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Article

Alleviating Effect of the Application of the Easily Decomposable Carbohydrate on Ammonium Toxicity in Chinese Cabbage (*Brassica rapa var. chinensis*)

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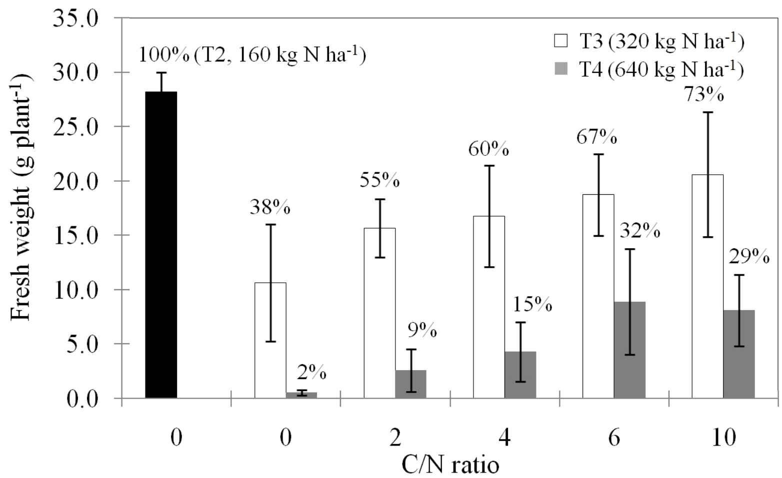
An excess application of N fertilizer causes physiological and morphological disorder known as ammonium (NH_4^+) toxicity in Chinese cabbage and it has been to be an issue for appropriate N fertilizer management. Hence, the pot experiment was conducted in order to evaluate the alleviating effect of the application of the easily decomposable carbohydrate on NH_4^+ toxicity in Chinese cabbage. Four levels of urea at 0, 160, 320, and 640 kg N ha^{-1}, represented as T1, T2, T3, and T4, respectively, were applied. In order to evaluate the alleviating effect of the application of the easily decomposable carbohydrate (sucrose) at T3 and T4 where NH_4^+ toxicity had occurred, five levels of sucrose were applied to meet C/N ratios of 0, 2, 4, 6, and 10, respectively. Our results showed that the NH_4^+ toxicity was observed at T3 and T4 at 5 days after treatment (DAT). NH_4^+ toxicity contributed to decrease fresh weight, length of leaves, length of root, and number of leaves significantly ($p<0.05$). The application of sucrose as a source of mitigating NH_4^+ toxicity had a good performance at T3 with the alleviating effect as 73 % and reduced in NH_4^+-N content in soil at 29 DAT. In the maximum N rate of T4, however, sucrose application recovered it as 32 % only compared to T2 even though the same C/N ratio was treated. Consequently, sucrose as the easily decomposable carbohydrate played crucial role to reduce NH_4^+ concentration in soil and finally alleviated NH_4^+ toxicity in plant.

Key words: Chinese cabbage, NH_4^+ toxicity, Easily decomposable carbohydrate, C/N ratio, Sucrose



An easily decomposable C source for the alleviating effect on NH_4^+ toxicity was applicable.
The maximum rate of the recovery effect was 73% relative to the healthy plant at 10 of C/N ratio.

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Introduction

In agricultural soils, an appropriate application of N is the most crucial for improving crop growth. However, excess application of N fertilizer resulted in not only physiological and morphological disorder such as ammonium toxicity symptom (Britto and Kronzucker, 2002), but also environmental pollution because most farmers applied it based on their empirical N practice without technical information of N dosage in relation to soil environmental condition (Savci, 2012).

Plant can uptake inorganic N (NH_4^+ and NO_3^-) formed through microbial N transformation such as hydrolysis, ammonification, nitrification, and mineralization in soils. Microbes also utilize N source for their population growth. Meanwhile, excess concentration of ammonium N causes the toxicity seriously when urea, fresh animal manure or unstable manure compost are applied to excessive level (Gerendás et al., 1997). For instance of visual symptoms, NH_4^+ toxicity inhibits seed germination, seedling establishment, the chlorosis of leaves, and yield depressions, and even plant dying (Kirkby and Mengel, 1967; Gerendás et al., 1997; de Graaf et al., 1998). In addition, ionic imbalances in the plant induced by NH_4^+ exposure result in total tissue depression, compared with NO_3^- -fed plants, which declines in essential cations such as potassium, calcium, and magnesium, physiologically (Kirkby, 1968; Gloser and Gloser, 2000).

