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Original article

The combined effect of nitrogen and biochar amendments on the yield and glucosinolate contents of the Chinese cabbage

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ABSTRACT

Nitrogen plays an important role in plant growth as an essential nutrient. When crops are grown on biochar applied soil, their growth be positive affected. Biochar has been announced as a soil amendment to improve nitrogen (N) use efficiency. The present study aimed to investigate the combined effect of nitrogen fertilizer and biochar amendment on the growth of Chinese cabbage including its glucosinolates (GSLs) functional compounds. Acidic (AB), neutral (NB) and basic (BB) biochars produced at 330 °C, 400 °C and at 600 °C, respectively were employed in the study with each of them applied to the soil at a rate of 1% (w/w). N fertilizer in form of urea was split applied to the soil at three different rates of 160, 320, 640 kg ha^{-1}. The Chinese cabbage yield was highest in the 320 kg ha^{-1} nitrogen amendment and all the biochar amendments decreased yield in comparison to the 320 kg ha^{-1} nitrogen amendment. The Chinese cabbage yield in the biochar amended soils increased with increasing amount of nitrogen applied to the soil. The GSLs content was highest in the Chinese cabbage grown on the 320 kg ha^{-1} N amendment in all the treatments. Except, BB, biochar amendments generally produced Chinese cabbage with higher GSLs content than the urea only amendment.

1. Introduction

Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) is one of the Brassica vegetables, very important as ingredient of kimchi, which is mainly used in Korea (Cartea et al., 2011; Cho et al., 1999). Chinese cabbage has several classes of secondary metabolites such as glucosinolates (GSLs), carotenoids and other phytochemicals. Their compositions and contents were depended on the genotype and agricultural factors (Podsedek et al., 2007; Reif et al., 2013; Chun et al., 2017; Cuong et al., 2017). In particular, interest in the study of GSLs is increasing, because of its health-promoting effects (Axelsson et al., 2017). GSLs are converted to isothiocyanates, thiocyanates, or nitriles by enzymatic hydrolysis with myrosinase and responsible for the flavor, pathogen defense system, anticarcinogenic, and other pharmacological effects (Mithen et al., 2000; Seo et al., 2014;). GSLs were classified as aliphatic, indolic, aromatic contributing with their amino acid precursor methionine, tryptophan, and phenylalanine respectively (Fahey et al., 2001; Lee et al., 2016; Arasu et al., 2017; Kwak et al., 2017). Because GSLs are nitrogen-(N) and sulfur-(S) containing secondary metabolites derived from amino acids, their metabolism in vegetables is influenced by the N and S fertilization and also by the balance between them (Falk et al., 2007; Kim et al., 2015; Kim et al., 2016; Groenbaek et al., 2016; Jeon et al., 2017).

In soil-crop system, N fertilizer is the most widely used fertilizer and the primary source. However, either shortage or excess of N fertilizer can result in low product quality, the amount of N fertilizer rates should be carefully determined (Bergman, 1986; Li et al., 2017; Park et al., 2016a,b,c). Insufficient supply of N results in lower yields and smaller vegetable heads, while excess of this mineral nutrient leads to a high concentration of nitrates in the heads (Magnusson, 2002; Wang & Li, 2004; Min et al., 2015). Chinese cabbage is particularly an N fertilizer demanding crop (Vavrina and Obreza, 1993; Park et al., 2017a; Park et al., 2017b).

Rice is the most consumed crop in Korea and cultivated in many countries. As a result, more than 160 million tons of rice hulls are produced around the world annually. Effective recycling of rice hulls is important to solve the problem of agricultural waste

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(Park et al., 2017; Ebe et al., 2019). There are many advantages of converting these rice hull to biochar such as energy production carbon sequestration, improvement of soil quality, and enhancement of crop yields (Abrishamkesh et al., 2015). Biochar has been proven to increase crop productivity, soil structure and nutrient adsorption. In particular, biochar has been found to alter soil N dynamics and water availability affecting N uptake by plants and plant growth and nutritional status (Fiorentino et al., 2019; Liu et al., 2017; Park et al., 2018). However, little information is available about how biochar could affect plant secondary metabolism (Viger et al., 2015).

The objective of this study was to assess the combined effect of biochar and nitrogen fertilization on the Chinese cabbage yield and its GSL contents.

