

**ENHANCEMENT OF ANTIOXIDANT CAPACITY IN WHEAT
(*TRITICUM AESTIVUM* L.) APPLYING SALICYLIC ACID
AT LOW TEMPERATURE STRESS CONDITION**

**YANJING WANG¹, BAOTING FANG¹, JUNQIN YUE¹, SIMENG DU¹, HAIYANG JIN¹,
CHENG YANG¹, DEQI ZHANG¹, HANFANG WANG¹, YUNHUI SHAO¹ AND XIANGDONG LI^{1*}**

*Life science laboratory, Zhengzhou Normal University, Zhengzhou city,
Henan province, China 450002*

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Abstract

Low temperature stress is one of the important environmental factor affecting the growth and development of wheat. As a widely existing hormone, salicylic acid do not only improve the immunity of wheat, but also improve the cold resistance capability of wheat. In the present study, effects of foliar spray of 0.3 g/l Salicylic acid (SA) on the cold resistance of wheat were studied under low temperature stress condition. Results showed that exogenous salicylic acid could enhance the activity of wheat antioxidant enzymes, reduce the content of Malondialdehyde (MDA) and change its own starch and sucrose synthesis, amino acid synthesis, carbon metabolism and glycolysis by affecting the differential expression of different hormone signal transduction genes and thereby improving the cold resistance of wheat.

Introduction

Wheat is one of the main food crops in the world. With the continuous emergence of extreme weather in recent years, the cultivation and production of wheat has been affected by different disasters. Low temperature and freezing injury is one of the main disasters affecting the growth and development of wheat. Through improvement of the cold resistance capability of wheat production and ensuring global food security can be ensured (Hassan *et al.* 2021).

As a hormone widely existing in plants like *Salix alba* Salicylic acid (SA) do not only regulate the growth and development of plants, but also participate in physiological processes such as plant immunity and disease resistance. It has a wide range of application value (Wani *et al.* 2017). Furthermore, with the wide application of modern information technology in biological research, and the deepening of human understanding of the relationship in plants through the interaction between hormones and the mechanism of improving their own stress resistance, such research has received increasing attention (Tolani *et al.* 2021, Yang *et al.* 2021). On the other hand, plants can maintain their normal growth and development by increasing the activity of antioxidant enzymes and reducing the content of peroxides. Low temperature stress can lead to the destruction of cell membranes and the degradation of metabolic pathways, which in turn promotes frostbite of cells and plant organs, consequently inhibiting the growth and development of plants. Exogenous SA (100 μ M) treatment can increase the activities of wheat CAT and other enzymes, increase the content of free proline, and reduce the content of MDA and H₂O₂ (Ignatenko *et al.* 2019). In addition, some studies have shown that the improvement of the cold tolerance of bananas by SA is related to the improvement of metabolites such as proline and unsaturated fatty acids (Chen *et al.* 2020). Thus, SA play an important regulatory role in the growth and development of wheat. In the

*Author for correspondence: <tgzy123456@126.com>. Yanjing Wang and Baoting Fang are equally for this paper. ¹Wheat Research Institute, Henan Academy of Agricultural Sciences, Zhengzhou city, Henan province, China 450002.

present study the molecular regulation mechanism of SA in inducing wheat cold resistance was also investigated.

Materials and Methods

In the present study Zhengmai 1860 was used as the test material, which was provided by the Wheat Institute of Henan Academy of Agricultural Sciences. The volume of the pot is a narrow cylinder with a bottom diameter of 10 cm and a height of 15 cm. The soil is organic nutrient soil. After the plants were sown in pots, they were sown in an artificial climate box for cultivation. The cultivation conditions were 12000 lux light intensity, 12 hrs/12 hrs light/dark, 25/22°C temp., 50 % humidity, and the soil was kept moist. After germination, when the seedling attain three leave stage, two side leaves and one culture leaf or top leaf , then the central leaf, that is, the top leaf, treated at a low temperature at 0 and 15 °C. After one day of treatment, the leaves were sprayed with SA and control seedlings were sprayed with water. Each treatment was replicated three times. After spraying with SA 0.3g/l, samples were collected at 6 and 24 hrs later to determine MDA, SOD and CAT activity. The samples were collected from the middle leaves of wheat at the seedling stage, weighed, and frozen in liquid nitrogen to determine various indicators. The specific test settings are presented in Table 1.

