ISSN:3134-8157

INVESTIGATION OF LIFE SCIENCE

March 1,2026
Volume 1
Issue 1

I O L S

Hong Kong Sinovita Group Limited

www.investigationoflifescience.com

INVESTIGATION OF LIFE SCIENCE

1 March 2026 | Volume 1, Issue 1

Print ISSN: 3134-8157 | Online ISSN: 3134-8165

Published by: Sinovita (Hong Kong) Group Co. Ltd.

Address: Unit 8, 26/F, Billion Plaza 2, 10 Cheung Yue Street, Cheung Sha Wan, Kowloon, Hong Kong

Printed by: SINOVITA

Price: 50EUR

Editorial Office E-mail: editor@investigationoflifescience.com

Official Website: https://www.investigationoflifescience.com

INVESTIGATION OF LIFE SCIENCE

1 March 2026 | Volume 1, Issue 1

Dietary Nutrition and Dietary Design for Hypertensive Patients

....Page 1–19

The Development and Application of Food Fiber in Food

..... Page 20–27

Molecular cloning and subcellular localization of GLOBOSA gene in Nicotiana tabacum

..... Page 28–39

Treatment of Multifidus Muscle Injury in Posterior Lumbar Spine Surgery: A Comprehensive Review

..... Page 40–58

Review:Synthesis and metabolism of tea aroma compounds

..... Page 59–73

Tea Tree NOX Amplification of gene families and their expression analysis in tea plant stress

..... Page 74–91

Dietary Nutrition and Dietary Design for Hypertensive Patients

ZHAO Xianlin [1*] TANG Xiaozhen[2] WEI Siqing[3] WEI Xuelian[4] HUANG Chao[1,5*]**

(1 Jinan Daxin Biotechnology Co., Ltd., Jinan, Shandong 250000, China)
(2 School of Food Science and Engineering, Shandong Agricultural University, Tai'an, Shandong 271099, China)
(3 Shandong Vocational College of Foreign Trade, Tai'an, Shandong 271000, China)
(4 State Power Investment Group Intelligent Technology Development Co., Ltd., Haidian, Beijing 100000, China)
(5 School of Academy, Jiaying College, Meizhou, Guangdong 514015, China)

Abstract With the rapid development of the social economy, the transformation of people's lifestyles, and the continuous improvement of their quality of life, the number of deaths caused by cardiovascular diseases such as stroke, angina, and myocardial infarction accounts for 30% of all deaths. Among them, 62% of strokes and 49% of myocardial infarction-related deaths are associated with hypertension. Currently, the prevalence of hypertension in China is high, with a clear growth trend and increasing harm. Therefore, it is becoming increasingly important for the general population to have a correct understanding of hypertension and to effectively implement early prevention, timely treatment, and dietary improvement. By analyzing and summarizing the hazards, complications, causes, and nutritional principles for hypertensive patients, a survey questionnaire was used to investigate 10 hypertensive patients to understand their dietary and lifestyle habits and analyze the causes of hypertension. A weekly nutritional diet was designed for hypertensive patients, and the most representative day was selected for nutritional analysis. Corresponding nutritional recommendations are provided for hypertensive patients.

Keywords hypertension; nutritional diet; dietary design

Hypertension is one of the main factors leading to premature death and disability worldwide. In addition to genetic factors, diet and lifestyle also have an important impact on blood pressure levels. Dietary nutrition and meal design are of great significance for blood pressure control and complication prevention in hypertensive patients. Therefore, this article will explore the dietary nutrition and meal

design for hypertensive patients, aiming to provide references for clinical nutritional support and health management.

This article discusses the dietary nutritional needs and meal design principles for hypertensive patients, formulates corresponding nutritional recommendations and recipes for different situations, and proposes nutritional education strategies to help hypertensive patients scientifically manage their diet and improve their health status. Finally, the main viewpoints and conclusions of the full text are summarized, aiming to provide practical guidance for the dietary nutrition and meal design of hypertensive patients.

1 Overview of Hypertension

1.1 Definition

In fact, the disease known as "hypertension" mainly refers to the condition where blood pressure in the heart and brain is excessively high. When an adult's diastolic blood pressure exceeds 90 mmHg, or systolic blood pressure exceeds 140 mmHg, they can be confirmed to have hypertension. Hypertension is characterized by increased systemic arterial blood pressure (SBP and DBP) as the main clinical manifestation. It coexists with other cardiovascular risk factors and can lead to functional or organic damage to organs such as the heart, brain, and kidneys [1]. Primary hypertension accounts for about 95% of all hypertensive patients in China. It is a disease with increased blood pressure as the main clinical symptom and is also one of the main risk factors for cardiovascular diseases [2], causing serious interference with the normal structure and function of major organs such as the myocardium, brain, and kidneys, and even leading to decreased organ function. Secondary hypertension refers to increased blood pressure due to a specific disease or cause, accounting for about 5% of all hypertensive patients.

1.2 Diagnosis

Blood pressure values measured at the brachial artery site during quiet rest can be used to diagnose hypertension, but hypertension cannot be determined based on one or two blood pressure readings alone. Long-term follow-up is needed to observe changes in blood pressure and the overall level. Once hypertension is diagnosed, it is important to promptly distinguish whether it is primary or secondary. The classification of primary hypertension in adults is shown in Table 1.

Table 1 Classification of primary hypertension in adults

Category	Systolic Pressure / mmHg	Diastolic Pressure / mmHg
Normal Blood Pressure	<120	<80
High Normal	120~139	80~89
Hypertension		
Grade 1 (Mild)	140~159	90~99
Grade 2 (Moderate)	160~179	100~109
Grade 3 (Severe)	≥180	≥110
Isolated Systolic Hypertension	≥140	<90

1.3 Clinical Manifestations

Generally, the specific clinical symptoms of hypertensive patients are as follows:

Early Stage: When patients first notice a sudden increase in blood pressure, there are no obvious symptoms. However, once this condition persists, mild symptoms such as headache, neck discomfort, dizziness, decreased sleep quality, and anxiety may occur. A few people may also experience chest tightness and palpitations. However, patients often do not realize they have hypertension and mistake these symptoms for neurasthenia.

Mid-stage: This refers to the stage when the hypertensive patient's condition has progressed to the middle phase. Symptoms start with headache, followed by dizziness, and finally nausea and vomiting. In severe cases, syncope and shock may occur.

Late Stage: This refers to the stage when the hypertensive patient's condition has progressed to the advanced phase. The condition evolves from initial external symptoms to complications involving major

organs throughout the body [3], including coronary heart disease, cerebral arteriosclerosis, cerebrovascular infarction, and renal arteriosclerosis. Severe cardiovascular and cerebrovascular diseases can seriously threaten the patient's life.

1.4 Hazards and Complications of Hypertension

Hypertension is a common cardiovascular disease, but its pathogenesis is not fully understood. Long-term hypertension can accelerate the development of medium and large arteries by affecting their structure and function [4].

1.4.1 Heart Disease

The harm of hypertension is mainly reflected in the damage to the cardiovascular system. Coronary heart disease, also known as ischemic heart disease, is caused by the narrowing of coronary arteries due to hypertension, leading to insufficient blood supply. The harm of hypertension is mainly reflected in the damage to the heart. When hypertension affects the heart, it often causes changes in cardiac structure and function. If blood pressure remains elevated for a long time, it increases the load on the left ventricle, causing left ventricular hypertrophy and dilation, which can eventually lead to heart failure.

1.4.2 Cerebral Hemorrhage

Damage to the brain caused by hypertension mainly manifests as damage to cerebral blood vessels. Long-term hypertension can lead to cerebral ischemia, degeneration, and may even form cerebral aneurysms, which can trigger cerebral hemorrhage. Increased blood pressure can also lead to arteriosclerosis and the formation of blood clots. Intracerebral hemorrhage (ICH) is one of the most common complications in patients with advanced hypertension.

1.4.3 Kidney Disease

Chronic hypertension can increase pressure in the glomeruli, leading to glomerular fibrosis and atrophy, and subsequently causing renal arteriosclerosis. As the disease progresses, kidney function gradually deteriorates, eventually developing into renal failure. Therefore, early diagnosis, detection, and treatment of hypertensive patients are essential to minimize damage.

1.5 Risk Factors for Hypertension

1.5.1 Genetic Factors

Hypertensive patients show significant familial aggregation. If both parents have hypertension, their children have a prevalence rate as high as 46%; if one parent has hypertension, the probability of the child having hypertension is 28%; if both parents have normal blood pressure, the child's probability of having the disease is only 3%.

1.5.2 Dietary Factors

1.5.2.1 Sodium Intake

Sodium ions are an important factor causing hypertension. In traditional Chinese dietary habits, the daily intake of salt is relatively high, averaging 9-13 g per person per day [5]. Excessive sodium intake seriously affects residents' blood pressure, causing hypertension. Sodium retention can lead to increased extracellular fluid, increased cardiac output, and elevated blood pressure. For every 2 g increase in daily per capita salt intake, diastolic blood pressure increases by 1.2 mmHg and systolic blood pressure increases by 1.2 mmHg. A low-salt diet can effectively improve the symptoms of hypertension.

1.5.2.2 Excessive Energy Intake

The incidence of hypertension is significantly higher in obese people than in the normal population. The average Body Mass Index (BMI) for Chinese men is 21-24.5 kg/m², and for women is 21-25 kg/m². There is a significant positive correlation between BMI and blood pressure. Studies show that for every 1 kg/m² increase in baseline BMI, the risk of developing hypertension within 5 years increases by 9%. By controlling energy intake, weight can be reduced, thereby lowering blood pressure.

1.5.2.3 Fat and Cholesterol

Obesity is a major factor inducing diseases such as hypertension. Studies show that 65% of patients have a BMI above the normal level [6]. High-fat, high-cholesterol foods can easily induce atherosclerosis. Therefore, excessive consumption of animal fats or cholesterol is not conducive to the prevention and treatment of hypertension.

1.5.3 Excessive Alcohol Consumption

Studies show a linear relationship between alcohol consumption and blood pressure; both systolic and diastolic blood pressure increase with increasing alcohol consumption. The incidence of hypertension increases significantly among people who drink more than 50g of alcohol per day. Heavy drinking may also cause stroke. Studies show that long-term alcohol consumption increases the levels of catecholamines and corticosteroids in the body, increases the activity of renin-angiotensin, vasopressin, and aldosterone, leads to disorders in sodium-potassium pump activity, abnormal ion transport, increased intracellular Ca^{2+} concentration, intensified vasoconstriction response, increased peripheral resistance, and ultimately leads to increased blood pressure.

1.5.4 Smoking

Nicotine in cigarettes can cause peripheral vasoconstriction, thereby accelerating arteriosclerosis and leading to increased blood pressure. When smoking, the increase in adrenaline and noradrenaline can cause accelerated heartbeat and increased systolic and diastolic blood pressure. In addition, nicotine in tobacco has a certain impact on the efficacy of antihypertensive drugs.

1.5.5 Psychological and Work Pressure

In the process of social development, with increasing competition, people's pace of life continues to accelerate, which not only brings more pressure but also causes a series of changes in the body. For example, catecholamines in the body increase, leading to vasoconstriction, increased blood pressure, and increased burden on the heart. "Traditional Chinese Medicine" records: the seven emotions (joy, anger, worry, thought, sadness, fear, fright) are related to the internal organs; imbalance of yin and yang, poor circulation of qi and blood, leads to various diseases. Therefore, being in a state of tension, anxiety, and fear for a long time can easily lead to hypertension. In addition, unpleasant stimuli, such as indifference, nitpicking, criticism, annoyance, sadness, and noise, may also lead to hypertension.

2 Nutritional Principles for Hypertensive Patients

2.1 Limit Sodium Intake

The daily salt intake in Chinese people's daily life is generally very high, which is not conducive to lowering blood pressure. In fact, 3g of salt per day can meet the body's demand for sodium, but due to different dietary habits and preferences, salt intake is much higher than 3g. WHO recommends 3-6g of edible salt per person per day, while the Chinese Nutrition Society, based on China's national conditions, recommends that adults consume 6g of edible salt per day. Therefore, daily diet should be mainly light, eat less soy sauce, pickled foods (such as bacon, salted fish, pickles, pickled vegetables, etc.), monosodium glutamate, etc. At the same time, when cooking, you can add some vinegar, which can both enhance the flavor of the dish and make it easier to accept a low-salt diet.

2.2 Control Weight, Avoid Overweight or Obesity

High-energy, high-sugar foods are easily converted into fat in the human body, leading to increased blood sugar and obesity. Therefore, by reasonably controlling weight, risk factors related to obesity can be effectively reduced. At the same time, weight management also helps improve diseases such as insulin resistance, diabetes, and hyperlipidemia.

2.3 Supplement Adequate Calcium and Magnesium

Both calcium and magnesium can regulate vasodilation and contraction. Increasing calcium intake can significantly improve the excretion of sodium and water, which has a significant promoting effect on the prevention and treatment of hypertension [7]. Calcium and magnesium have antihypertensive effects; magnesium has the effect of dilating peripheral blood vessels. Both of these effects can protect the heart

and prevent arteriosclerosis [8]. Foods rich in calcium include milk, shrimp, fish, etc.; foods rich in calcium include wheat bran, red beans, soybeans, mushrooms, etc.; foods rich in magnesium include beans, shiitake mushrooms, longan, etc.

2.4 Choose Foods Containing High-Quality Protein

Protein is an important nutrient. Severe deficiency of protein in the human body may endanger life or even lead to death. Protein contains substances such as methionine and taurine, which have the function of regulating blood pressure, can increase sodium excretion in urine, thereby inhibiting the effect of sodium ions on blood pressure. Soybeans are rich in high-quality plant protein and can effectively prevent cardiovascular diseases. Therefore, hypertensive patients should eat more protein-rich foods, such as high-quality plant-based protein like soybeans, and high-quality animal-based protein like fish, chicken, beef, milk, etc. Plant-based protein intake should account for at most half of the total protein intake [9].

2.5 Reduce Fat and Cholesterol Intake

High-fat, high-cholesterol diets can easily lead to arteriosclerosis and obesity, so dietary fat should be controlled below 25% of total energy [10]. When cooking, the best ratio of vegetable oil to animal oil is 1:0.5. This is because the polyunsaturated fatty acids, vitamin E, and phospholipids contained in vegetable oil can inhibit cholesterol absorption, accelerate cholesterol decomposition and excretion, and play a significant role in preventing and treating cardiovascular diseases. Animal oil is rich in saturated fatty acids, and saturated fatty acids are rich in cholesterol, which can easily lead to arteriosclerosis and obesity. Therefore, in terms of diet, hypertensive patients should try to eat less animal oil and eat more vegetable oils, such as corn oil, soybean oil, rapeseed oil, peanut oil, olive oil, tea oil, etc., and try to minimize the consumption of foods high in cholesterol, such as animal offal, fat, abalone, butter, cream, egg yolk, etc. Cooking methods are suitable for steaming, boiling, and stewing.

2.6 Choose Fibrous Foods and Complex Carbohydrates

Foods rich in fiber can help gastrointestinal peristalsis, accelerate cholesterol excretion, and thus achieve the purpose of lowering blood lipids. Therefore, eat more foods high in fiber, such as corn, millet, whole wheat flour, root vegetables, algae, potatoes, vegetables and fruits, etc. [11]. When consuming sugar, try to choose complex carbohydrates such as starch, corn, millet, etc., and use less glucose, fructose, and sucrose.

2.7 Quit Smoking and Limit Alcohol Consumption

Smoking is a major cause of hypertension, and long-term smoking can also cause large artery atherosclerosis; the intima of small arteries will gradually thicken, causing the entire blood vessel to slowly harden. Therefore, it is best for people with hypertension not to smoke. Excessive drinking can cause harm to the body and also increase blood pressure. The Chinese Nutrition Society recommends the standard for "moderate drinking" for adults: men should not drink more than 25g of alcohol per day, and women should not drink more than 15g of alcohol per day [12].

3 Questionnaire and Analysis

3.1 Basic Information

3.1.1 Survey Subjects

10 people with hypertension from different regions and different industries.

3.1.2 Survey Method

The questionnaire survey method was used. 10 questionnaires were distributed and 10 were recovered, with a recovery rate of 100%. The questionnaire was based on reality to understand the problems in the physical condition, dietary habits, and lifestyle habits of hypertensive patients, thereby reflecting the bad tendencies of hypertensive patients in diet and life.

3.2 Basic Information Entry

The basic information of the survey subjects is shown in Table 2.

Table 2 Basic Information Survey of Hypertensive Patients

No.	Gender	Height / cm	Age / Years	Weight / kg
1	Male	175	25	84
2	Female	158	45	67
3	Male	180	45	90

No.	Gender	Height / cm	Age / Years	Weight / kg
4	Male	170	48	89
5	Female	160	20	60
6	Female	165	55	69
7	Male	179	23	88
8	Female	180	30	79
9	Female	163	71	70
10	Female	162	69	65

3.3 Cause Analysis

Among the 10 questionnaires surveyed, the survey results show the following characteristics. One is genetics. About 60% of hypertensive patients have a family history, and if there are hypertensive patients in the family, the likelihood of offspring developing hypertension will increase. The second is obesity and overweight. In today's society, people's living standards are constantly improving, their dietary standards are gradually increasing, and food is becoming more diverse. People eat more but exercise less, which leads to an increasing number of obese people. Obesity is more likely to cause high blood pressure, with a 25% higher risk than non obese people. Thirdly, excessive intake of sodium. Many people enjoy salty foods, as salt can raise blood pressure and damage the cardiovascular system, making it a recognized killer of cardiovascular diseases. Fourthly, drinking alcohol. More than 40% of people who drink heavily for a long time will suffer from hypertension, and are also prone to various diseases such as stroke, myocardial infarction, and pancreatitis. Five are environmental and spiritual factors. Noise, weather changes, mental stress, and psychological trauma can all increase the risk of hypertension. Therefore, a stable and peaceful living environment is very important for people's health. Living in a noisy and noisy environment for a long time will inevitably affect the quality of rest and sleep. People with long-term sleep disorders or poor sleep have a much higher risk of developing hypertension than normal people, and excessive mental labor can also easily lead to hypertension.

4 Nutritional Meal Planning for Hypertensive Patients

4.1 Meal Planning Principles

First, control thermal energy, mainly control the intake of staple foods and fats, try to eat less or no candy, pastries, sweet drinks, fried foods and other high-energy foods [13]. Second, reduce the amount of salt used in cooking, try to eat less pickled vegetables and other salty foods. Third, eat less fatty meat and various animal fats, and control the intake of high-cholesterol foods such as animal brains and fish roe. Try to use vegetable oils such as soybean oil, peanut oil, and sunflower seed oil for cooking oil. Fourth, eat more vegetables and fruits, especially dark-colored vegetables, and appropriately increase the intake of seafood, such as kelp, laver, marine fish, etc. [14].

4.2 1-Week Recommended Recipes and Evaluation

As can be seen from Table 3, hypertensive patients should pay special attention in their daily diet: control the intake of thermal energy, try to eat complex carbohydrates, such as foods with more plant fiber in the recipes like corn, millet, oats, etc., use a combination of coarse and fine grains to make nutrition more balanced; the recipes are rich in potassium and calcium, such as potatoes, eggplant, kelp, lettuce, winter melon, etc. Potassium salts can promote cholesterol excretion, increase blood vessel elasticity, and help improve myocardial contraction ability; foods rich in magnesium are abundant, such as green leafy vegetables (celery, lettuce), buckwheat noodles, beans and bean products. Magnesium salts can achieve blood pressure lowering effects by dilating blood vessels; cooking methods should mainly involve less oil, such as tossing, stir-frying, braising, and stewing.

4.3 1-Day Recipe Nutritional Analysis

One patient was selected from the basic information survey form: height 160 cm, weight 60 kg, an office clerk, belonging to a light physical laborer.

Calculation of energy and nutrient intake: Standard weight = 160 - 105 = 55 kg; BMI = Actual weight (kg) / Height squared (m^2) = 60 / 1.60^2 = 23.4 kg/m^2 (Normal body type); Checking the adult daily energy supply table, the energy supply per unit standard weight for normal weight, light physical laborers is 30 kcal/kg, so the full-day energy supply = 60 kg × 30 kcal/kg = 1,800 kcal; Among the three major heat-producing nutrients, protein accounts for 15% (1~1.2 g/kg standard weight), fat accounts for 25%, carbohydrates account for 60%~65%, cholesterol is less than 300 mg/d, potassium 2.5 g/d, sodium 4~6 g/d, other inorganic salts and vitamins are based on the Dietary Reference Intakes.

Taking Tuesday's recipe as an example, nutritional analysis and calculation were performed. The specific results are shown in Tables 4~7.

Table 3 1-Week Recommended Recipes for Hypertensive Patients

Day	Breakfast	Lunch	Dinner
Monday	Mantou (50g wheat flour), oatmeal Congee (15g oatmeal 30g of glutinous rice, 100g of mixed lettuce, boiled chicken Egg (50g red skin egg)	Red bean rice (90g glutinous rice, 10g adzuki beans), garlic Mud eggplant (150g eggplant, 10g garlic), braised in red Carp chunks (75g carp), 50g citrus fruits	Two rice porridge (15g japonica rice, 10g millet), fried celery with black fungus Vegetables (30g black fungus, 150g celery), garlic sprouts and meat slices (garlic) 80g seedlings, 20g pork, 70g bananas
Tuesday	Cabbage Bun (25g chives, 50g wheat flour, small) 50g cabbage, 25g pork), Congee with corn flour (corn 25g noodles), mixed with melon strips (100g cucumber, 5g chili) Vinegar peanuts (10g peanut kernels, 2g white vinegar)	Gold and silver rice (120g glutinous rice, 30g corn grits) Stir fried Tofu with Scallion (100g Southern Tofu, Scallion) 10g)、 Sauce beef 25g, stir fried tomatoes, chili peppers	Coarse grain Congee (10g glutinous rice, 5g job's tears, 5g kidney beans), Mantou (50g wheat flour, 1g dry yeast), stir fried eggplant and green pepper (eggplant) 100g、 15g chili pepper, 10g carrot, fruit platter (citrus) 100g orange, 50g red Fuji apple, 100g Jingbai pear, beans

Day	Breakfast	Lunch	Dinner
		(50g tomato, 25g chili), 10g soybean oil	Oil 8g
Wednesday	Cabbage Bun (50g Wheat Flour, 50g Cabbage, Pig) Meat 25g), corn flour Congee (corn flour 25g), mixed yellow 100g melon, 10g sweet and sour peanuts (10g peanut kernels)	Red bean rice (30g red beans, 100g glutinous rice), mixed Tofu (100g Southern Tofu), Stir fried Three Fresh, Lettuce 75g、 Sauce beef (25g beef), 15g pear	Coarse grain Congee (10g japonica rice, 5g glutinous rice, 5g job's tears, kidney beans 5g）、 Mash sauce mixed with lettuce (100g of lettuce), crispy and fragrant Carp (Carp 50), Red Extract 50g
Thursday	Sweet potato corn flour Congee (20g corn, 30g sweet potato) Spinach and mung bean sprouts (100g mung bean sprouts, 100g spinach)	Rice (90g glutinous rice), shiitake mushrooms and rapeseed (shiitake mushrooms) 50g、 100g rapeseed), shredded pork beans (beans)	Mantou (wheat flour 100g), beef ball and shredded radish soup (beef 25g、 White radish 25g), fried Rolls of dried bean milk creams and celery (celery 100g Rolls of dried bean milk creams 5g,

Day	Breakfast	Lunch	Dinner
	Five spice tofu rolls (25g per thousand sheets)	150g、 Pork 25g, Shrimp Slippery (Shrimp 25g), Grass Berry 35g	shredded carrot 25g), peach 50g
Friday	French bread (100g French pairing bread), milk 200mL、 Roasted broccoli (100g broccoli)	Green bean rice (20g green beans, 80g glutinous rice), stir fried meat Onion (100g onion, 25g pork), stir fried beans Sprout (100g mung bean sprout), 50g orange	Steamed Chinese sponge cake (100g wheat), tremella and lotus seed soup (5g tremella 10g lotus seeds), stir fried octopus shreds with garlic sprouts (100g garlic sprouts) Octopus 25g, Kiwi 35g
Saturday	Century egg lean meat Congee (8g lean pork, 25g japonica rice), steamed bread Head (75g wheat flour), mixed with sesame and spinach (spinach)	Rice (90g glutinous rice), stewed yellow croaker (yellow croaker) 75g fish), Hetang stir fry (50g lotus root, celery)	Chicken Shredded Buckwheat Noodles (75 g Buckwheat Noodles, Chicken Breast Meat) 37.5g)、 Cleverly mixed lettuce (50g lettuce, 100 red tomatoes)

Day	Breakfast	Lunch	Dinner
Sunday	100g、 Sesame 5g)	100g、 Cabbage 25g, Mulberry 50g	g、 5g black fungus and 50g banana
	Bean paste roll (40g wheat flour), 10g Red bean soup, corn Noodle Congee (corn flour 25g), sauce beef (beef 25g) Mix seaweed shreds (100g seaweed, 10g garlic)	Green Bean Rice (120g Japonica Rice, 30g Green Bean), Sauce Pork ribs (75g pork chops), stewed pumpkin and potatoes (South) 100g melon, 50g potato, 50g apple	Steamed Chinese sponge cake (30g wheat flour, 15g corn flour), Congee (10g glutinous rice, 5g glutinous rice, 5g Job's tears, 5g kidney beans), meat Stir fried black fungus and cabbage (100g Chinese cabbage, 10g carrot, black) 5g fungus, 15g pork, 50g orange

Table 4 Breakfast Nutritional Composition Calculation Table

Nutritional Composition	Nutrient Name	Content
	energy/kcal	418

	protein/g	14
	fat/g	12
	carbohydrate/g	65
13% of energy is provided by protein, and 27% by fat 60% energy supply from carbohydrates	Vitamin E/mg α-TE	6
	Vitamin C/mg	50
	sodium/mg	89
	calcium/mg	118

Table 5 Lunch Nutritional Composition Calculation Table

Nutritional Composition	Nutrient Name	Content
	energy/kcal	760
Protein provides 14% energy, fat provides 20% energy, and carbohydrates provide 66% energy	protein/g	27
	fat/g	17
	carbohydrate/g	126

	Vitamin E/mg α-TE	12
	Vitamin C/mg	56
	sodium/mg	362
	calcium/mg	202

Table 6 Dinner Nutritional Composition Calculation Table

Nutritional Composition	Nutrient Name	Content
Protein provides 15% energy, fat provides 20% energy, and carbohydrates provide 65% energy	energy/kcal	577
	protein/g	23
	fat/g	13
	carbohydrate/g	100
	Vitamin E/mg α-TE	272
	Vitamin C/mg	41
	sodium/mg	1835

calcium/mg	257

Table 7 Full-Day Nutritional Composition Calculation Table

Total Energy	Protein (% of Energy)	Fat (% of Energy)	Carbohydrate (% of Energy)
1,815 kcal	65 g (14%)	43 g (21%)	292 g (65%)
Energy Distribution	Breakfast 24%	Lunch 43%	Dinner 33%

Health guidance recommendations for hypertensive patients are: on the premise of ensuring adequate nutrient supply, control total energy intake; increase the intake of vegetables and fruits, consume 400g of vegetables and 200~300g of fruits daily, which can prevent blood viscosity and prevent cardiovascular and cerebrovascular diseases; appropriately increase the intake of coarse grains, such as buckwheat, oatmeal, corn, millet, etc., and promote the consumption of fresh corn in summer and autumn; appropriately increase the intake of edible fungi, such as black fungus, white fungus, etc.; the diet should be light; life should be regular, maintain a relaxed and happy mood, and avoid anger and melancholy. A good and calm mood is conducive to maintaining stable blood pressure; regular physical examinations should pay attention to abnormal changes in indicators such as blood pressure, blood lipids, and blood viscosity to prevent the occurrence of cardiovascular and cerebrovascular diseases.

