To Improve Crop Biomass by 2-keto-L-Gulonic Acid supplementation: A Preliminary Study with Non-Heading Chinese Cabbage

Haozhe Xu [1], Ying Wang [2], Hui Xu [3*]

[1] Northeast Yucai Foreign Language School, Shenyang, 110179, China; haozhe_xu@yeah.net;
[2] Liaoning agricultural development service center; xyxk20052008@163.com
[3] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, 110016, China; xuhui@iae.ac.cn
* Correspondence: xuhui@iae.ac.cn; Tel.: +86-24-83970369

Abstract: 2-Keto-L-gulonic acid (2KGA), produced from a mixed-microbial fermentation, is a precursor of vitamin C (Vc) in its industrial synthesis. In this study, we try to verify a hypothesis: crop's biomass can be enhanced by exogenous 2KGA supplementation. The results from a trial of non-heading Chinese cabbage (*Brassica campestris ssp. chinensis*) cultivation showed that biomass of the cabbage was increased significantly from 21.87±6.25 g/dish to 33.64±10.39 g/dish by 2KGA application, namely a 53.82% increase in biomass accumulation. Meanwhile, the fresh weight of leaf and root were increased by 50.68% and 63.91%, respectively. To the best of our knowledge, it is a novel approach to improving biomass of crops. We speculated that 2KGA was an effective carbon resource and a biostimulant for plant. Due to ready accessibility of 2KGA from industrial microbial fermentation, there is a promising potential to utilize 2KGA for crop's yield enhancement in agricultural practices.

Keywords: Vitamin C; L-ascorbic acid; 2-keto-L-gulonic acid; non-heading Chinese cabbage

1. Introduction

Vitamin C, also named as L-ascorbic acid (ASA or Vc), is an excellent free radical scavenger and essential for humans, non-human primates, and a few other mammals [1]. ASA can only be ingested from outside, such as foods and pharmaceuticals, for maintaining human health. Nowadays, low levels of vitamin C are present in main crops, with a consequence that diet does not provide enough intake of this vitamin[2,3]. Currently, ASA has been produced on a large scale by a two-step microbial fermentation and a subsequent chemical synthesis process[4]. The global production of synthetic ASA is estimated to be 160,000-180,000 tons per year, and all of the synthetic ASA is produced in China. However, simultaneously, there is a fermentation residue after evaporation (RAE) discarded in a large amount (about 50,000-60,000 tons/year) from the ASA industry[5].

2-Keto-L-gulonic acid (2KGA), a precursor of vitamin C in its industrial synthesis, is produced from a mixed-microbial fermentation process. Meanwhile, the RAE is a remaining mother liquor in the reaction kettle after ultrafiltration, vacuum decompression evaporation of the fermentation liquid, for purpose of crystallization and extracting of 2KGA. The COD of the RAE is about 800000 mg/L. The main component of the RAE is 2KGA (concentration of 2KGA is 25%~30%, oxalic acid is 10~15%, formic acid is 2~4%, water content is about 50%). The pH of RAE is 0.3 and RAE had been considered as a hazardous waste in China, due to its lower pH value and corrosivity. Currently, RAE was treated as a waste water in the sewage treatment plant, or alternatively used as an imperfect raw material for oxalic acid production[5].

Our previous study showed that this residue could increase the Vc content, as well as the biomass, of non-heading Chinese cabbage in a saline soil [6]. Therefore, we speculated that there is a potential for recycling the RAE as a nutrient of crops and further one of fertilizer's materials in agriculture.

In this study, we speculated that 2KGA was a key compound for enhancing crop biomass accumulation, and try to verify this hypothesis with a trial of non-heading Chinese cabbage cultivation. It is expected to provide a scientific basis for the future application of 2KGA or RAE in agricultural practices.