2. Materials and methods

2.1. Preparation and analysis of the soil and biochar

The study was conducted through a field experiment that was set up at Chungnam National University experimental farm, located at the Daejeon, Korea (latitude, 127°35'E; longitude, 36́36́N). The soil at the farm is a sandy loam and belongs to the Inceptisol and Udepts order and suborder, respectively according to the IUSS working group WRB classification (Table 1). Soil pH and EC were determined in water by paying strict adherence to the method outlined by Benchtop Meter (ORION™ Versa Star Pro™, Thermo Scientific Inc., Waltham, Massachusetts, USA). The soil cations were determined with ICP-OES (ICAP 7000 series ICP spectrometer, Thermo Scientific Inc., Waltham, Massachusetts, USA) after extraction from the soil with 1.0 M neutral ammonium acetate solution (pH 7.0). Total carbon and nitrogen were assessed with the CHN Elemental Analyzer (TruSpec Micro, Leco, Michigan, USA). Both total and available phosphorus were determined by UV/Vis-spectrophotometer (GENESYS 50, Thermo Scientific Inc., Waltham, Massachusetts, USA) using a Lancaster method (RDA, 1988).

The biochar used in the study was purchased from Purnnature (Suncheon, Korea) and Yoogi Lnd (Gochang, Korea) and was prepared by charring rice hull, a readily available agricultural waste in South Korea, at different temperatures of 330 °C, 400 °C and 550 °C. The biochars produced at 330 °C were acidic while those produced at 400 °C and 550 °C were neutral and basic, respectively. The pH and EC were determined by Benchtop Meter with pH and EC (ORION™ Versa Star Pro™, Thermo Scientific Inc., Waltham, Massachusetts, USA) in distilled water at a ratio 1:5 (w/w) and stirring for 1 h. The total carbon and nitrogen were assessed with the CHN elemental analyzer (LECO, TruSpec, USA). The total phosphorus was determined by Vanadate method (Tandon et al., 1968) and UV/Vis-spectrophotometer (GENESYS 50, Thermo Scientific Inc., Waltham, Massachusetts, USA). The concentration of cations in the biochar was quantified by ICP-OES ICAP 7000series ICP spectrometer, Thermo Scientific Inc., Waltham, Massachusetts, USA). The properties of biochar obtained from the analysis are given in Table 2.

2.2. Experimental set up and crop management

The study was set up in a completely randomized design and each treatment was replicated thrice. Each replicate was set up on a 3 m × 2.5 m plot which translates to an area of 7.5 m^2. The protection bands, 1 m in width, were left to prevent the contamination of the plots with fertilizers from the neighboring sectors. The Chinese cabbage variety grown was 'Chunkwang' which was purchased from SAKATA KOREA (Seoul, Korea) and the spacing adopted was 0.4 m within the plants rows. Each plot was planted with only one row of Chinese cabbage. The study was laid out with twelve treatments which included the following; Nitrogen fertilizer applied at the recommended rate (1.0 N), nitrogen fertilizer applied at half the recommended rate (0.5 N), nitrogen fertilizer applied at double the recommended rate (2.0 N) as well as the combined applications of biochars with nitrogen fertilizers i.e. AB + 1.0 N, AB + 0.5 N, AB + 2.0 N, NB + 1.0 N, NB + 0.5 N, NB + 2.0 N, BB + 1.0 N, BB + 0.5 N and BB + 2.0 N. Biochar was applied to the soil at a rate of 1% (w/w) following the recommendations of the previous study by Oh et al. (2017). Urea, phosphorus pentoxide and potassium oxide were utilized to supply nitrogen, phosphorus and potassium, respectively and for the 1.0 N amendment, the quantities of the nutrients applied were 320, 78 and 198 kg/ha of nitrogen, phosphorus and potassium, respectively. Split application of nitrogen and potassium was adopted where a third of the nutrients were applied at the transplanting stage. The next 1/3rd of the nutrients were applied 15 days after transplanting and the remaining 1/3rd were applied ~~at~~ 30 days after transplanting. The plots were irrigated after each fertilizer application to prevent water stress. The parameters studied included; plant weight, water content, head diameter, head length, leaves diameter, leaves length, chlorophyll (SPAD). The weight of Chinese cabbages was measured after harvest. Plant height was determined by ruler, the three highest leaves were used to measure leaf length and diameter. After cutting the head in half, diameter and length were estimated. The chlorophyll contents of plant were determined by MINOLTA Chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan).

Table 1
Chemical properties of soil before this experiment.

Treatment	pH (1:5, D.W)	EC (ds m^{-1})	Av. P_2O_5 (mg kg^{-1})	T-C (%)	T-N	Ca^{2+} ($cmol_c$ kg^{-1})	K^+	Mg^{2+}	Na^+
Initial soil	7.0 ± 0.2	0.35 ± 0.05	94.10 ± 21.08	0.71 ± 0.19	0.11 ± 0.05	4.50 ± 0.21	0.21 ± 0.03	1.24 ± 0.11	0.17 ± 0.00

Abbreviations: D·H_2O, Distilled water; EC, Electrical Conductivity; Av. P_2O_5, Available Phosphate; T-C, Total Carbon; T-N, Total Nitrogen.