There were several evidences that increasing N applications usually tended to increase the risk of the physiological disorder known as internal tip-burn and restricted root growth (Lee and Lim, 1984; Marschner and Marschner, 2012). The optimum N addition for marketable yield in Chinese cabbage has been suggested as low as 200 kg N ha^{-1}, or between 200 and 300 kg ha^{-1} in cases (Goodlass et al., 1997). Probably, the actual optimal N rate might be expected to depend upon soil physical and chemical properties in relation to the availability of other nutrient elements.

For alleviation of NH_4^+ toxicity, there were diverse research results; for instance, maintaining neutral to slightly alkaline pH can prevent it (Goodchild and Givan, 1990), optimization of light regimes to avoid high light effects is more critical with NH_4^+ grown plants than with NO_3^- or organic N grown plants (Britto and Kronzucker, 2002), maintaining high levels of cations known to be depressed in plant tissue when NH_4^+ is used as a sole N source, the supply levels of potassium have been shown to alleviate toxicity both in solution and field (Barker, 1995), and co-provision of NH_4^+ with NO_3^- can alleviate it (Gill and Reisenauer, 1993).

Ku et al. (2010) reported that easily decomposable carbohydrates (i.e. glucose, sucrose, or starch) contributed to rapid decrease of soil inorganic N content (i.e. NH_4^+ and NO_3^-) within three days through immobilization by microbial process in soil. Based on the results, it assumed that the microbial process alleviated NH_4^+ availability for plant uptake, contributing recovery effect on NH_4^+ toxicity. Thus, this experiment was conducted in order to examine the hypothesis on the alleviating effect of the application of the easily decomposable carbohydrate on the NH_4^+ toxicity of Chinese cabbage under excess N application.

Materials and Methods

Pot preparation and treatment Pot experiment was conducted at glasshouse in Hankyong National University (HKNU) in order to evaluate the effect of the application of easily decomposable carbohydrate to the soil on the growth of Chinese cabbage affected by NH_4^+ toxicity.

A soil used in this experiment was collected from the surface layer (0-30 cm) of the field at experimental farm station in HKNU, and air-dried. The prepared soil was filled up at 20 cm depth of 1/5000 a Wagner pot (16 cm diameter and 25 cm height). The soil chemical properties had 7.6 of pH (1:5), 1.1 dS m^{-1} of electrical conductivity (EC), and inorganic nitrogen (N) contents: NH_4^+-N, 38.6 mg kg^{-1} and NO_3^--N, 12.4 mg kg^{-1}, respectively.

Four levels at 0, 160, 320, and 640 kg N ha^{-1} of urea fertilizer as a NH_4^+ source were applied to the pot soil for the observation of NH_4^+ toxicity in Chinese cabbage plants. When two N levels at 320 and 640 kg N ha^{-1} showed typical symptom of NH_4^+ toxicity at Chinese cabbage plants, five levels of carbon to meet C/N ratio of 0, 2, 4, 6, and 10 were treated immediately. The amounts of C were calculated based on the N rates of treatments. Sucrose was used as a carbon source and dissolved in deionized water of 400 mL prior to the application. The solutions were treated into each pots at 5 DAT (Table 1).

Cultivation of Chinese cabbage and NH_4^+ toxicity Seedlings of Chinese cabbage (*Brassica rapa var. chinensis*) grown at a fertilized seed bed were transplanted into the pots 25 days after sowing (DAS). One seedling per pot was planted with triplicates.

Based on P_2O_5-K_2O (78-198 kg ha^{-1}) fertilizers application rate for Chinese cabbage, super single phosphate (SSP) and potassium chlorice (KCl) as PK sources were applied. And boric acid (H_3BO_3) was also applied at 15 kg ha^{-1} to prevent the physiological B deficiency during early growth. KCl was basally applied by 60 % of the total doses at one day before transplanting. SSP and H_3BO_3 were also applied basally at one day after transplanting (DAT).