2. Materials and Methods

Cultivation of non-heading Chinese cabbage

Eighty seeds of non-heading Chinese cabbage (*Brassica campestris ssp. chinensis*) were placed into a culture dish (15 cm in diameter and 4 cm in height) filled with pearlite particles (Figure 1). Fifteen dishes with seedlings were divided into three groups: Group Control, abbreviated as CK, seedlings was irrigated with MS medium solution; Group KCl, as KCl, was irrigated with MS medium solution (Beijing Solarbio Science & Technology Co., Ltd.) added with KCl solution (the final concentration was 0.3 mmol/L); Group KGA, as KGA, were irrigated with the MS medium solution added with both 2KGA solution (the final concentration was 1.0 mmol/L) and KOH solution (the final concentration was 0.3 mmol/L). 2KGA product (with a purity > 99%) was provided by Northeast Pharmaceutical Group Co., Ltd. Addition of KOH in Group KGA was due to a lower pH value of 2KGA solution. Meanwhile, KCl addition in Group KCl was due to keeping the same amount of potassium as that in group KGA. The final pH values of nutrition solutions were between 6.5 to 7.0. All treatments were 5 repetitions. The cultivation conditions of seedlings referred to the previously reported method [7] and seedlings were watered with the solutions every 3 to 5 days. Samples of non-heading Chinese cabbage with leaves and roots were collected on the 25th day of the cultivation and their fresh weights were measured.

2.2 Statistical analysis

Statistical significances among groups of control and treatment were calculated using T-test or one-way analysis of variance (ANOVA).

3. Results

On the 25th day of cultivation, the non-heading Chinese cabbages in dishes (Figure 1) were harvested and sampled (Figure 1). Fresh weights of total plant, leaf and root of cabbages were measured immediately. Fresh weights of the Chinese cabbage of group CK, KCl and KGA were 19.34±4.96, 21.87±6.25 and 33.64±10.39 g/dish (Table 1). Meanwhile, fresh weights of leaf were 15.47±4.23, 17.52±5.78 and 26.40±9.50 g/dish, fresh weights of root were 3.86±1.14, 4.35±1.31 and 7.13±1.97 g/dish for group CK, KCl and KGA, respectively (Table 1). Comparing to KCl group, the fresh weight of leaf and root were increased by 50.68% and 63.91%, with the 2KGA application. The difference in biomass between Group CK and Group KCl was not significant ($p > 0.05$), indicating no significant influence of potassium chloride on biomass of cabbage. These results demonstrated that the biomass of non-heading Chinese cabbage was considerably strengthened by exogenous 2KGA supplementation.

Figure 1. Cultivation of non-heading Chinese cabbage

Table 1. The results of Vc concentrations and weights of non-heading Chinese cabbage.

Groups	Fresh weight of plant (g/dish)	Increase (%)	Fresh weight of leaf	Increase (%)	Fresh weight of root	Increase (%)
CK	19.34 ± 4.96^{a}	-11.57	15.47 ± 4.23^{a}	-11.70	3.86 ± 1.14^{a}	-11.26
KCl	21.87 ± 6.25^{ab}	0	17.52 ± 5.78^{ab}	0	4.35 ± 1.31^{ab}	0
KGA	33.64 ± 10.39^{c}	53.82	26.40 ± 9.50^{c}	50.68	7.13 ± 1.97^{c}	63.91

[1] Abbreviations: Group CK, cabbage seedlings were watered with nutrition solution; Group KCl, watered with the nutrient solution added with KCl solution; Group KGA, watered with the nutrient solution added with both 2KGA solution and KOH solution.
Values are the means of 3 replicated samples ± SD. Values in the same Column with different letters in lower case were significantly different at level of $p<0.05$.

4. Discussion

In this study, the results showed that biomass of non-heading Chinese cabbage was overwhelmingly enhanced by 53.82% through 2-keto-L-gulonic acid application. In our previous study, an enhanced biomass (increased by 28.13%) by application of residue after evaporation (RAE) from industrial vitamin C fermentation was stated [6]. However, the RAE is a mixture of many compounds, such as 2KGA, oxalic acid, formic acid, acetic acid, etc. Although it's a fact that the major component of the residue was 2KGA (concentration of 2KGA is 25%~30%, oxalic acid is 10~15%, formic acid is 2~4%, water content is about 50%), it's hard to exclude a possibility that the enhanced biomass was derived from other components in the residue. Therefore, in this study, a 2KGA product with a purity of > 99% was added to medium of non-heading Chinese cabbage cultivation for the purpose of verifying our hypothesis: the crop's biomass can be enhanced by 2KGA supplementation. Consequently, the result of increased biomass in cabbage through 2KGA application provided a convincing evidence for the hypothesis. Meanwhile, the results from our field trials with 16 species of crops showed the yield of these crops were significantly increased by application of residue after evaporation (RAE) (from 7.7% to 113%, with an average of 28.6%, unpublished data).