Table 2
Pyrolysis conditions and chemical properties of rice hull biochar.

Treatment	Temp.(°C)	Time (min)	pH (1:10, D.W)	EC (dS m^{-1})	T-C (%)	T-N	T-P_2O_5	CaO	K_2O	MgO	Na_2O
Biochar	330	15	6.1	11.49 ± 1.62	41.3 ± 0.0	0.4 ± 0.0	0.14 ± 0.03	0.09 ± 0.03	0.36 ± 0.12	0.04 ± 0.02	0.03 ± 0.01
	400	15	7.1	9.50 ± 0.83	44.1 ± 0.0	0.4 ± 0.0	0.06 ± 0.01	0.08 ± 0.02	0.47 ± 0.07	0.04 ± 0.02	0.03 ± 0.01
	600	30	11.0	6.59 ± 0.13	54.9 ± 0.2	0.6 ± 0.0	0.21 ± 0.00	0.16 ± 0.05	0.78 ± 0.29	0.07 ± 0.04	0.04 ± 0.01

Abbreviations: Temp., Temperature; D·H_2O, Distilled water; EC, Electrical Conductivity; T-C, Total Carbon; T-N, Total Nitrogen; T-P_2O_5, Total Phosphate.

2.3. Glucosinolate analysis using by HPLC

Desulfo (DS) – GSLs were extracted according to the procedure of Chun et al. (2018) and ISO 9167-1 (1992). Freeze-dried plant powders (100 mg) were extracted by 1.5 ml of 70 % (v/v) boiling methanol in water bath at 5 min. After centrifugation at 12,000 rpm for 10 min, the resulting supernatant was collected, and the residues were re-extracted twice by repeating the above mentioned process. The combined supernatant was taken as the crude of GSLs. Separately 0.5 mg of sinigrin (external standard) was dissolved in 5 ml water. Desulfation of the crude extracts and sinigrin were performed on DEAE anion exchange column which was prepared by Sephadex A-25 previously activated with 0.5 M sodium acetate. The crude GSL extracts were loaded into a pre-equilibrated column and rinsed two times with 1 ml of water. 75 μl of Aryl sulfatase solutions (E.C.3.1.6.1) was then loaded into each column. After 16 hrs of desulfation reaction at room temperature, the desulfated GSLs were eluted with 1.5 ml of water. The supernatants were filtered through a 0.45 μm PTFE syringe filter and analyzed by HPLC. GSLs were analyzed by 1260 Infinity HPLC system (Agilent Technologies, CA, USA) equipped with an Inertsil ODS-3 column (150 × 3.0 mm ID, particle size 3 μm) (GL Science, Tokyo, Japan). The HPLC analysis was carried out with a column oven temperature of 40 °C and a wavelength of 227 nm. The solvent system employed was solvent (A) water and (B) 100 % acetonitrile. The gradient elution program was as follows with a flow rate of 0.4 ml/min, 0–2.0 min, 0% B; 2.0–7.0 min, 10% B; 7.0–16 min, 31% B; 16–19 min, 31% B; 19–21 min, 0% B and 21–

Table 3
Chemical properties of soil in different biochar treatments.