Table 1. Different treatments and their codes.

Treatments	Code
0°C+6hrs+water	CK06
0°C+6 hrs +SA	SA06
0°C+24 hrs +water	CK024
0°C+24 hrs +SA	SA024
15°C+6 hrs +water	CK156
15°C+6 hrs +SA	SA156
15°C+24 hrs +water	CK1524
15°C+24 hrs +SA	SA1524

In the present study, the activity of three peroxidase enzymes (MDA, SOD and CAT) in wheat leaves at seedling stage was determined to determine the degree of low temperature-induced wheat membrane lipid peroxidation. SOD was determined by NBT photoreduction method, and the amount of enzyme required to inhibit NBT photoreduction by 50 % was one unit of enzyme activity; Determination of MDA content was carried out by thiobarbituric acid colorimetric method (LI 2000).

The experiment refers to the cultivated wheat genome, obtains high-throughput data through second-generation sequencing, and carries out bioinformatics analysis. The detailed test methods refer to different similar articles (Bolger *et al.* 2014, Kim *et al.* 2015). The methods could be divided into two steps. RNA isolation and library preparation and RNA sequencing and differentially expressed gene analysis were done by following the methods used by the following authors (Kanehisa *et al.* 2008, Trapnell *et al.* 2010, Roberts *et al.* 2011, Langmead and Salzberg 2012, Anders and Huber 2013, Roberts and Pachter 2013, Bolger *et al.* 2014, Anders *et al.* 2015, Kim D *et al.* 2015).

For statistical analysis and graphing R, Excel and other software were used.

Results and Discussion

Superoxide dismutase (SOD) and CAT are two main peroxide scavenging enzymes in plants. The level of their activity levels can directly reflect the level of stress resistance in crops, and the change of MDA content in leaf cells directly reflects the changes in plant leaf cells. The level of adversity coercion received. The MDA content of CK was higher than that of SA treatment at 6hrs and different temperatures, while at 24hrs, SA treatment was lower than CK at 15°C, while at 0°C SA treatments MDA was slightly higher CK at 24 hrs but the difference was not significant (Fig. 1). Secondly, in the change graph of SOD activity, under different temperature conditions, except that of SA at 0°C was lower than CK and the difference reached a significant level, there was no significant difference between SA and CK at the same temperature and time period. It is speculated that exogenous SA may have a certain effect on SOD, but the effect is not obvious in the selected time period under the experimental conditions. Finally, the change of SOD activity, showed a large difference than change of CAT activity.