5 Conclusion

In recent years, there have been more and more hypertensive patients, and the probability of primary hypertension occurring during adolescence is also increasing. The cause of hypertension is not a single factor, but the result of a combination of multiple factors. Nowadays, besides genetic factors, most hypertensive patients are caused by poor dietary habits, smoking, excessive alcohol consumption, and long-term mental stress.

Through the investigation of hypertensive patients, it can be seen that under the influence of multiple factors such as heredity, diet, excessive alcohol consumption, smoking, psychological and work pressure, some people are prone to hypertension. It is recommended that hypertensive patients follow the nutritional principles of limiting sodium salt, controlling weight, supplementing with sufficient calcium and

magnesium, choosing foods containing high-quality protein, reducing fat and cholesterol intake, choosing fibrous foods and complex carbohydrates, quitting smoking, and limiting alcohol consumption. They should eat more foods such as potatoes, eggplant, kelp, lettuce, winter melon, buckwheat noodles, soy milk, and bean products. Develop healthy eating and living habits, strengthen blood pressure regulation, maintain a relaxed and happy mood, and avoid anger and melancholy.

Fund Project: 2020 Shandong Provincial Science and Technology Commissioner Action Plan, Colored Wheat Planting and its Healthy Food Achievement Transformation and Demonstration (2020KJTPY079).

References

[1] Liu Bin, Zhang Shuo. Observation on the Effect of Dixing Haidai in Treating 48 Cases of Vertigo with Hypertension of Wuzheng Type [J]. Smart Healthcare, 2021,7(13):26-28.
[2] KARINA A Z, SANTIAGO U C, PATRICIA R G, et al. Genetic diet interactions of ACE: the increased hypertension predisposition in the Latin American population13[J]. Frontiers in Nutrition, 2023,10:1 241 017-1 241 017.

[3] Li Hao. Thoughts Based on Clinical Treatment of Elderly Hypertension [J]. Journal of Integrated Chinese and Western Medicine, 2009,26(20):285-286.

[4] Ma Wenjun. Common Misconceptions in Elderly Hypertensive Patients [J]. Chinese Journal of Geriatric Medicine, 2005,14(15):10-11.

[5] Shen Long. Research Progress on Dietary Nutrition and Hypertension [J]. Nongjia Canmou, 2019(21):205.

[6] Tang Jiqiu. Research Progress on the Relationship between Hypertension and Dietary Nutrition [J]. Modern Food, 2017(10):65-67.

[7] Li Zhichao, Huang Peng, Liao Xiaoyang. Research Progress on the Relationship between Dietary Nutrition and Hypertension [J]. Modern Preventive Medicine, 2016,43(11):1959.

[8] Liu Lisheng. Chinese Guidelines for the Prevention and Treatment of Hypertension [J]. Chinese Journal of Hypertension, 2008,27 (17):4-5.

[9] Xiong Jie. Dietary Care for Hypertensive Patients [J]. Journal of Tongren Vocational and Technical College, 2008(4):3.

[10] Wen Bao. The Protracted Battle of Preventing and Controlling Hypertension [J]. Laoren Tiandi, 2006,34 (15):16-17.

[11] Liu Zhongxing. Exercise Suitable for Hypertensive Patients [J]. Fitness Science, 2006,4 (6):30-31.

[12] Chinese Nutrition Society. Dietary Guidelines for Chinese Residents [M]. Beijing: Tibet People's Publishing House, 2009.

[13] Chen Chun. The Role of Comprehensive Nursing Intervention in Improving the Compliance and Quality of Life of Middle-aged and Elderly Hypertensive Patients [J]. Contemporary Clinical Medicine, 2020,33(4):366;378.

[14] Chinese Nutrition Society. Dietary Reference Intakes for Chinese Residents [M]. Beijing: China Light Industry Press, 2010.

The Development and Application of Food Fiber in Food

Zhao Xianlin[2] Tang Xiaozhen[3] Wei Siqing[4] Wei Xuelian[5] Huang Chao[1]※

1 School of Medicine, Jiaying College, Meizhou, Guangdong 514015, China

2 Jinan Daxin Biotechnology Co., Ltd., Jinan, Shandong 250000, China

3 School of Food Science and Engineering, Shandong Agricultural University, Tai'an, Shandong 271099, China

4 Shandong Vocational College of Foreign Trade, Tai'an, Shandong 271000, China

5 State Power Investment Group Green Energy Technology Development Co., Ltd., Haidian, Beijing 100000, China

Abstract: Dietary fiber is essential to human health as the seventh source of nutrients. In recent years, with changes in dietary habits, dietary fiber has become increasingly important for human health and has begun to be included in dietary studies. This article briefly introduces the physiological functions of dietary fiber, its development in food, the importance of its application, and also discusses the understanding regarding the development and comprehensive utilization of dietary fiber.

Keywords: dietary fiber; food; development; application

Introduction

Dietary fiber is defined as an indigestible food nutrient, a substance present in plant cell walls. With the scientific development of food nutrition and health concepts, it has become increasingly clear that dietary fiber is not only not a waste but also makes significant medical contributions to disease prevention, thus appearing particularly important. China is rich in dietary fiber sources, which are extensive, but the per capita intake is severely insufficient. The number of people suffering from various diseases due to a lack of dietary fiber is increasing year by year. As people's understanding of the nutritional value of dietary fiber grows, foods rich in dietary fiber are receiving more attention from consumers and have broad market prospects. This article briefly introduces the significance of the development of dietary fiber in the food industry and its various applications in food, and looks into the market prospects of dietary fiber.

1 The Development of Dietary Fiber in Food

1.1 Definition of Dietary Fiber

Dietary fiber typically possesses the following characteristics: it is a carbohydrate found in plant-based foods that cannot be digested by the body's starch-digesting enzymes. Although dietary fiber has no nutritional function and cannot provide energy for the body, it is an essential nutrient for the body. In summary, the definition of dietary fiber includes the following concepts: [1]

(1)The source must be a proven food source.

(2)It cannot be digested and absorbed by digestive enzymes in the body.

(3) Its chemical nature is that of a sugar, but it basically provides no energy under environmental.

(4) Other physiological functions can be clearly detected in the human body.

1.2 Physiological Functions of Dietary Fiber

(1) Dietary fiber helps Escherichia coli in the intestines synthesize various vitamins, improves intestinal function, prevents colon cancer, and reduces serum cholesterol, which can prevent heart disease.

(2) Dietary fiber has a relatively low density and large volume, occupying more space in the human stomach and intestines, making people feel full and helping to prevent energy surplus and obesity.

(3) As a fibrous food material, after consumption, it stimulates an increase in gastrointestinal waste, accelerates gastrointestinal peristalsis, prevents the formation of gallstones, and can also prevent and treat constipation and diabetes.

(4) A high-fiber diet can reduce the stimulation of intestinal factors on pancreatic β-cells, reduce insulin release, increase the sensitivity of insulin receptors, and enhance glucose metabolism [2].

1.3 Significance of the Development of Dietary Fiber in Food

Scientific research has found that dietary fiber has good effects in inhibiting the rapid rise of postprandial blood sugar and improving glucose tolerance. Dietary fiber can delay the absorption of glucose, slow down the absorption of digestible sugars and starches, avoid a sharp increase in postprandial blood sugar, enhance sensitivity to insulin, improve the regulation of insulin in the blood, and increase the body's glucose tolerance, which is beneficial for the treatment and rehabilitation of diabetes. Foods with added high fiber have special effects in both preventing and treating diabetes [3].

The significance of the development of dietary fiber in food is mainly reflected in the following aspects:

Supplementing the amount of dietary fiber required by the human body: Dietary fiber is a polysaccharide that can be derived from various foods such as grains, vegetables, and fruits. In food, beverages, health products, and pharmaceutical products, dietary fiber can be used as an additive to supplement the physiological requirement for dietary fiber.

Improving food texture and stability: Dietary fiber can affect the texture and stability of products. In some foods, such as ice cream and milkshakes, dietary fiber can act as a stabilizer, maintaining the product's structure and stability, and extending its shelf life.

Promoting digestion, cardiovascular health, and boosting immunity: Due to its unique physical and chemical properties, dietary fiber is considered to have various physiological functions, such as promoting digestion, lowering cholesterol, and controlling blood sugar. Therefore, in new functional food and beverage products, dietary fiber has become an important raw material that can add benefits such as promoting digestion, cardiovascular health, and boosting immunity to the product.

As people's attention to healthy eating continues to increase, the application fields of dietary fiber will become increasingly wider. Especially against the backdrop of an aging population, following the dietary principles for the elderly and developing foods that are low in salt, fat, and energy, but high in dietary fiber, can reduce the incidence of nutritional diseases among the elderly and improve their quality of life and health levels. Therefore, the development prospects of dietary fiber in the food industry are promising.

Application of Dietary Fiber in Food

2.1 Application of Dietary Fiber in Staple Foods

In staple foods, it mainly involves adding a certain amount of dietary fiber to rice, steamed buns, and noodle products. Foods with added dietary fiber have toughening, freshness-enhancing, and flavor-enhancing effects, which are beneficial for people's physical and mental health. Adding dietary fiber to rice can enhance its fluffy and fragrant taste. Adding 5% dietary fiber to steamed buns results in buns with a special aroma and good texture, similar to those made from whole wheat flour. Adding 4% dietary fiber to noodles increases their strength after cooking, resulting in good toughness, resistance to overcooking and soaking, and a more refreshing taste [4].

The application of dietary fiber in staple foods mainly involves grain-based staples. The following are some main applications of dietary fiber in staple foods:

Whole Grain Staples: Whole grains, such as whole wheat, brown rice, and corn, are primary sources of dietary fiber. They are rich in dietary fiber, which can help maintain intestinal health, control weight, and lower cholesterol. Whole grain staples are also believed to improve insulin sensitivity, lower blood

sugar and triglycerides. Among whole grain staples, products like whole wheat flour and whole wheat bread are common sources of dietary fiber.

Oats: Oats are a staple food rich in soluble dietary fiber, which helps control blood sugar and lipids, and improves intestinal health. Oats can be used as a staple for breakfast, lunch, or dinner, and can also be made into oatmeal, oat bread, and other foods.

Legume Staples: Legume staples such as red beans, mung beans, and black beans are also good sources of dietary fiber. They are rich in dietary fiber, which helps maintain intestinal health and lower cholesterol. Legume staples can serve as the main food source for vegetarians or be consumed with meat or grains.

Sweet Potatoes and Potatoes: Although not strictly staple foods, sweet potatoes and potatoes are good sources of dietary fiber. Sweet potatoes are rich in pectin and dietary fiber, which can improve intestinal health. Potatoes are rich in resistant starch, which is beneficial for maintaining intestinal health.

2.2 Application of Dietary Fiber in Baked Goods

Baked goods are foods made primarily from basic cereal wheat flour and matured through a high-temperature baking process, mainly dominated by the baking industry. China is a major producer and consumer of barley products. With the advancement of science and technology and the increased refinement of flour processing, many nutrients in food are lost. Therefore, adding dietary fiber to products can not only enhance nutrition but also improve people's dietary structure. Adding a certain amount of dietary fiber to baked goods affects their taste and quality. For example, a high content of dietary fiber and protein from potato peel significantly improves the quality of cakes, greatly enhancing their texture and color. If potato peel is used instead of wheat flour, the hardness of the cake decreases significantly, and the color value of the dough also decreases. Furthermore, 6% potato peel powder can produce good quality, protein-rich, and fiber-rich pastries compared to wheat flour [5].

Improving the taste and texture of baked goods: Dietary fiber can serve as a natural thickener and stabilizer in baked goods, increasing the volume and mouthfeel of the food while improving its texture. For example, adding dietary fiber when making bread can make it softer, more porous, and easier to digest.

Increasing the nutritional value of baked goods: Adding dietary fiber to baked goods can increase their nutritional value. For instance, adding dietary fiber, vegetables, or fruits when making cakes can increase the content of vitamins, minerals, and dietary fiber in the cake.

Controlling the calorie and fat content of baked goods: Dietary fiber can help control the calorie and fat content of baked goods, aiding in the management of dietary intake and blood sugar levels. For example, adding dietary fiber when making cookies can reduce their calorie and fat content.

Promoting the digestion and absorption of baked goods: Dietary fiber can promote the digestion and absorption of baked goods, increase the transit time and water content of food in the intestines, and help improve intestinal health. For example, adding dietary fiber when making biscuits can promote intestinal peristalsis and digestion and absorption.

2.3 Application of Dietary Fiber in Meat Products

Meat is nutritious, providing high-quality protein, various minerals, and vitamins. However, meat products are rich in fat and cholesterol and contain no dietary fiber. Adding dietary fiber to meat products can not only improve their water-holding capacity and emulsion stability but also enhance their health-care functions [6].

The polyglycans in dietary fiber are low molecular weight polymers that are difficult to digest and absorb in the body. Adding dietary fiber to meat products imparts a thick, smooth mouthfeel and sensory characteristics similar to fat. Moreover, the addition of dietary fiber, through the action of salt and some hydrophobic bonds, allows the dietary fiber and protein to form a more stable coagulant at high temperatures, greatly increasing the yield of meat products.

2.4 Application of Dietary Fiber in Beverages

Fiber-based beverages are very popular in the West. They can quench thirst, replenish water, and provide the body with needed cellulose. These products, especially those containing water-soluble dietary fiber, are more common in developed countries like Europe and the United States. For example, Japanese companies produce fiber-based mineral water; in addition, Western European countries produce high-fiber orange juice, high-fiber tea, etc., which is quite common [7]. In the long term, drinking water rich in dietary fiber can prevent constipation and gallstones, and lower cholesterol. It is also possible to regulate blood lipids and blood sugar by helping to reduce weight, making it especially suitable for people with diabetes and obesity. Flavored beverages have been sold on domestic and international markets for many years and are very popular. Besides the milky taste, they also taste fruity and have a good mouthfeel. Adding dietary fiber to dairy-containing beverages can significantly improve their nutritional and health functions. Water-insoluble dietary fiber is a good choice for beverage manufacturers because they are very effective in terms of health benefits.

2.5 Application of Dietary Fiber in Dairy Products

Studies have shown that dietary fiber can be used as a functional food ingredient in fermented milk to improve its quality and antioxidant activity. Adding dietary fiber to dairy products can meet people's needs for protein, animal nutrients, and phytonutrients, further enhancing the nutritional value and application

scope of dairy products. Dietary fiber can improve the flavor and stability of dairy products, and none of its components cause adverse physical or chemical reactions in the human body. Long-term consumption of milk containing dietary fiber can make the intestines feel comfortable, lower cholesterol, regulate blood lipids and blood sugar, making it particularly suitable for the elderly. Adding dietary fiber to dairy products can meet the demand for protein, vitamin A, and other animal nutrients, further improving the nutritional value of dairy products [8].

The application of domestic dietary fiber in dairy products is also developing rapidly and has huge market potential. For example, the national dairy market is transitioning from the era of pure milk to the era of high-end functional dairy products. As Chinese people's awareness of the importance of dietary fiber continues to increase, dietary fiber diets will become a new trend in China's functional health foods, and dietary fiber milk will also become a new highlight in functional dairy products.

2.6 Application of Dietary Fiber in Health Products

In today's society, the application of dietary fiber in health products and medical products is becoming more and more widespread. The main purposes of its application are to supplement the physiological requirement for fiber in the human body, enhance the health functions of products, improve product flavor, and increase product quality.

Domestically, functions such as lubricating the intestines, relieving constipation, detoxifying, and beautifying the skin are particularly popular in the health product industry, and dietary fiber has particularly significant effects in these aspects, so its market prospects are very optimistic. The main components of dietary fiber products can be widely used, for example, to supplement calcium and blood with good effects. Moreover, adding dietary fiber can stimulate the body to produce lactic acid, which in turn can dissolve minerals and promote their absorption by the body [9].

2.7 Application of Dietary Fiber in Other Foods

Dietary fiber is not only widely used in staple foods, beverages, and other products, but its application in other foods also has great practical significance. For example, dietary fiber is used in fillings, soups, and other foods. To improve the appearance quality of pasta, mixing dietary fiber with trace elements and heating it to make fillings yields good results. Salad dressings, condiments, pastries, etc. Adding dietary fiber to these foods can increase their nutritional value and taste, while controlling their calorie and fat content. Dietary fiber can be directly added to some soups to supplement the body's required cellulose. It can also be added to fried foods to make tasty balls; these products have good flavor and nutritional effects and can extend the shelf life of such foods [10].

3 Conclusion and Outlook

3.1 Conclusion

Dietary fiber is an important nutrient with unique physiological functions. Currently, China not only applies dietary fiber in various foods but also uses fiber as an additive to produce capsule and tablet products, achieving significant social benefits. As people's understanding of dietary fiber deepens and research advances, it will play a huge role in future development.

3.2 Outlook

Currently, the development trends in dietary fiber research mainly include the following aspects:

The development and utilization of dietary fiber resources, including further utilization of existing resources on one hand, and the investigation and development of unexplored resources on the other hand.

(2) Establishing efficient methods for determining dietary fiber to promote research in this field.

(3) Improving the scale of instrumentation for industrial production of dietary fiber, finding feasible industrial preparation methods, strengthening research on dietary fiber modification, and promoting the industrial production of dietary fiber.

(4) Encouraging in-depth research on the functional properties and nutritional significance of dietary fiber.

(5) Through the development of dietary fiber applications in food, further expand the scope of dietary fiber development. China should fully utilize its resource advantages in dietary fiber, vigorously develop the dietary fiber industry, meet the needs of the food market, and optimize and improve the dietary structure of our people.

As more and more people domestically and internationally increase their awareness of the nutritional value of dietary fiber and conduct in-depth research on its application in food, the use of dietary fiber in food will have important practical significance and broad market prospects.

Fund Project:

Shandong Provincial Natural Science Foundation, China, ZR2021MC004, Chain Conformational Changes of Ginger Triple-Helix Polysaccharide in Aqueous Solution and the Structural Basis of Its High Immunological Activity, 2022.1-2024.12, Ongoing, Host.

References

[1]Zhang Xiaoqiang. Definition, Classification and Physicochemical Properties of Dietary Fiber [J]. Technology Outlook, 2016, 26(20): 285-286.

[2]Huang Qiang, Shu Ting, Wang Chuan, Ouyang Man, Zheng Min. A Brief Discussion on Dietary Fiber and Health [J]. China Food Safety Magazine, 2018, (27): 68.

[3]Wang Lili. A Brief Discussion on the Medical Value of Dietary Fiber [J]. Everyone's Health (Academic Edition), 2015, 12: 42-43.

[4]Li Dandan. A Brief Discussion on the Physiological Functions and Applications of Dietary Fiber [J]. Food Engineering, 2015, (1): 20-21.

[5]Zhang Song. Functional Properties of Dietary Fiber and Research Progress in the Food Field [J]. Food Research and Development, 2018, 39(17): 214-218.

[6]Zhang Bohua, Zhang Ming, Yang Lifeng, et al. Study on the Extraction of Dietary Fiber and Its Application in Food [J]. China Fruits and Vegetables, 2019, Vol. 39(4): 1-5.

[7]Sun Guangyan. Application of Water-Soluble Dietary Fiber in Functional Foods [J]. Technological Innovation and Application, 2012, (3): 249.

[8]Yang Zhiyuan, Yang Haijun. Functional Properties of Dietary Fiber and Its Application in Dairy Products [J]. China Dairy, 2003, 11: 33-34

[9]Cao Rong'an. Physiological Functional Characteristics of Dietary Fiber and Its Application in the Food Industry [J]. Meat Research, 2010, 2: 76-78.

[10]Zhao Li, Li Qian, Zhu Dashi. Research Status and Prospects of Dietary Fiber [J]. Food and Fermentation Technology, 2014, 50(5): 76-82.

Molecular cloning and subcellular localization of *GLOBOSA* gene in *Nicotiana tabacum*

Cui Fangfang [1] Zheng Yun [1] Deng Lingfan [1] Yang Xiangfei [1] Zheng Jiuzhou [1] Meng Linfeng [1] Wang Jiange [2*] Liu Qiyuan [1]

1 The Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Ministry of Education, College of Agriculture, Jiangxi Agricultural University, Nanchang 330045;

2 College of Forestry, Jiangxi Agricultural University, Nanchang 330045

Corresponding author, wjgsd@126.com

Abstract Cytoplasmic male sterility is widely used for tobacco hybrid production. In order to study the mechanism of cytoplasmic male sterility (CMS) with carperlloid stamens, the *GLOBOSA* gene was cloned and sequenced from the flower buds of tobacco K326. The physicochemical properties ,functional domains and subcellular localization of its coding protein was predicted. The phylogenetic relationship was analyzed based protein sequences by maximum likelihood method. The recombinant expression vector of *GLOBOSA* gene and green fluorescent protein expression (GFP) was constructed, and transformed into *agrobacterium tumefaciens* and directly injected into tobacco leaves for transient expression. 36h later after the injection, the subcellular localization of *GLOBOSA* gene were observed by fluorescence microscope. The results showed that *GLOBOSA* was 768bp in length, coding 203aa. It was a hydrophobic protein with two domains of SRF-TF and K-box, and had no transmembrane region and signal peptide. Fluorescence microscopy showed that the protein was localized in cytoplasm and nucleus, and more was in the nucleus.

Keywords Tobacco; *GLOBOSA* genes; Cloning; Subcellular localization; Cytoplasmic male sterility

Cytoplasmic male sterility refers to the phenomenon in which plants cannot produce functional pollen but have normal pistils, exhibiting maternal inheritance and commonly occurring in flowering plant populations (Wang et al., 2019) . Cytoplasmic male sterility in many crops is a tool for utilizing heterosis . Phenotypically , in addition to pollen abortion, cytoplasmic male sterility presents a series of related

changes: pollen abortion, anther degeneration, stamen carpelization, and stamen petalization (Fan et al., 2016) . Studies have shown that environmental factors, natural variation, heterocytoplasm, and cytoplasmic fusion can all lead to cytoplasmic male sterility (Fang et al., 2019) , and sterility can be maintained or restored by nuclear-encoded genes (Wang et al., 2021) .

To date, cytoplasmic male sterility (CMS) has been studied in 150 species (Zhang, 2021) . Molecular biology research has found that mitochondrial DNA rearrangements are frequently observed in all sterile lines, producing new open reading frames (ORFs) or chimeric ORFs, leading to cytoplasmic male sterility (Wang et al., 2020) . However, not all of these variations are associated with the cytoplasmic male sterility phenotype, as some ORFs associated with CMS are hypothetical or chimeric. The petalization and carpelization of stamens in cytoplasmic male sterility are very similar to the flower developmental homeomorphism caused by nuclear genes, and are also referred to as cytoplasmic homeomorphism (Yao et al., 2021) . This type of cytoplasmic male sterility cannot form anthers and therefore cannot produce pollen. In cytoplasmic male sterile lines with carpel-like and petal-like stamens across multiple species, not only were mitochondrial DNA rearrangements detected (Liu, 2019) , but also downregulation of class B or C genes was observed (Chen et al., 2018; Linke et al., 2003; Rijpkema et al., 2006) . However, how cytoplasmic signaling retrogradely regulates cellular gene expression remains unclear.

Our research group, through years of continuous backcrossing, has cultivated the cytoplasmic male sterile line tobacco K326 with carpel-like stamens. Sequencing revealed downregulation of the class B gene *GLOBOSA* . Downregulation of class B genes is also present in cytoplasmic male sterile lines with carpel-like stamens in many species. Therefore, what impact would overexpression of class B genes, leading to restoration of the carpel-like phenotype, have on male sterility? Gene cloning and subcellular localization are prerequisites for studying gene function. Therefore, the objectives of this study are: 1) What are the physicochemical properties of the GLOBOSA protein? 2) Where is the encoded protein located in the cell? This research will provide data for elucidating the mechanism of cytoplasmic male sterility in tobacco.

