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Full Length Research Paper

Evaluating the Effects of HCO3–, K+, and HSO3– on Rubisco Subunit Gene Expression (rbcL and rbcS)

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$KHCO_3$ and $NaHSO_3$ were sprayed on the leaves of cucumber, and HCO_3^-, K^+, HSO_3^- in liquor can accelerate the expression of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) large-subunit (*rbcL*) and RUBISCO small-subunit (*rbcS*) genes. These results proved that, mRNA content in the leaves of *rbcL* and *rbcS* was increased and the carboxylation activity of RUBISCO was also improved obviously, the content of RUBISCO has not increased but decreased. According to the analysis, we obtain that K^+ ,HCO_3^-, HSO_3^- could affect the expression of *rbcL* and *rbcS* in a transcription way.

Key words: RUBISCO large-subunit (*rbcL*), RUBISCO small-subunit (*rbcS*) expression, RUBISCO.

INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) is a key enzymes of all aphotosynthesizing organism on photosynthetic carbon assimilation. It catalyses RuBP carboxylation and add oxygen reaction, and adjusts the relationship between them. It is composed of eight large-subunit and eight small-subunit; the large-subunit is encoded by chloroplast genes, small-subunit by karyogene (Guo Zhang et al., 2004). The large-subunit acts as a catalytic function and the small controls the activity of RuBPCase. The catalytic effect of RUBISCO is slow, which cannot reach to 0.33% of usual enzyme. As the plant has to maintain its total activeness, RUBISCO needs massive copies. Therefore, the studies on rbcL and the rbcS expression and the environmental factor to its influence have both theoretical and practical significance. Usually, we think this enzyme is easily adjusted by CO_2, Mg^{2+}, light and so on (Coruzzi et al., 1984; Knight and JenkinsGI, 1992; Shirley et al., 1987; Ruddle and Zielinslki, 1991; Pilgrim and McClung, 1993). This study indicates that the rbcS expression mainly centralizes in the light, the photosensitive pigment and in the biological clock regulation. Transfering gene research further indicates that as the light induction's promoter or enhancer, rbcS5'flanking sequence controls its mRNA and the code protein level (Shirley et al., 1987). However, the research of K^+, HSO_3^-, HCO_3^- regulating on rbcL, the rbcS expression is reported rarely. This study aimed at using $KHCO_3$ and $NaHSO_3$ to spray on cucumber leaves, discusses the influence of K^+, HCO_3^-, HSO_3^- in water-soluble fluid on expression of the RUBISCO large-subunit and small-subunit, and explores the molecular mechanism by which $KHCO_3$ and the $NaHSO_3$ regulate on cucumber seedling photosynthesis.

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MATERIALS AND METHODS

Reagent, biological software and main instrument

The experimental product was Tianjin spring 4 cucumber seedlings. Reagent used were, RNA extraction reagent, total RNA extraction reagent box of RNAprep plant, the cDNA first chain synthesis reagent box from TIANGEN Corporation, 2×Taq PCR Master Mix from TIANGEN Corporation. Other reagents were purchased from TIANGEN Corporation. Applied biological software; Primer 5.0 and DNAMAN. The main instrument used were BIO-RAD PTC-200 PCR instrument, TECHNE TC-512 PCR instrument, BIO-RAD gelatin image formation instrument, Vortex oscillator, HERMLE Z 233 MK-2 table-model micro freezing centrifug, HITACHI SCR20BA high-speed freezing centrifuge, Hitachi UV-3010 spectrophotometer; constant temperature –revealing water-bath.

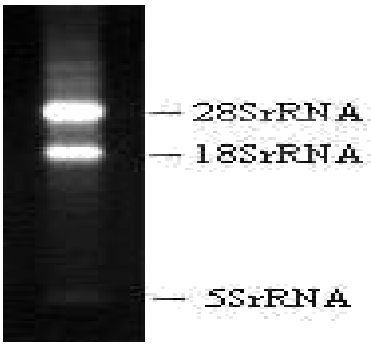

Figure 1. Agarose electrophoresis of total RNA.