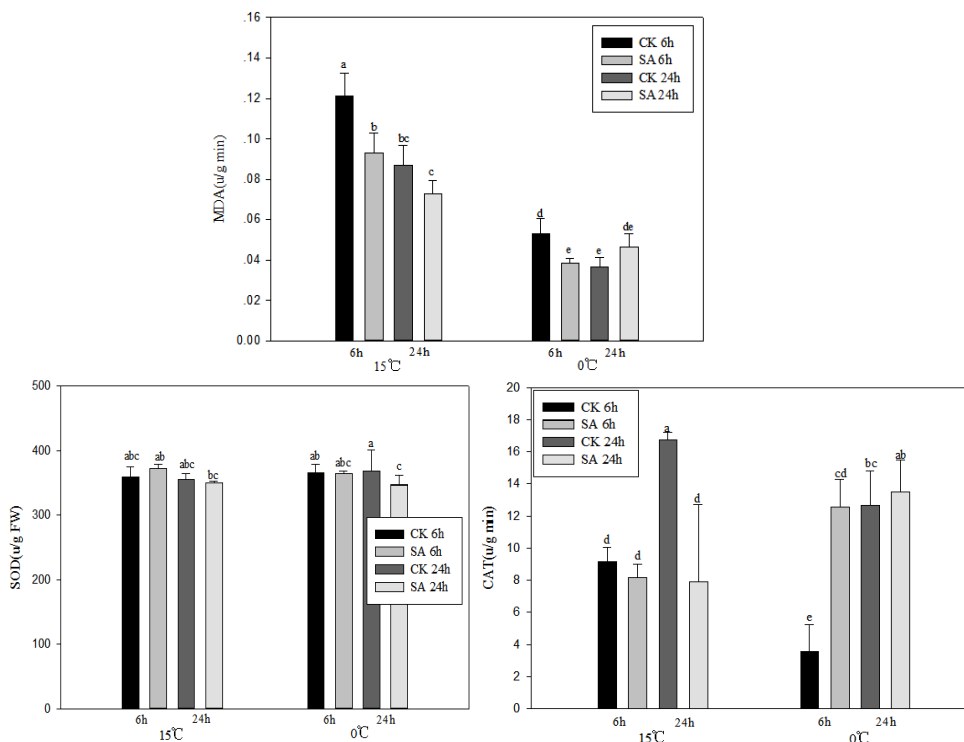


Fig. 1. Membrane lipid antioxidants in wheat leaves at 6 and 24 hrs.
 Notes: a, b, c means the significance of difference, p < 0.05.

The CAT activity of SA treatment was lower than that of CK at 15°C, but the activity of SA treatment at 0°C was higher than that of control at 6 hrs, and it was significantly higher. However, it should also be pointed out here that at 24hrs at 15°C, the activity of CK was higher than that of SA treatment, and there was a large error in SA treatment at 24h. It was more pronounced at 0°C. SA treatment can significantly increase the activity of CAT, but has no obvious effect on the activity of SOD, but reduces the content of MDA, especially under the condition of 0°C treatment, the effect was more obvious.

The different spatiotemporal transcriptomes of wheat leaves were sequenced on the Illumina Novaseq™6000 platform, and a total of 236.79G of raw_bases were obtained. After data cleaning, the available clean_bases 213.45G were filtered. The Q30 value (base amount $\geq 30\%$) was 94.66 to 95.08%, and the GC content was 54.25%, indicating that the base quality were good and the data were reliable, which could be used for subsequent analysis.

As shown in Fig. 2, through principal component analysis between different treatments, it can be seen that SA024 was quite different from CK024, SA06, and CK06 at 0°C. Under the condition of 15°C, the distances between SA1524, CK1524, SA156 and CK156 were close, and the difference was not obvious. From the above analysis, it can be seen that under the condition of lower temperature, the effect of SA on the growth and development of wheat was more obvious.