1. Results and Analysis

1.1 Tobacco CLOBOSA Sequence Analysis

The tobacco *GLOBOSA* gene sequence is 768 bp, with a coding region of 635 bp, encoding a total of 209 amino acids (Figure 1). The molecule has a mass of approximately 24 kD , a theoretical isoelectric point (pI) of 8.94, and contains a total of 3,451 atoms ($C_{1060}H_{1729}N_{325}O_{328}S_{13)}$. Among the 20 amino acids that make up the protein, glutamic acid (Glu) has the highest proportion at 9.1%, while cysteine (Cys) has the lowest proportion at 0.5%. The instability index is 56.79, classifying it as an unstable protein, while the lipid index is 77.89, and the overall average hydrophilicity is -0.890, classifying it as a hydrophilic protein.

```
ATGGGAAGAGGAAAGATAGAGATCAAAAGAATAGAGAACTCAAGCAACAGGCAGGTCACT   60
 M  G  R  G  K  I  E  I  K  R  I  E  N  S  S  N  R  Q  V  T
TACTCAAAAAGAAGAAATGGGATCTTGAAAAAAGCTAAGGAAATCAGTGTTCTTTGTGAT  120
 Y  S  K  R  R  N  G  I  L  K  K  A  K  E  I  S  V  L  C  D
GCTCGTGTTTCTGTCATCTATTTTGCTAGTTCTGGCAAGATGCATGAGTTCTCCTCTACT  180
 A  R  V  S  V  I  I  F  A  S  S  G  K  M  H  E  F  S  S  T
TCGTTGGTTGATATTTTGGATCAATACCACAAGCTTACTGGAAGAAGATTGTGGGATGCT  240
 S  L  V  D  I  L  D  Q  Y  H  K  L  T  G  R  R  L  W  D  A
AAGCATGAGAACTTGGACAATGAAATCAACAAAGTCAAGAAAGACAATGACAACATGCAA  300
 K  H  E  N  L  D  N  E  I  N  K  V  K  K  D  N  D  N  M  Q
ATTGAACTCAGGCACCTAAAGGGTGAAGACATCACATCTTTGAACCACAGAGAGCTCATG  360
 I  E  L  R  H  L  K  G  E  D  I  T  S  L  N  H  R  E  L  M
ATGTTGGAAGATGCCCTTGATAATGGACTCACTAGTATCCGTAACAAGCAGAATGACCTT  420
 M  L  E  D  A  L  D  N  G  L  T  S  I  R  N  K  Q  N  D  L
CTGAGGATGATGAGGAAAAAGACTCAAAGTATGGAGGAGGAGCAAGACCAACTTAATTGG  480
 L  R  M  M  R  K  K  T  Q  S  M  E  E  E  Q  D  Q  L  N  W
CAATTGCGGCAACTAGAGATAGCAAGCATGAATAGGAATATGGGAGAAATAGGGGAAGTG  540
 Q  L  R  Q  L  E  I  A  S  M  N  R  N  M  G  E  I  G  E  V
TTTCACCAAAGGGAGAATGAATACCAAACTCAGATGCCTTTTGCCTTCCGAGTTCAGCCA  600
 F  H  Q  R  E  N  E  Y  Q  T  Q  M  P  F  A  F  R  V  Q  P
ATGCAGCCTAATTTGCAGGAGAGATTCTAAACACCTTGATTTATTTGGTGAATTATGACC  660
 M  Q  P  N  L  Q  E  R  F  *
TTTTATTAATGTTGTGCTATCAAAAAACTTGGTGTGTAATATCAATACTCATCTACCTTA  720
GCTTATGGTTTAAGTGACATTAATTATATATTTATCTATAACCATTAC               768
```

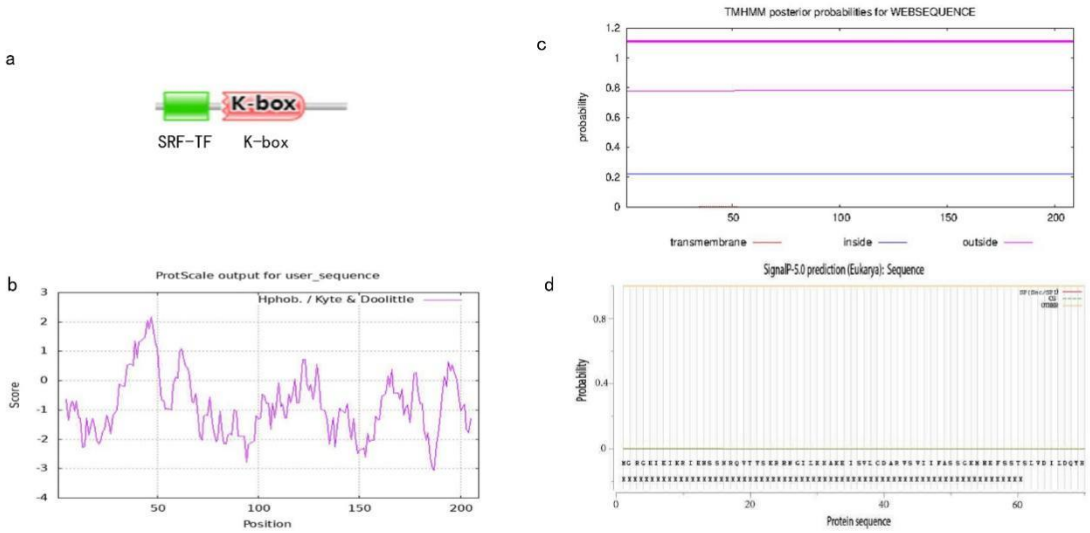
Figure 1. Sequence of tobacco GLOBOSA gene and its encoded protein

Figure 1 Nucleotide sequence and its coding protein sequence of *CLOBOSA* gene in *Nicotiana tabacum*

1.2 Analysis of the structure of the tobacco GLOBOSA gene

Structural analysis of the tobacco *GLOBOSA gene* (Figure 1) . It can be seen that the tobacco *GLOBOSA* gene has two domains: SRF-TF (PF00319) and K-box (PF01486). The SRF-TF domain is located at positions 10–57 aa, and the K-box domain is located at positions 75–164 aa. The SRF-TF domain protein has two antiparallel facultative α-helices, which can dimerize the protein and determine the binding of transcription factors to specific DNA (Ratcliffe et al., 2001). The K-box domain protein sequence has three α-helices, forming affinity helices within the protein dimer, which may be related to protein-protein interactions (Melzer et al., 2010). MADS-box genes are widely distributed in animals, plants, and fungi, participating in the regulation of growth and development processes; the *GLOBOSA* gene

is one such member. In the four-factor model of flower development, the ability to form polymers is fundamental to floral allotropy and determines floral organ specificity (Hanano et al., 2011). *The GLOBOSA* gene structure also suggests that these polymers may bind to target genes during flower development, participating in floral organ formation. (This information was obtained through ExPASy...) ProtScale results indicate the absence of hydrophobic regions in the encoded protein (Figure 1), consistent with TMHMM analysis. No transmembrane regions were found in the Figure 1). SignalP-5.0 results show the absence of a signal peptide in the encoded protein (Figure 1), and Cell -PLoc 2.0 prediction indicates its subcellular localization to the nucleus. Therefore, *GLOBOSA* is a MIKC $^{C-}$ type MADS-box gene with SRF-TF and K-box domains, capable of dimerizing to form a complex that binds to specific DNA and participates in gene regulation. The encoded protein lacks hydrophobic regions and a signal peptide, and is located in the nucleus.



a:预测的GLOBOSA结构域；b：预测的GLOBOSA疏水性；c：预测的GLOBOSA跨膜域；d：预测的GLOBOSA信号肽。

a:Predicted motifs of GLOBOSA;b:Predicted hydrophobicity of GLOBOSA;c:Predicted transmembrane domain of GLOBOSA;d:Predicted dicted signal of GLOBOSA.

Figure 1 Tobacco *GLOBOSA* gene analysis

Figure 2. An analysis of *GLOBOSA* gene i in *Nicotiana tabacum*

1.3 System Evolution Analysis

Using the tobacco *GLOBOSA* sequence, protein data from six other species were scanned. Under the criteria of a threshold of 1e-10 and a score greater than 120, ten homologous protein sequences were obtained. After Hmmer identification and removal of sequences containing only SRF-TF but no K-box, eight *GLOBOSA* homologous sequences were finally obtained. These sequences, along with the *GLOBOSA* sequences, were used to construct a phylogenetic tree based on maximum likelihood using IQTREE, as Figure 2shown in Figure 3. It can be seen that the basal group *Cinnamomum camphora* is at

the outermost end, followed by the monocotyledonous rice , then the eudicotyledonous * Arabidopsis thaliana*, with potato and tomato at the other end. In the phylogenetic tree, Solanaceae plants cluster together, with *Nicotiana benthamiana* and *Tobacco Bungei* initially clustering together, while potato and tomato are at the top of the evolutionary hierarchy. Although the phylogenetic tree was constructed using *GLOBOSA* homologous protein sequences, the relationships reflected are consistent with the evolutionary positions of the species. The figure also shows that rice and * Nicotiana benthamiana* each have two homologous protein sequences, reflecting gene duplication events during evolution.

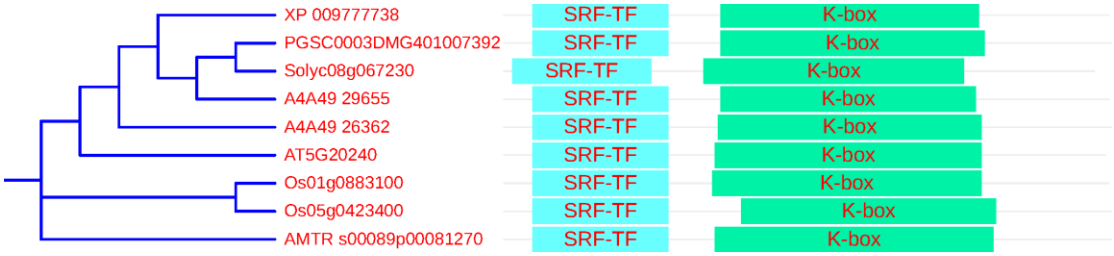


Figure 2 *GLOBOSA* phylogenetic tree

Figure 3 Phylogenetic tree of *GLOBOSA*

1.4 Construction of tobacco GLOBOSA vector

The expression vector pCAMBIA1302 is an expression vector carrying the GFP gene. It has a strong 35S promoter before its multiple cloning site and carries hygromycin (HygR) and kanamycin (Kan) resistance genes, making it a good vector for gene expression studies. The *GLOBOSA* gene was inserted between the NcoI and SpeI restriction sites to construct a recombinant expression vector carrying the 35S: *GLOBOSA* :GFP gene (Figure 3) . During vector construction, the stop codon needs to be removed, and protective bases are added. Therefore, the target gene on the vector cannot be cleaved by enzymes due to the altered enzyme recognition site at the *GLOBOSA gene insertion position. Thus, PCR amplification was used to obtain the target gene. After successful vector construction,* the vector was verified by double digestion with Figure 3NcoI / BsiWI . The verification results are shown in Figure 4b. It can be seen that the recombinant expression vector initially showed one band, and after enzyme digestion, two bands appeared, indicating successful construction of the recombinant expression vector. Before transient expression, the recombinant expression vector needs to be transformed into Agrobacterium. Positive clones are selected and PCR is used to verify whether the target gene has been successfully transformed into Agrobacterium. The results Figure 3are shown in Figure 4c. As can be seen from the figure, the target gene band is as expected, indicating that the recombinant expression vector carrying the target gene has been successfully transformed into Agrobacterium.

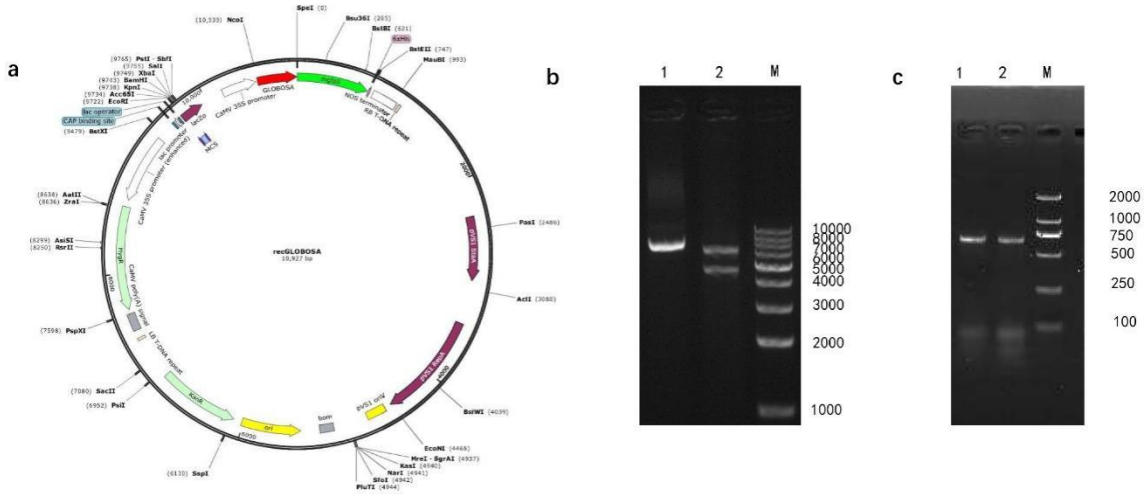


Figure 3 A: Recombinant expression vector containing *GLOBOSA* ; B: Recombinant expression vector enzyme digestion verification ; C: Recombinant expression vector PCR verification.

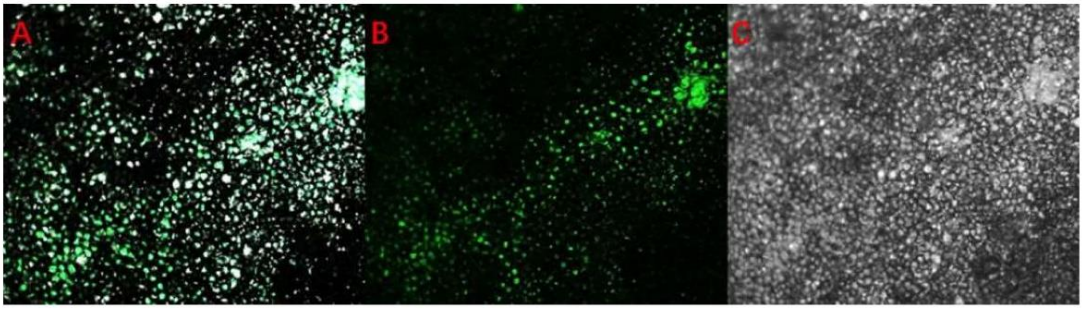
Figure 4 A: Recombinant expression vector containing *GLOBOSA* ; B: Validation of recombinant expression vector

by restriction enzyme digestion ; C: Validation of recombinant expression vector by PCR

1.5 Transient expression and subcellular localization of the target gene

The function of a gene is closely related to the subcellular localization of its encoded product. In the recipient, the reporter gene GFP expressed on the recombinant expression vector emits green fluorescence, which can be located using fluorescence microscopy to determine the subcellular localization of the target gene. When tobacco K326 reached the 6-leaf stage, Agrobacterium tumefaciens with the successfully introduced gene recombinant expression vector was injected into the tobacco leaves. After 36 days... Transient expression results can be observed after h (picture 4) . In Cell- PLoc prediction, the *GLOBOSA* gene is located in the cell nucleus. As can be seen, there is strong fluorescence in the cell nucleus,

indicating that the target gene is subcellularly located within the cell nucleus, consistent with Cell- PLoc prediction.



A：合并图像；B：荧光图像；C：明场。
a：Merged image；b：Fluorescence image；C：Bright-field image

picture 4 Subcellular localization of *GLOBOSA*

Figure5 Subcellular localization of *GLOBOSA*

2 Discussion

investigated the *GLOBOSA gene* in the K326 tobacco line, a male-sterile line with carpel-derived cytoplasm . The gene contains a total of 768... bp, encoding 209 amino acids, the encoded protein is mainly composed of two domains, SRF-TF and K-box, has no transmembrane region, no signal peptide, and is subcellularly located on the cell nucleus.

The formation mechanism of cytoplasmic male sterility is a question worthy of exploration. Cytoplasmic male sterility refers to the inability to produce functional pollen. Although the manifestations differ, they are all maternally inherited, indicating that they are influenced by cytoplasmic genes. Mitochondrial gene rearrangements have been found in all types of cytoplasmic male sterility (Yan et al. , 2022) , which supports this view. Among cytoplasmic male sterility, pollen abortion and anther abortion are types of cytoplasmic male sterility in many crops , while cytoplasmic male sterility caused by stamen degeneration, stamen petalization, and stamen carpelization is another type of sterility caused by the inability to form male organs . Cytoplasmic male sterility lines with stamen carpelization and stamen petalization not only show mitochondrial DNA rearrangements but also show downregulation of class B or C genes. According to the flower ABC model, the interaction between class B genes and class A genes determines petal development, while the interaction between class B genes and class C genes determines stamen development (Yang et al., 2017) . In Arabidopsis , B functional genes also act as transcriptional activators. The GLO/DEF heterodimer directly binds to the CAR -ArG motif, leading to promoter activation (Mahajan and Yadav, 2014) . In tomato, there are four B genes: TAP3 and TM6 from the AP3 branch, and SLGLO1 and TPI from the PI branch. Different B genes have different functions, resulting in

different loss-of-function phenotypes. Loss of TM6 function causes stamen defects, while silencing TPI leads to flower homeomorphism defects (Cao et al., 2019) .

From the perspective of origin, heterocytoplasmic male sterility can be caused by heterocytoplasmic male sterility, cytoplasmic fusion, and natural mutations. Considering heterocytoplasmic male sterility alone, the same cytoplasm under different nuclear backgrounds , exhibiting stable performance across different years and environments, suggests that nuclear gene function is intact. A reasonable explanation is that cytoplasmic genes influence nuclear gene expression. The question then becomes: how do mitochondrial genes affect nuclear gene expression?

Nucleocytoplasmic coordination is a prerequisite for correct gene expression, and cytoplasmic male sterility is one of the consequences of nucleocytoplasmic imbalance. Cytoplasmic genes can only encode a small portion of the proteins they need; most of the required proteins are encoded by nuclear genes. Nucleocytoplasmic imbalance likely occurs when the nuclear genes providing proteins to mitochondria do not encode the proteins required by the mitochondria. The mitochondria then send feedback signals affecting the nuclear genes. Simultaneously, the accumulation of these nuclear proteins becomes toxic to the cell, triggering mechanisms to degrade them. These cellular activities ultimately result in cytoplasmic male sterility. In cytoplasmic male sterility, flower development genes also change, affecting male organogenesis. If overexpression of class B or class C genes restores normal male organogenesis, what will their fertility be like?

In the tobacco carpel-derived cytoplasmic male sterile line K326, transcriptome sequencing revealed not only mitochondrial genome rearrangement but also downregulation of the class B gene *GLOBOSA* . *This study aims to observe* phenotypic and fertility changes in the sterile line K326 by overexpressing the *GLOBOSA gene; the final results await confirmation* by transgenic studies.

3 Materials and Methods

3.1 Test Materials

The tobacco maintainer line K326 used in the experiment was planted in the experimental field of Jiangxi Agricultural University Science and Technology Park. Field management methods for high-quality tobacco production were applied. At the bud stage, K326 flower buds were immediately immersed in liquid nitrogen and stored at -80℃ for RNA extraction. The transient expression material was tobacco K326, which was used for Agrobacterium-mediated transient expression 60 days after sowing, when 5-6 true leaves had emerged .

3.2 Cloning of the tobacco *GLOBOSA* gene

The GLOBOSA gene cloning method followed the method described by Qiu et al. (2021) . TaKaRa The MiniBEST Plant RNA Extraction Kit , PrimeScript™ 1st Strand cDNA Synthesis Kit , and pGME -T Easy vector were purchased from TAKARA. RNA extraction and reverse transcription were performed according to the kit instructions.

GLOBOSA gene amplification were: forward primer : 5′-ATGGGAAGAGGAAAGAT-3′, reverse primer : 5′-TCACTTAAACCATAAGCTAA-3′. The reaction mixture was 2×T5 Super PCR Mix for PAGE 22. μL , forward primer 1 μL , reverse primer 1 μL , template 1 μL . The PCR reaction program was as follows: 94℃ for 5 min pre-denaturation, 94℃ for 30 s denaturation, 58.7℃ for 30 s, 72℃ for 50 s, for a total of 35 cycles, with a final 72℃ for 5 min followed by incubation at 4℃. The amplified products were verified by agarose gel electrophoresis , and the target band was recovered via a low-melting-point agarose gel . The recovered product was ligated into the pGME -T Easy vector , transformed into *E. coli* DH5α , and cultured on LB agar plates containing kanamycin . After 24 h , clones were selected for PCR verification. Positive clones were sent to Kexin Technology Co., Ltd. for sequencing.

3.3 Bioinformatics Analysis

Analyzing the physicochemical properties of gene-encoded proteins can help study gene function and its location. Bioinformatics analysis methods were performed according to those of Qiu et al. (2021) . To understand the physicochemical properties and subcellular localization of the protein encoded by the *GLOBOSA gene,* ProtParam (http://web.expasy.org/protparam/) was used. The physicochemical properties of the protein were inferred ; the domains of the encoded protein were analyzed using the Pfam database (http://pfam.xfam.org/) , and the results were further validated using hmmer (https://www.ebi.ac.uk/Tools/hmmer) ; the hydrophobicity of the encoded protein was inferred using ExPASy (https://web.expasy.org/protscale/) ; the transmembrane region was predicted using TMHMM (https://dtu.biolib.com/DeepTMHMM) ; the signal peptide was determined using SignalP 5.0 (http://www.cbs.dtu.dk/services/) ; and the subcellular localization prediction of the protein was based on Cell- Ploc (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/) .

3.4 Evolutionary Analysis

To investigate the evolutionary relationships of *GLOBOSA* genes among different species, a phylogenetic tree was constructed based on *GLOBOSA homologous protein sequences* , following the method described by Qiu Shanshan (2021) . When studying interspecies relationships, wild tobacco, potato, and tomato, all belonging to the Solanaceae family, were considered first. The model species Arabidopsis and rice were used as references. Furthermore, to trace earlier origins, the earliest angiosperm, *Cinnamomum camphora*, was included. Genomic data were downloaded from NCBI

(https://blast.ncbi.nlm.nih.gov/) and stored locally. A blast search was then performed on the local library using tobacco *GLOBOSA* protein sequences, with a threshold of 1e-10 and a scroe count greater than 200. The obtained sequences were further identified using hmmer (https://www.ebi.ac.uk/Tools/hmmer). A phylogenetic tree was constructed using the maximum likelihood method with IQTREE software, with 1000 bootstrap iterations. Results were presented using Evolview (https://www.evolgenius.info/evolview) .

3.5 Construction of expression vectors

The expression vector was constructed according to the method described by Qiu Shanshan. Plasmid extraction kits, expression vector pCAMBIA1302, and *Nco* I enzyme were purchased from Qingke Biotechnology Co., Ltd. DH5α containing the expression vector was cultured in LB liquid medium (Tryptone 10 g/L, Yeast extract 5 g/L, NaCl 10 g/L) . Six hours later, plasmids were extracted using a kit. The cloning vector containing the target fragment pGME -T Easy was digested with *Nco* I , and the target fragment was recovered . Simultaneously, the expression vector pCAMBIA1302 was digested with *Nco* I , and the target fragment was then ligated into the *Nco* I- *Spe* I multiple cloning site of the expression vector. After transformation into DH5α , the vector was cultured and amplified in LB medium . After 24 hours , the recombinant expression vector was extracted and the ligation of the recombinant vector was verified by digestion with *Nco* I / *BsiWI* .

3.6 Subcellular localization

The subcellular localization method for GLOBOSA was performed according to the method of Qiu et al. (2021) . Competent Agrobacterium GV3101 was purchased from Shanghai Weidi Biotechnology Co., Ltd., and the method was performed according to the reagent instructions . The expression vector was transformed into competent Agrobacterium GV3101 using the freeze-thaw method. After shaking culture at 28 °C for 2–3 h, the Agrobacterium was further cultured on LB solid medium (containing kanamycin and hygromycin resistance). Positive clones were selected and cultured on LB liquid medium (containing kanamycin and hygromycin resistance) at 28 °C with shaking . When $_{\text{the OD600}}$ *reached* 1.0, the bacterial solution was directly injected into tobacco K326 leaves . After 36 h , Olympus fluorescence microscopy was used for detection, examining 6 leaves with 3 fields of view per leaf.

Author's Contribution

Cui Fangfang was the main executor of the experimental research in this study and completed the data processing and writing of the first draft of the paper; Zheng Yun, Deng Lingfan, Yang Xiangfei, Zheng

Jiuzhou , and Meng Linfeng participated in some of the experiments; Liu Qiyuan and Wang Jiange were the conceivers and leaders of the project, and guided the experimental design, data analysis, paper writing and revision. All authors agreed on the final text.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (31960418).

References

Cao X., Liu 2125-2135 .

Chen G. , Ye

Fang C., Li L., He R., Wang D., Wang M., Hu Q., Ma Q., Qin K., Feng X., Zhang G., Fu X., and Liu Z., 2019, Identification of S23 causing both interspecific hybrid male sterility and environment-conditioned male sterility in rice, Rice (NY), 12(1):10. doi: 10.1186/s12284-019-0271-4 .

Yj , Wang Y., Liu QY, Zhou QM, and Zhou W., 2016, Advances in Cytoplasmic Male Sterility in Plant, Zhongguo Nongxue Tongbao (Chinese Agricultural Science Bulletin) , 32 (18) : 70-75 .

Liu ZH, 2019, Establishment of Abortion Regulatory Network and Function Analysis of *TaUGP1-6A* in Isonuclear Alloplasmic Male Sterility Wheat (*Triticum Aestivum* L.), Dissertation for Ph.D. , Northwest A&F University.

Linke B., Nothnagel T., and Borner T., 2003, Flower development in carrot CMS plants: mitochondria affect the expression of MADS box genes homologous to GLOBOSA and DEFICIENS, Plant J, 34(1): 27-37 . doi: 10.1046/j.1365-313x.2003.01703.x.

Mahajan M., and Yadav SK, 2014, Gain of function mutation in tobacco MADS box promoter switch on the expression of flowering class B genes converting sepals to petals., Molecular Biology Reports, 41(2) 705-12 . doi: 10.1007/s11033-013-2909-4.

Qiu SS, Deng LF, Liu MM, Zhang JQ, Wang YB, Liu QY and Wang JG, 2021, Molecular cloning and subcellular localization of *SRK* Gene in *Sinomanglietia glauca* , Acta Agriculturae Universitatis Jiangxiensis, 43(6):1339-1347. (邱珊珊，邓凌帆，刘苗苗，张建强，王炎波，刘齐元，王江革, Cloning and subcellular localization of the *SRK gene of* the rare plant Magnolia grandiflora , Journal of Jiangxi Agricultural University 2021, 43(6): 1339-1347.)

Rijpkema AS, Royaert S., Zethof J., van der Weerden G., Gerats T., and Vandenbussche M., 2006, Analysis of the Petunia TM6 MADS box gene reveals functional divergence within the DEF/AP3 lineage, Plant Cell, 18(8): 1819-1832 .

BM , Chen YZ, Li HB, Mo H., Huang LB, 2019, Progress of Mechanism of Male Sterility of Plants and Its Application, Henan Nongye Kexue (Journal of Henan Agricultural Sciences) , 48 (05): 1-9 .

Wang Q., Zhang X., Lin S., Yang S., Yan X., Bendahmane M., Bao M., and Fu X., 2020, Mapping a double flower phenotype-associated gene DcAP2L in Dianthus chinensis, J Exp Bot, 71(6): 1915-1927.

Wang T., He T., Ding X., Zhang Q., Yang L., Nie Z., Zhao T., Gai J., and Yang S., 2021, Confirmation of GmPPR576 as a fertility restorer gene of cytoplasmic male sterility in soybean, J Exp Bot, 72(22): 7729-7742 .

an HX, Lin CJ, Fan YJ, Zhang CB, 2022, Research Progress on Cloning and Molecular Mechanism of Cytoliasmic Male Sterility Genes in Plants, ZHIwu Shengli Xuebao (Plant Physiology Journal), 58(01): 61-76 （燕厚兴，林春晶，范亚军，张春宝， 2022， 生进 植种霜男死基因衣衣及分子机制研究前进， 植植孔学报， 58(01): 61-76 ）

Yang W., Lou

Yao XF, Dong JJ, Liu SF, Zhang Y., Tang RM, Wang WB, Xie HE, Wu YH, Wu ZX, Li RZ, and Jia XY, 2021, Genome-wide Identification and Tissue-specific Expression Analysis of MADS-box Gene Family in Ipomoea trifida, Jiguang Shengwu Xuebao (Acta Laser Biology Sinica), 30(03): 223-235 (姚旭峰,董静静, 刘世芳, 张毅, 唐锐敏, 王文斌, 解红娥, 吴宇浩, 吴宗信, 贺立恒, 李润植, 贾小云, 2021, Genome-wide Identification and Tissue-specific Expression Analysis of MADS-box Gene Family in Ipomoea trifida, Journal of Laser Biology, 30(03): 223-235)

Zhang YM, 2021, Analysis of Anther Proteomics and Ubiquitinome and Functional Analysis of E3 Ligase TaHUB1 */2* Genes in P-type Cytoplasmic Male Sterility in Wheat, Dissertation for Ph.D., Northwest A&F University .