Table 1. Detail information of N and C levels with various C/N ratios applied in the treatments.

Treatment	N rates		C/N ratio (C rate, kg C ha^{-1})†				
	(kg N ha^{-1})	(g N pot^{-1})	0	2	4	6	10
T1	0	0	not applied				
T2	160	0.73					
T3	320	1.46	(0)	(384)	(768)	(1,152)	(1,920)
T4	640	2.92	(0)	(768)	(1,536)	(2,304)	(3,384)

†The sucrose was applied at 5 DAT.
Note: boric acid of 15 kg ha^{-1} was also applied at -1 DAT in all treatments.

(A) 5 DAT (B) 29 DAT

Fig. 1. The symptom of NH_4^+ toxicity occurred at 320 (T3) and 640 (T4) kg N ha^{-1} by excess N applications, but no symptom at 0 (T1) and 160 (T2) kg N ha^{-1} at 5 and 29 DAT, respectively.

Soil analysis and crop growth measurement Triplicate soil samples at 5 and 29 DAT were taken from the depth of 15 cm of the pot, air-dried for 1 week, and sieved through 2 mm aperture prior to the analysis. Inorganic nitrogen (NH_4^+ and NO_3^-) in the soil extracts with 2M KCl solution was determined by Kjeldahl method (Bremner, 1996).

For the determination of crop growth, fresh weight (FW), length of leaves (LOL), length of root (LOR), and number of leaves (NOL) per plant were measured at 29 DAT with triplicates. For measurement of LOR, whole soil and plant in the pot were pulled out then washed with flowing water.

Statistical analysis One-way analysis of variance (ANOVA) for statistical evaluation of analytical results and Duncan's multiple range test (DMRT) at 5% of probability for significant difference between mean values of treatments was employed by using the STAR V. 2.0.1 (IRRI, 2013) computer program.

Results and Discussion

The symptom of NH_4^+ toxicity in Chinese cabbage The symptom of NH_4^+ toxicity in the treatments was observed in T3 and T4 where leaves turned dark green and poorly developed as compared with T1 and T2 at 5 DAT (Fig. 1A), and was continued until 29 DAT (whole experiment period) (Fig. 1B). The significant difference of NH_4^+-N concentration in soils of 5, 81, 175, and 227 mg N kg^{-1} at T1, T2, T3, and T4 was observed, respectively. The more N application resulted in the higher amount of NH_4^+-N in soil ($p<0.05$). Moreover, amount of NO_3^--N which is another form of inorganic N in soil tended to increase, but there was no significant difference between T3 and T4 treatments (Table 2).

Table 2. Inorganic N concentrations affected by N rates at 5 DAT in the treatment.

Treatment	N rates (kg ha^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
T1	0	5d†	1c
T2	160	81c	27b
T3	320	175b	74a
T4	640	227a	67a

†Denotes that mean values of three replicates with the same letter within the column are not significantly different at $p<0.05$.

In Table 3, as compared to the treatment of T1 and

Table 3. Effect of excess N rates and occurrence of NH_4^+ toxicity on the growth of Chinese cabbage at 29 DAT in T3 and T4 treatments.

Treatment	N rates (kg ha^{-1})	FW† (g plant^{-1})	LOL (cm plant^{-1})	LOR (cm plant^{-1})	NOL (plant^{-1})
T1	0	20b‡	14a	15b	11a
T2	160	28a	16a	24a	11a
T3	320	11c	11b	18b	9b
T4	640	1d	4c	7c	3c

†FW: fresh weight; LOL: length of leaves; LOR: length of root; NOL: number of leaves.
‡Denotes that mean values of three replicates with the same letter within the column are not significantly different at $p<0.05$.

T2, T3 and T4 remarkably suppressed FW, LOL, LOR, and NOL ($p<0.05$). At highest N application rate of 640 kg N ha^{-1} (T4), Chinese cabbage is faced with severe growth restriction (Fig. 1B). This result supported that the N application rates at T3 and T4 caused NH_4^+ toxicity in Chinese cabbage plant. For the visual symptom of NH_4^+ toxicity, Gerendás et al. (1997) reviewed that high concentration of NH_4^+ led to the marked reduction in crop growth, smaller leaves, and a stunted root system. Meanwhile, Lee and Lim (1984) and Magnusson (2002) revealed that plant roots grown under high concentration of NH_4^+-N in soil were severely damaged due to NH_4^+ toxicity which accelerated tip-burn incidence at head formation stage of Chinese cabbage.