Treatment		pH (1:5, H_2O)	EC (dS m^{-1})	T-C (%)	T-N	Av. P_2O_5 (mg kg^{-1})	Ca^{2+} ($cmol_c$ kg^{-1})	K^+	Mg^{2+}	Na^+
Only urea	0.5 N	7.16 ± 0.36	1.49 ± 1.00	0.61 ± 0.19	0.06 ± 0.02	133.38 ± 13.90	4.59 ± 0.10	0.23 ± 0.04	1.86 ± 0.55	0.28 ± 0.12
	1.0 N	6.80 ± 0.17	2.25 ± 0.58	0.66 ± 0.13	0.09 ± 0.03	117.40 ± 57.31	4.62 ± 0.05	0.77 ± 0.45	1.51 ± 0.17	0.20 ± 0.01
	2.0 N	6.59 ± 0.18	2.33 ± 1.07	0.74 ± 0.18	0.11 ± 0.03	122.74 ± 19.97	4.09 ± 0.30	0.94 ± 0.08	1.48 ± 0.18	0.22 ± 0.02
Acidic Biochar	0.5 N	7.19 ± 0.18	0.52 ± 0.09	1.03 ± 0.12	0.07 ± 0.02	140.69 ± 39.79	4.60 ± 0.13	0.63 ± 0.52	1.74 ± 0.12	0.21 ± 0.06
	1.0 N	7.09 ± 0.49	1.15 ± 0.34	1.19 ± 0.06	0.08 ± 0.02	159.69 ± 29.63	4.93 ± 1.05	0.73 ± 0.38	1.76 ± 0.29	0.22 ± 0.04
	2.0 N	7.22 ± 0.43	1.90 ± 0.97	1.44 ± 0.17	0.10 ± 0.03	112.08 ± 21.34	4.90 ± 0.37	0.58 ± 0.04	1.59 ± 0.08	0.22 ± 0.01
Neutral Biochar	0.5 N	7.26 ± 0.22	1.62 ± 0.65	1.64 ± 0.37	0.07 ± 0.01	107.99 ± 27.98	5.54 ± 0.57	0.41 ± 0.09	1.72 ± 0.03	0.20 ± 0.00
	1.0 N	7.04 ± 0.36	2.05 ± 0.74	1.78 ± 0.68	0.08 ± 0.01	140.56 ± 21.74	4.66 ± 0.21	1.27 ± 0.73	1.55 ± 0.25	0.22 ± 0.04
	2.0 N	7.13 ± 0.69	2.33 ± 1.01	2.26 ± 0.49	0.10 ± 0.01	143.44 ± 56.80	4.76 ± 0.52	1.13 ± 0.94	1.45 ± 0.08	0.25 ± 0.09
Basic Biochar	0.5 N	7.45 ± 0.21	0.87 ± 0.16	3.69 ± 0.58	0.10 ± 0.01	113.42 ± 6.23	5.33 ± 0.07	0.32 ± 0.04	1.65 ± 0.02	0.19 ± 0.02
	1.0 N	7.84 ± 0.22	0.53 ± 0.04	4.06 ± 1.23	0.12 ± 0.03	154.40 ± 54.19	5.01 ± 0.34	1.51 ± 0.75	1.58 ± 0.11	0.27 ± 0.06
	2.0 N	7.24 ± 0.64	1.41 ± 0.48	4.76 ± 1.44	0.11 ± 0.02	137.17 ± 34.87	4.57 ± 0.55	0.40 ± 0.16	1.37 ± 0.08	0.20 ± 0.02

Abbreviations: EC, Electrical Conductivity; T-C, Total Carbon; T-N, Total Nitrogen; Av. P_2O_5, Available Phosphate.

Table 4
Chinese cabbage growth according to the use of nitrogen fertilizer and biochar.

Treatment		Head			Leaf		Chlorophyll	Water contents
		Fresh weight	Height	Width	Length	Width		
		(g)	(mm)		(mm)		(SPAD)	(%)
Only urea	0.5 N	3077.3 ± 138.4b	87.7 ± 0.7ab	143.0 ± 2.9bc	332.6 ± 4.6avc	230.2 ± 6.7ab	36.2 ± 0.1d	87.7 ± 0.7ab
	1.0 N	3859.3 ± 110.0a	87.7 ± 2.9ab	168.7 ± 3.7a	333.9 ± 21.7abc	228.1 ± 17.8abc	39.2 ± 4.0 cd	87.7 ± 2.9ab
	2.0 N	3635.3 ± 419.4a	86.8 ± 1.1abc	153.3 ± 10.9b	348.1 ± 9.4ab	249.0 ± 13.3a	41.7 ± 3.7bcd	86.8 ± 1.1abc
Acidic Biochar	0.5 N	1943.2 ± 41.8e	84.0 ± 0.4c	111.7 ± 4.8f	315.2 ± 7.6c	193.9 ± 3.0d	36.2 ± 1.0d	84.0 ± 0.4c
	1.0 N	2572.7 ± 36.0c	86.1 ± 1.4abc	127.3 ± 5.4 cd	322.3 ± 14.8bc	210.0 ± 12.2abc	40.9 ± 0.9bcd	86.1 ± 1.4abc
	2.0 N	2473.5 ± 83.4 cd	85.6 ± 2.0bc	126.0 ± 7.1de	319.2 ± 10.6c	207.3 ± 6.1abc	48.6 ± 4.7a	85.6 ± 2.0bc
Neutral Biochar	0.5 N	2210.7 ± 203.0de	85.4 ± 1.1bc	127.7 ± 6.3 cd	323.2 ± 12.3bc	203.8 ± 5.9 cd	38.1 ± 2.5d	85.4 ± 1.1bc
	1.0 N	2777.3 ± 73.1bc	86.6 ± 0.9abc	130.9 ± 12.0 cd	332.7 ± 19.6abc	211.6 ± 16.8abc	39.3 ± 2.4 cd	86.6 ± 0.9abc
	2.0 N	2846.5 ± 84.1bc	86.6 ± 1.1abc	141.3 ± 6.8bcd	342.8 ± 7.9abc	225.6 ± 10.2abc	46.0 ± 1.5ab	86.6 ± 1.1abc
Basic Biochar	0.5 N	2634.1 ± 130.7c	86.2 ± 2.1abc	129.2 ± 7.8 cd	326.3 ± 10.6bc	200.1 ± 7.7d	36.1 ± 1.2d	86.2 ± 2.1abc
	1.0 N	3628.5 ± 153.1a	89.1 ± 0.3a	151.7 ± 1.7b	357.2 ± 5.5a	243.7 ± 5.5a	40.3 ± 2.8bcd	89.1 ± 0.3a
	2.0 N	3647.3 ± 134.5a	85.1 ± 1.2bc	15.7 ± 7.1ab	360.8 ± 6.1a	244.9 ± 9.9a	44.8 ± 2.5abc	85.1 ± 1.2bc