Design and synthesis of primers

The design of primer refers to the methods of Wang Chong et al. (2006), using the DNAMAN biology software to analyze rbcL, the rbcS analysis which have been reported and refers to the GenBank database information, using the designing software of Primer 5.0 to design the upstream and the downstream: The internal reference of the primer: 18 SF: CATGCATTYCACAAYGTTTG, 18 SR: GTCATCCAAGXGCCATACC, RUBISCO large-subunit gene: (gene ID: DQ535747), rbcL F: 5-CCGATGGGCTTACCAGTCT-3, rbcL R: 5-CCGCACAAAATAGGAAACG-3, RUBISCO small-subunit gene: (gene ID: EF208124), rbcS F : 5-ATGGGTTCCCTGCGTTGA-3, rbcS R: 5-GGGGCTTGTAGGCGATGA-3. The primer was synthesized by SaiBaisheng bio-engineering company.

Experiment design

Soaking seeds the expediting sprout

Firstly, the seeds were soaked in 55-60℃ lukewarm water for disinfection, and then the seed was poured into a beaker containing the lukewarm water and stirs unceasingly until the water temperature drop to below 30°C. The seeds were clean and taken out after it has been soaked for 6 h; evenly tiles them (5-6 wet gauze are laid in the bottom) in the big cultivating dish and 2 wet gauzes are spread on the upper after the lid covers, the sprout will be expedited in the incubator having 28℃ constant temperature, which will be germinated after 24 h.

Sowing seeds and dividing the seedling

Seeds with the same length of bud were choosen and planted in the lymph plate which had 16 holes; they are cultivated in the artificial incubator. When the cucumber seedling grows to the degree of covering neighboring adult plants, the leaf blade divides the seedling plate; the leaf blade can be used as experimental material when it spread completely.

Six treatments of the experiment (act three times)

1. 1500 mg/L $KHCO_3$ water-soluble fluid treatment;
2. 3.33 mmol/L $NaHSO_3$ water-soluble fluid treatment;
3. 1500 mg/L $KHCO_3$ water-soluble fluid and 3.33 mmol/L $NaHSO_3$ water-soluble fluid compound treatment;
4. Clear water comparison treatment;
5. 1500 mg/L $NaHCO_3$ water-soluble fluid treatment;
6. 3.33 mmol/L NaCl water-soluble fluid treatment.

To spray on the leaves of the first and second period of the cucumber separately with watering can (causing solution to form even and close distribution on leaf blade surface), the third piece of leaf spreads completely taking the fresh leaf blade to mensurate rbcL and the rbcS expression, the RUBISCO content, carboxylation activity and so on.

RESULTS

Influences of all treatments on cucumber seedling leaf blade rbcL and rbcS expression

Quality examination of total RNA

From Figure 1, we obtain that total RNA has 3 clear electrophoresis bandings; they were 28SrRNA, 18SrRNA and 5SrRNA. Among them, 28SrRNA banding was brighter than that of 18SrRNA. There was no obvious dissemination phenomenon in the electrophoresis banding area, which shows that RNA has not degraded quite completely. RNA extinction value was: OD260 = 0.162, OD280 = 0.083, OD310 = 0.011 and OD260/ OD280 = 1.95, respectively, this value conforms to the requests of pure RNA solution OD260/OD280 which was situated between 1.7 and 2.0; so the smaller the OD310 value, the smaller the salts material pollution is.

The aforementioned analysis indicates that adopting this method will get a very good purity from total RNA. Analyzed from the purity and the integrality, this RNA conforms to the experimental requirement. RNA density can be obtained by using the RNA density formula = (0.162-0.011)×200×0.04 = 1.208 μg/μl.

Determination of template quantity

This experiment takes cDNA as the template; 18SF, 18SR were the materials for internal reference primer to carry on PCR amplification, and it obtains a special banding which was bigger than 200 bp. In Figure 2, the brightness of six kinds of treatment sample bandings are consistent generally, so we can use the adding quantity of the cDNA template to carry on the next step of PCR amplification.