The fresh weight of root was increased by 63.91% under 2KGA application, which is higher than that of fresh weight of leaf (50.68%). This implied that root was more sensitive to 2KGA application than leaf. In a previous study, we evaluated the potential of using 2KGA to improve crop resistance to salt stress through a cultivation experiment of non-heading Chinese cabbage and found the leaf and root biomass were significantly improved by 2KGA application[8]. In that paper, we also demonstrated that exogenous 2KGA application can relieve the inhibitory effect of salt stress on plant growth, and more importantly, we indicated the promotion of ASA synthesis may represent a critical underlying mechanism[7]. Therefore, we speculated that 2KGA was a effective carbon resource and a biostimulant of plant, and this could be an explanation for the biomass improvement in this study.

It is certainly a preliminary study on this new phenomenon and only minimal information was obtained. More understanding of the phenomenon is imperative, such as the influence of crop's species and varieties, measures of RAE and 2KGA fertilization and environmental conditions, etc, on crop biomass. Furthermore, from our point of view, the metabolism of biomass enhancement by 2KGA application would be an important and attractive topic in the future research for scientists.

2KGA is a key intermediate in the process of industrial fermentation of vitamin C. Currently, there is a large amount of RAE from Vc industry (the global production of RAE is approximately 50,000-60,000 tons per year) and RAE is now regarded as a waste water. The results in this article provided a solid evidence for enhancement in plants biomass by 2KGA supplementation. Consequently, there is a promising potential to utilize RAE (the wasted fermentation residue rich in 2KGA) for crops in agricultural production.

5. Conclusions

2-Keto-L-gulonic acid (2KGA) is a precursor of industrial Vc synthesis. A new phenomenon was reported: 2KGA can significantly increase biomass of plants. There is great potential to utilize 2KGA and RAE for increasing crop yield in agricultural practice.

Funding: This research was funded by National Key Research and Development Program of China (Grant number 2020YFA0907800) and Science and Technology Plan Project of Shenyang city, China (Grant number 20-203-5-48).

Acknowledgments: The authors are grateful to Mrs. Xiaohuan Lv and Mrs. Shuang Kong for their technical assistance in HPLC analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Padh H. Vitamin C: newer insights into its biochemical functions. Nutr Rev 2010; 49: 65-70. DOI:10.1111/j.1753-4887.1991.tb07407.x.
2. Frei B, Birlouez-Aragon I & Lykkesfeldt J. Authors' perspective: what is the optimum intake of vitamin C in humans? Crit Rev Food Sci Nutr 2012; 52: 815-829. DOI: 10.1080/10408398.2011.649149.
3. Levine M, Conry-Cantilena C, Wang Y, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. P Natl Acad Sci USA 1996; 93: 3704-3709.

4. Weichao Yang, Hui Xu, Industrial Fermentation of Vitamin C, in: Industrial Biotechnology of Vitamins, Biopigments, and Antioxidants, Editors: Vandamme, Erick J., Revuelta, José Luis, WILEY-VCH, 2016, p161-192.
5. Xu, H., Yang, W., and Li, J. (2021). New progress on the second step of the mixed fermentation for vitamin C. China J. Microbiol. 41, 1–9. doi: 10.3969/j. issn.1005-7021.2021.02.001.
6. Kong T, Xu H, Wang ZY, et al., Effect of a residue after evaporation from industrial vitamin C fermentation on chemical and microbial properties of alkali-saline soil. Pak J Pharm Sci 2014; 27:1069-1074. DOI: 10.1002/smll.200900562
7. de Lacerda CF, Cambraia J, Oliva MA, Ruiz HA, and Prisco, JTN. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environ Exp Bot 2003; 49: 107-120. DOI: 10.1016/S0098-8472(02)00064-3
8. Mingfu Gao, Sun Hao, Meijun Shi, Hui Xu, Weichao Yang. 2-Keto-L-Gulonic Acid Improved the Salt Stress Resistance of Non-heading Chinese Cabbage by Increasing L-Ascorbic Acid Accumulation. Frontiers in Plant Science. 2021, DOI: 10.3389/fpls.2021.697184]