Within each column, values followed by the same letters are not significantly different at $p < 0.05$, using Duncan's multiple-range test ($n = 3$).

Table 5
Glucosinolates identified in Chinese cabbage.

No.	Retention time	Trivial name	Chemical name	Structure of R group	Compound group	Response factor
1	9.11	Progoitrin	2-Hydroxy-3-butenyl-	CH_2=CH-CH(OH)-CH_2-	Aliphatic	1.09
2	9.87	Sinigrin	2-Propenyl-	CH_2=CH-CH_2-	Aliphatic	1.00
3	10.76	Glucoalyssin	5-Methylsulfinylpentyl-	CH_3-SO-CH_2-CH_2-CH_2-CH_2-CH_2-	Aliphatic	1.07
4	11.13	Gluconapoleiferin	2-Hydroxy-4-pentenyl-	CH_2=CH-CH_2-CH(OH)-CH_2-	Aliphatic	1.00
5	12.42	Gluconapin	But-3-enyl-	CH_2=CH-CH_2-CH_2-	Aliphatic	1.11
6	14.72	Glucobrassicanapin	Pent-4-enyl-	CH_2=CH-CH_2-CH_2-CH_2-	Aliphatic	1.15
7	15.86	Glucobrassicin	Indol-3-ylmethyl-	Indole-3-CH_2-	Indolic	0.29
8	16.77	4-Methoxyglucobrassicin	4-Methoxy-indol-3-ylmethyl-	Indole-4-OCH_3-	Indolic	0.25
9	17.15	Gluconasturtiin	2-Phenylethyl-	C_6H_5-CH_2-CH_2-	Aromatic	0.95
10	18.91	Neoglucobrassicin	1-Methoxy-indol-3-ylmethyl-	Indole-1-OCH_3-	Indolic	0.25

No., the elution order of glucosinolates from HPLC chromatograms in Fig. 2.

Fig. 1. Chinese cabbage by biochar treatment. a), Only urea; b), Acidic Biochar; c), Neutral Biochar; d), Basic Biochar.

27 min, 0% B. Individual GSLs were identified with previously data (Chun et al, 2018) and quantified according to their peaks of HPLC area and response factor by comparison to those of an external standard sinigrin solution (ISO 9167-1, 1992; Clarke, 2010).

2.4. Statistical analysis

All the data were subjected to a one-way analysis of variance (ANOVA) using IBM SPSS statistical software (version 26 for Windows, SPPS Inc., Chicago, IL, USA). The significantly different data at $P \leq 0.05$ were subjected to the Duncan's multiple range test to quantify the differences between the different treatments.

3. Results and discussions

3.1. Changes in the chemical characteristics of soil

Chemical properties of soil after experiment were shown in Table 3. The pH and EC of soli are important factors of the nutrient availability of plant. The soil pH of BB + 1.0 N amendment was the highest value (pH 7.8). In all biochar amendments were increased to compare with initial soil and urea fertilizer only treatments. The pH of soils tended to decrease as the inorganic fertilizer application (Eo et al., 2016). Shin et al (2019) reported rice hull biochar could be used to improve acid soil effectively increasing soil pH and CEC. Soil EC values has increased by amendments with biochars (Park et al, 2020). But, soil EC values are thought to have no effect by biochar, increasing N tends to increase the EC. Total carbon contents in initial soil were 0.99%, and after the experiment, it decreased in the urea fertilizer only treatments and as the total carbon contents of biochar amendment was increased. The exchangeable cations, Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} were increased after treatment with fertilizer and biochar.

