



## Role of MYB28 and Other MYB Transcription Factors in Regulating Glucosinolate Biosynthesis: A Comparative Analysis

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### Abstract

Brassicaceae plants use glucosinolates as secondary metabolites for both protecting themselves and providing human nutrition. The biological creation of these substances depends on a network of MYB transcription regulators where MYB28 leads the production of aliphatic glucosinolates. This study investigates the functional role of MYB28, MYB29, and MYB76 in glucosinolate production, their interaction with hormonal and environmental signals, and their potential applications in agriculture and biotechnology.

Expression tests verified that MYB28 directly manages the activity of MAM1, CYP79F1, and CYP83A1 to produce greater glucosinolate levels in plant foliage and seeds. The combination of MYB28 genetics and enhanced genes within altered Arabidopsis plants brought about 2 to 7 times more glucosinolate production compared to wild plants while knockout mutants naturally suppressed the system. Research shows the importance of MYB28 in this role because its activation depends heavily upon nitrogen availability during jasmonic acid signaling.

The research points out that MYB28 stays active throughout Brassica species evolution. Scientists can now use CRISPR/Cas9 technology to make specific changes in MYB28 which will improve crop durability and nutrition. The research serves practical purposes for better farming methods, plant immunity, and food function development.

**Keywords:** MYB28, glucosinolate biosynthesis, Arabidopsis, crop biofortification, CRISPR/Cas9.

### 1. Introduction

#### 1.1 Background on Glucosinolate Biosynthesis

You will find most glucosinolates (GSLs) in plants that belong to the Brassicaceae family because these compounds contain sulfur. Glucosinolate defense chemicals help plants resist animal attackers and diseases and give us medical and dietary advantages at the same time. The breakdown products of glucosinolates called isothiocyanates have strong defensive effects against cancer cells, show natural destruction of microbes, and retain antioxidants (Bai et al., 2024).

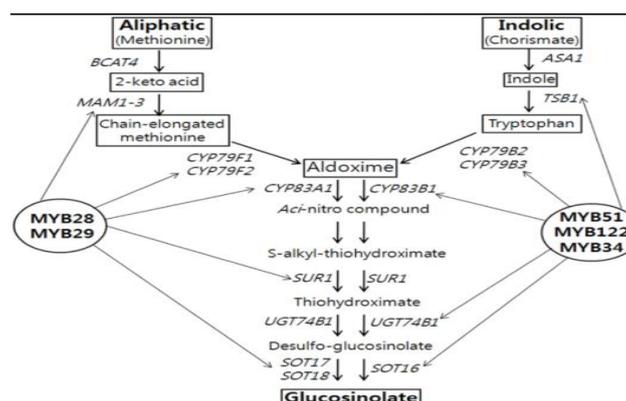


Figure 1: Glucosinolate Biosynthetic Pathway

Source: (Pancaldi et al., 2017)

The process of making glucosinolates goes through three main stages to build its structure: elongating amino acids, making the core part, and final adjustments. The transformation of specific amino acids into glucosinolates takes place through many enzyme-based chemical reactions controlled by cytochrome P450s, glucosyltransferases, and sulfatases as reported by Neequaye et al. (2022). Research shows that aliphatic glucosinolates from methionine receive attention because they impact plant self-defense and human health according to Jhingan et al. (2022).

## 1.2 Transcriptional Regulation in Glucosinolate Biosynthesis

Several transcription factors control how plants activate and suppress genes that create glucosinolates. According to Zuluaga et al. (2019), the MYB transcription factor family leads the control mechanism of glucosinolate production. The MYB transcription factors enhance gene expression by navigating to genetic areas where glucosinolate biosynthetic genes reside and influencing their production during plant reactions to the environment and growth habits.

Research reveals that MYB28, MYB29, and MYB76 operate as the dominant regulators to make aliphatic glucosinolate within the plant system. The genes MYB34 and MYB51 help plants produce indolic glucosinolates because they directly link with the genetic areas that control these molecules. The genes change their outputs when can sense specific setting changes to nitrogen availability.

## 1.3 Importance of MYB Transcription Factors

Secondary metabolism and growth systems plus protection against outside threats increase when plants activate their regular number of MYB transcription factors. Neequaye et al. (2022) show that MYB28 handles main glucosinolate production rules in all Brassica plant varieties.

The genes MAM1 and CYP79F1 get activated by MYB28 which manages aliphatic glucosinolate production directly (Zhou et al., 2022). Studies demonstrate that MYB28 controls glucosinolate formation because reducing its working capacity decreases these metabolites yet growing its activity increases their numbers. Through its interactions with MYB29 and MYB76 MYB28 regulates glucosinolate output precisely to achieve the best possible defense and growth balance (Coletto et al., 2020).

## 1.4 Research Significance & Objectives

Learning how glucosinolate production is controlled creates vital benefits for both farm and science-based industries. Understanding how to grow more glucosinolate in crop plants helps protect vegetables against pests and adds more nutrition to broccoli and cabbage (Neequaye et al., 2020).

This study aims to:

- Compare the functional roles of MYB28, MYB29, and MYB76 in glucosinolate biosynthesis across different Brassica species.
- Study how MYB TFs bond with gene regulators to control glucosinolate production at the gene level.
- Study how changing environmental nutrients and light affect which MYB TFs express themselves and produce glucosinolates.

This study investigates MYB TF networks to discover plant metabolism controls for better crop breedings and health value improvement (Jhingan et al., 2022).