Treatment of Multifidus Muscle Injury in Posterior Lumbar Spine Surgery: A Comprehensive Review

Huixian Li[1], Keming Xie[2], Shiting Wang[3], Yinuo Wu[1], Xiaona Chen[1], Ziling Weng[2], Jialin Li[2], Tong Liu[1*], Chundi Wen[1*]

[1] Fifth Clinical School of Medicine, Guangzhou University of Traditional Chinese Medicine, Guangzhou 510095, Guangdong, China

[2] Medical College of Jiaying University, Jiaying University, Meizhou 514031, Guangdong, China

[3] The Eighth Clinical Medical College of Guangzhou University of Chinese Medicine, Foshan 528000, Guangdong, China

***Correspondence**

Chundi Wen: Department of Acupuncture and Rehabilitation, Guangdong Second Hospital of Traditional Chinese Medicine, 60 Hengfu Road, Yuexiu District, Guangzhou, Guangdong, China. Email: wen13719261878@163.com

Tong Liu: Department of Acupuncture and Rehabilitation, Guangdong Second Hospital of Traditional Chinese Medicine, 60 Hengfu Road, Yuexiu District, Guangzhou, Guangdong, China. Email: liutongmama@163.com

Abstract Posterior lumbar surgery (PLS) is widely performed to treat lumbar spine disorders; however, it frequently results in iatrogenic injury to the multifidus muscle due to surgical detachment, prolonged retraction, and nerve manipulation, subsequently contributing to postoperative pain, impaired function, and delayed recovery. Accumulating evidence indicates that inflammation plays a central role in the pathophysiological cascade initiated by multifidus injury and is a major contributor to postoperative pain. Accumulating evidence indicates that inflammation plays a central role in the pathophysiological cascade initiated by multifidus injury and is a major contributor to postoperative pain. This review summarizes current findings regarding the mechanisms of multifidus injury and surgery-induced inflammation in PLS, highlights the impact of inflammation on skeletal muscle regeneration, and discusses integrative

therapeutic strategies involving both Western medicine and traditional Chinese medicine. The objective is to provide a mechanistic foundation and therapeutic perspective to optimize perioperative protection of the multifidus muscle and improve clinical outcomes after PLS.

Keywords: Posterior lumbar surgery; Multifidus muscle; Rehabilitation therapy; Muscle satellite cells

Posterior lumbar surgery (PLS) is one of the most frequently employed approaches for treating degenerative lumbar spine disorders. It is primarily indicated for conditions involving lumbar instability, neural compression, and mechanical deformity. For patients with common lumbar diseases, such as lumbar disc herniation, lumbar spinal stenosis, and lumbar spondylolisthesis, in whom non-surgical treatments are ineffective, posterior lumbar surgery is a highly selective option and is considered the first-line treatment[1-2].

Despite its therapeutic benefits, the procedure often involves excessive muscle detachment or retraction, which may cause multifidus ischemia, edema, necrosis, and subsequent atrophy. These pathological changes constitute a major source of recurrent low back pain and postoperative dysfunction. In some cases, these postoperative symptoms can even exceed the preoperative level. This phenomenon is referred to in the literature so-called ‘fusion disease’ [3] or ‘failed back surgery syndrome’ (FBSS)[4]. Multifidus muscle injury is a key factor contributing to poor postoperative recovery and the occurrence of various adverse symptoms in patients following posterior lumbar surgery. However, few studies have systematically summarized the inflammatory mechanisms and integrative treatments specifically targeting multifidus injury following PLS. Therefore, in-depth research on multifidus muscle injury and its subsequent effects holds significant clinical importance. This article will provide a review of the mechanisms of the inflammatory response following multifidus muscle injury in PLS, as well as the treatment approaches, including both traditional Chinese and Western medicine.

1. Anatomy and Function of the Multifidus Muscle

The multifidus muscle lies deep within the paraspinal region and possesses the largest attachment area among the lumbar extensors. It is composed of multiple bundles, originating from the lamina and spinous processes of the lumbar vertebrae and inserting into the dorsal surface of the sacrum. It is well developed in the lumbar region and is considered the primary posterior stabilizing muscle of the spine [5]. The muscle extends from the sacrum to the cervical vertebrae, spanning from the sacrum to the second cervical vertebra, and is more developed in the lumbar and cervical regions. It is the only muscle that spans from the lumbosacral region to the back and is a small, deep intervertebral muscle of the spine. It is located

deep to the semispinalis muscle and is similar in shape to the semispinalis but shorter. Compared with other paraspinal muscles, the multifidus exhibits a unique short-fiber, large-cross-sectional architectural design optimized for segmental stabilization rather than large-amplitude movement. It has a relatively large physiological cross-sectional area but short fiber length, an architectural design that allows the multifidus to generate significant force over a relatively short distance. [6-7]

From an anatomical perspective, the multifidus muscle consists of superficial and deep layers. The superficial layer originates from the spinous processes of the lumbar vertebrae and inserts into the iliac bone or sacrum. The deep layer originates from the lamina of each lumbar vertebra. Except for the deep muscle fibers originating from the lamina of L5, all other fibers extend caudally, crossing two vertebral levels, and insert into the mammillary processes and the joint capsule of the facet joints. The deep fibers originating from the L5 lamina ultimately insert above the sacral foramen between S1 and S2. The multifidus muscle is composed of five groups of muscle fiber bundles. These fibers extend from the lumbar vertebrae caudolaterally to the coccyx, iliac bone, and sacrum. These five groups partially overlap. Compared with fibers originating from lower lumbar vertebrae, those from upper lumbar levels are more superficially located and positioned more laterally. The multifidus muscle has close anatomical relationships with surrounding structures. It lies bilaterally adjacent to the spinous processes and is enclosed within a myofascial sheath formed by the superficial and middle layers of the thoracolumbar fascia. It is also in proximity to structures such as the lamina and longissimus muscle. In terms of origin and insertion, the lumbar multifidus originates from the mammillary processes and inserts into the inferior margins of the spinous processes two to three levels above.

The function of a muscle is determined by the orientation and morphology of its fibers. The multifidus muscle plays a crucial role in maintaining lumbar spine stability. The superficial multifidus originates from the spinous processes and extends inferolaterally to insert into the mammillary processes, posterior sacrum, or iliac crest, generating an inferolateral force that primarily controls spinal flexion and directional movement. In contrast, the deep multifidus primarily regulates intersegmental motion. Morphologically, the multifidus is short with a large cross-sectional area of the muscle belly and a high density of muscle fibers. These structural characteristics enable it to generate substantial force to support spinal stability. Functionally, the multifidus provides compressive force between vertebral bodies. The orientation of its fibers allows it to apply compressive loads on the lumbar spine. Muscle fibers that insert into the iliac and sacral bones contribute to lumbar extension and help resist excessive spinal rotation and translation. Bilateral contraction of the multifidus prevents vertebral rotational dislocation, thus maintaining the lumbar lordotic curvature [8]. Compared with other paraspinal muscles such as the iliocostalis lumborum and longissimus, the multifidus muscle is more closely connected to the lumbar vertebrae and is located nearer to the spinal axis. When the spine is moved by larger surrounding muscles, the multifidus helps ensure that individual vertebrae do not bend or rotate excessively. In particular, the deep layer of the multifidus plays a critical role in controlling intersegmental spinal rotation, while the superficial layer spans multiple vertebral levels and functions to guide spinal motion in an appropriate

direction. This reduces segmental displacement in the lumbar spine, maintains the spine's physiological alignment, and contributes to lumbar stability.

2. Multifidus Muscle Injury and Inflammatory Response Caused by PLS

Multifidus muscle injury commonly occurs in patients undergoing lumbar spine surgery [9]. Radiologically, such injury is typically characterized by muscle atrophy and fatty infiltration, often accompanied by an inflammatory response [10]. Due to its anatomical proximity to the spine, the lumbosacral multifidus is the most severely affected during posterior lumbar surgery (PLS). The primary factors contributing to multifidus injury in PLS include muscle detachment, retraction, and nerve injury.

2.1 Injury Caused by Muscle Detachment

During PLS, subperiosteal detachment of paraspinal muscles disrupts the tendinous attachments and vascular supply of the multifidus, resulting in direct structural and ischemic injury. The procedure typically involves a midline posterior incision, followed by bilateral dissection of the paraspinal muscles to expose the lamina, facet joints, and other anatomical structures necessary for decompression, fusion, and fixation. During this process, the tendinous attachments between the multifidus muscle and the spinous processes or lamina are severed, directly disrupting its original vascular supply and structural integrity. Studies have shown that scar tissue is prone to form at the site of the multifidus postoperatively, with fatty degeneration observed within the muscle, resulting in a reduction of its contractile function [11]. Macintosh et al. [12] reported that creatine kinase levels are significantly higher after posterior lumbar surgery compared to anterior approaches, indirectly reflecting greater multifidus muscle damage. The injury caused by muscle detachment substantially impairs the physiological function of the multifidus and is considered a major contributor to postoperative pain and functional impairment.

2.2 Injury Caused by Retraction

During surgery, the use of self-retaining retractors or laminar retractors to retract the detached multifidus muscle can also cause significant injury. Sustained retraction markedly compromises intramuscular perfusion, and the extent of ischemic damage is strongly correlated with retraction duration. Kawaguchi et al. [13], in a simulated posterior lumbar surgery on pigs, found that retraction of the paraspinal muscles using retractors caused a sharp reduction in local muscle blood flow, with evidence of multifidus necrosis as early as 3 hours postoperatively.

A prospective clinical study by Gejo et al. [14]demonstrated a direct relationship between muscle retraction and muscle injury, particularly in the multifidus. When retraction time exceeded 80 minutes, delayed muscle strength recovery and a higher incidence of postoperative low back pain were observed. In a randomized controlled clinical trial, Kotil et al. [15] reported that relaxing the retraction every 15 minutes for 3 minutes during lumbar discectomy significantly reduced muscle injury. These findings underscore the severity of retraction-induced injury: the longer the retraction time, the greater the damage to the multifidus muscle, which in turn negatively impacts postoperative recovery.

2.3 Nerve Injury

The multifidus muscle is innervated by the medial branch of the dorsal ramus of the lumbar spinal nerve. This anatomical feature makes it particularly susceptible to injury during surgery. During muscle dissection, the medial branch travels along the deep surface of the muscle and is highly vulnerable to damage during surgical manipulation. Once the medial branch nerve is damaged, the muscle undergoes denervation changes postoperatively. In a study by Hodges et al. [16], transection of the medial branch of the dorsal ramus in animal models led to rapid atrophy of the multifidus muscle within a short period. Moreover, the multifidus is innervated by a single nerve branch without communicating branches between adjacent segments, making it more susceptible to denervation-induced atrophy following medial branch injury. This atrophy compromises the normal function of the spine and negatively affects postoperative lumbar mobility and functional outcomes in patients.

3. Inflammatory Response and Skeletal Muscle Regeneration and Repair After Injury

Inflammation orchestrates the early stages of skeletal muscle regeneration; however, excessive or prolonged inflammatory signaling may impair satellite-cell-mediated repair[17-18]. However, excessive inflammation may inhibit the proliferation and differentiation of MSCs and can even exacerbate muscle tissue damage. Although the etiology of skeletal muscle injury may vary, the regenerative process generally follows a consistent pattern, consisting of three overlapping phases: the inflammatory phase, the regeneration and repair phase, and the fibrosis and scar formation phase [19-20].

3.1 Muscle Satellite Cells

In 1961, Mauro was the first to identify the anatomical location and morphological characteristics of MSCs in the tibialis anterior muscle of frogs, laying the foundation for subsequent research on skeletal muscle [21]. MSCs originate from the central portion of the dermomyotome and are undifferentiated mononuclear cells characterized by a large nucleus and relatively scant cytoplasm. Within the cytoplasm,

various organelles can be observed, including free ribosomes, microfilaments, intermediate filaments, microtubule vesicles, and occasionally glycogen granules, Golgi apparatus, rough endoplasmic reticulum, and tendon sheaths. Additionally, small mitochondria are typically present around the nucleus, along with centrosomes arranged in pairs adjacent to the nuclear membrane.

After muscle injury, the activation of MSCs is a prerequisite for muscle regeneration. MSCs reside beneath the basal lamina of skeletal muscle fibers and are myogenic precursor cells with the capacity for proliferation and self-renewal. The repair of injured skeletal muscle primarily depends on the proliferation and differentiation of these cells. Under normal physiological conditions, MSCs remain in a quiescent state; however, under certain stress conditions—such as mechanical loading, exercise, or trauma—they become activated. Once activated, MSCs undergo a cascade of events including proliferation, differentiation, and fusion to form myotubes, which subsequently mature into functional muscle fibers. Following activation, the majority of MSCs differentiate and fuse to form new myofibers, thereby repairing the damaged area. A smaller subset of these cells return to a quiescent state to replenish the satellite cell pool, ensuring the capacity for future regeneration [22].

3.2 Effects of Cytokines on Muscle Satellite Cells

Multiple cytokines are involved in the proliferation and differentiation of MSCs. Cytokines released during the inflammatory response influence the expression of members of the myogenic regulatory factors (MRFs) family. Among them, myogenic differentiation factor (MyoD) plays a pivotal role in muscle cell regeneration and is considered the most critical myogenic regulator during myogenesis. MyoD serves both as a marker of MSCs proliferative activity and as a promoter of early myogenic differentiation, making it a key indicator of muscle regeneration. Paired box protein 7 (Pax7) is recognized as the most important marker protein for identifying MSCs. Co-expression of Pax7 and MyoD is typically observed during the proliferation and early differentiation phases of MSCs [23]. Myogenin (MyoG) is expressed during the fusion of MSCs into myotubes and is essential for skeletal muscle development and maturation. It represents a key factor in terminal muscle cell differentiation. MyoD and MyoG can mutually activate each other and bind to E-box elements within promoter or enhancer regions, thereby inducing the expression of a subset of muscle-specific genes. Inhibition of MyoD or MyoG activity can effectively block the myogenic differentiation process [24]. Myosin heavy chain (MyHC) is a key determinant of muscle fiber type (fast or slow) and exhibits temporally regulated expression during the myogenic differentiation process. It is one of the primary markers used in the study of myogenic differentiation [25]. These myogenic regulatory factors are critical for the differentiation, development, and regeneration of skeletal muscle cells. Changes in their expression levels directly affect the progression of skeletal muscle regeneration [26].

3.3 Effects of Inflammatory Cytokines on Muscle Satellite Cells

Following multifidus muscle injury, an inflammatory response is rapidly initiated, with macrophages acting as key inflammatory cells playing an early role. In the initial phase of injury, macrophages migrate to and accumulate in the injured area under the guidance of chemotactic factors [27], predominantly polarizing into M1-type macrophages. M1-polarized macrophages predominate initially and release pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which stimulate satellite cell activation but may hinder differentiation when excessively sustained [28]. In single-limb perfusion, TNF-α exerts dual effects on blood vessels: on one hand, TNF-α can moderately increase vascular permeability, accelerating the transport and absorption of inflammatory exudates; on the other hand, excessive TNF-α selectively damages endothelial cells involved in angiogenesis, leading to vascular rupture [29].Studies have also shown that TNF-α can inhibit skeletal muscle differentiation under cachectic conditions through the MyoD or caspase pathway, thereby mediating skeletal muscle repair [30]. After interference with IL-6 mRNA, the gene expression of MyoG and α-actin in myogenic cells is also reduced to some extent, suggesting that IL-6 may have potential myogenic activity [31]. Through skeletal muscle cell culture experiments, some researchers have found that activated mononuclear cells can secrete growth factors that stimulate myoblasts to induce the production of IL-6. It is believed that IL-6 secreted by skeletal muscle cells in an autocrine manner can regulate the proliferation of myoblasts and promote the nuclear fusion of myoblasts [32].Double immunofluorescence staining has shown co-localization of IL-6 and Pax7, indicating that muscle satellite cells are capable of producing IL-6 [33].Following tissue injury, the release, transduction, and transmission of inflammatory signals can further activate inflammasomes, stimulating the secretion of IL-1β. A small amount of IL-1β expression can induce an appropriate inflammatory response program, activating specific immune responses and maintaining homeostasis of the injury microenvironment. However, excessive IL-1β expression can expand the scope of the inflammatory response and exacerbate inflammatory injury [34].

With injury resolution, macrophages transition toward an M2 phenotype that secretes IGF-1 and other pro-regenerative factors, promoting tissue remodeling and myofiber maturation. Among them, IGF-1 is a key factor in the resolution of inflammation and macrophage programming during muscle regeneration [35]. It can activate skeletal muscle satellite cells [36], and its overexpression can accelerate the resolution of the inflammatory phase following muscle injury. MSCs are activated and proliferate following skeletal muscle injury, and are capable of differentiating and fusing to generate new myogenic cells. However, TGF-β can inhibit the proliferation and differentiation of MSCs and is involved in the regulation of muscle mass [37]. MRFs promote muscle formation and development through various regulatory mechanisms [38], and TGF-β can modulate the phased proliferation of MSCs by regulating MRFs, thereby reducing transcriptional activity and suppressing skeletal muscle-specific gene expression.

3.4 Relationship Between Extracellular Matrix and Skeletal Muscle Injury Repair

Cells reside within the extracellular matrix (ECM), a complex structure composed of various proteins and polysaccharides. The ECM provides a structural scaffold for cells and tissues, serving as a substrate for cell migration and playing a critical role in cellular signal transduction and responses to pathological stimuli [39].

Inflammatory responses are closely associated with remodeling of the extracellular matrix (ECM) in skeletal muscle. During the injury repair process, the ECM provides structural and biochemical support to muscle cells, with its abundant active molecules serving as a foundation for cellular activities. Transforming growth factor-beta, a multifunctional cytokine, has been demonstrated in studies by Dandan Shi et al. [2,40] to play a role in regulating ECM synthesis and deposition, thereby aiding in the remodeling of skeletal muscle tissue structure to facilitate better functional recovery. Skeletal muscle injury can induce changes in niche signaling, including damage to muscle fibers, which typically affects the basal lamina. This exposes MSCs to altered signals, as the MSC basal lamina can form ECM networks that split from the muscle fiber interstitium [41], impacting the quality of skeletal muscle regeneration and repair.

4. Assessment Methods for Multifidus Muscle Injury after PLS

Currently, there is no dedicated method for independently assessing multifidus muscle injury specifically after PLS. However, common assessment methods for the lumbar multifidus muscle are as follows:

4.1 Ultrasonography

Most current studies focus on morphological changes of the multifidus muscle following treatment of lumbar disc herniation. Zhang Lihong et al. [42] investigated the anteroposterior diameter (A-P), lateral diameter (Lat), and cross-sectional area (CSA) of the multifidus muscle before and after lumbar treatment. Other studies [43] using ultrasonography have found that the CSA, fatty infiltration (FI), and bone mineral density (BMD) of the lumbar multifidus muscle are closely related to the occurrence of adjacent segment disease (ASD). Comparative studies between mature ultrasonography equipment and handheld ultrasound devices have demonstrated that handheld devices also possess reliable measurement capabilities [44]. The widespread application of real-time ultrasound imaging technology has thus become feasible. Moreover, musculoskeletal ultrasound (MSKUS) is a commonly used imaging tool for evaluating the morphology, structure, and function of the multifidus muscle, with advantages including being non-invasive, real-time, dynamic, and high-resolution.

4.2 Magnetic Resonance Imaging (MRI) Assessment

Magnetic resonance imaging (MRI) utilizes magnetic fields and radio waves to generate images of internal body structures. Zhou Min et al. [45] investigated the effect of different postures on the assessment of the multifidus muscle, finding that multifidus degeneration is one of the causes of lumbar disc herniation (LDH), which is significant for studying the etiology and pathogenesis of LDH. In terms of computational methods, image processing software such as ImageJ can be used to quantify CSA of muscles on MRI scans [46]. Clinical studies[47] have shown that multifidus muscle degeneration is most closely associated with lumbar disc herniation, and MRI parameters are commonly used as primary observational indicators. These include measurements of fatty infiltration ratio, multifidus muscle CSA, and vertebral body CSA at the superior endplate levels of L5 and S1 vertebrae to evaluate the degree of postoperative multifidus muscle atrophy [48].

4.3 Other Assessments

In clinical examinations, palpation is commonly used to assess muscle tenderness, tone, atrophy, and to evaluate muscle strength. Electrophysiological assessment is also an important method to evaluate multifidus muscle function, primarily through electromyography (EMG) technology, which provides real-time feedback to guide patients in activating the multifidus muscle during core stabilization training, playing a critical role in rehabilitation [49]. Some studies have reported pathological biopsy analyses of the multifidus muscle to assess muscle fiber types, fatty infiltration, and inflammatory cell infiltration [50], greatly improving the accuracy of evaluation.

5 Treatment of Multifidus Muscle Injury After PLS: Integrative Medicine Approach

5.1 Western Medicine Treatment

5.1.1 Pharmacological Treatment

Pharmacological treatment is one of the commonly used approaches in managing multifidus muscle injury following PLS. Common Western medications include nonsteroidal anti-inflammatory drugs (NSAIDs), muscle relaxants, corticosteroids, and others. These drugs work through different mechanisms to alleviate the various complications arising from multifidus muscle injury and to promote muscle repair.

NSAIDs reduce prostaglandin synthesis and are widely used for managing postoperative inflammatory pain. Ibuprofen, in particular, can achieve analgesic effects by inhibiting or decreasing the production of pain-inducing substances at the affected site [51], which greatly facilitates early intervention for patients experiencing low back pain. However, if the patient’s symptoms do not improve after a period of medication use, discontinuation is recommended to avoid potential adverse effects caused by prolonged or excessive use.

Muscle relaxants are a class of drugs that promote muscle relaxation by acting on the central nervous system and neuromuscular junctions to suppress muscle spasms and reduce muscle tone. Shen Yingchao [52] found that eperisone can effectively reduce muscle tension in patients with low back pain and restore peripheral blood flow, thereby alleviating back pain. However, some patients may experience drowsiness while taking this type of medication [53], and clinical use should be considered with caution.

Corticosteroids possess potent anti-inflammatory effects and can effectively alleviate inflammation in muscle tissues, thereby reducing low back pain. For example, prednisone has demonstrated good efficacy in reducing inflammation and relieving pain. However, despite their strong anti-inflammatory properties, long-term use of corticosteroids may lead to adverse effects such as increased risk of infection, insomnia, and osteoporosis. Therefore, their use should be tailored and adjusted according to the patient's specific condition.

5.1.2 Physical Therapy

Physical therapy primarily refers to treatment modalities that utilize infrared light, electromagnetic waves, and low-frequency electrical currents generated by modern therapeutic devices to irradiate or electrically stimulate the affected area. Clinically common devices include the Thermal Design Power (TDP) electromagnetic spectrum therapy instrument, which emits electromagnetic waves of specific wavelengths. These waves are absorbed by biological tissues with matching absorption spectra in human cells, leading to biological effects through transmission, transformation, and utilization. These effects include enhancing microcirculation, promoting metabolism, inhibiting inflammatory factors, enhancing tissue repair, increasing bioavailability, improving endothelial function, lowering regional nerve excitation thresholds, and increasing sensitivity to pain stimuli, thus exerting a definite analgesic effect on multifidus-related pain [54].Electrical stimulation therapy devices (medium- and low-frequency) apply electrode pads to the affected area. When current is applied, the electrodes emit weak electrical currents of specific frequencies, which can inhibit nerve impulses, relieve local muscle spasms and tension, reduce fascial adhesions, suppress inflammatory cell activity, and reduce the production of inflammatory factors—ultimately relieving pain [55]. Magnetic therapy can alter the magnetic field of local tissues, exerting a calming effect on local nerves, improving microcirculation, reducing cellular immune responses, and thereby achieving pain relief [56].

5.1.3 Exercise Therapy

Also known as core strength training or functional training, exercise therapy in rehabilitation medicine typically involves strengthening the lumbar and back muscles in the affected area. This enhances muscle strength, regulates muscle tone, improves lumbar structural stability, and reduces local nerve sensitivity [57]. Different training focuses are applied at various stages of lumbar muscle injury recovery.

In the early stages, simple activation exercises can be performed, such as diaphragmatic breathing in the supine position, which activates core muscle groups of the abdomen and lower back, including the multifidus, laying the foundation for further rehabilitation. As recovery progresses, the intensity of training

can be gradually increased, incorporating lumbar stability exercises in a standing position, such as single-leg stance or side plank holds, to progressively strengthen the multifidus muscle's power and control, promoting its regeneration and repair.Core strength training can enhance the stability of the lumbar skeletal muscles and restore muscle strength. Targeted functional exercises aimed at the site of injury can help alleviate pain by addressing the underlying muscular deficiencies.

5.2 Traditional Chinese Medicine (TCM) Treatment

5.2.1 Herbal Formula Therapy

In the treatment of multifidus muscle injury following posterior lumbar surgery (PLS), traditional Chinese herbal formulas play a unique role. Feng Ning et al. [58] found that Shen Tong Zhu Yu Tang can improve local blood circulation at the site of injury, promote the dissipation of blood stasis, and relieve pain caused by qi and blood stagnation resulting from multifidus muscle injury. This has a positive effect on improving the postoperative local qi-blood circulation around the multifidus.

Additionally, animal studies have shown that the use of Bu Yang Huan Wu Tang in rats with denervated skeletal muscle fibrosis can improve local blood circulation and nutritional supply, reduce the degree of muscle fibrosis, delay the progression of denervation-induced muscle atrophy, and promote skeletal muscle functional recovery [59].

5.2.2 Acupuncture Therapy

Acupuncture is a representative external treatment method in traditional Chinese medicine. Its efficacy in treating muscle injuries and relieving pain has been widely recognized by clinical practitioners [60]. Acupuncture techniques include filiform needle therapy, electroacupuncture, fire needle therapy, and the gradually evolved acupotomy therapy. Relevant studies have shown that acupuncture has significant clinical effects in treating muscle injuries, and even when used alone, it can effectively alleviate pain—sometimes even achieving acupuncture anesthesia effects [61]. Liu Tong et al. [62]found that the use of "bone-penetrating and muscle-regulating" acupuncture techniques had a good therapeutic effect on multifidus muscle injury after PLS, improving long-term pain outcomes.