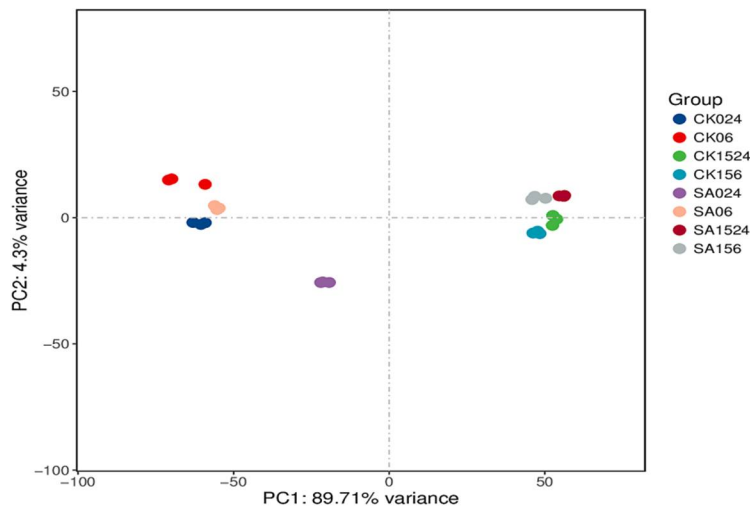
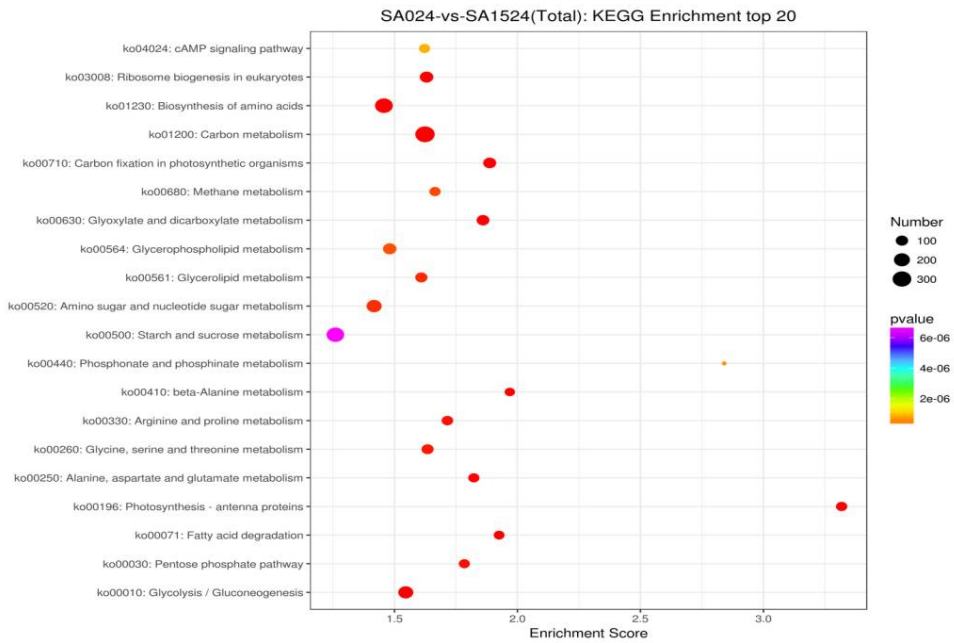
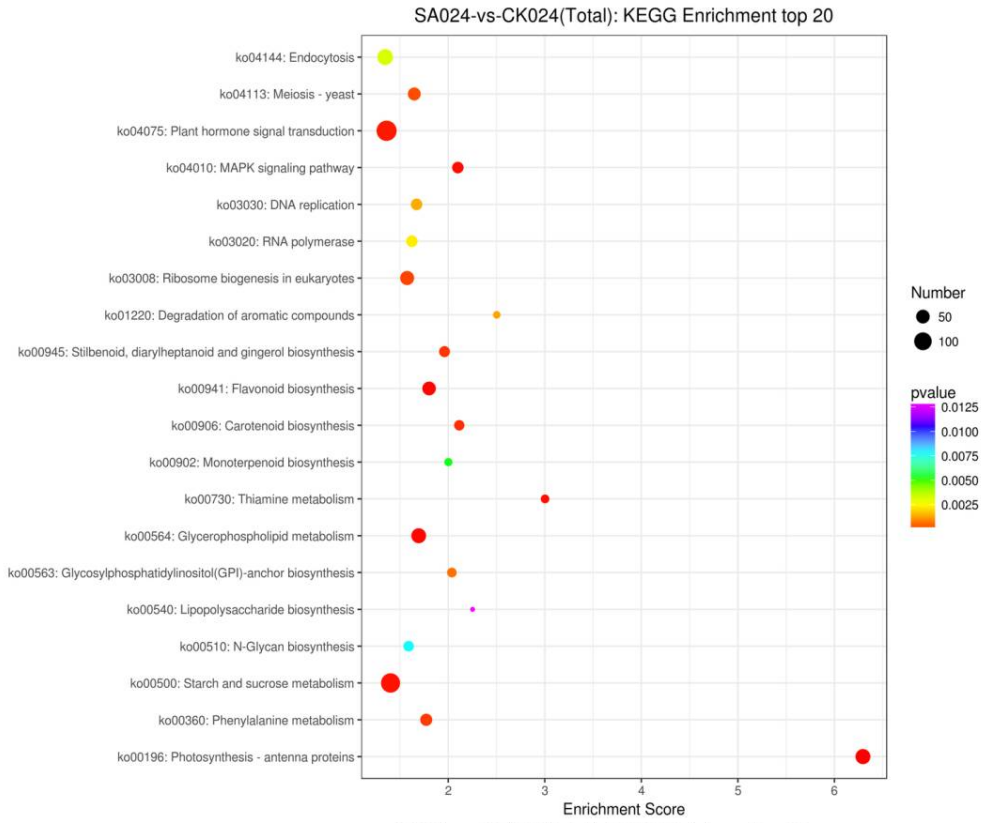