3.2. Effects of the different treatment on yield

The comparative effects of the different amounts of nitrogen fertilizer on Chinese cabbage growth and different rice hull biochar amendments were assessed and the result are shown in Table 4. While the 1.0 N amendment produced higher yields than the 2.0 N treatment, there was no statistical significance difference between the yield produced with the recommended and the double rates of nitrogen treatments, but applying nitrogen at half the recommended rate reduced yield. Lee et al (2012) reported that

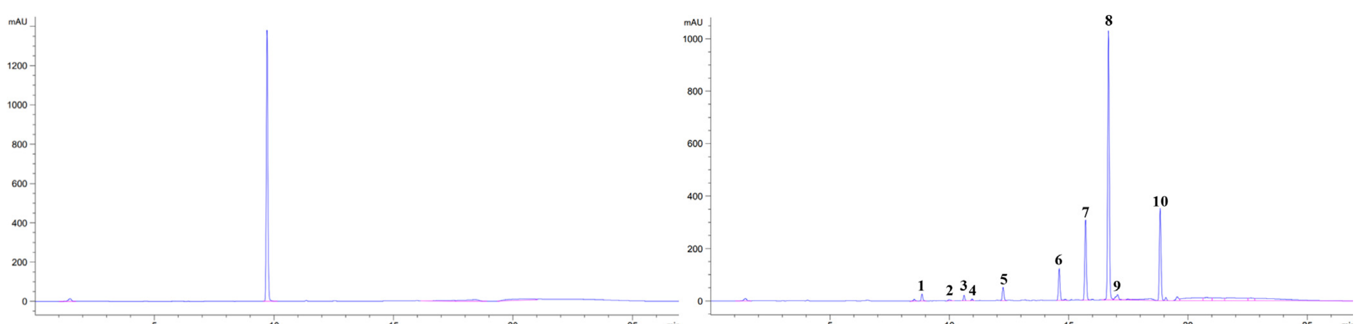

Fig. 2. HPLC chromatogram of sinigrin standard and Glucosinolate in Chinese cabbage.

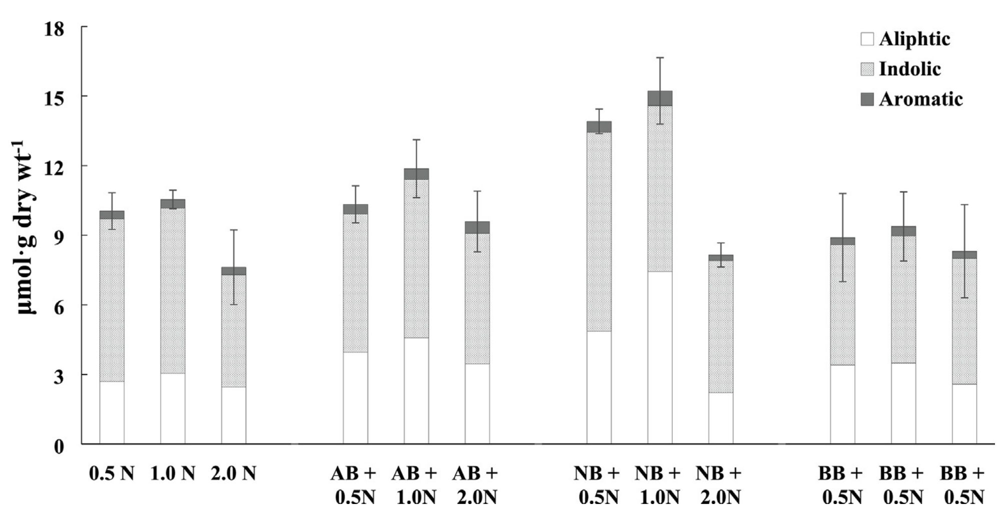

Fig. 3. Glucosinolate portions divided into three groups in Chinese cabbage. Within each column, values followed by the same letters are not significantly different at $p < 0.05$, using Duncan's multiple-range test ($n = 3$). Abbreviations: N, Nitrogen fertilizer; AB, Acidic Biochar; NB, Neutral Biochar; BB, Basic Biochar.

there was no significant difference in the yield when plants were grown with different levels of nitrogen fertilizer. And also, Staugaitis et al (2008) investigated that lower nitrogen rates the yield decreased and the Chinese cabbage heads were smaller and the yield was lower. Similarly, in our study the recommended and the double rates of nitrogen treatments produced well in the width and length of the leaves and heads, and the chlorophyll contents of the leaves increased.