In modern times, electroacupuncture has been developed by combining traditional acupuncture with electrical stimulation. Yan et al. [63]found that electroacupuncture at the Zusanli (ST36) acupoint could promote macrophage polarization after skeletal muscle contusion, thereby contributing to the regeneration of contused skeletal muscle. Animal experiments have confirmed that acupuncture can promote the proliferation, differentiation, and repair of satellite cells in the multifidus muscle. In a rat model of lumbar multifidus muscle injury, Xia et al. [64]demonstrated that electroacupuncture facilitates the repair of multifidus muscle cells by regulating mitochondrial function. Zhang Jiayi et al. [65] studied the timing of electroacupuncture intervention in a rat model of multifidus muscle injury and found that electroacupuncture may be most effective when applied 24 hours after injury, as it enhanced the expression

of various muscle proteins. Electroacupuncture also modulates pain transmission pathways and improves local microcirculation, creating favorable conditions for the repair of the multifidus muscle.

5.2.3 Moxibustion Therapy

As recorded in *Lingshu · Guan Neng*, "What cannot be treated by acupuncture may be treated by moxibustion." Moxibustion is believed to have functions such as warming the meridians and dispelling cold, expelling wind and dampness, supporting Yang and preventing collapse, unblocking channels and collaterals, and harmonizing the viscera to balance Yin and Yang. When acupuncture is limited in its application, moxibustion can serve as an effective alternative. As an important modality of acupoint therapy, moxibustion exerts its therapeutic effects primarily through thermal stimulation, neural regulation, and immune modulation. It can activate the body's immune system and enhance its resistance to disease.

Moxibustion therapy has been widely recognized by clinicians for its advantages in treating lumbar muscle injuries, including minimal adverse effects and significant clinical efficacy. Studies have shown that Du Meridian moxibustion can improve the strength of core lumbar muscle groups and enhance mobility in patients with lumbar disc herniation caused by kidney Yang deficiency [66]. Lumbar muscle injuries are primarily associated with inflammatory responses, and moxibustion has demonstrated a distinct advantage in modulating inflammatory cytokines. Hu Xiuwu et al. [67] found that long snake moxibustion in patients with cold-dampness type low back pain may exert its therapeutic effect by reducing TNF-α levels. In addition, Zhenni et al. [68] analyzed that moxibustion effectively reduces the levels of pro-inflammatory cytokines, thereby achieving anti-inflammatory effects, providing theoretical support for its clinical application. Moxibustion also exhibits anti-inflammatory, analgesic, immune-enhancing, and circulatory-improving properties. Basic research has confirmed that moxibustion suppresses the expression of inflammatory factors, reduces cellular autophagy, and promotes cell proliferation [69].

5.2.4 Other Traditional Chinese Medicine Therapies

Other TCM treatments for multifidus muscle injury include cupping therapy, acupoint catgut embedding, bloodletting therapy, auricular acupressure, and health-preserving exercises such as Baduanjin, Tai Chi, and Wuqinxi. These modalities are commonly used as adjunctive therapies. For instance, cupping is often combined with Tui Na (Chinese therapeutic massage), acupuncture is paired with catgut embedding or bloodletting, and patients may be instructed to practice health-preserving exercises alongside their primary treatments. While these methods are frequently employed as complementary approaches, their clinical applications and therapeutic efficacy require further exploration and validation.

6. Discussion and Summary

Growing evidence underscores the essential role of the multifidus in maintaining spinal stability, and its vulnerability to surgical injury has become increasingly appreciated. Currently, although numerous achievements have been made regarding multifidus muscle injury caused by PLS, some deficiencies remain. In terms of injury mechanisms, while studies have identified factors leading to multifidus muscle damage and the impact of post-injury inflammatory responses on skeletal muscle regeneration, the more detailed injury mechanisms are not yet fully elucidated, especially regarding the involved signaling pathways, which require further in-depth investigation.

In summary, the multifidus muscle is indispensable for maintaining spinal stability, and direct injury from PLS is the primary cause of multifidus muscle damage. The factors and specific mechanisms underlying multifidus muscle injury warrant further exploration. Alongside improving and innovating minimally invasive posterior lumbar surgical techniques, efforts should be made to reduce multifidus muscle injury from the outset. On one hand, combining cell-tracing techniques to explore the intrinsic mechanisms of multifidus muscle injury and repair may help identify more effective therapeutic targets; on the other hand, actively conducting multicenter, large-sample, and long-term clinical studies to optimize the current integrative treatment strategies of traditional Chinese and Western medicine is necessary. These measures aim to better address the clinical challenge of multifidus muscle injury after posterior lumbar surgery, improve postoperative patient quality of life, and reduce the incidence of complications.

Abbreviations

PLS	Posterior lumbar surgery
MSCs	Muscle satellite cells
MRFs	Myogenic regulatory factors
MyoD	Myogenic differentiation factor
MyoG	Myogenin
Pax7	Paired box protein 7
MyHC	Myosin heavy chain

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Conflicting interests

The authors declare that there is no conflict of interest.

Funding

Not funding.

Author contributions

All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Acknowledgments

Not applicable.

References

1 Yizhong Ma, Guanyi Liu, Lihua Hu, et al. Research progress of posterior median lumbar spinous process splitting approach[J]. *Chinese Journal of Bone and Joint Injury*, 2024, 39(11): 1171-1174. (in Chinese)

2 Pan D, Liu K, Huang M, et al. Multifidus lesions: A possible pathological component in patients with low back pain after posterior lumbar surgery[J]. *Medicine (Baltimore)* , 2024, 103(9):e37239.

3 Snider RK. A prospective, randomized study of lumbar fusion: preliminary results[J]. *Spine*, 1994, 19(1):109.

4 Daniell JR, Osti OL. Failed Back Surgery Syndrome: A Review Article[J]. *Asian Spine J*, 2018, 12(2):372-379.

5 Bogduk N. Proceedings: the posterior lumbar muscles and nerves of the cat[J]. *J Anat,* 1973, 116:476–7.

6 Ward SR, Kim CW, Eng CM, et al. Architectural analysis and intraoperative measurements demonstrate the unique design of the multifidus muscle for lumbar spine stability[J]. *J Bone Joint Surg Am*, 2009, 91:176–85.

7 Cholewicki J, Panjabi MM, Khachatryan A. Stabilizing function of trunk flexor-extensor muscles around a neutral spine posture[J]. *Spine (Phila Pa 1976)* , 1997, 22:2207–12.

8 Macintosh E, Bogduk N. The biomechanies of the lumbar multifi.dus[J]. *Clin Biomech(Bristol, Avon)* , 1986, 1 (4):205-213.

9 Sun XY, Kong C, Zhang TT, et al. Correlation between multifidus muscle atrophy, spinopelvic parameters, and severity of deformity in patients with adult degenerative scoliosis: the parallelogram effect of LMA on the diagonal through the apical vertebra[J]. *J Orthop Surg Res,* 2019, 14(1):276.

10 Linzer P, Filip M, Šámal F, et al. Comparison of Biochemical Markers of Muscle Damage and Inflammatory Response Between the Open Discectomy, Microsurgical Discectomy, and Microsurgical Discectomy Using Tubular Retractor[J]. *J Neurol Surg A Cent Eur Neurosurg,* 2015, 76(5):384-91.

11 Fan S, Hu Z, Zhao F, et al. Multifidus muscle changes and clini-cal effects of one-evel posterior lumbar interbody fusion: minimallyinvasive procedure versus conventional open approach[J]. *EurSpine J*, 2010, 19(2):316-324.

12 Macintosh E, Bogduk N. 1987 Volvo award in basic science. Themorphology of the lumbar erector spinae [J]. *Spine (Phila Pa1976)* , 1987, 12(7):658-668

13 Kawaguchi Y, Matsui H, Tsuji H. Back muscle injury after posterior lumbar spine surgery[J]. *Spine*, 1996, 21(8):941-944.

14 Gejo R, Matsui H, Kawaguchi Y, et al. Serial changes in trunkmuscle performance after posterior lumbar surgery [J]. *Spine (Phi-la Pa 1976)* , 1999, 24(10):1023-028

15 Kotil K, Tunckale T, Tatar Z, et al. Serum creatine phosphokinaseactivity and histological changes in the multifidus muscle: a prospective randomized controlled comparative study of discectomy with orwithout retraction[J]. *J Neurosurg Spine*, 2007, 6(2):121-25

16 Hodges P, Holm AK, Hansson T, et al. Rapid atrophy of the lum-bar multifidus follows experimental disc or nerve root injury[J]. *Spine(Phila Pa 1976)* , 2006, 31(25):2926-2933.

17 Han WM, Jang YC, García AJ. Engineered matrices for skeletal muscle satellite cell engraftment and function[J]. *Matrix Biol*, 2017, 60-61:96-109.

18 Sacco A, Puri PL. Regulation of Muscle Satellite Cell Function in Tissue Homeostasis and Aging[J]. *Cell Stem Cell*, 2015, 16(6):585-7.

19 Urso ML. Anti-inflammatory interventions and skeletal muscle injury: benefit or detriment?[J]. *J Appl Physiol (1985)*, 2013, 115(6):920-8.

20 Kääriäinen M, Kauhanen S. Skeletal muscle injury and repair: the effect of disuse and denervation on muscle and clinical relevance in pedicled and free muscle flaps[J]. *J Reconstr Microsurg*, 2012, 28(9):581-7.

21 Shinin V, Gayraud-Morel B, Tajbakhsh S. Template DNA-strand co-segregation and asymmetric cell division in skeletal muscle stem cells[J]. *Methods Mol Biol,* 2009, 482:295-317.

22 Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases[J]. *Microbiol Mol Biol Rev*, 2011, 75(1):50-83.

23 Dumont NA, Rudnicki MA. Characterizing Satellite Cells and Myogenic Progenitors During Skeletal Muscle Regeneration[J]. *Methods ol Biol*, 2017, 1560:179-188.

24 Huang YZ, Lai XS, Hu LY, et al. Over-expression ofDECl inhibits myogenic differentiation by modulating MyoG activity in bovine satellite cell[J]. *J Cell Physiol*, 2018, 233(12):9365-9374.

25 Čebašek V, Ribarič S. Changes in local capillarity of pure and hybrid MyHC muscle fiber types after nerve injury in rat extensor digitorum longus muscle (EDL)[J]. *Histochem Cell Biol,* 2019, 152(2):89-107.

26 Zammit PS. Function of the myogenic regulatory factors Myf5, MyoD, Myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis[J]. *Semin Cell Dev Biol,* 2017, 72:19-32.

27 Jianhong Cheng, Li Hong, Shasha Hong, et al. Research progress of macrophages in skeletal muscle injury and regeneration[J]. *Medical Review,* 2021, 27(16): 3125-3129. (in Chinese)

28 Wehling-Henricks M, Welc SS, Samengo G, et al. Macrophages escape Klotho gene silencing in the mdx mouse model of Duchenne muscular dystrophy and promote muscle growth and increase satellite cell numbers through a Klotho-mediated pathway[J]. *Hum Mol Genet*, 2018, 27(1):14-29.

29 Lejeune FJ, Liénard D, Matter M, et al. Efficiency of recombinant human TNF in human cancer therapy[J]. *Cancer Immun*, 2006, 6:6.

30 Song J, Saeman MR, De Libero J, et al. Skeletal Muscle Loss is Associated with TNF Mediated Insufficient Skeletal Myogenic Activation After Burn[J]. *Shock*, 2015, 44(5):479-86.

31 Tomiya A, Aizawa T, Nagatomi R, et al. Myofibers express IL-6 after eccentric exercise[J]. *Am J Sports Med*, 2004, 32(2):503-8.

32 Lee GL, Wu JY, Yeh CC, et al. TLR4 induces CREB-mediated IL-6 production via upregulation of F-spondin to promote vascular smooth muscle cell migration[J]. *Biochem Biophys Res Commun*, 2016, 473(4):1205-1210.

33 Chiche A, Le Roux I, von Joest M, et al. Injury-Induced Senescence Enables In Vivo Reprogramming in Skeletal Muscle[J]. *Cell Stem Cell*, 2017, 20(3):407-414.e4.

34 Yang Y, He J, Suo Y, et al. Anti-inflammatory effect of taurocholate on TNBS-inducedulcerative colitis in mice[J]. *Biomed Pharmacother,* 2016, 81: 424-430.

35 Tonkin J, Temmerman L, Sampson RD, et al. Monocyte/Macrophage-derived IGF-1 Orchestrates Murine Skeletal Muscle Regeneration and Modulates Autocrine Polarization[J]. *Mol Ther,* 2015, 23(7):1189-1200.

36 Lee W, Lee JY, Lee HS, Yoo Y, Shin H, Kim H, Min DS, Bae JS, Seo YK. Thermosensitive Hydrogel Harboring CD146/IGF-1 Nanoparticles for Skeletal-Muscle Regeneration[J]. *ACS Appl Bio Mater,* 2021, 4(9):7070-7080.

37 Ahmad SS, Chun HJ, Ahmad K, et al. The roles of growth factors and hormones in the regulation of muscle satellite cells for cultured meat production[J]. *J Anim Sci Technol*, 2023, 65(1):16-31.

38 Shi LL, Zhu KC, Wang HL. Characterization of myogenic regulatory factors, myod and myf5 from Megalobrama amblycephala and the effect of lipopolysaccharide on satellite cells in skeletal muscle[J]. *Gene,* 2022, 834:146608.

39 Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance[J]. *J Cell Sci,* 2010, 123(Pt 24):4195-200.

40 Shi D, Xiao J, Gu R, et al. Polyurethane conjugating TGF-β on surface impacts local inflammation and endoplasmic reticulum stress in skeletal muscle[J]. *J Biomed Mater Res A*, 2017, 105(4):1156-1165.

41 Brightwell CR, Latham CM, Thomas NT, et al. A glitch in the matrix: the pivotal role for extracellular matrix remodeling during muscle hypertrophy[J]. *Am J Physiol Cell Physiol,* 2022, 323(3):C763-C771.

42 Lihong Zhang, Huixu Wang. Correlation between changes in multifidus muscle morphology before and after electroacupuncture treatment and chronic lumbar disc herniation using ultrasound[J]. *Zhejiang Journal of Traditional Chinese Medicine*, 2025, 60(03): 252-253. (in Chinese)

43 Gong Z, Li D, Zou F, et al. Low lumbar multifidus muscle status and bone mineral density are important risk factors for adjacent segment disease after lumbar fusion: a case-control study. *J Orthop Surg Res*. 2022 Nov 16;17(1):490.

44 Elliott-Burke T, Dillon T, Bailey J, et al. Lumbar multifidus muscle ultrasound imaging: Is handheld technology reliable? *Musculoskelet Sci Pract*. 2023 Jun;65:102771.

45 Min Zhou, Na Su, Chunli Yan, et al. Study on MRI examination of erector spinae and multifidus muscles in different positions in lumbar disc herniation[J]. *Journal of Clinical Radiology,* 2024, 43(03): 426-431. (in Chinese)

46 Osterhoff G, Asatryan G, Spiegl UJA, et al. Impact of Multifidus Muscle Atrophy on the Occurrence of Secondary Symptomatic Adjacent Osteoporotic Vertebral Compression Fractures. *Calcif Tissue Int.* 2022 Apr;110(4):421-427.

47 Wei Wang, Guanghui Chen, Chunyan Ling, et al. Correlation between MRI indicators and clinical indicators of multifidus muscle degeneration in patients with unilateral lumbar disc herniation[J]. *Chinese Journal of Rehabilitation Medicine*, 2024, 39(11): 1594-1599.

48 Ahn JS, Lee HJ, Park EJ, et al. Multifidus Muscle Changes After Biportal Endoscopic Spinal Surgery: Magnetic Resonance Imaging Evaluation. *World Neurosurg*. 2019 Oct;130:e525-e534.

49 Lothe LR, Raven TJL, Sandbæk G, et al. Single-motor-unit discharge characteristics in lumbar multifidus muscle of acute low back pain patients. *J Neurophysiol*. 2019 Oct 1;122(4):1373-1385.

50 Chen X, Hodges PW, James G, et al. Do Markers of Inflammation and/or Muscle Regeneration in Lumbar Multifidus Muscle and Fat Differ Between Individuals with Good or Poor Outcome Following Microdiscectomy for Lumbar Disc Herniation? *Spine (Phila Pa 1976)*. 2021 May 15;46(10):678-686.

51 Bo Xuan. A comparative study of the efficacy of cupping and dexibuprofen in the treatment of nonspecific low back pain[J]. *Journal of Anhui Health Vocational and Technical College,* 2010, 9(01): 19-21. (in Chinese)

52 Yingchao Shen, Yixin Shen. Effect of eperisone on paraspinal muscle blood flow in chronic low back pain: a randomized controlled observation[J]. *Chinese Journal of Tissue Engineering Research and Clinical Rehabilitation*, 2009, 13(07): 1293-1296. (in Chinese)

53 Wannian Liang. Neck, shoulder, waist and back pain[J]. *Continuing Medical Education*, 2005(03):18-24. (in Chinese)

54 Lixia Yuan, Yue Sun, Chenghua Ding, et al. Exploring the biological effects of far infrared ray in treating chronic pain based on the theory of warming meridians and unblocking collaterals in traditional Chinese medicine [J]. *World Science and Technology - Modernization of Traditional Chinese Medicine*, 2022, 24(01): 283-288. (in Chinese)

55 Huiting Chen. Study on the clinical value of computer low-medium frequency massage therapy device in treating traumatic low back pain[J]. *Primary Medicine Forum*, 2021, 25(26): 3846-3847. (in Chinese)

56 Chaoxue Ren, Daping Qiang, Menggang Sang. Observation on the therapeutic effect of manipulation and magnetic field in treating lumbar muscle strain[J]. *Shaanxi Traditional Chinese Medicine*, 2014, 35(09):1182-1184. (in Chinese)

57 Manyi Jiang, Simao Xu, Enming Bin. Core strength training and electroacupuncture intervention have a synergistic therapeutic effect on lumbar muscle strain in college students[J]. *Chinese Journal of Tissue Engineering Research*, 2019, 23(27): 4315-4320. (in Chinese)

58 Yu Feng, Dongqing Xie. Analysis of the efficacy of Shentong Zhuyu Decoction in reducing pain caused by acute skeletal muscle injury and its effect on serum inflammatory factors[J]. *Chinese Journal of Health Care Medicine*, 2024, 26(04): 511-513. (in Chinese)

59 Hongwen Liu, Jie Xu, Li Yin, et al. Effects of Buyang Huanwu Decoction on skeletal muscle fibrosis and TGF-β1/Smad3 signaling pathway in denervated rats[J]. *Chinese Journal of Gerontology*, 2023, 43(07): 1655-1659. (in Chinese)

60 Hongna Yin, Jianuo Li, Quan Li, et al. The development, inheritance and innovation of traditional Chinese medicine acupuncture. *Chinese Journal of Traditional Chinese Medicine*, 2019, 34(10): 4467, 4470. (in Chinese)

61 Dandan Zhao, Chenglin Tang, Siqin Huang, et al. Effects of electroacupuncture intervention on muscle satellite cell differentiation and muscle fiber type transformation in denervated muscular atrophy rats[J]. *Acupuncture Research*, 2019, 44(01): 37-42. (in Chinese)

62 Tong Liu, Yue Liu, Weichuan Kuang, et al. Clinical efficacy of bone-piercing and tendon-regulating acupuncture in the treatment of lumbar spine surgery failure syndrome[J]. *Chinese Journal of Traditional Chinese Medicine*, 2018, 36(12): 2914-2917. (in Chinese)

63 Yan M, Wang R, Liu S, et al. The Mechanism of Electroacupuncture at Zusanli Promotes Macrophage Polarization during the Fibrotic Process in Contused Skeletal Muscle[J]. *Eur Surg Res*, 2019, 6:1-12. (in Chinese)

64 Li X, Zhao J, Lv Q, Tian Y, et al. Electroacupuncture alleviates multifidus muscle injury by modulating mitochondrial function and Ca2+ uptake[J]. *Anat Rec (Hoboken)*, 2023, 306(12):3060-3072. (in Chinese)

65 Jiayi Zhang, Tong Wang, Bai Yuzhuo, et al. Effect of electroacupuncture intervention timing on the expression of Foxo1, Myostatin and Myod proteins in rats with lumbar multifidus muscle injury model[J]. *World Chinese Medicine*, 2019, 14(03): 598-601+607. (in Chinese)

66 Haizhu Fan. Observation on the efficacy of lumbar core muscle rehabilitation combined with Governor Vessel moxibustion on patients with lumbar disc herniation due to kidney yang deficiency syndrome[J]. *Chinese Journal of Metallurgical Industry Medicine*, 2022, 39(01): 22-24. (in Chinese)

67 Xiuwu Hu, Chenying Deng, Hui Huang, et al. Effects of long snake moxibustion on pain symptoms and serum IL-1β and TNF-α levels in patients with cold-dampness type low back pain[J]. *Modern Distance Education of Traditional Chinese Medicine*, 2020, 18(10): 92-95. (in Chinese)

68 Zhao Z, Li J, Wen J, et al. Effect of Moxibustion on Inflammatory Cytokines for Low Back Pain: A Systematic Review, Meta-Analysis and Meta-Regression[J]. *Ther Clin Risk Manag*, 2023, 19:811-827.

69 Li Q, Wang W, Ma Q, et al. Moxibustion Improves Chronic Heart Failure by Inhibiting Autophagy and Inflammation via Upregulation of mTOR Expression[J]. *Evid Based Complement Alternat Med*, 2021, 2021:6635876.

Review:Synthesis and metabolism of tea aroma compounds

Long Yuanfu[1]，He Yufan[1]，Wu Siyu[1]，Mo Shiqin[2]，Zhang Zaibao[1,*]

1 School of Life and Health Sciences, Huzhou University, Huzhou, Zhejiang, China

2 Changxing Bai Suiye Tea Industry Co., Ltd., Huzhou, Zhejiang, China* Corresponding author: Zhang Zaibao,zaibaozhang79@163.com

Synthesis and metabolism of tea aroma compounds

Abstract tea aroma is an important index of tea quality evaluation, and its formation mainly derives from the metabolites formed in the process of tea growth and processing. These metabolites mainly include phen olic compounds, amino acids and aromatic compounds. The type and content of these compounds are very important for the formation of tea aroma type, but also an important factor affecting the quality of tea. Both the preharvest growth and postharvest processing of tea affected the formation and metabolism of tea arom a. Biological stresses
(tea garden pests, pathogens) and abiotic stresses (light, temperature, mechanical damage) have important effects on the synthesis and release of aroma substances.

keywords tea aroma; volatile compounds; stresses

1 Composition and content of aroma substances in tea

The content of aroma substances in tea is very low, but there are many types, including volatile fatty acid derivatives (VFADs), volatile terpenes (VTs) and volatile phenylpropionic acid/benzene ring compounds (VPBs) [1]. Volatile aromatic substances, such as alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, phenols and nitrogen, oxygen and sulfur compounds (Table 1), account for 0.03% to 0.05% of the dry matter mass of tea, and are the main factors affecting the aroma of tea [2]. According to different chemical structures and characteristics, alcohols can be divided into aliphatic alcohols, aromatic alcohols and terpene alcohols. Among them, aliphatic alcohols account for 12.76% of the aromatic oil in the dry matter of tea after processing, accounting for about 60% of the aromatic oil content of fresh tea leaves (these substances have low boiling points and will evaporate in large quantities with water vapor during tea processing). In oolong tea, the content of nerolidol was as high as 25.06% [3]. Aldehydes account for about 3% of the aromatic oil in fresh tea leaves and about 10.30% of the aromatic oil in dried tea leaves, meaning that the content of aldehydes in finished tea is higher than that in fresh tea leaves. Ketones account for 15.35% of the aromatic substances in tea. There is no significant difference in the content of ketone compounds among different types of tea, meaning that ketones are commonly present in tea and are not the main factor causing significant differences in the aroma of various types of tea [3]. Esters and lactones account for 12.44% of the aromatic substances in tea. Nitrogen-containing compounds (pyridine, indole, etc.) are components with roasted aroma produced through thermophysical and chemical processes [4], accounting for about 13.41% of the aromatic substances in tea. The remaining components exist in trace amounts in volatile aromatic substances, but the role of these substances in the formation of tea aroma is not negligible.

	Main ingredients	content
alcohols	(Z)-3-Hexen-1-ol , benzyl alcohol, phenylethanol, phenylpropanol, linalool, geraniol, nerol, Vanillyl alcohol , nerolidol	12.76%
aldehydes	acetaldehyde, n-pentanal, n-butyraldehyde, hexenal, benzaldehyde, cinnamaldehyde, neraldehyde, vanillin	10.30%
ketones	acetophenone, α-ionone, β-ionone, jasmone, theanone	15.35%
esters	geraniyl acetate, vanillyl acetate, linalyl acetate, neryl acetate, phenylacetic acid methyl ester, methyl salicylate, methyl anthranilate	12.44%
lactones	jasmine lactone, dihydroanionolide	

nitrogen compounds	indole, pyridine, quinoline, 2,5-dimethylpyrazine, 2-formylpyrrole, phenylacetonitrile	13.41%
carboxylic acids	acetic acid, propionic acid, isovaleric acid, butyric acid, isobutyric acid, salicylic acid, palmitic acid	
phenols	2-ethylphenol, 4-ethylguaiacol, eugenol, thymol	
oxygen- and sulfur-containing compounds	anethole, anethole, 2-ethylfuran, 1,1-dimethoxyethane, dimethyl sulfide, thiophene, 2,5-dimethylthiazole, benzothiazole	

Table 1. Composition and content of volatile aromatic substances in tea aroma

2. The Relationship Between Tea Aroma and Tea Quality

Among the color, aroma, and flavor of tea, aroma is one of the most important factors affecting tea quality. The combined effect of the types and contents of volatile aromatic substances in tea is the key to restricting the aroma quality of tea [5]. The precursor substances of tea aroma determine the characteristics and properties of aroma, including aromatic hydrocarbons and their oxides, terpenes (monoterpenes and sesquiterpenes), amino acids, carotenoids, sugars, etc. in fresh tea leaves, as well as enzymes necessary for the formation of aroma during processing [6-7]. The unique aroma types formed by the combination of different aromatic substances in different proportions are one of the characteristics of the aroma of various teas. The types and proportions of substances that make up the aroma of the six major tea categories are different, and their aroma characteristics are also different. For example, the main aroma components of green tea are chlorophyll, linalool and its oxides, indole, etc. Fresh tea leaves are processed through methods such as fixation (pan-frying or steaming), rolling, and drying (or baking, sun-drying) to form the floral and fruity aroma and roasted aroma of tea [2,8]. Black tea is a fermented tea and generally has floral and fruity aroma. The main aroma components are geraniol, benzyl alcohol, linalool and its oxides, etc. Some of these are originally present in fresh tea leaves, while others are formed by precursor substances (esters, terpenes, etc.) during processing [9-10]. Fresh black tea leaves undergo four basic processes: withering, rolling, fermentation and drying. The changes in various components are very complex, such as polyphenol compounds. The degradation and oxidation of substances eventually form the unique red soup and red leaves, sweet and mellow aroma of black tea [11]; Oolong tea is a semi-fermented tea, and neroli is the characteristic component of oolong tea aroma [12]; Black tea has different characteristic aromas depending on the place of origin, such as Hunan Xiangjian with pine resin, Sichuan Nanlu

Biancha with oily aroma, and Yunnan Pu'er with agarwood aroma [13]; Yellow tea converts sugar into caramel under dry heat, and low-boiling-point aromatic substances will volatilize or isomerize at higher temperatures, forming a fresh aroma; White tea is a lightly fermented tea, and the abundant white hairs on the surface are an important factor that gives it aroma quality characteristics, constituting the sweet and pure aroma of white tea [14]. The different composition and proportion of aroma substances between different tea varieties, as well as the differences in processing methods, form the representative characteristic aromas between different teas. Even among the same type of tea, the difference in aroma will affect the quality of the tea. Therefore, the aroma characteristics of tea determine the quality of tea and meet different needs.