Fig. 2. PCA diagrams of different treatments.

In the present study the pathway entries with the number of corresponding differential genes were greater than 2, and were screened sorted them according to the $-\log_{10}P$ value corresponding to each entry from large to small (Fig. 3). Compared with CK1524, SA154 had obvious changes in the regulation pathway of phenylalanamide biosynthesis. Compared with SA1524, SA024 had greater changes in amino acid biosynthesis and carbon metabolism, and the changes of other pathways were scattered. Significant changes in plant hormone signal transduction pathways and starch and sucrose metabolism were observed. It is speculated that SA-induced wheat cold resistance is related to the induced signal cross-response and the metabolic components, such as sugar and protein content changes.

The differential genes of the pathways of major endogenous hormone signal transduction induced by exogenous SA were analyzed (Fig. 4). Its effect on auxin was not only on the transport across the cell membrane, but also on the changes of gene expression in the nucleus, among which there was an inhibitory effect on AUX1, TIR1 and ARF, and a promoting effect on the expression of GH3.

Secondly, its effects on Cytokinins, except for inhibiting the expression of CRE1, were different for other genes. And it has certain inhibitory effect on gibberellin gene OID1, DELLA and GID2 in the nucleus. Furthermore, it has a certain inhibitory effect on PP2C and SARK2 in the abscisic acid regulation pathway.