Expression analysis of rbcL gene

From Figure 3, we can get that the cucumber leaf blade rbcL gene obtains differential amplification product which was consistent with the size of anticipated fragment of 456 bp. In contrast with CK treatment, the rbcL gene amplification strap in each treatment cucumber leaf blade is brighter than CK. There is much brightness in the

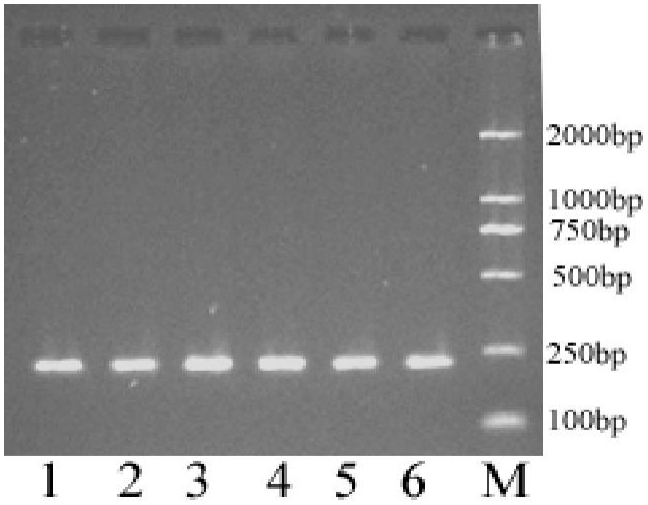

Figure 2. Agarose electrophoresis of 18S rRNA from cucumber seedlings. 1: $KHCO_3$; 2: $NaHSO_3$; 3: $KHCO_3$+$NaHSO_3$; 4: CK; 5: $NaHCO_3$; 6: NaCl; M: DNAMolecular weight standard.

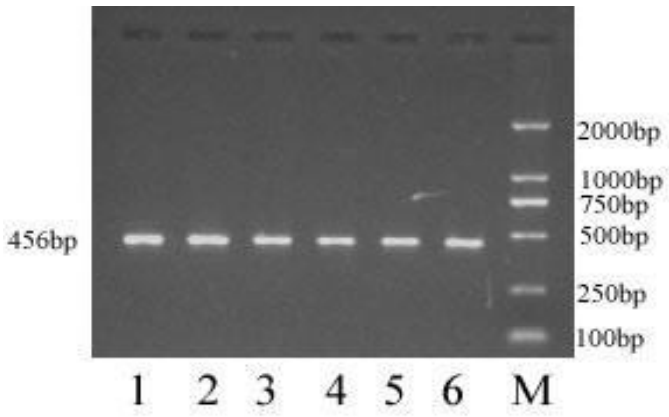

Figure 3. Agarose electrophoresis of *rbcL* from cucumber seedlings. 1: $KHCO_3$; 2: $NaHSO_3$; 3: $KHCO_3$+$NaHSO_3$; 4: CK; 5: $NaHCO_3$; 6: NaCl; M: DNAMolecular weight standard.

$KHCO_3$ treatment cucumber leaf blade amplification strap. The next place is $NaHSO_3$ treatment. $KHCO_3$ treatment was the brightest, so the expressive quantity of rbcL gene on cucumber leaf blade which was treated by $KHCO_3$ is more than that of other treatment. $KHCO_3$ accelerates the expression of rbcL gene.

In contrast with CK, the brightness of $NaHCO_3$ was more obvious, so HCO_3^- has the function of acceleration. As $KHCO_3$ expression is much brighter than $NaHCO_3$, so K^+ affects the acceleration of the expression of rbcL gene most. We can see $NaHSO_3$ is brighter than the NaCl strap clearly from $NaHSO_3$ and NaCl which have the same Na^+; this means that HSO_3^- plays more important role on the cucumber leaf blade rbcL gene expression compared to Cl^-. The compound treatment of $KHCO_3$ and

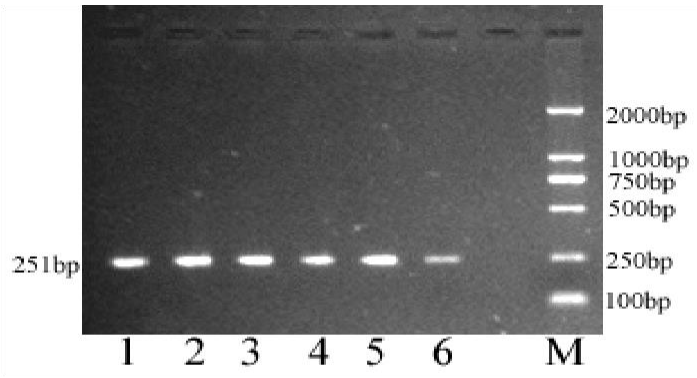

Figure 4. Agarose electrophoresis of *rbcS* from cucumber seedlings. 1: $KHCO_3$; 2: $NaHSO_3$; 3: $KHCO_3$+$NaHSO_3$; 4: CK; 5: $NaHCO_3$; 6: NaCl ; M: DNAMolecular weight standard.

the $NaHSO_3$ has no obvious effective function, compared to $KHCO_3$ and $NaHSO_3$ respectively, the expression is weak. The reason needs further studies.

