lead to significant nitrogen losses through nitrification and subsequent leaching. For instance, heavy rainfall can accelerate nitrogen leaching, while prolonged dry periods may reduce microbial activity, potentially limiting the effectiveness of inhibitors. Such positive effects of nitrification and urease inhibitors on yield were previously reported for various vegetables (Min et al., 2021; Pasada et al., 2001). An expansion on the implications for field conditions may further highlight the economic advantages of reduced nitrogen application, such as savings on fertilizers. This would further underline the practical relevance of the findings.

Does the use of urea and urease inhibitor change glucosinolates quality?

The glucosinolate concentrations and profiles in *Brassica* plants are influenced by various factors including nutrition, temperature, irradiation, and irrigation (Maschada et al., 2019). As one of health-promoting secondary metabolites, glucosinolates not only serve as one of the important quality indicators of kohlrabi, but also as an indicator of important nitrogen fertilizer changes in this brassica family of vegetables (Chun et al., 2017; Grant et al., 2011; Sathasivam et al., 2021). As nitrogen levels increased, the content of glucosinolates rose (Lie et al., 2007; Mashabela et al., 2019). However, individual glucosinolates exhibited distinct responses to the changes in nitrogen availability. Studies on broccoli have reported an increase in glucobrassicin concentrations with higher nitrogen input (Fabek et al., 2012), which aligns with our findings using CAN, PIAGRAN, and ALZON fertilizers in kohlrabi (Fig. 4A). In contrast, the concentrations of NGB decreased with increasing nitrogen fertilization for CAN and PIAGRAN (Fig. 4B). In agreement with the results observed in broccoli, higher nitrogen fertilization leads to lower glucoiberin concentration in kohlrabi (Fig. 5). In this study the increases in nitrogen levels did not result in significant differences in the concentrations of 4-ME and sinigrin (data not shown). The amount and type of N fertilizer available during growth had an effect on glucosinolate concentrations in different Brassica species, however consistent results were not found by the literature (Fallovo et al., 2011; Groenbaek et al., 2014; Mashabela et al., 2019). On the one hand, even within the cauliflower family, different varieties exhibit completely opposite responses. Sinigrin levels increased in the cauliflower variety ‘Largardo’ with higher nitrogen input, while they decreased in the variety ‘CF-744’ under the same conditions (Mashabela et al., 2019). On the other hand, it was confirmed that suitable ratio of sulfur/nitrogen (1/5~1/10), plays an important role in influencing glucosinolate concentrations (Kim et al., 2002; Schonhof et al., 2007; Omirou et al., 2009; Groenbaek et al., 2014). It has been suggested that, under conditions of sufficient sulfur availability, variations in N levels do not affect alphatic glucosinolate concentrations (Omirou et al., 2009). Based on these two factors, it can be speculated that the unchanged sinigrin content observed in this experiment may be attributed to varietal characteristics and the presence of sufficient sulfur. The stable concentrations of 4-ME, which did not respond to nitrogen fertilization in broccoli and cabbage, have also been reported by Rosen et al. (2005) and Omirou et al. (2009). Nevertheless, with the 20% reduction of fertilization, the quality maintains same level with full fertilization in the types of CAN and PIAGRAN. Nitrogen level takes an important role in glucosinolates quality of kohlrabi but depends on fertilizer type.

The patterns of glucosinolate in kohlrabi under CAN and PIAGRAN fertilizers showed similar trends as nitrogen levels changed (Fig. 4, Fig. 5). However, significant differences of NGB and glucoiberin concentrations were observed in CAN-N80 and PIAGRAN-N80. The kohlrabi bulbs treated with Piagran-N80 have higher NGB and lower glucoiberin levels compared to those treated with CAN-N80 (Fig. 4B, Fig. 5), resulting in a flavor that is less bitter, less pungent, and more spicy (Wieczorek et al., 2018; Wieczorek et al., 2022). This difference in flavor could offer economic benefits in production and sales. While ALZON contains two inhibitors designed to reduce nitrogen loss, its effect on the NGB and glucoiberin levels at N80 differs significantly from that at N100 (Fig. 4 B, Fig. 5). This suggests that the inhibitors in ALZON may require an optimal concentration or ratio to achieve their maximum effect in kohlrabi cultivation. Judging from the available results with kohlrabi, reducing the nitrogen fertilizer application rate by 20% in all fertilizer types did moderately change the glucosinolates pattern. It might be assumed that the combination of urea and urease inhibitor (Piagran) improved the absorption of nitrogen in kohlrabi.

Conclusion

A reduction of about 20% of the usual N fertilization practice might be feasible. This reduction may however lead to a changed glucosinolate pattern but also to slightly reduced nitrate concentrations in kohlrabi bulbs. Moreover, a reduction leads to lower entry of nitrate in soil and soil water fractions and may reduce fertilizer costs. The greenness of the kohlrabi is not changed therefore consumers would not detect a difference. The slightly altered glucosinolate pattern might influence the taste, but it is difficult to assess whether this would be perceived positively, as it could result in a lower sensory intensity of glucosinolates, which might then correlate with individual taste preferences. Considering factors like fertilizer application limitations, PIAGRAN remains a viable option for kohlrabi production, as it does not cause a significant decline in yield or quality even with a 20% reduction in fertilizer application.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Supplementary material

S1: Manufacturer's data of the seedling substrate used in this trial.

Seedling substrate	
pH value	5.5
Nitrogen	90 mg L^{-1}
Phosphate	100 mg L^{-1}
Potassium	250 mg L^{-1}
Magnesium	100 mg L^{-1}
Sulfur	100 mg L^{-1}

S2: Two-way ANOVA of fertilizer type with nitrogen level

Indicators	Fertilizer type	N level	F×N
FW bulbs (g)	ns	***	ns
SPAD value	ns	ns	ns
Nitrate concentration (mg/kg FW)	ns	***	ns
Glucoiberin (mg/kg DM)	*	**	ns
Glucobrassicin (mg/kg DM)	ns	**	ns
NGB (mg/kg DM)	**	**	*

S2: Two-way ANOVA of eight indicators. FW: fresh weight; 4-ME: 4-Methoxyglucobrassicin; NGB: 1-Methoxyglucobrassicin (Neoglucobrassicin); Two-way ANOVA was analyzed by R; *, $p \le 0.05$; **, $p \le 0.01$; ***, $p \le 0.001$.