To confirm the effectiveness of biochar amendment in growing Chinese cabbage, a comparison to urea fertilizer only treatments was performed and it was found that the Chinese cabbage yield was reduced in acidic and neutral biochar treatments whereas there were no statistical significant differences between the urea fertilizer only and basic biochar treatments. Biochar has generally a very high pH and contains nutrient elements. These elements could be used for plant nutrition. However, only in few plant growth trials the nutrients presented in biochar were taken into account when it was used as a growing medium (Prasad et al., 2020). Numerous papers have evaluated biochar's positive effects on plant growth but, few authors have reported negative effects (Woo, 2013, Lee et al., 2018).

3.3. Glucosinolates profile and contents

GLSs content in the Chinese cabbage leaves was quantified and the results are shown Table 5. Six aliphatic GSLs including progoitrin, sinigrin, glucoalyssin, gluconapoleiferin, gluconapin and glucobrassicanapin, three indolyl GSLs including glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin as well as one aromatic GSLs (gluconasturtiin) were identified based on the HPLC chromatogram. Each of the peaks and retention time coincided with those reported in Chun et al (2018). In Chinese cabbage leaves, a statistically significant increase in total GSLs were observed in biochar treatment compared to the urea only treatment except for BB as shown in Fig. 1. The aliphatic GSL contents were increased in all biochar treatments, however, in the case of sinigrin, known as a functional component, there was no statistically significant difference. Indolic GSLs containing glucobrassicin were increased significantly in the NB treatment. Indole-3-carbinol is a phytochemical that is derived from the breakdown of the glucobrassicin. It has been reported to contain diverse promising biological properties, with anti-atherogenic, antioxidant, anti-carcinogenic, and anti-inflammatory activities by Kim and Park (2018). The contents of aromatic GSL, gluconasturtiin which is precursor of phenethyl isothiocyanate with efficient therapeutic properties (Soundararajan and Kim, 2018; Thwe et al., 2016) was increased in the biochar treatments. Garcia-Ibañez et al. (2020) reported that the biochar amendments enhanced the GSLs concentration in broccoli. In our study, NB and AB amended soils produced the Chinese cabbage with the highest contents of GSL (Table 6).

The GSL contents were the highest in all treatments that were fertilized with nitrogen at a recommended rate of 320 kg ha^{-1}, followed by half and double the recommended rates of nitrogen application. Chen et al (2006) and Li et al (2007) reported that increasing N tends to decrease the total GSL contents in Brassica crops. Generally, the content of GSLs and the bitter taste have a significant relationship with the degradation products. Above all, the amount of gluconapin and glucobrassicanapin in B. rapa is related to the bitterness (Padilla et al., 2007). Their content accounted for approximately 73% of the aliphatic GSL contents, and the tendency to increase and decrease due to nitrogen fertilizers was similar to that of the total and aliphatic GSLs. The indolic GSLs tended to decrease with increasing nitrogen application, while the aromatic GSL contents did not exhibit any relationship to the rates of nitrogen applied to the soil (Fig. 3).

Table 6
Glucosinolate contents in Chinese cabbage effected by nitrogen fertilizer and biochar.