Expressive analysis of rbcS gene

Figure 4 illustrates that the cucumber leaf blade rbcS gene obtain differential amplification product which is consistent with the size of the anticipated fragment 251bp size. The rbcS gene which expands in the $NaHCO_3$ treatment cucumber leaf blade were brighter than all other treatments; this shows that the rbcS gene has more expressive quantity in cucumber leaf blade, which was treated by the $NaHCO_3$ than that of other treatments. Under the same function of the HCO_3^- , the $KHCO_3$ treatment is weaker than $NaHCO_3$, which means that the expression of Na^+ on rbcS gene is superior to K^+, but these two kinds of treatments are better than comparison and it indicates HCO_3^- played the main role; it may increase the duplication of rbcS. The expanding belt of $NaHSO_3$ treatment is brighter than that of NaCl, which shows HSO_3^- plays more important role than Cl^- when they all include the Na^+ situation and HSO_3- plays the main role in duplication. $KHCO_3$ and the $NaHSO_3$ compound treatment are more obvious on efficiency.

Influence of all treatments on RUBISCO content of the cucumber seedling leaf blades

According to Table 1, the $NaHCO_3$ treatment plays more important role than CK in increasing the RUBISCO content; the increasing extent is 23.20%. The $KHCO_3$ treatment causes the RUBISCO content reduction of 8.82% compared to the CK treatment; the $KHCO_3$ treatment compared with the $NaHCO_3$ treatment affects greater on the RUBISCO content reduction, that is 25.99%, and it shows that Na^+ plays the main role in

Table 1. The influence of all treatments on RUBISCO content of the leaf of cucumber seedlings (unit: $mg \cdot g^{-1}$).

Treatment	RUBISCO content	%	RUBISCO percentage of soluble protein	%
$KHCO_3$	5.58	-8.82	32.69	-25.48
$NaHSO_3$	6.29	2.78	38.33	-12.63
$KHCO_3$+$NaHSO_3$	7.18	17.32	42.46	-3.21
CK	6.12	——	43.87	——
$NaHCO_3$	7.54	23.20	45.23	3.10
NaCl	6.65	8.66	42.93	-2.14

Table 2. The influence of all treatments on the RUBISCO carboxylation active of cucumber seedling leaf blade.

Process	RuBPCase vigor ($nmolNADH \cdot ml^{-1} \cdot min^{-1}$)	%	RuBPCase specific activity ($nmolNADH \cdot \mu g^{-1} \cdot min^{-1}$)	%
$KHCO_3$	22.51	86.67	2.24	111.3
$NaHSO_3$	14.47	19.98	1.69	59.43
$KHCO_3$+$NaHSO_3$	18.49	53.33	1.75	65.09
CK	12.06	——	1.06	——
$NaHCO_3$	17.69	46.68	1.62	52.83
NaCl	12.86	6.63	1.54	45.28

increasing RUBISCO content. On the contrary, the function of K^+ is very small. The $NaHCO_3$ treatment makes RUBISCO in the cucumber seedling leaf blade increase its percentage in the soluble protein. The $NaHCO_3$ treatment compared with the CK treatment causes the RUBISCO containing in the soluble protein to reduce 8.82%. From the aforementioned statement, we can obtain that K^+ possibly increases other protein synthesis, but suppresses the RUBISCO biosynthesis in the translation level. So this needs further research; RUBISCO in $NaHSO_3$ and NaCl treatment is higher than CK which means Na^+ may enhance the content of the enzyme. However, the latter is higher than the former, so the auxo-action of Cl^- is higher than HSO_3^-.

