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Inquiry on Abscisic Acid (ABA) Synthesis Methods

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Abstract. In this paper, we aim to find a suitable product to solve the problem of food production through agriculture and find a suitable host and matching feedstock to enhance yield. ABA was selected as the target product to alleviate the frost damage on the crops due to its famous role in alleviating cold stress damage. Saccharomyces cerevisiae has excellent genetic traits and is able to grow in fermentation tanks at scale, hence it was chosen to be the microbial host for synthetic production of ABA through research on ABA pathways. The galactose induction medium was specially designed to produce ABA, ensuring a large volume. Therefore, this article proposed an integrated solution with the goal of avoiding losses caused by frost damage in agriculture and improve the security of food supplies.

Keywords: Abscisic acrid, Genetic engineering, biomodification, synthetic biology

1. Introduction

Recent developments have highlighted about the relations between foods. "As a result of the 'industrial agriculture'", there is very anxious situations about the losses of nutrient vitamins, for example. A very terrible loss comes in production in that for some crops and fruits, the protein drops by 10~15%, which directly leads to a falling down the nutrition level for human. Meanwhile, there are problems with economy: "The high cost of raw material and delivery and so on makes the person very worried if he cannot afford the price for his eating everyday as individuals [1]. As for the products, the price of sugar has reached ¥7,200 per ton [2], and this record of an 11-year high [3] has made it a hot issue in circle". In view of many people still suffering from hunger and insufficient yields regarding cereal output; while at the same time, it becomes clear that many demands of cereals exist in areas of agricultural produce.

By the year 2024, the world's population will reach nearly 8.1 billion people. The average annual per capita food requirement measured by mass would be 400–500 kg per person [3]. Therefore, an enormous lack of food will occur in the world in the future [4]; as of the year 2023, more than 735 million people suffered from acute hunger [2]. Frosting damage is one of the serious problems for crop growth in areas of agricultural produce, which will cause adverse situations, such as elongating growing periods, decreased stress resistance, drastic decline or even total failure to harvest due to

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frost damage [5], and severe reduction in the quality of crops. But anti-freezing methods, especially those applied through introducing AFPs, can cause some problems about food safety due to introduction of foreign proteins. Therefore, we thought of using abscisic acid (ABA) to strengthen the stability of the bio-membrane structure [6] of cell organs and protect [7] them from damage caused by freezing frost [8].

Regarding the synthesis of abscisic acid (ABA), traditional plant tissue extraction methods have many issues, such as extremely low yields and supply chain challenges. Although chemical synthesis offers relatively higher production capacity, this method is subject to significant fluctuations in raw material prices, high operating costs, and unsatisfactory purity [9]. Therefore, the aim of this study is to explore a biosynthetic method to efficiently produce abscisic acid (ABA) and to mitigate frost damage in agricultural production.

To achieve the final goal of frost protection, we first studied ABA biosynthetic pathways and identified the key genes [10] and mutants involved in this pathway [11], so that several methods for increasing ABA biosynthesis through gene modification can be found. Second, yeast (S. cerevisiae) was selected as a microbial host that could efficiently produce ABA because this yeast has well-known genetic background and is easy to adapt to fermentation. Third, suitable feedstock — a galactose-induced medium for S. cerevisiae — is the means of large-scale production [12]. Finally, we predicted that this study would have certain strategy and theoretical implications for overcoming agricultural losses caused by frosts and ensuring food security.

2. Research results and result discussions

2.1. Product

The product of this study is Abscisic acid [8], a Phytohormone that can be used to solve food problems, it carries out a function that enhance the stability of biological membranes and further reduce damage to membrane structures.

ABA is chosen as the product for different reasons.

It is used to solve food problems by increasing the yield by improving crop frost resistance [10]. Because one of ABA's properties is that it can protect the membrane of the cells from freezing [6]. In this case, ABA promotes the expression of antifreeze proteins (such as AFP), which can bind to intracellular ice crystals, inhibit ice crystal growth, and protect cell structure. It also decreased the risk of having a poison problem due to the fact that ABA is a natural chemical so that it will do no harm to the human body [5]. It decreases the possibility of the plants dying out. Further eliminates the damage to other parts, in example, economy.

However, there are some problems with traditional ABA synthesis, in which the traditional passway limits the production of ABA from product and cost [9]. The first way is to extract from plants, but in this way, there are two problems. One is that it had a very low yield and with supply chain challenges. The other is through chemical synthesis, it had the problems of having a material price volatility, a sign of unstable, also with a high cost and low purity issue.

2.2. Pathway

There are two common pathways in the catabolism of Abscisic acid in plants [9].

Initially, oxidative degradation is the main pathway happened in the Abscisic acid (ABA), this is mainly because under the extreme environment; to protect plants, the content of the Abscisic acid will increase significantly. By contrast, in the normal circumstance, the content of Abscisic acid will

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be lower, so the oxidative degradation will be activated and lead the CYP707A gene [9]'s expression. In this pathway, the Abscisic acid will be broken down in three steps. First is the 8'-hydroxylation reaction, which is the rate limiting step [11]. The Abscisic acid will transfer to the 8'-hydroxy Abscisic acid. Then as we all know the 8'-hydroxy Abscisic acid is not stable. Second, as 8'-hydroxyabscisic acid is unstable, it spontaneously undergoes isomerization; intramolecular rearrangement then occurs and integrate to form the phaseic acid. At last, under the catalysis of reductase, the double bond in phaseic acid is reduced next produce dihydrophaseic acid. Because in the experiment scientists discovered DPA has no biological activity, therefore, we can determine that the dihydrophaseic is the final products of the oxidative degradation pathway.