The alleviating effect of the easily decomposable carbohydrate on the NH_4^+ toxicity To alleviate the NH_4^+ toxicity of Chinese cabbage, sucrose as an easily decomposable carbohydrate was applied with rates to meet C/N ratio of 0, 2, 4, 6, 10 in the treatments of T3 and T4 where NH_4^+ toxicity had occurred. In Table 4, the concentrations of NH_4^+-N in soils at low N rates of T1 and T2 without showing NH_4^+ toxicity were ranged from 2 to 35 mg N kg^{-1} at 29 DAT, whereas it was more than 50 mg N kg^{-1} at higher N treatments (T3 and T4). With the increasing sucrose application, the concentrations of NH_4^+-N were significantly reduced to the level of no N fertilization (T1) ($p<0.05$). NO_3^- contents and EC values showed similar trends.

The results indicated that sucrose application could alleviate the NH_4^+ toxicity of Chinese cabbage and induce the regrowth of plants in this experiment. In Fig. 2, the alleviating effects through sucrose application were identified. Shoot fresh weights of Chinese cabbage were significantly decreased to 27~62% at T3 and 68~98% at T4, respectively, as compared with T2 which showed highest

Table 4. Effect of the control of C/N ratios by the application of sucrose as a carbon source on reduction of the NH_4^+-N and NO_3^--N concentrations in soils at 29 DAT .

Treatment	C/N ratio	EC (dS m^{-1})	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})
T1	0	0.7b	2f†	1f
T2	0	0.8ab	35cd	22de
T3	0	1.6a	60a	36abcd
	2	1.2ab	44bc	35abcd
	4	1.1ab	26de	25cde
	6	1.0ab	13ef	23cde
	10	0.9ab	8f	20e
T4	0	1.4ab	51ab	37abc
	2	1.4ab	44bc	45a
	4	1.5ab	33cd	41ab
	6	1.3ab	16ef	30bcde
	10	0.7b	11f	26cde

†Denotes that mean values of three replicates with the same letter within the column are not significantly different at $p<0.05$.

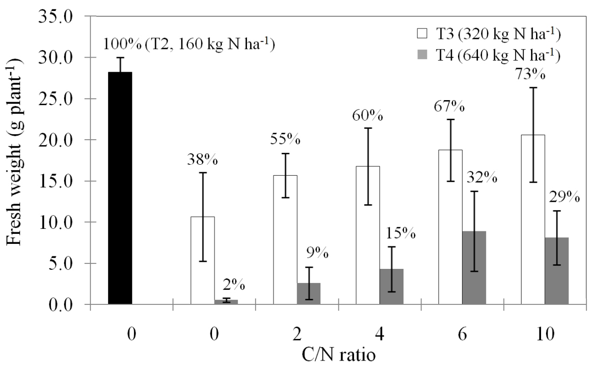

Fig. 2. Effect of sucrose application for controlling of soils on the recovery of fresh weight of Chinese cabbage at 29 DAT, which had been damaged by ammonia toxicity at high N rates.

FW without NH_4^+ toxicity. However, in T3 sucrose application increased FW with increase of C/N ratio, and the maximum alleviating effect was at 10 of C/N ratio which showed 73% of FW as compared to T2. The growth of Chinese cabbage in T4 was also recovered with sucrose application and the maximum recovery effect at 6 of C/N ratio was obtained with 32% of FW. The results showed that over application of sucrose resulted in reduction of the alleviating effect.

Brady and Weil (2002) reviewed that soil microbes require a balance of nutrients such as C and N in order to metabolize and synthesize organic compounds. On the average, the microbes must incorporate into their cells about eight parts of C for every one part of N (i.e., assuming the microbes have an average C/N ratio of 8). Ku et al. (2010) reported that when C/N ratio was 10 the activity of microbes was stimulated by carbon source. In turn NH_4^+-N in soil is utilized by microbes according to the C/N ratio. This is the mechanism that easily decomposable carbohydrate could alleviate NH_4^+ toxicity occurring in soil by high N rates.