Influence of all treatments on the cucumber seedling leaf blade's RUBISCO carboxylation active

From Table 2, $KHCO_3$ treatment and $NaHSO_3$ treatment causes the leaf blade RUBISCO carboxylation activeness and carboxylation specific activity increase. The $KHCO_3$ treatment compared with CK processes have more increase in carboxylation activeness and carboxylation specific activity to the RUBISCO; their enhancement rate is 86.67 and 111.32% respectively. The $NaHSO_3$ treatment compared with CK process is low and their enhancement rate is 19.98 and 59.43% respectively. The $KHCO_3$ treatment compared with $NaHSO_3$ processes is more; their enhancement rate is 55.56 and 32.54% respectively. The $NaHSO_3$ treatment compared with CK processes, its enhancement rate in carboxylation activeness to the RUBISCO is 46.68% and this explains HCO_3^- promotive is biggest; K^+ is stronger than Na^+. Although, NaCl treatment RUBISCO carboxylation activeness and carboxylation specific activity is higher than CK, but it is similar, otherwise the $NaHSO_3$ two targets are higher than CK; and this explains HSO_3^- also has promotive in carboxylation activeness of RUBISCO.

DISCUSSION

According to the study of Chaoying et al. (2001). rbcS has the obvious response to many outside factors. This experiment uses outside ion induction to prove that HCO_3^-, K^+, HSO_3^- have the positive promotive function on rbcL, rbcS expression of cucumber leaf blade. In this study, the change of RUBISCO content is not synchronous with rbcL and rbcS expression; there are maybe four reasons: First, RUBISCO content and air CO_2 are related. The air CO_2 density ascension causes the plant of RUBISCO content reduce (Chen, 2005; Spencer and Bowes, 1986), this possibly is the adjustment of plant to RUBISCO content in the process of adapting to external environment, so CO_2 assimilation and the electron transferring ability become balanced (Zhenhua, 2007).

In this study, HCO_3^- was spurted on the material of leaf blade, which increases CO_2 density indirectly. Thus, the RUBISCO content dropped. Secondly, it is related with trans-membrane transportation of rbcS expression product entering the chloroplast. K^+, HCO_3^- promote the rbcS expression and form the big precursor in the

cytoplasm (prSS), then, enter the chloroplast through the transportation peptide. However, K^+, HCO_3^- do not promotes the precursor to cross membrane transportation and the transportation peptide to duplicate and translate. Thirdly, the research of Hubbs and Roy (1993) proves that the existence of excessive K^+ has the inhibitory action over the final form from the intermediate to the holoenzyme, which is completely consistent with our test result. Namely, big and small subunit holoenzymes are affected by excessive K^+ in the process of holoenzyme assembly or participating in organizing the chloroplast guardianship protein, so it causes the rubico content to reduce. Fourthly, K^+ possibly increases other protein synthesis, but suppresses the RUBISCO biosynthesis in the translation level. K^+, HCO_3^- treatment may increase RUBISCO carboxylation activeness and the effect is obvious, rbcS affects RUBISCO carboxylation activeness.

There may be many reasons for K^+ to enhance RUBISCO carboxylation ability; K^+ may enhance the fixed speed of CO_2, and then affect the RUBISCO activeness. K^+ may activate the related enzyme, strengthen the assimilation and the assimilation product transportation of CO_2, and enhance the photosynthetic phosphorylation function and the photosynthesis intensity and efficiency, so that it affects the RUBISCO activeness. This test result indicated that K^+ has certain influence on the RUBISCO activeness, which is consistent with the findings of Yamashita et al. (1988) on mulberry tree leaf blade.

HCO_3^- can also enhance RUBISCO carboxylation activeness through several aspects; first, RUBISCO enzyme is suitable to pH≈8 most, HCO_3^- provides a leaning alkalinity environment, which is good for the RUBISCO activation. Secondly, HCO_3^- is formed into malic acid fixed by the PEP carboxylase in seedling and transported to RUBISCO nearby. Decarboxylation releases CO_2 to cause the CO_2 differential pressure ascension around RUBISCO, reduces the auxo-oxygen activeness of RUBISCO, and promotes carboxylation activeness. Thirdly, HCO_3^- may act as the substrate of RUBISCO carboxylation and supplement atmosphere which is insufficient in the CO_2. Thus, it promotes its carboxylation activeness and the RUBISCO carboxylation speed suppresses the auxo-oxygen response of RUBISCO, and promotes photosynthesis carbon assimilation.

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