Treatment		Aliphatic GSL						Indolic GSL			Aromatic GSL	Total
		PRO	SIN	GAL	GNP	GNA	GBN	GBS	4-MGBS	NGBS	GNST	
Only urea	0.5 N	0.41 ± 0.08bc	0.12 ± 0.02a	0.32 ± 0.03bc	0.17 ± 0.07a	0.43 ± 0.09d	1.23 ± 0.19d	1.86 ± 0.20ab	3.35 ± 0.36ab	1.82 ± 0.30bcd	0.33 ± 0.07bcd	10.04 ± 0.79cde
	1.0 N	0.33 ± 0.08bcde	0.09 ± 0.02a	0.22 ± 0.08bc	0.09 ± 0.02bcd	0.64 ± 0.19d	1.66 ± 0.43bcd	1.24 ± 0.16 cd	3.95 ± 0.12a	1.92 ± 0.83abc	0.37 ± 0.16bcd	10.54 ± 0.40 cd
	2.0 N	0.22 ± 0.09e	0.11 ± 0.03a	0.29 ± 0.08bc	0.06 ± 0.01 cd	0.57 ± 0.21d	1.21 ± 0.30d	0.91 ± 0.40d	3.00 ± 0.44ab	0.92 ± 0.14de	0.33 ± 0.07bcd	7.63 ± 1.61d
Acidic Biochar	0.5 N	0.44 ± 0.05b	0.06 ± 0.03a	0.32 ± 0.04bc	0.12 ± 0.02abc	0.93 ± 0.05bcd	2.11 ± 0.13bcd	1.04 ± 0.18d	3.49 ± 0.34ab	1.42 ± 0.20bcde	0.42 ± 0.05bcd	10.33 ± 0.80 cd
	1.0 N	0.43 ± 0.05b	0.08 ± 0.01a	0.43 ± 0.20bc	0.10 ± 0.01bcd	1.21 ± 0.34bc	2.33 ± .50bc	1.40 ± 0.21bcd	3.62 ± 0.62ab	1.80 ± 0.63bcd	0.47 ± 0.03bcd	11.87 ± 1.25bc
	2.0 N	0.40 ± 0.09bcd	0.10 ± 0.06a	0.55 ± 0.51ab	0.10 ± 0.02bcd	0.78 ± 0.16 cd	1.53 ± 0.28bcd	1.03 ± 0.15d	3.04 ± 0.91ab	1.54 ± 0.48bcde	0.52 ± 0.15ab	9.59 ± 1.31cde
Neutral Biochar	0.5 N	0.43 ± 0.11bc	0.07 ± 0.05a	0.37 ± 0.07bc	0.07 ± 0.04 cd	1.36 ± 0.71b	2.56 ± 1.29b	2.04 ± 0.77a	3.79 ± 0.36ab	2.75 ± 0.64a	0.47 ± 0.15abc	13.91 ± 0.53ab
	1.0 N	0.61 ± 0.10a	0.08 ± 0.02a	0.79 ± 0.18a	0.11 ± 0.01bc	2.05 ± 0.24a	3.80 ± 0.57a	1.68 ± 0.43abc	3.35 ± 0.71ab	2.11 ± 0.81ab	0.64 ± 0.11a	15.22 ± 1.43a
	2.0 N	0.26 ± 0.06cde	0.11 ± 0.02a	0.18 ± 0.04c	0.06 ± 0.04 cd	0.50 ± 0.30d	1.10 ± 0.37d	1.20 ± 0.07 cd	2.63 ± 0.67b	1.85 ± 0.48abcd	0.25 ± 0.03d	8.16 ± 0.52 cd
Basic Biochar	0.5 N	0.48 ± 0.18ab	0.07 ± 0.08a	0.33 ± 0.19bc	0.14 ± 0.05ab	0.72 ± 0.37 cd	1.66 ± 0.74bcd	1.16 ± 0.16 cd	2.93 ± 0.36ab	1.10 ± 0.33cde	0.31 ± 0.13 cd	8.90 ± 1.90 cd.
	1.0 N	0.36 ± 0.07bcde	0.10 ± 0.07a	0.25 ± 0.05bc	0.09 ± 0.02bcd	0.84 ± 0.10bcd	1.85 ± 0.37bcd	0.99 ± 0.11d	3.66 ± 0.54ab	0.83 ± 0.21e	0.41 ± 0.09bcd	9.39 ± 1.49 cd
	2.0 N	0.24 ± 0.04de	0.09 ± 0.02a	0.28 ± 0.03bc	0.04 ± 0.04d	0.61 ± 0.13d	1.33 ± 0.38 cd	0.92 ± 0.37d	3.73 ± 1.06ab	0.76 ± 0.30e	0.32 ± 0.09bcd	8.30 ± 2.01 cd

Within each column, values followed by the same letters are not significantly different at $p < 0.05$, using Duncan's multiple-range test ($n = 3$).
Abbreviations: GSL, Glucosinolate; PRO, Progoitrin; SIN, Sinigrin; GAL, Glucoalyssin; GNP, Gluconapoleiferin; GNA, Gluconapin; GBN, Glucobrassicanapin; GBS, Glucobrassicin; 4-MGBS, 4-Methoxyglucobrassicin; NGBS, Neoglucobrassicin; GNST, Gluconasturtiin.

4. Conclusions

Chinese cabbage is an important ingredient in the diet of Koreans. Strategy of improving its productivity and quality is important. This study was conducted to explore the optimal nitrogen fertilization regimes in order to increase productivity and quality of the Chinese cabbage with or without biochar amendments. The results revealed that the yield was applying nitrogen at half the recommended rate negatively impacted yield while doubling the recommended nitrogen rate had no significant effect on yield in comparison with the recommended nitrogen. Additionally, acidic and neutral biochar amendments had negative impacts on the Chinese cabbage yields while the basic biochars didn't have any impact on yield in comparison with the recommended nitrogen rate. The GSL content, known as the functional ingredient in brassica vegetables, was highest in the recommended rate of nitrogen treatments, and decreased as nitrogen input increased. When treated with neutral biochars, the amount of GSL, including glucobrassicin and gluconsturtiin, which have anticancer effects, increased. The effects of biochar amendment on soil physico-chmical properties, alters soil nutrition dynamic including nitrogen and have agronomic benefits. According to this study, biochar and nitrogen fertilizer can be effectively used to improve the quality and yield of Chinese cabbage.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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