3. Tea aroma formation and metabolism

Volatile fatty acid derivatives (VFADs), volatile terpenes (VTs), volatile carotenoid derivatives and volatile phenylpropionic acid/benzene ring compounds (VPBs) are characteristic aromatic compounds commonly found in tea trees[1]. There are also some non-aromatic substances (such as amino acids) that play an important role in the formation of tea aroma substances. The metabolic changes of these compounds during the pre-harvest (tea growth process) and post-harvest (processing) process are key factors in the formation of tea aroma[6].

Aroma substances of tea

Most fatty acid derivatives are aldehydes and esters, while unsaturated fatty alcohols (such as quinol) are abundant in fresh tea leaves. Quinol and its precursor quinol not only contribute to the grassy aroma of tea, but quinol also provides precursors for the formation of other aroma compounds. Fatty acid metabolism in tea mainly occurs through two pathways: enzymatic reactions and auto-oxidation. Unsaturated fatty acids are catalyzed by lipoxygenases to generate peroxides, which are then catalyzed by peroxidases to form quinol and aldehydes. Finally, dehydrogenases catalyze the reduction of aldehydes to the corresponding alcohols (Figure 1).

图1. 青叶醛、青叶醇的生物合成

Fig 1. Biosynthesis of trans-2-hexenal and (Z)-3-hexene-1-ol leaf

Terpenes are an important class of volatile aroma components in tea and belong to hydrocarbons. The terpenes present in tea trees are mainly monoterpenes and sesquiterpenes, which often exist in the form of glycosides (such as linalool and nerol), thus reducing their volatility. Therefore, under normal circumstances, they do not produce aroma in fresh leaves[15]. There are two pathways for the biosynthesis of terpenes: the mevalonate pathway (MVA) and the methyl erythritol phosphate pathway (MEP)[18]. Both of these pathways can produce isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which further synthesize terpenes. The key enzyme in the MVA pathway is 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), which catalyzes the formation of mevalonic acid for further synthesis of IPP; 1-deoxy-D-xylitol-5-phosphate reductase (DXR) catalyzes the formation of 2-C-methyl-D-erythritol-4-phosphate (MEP), which is also a key rate-limiting step in the MEP pathway [16-17].

In the shikimic acid pathway, volatile phenylpropionic acid/phenyl ring derivatives can be generated through a series of processes. In tea, VPBs are also an important class of aroma compounds, such as benzyl alcohol and phenylethyl alcohol, which can give tea a "fruity" aroma. The biosynthesis of these substances is regulated by the level of precursor substances (such as phenylalanine), but their biosynthetic pathways and corresponding formation mechanisms still need further research to clarify.

Carotenoids directly participate in the composition of tea aroma. The oxidative degradation of carotenoids in tea mainly occurs during processing and storage [19], and the formation of tea aroma depends on its transformation products. The oxidative degradation of carotenoids in tea is carried out through two pathways: biodegradation and physical degradation. Its degradation products are mainly C9-C13 isoprene aroma substances [20]. Among them, the degradation products of C13 include β-ionone, β-damascene, etc. [22], and these substances have very low thresholds in aqueous solution [21]. Using gas chromatography-olfaction determination and aroma extract dilution analysis, Schuh et al. analyzed the main aroma substances of Darjeeling black tea and found that the aroma threshold of β-damascene was as low as 0.004 μg/L [22]. Although these volatile substances are present in very low amounts in tea, they have a unique influence on the aroma quality of tea. Therefore, it is believed that β-damascene plays a very important role in the formation of the aroma of Darjeeling black tea.

In addition to some aromatic substances participating in the formation of tea aroma, some non-aromatic substances (such as amino acids) can also undergo Strecker degradation and Maillard reaction under heat to transform into aromatic substances, or react with sugars to produce caramel aroma substances [15, 23]. Catechins in tea trees can produce ortho-quinones through oxidation, and amino acids formed after protein hydrolysis can react with them to produce a similar apple aroma. Sugars can also emit a sweet aroma or roasted chestnut aroma during the roasting process of tea [12].

Multiple reactions involved in the formation of tea aroma

Tea processing involves various types of reactions: oxidative degradation of carotenoids and fatty acids, carbonyl-amine reaction (Maillard reaction [23]), caramelization, glycoside hydrolysis and microbial fermentation. The products formed by these reactions constitute important components of tea aroma. Ionone series compounds can participate in the formation of tea aroma and can be formed by the degradation of carotenoids during tea processing. Through oxidative degradation, carotenoids can produce a series of aroma substances, including β-ionone, β-damasone, tea spirone and their oxidized derivatives. These are compounds with floral and fruity aromas and are key aroma substances in oolong tea and black tea [24-25]. Fatty acid substances can lead to the breakage of fatty acid chains through enzymatic oxidation or non-enzymatic oxidation pathways. The degree and rate of oxidation are related to the degree of unsaturation of fatty acids [23]. For example, unsaturated fatty acids undergo a series of enzymatic reactions to eventually form corresponding alcohols, which can then participate in the formation of tea aroma. The Maillard reaction is common in food processing and is also a common browning process in tea drying. During tea processing, amino and carbonyl groups undergo Maillard reactions at high temperatures. Many heterocyclic compounds, such as furan, pyrrole, thiophene, and their derivatives, are also mainly derived from Maillard reactions [23]. Sugars can undergo caramelization at high temperatures. Maillard and caramelization reactions during tea processing can promote the formation of pyrazine and furan derivatives, which in turn can give tea a roasted aroma. Terpenoids and linalools in tea are bound aroma compounds. These two types of compounds are mainly present in the form of glycosides in fresh tea leaves [26], and can participate in the formation of alcoholic aromas in tea. In addition to bound compounds, alcoholic aromas are also formed from free compounds, but bound compounds contribute more to alcoholic aromas. Glycoside aroma precursors can be hydrolyzed by glycosidases, thereby releasing the corresponding alcoholic aromas [27]. In addition, acid hydrolysis can also effectively hydrolyze glycoside aroma precursors and release volatile aglycones[28]. The aglycones released by acid hydrolysis and enzymatic hydrolysis can be detected by gas chromatography (GC) and GC-mass spectrometry (GC-MS) to infer the types and contents of glycosides[29]. Microbial fermentation mainly occurs in the production process of black tea, and it can produce characteristic aroma substances. Taking the pile fermentation process of Pu'er tea as an example, this process uses sun-dried green tea as raw material. Various extracellular enzymes secreted by microorganisms can lead to a series of reactions, which change some chemical components in the tea leaves and form quality characteristics with aged aroma and bright red and yellow liquor.

Aliphatic alcohols, aromatic alcohols, and terpenoids are important components of tea aroma. A small portion exists in free form, while the vast majority are stored in cells by combining with monosaccharides and disaccharides to form glycosides. Because glycosides have stronger water solubility and weaker activity than their free glycoside ligands, aromatic compounds in tea usually accumulate in glycoside form. Glycosidases participate in the formation of alcohol-based aromas in

tea. The isolation, purification, and characterization of glycosidases can help study the formation mechanism of alcohol-based aroma components. Glycoside aroma precursors (primrose glycosides, glucosides) can be hydrolyzed by β-glucosidases (β-primrose glycosidase, β-glucosidase) to release various free volatile aroma substances [6-7]. To date, through the separation and identification of aroma substances in tea, a variety of monoterpene alcohol glycosides and aromatic alcohol glycosides have been discovered. They are mostly present in the form of disaccharides and monosaccharides. Among them, the disaccharides are mostly β-primrose glycosides, and the monosaccharides are mostly β-glucosides[27]. The content of β-primrose glycosides is relatively high. In fresh tea leaves, glycosidases and their substrates are compartmentalized by cells and do not react. Due to a series of reasons (such as picking, mechanical damage, insect feeding, and pathogen infection), endogenous glycosidases in tea leaves bind to their substrates, and glycoside aroma precursors are hydrolyzed, releasing volatile aglycones[6], thus exhibiting aroma characteristics.

Besides glycoside hydrolysis, many aromatic compounds are formed through resynthetic pathways. In the past decade, most of the relevant genes have been cloned and their functions verified in vitro. In 2002, Mizutani et al. [29] isolated and purified β-primrose glycosidase from fresh tea leaves. Based on its partial amino acid sequence, they designed degenerate primers and cloned the β-primrose glycosidase sequence from tea leaves. The enzyme gene was introduced into Escherichia coli for expression. The results showed that the enzyme sequence had 50%-60% similarity to the β-primrose glycosidase sequence in other plants. The enzyme could hydrolyze β-primrose glycosides in vitro to generate primrose sugar and the corresponding ligand, but could not act on 2-phenylethyl-β-D-glucoside. This result indicates that β-primrose glycosidase can only specifically recognize β-primrose glycosides and only hydrolyzes the glycosidic bond between primrose sugar and the ligand.

4. The Influence of Post-Harvest Processing Stages on the Aroma Composition of Tea

According to the different processing and manufacturing methods of tea, it canbeividedinto six types, including non-fermented green tea, slightly fermented white tea, semi-fermented oolong tea, fully fermented black tea, post-fermented yellow tea, and dark tea[30]. Tea is subjected to multiple stresses (damage, low temperature, water loss, etc.) during the production process. For example, the production of black tea requires seven steps: indoor withering, rolling, breaking up clumps, replenishing fermentation, drying, refining and baking. Among them, the withering process requires attention to time, temperature, light and other conditions. Low temperature (26℃) withering is conducive to aroma formation [31]. Shortening the withering time is not conducive to increasing the activity of enzymes (polyphenol oxidase, β-glucosidase), which to some extent inhibits the formation of tea aroma substances [31-32]. During the fermentation process, polyphenol oxidase is

released from the epidermal cells of the leaves and encounters polyphenol compounds to produce an enzymatic reaction [33]. It is also accompanied by the degradation of carotenoids, oxidation and reduction of catechins, decarboxylation of amino acids and decomposition of fatty acids [1]. The synthesis of indole is terminated. After refining and baking, the quality of tea is purified, the leaf color is reddish-brown, and the grassy smell is transformed into a sweet taste and floral and fruity aroma. The production process of oolong tea is relatively complex. It involves a series of processing steps, including withering (sunlight or hot air), indoor resting, stirring, pile fermentation, fixation, initial rolling, initial drying, rolling, breaking up clumps, and drying. The leaves rub against each other, leaf cells are damaged, moisture is released, enzyme activity is increased, internal contents are transformed, and the formation of aroma substances (nerolidol, geraniol, linalool and their oxides) is promoted[34]. The grassy smell is removed, and a high aroma is formed, which is fragrant. The stress involved mainly includes mechanical damage from stirring and fixation, water loss from withering and ultraviolet radiation[1]. Damage stress forces water to evaporate, promotes a series of enzymatic reactions, and is a key factor affecting the aroma formation of oolong tea[35]. In addition, low temperature stress is also an important factor inducing the formation of various aroma components in tea. The dual stress (damage and low temperature) not only regulates the formation of volatiles, but also has a synergistic effect[36]. Green tea is processed through withering, fixation, rolling, and drying after picking to produce finished tea, which forms a green aroma and floral aroma[37]. During the withering stage, green and floral aroma compounds accumulate in large quantities[37], which may be related to the large amount of abscisic acid induced by dehydration stress during the withering process[38]. The formation of floral and green aroma in green tea is not only related to the withering stage, but also affected by the fixation and drying stages. The manufacturing process of white tea is relatively simple. White tea is processed by picking tender green leaves, withering indoors, piling, and drying to form a unique downy aroma. During the withering process of white tea, the components contained in the fresh leaves undergo a series of enzymatic and chemical reactions, such as the degradation of glycosides, the peroxidation and degradation of fatty acids, resulting in the generation of a large amount of aroma substances[39].

5. Stress and the synthesis of tea aroma

Tea leaves are subject to various stresses before and after picking (Figure 2), such as climate conditions, soil factors, feeding by leaf-eating pests and infection by pathogens. These stress factors promote the formation, accumulation and release of aroma compounds in tea leaves. On the one hand, the accumulated volatile aromatic substances are conducive to improving the aroma quality of tea leaves, and on the other

hand, they are also conducive to the generation of stress response mechanisms in tea trees [1].

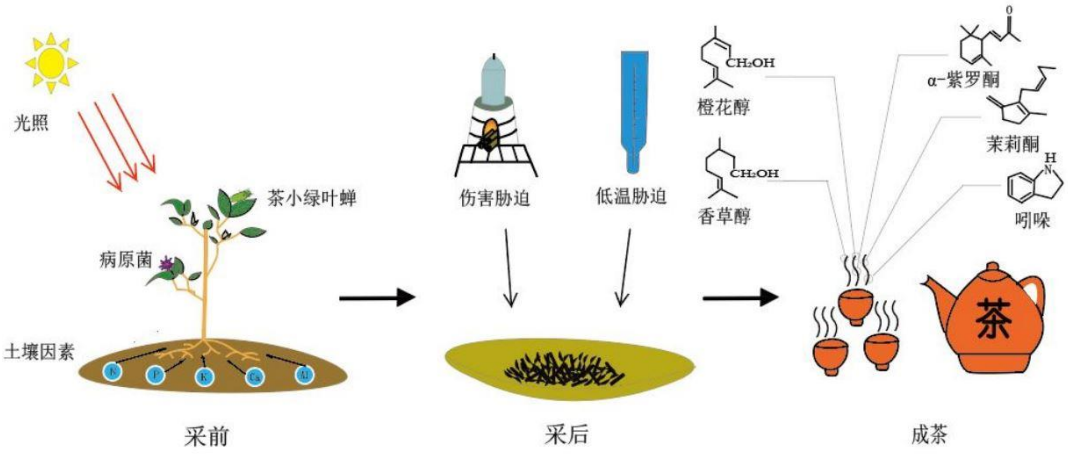


图 2. 胁迫与香气的关系

Fig 2. Relationship between stress and aroma

The effects of biological stress on tea aroma formation

Under normal conditions, most volatile compounds exist in plants primarily in liquid form [40]. When plants are subjected to biological stresses such as those from the external environment, they usually synthesize and release some volatile substances, which may have the function of mitigating the negative effects of stress on plants. Biological stress is particularly important for the formation and release of plant volatiles. During the growth of tea trees, some tea garden pests (such as tea green leafhoppers, tea geometrid moths, tea aphids, tea leafrollers, etc.) will attack tea trees [41]. In addition, some tea diseases such as tea anthracnose, leaf blight, and leaf swelling will also affect the yield, appearance, and quality of tea. These biological stress factors will lead to the production of aroma substances such as linalool, geraniol, α-farnesene, and indole in tea.

Attacks by the green leafhopper [1,42] can increase the release of (S)-linalool in tea leaves, and damage caused by continuous feeding is a key factor in the formation and release of (S)-linalool induced by green leafhopper attacks. In addition, green leafhopper feeding can also induce the release of some monoterpenes, such as 2,6-dimethyl-3,7-octadien-2,6-diol (diendiol I). The production of Diendiol I is a marker of green leafhopper infestation of tea leaves. Plant hormones, especially jasmonic acid, are involved in the formation and release of volatile substances in plants. Since damage stress from some tea garden pests can enhance the release of jasmonic acid in tea leaves, the formation of diendiol I may depend on jasmonic acid signaling [36].

Infection by pathogens can also affect the formation of tea aroma. For example, tea infected with tea anthracnose[43] has increased activity of phenylalanine ammonia-lyase and activated lipoxygenase metabolic pathway, which ultimately leads to the formation and release of volatile substances[44]. Tea cake disease is a low-temperature and high-humidity disease[45]. When tea trees are infected with tea cake disease, the content of some tea volatiles (terpenes, aromatic compounds, etc.) increases significantly. These substances have a certain inhibitory effect on the infection of pathogens[1].

The effects of abiotic stress on tea aroma formation

Not only do biological factors promote the formation of tea aroma, but many more abiotic factors can positively regulate tea aroma before and after harvest. In the pre-harvest stage, abiotic factors such as soil, temperature, light, and mechanical damage during processing can lead to a variety of plant responses.

Tea trees are perennial deep-rooted plants, and their growth is adaptable to a wide range of soil textures. They are generally planted in loose, well-drained sandy soil [46]. However, they have high requirements for soil acidity and alkalinity. Tea trees prefer acidic soil, and their soil pH range is mostly between 4.0 and 6.5. If the soil is too acidic and the pH is below 4.0, it will cause the tea seedlings to be infected with hydrogen ion poisoning, causing the tea leaves to turn from green to dark and then to red. In severe cases, it will lead to the death of the tea tree. If the soil is too alkaline, i.e., the pH is above 6.5, it will cause the tea leaves to turn yellow and be infected with chlorosis. In severe cases, it will cause the roots to turn black, thus leading to decay and death. pH mainly affects the aroma quality of tea by influencing the absorption of elements such as nitrogen, phosphorus, potassium, aluminum, and calcium by the roots of the tea tree. For example, when potassium is rich [46], it is beneficial to regulate water evaporation and can also increase the content of flavor and aroma components in tea.

The growth and development of tea trees also have certain requirements for temperature. Temperature restricts the growth rate of tea trees. It is usually divided into the maximum temperature, the optimal temperature and the minimum temperature. When the temperature gradually increases, its physiological reaction will gradually accelerate[47], and the tea tree grows faster. When the optimal temperature is reached, the growth rate of tea trees reaches its fastest. Then, as the temperature gradually increases, its physiological reaction will decrease. When the maximum temperature is exceeded, the tea tree will be damaged due to lack of water. Therefore, excessively high or low temperatures are not conducive to the growth and development of tea trees[48]. Low temperature can affect the light reaction of photosynthesis, inhibiting the photosystem II in thylakoids. Low temperature stress can significantly promote the synthesis of aromatic substances in fresh tea leaves[49]. High temperature conditions will reduce the enzyme activity in the tea tree, causing the

tea tree to stop growing or even wither. Therefore, in actual production, measures such as early spring cultivation of tea gardens, summer and autumn mulching between rows, and application of organic fertilizer are often used to improve the soil temperature of tea gardens in order to achieve the best environment for tea tree growth.

As an important environmental factor, light can cause stress response in tea trees, which leads to the formation and release of aroma substances in tea[50]. Light quality, light intensity and light duration not only affect the metabolic status of tea trees, but also affect other physiological processes and developmental stages of tea trees. Tea trees are shade-tolerant plants with a low light compensation point, so in actual production, shading treatment (almost no light treatment) is often used to improve the flavor quality of tea. In addition, shading treatment also accelerates the conversion of related ascending metabolites, especially the conversion of shikimic acid, prephenylacetic acid and phenylpyruvic acid (PPA) to VPBs. In terms of light quality, tea trees absorb a large proportion of blue-violet light and red light. Generally, the photosynthetic rate under red light is higher than that under blue-violet light, which is conducive to the synthesis of sugars. Blue-violet light can accelerate the formation of amino acids and proteins, while red light has an important influence on the formation of tea polyphenols and carbohydrates, which is the basis for the accumulation of substances[51]. Blue and red light can significantly increase the formation of most volatiles in tea leaves before harvest, including VFADs, VPBs, and VTs, thereby enriching the aroma of the tea.

Tea is subjected to multiple stresses during production and processing[1], such as low temperature and water loss. In the processing of green tea, different fixation and drying processes have different effects on the formation and release of aroma in the finished green tea; the relatively complex processing of oolong tea gives it a special honey aroma and floral and fruity aroma.

6. Problems and Prospects

Tea aroma is an important component of tea quality, and its formation is closely related to both the pre-harvest growth stage and the post-harvest processing stage. This article reviews the biosynthesis of tea aroma substances and the regulatory mechanisms of tea under stress responses. It introduces several characteristic aroma substances in tea and their biosynthetic pathways. Furthermore, this article also explores the effects of biotic stress (tea garden pests, pathogens) and abiotic stress (light, temperature, mechanical damage) on the formation of tea aroma substances.

Currently, the isolation, purification, synthesis pathways, and enzymatic or non-enzymatic reactions involved in the aroma substances produced during tea processing require further investigation. For example, the mechanisms by which catechins and caffeine participate in aroma

reactions remain unclear. In future research, we hope to artificially control stress mechanisms to alter the composition and content of aroma substances in tea, aiming to obtain our desired aroma and improve tea quality.

References

[1]YAN Zhen, WANG Dengliang. Progress in Understanding the Stress Response Mechanisms and Functions of Aroma Compounds in Tea[J]. Food Science, 2021, 42(15): 337-345.

[2]WAN Xiaochun. Biochemistry of Tea. 3rd Ed. 2003, Beijing: China Agriculture Press.

[3]LV Shidong. Study on volatile components of tea by GC-MS combined with stoichiometry[D]. Kunming University of Science and Technology, 2014.

[4]ZHAO Hetao, WANG Chunyuan. The composition of main aromatic substances in tea in China and their changes during processing[J]. Natural Products Research and Development, 1990(03): 42-48.

[5]WANG Rangjian, YANG Jun, KONG Xiangrui. Research progress on volatile terpenoids in tea plant[J]. Acta Tea Sinica, 2018, 59(03): 149-154.

[6]ZHOU Jie, DING Peng, WANG Guiyu, et al. Advancesin Glycosidically Bound Aroma Precursors in Tea(*Camellia Sinensis*). Journal of Xinyang Normal University (Natural Science Edition), 2018, 31(01): 152-159.

[7] ZHANG Zhengzhu, WAN Xiaochun, KANZO Sakata. Purification by Affinity Chromatography and Characterization of Tea Leaf -Glucosidase[J]. Journal of Tea Science, 2005, 25(1): 16-22.

[8]WANG Huafu. Flavor types and aromatic substances of tea[J]. Chinese tea, 1989(02): 16-17.

[9]PENG Yun, LI Guo, LIU Xueyan, et al. SPME/GC-MS Analysis of Aroma Quality of Black Tea from Different Producing Areas[J]. Science and Technology of Food Industry, 2021, 42(9): 228-235.

[10]ZHANG Chao, LU Yan, LI Jixin, et al. Advances in research on aroma components and mechanism of aroma formation in tea[J]. Fujian tea, 2005(03): 17-19.

[11] Introduction to the Processing of High Quality Black Tea[J]. Tea Bulletin, 2017, 44(03): 53.

[12]YAO Zhaopeng, HUANG Jianan. Tea review and inspection. 4th Ed. 2010, Beijing: China Agriculture Press.

[13]JI Jieli, YUAN Dandan, LIU Fei, et al. Research progress on the quality formation mechanism of dark tea[J]. Food and Drug, 2016, 18(01): 52-60.

[14]ZHANG Yinggen, CHEN Lin, CHEN Quanbin, et al. Aromatics-based Model for Quality Evaluation of White Teas. Journal of Tea Science, 2017, 58(04): 149-156.

[15]ZHANG Zhengzhu, SHI Zhaopeng, WAN Xiaochun. Terpenoids and Tea Aroma (Review)[J]. Journal of Anhui Agricultural University, 2000(01): 51-54.

[16]ZHANG Dongtao, SUN Jun, YE Naixing, et al. Research Progress of Enzymes Associated with Terpene Synthesis in *Camellia sinensis*. Journal of tea industry, 2015, 56(02): 68-79

[17]MA Liang, DING Peng, YANG Guangxiao, et al. Advances on the Plant Terpenoid Isoprenoid Biosynthetic Pathway and Its Key Enzymes. Biotechnology Bulletin, 2006(S1): 22-30.

[18]HE Zhirong, XIANG Wei, XU Yan, et al. Progress in the Research of Biosynthesis of Volatile Terpenoids and Their Glycosides in Tea Plant[J]. Journal of Tea Science, 2012, 32(1): 1-8.

[19]ZENG Xiaoxiong. Oxidative degradation of carotenoids in tea and its relationship with tea quality[J]. Tea communication, 1992(01): 31-33.

[20]CHEN Li, YE Yulong, WANG Chunyan, et al. Research progress on carotenoid aroma precursors in tea. Food and fermentation industry.

[21]WAN Xiaochun, LI Daxiang, ZHANG Zhengzhu, XIA Tao, LING Tiejun, CHEN Qi. Research Advance on Tea Biochemistry[J]. Journal of Tea Science, 2015, 35(1): 1-10.

[22]Schuh Christian, Schieberle Peter. Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: quantitative differences between tea leaves and infusion[J]. Journal of Agricultural and Food Chemistry, 2006, 54(3): 916-924.

[23]ZHA Minyu, WU You, ZHANG Liang. Research advances on volatile aroma compounds of tea[J]. Journal of Food Safety and Quality Inspection, 2020, 11(13): 4298-4303.

[24]Kawakami Michiko, Kobayashi Akio. Carotenoid–derived aroma compounds in tea[J]. Am Chem Soc, 2001, 802(11): 145-159.

[25]Ravichandran Ramaswamy. Carotenoid composition, distribution and degradation to flavour volatiles during black tea manufacture and the effect of carotenoid supplementation on tea quality and aroma[J]. Food Chem, 2002, 78(1): 23-28.

[26]Wang Huafu, You Xiaoqing. Free and glycosidically bound monoterpene alcohols in Qimen black tea[J]. Food Chemistry, 1996, 56(4).

[27]DENG Huili, SUN Yun, LAI Zhongxiong. Progress on the effects of β-primeverosidase on tea alcoholic aroma. Subtropical Agricultural Research, 2015, 11(02): 139-143.

[28]KU Xiuping, LIU Kun, DENG Weiwei, et al. Research progress of glycoside aroma precursors and related endogenous glycosidases in tea. Tea Industry Bulletin.

[29]Mizutani M et al. Cloning of β-primeverosidase from tea leaves, a key enzyme in tea aroma formation. Piant Physiology, 2002, 130: 2164-2176.

[30]Susanne Baldermann, Ziyin Yang, Tsuyoshi Katsuno, et al. Discrimination of Green, Oolong, and Black Teas by GC-MS Analysis of Characteristic Volatile Flavor Compounds[J]. American Journal of Analytical Chemistry, 2014, 5(9).