Conclusion

An excess N application leads to NH_4^+ toxicity as well as the reduction in growth and yield of Chinese cabbage, and it is one of the most important problems in agriculture in Korea. Our results showed that sucrose as the easily decomposable carbohydrate played crucial role to reduce NH_4^+ concentration in soil and finally alleviated NH_4^+ toxicity in the plant. When C source was applied to soil where NH_4^+ toxicity had occurred, the increase of C rate to meet C/N ratio up to 10 resulted in significant increase of plant growth. Consequently, the easily decomposable carbon sources such as sucrose, starch, molasses, etc. could be used effectively to alleviate the NH_4^+ toxicity.

References

Barker, A.V. 1995. Laboratory experiment to assess plant responses to environmental stresses. J. Nat. Resour. Life Sci. Educ. 24:145-149.

Brady N.C. and R.R. 2002. The Nature and Properties of Soils. 13th ed. pp 498-511., Pearson Education, Inc., Upper Saddle River, NJ, USA.

Bremner, J.M. 1996. Nitrogen-total. Methods of soil analysis. Part 3, 1085-1121.

Britto, D.T. and H.J. Kronzucker. 2002. NH_4^+ toxicity in higher plants: a critical review. J. Plant Physiol. 159:567-584.

de Graaf, M.C.C., R. Bobbink, J.G.M. Roelofs, and P.J.M. Verbeek. 1998. Differential effects of ammonium and nitrate on three heathland species. Plant Ecol. 135:185-196.

International Rice Research Institute (IRRI). 2013. Statistical Tool for Agricultural Research (STAR) Version: 2.0.1.

Gerendás, J., Z. Zhu, R. Bendixen, R.G. Ratcliffe, and B. Sattelmacher. 1997. Physiological and biochemical processes related to ammonium toxicity in higher plants. Z. Pflanzenernähr. Bodenkd. 160:239-251.

Gill, M.A. and H.M. Reisenauer. 1993. Nature and characterization of ammonium effects on wheat and tomato. Agron. J. 85:874-879.

Gloser, V. and J. Gloser. 2000. Nitrogen and base cation uptake in seedlings of *Acer pseudoplatanus* and *Calamagrostis villosa* exposed to an acidified environment. Plant Soil 226: 71-77.

Goodchild, J.A. and C.V. Givan. 1990. Influence of ammonium and extracellular pH on the amino and organic acid contents of suspension culture cells of *Acer pseudoplatanus*. Physiol. Plant. 78:29-37.

Goodlass, G., C. Rahn, M.A. Shepherd, A.G. Chalmers, and F.M. Seeney. 1997. The nitrogen requirement of vegetables: comparisons of yield response models and recommendation systems. J. Hort. Sci. 72:239-254.

Kirkby, E.A. 1968. Influence of ammonium and nitrate nutrition on the cation-anion balance and nitrogen and carbohydrate metabolism of white mustard plants grown in dilute nutrient solutions. Soil Sci. 105:133-141.

Kirkby, E.A. and K. Mengel. 1967. Ionic balance in different tissues of the tomato plant in relation to nitrate, urea, or ammonium nutrition. Plant Physiol. 42:6-14.

Ku, H.H., W.J. Lim, and S.E. Lee. 2010. Effect of the application of sucrose on rapid decrease of soil inorganic nitrogen. Korean J. Soil Sci. Fert. 43:424-429.

Lee, S.E., and S.G. Lim. 1984. Studies on tip-burn of chinese cabbage by ammonium toxicity. Korean J. Soil Sci. Fert. 17:389-398.

Magnusson, M. 2002. Mineral fertilizers and green mulch in Chinese cabbage [*Brassica pekinensis* (Lour.) Rupr.]: Effect on nutrient uptake, yield and internal tipburn. Acta. Agric. Scand., Sect. B, Soil Plant Sci. 52:25-35.

Marschner, H. and P. Marschner. 2012. Marschner's mineral nutrition of higher plants. London, Academic press. 6th ed.

Mattson, N. 2011. Ammonium toxicity: avoid getting burned this winter. Grower talks 75(6):36.

Savci, S. 2012. An agricultural pollutant: chemical fertilizer. Int. J. Environ. Sci. Dev. 3:77-80.