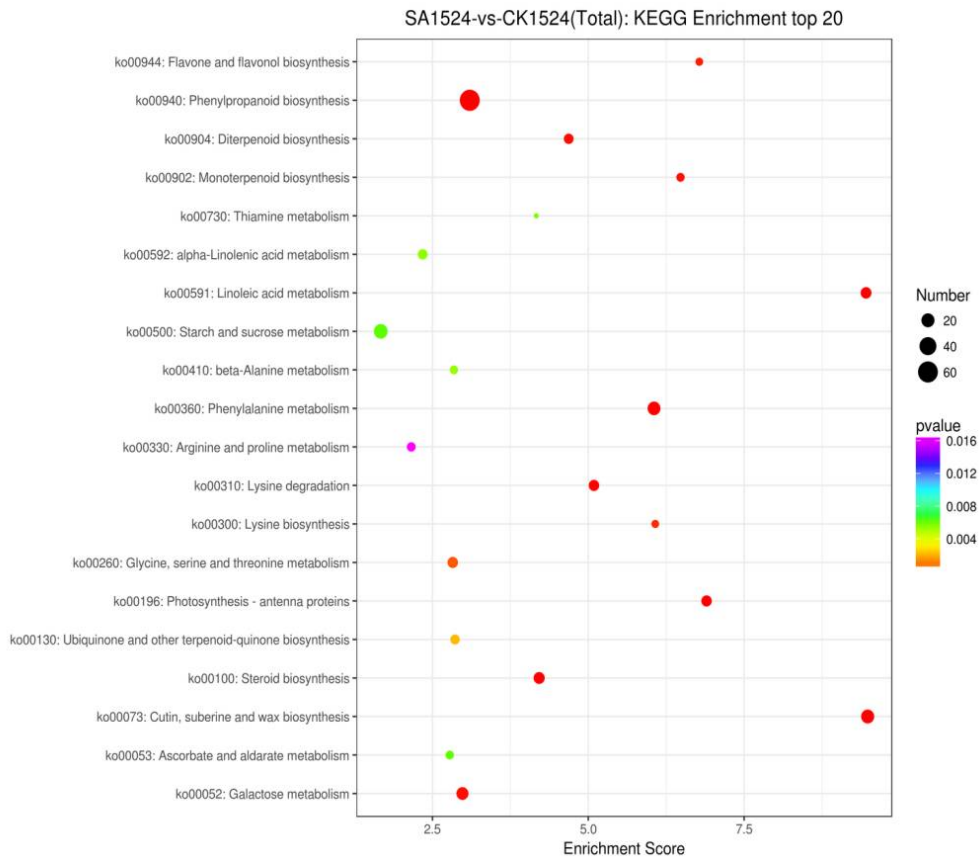


Fig. 3. KEGG enrichment top20 bubble chart image. The X-axis Enrichment Score in the figure is the enrichment score. The larger the bubble, the more the number of differential protein-coding genes contained in the entry. The bubble color changes from purple-blue-green-red. The smaller the enrichment pvalue, the greater the significance.

Thirdly, its effect on ethylene and Brassinosterol is more complicated. Among them, in the transduction pathway of ethylene, there was an inhibitory effect on the transduction mechanism from the endoplasmic reticulum to the nucleus, such as inhibition of CTR1 and MPK6. And there was a significant inhibitory effect on BRI1 in the process of brassin transmembrane into the cytoplasm.

Exogenous SA can improve the cold resistance of wheat, but its internal molecular regulation mechanism in wheat plants is still not clear, especially the cross-response pathway with other major hormones.

In the present study, the induction effect of SA on wheat cold resistance was stronger at lower temperature. Among them, SA treatment can improve the activities of SOD and CAT, especially at 0°C, the content of MDA treatment was lower than 15°C, and its effect on CAT activity was more obvious, which is basically consistent with the research results reported by Agwarwal *et al.* (2005). It indicated that exogenous SA had a stronger effect on improving the cold resistance of wheat in the selected time period and under the condition of lower temperature.