[31]XIA Tao, TONG Qiqing, DONG Shangsheng, et al. Studies on the Change of β-Glucosidase Activity During the Withering and Fermentation of Black tea. Tea Science, 1996(01): 63-66.

[32]XIA Tao, TONG Qiqing. Effects of freeze withering on polyphenol oxidase and β-glucosidase activity of tea leaves. Journal of Zhejiang Agricultural University, 1997, 23(1): 108-110

[33]TAO Linlin, ZHANG Yanan, YAN Zhen, et al. Research progress of fermentation technology in black tea processing[J]. Guangdong Tea Industry, 2020(01): 2-6.

[34]ZHANG Wanting, ZHANG Lingzhi, WANG Dengliang. Research progress on the influence of oolong tea processing technology on aroma components[J]. Guangdong Tea Industry, 2010(Z1): 21-27.

[35]CHENG Liu, WU Yun. Processing technology of oolong tea in dachouding tea farm[J]. Guangdong tea industry, 2010(Z1): 43-44.

[36]ZENG Lanting, Naoharu Watanabe, YANG Ziyin. Understanding the biosyntheses and stress response mechanisms of aroma compounds in tea (Camellia sinensis) to safely and effectively improve tea aroma[J]. Critical Reviews in Food Science and Nutrition, 2018, 5.

[37]LIU Zhenzhen, CHEN Xiaohua, LI Chongyong, et al. Study on the important aroma components of Hanzhong green tea and their changes during processing[J]. Tea communication, 2021, 48(01): 71-79.

[38]CHAO Jeong-Yong, MIZUTANI Masaharu, SHIMIZU Bun-ichi, et al. Chemical Profiling and Gene Expression Profiling during the Manufacturing Process of Taiwan Oolong Tea “Oriental Beauty”[J]. Bioscience, Biotechnology, and Biochemistry, 2007, 71(6).

[39]CHEN Zhida. Research on the material basis and quantitative evaluation of the flavor quality of white tea. Hangzhou: Zhejiang University, 2019.

[40]Eran Pichersky, Jonathan Gershenzon. The formation and function of plant volatiles: perfumes for pollinator attraction and defense[J]. Current Opinion in Plant Biology, 2002, 5(3): 237-243.

[41]CHEN Zongmao, XU Ning, HAN Baoyu, et al. Chemical Communication Between Tea Plant-Herbivore-Natural Enemies[J]. Journal of Tea Science, 2003, 23(s1): 38-45.

[42]SUN Yichao, CAO Fanrong. Effects of Leafhopper Infection on Defensive Response of Tea Plants[J]. Guangdong Tea Industry, 2018(02): 2-5.

[43]ZHANG Zhengzhu, LI Yingbo, QI Li, WAN Xiaochun. Antifungal activities of major tea leaf volatile constituents toward Colletorichum camelliae Massea[J]. Journal of agricultural and food chemistry, 2006, 54(11): 3936-3940.

[44]CHEN Shenglong, ZHANG Liping CAI Xiaoming, et al. (E)-Nerolidol is a volatile signal that induces defenses against insects and pathogens in tea plants[J]. Horticulture Research, 2020, 7: 52.

[45]LIU Hui, ZHOU Yufeng, ZHOU Luona, et al. Research progress on interaction mechanism between pathogenic bacteria of tea cake and tea plant[J]. Guizhou Agricultural Sciences. 2021, 49(01).

[46]DENG Xihai, JIANG Qiao, ZHOU Lingyun. Habitat Environment and Chemical Composition of Major High Quality Black Teas in the World[J]. Soils,2008,40(4):672-675

[47]YE Cui. Effect of temperature change on plant growth period. Tianchang Anhui, 2020, 54(10): 7-8.

[48]SHENG Zhonglei, ZHANG Ying, LI Zhonglin, et al. Effects of microenvironment on the growth and development of tea tree cuttings[J]. Agricultural Engineering Technology, 2018, 38(32): 24-25.

[49]ZHOU Hanchen, SHAMALA Lubobiferdinand, YI Xingkai, et al. Analysis of terpene synthase family genes in Camellia sinensis with an emphasis on abiotic stress conditional[J]. Scientific Reports, 2020, 10(1): 933.

[50]TAO Hanzhi, WANG Xinzhang. The Relationship between Photosynthesis and Light Quality in Tea Plants[J]. Plant Physiology Communication, 1989(1): 19-23.

[51]FANG Shimao, HUANG Wenjing, LIU Zhongying, et al. Research progress of light quality withering on quality formation of tea. Agricultural technology services, 2020, 37(10): 40-45.

Tea Tree NOX Amplification of gene families and their expression analysis in tea plant stress resistance

He Yufan ¹ Wu Siyu¹ Mo Shiqin² Zhang Zaibao¹, *

1. School of Life and Health Sciences, Huzhou University, Huzhou, Zhejiang, China

2. Changxing Bai Suiye Tea Industry Co., Ltd., Huzhou, Zhejiang, China

*Corresponding author: Zaibao Zhang, zaibaozhang79@163.com

Abstract Reactive oxygen species (ROS), as key signaling molecules in response to stress, play an important role in plant signal transduction and developmental regulation. Plasma membrane NADPH oxidase (NOX) is the main source of ROS production and plays a crucial role in plant-pathogen interactions. This study systematically analyzed *the NOX* gene family in the tea plant genome, identifying nine *NOX* (*CsNOX*) genes. Phylogenetic, gene structure, conserved domain, motif, and collinearity analyses revealed that the tea plant *NOX* gene family can be divided into three subfamilies, exhibiting high sequence conservation and containing three pairs of repetitive gene segments. The ratio of nonsynonymous mutation rate to synonymous mutation rate (Ka/Ks) is less than 1. Promoter element analysis showed that tea plant *NOX* genes contain not only numerous stress-response elements but also various hormone-related response elements. Expression pattern analysis revealed that tea plant *NOX* genes exhibit tissue-specific expression characteristics and participate in various abiotic stress response processes. This study, through bioinformatics analysis of the tea plant *NOX gene family, provides a theoretical basis and reference value for further exploration of its functions.*

Keywords tea plant; NOX; ROS; evolution

Foreword

NADPH oxidase (NOX) is a nicotinamide adenine dinucleotide phosphate-dependent oxidase that can use electrons provided by NADPH to catalyze the generation of superoxide anions from oxygen molecules. It is an important enzyme in organisms that produces reactive oxygen species (ROS) and is widely distributed in nature. This enzyme was first discovered in phagocytes of the human immune system and exists in mammalian neutrophils and plant cells. It is a multi-enzyme complex located on the cell membrane. NOX is mainly composed of two parts: the core enzyme part (including p40phox, p47phox, p67phox, p22phox and gp91phox) and small molecular weight guanylate-binding proteins (such as Rac2 and Rap1A) [1] . In the resting state, NOX remains inactive. When cells are stimulated by external factors, the complex composed of the core enzymes p40phox, p47phox and p67phox interacts with Rac -GTP through the proline-rich tail of p22phox, which leads to a conformational change of gp91phox, thereby activating NOX and catalyzing the generation of reactive oxygen species from oxygen molecules [2] .

Reactive oxygen species (ROS) can act as signaling molecules and play an important role in the stress response of plants. Studies have shown that when plants are subjected to stress, the burst of ROS is a common phenomenon [3] , and the ROS produced by plants in response to stress is mainly generated by NOX catalysis [4-6] . When plants are subjected to external signal stimulation, NOX rapidly regulates the level of ROS in the plant through changes in its own conformation in response to biotic or abiotic stress. Under biotic stress, pathogen invasion will promote the enhancement of NOX activity, leading to an increase in the concentration of ROS in the plant. Subsequently, ROS directly kills pathogens through its oxidative toxicity or induces programmed cell death, thereby inhibiting the further spread of pathogens [7] . Under abiotic stress, Arabidopsis *thaliana AtNOX C and* AtNOX *D participate* in the mechanical damage response by regulating the level of ROS [8,9] . Under salt stress, NOX activity in maize leaves decreases, leading *to* a reduction in the level of ROS in cells, which in turn affects the elongation growth of leaves [9] .

In plants NOX also has other biological functions, for example, in Arabidopsis thaliana, NOX participates in ABA signal transduction [10] ; in rice, the NOX gene shows obvious spatiotemporal and climatic regulation characteristics in response to drought (*OsNOX1-3, OsNOX5, OsNOX9, OsFRO1 and OsNOX6*) , salt *stress* (*OsNOX2* , *OsNOX8* , OsFRO1 , OsFRO7 , OsNOX1 , OsNOX3 , OsNOX5 *and* OsNOX6 *)* and *high* temperature *treatment* (OsNOX5-9 , OsNOX1-3 and *OsFRO1)* [11] . In addition , *NOX* Besides participating in responses to abiotic stress, it also participates in the regulation of plant growth and development. For example, in maize leaves... NOX It participates in the elongation process of maize leaves[12] ; Arabidopsis thaliana *AtNOXC* It participates in cell elongation and root hair growth processes [13-15] ; AtNOXH and AtNOXJ It is essential for pollen tube growth and fertilization in Arabidopsis thaliana [16-19] . Further research has shown... NOX It also participates in a series of developmental processes, including seed germination, growth and stomatal closure [20] .

Tea (*Camellia sinensis*) is one of the important economic crops in China, Japan, India and Africa. It is suitable for growing in warm and humid environments. However, adverse factors such as high

temperature, drought, high salt and pests often have an adverse effect on the growth and development of tea trees, thereby reducing the yield and quality of tea. In recent years, with the continuous progress of sequencing technology, the genome sequencing of many tea tree varieties has been completed one after another. However, as of now, there are no reports on the study of *NOX genes in tea trees* [37] . Therefore, we identified *NOX genes* in the tea tree genome and conducted a comprehensive analysis of their phylogenetic relationship, gene structure, protein structure, chromosome distribution, homology, promoter elements and expression characteristics. Through these studies, we have preliminarily revealed the functional mechanism of *NOX* genes in tea trees to resist adverse stress, providing a theoretical basis and reference for subsequent functional verification and resistance breeding research.

Materials and Methods

1: Tea Tree NOX Identification of genetic family members

First, the domains of Arabidopsis *NOX* gene family members were predicted and analyzed using SMART (Simple Modular Structure Study Tool, https://smart.embl.de/). The results showed that the domain unique to *NOX genes was* NADPH_Ox (PF08414) [21] . Then, the hidden Markov file of the domain NADPH_Ox was downloaded from the Pfam database (http://pfam.Xfam.org) [22] , and the genome and proteome data of tea plants were obtained from the TPIA (tea plant information archive) database. Then, the hidden Markov model (HMMsearch , E value set to 10-10) and the basic local alignment search algorithm (BLASTP, E value set to 10-10) were used to search for NADPH gene family members in the tea plant proteome. The obtained protein sequences were then used to predict and verify the domains using the SMART tool to ensure that all selected tea plant *NOX* genes contained the NADPH_Ox domain. The identified *NOX* gene in the tea plant was used for further analysis.

2: Tea Tree NOX Phylogenetic analysis of gene families

First, the obtained NOX protein sequence was aligned using Muscle 5 in TBtools . Then, the sequence was pruned using the Multiple Alignment TrimAL module. Finally, a phylogenetic tree was constructed using the IQ-Tree wrapper module, and the results were visualized using iTOL (https://itol.embl.de/) [23] .

3: Tea Tree NOX Statistical analysis of physicochemical properties, gene structure analysis and motif analysis

The physicochemical properties of the *NOX gene* in tea were statistically analyzed using the Protein Parameter Calc module in TBtools ; the motif of the NOX protein sequence in tea was searched using the

Simple MEME Wrapper module [23,24] . Gene annotation files of tea were downloaded from the TPIA database and visualized using TBtools ' Visualize function. gene Visualize the *NOX gene structure of tea trees using* the Structure (from GTF/GFF File) module .

4: Tea Tree NOX Analysis of cistropic elements in gene promoter regions

Based on the genomic data and gene annotation files of tea plants, the upstream 2000 bp sequence of the *NOX gene in tea plants was extracted using the* Gtf / Gff Sequence Extract module of TBtools and defined as the promoter region of the *NOX gene. Then, the online tool* PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to perform cis-regulatory element prediction analysis on this promoter region.

5: Chromosomal localization and collinearity analysis of *NOX genes in tea plants*

Based on the gene annotation file of tea plant, the location of the *NOX gene on the chromosome* of tea plant was visualized using the Gene Location Visualize from GTF/GFF module of TBtools . At the same time, using genomic data and gene annotation file, the collinearity relationship between the *NOX gene* of tea plant itself and between tea plant and Arabidopsis thaliana was analyzed using the One Step MCScanX - Super Fast module of TBtools , and the results were visualized using the Multiple Synteny Plot module. In addition, the Ks/Ka value of duplicated genes was calculated using the Simple Ka/Ks Calculate (NG) module [23,25] .

6: Expression analysis of *NOX gene in tea plants*

Gene expression data of the *NOX* gene in tea plants under eight tissues (terminal bud, flower, fruit, young leaf, mature leaf, senescent leaf, root, and stem) and four abiotic stresses (cold, NaCl, drought (PEG) , and methyl jasmonate (MeJA)) were obtained from the TPIA database. These data were then analyzed using TBtools . HeatMap Plate drawing expresses heat map [23] .

Result

1: Identification of NOX genes in tea plants

Through HMMsearch And BLASTP search and SMART Predictive testing identified nine NOX gene family members in the tea plant proteome. These genes encode between 757 and 969 amino acids, with CsNOX 4 encoding the most and CsNOX 9 the fewest . The predicted relative molecular weights range

from 86.8 kDa to 110.11 kDa , with CsNOX 4 having the highest molecular weight and CsNOX 9 the lowest . All CsNOX... The predicted isoelectric points of all proteins were greater than 8, and all were basic proteins (Table 1).

2 : Evolutionary analysis of the NOX gene family in tea plants

To further investigate the evolutionary relationships and clustering of the NOX gene family in tea, aphylogenetic tree was constructed using the protein sequences encoded by NOX genes from tea, Arabidopsis thaliana, rice, soybean, alfalfa , and bird's eye (Figure 1). Based on the topological structure of the phylogenetic tree, the gene families were divided into three subfamilies (Group-I, Group- II, and Group - III) . *The CsNOX 6/9 gene* in tea ... Belongs to Group - I Subfamily, *CsNOX 2 - 5* Belongs to Group - II Subfamily, *CsNOX 1 / 7/8* Belongs to Group - III Sub-family. In Group - II In the subfamily, *CsNOX 2* and *AtNOXE* and *CsNOX 3-5* and *AtNOXI /F* They have high homology; in Group III CsNOX *1 / 7/8* and *AtNOXA /C/D/G* They exhibit high homology. However, in Group I ... In China, *AtNOX 6/9* and *AtNOXJ /H* Although they belong to the same subfamily, they are located on two different branches.

3: Chromosomal localization and collinearity analysis of the NOX gene family in tea plants

Chromosomal mapping results of NOX gene family members in tea plants showed that 9 *CsNOX genes ...* Genes can be located on four chromosomes and two contigs. On *the* fragment (Figure 2). CsNOX *1/2* is located on chromosome 2, *CsNOX 3/4* Located on chromosomes 1 and 2 , *CsNOX 5* Located on chromosomes 1 and 4 , *CsNOX 6/7* Located on chromosomes *1 and* 5 *, and CsNOX 8/9* They were located on Contig 464 and 581 segments, respectively . *CsNOX cells* were distributed over a wide area on the chromosome, and no clustering occurred.

To further investigate *CsNOX* Homologous relationships between genes were analyzed using MCScanX. Collinearity analysis was performed (Figure) 2). The results showed that *the CsNOX content in tea plants...* There are 3 pairs of duplicate gene segments (*CsNOX 1 -CsNOX 7* , *CsNOX 3- CsNOX 5)* . And *CsNOX 6- CsNOX 9). Ka* /Ks analysis of the three pairs of duplicate genes showed that the Ka/ Ks values were all less than 1, and the Ka /Ks values of two pairs of genes were only 0.15 and 0.13 (Table 2), indicating that they were subjected to strong purifying selection during evolution. In the collinearity analysis of Arabidopsis and tea, *CsNOX 4- AtNOXI /F* , *CsNOX 5- AtNOXI /F* and *CsNOX 7- AtNOXC* They have a common origin.

4: Tea tree NOX Gene structure and protein sequence analysis of gene families

Using SMART Predicting CsNOX structure domain and through TBtools Visualization was performed (Figure 3A). A total of five protein domains were predicted: NADPH_Ox (PF08414),

Ferric_reduct (PF01794), FAD_binding_8 (PF08022), NAD_binding_6 (PF08030) , and EF - hand . The four domains NADPH_Ox , Ferric_reduct , FAD_binding_8 , and NAD_binding_6 are all CsNOX domains. Common to all proteins. Except for CsNOX 6/9 , the rest of CsNOX... Both have two EF - hand structural domains for binding with Ca^{2+} . However, in CsNOX 6/9 , NADPH_Ox Two low-confidence EFs were found at the C-terminus of the structural domain - hand Domains (not shown in the figure). Sequences containing four common domains were extracted and multiple sequence alignment revealed CsNOX. The sequences of the protein's four domains are highly conserved (Figure 3C).

Tea Tree NOX The gene structure of the gene family shows that most *CsNOXs* have 12-14 exons, with 14 exons being the most common type, and there are a total of 6 *CsNOXs.* With 14 exons, the most *CsNOX 1 It* has 17 exons, with the fewest being *CsNOX 7/8* . There are 1 or 2 exons. Classified by group, only members of Group - II have a different number of exons (Figure 3B).

To further investigate CsNOX The protein sequence was obtained using TBtools . Motif analysis was performed (Figure 4). A total of 8 different motifs were retrieved in CsNOX , and each motif... Both are CsNOx Shared by all. The Motif logo . The image shows each motif They all have some completely conserved amino acid sites.

5 : Tea trees NOX Analysis of cistropic elements in promoter regions of gene families

CsNOX from tea trees The upstream 2kbp sequence of the gene was used as the promoter region for prediction of cisfunctional elements, and the results are shown in Figure 5. *CsNOX* in tea plants. Genes contain multiple response elements with complex functions, participating in various physiological processes in tea plants, including responses to biotic and abiotic stresses, plant growth and development, and hormone regulation. The most numerous element is... Box 4 , this element is related to the light response. The second most common element is ARE, which is related to anaerobic induction in plants.

6 : Tea Tree NOX Gene family expression analysis under different treatments and tissue-specific expression analysis

From TPIA Downloaded tea tree *CsNOX* Expression data were used to create an expression heatmap (Figure 6). Under drought conditions, *CsNOX 1/4/5/9/2* In the early stages, the expression levels were all upregulated, but gradually decreased with increasing treatment time. Under methyl jasmonate treatment, *CsNOX 1/6* Expression levels gradually increased with increasing processing time, *CsNOX 2 / 7 CsNOX expression levels are high in the early stages and low in* the later stages. Under salt treatment, *CsNOX 3/4/9* The expression level gradually increased over time, *CsNOX 2* Expression levels gradually decreased over *time* . Under cold conditions *, CsNOX 2/3/5/6/8* The expressiongradually decreased over time.

Tissue expression analysis showed (Figure 7) that *C sNOX 1* This was expressed in all eight organizations, *CsNOX 6/9 Expression* levels were low in all eight tissues. Furthermore *, CsNOX 7/8 CsNOX 3/4* was expressed at relatively high levels in flowers, roots, and stems. The expression levels *of CsNOX* are relatively high in flowers and stems, *2/5* . The expression level is relatively high in the roots and stems.

Discuss

Plant NOX NOx plays a vital role in plant growth, development, and abiotic stress. To date, NOx... Gene families have been identified and functionally studied in multiple species, including Arabidopsis thaliana, rice, wheat, sweet orange, grape, pigeon pea, cassava, and strawberry [26-28] . However, the tea plant... NOX Gene family studies are scarce. With the development of sequencing technology, genome sequencing of different tea varieties has been completed successively. Therefore, utilizing bioinformatics to study tea varieties... NOX Whole-genome identification and expression analysis of gene families are necessary. Studies have found nine NOX genes in the tea plant genome, a number similar to that of Arabidopsis thaliana and rice, but fewer than in legumes such as soybean. This may be due to… NOX The number of gene families increases with the expansion of the entire legume genome (Table 1). Motif analysis shows that each NADPH gene family has the same motif, indicating high conservation of NOX protein sequences in tea (Figure 4). Furthermore, protein domain and gene structure analysis shows that the number of introns and exons in tea NOX is not significantly different, and domain predictions also show high consistency. Although CsNOX6/9 lacks two EF-Hand domains for binding Ca^{2+} , SMART prediction still found two low-confidence EF-Hand domains at the C-terminus of the NADPH domain. Gene structure and protein domain analysis further demonstrate the high conservation of the tea NOX gene sequence (Figure 3).

When a plant is stimulated by external signals, NOx located on the plasma membrane... It can change its own activity through conformational changes, thereby rapidly regulating the level of reactive oxygen species in intracellular parts to respond to stress and regulate its own growth and development. In Group-I, *CsNOX6/9* and *AtNOXH /J* have high homology. Previous studies have shown that *AtNOXH/J* is mainly expressed in floral organs and is essential for pollen tube elongation and fertilization [16-19] . Combined with RNA-seq expression analysis, it was found that although *CsNOX6/9* was expressed at low levels in all eight tissues, it was expressed at high levels in floral organs, and was also expressed in roots and stems (Figures 1 and 7) . Therefore, *CsNOX6/9* may have similar functions to *AtNOXH /J* , while also playing other roles in roots and stems. In Groups II and III, *CsNOX2* and *AtNOXE* , *CsNOX3/4/5* and *AtNOXI /F* , and *CsNOX1/7/8* and *AtNOXA /C/D/G* showed high homology. Previous studies have found that *AtNOXC /D/F* are involved in the salt stress response [29,30] . RNA-seq expression analysis revealed that *CsNOX1/7* was indeed upregulated in the early stages of salt stress, but downregulated at 72 hours of salt stress. CsNOX5 was downregulated at 24 hours of salt treatment, upregulated at 48 hours, and downregulated at 72 hours. *CsNOX3* expression gradually increased with treatment time, while *CsNOX4 was downregulated*

at 24 hours of salt treatment, with little change at 48 and 72 hours (Figures 1 and 6). These results indicate that CsNOX1 /7 exhibit significant spatiotemporal characteristics in response to salt stress. It is expressed in the roots, produces ROS, and controls root hair growth [13] . RNA-seq expression analysis found that *CsNOX1/7* are highly expressed in the roots. *AtNOXD,* as a putative housekeeping gene in Arabidopsis, does not show significant differences in expression across different tissues, which is similar to the expression pattern of CsNOX1 [3] (Figure 1, Figure 7) . Therefore, *CsNOX1* may have similar functions to *AtNOXD* , participating in plant stomatal closure, pathogen stress, mechanical damage response, and signal transduction [4] , and together with *CsNOX7 ,* it participates in controlling the growth of tea tree root hairs.

Collinearity analysis revealed that *CsNOX4/5* and *AtNOXI /F* were collinear, suggesting some functional similarity; however, their specific functions require further investigation (Figure 2). *CsNOX7* and *AtNOXC* were also collinear. Furthermore, *CsNOX7* and *CsNOX1* belong to gene pairs resulting from fragment duplication events, with Ka/Ks values significantly less than 1. This indicates that *CsNOX1/7* underwent intense purifying selection during evolution, further suggesting that *AtNOXC* and *CsNOX1/7* should be functionally similar (Figure 2, Table 2). *CsNOX3/5* and *CsNOX6/9* are also gene pairs resulting from fragment duplication events, with Ks/Ks values similarly significantly less than 1 (Table 2). Promoter element analysis showed that *CsNOX* contains numerous stress-responsive elements as well as hormone-responsive elements (Figure 5). For example, the ABRE element contained in CsNOX1/2/7/8 participates in the response to ABA. The response. In Arabidopsis, maize, and tobacco, ABA can induce corresponding NOx. Gene expression is upregulated [31] .

In summary, the NOX gene family in tea plants plays an important role in stress resistance and growth and development regulation, but its specific biological functions require further research.

Acknowledgments

This research was supported by the National Undergraduate Innovation and Entrepreneurship Training Program (202313287003) , the General Scientific Research Project of Zhejiang Provincial Department of Education (Y202351039), the Huzhou Science and Technology Commissioner Program (2023KT44), and the Huzhou Higher Education Scientific Research Program (2023HXKM09) .

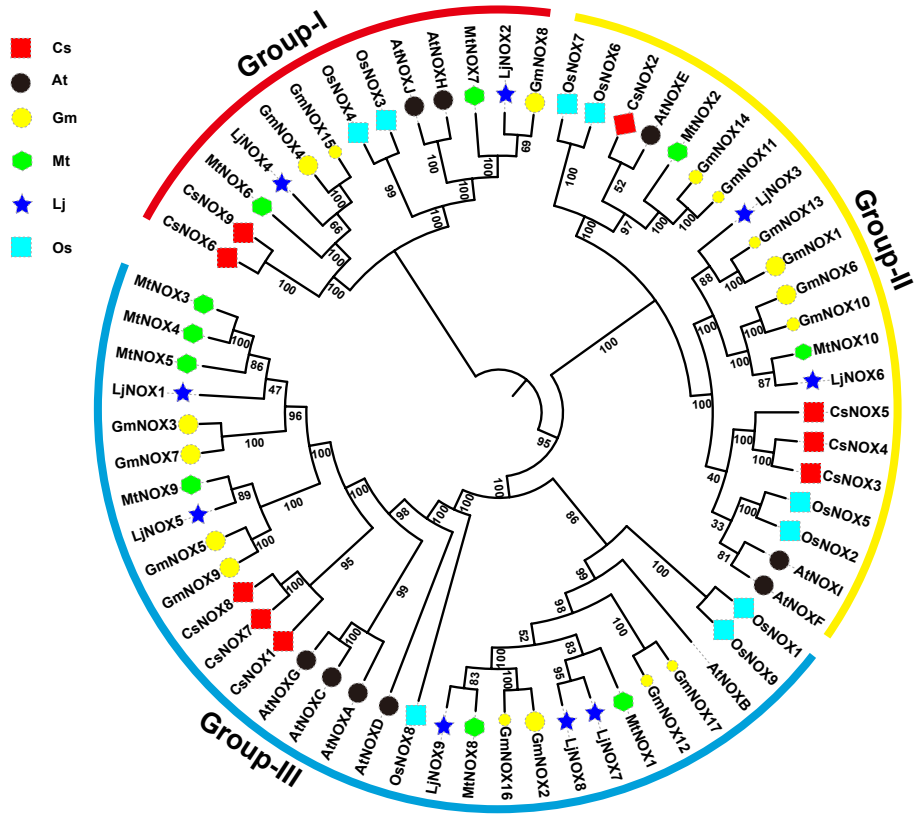


Figure 1. Phylogenetic tree and grouping of *NOX genes* in tea, Arabidopsis thaliana, rice, soybean, alfalfa and birdsfoot .

