Bioinformatic Analysis of Strawberry Rbsc Gene

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Abstract. RbsC has always been known as a key factor in plant testa. Recent studies show that rbsC (proton gradentregulation 5) is a proton gradient regulatory protein, which transfers cycle electronic I encoding thylakoid membrane proteins and optical systems, and helps to induce pH heat dissipation. In this research we chosen rbsC gene of 10 kinds species, downloaded their nucleotide and protein sequence from NCBI as the research object, found strawberry rbsC gene via bioinformation analyses, constructed phylogenetic tree. At the same time, we analyzed the strawberry rbsC gene of physical and chemical properties and its protein structure and so on. The phylogenetic tree showed that strawberry and Rosa roxburghii were closest relative. By the protein prediction, we found that the protein owed one proper signal peptide without obvious transmembrane regions.

1. Introduction

Rubisco is the key enzyme for the immobilization of CO_2 in plant photosynthesis, which catalyzes the first major carbon fixation reaction in the Calvin cycle. Different from the activation of CO_2, Rubisco in the substrate during carbon fixation is ribulose -1,5- two phosphate and CO_2 carboxylation reaction catalyzed by Rubisco and RuBP carbon dioxide, light respiration metabolism in plants at the same time, the catalytic ribulose -1,5- two phosphate and O_2 reaction, organic matter consumption [1] plant photosynthesis. 1,5- two ribulose monophosphate form 2 molecules of 3- and phosphoglycerate, a series of chemical reaction, in ATP can be transformed into glucose in [2]. When substrate is carbon dioxide, the product of carboxylase reaction is a highly unstable six carbon phosphorylation intermediate called 3- ketone -2- carboxyl Arabia sugar alcohol -1,5- two phosphate. It almost decays to two molecule glyceric acid -3- phosphate and phosphate. 3- phosphoglycerate can be used to produce larger molecules such as glucose. The side effects of Rubisco may lead to useless or inhibition of byproducts, one of which is a product of lignan -1,5- two phosphoric acid, which inhibits Rubisco activity [3]. When the substrate is molecular oxygen, the products of the oxygenase reaction are phosphoric acid glycolate and 3- phosphoglycerate. Glycolic acid is a series of reaction cycles known as photorespiration, which involves enzymes and cytochrome in mitochondria and peroxisomes. In this process, two molecules of phosphoglycollate salt into a molecule of carbon dioxide and one molecule of 3- phosphoglycerate, it re-enter the Calvin cycle. Some phosphoric acid esters that enter this way can be preserved by plants to produce other molecules, such as glycine. At the ambient level of carbon dioxide and oxygen, the ratio of reaction is about 4 to 1, which results in only 3.5 of the net carbon dioxide fixed. Therefore, the enzyme does not prevent the reaction with oxygen, which greatly reduces the photosynthetic capacity of many plants. Some plants, many

algae and photosynthetic bacteria by improving the concentration of carbon dioxide around enzyme method overcomes this limitation, including C4 carbon fixation, the use of Crassulacean acid metabolism and carotenoids.

2. Result and Analysis

2.1 Strawberry Rbsc Find and Phylogenetic Tree Construction of Rbsc

Run BLAST in the Fragaria ×ananassa database of GDR and Strawberry GARDEN respectively using Arabidopsis thaliana rbsC as a sample. Select the sequence with ideal S and C value, we find strawberry rbsC.

We have chosen rbsC-like gene of 10 kinds species (Table 1), downloaded their nucleotide and protein sequence from NCBI as the research object, analyzed sequences via MEGA6.0, constructed phylogenetic tree on the genetic distance base (Figure 1). The relationship between strawberry and petunia is closer than others, due to they all contain a lot of anthocyanins probably.

Table 1. Basic information of rbsc gene in different species

| Species	mRNA Accession Number	Protein Accession Number	Amino Acid Number
Fragaria vesca	XM_004303089.2	764604480	969
Rosa roxburghii	HM071984.1	327323114	879
Malus x domestica	AB627321.1	358350593	931
Pyrus x bretschneideri	XM_009359537.2	1079218129	979
Pyrus pyrifolia var. culta	D00572.1	218020	844
Pyrus x bretschneideri	XM_009344381.1	694430338	869
Prunus mume	XM_008246284.2	1027086152	887
Prunus avium	XM_021972039.1	1220082194	856
Theobroma cacao	XM_007033208.2	1063488178	944
Carica papaya	XM_022054743.1	1227948693	830
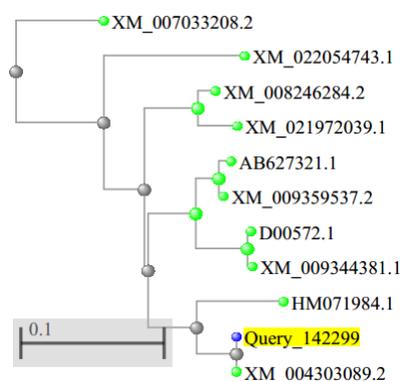
Figure 1. Phylogenetic Tree of Strawberry rbsC.