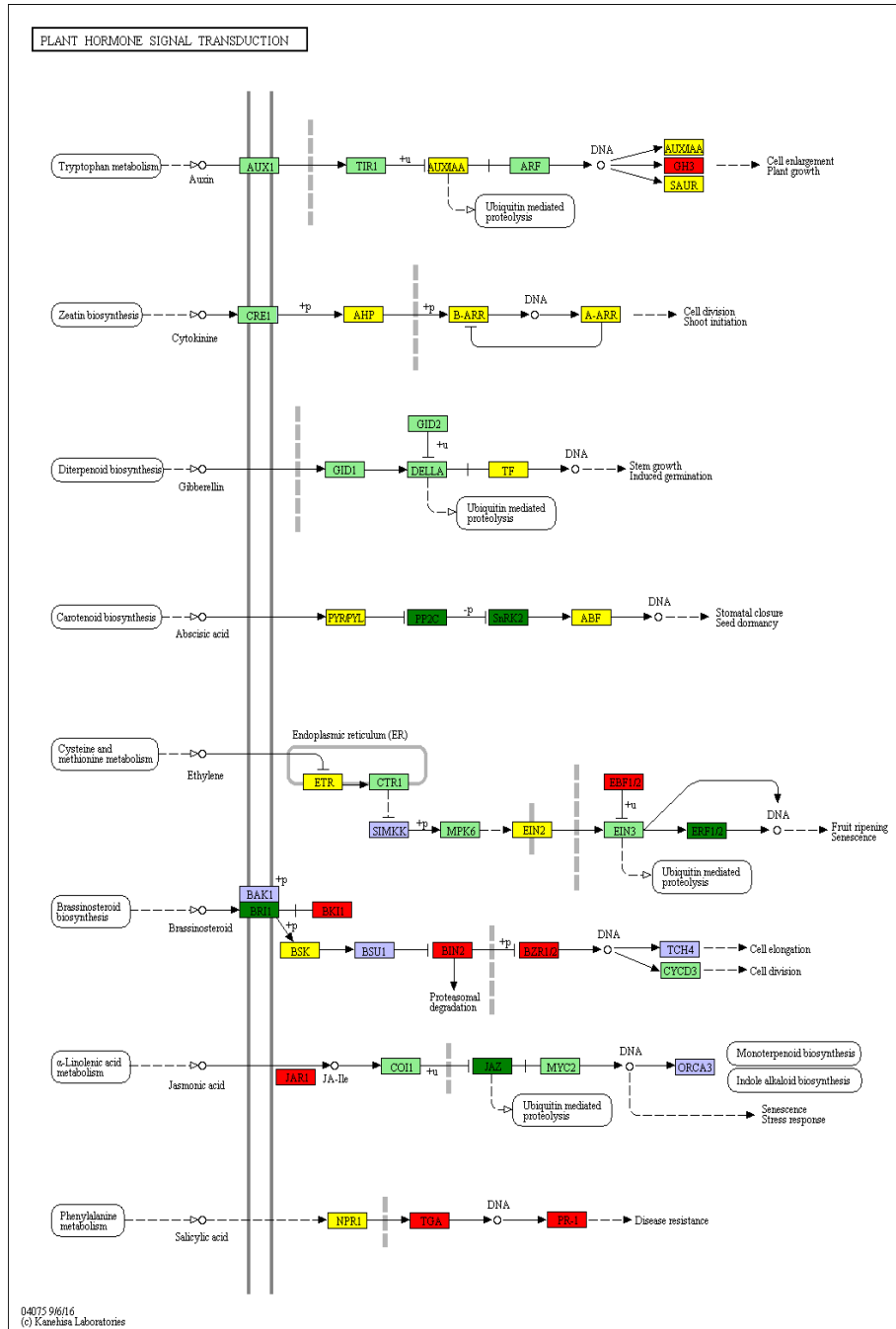


Fig. 4. The signal transduction pathway of wheat stress resistance hormone induced by exogenous SA under low temperature. On the KEGG pathway map, red indicates up-regulated genes, green indicates down-regulated genes, and yellow indicates that the corresponding genes are both up-regulated and down-regulated. Putting it on the corresponding protein-coding gene will automatically display the gene annotated with the protein-coding gene and the corresponding FC value and up-regulation information.

Furthermore, with the decrease of temperature, the effect of SA on the expression of wheat cold resistance genes was enhanced. Through gene location and pathway analysis, it can be seen that the number of differentially expressed genes induced by SA increases at lower temperature, and the number of changes in biological processes was the maximum, while the binding and catalytic functions of molecular functions have much changes. This indicates that SA can improve the cold resistance of wheat by affecting the stability of wheat structure and the synthesis or decomposition of metabolites under low temperature conditions. In addition, SA can participate in the regulation of plant cell division, elongation, stomatal switch, fruit ripening and plant senescence, and plant resistance by regulating genes in different hormone response pathways such as GH3, CRE1, DELLA, PP2C, CTR1, BRI1, COI1 and TGA. Sickness and other activities (Rodriguez *et al.* 2014, Manohar *et al.* 2017, Setsungnern *et al.* 2020, Kim *et al.* 2022).

The present study focused on the molecular mechanism of SA-induced cold resistance in wheat. By analyzing the signal cross-response pathway induced by SA, the relationship between SA and the expression of different hormones and the changes in the metabolic balance of different hormones in wheat were preliminarily discussed and changes in physiological activities, and preliminary screening to identify several key regulatory genes. This study not only would provide favorable support for the application of salicylic acid in the actual cultivation and production of wheat, but also provide a reference for the breeding of cold-resistant wheat.

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