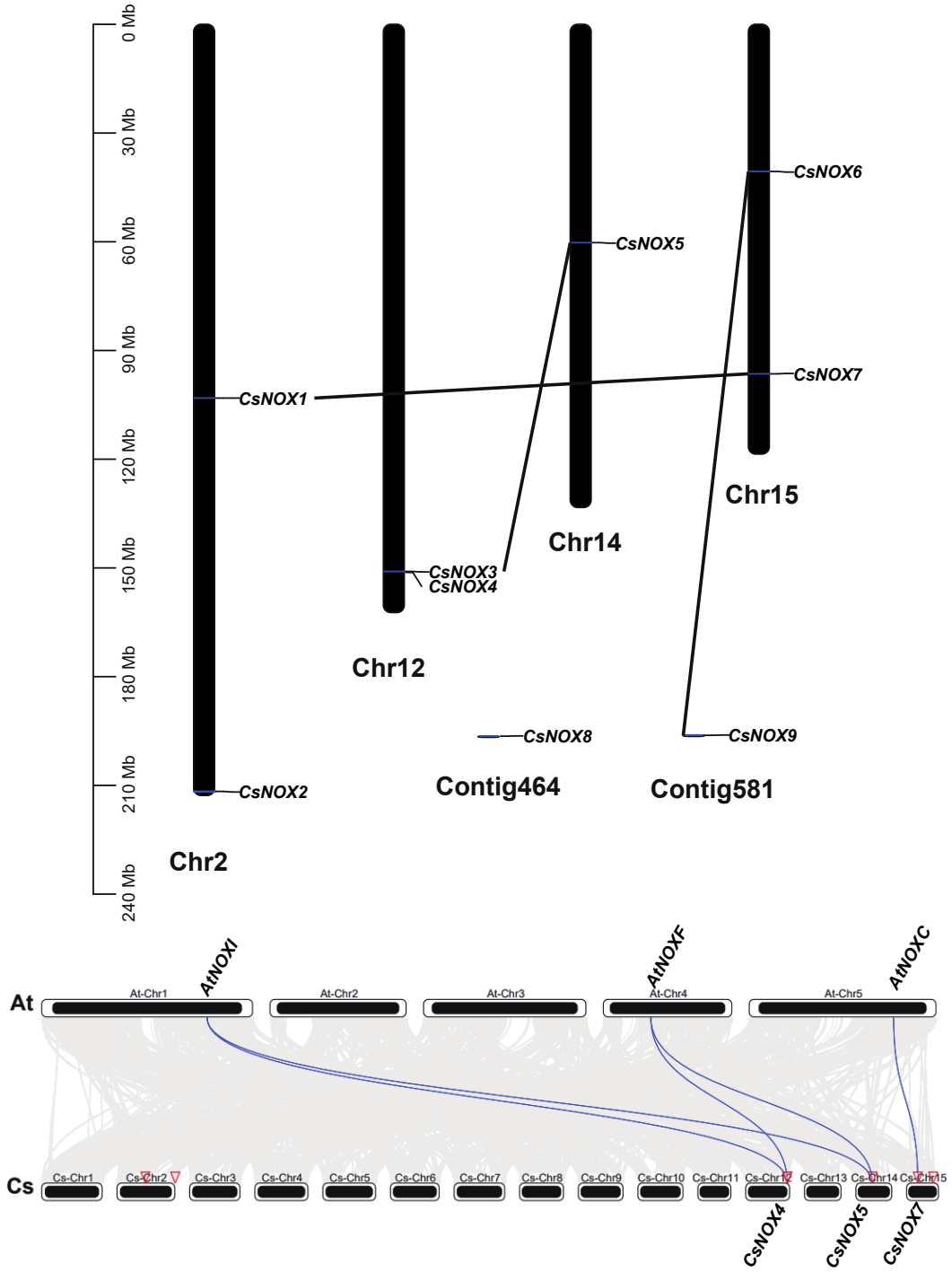


Figure 2. Chromosomal localization of *NOX genes* in tea and collinearity analysis of *NOX genes in tea and Arabidopsis thaliana.*

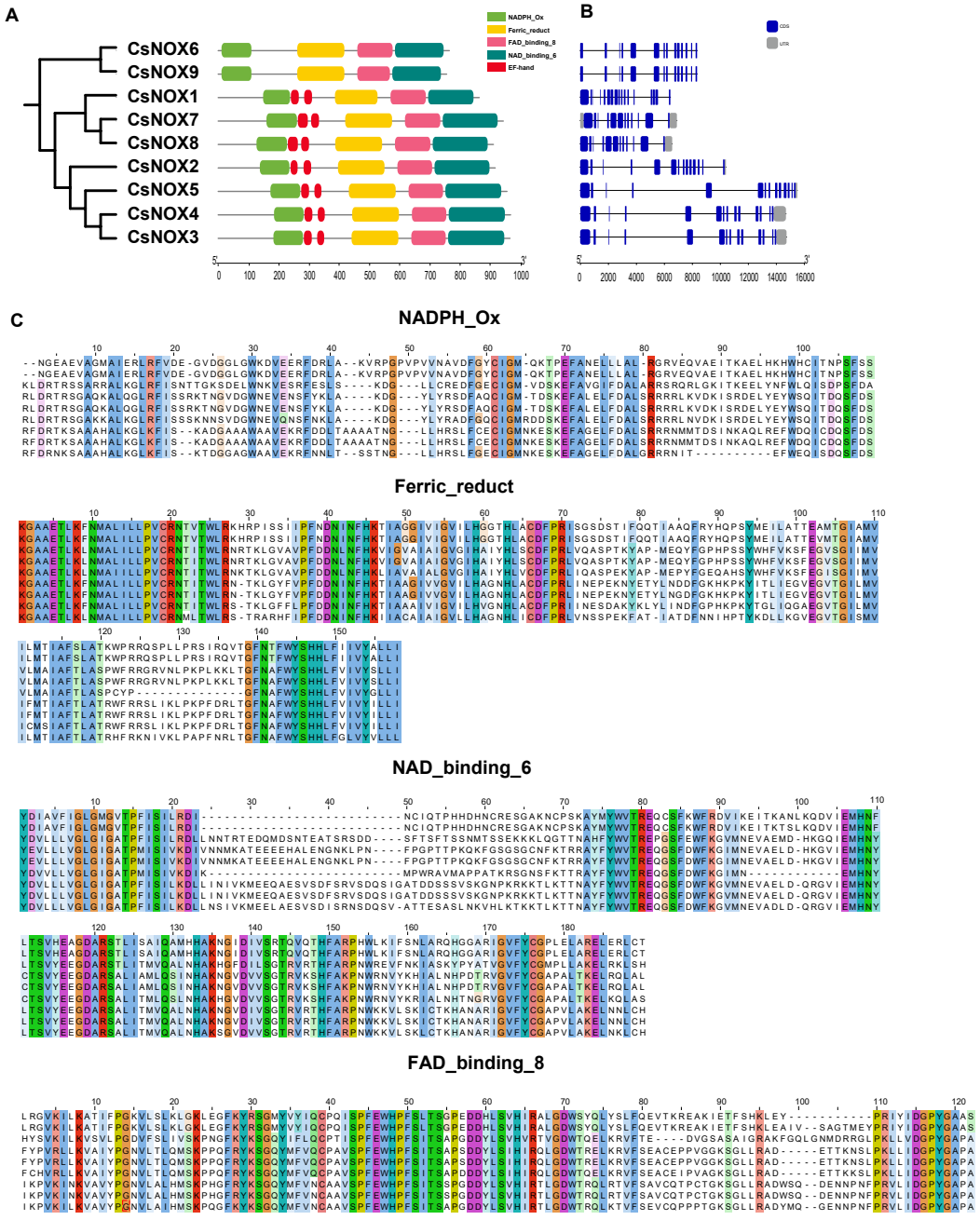


Figure 3. Protein domains (A), intron/exon structures (B), and sequence similarity analysis of the *NOX gene* in tea plants (C).

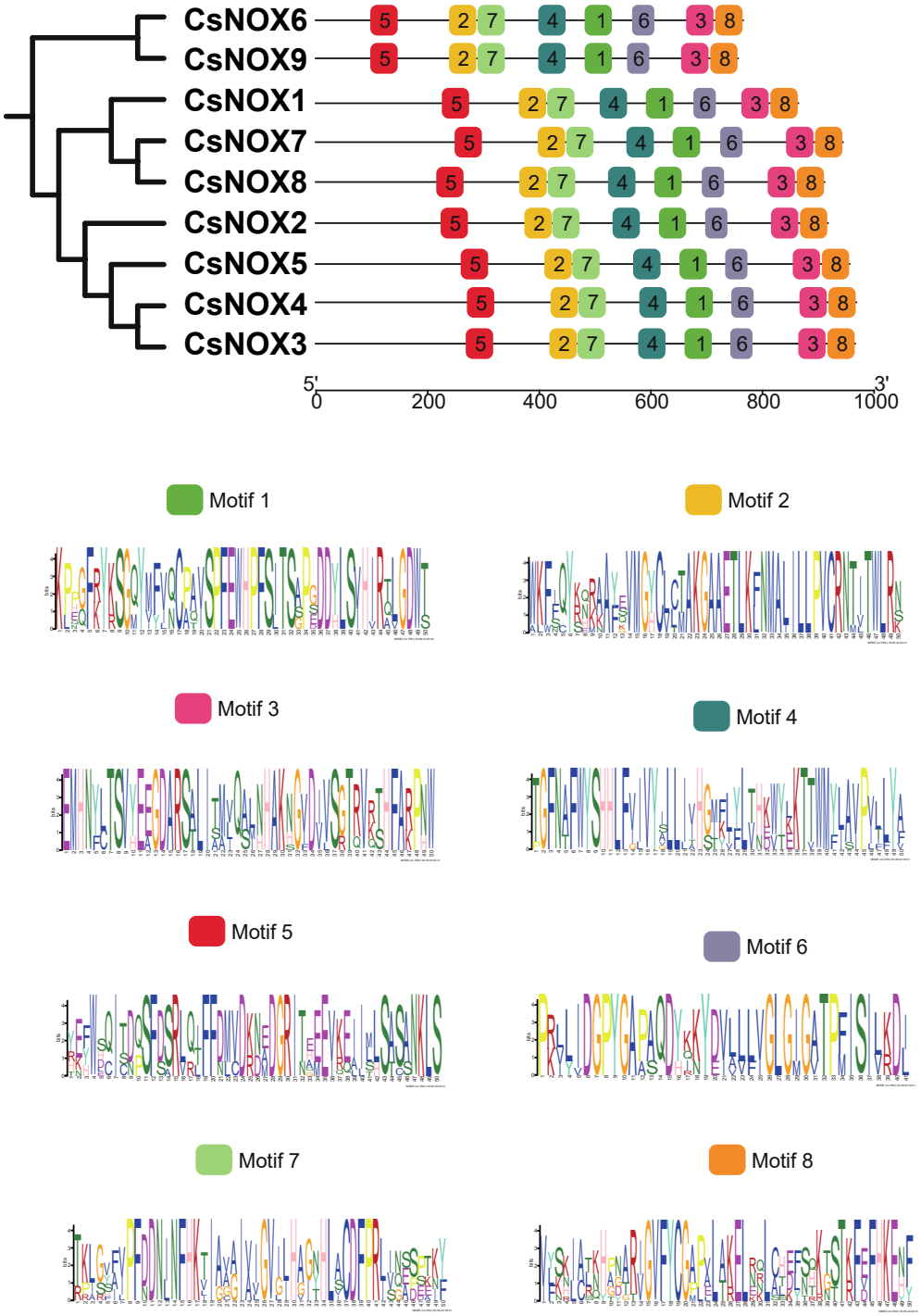


Figure 4. Protein meta- if analysis of NOX from tea plants .

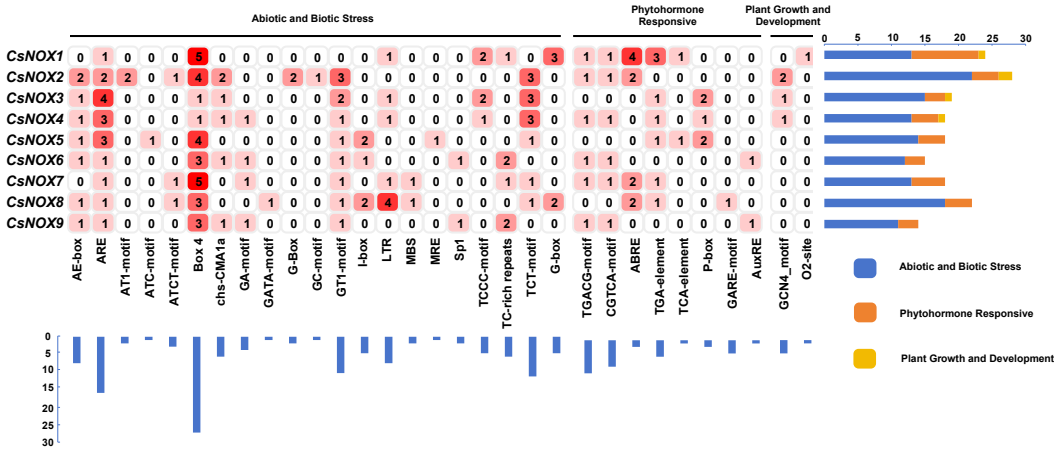


Figure 5. Promoter element analysis of *NOX gene* in tea plant .

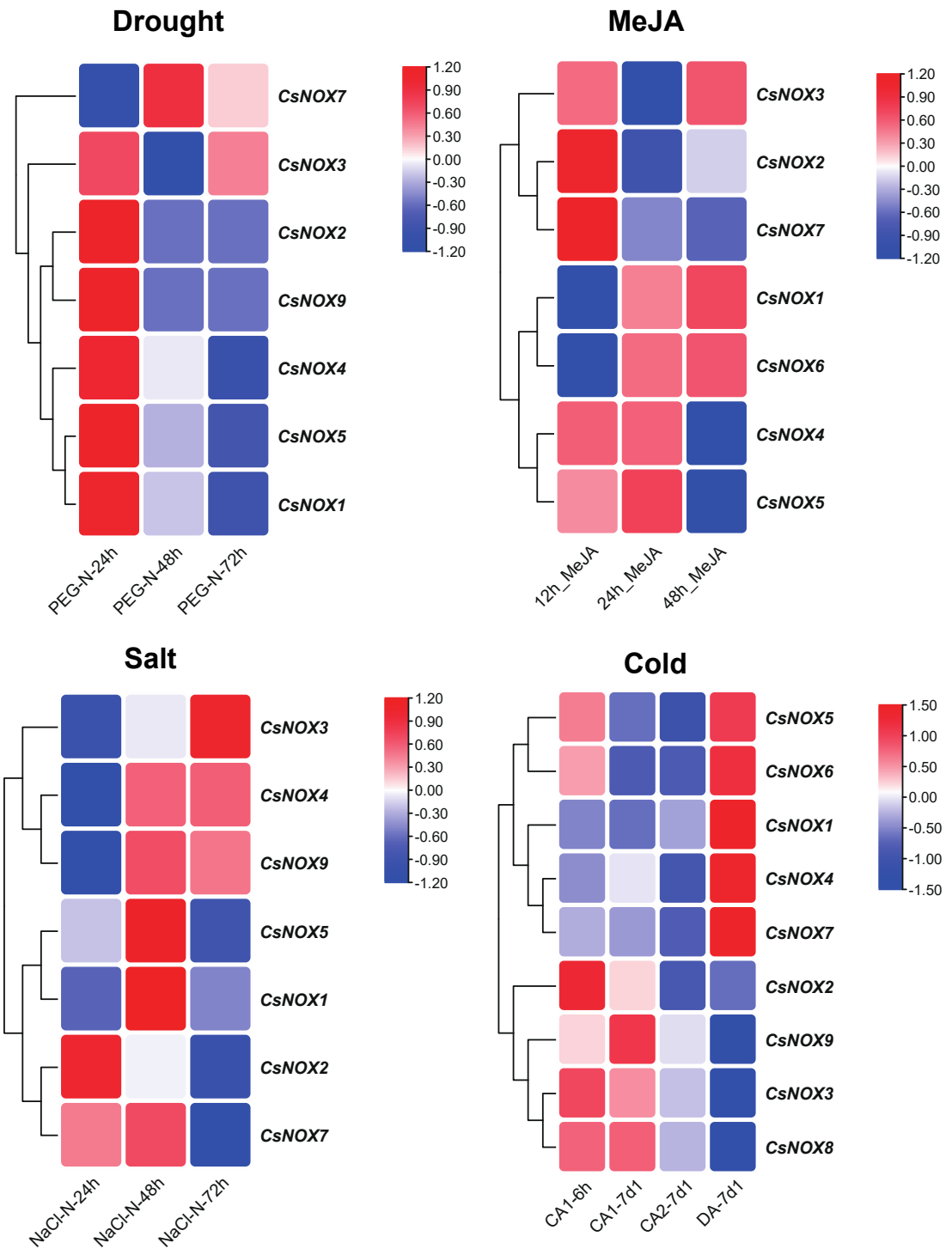


Figure 6. Expression heatmap of *NOX gene in* tea plant under drought, JA , salt and cold stress.

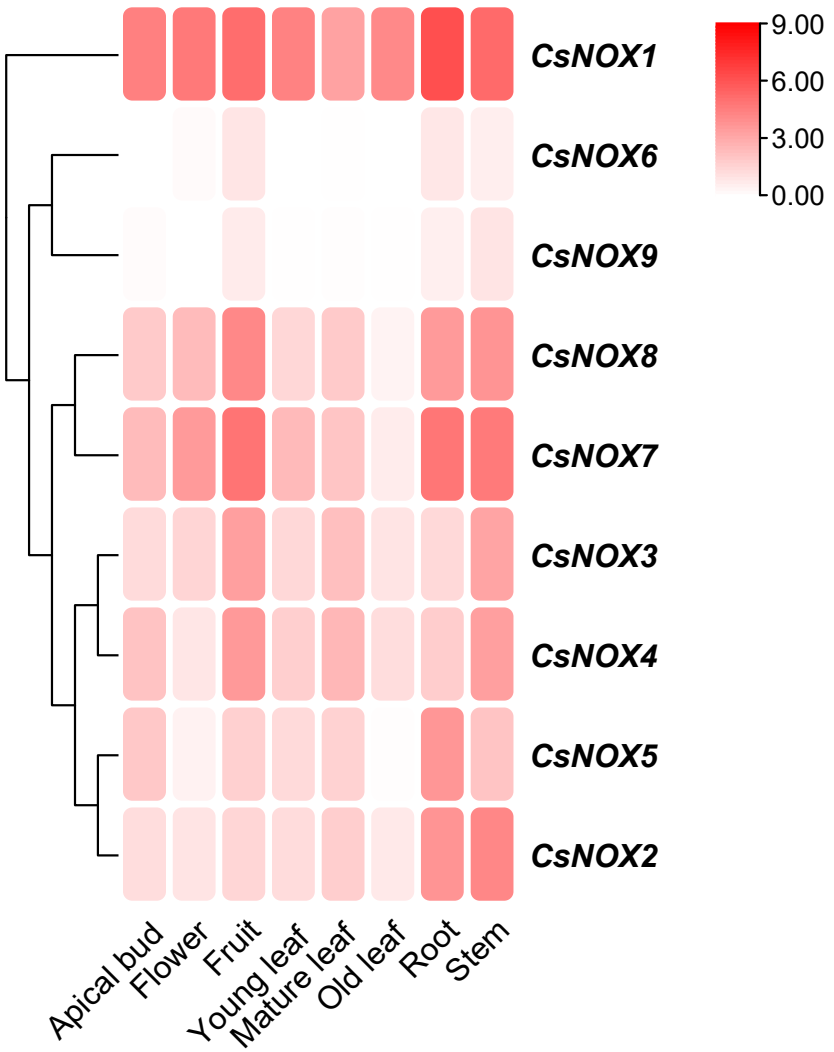


Figure 7. Expression profile analysis of *NOX* gene in important tissues and organs of tea plant .

Table 1: Physicochemical Properties Analysis of NOX Protein

Serial Number	Gene Name	Origin name	Molecular Weight (kDa)	Protein Length (aa)	Pl
1	CsNOX1	CSS0039845	97.01	865	9.39
2	CsNOX2	CSS0046357	104.24	918	8.36
3	CsNOX3	CSS0043661	109.9	967	9.22
4	CsNOX4	CSS0001287	110.11	969	9.19
5	CsNOX5	CSS0047680	108.378	957	9.19
6	CsNOX6	CSS0029939	87.69	766	8.9
7	CsNOX7	CSS0008067	106.4	945	9.11
8	CsNOX8	CSS0045221	102.7	912	9.27
9	CsNOX9	CSS0027498	86.8	757	8.95

Table 2 : Ka /Ks analysis of Cs NOX replication gene

Serial Number	Paralogous Pairs	Ka	Ks	Ka/Ks	Duplication
1	CsNOX3-CsNOX5	0.075158599	0.483189957	0.155546692	SD
2	CsNOX1-CsNOX7	0.099370823	0.723981409	0.137256042	SD
3	CsNOX6CsNOX9	0.001717394	0.003840008	0.447237077	SD

*SD: Segmental Duplication

References

[1] Ding Kunfeng, Tan Xiaorong . Research progress on plant NADPH oxidase %J Life Sciences . 2010 22, 723-728.

[2] Hao Fushun, Chen Jia . Research progress on NADPH oxidase in plant cell membranes %J Bulletin of Botany . 2005, 1-10.

[3] Marino D.Dunand C.Puppo A. et al. A burst of plant NADPH oxidases . Trends Plant Sci 2012 17, 9-15.

[4] Bedard K. Krause KH The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology . Physiol Rev 2007 87, 245-313.

[5] Van Breusegem F.Bailey-Serres J. Mittler R. Unraveling the tapestry of networks involving reactive oxygen species in plants . Plant Physiol 2008 147, 978-984.

[6] D J. LightfootBoettcher A.Little A. et al. Identification and characterization of barley (Hordeum vulgare) respiratory burst oxidase homologue family members . Funct Plant Biol 2008 35, 347-359.

[7] Torres MAJones JD Dangl JL Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in Arabidopsis thaliana . Nat Genet 2005 37, 1130-1134.

[8] Takahashi F.Mizoguchi T.Yoshida R. et al. Calmodulin-dependent activation of MAP kinase for ROS homeostasis in Arabidopsis . Mol Cell 2011 41, 649-660.

[9] Monshausen GBBibikova TNWeisenseel MH et al. Ca2+ regulates reactive oxygen species production and pH during mechanosensing in Arabidopsis roots . Plant Cell 2009 21, 2341-2356.

[10] Kwak JMMori ICPei ZM et al. NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in Arabidopsis . Embo j 2003 22, 2623-2633.

[11] Wang GFLi WQLi WY et al. Characterization of Rice NADPH oxidase genes and their expression under various environmental conditions . Int J Mol Sci 2013 14, 9440-9458.

[12] Rodríguez AAGrunberg KA Taleisnik EL Reactive oxygen species in the elongation zone of maize leaves are necessary for leaf extension . Plant Physiol 2002 129, 1627-1632.

[13] Foreman J.Demidchik V.Bothwell JH et al. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth . Nature 2003 422, 442-446.

[14] Carol RJTakeda S.Linstead P. et al. A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells . Nature 2005 438, 1013-1016.

[15] Takeda S. Gapper C. Kaya H. et al. Local positive feedback regulation determines cell shape in root hair cells . Science 2008 319, 1241-1244.

[16] Sagi M. Fluhr R. Production of reactive oxygen species by plant NADPH oxidases . Plant Physiol 2006 141, 336-340.

[17] Boisson-Dernier A.Lituiev DSNestorova A. et al. ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases . PLoS Biol 2013 11, e1001719.

[18] Kaya H.Nakajima R.Iwano M. et al. Ca2+-activated reactive oxygen species production by Arabidopsis RbohH and RbohJ is essential for proper pollen tube tip growth . Plant Cell 2014 26, 1069-1080.

[19] Lassig R.Gutermuth T.Bey TD et al. Pollen tube NAD(P)H oxidases act as a speed control to dampen growth rate oscillations during polarized cell growth . Plant J 2014 78, 94-106.

[20] Müller K.Carstens ACLinkies A. et al. The NADPH-oxidase AtrbohB plays a role in Arabidopsis seed after-ripening . New Phytol 2009 184, 885-897.

[21] Letunic I.Doerks T. Bork P. SMART: recent updates, new developments and status in 2015. Nucleic Acids Res 2015 43, D257-260.

[22] Finn RDCoggill P.Eberhardt RY et al. The Pfam protein families database: towards a more sustainable future . Nucleic Acids Res 2016 44, D279-285.

[23] Chen C.Chen H.Zhang Y. et al. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data . Mol Plant 2020 13, 1194-1202.

[24] Bailey TLBoden M.Buske FA et al. MEME SUITE: tools for motif discovery and searching . Nucleic Acids Res 2009 37, W202-208.

[25] Wang Y.Tang H.Debarry JD et al. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity . Nucleic Acids Res 2012 40, e49.

[26] Zhang Y.Li Y.He Y. et al. Identification of NADPH oxidase family members associated with cold stress in strawberry . FEBS Open Bio 2018 8, 593-605.

[27] Fan Yuxin, Li Na, Song Zhihua et al. Response of the pigeon pea NADPH oxidase gene family to abiotic stress %J Journal of Agricultural Biotechnology . 2022 30, 284-295.

[28] Hu Guangliang Dacheng . Bioinformatics and expression analysis of cassava NOX gene family members %J Journal of Southern Agriculture . 2019 50, 2178-2187.

[29] Leshem Y. Melamed-Book N. Cagnac O. et al. Suppression of Arabidopsis vesicle-SNARE expression inhibited fusion of H2O2-containing vesicles with tonoplast and increased salt tolerance . Proc Natl Acad Sci USA 2006 103, 18008-18013.

[30] Sakamoto H.Matsuda O. Iba K. ITN1, a novel gene encoding an ankyrin-repeat protein that affects the ABA-mediated production of reactive oxygen species and is involved in salt-stress tolerance in Arabidopsis thaliana . Plant J 2008 56, 411-422.

[31] Li Ri. Identification of wheat NOX family genes and cloning of TaNOX12 gene , (2015).

INVESTIGATION OF LIFE SCIENCE
1 March 2026 | Volume 1, Issue 1

Print ISSN: 3134-8157 | Online ISSN: 3134-8165

Published by: SINOVITA (Hong Kong) Group Co. Ltd.

Address: Unit 8, 26/F, Billion Plaza 2, 10 Cheung Yue Street, Cheung Sha Wan, Kowloon, Hong Kong

Printed by: SINOVITA

Editorial Office E-mail: editor@investigationoflifescience.com

Official Website: https://www.investigationoflifescience.com