Furthermore, there is also a special catabolic pathway called glycosylation. In Figure 1, it is shown that the passway This pathway is usually used in severe weather conditions. When plants need to allocate or control is energy, the special enzyme which is called Uridine 5'-Diphospho-Glucuronosyl Transferase (UGT)will simply Catalyzes the Abscisic acid binds to glucose to form ABA-glucose ester [11] (ABA-GE [9]), in this way plants can store some Abscisic acid for unpredictable natural disasters, such as drought. In the process of glycosylation, it only includes two steps. First is that the forming of the ABA-glucose ester. Since the glucose cannot directly bond with, Abscisic acid, so it must be converted to the adverse weather uridine diphosphate glucose (UDP-glc [9]). Then when the material gets to the specific concentration, the UGT enzyme can accelerate the connecting of UDP-glc and the Abscisic acid and produce the ABA-glucose ester. In addition, the synthesized ABA-GE will be transported to the vacuole and the cell wall respectively by transfer protein ABC and diffusion. After that the Abscisic acid can be stored in the plants, when the adverse weather comes , the ABA-glucose ester can be activate by a specific enzyme called β-glucosidase (βG) or hydrolysis, then converting it back into active ABA [8].

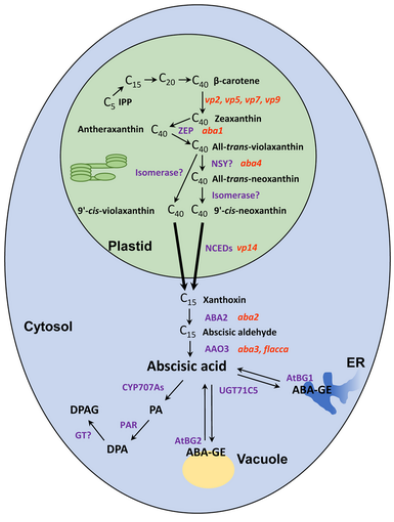

Figure 1. Process of synthesis [8]

2.3. Host

We eventually chose yeast as the host of the pathway. We consider this issue from an economic perspective. Yeast is a kind of microorganism which exhibits a short growth cycle and easy to culture. However, we finally choose yeast as the host instead of E. coli although it also contains these advantages [13]. The most immediate reason of this is that yeast is also eukaryotic and has structures which have high similarities with plants such as rough endoplasmic reticulum, Golgi body

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[13]. This plays a significant role in the construction of a suitable environment for the synthesis that, makes the complex enzymatic reactions especially enzymes like 9-cis-epoxycarotenoid dioxygenase are derived from plants, which activity depends on eukaryotic-specific post-translational modifications.

We have decided to use PCR techniques to produce ABA in yeast. To do this, we just need to ensure the yeast can act as a host that can produce 9-cis-epoxycarotenoid dioxygenase and Abscisic aldehyde oxidase [11]. So, we planned to import NCED and AAO genes into a functional plasmid in order to let yeast replicate this vector and express the enzyme inside. As for the primer design, initially we get NCED and AAO's gene sequence [11]. Followed by is the addition of the primers which contain restriction endonuclease recognition sequence in such as EcoRI to the 5'ends of both genes of genes to facilitates the cutting and insertion these gene segment into the original strands. In addition, we use high-fidelity DNA polymerase for the PCR reaction, which can make sure there is no mutation and keep the accuracy of the edited genes. Then we choose an expression plasmid containing origin of replication (ORI) and strong promoter to let this plasmid can replicate in yeast. After that we plan to insert the modified genes into the carrier still by restriction endonuclease digestion, using the designed primers on the sides of previous genes to cut the PCR products and carriers and induce recombination to obtain the desired yeast strain.

2.4. Feedstock

As for the feedstock, the main lines and principles of choosing the feedstock are that:

Initially, the feedstock should only provide the basic nutrients required for the yeast to grow, but it also provides ample precursors and cofactors such as Acetyl-Coa, pyruvate for the ABA synthesis pathway that may induce and enhance the expression of this pathway through controlled conditions.

Furthermore, the synthesis pathway especially MVA, which highly consumes energy (ATP) and reducing power (NADPH). So we need to stimulate the aerobic respiration, although glucose has priority to be degraded because it is the carbon resource which metabolize and produce energy the most easily, it can also inherit aerobic respiration and produce a large amount of alcohol which apply pressure on host, this is not conducive to produce ABA in yeast sustainably. So, we choose galactose as the feedstock because it not only has strong starting codons but also promote the yeast to produce energy high-efficiently [12]. The concentration of galactose does not affect the rate of aerobic respiration. After that, utilizing the inducible promoters such as GAL1, GAL10 to induce the expression of the genes of the ABA synthesis pathway [14]. Finally, using inexpensive glucose or glycerol as the carbon source allow the microorganisms to grow rapidly to high density, then transfer the yeast into the carbon sources which only contain galactose when the previous carbon sources run out, we can produce Galactose-Induction ABA Production Medium in only 3 steps:1. Dissolve succinic acid, YNB, and supplements in water. 2.Adjust the pH to 6. 3.Add galactose and make up to the final volume and sterilize. And just trigger the GAL to catalyst the pathway of ABA synthesis.

3. Conclusion

This article discussed on Abscisic acid synthesis, including pathway and host, through researching articles and websites. We come up ABA synthesis can be used to prevent the plants from frost damage, in addition, the project discussed in the article can create a way of synthesizing ABA more sufficiently. It can be used to increase the amount of product along with a decrease in the costs. In future work, the testing in the lab could prove that this method of synthesizing would be introduced

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in the agriculture system. Therefore, generalization of ABA usage and more ways of using ABA could be a field for future studies.

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