2.2 Strawberry Rbsc Protein Physical Analysis

Analyze strawberry rbsC protein physical feature using ProtPram. Result shows as following: the protein is composed by 549bp, molecular weight is 46255.84, theoretical pI is 5.13, electric neutrality, molecular formula is $C_{1644}H_{2741}N_{549}O_{679}S_{162}$. Because the N-terminal of the sequence considered is A (Ala), the estimated half-life is 30 hours in mammalian reticulocytes (in vitro), 20 hours in yeast (in vitro) and 10 hours in Escherichia coli (in vitro). The instability index (II) is computed to be 42.68, so the protein is unstable. The aliphatic index of the protein is 22.95. Grand average of hydropathicity (GRAVY): 0.890.

Amino acid composition result is settings as following (Table 2): the peptide chain consists many Ala as 23.0%.

Table 2. Strawberry rbsc gene amino acid composition

Amino Acid	Count	Percentage
Ala (A)	126	23.0%
Cys (C)	162	29.5%
Gly (G)	132	24.0%
Thr (T)	129	23.5%

2.3 Strawberry Rbsc Protein Signal Peptide Forecast

Forecast strawberry rbsC protein signal peptide using SignalP 4.1. Result shows as following (Figure 2). There is one signal peptide in the rbsC between 23th base and 24th base.
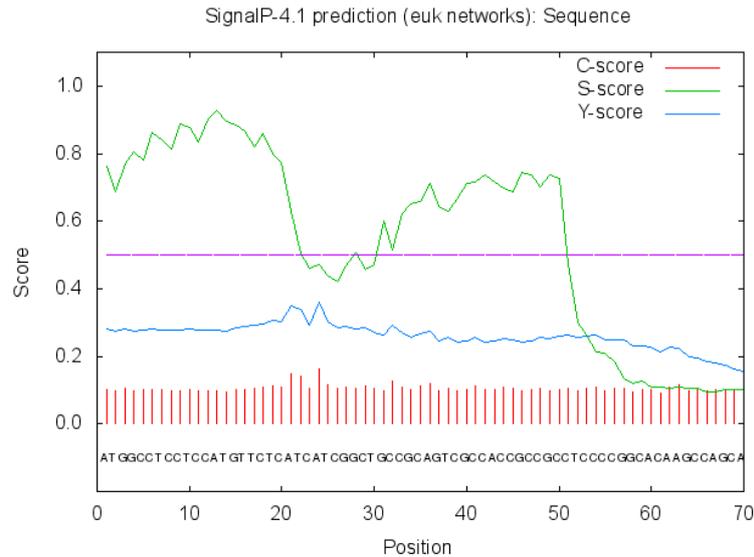
Figure 2. Signal peptide forecast of strawberry rbsC.

2.4 Strawberry Rbsc Protein Transmembrane Domain Forecast

Forecast strawberry rbsC protein transmembrane domain using TMHMM Server. Result shows as following (Figure 3). There isn't transmembrane domain in the strawberry rbsC, thus the protein is not cytomembrane receptor or located on cytomembrane.
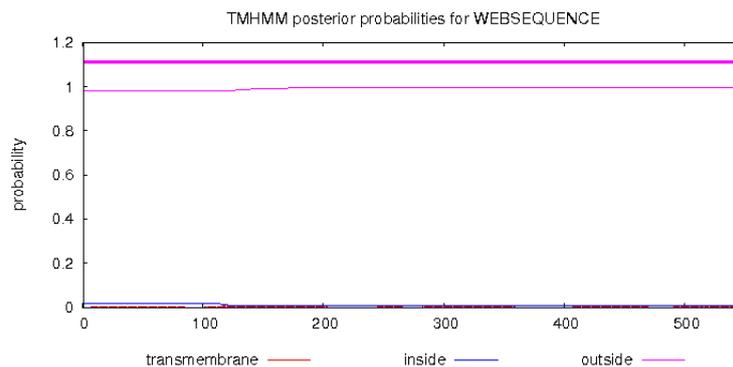

Figure 3. Transmembrane domain forecast of strawberry rbsC.

3. Materials and Methods

Sequences access from NCBI (https://www.ncbi.nlm.nih.gov/). BLAST run in GDR (https://www.rosaceae.org/), and Strawberry GARDEN(http://strawberry-garden.kazusa.or.jp/). Phylogenetic tree is constructed via MEGA6.0, forecast strawberry rbsC protein signal peptide using SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/), forecast strawberry rbsC protein transmembrane domain using TMHMM Server (http://www.cbs.dtu.dk/services/TMHMM/).

4. Conclusion

In plants, algae, cyanobacteria, Proteobacteria in light and chemoautotrophy Rubisco consists of 8 large subunit (rbcL) and 8 small subunit (rbcS) composition, which consists of large subunit of chloroplast genes encoding nucleotide sequence, the chloroplast genome is relatively stable; small subunit by nuclear gene encoding the small subunit, and through through the outer chloroplast membrane from the cytosol into the chloroplast stromal compartment [4,5] subunit plays a catalytic role in substrate, enzyme activity (ribulose phosphate 1,5- two) binding sites located in the formation of two dimers of the large subunit and small subunit, [6] has control of RuBisCO activity. The small subunit (rbcS) is responsible for the catalytic cycle of carbon Calvin in photosynthesis is fixed,

entrance [7] inorganic carbon into the biosphere, the plant at the right time and place to produce two Rubisco amount and in the right way is vital for photosynthesis [8]. The exploration on strawberry TT12, which is forward gene to strawberry rbsC, have been attempt in our lab, we would focus on the interaction between strawberry TT12 and rbsC so as to provide reference for further study and utilization.

Acknowledgments

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