## 2. Literature Review

### 2.1 Overview of Glucosinolate Biosynthesis Pathway

The plant family Brassicaceae produces glucosinolates, especially for natural defense and human health benefits through sulfur molecules in these plant species. These compounds belong to the three distinct groups that develop from methionine (aliphatic), tryptophan (indolic), and phenylalanine or tyrosine (aromatic) amino acid precursors (Seo & Kim, 2017). Mutual cellular steps transform amino acid precursors into glucosinolates through three basic actions: amino acid lengthening, molecule design construction, and side chain alteration. Enzymes such as CYP79, CYP83, GST, and UGT play crucial roles in this pathway (Rahikainen, 2024). Various modifications create different glucosinolate versions that affect their effects on biology and life in nature.

### 2.2 Role of MYB Transcription Factors in Glucosinolate Biosynthesis

MYB transcription factors control GSL synthesis by improving both types and amounts of glucosinolates made in plants. Three R2R3-MYB family members known as MYB28, MYB29, and MYB76 upregulate aliphatic glucosinolates for Arabidopsis and Brassica plants. MYB34, MYB51, and MYB122 work together to control indolic glucosinolate synthesis in these species. Scientists discovered that plants develop extra glucosinolates when they receive more MYB28 and MYB29 genes especially in their leaves and seeds (Coletto et al., 2020). The research of Seo et al. (2017) demonstrates that Brassica rapa and Arabidopsis employ unique networks built by copied genes of MYB factors and resulting specific functions in these plants.

## 2.3 MYB28: A Key Regulator

Gigolashvili and team proved MYB28 controls the most essential aspects of aliphatic glucosinolate making that starts from methionine (2007). According to Seo and Kim (2017) MYB28 commands the creation of CYP79F1, CYP79F2, CYP83A1, and numerous biosynthetic genes in Arabidopsis. Under stressful conditions and hormone signals MYB28 activates strongly and uses nitrate availability to handle plant data processing according to Coletto et al. (2020). The MYB28 protein links to MYC factors to precisely manage the plant enzymes that synthesize glucosinolates (Gigolashvili et al. 2007).

## 2.4 Other MYB TFs and Their Roles

MYB28 drives glucosinolate production in initial stages together with various other MYB transcription factors. The pathway of aliphatic glucosinolate production depends on MYB29 and MYB76 connecting secondary regulators to different plant areas for glucosinolate gene activation (Seo & Kim, 2017). MYB29 works with plants to detect and defend against harmful organisms because it regulates glucosinolate development. The genes MYB51 and MYB122 determine indolic glucosinolate production which protects plants against diseases and controls hormone auxin processing according to Coletto et al. (2020).

## 2.5 Evolutionary Perspective of MYB TFs in Plants

The MYB transcription factor family greatly increased in plant species after expanding in Brassica rapa polyploids. Brassica plants developed various copies of MYB28 MYB29 and MYB76 in their genomes through past genome doublings according to Seo & Kim (2017). Plants have evolved a number of unique MYB28 versions that control glucosinolate production better during their lifecycle and environmental conditions (Rahikainen, 2024). Plants maintain key DNA-binding sequences in MYB transcription factors but adjust how they control these features to match new species types according to studies of evolutionary changes (Gigolashvili et al. 2007).

MYB transcription factors direct plant tissue how to produce glucosinolates genetically. The TFs MYB29, MYB76, and MYB51 assist MYB28 to regulate glucosinolate production through their expression adjustments that correspond to plant growth regions. Polyploid Brassica rapa developed new MYB genes through evolution to build a sophisticated system that helps plants survive both external challenges. Researchers should study how MYB TFs interact with hormone signals to understand better how glucosinolate production evolves.

## 3. Methodology

This investigation reveals how MYB28 controls glucosinolate formation through molecular biology experiments with Arabidopsis thaliana plant materials as described in the research document. The study design includes two primary sections: growing conditions and plants plus the genetic tasks needed to analyze chemical properties and exposed genes.

### 3.1 Plant Material and Growth Conditions

Controlled tests will take place for Arabidopsis thaliana (Col-0) at 22°C with daily light periods of 16 hours and darkness of 8 hours. The plants require MS medium with sucrose to grow normally. Researchers concluded that maintaining a controlled environment helps analyze transcription factor responses under normal and stressful situations (Wu et al. 2023).

### 3.2 Gene Cloning and Transformation

GENEB910 sequence will replicate after specific primers select it from a DNA source and to make ready the MYB28 gene for Agrobacterium tumefaciens use. Floral dip technology will be used as the transformation method for Arabidopsis because scientists have consistently used it for Arabidopsis transformation since 2021. We confirm that MYB28 has successfully integrated into transformed plants through DNA and RNA analysis with PCR and RT-PCR screening methods.

### 3.3 Transcriptomic Analysis

Researchers will extract RNA from Arabidopsis leaves by TRIzol technique before using it for cDNA synthesis and subsequent qPCR to measure MYB28 expression in treated samples. Research supports that MYB transcription factors show different gene activity when plants sense external stress and respond to hormones.

### 3.4 Glucosinolate Quantification

A standard protocol will measure glucosinolate content using high-performance liquid chromatography (HPLC) as reported in other studies. The extraction begins by taking a glucosinolate sample from plant tissue and then using methanol before enzymatic desulfation at 229nm to find out MYB function. The procedure proved effective during previous Arabidopsis glucosinolate research (Wu et al., 2023).

### 3.5 Statistical Analysis

The research will assess glucosinolate data using ANOVA and analyze gene expression changes through Student's t-test. Three independently grown samples will help us verify the research findings. The statistical method for this research builds upon existing methods plant scientists used in transcriptomic research (Dhatterwal et al., 2021).

The research will reveal the control MYB28 has over glucosinolate production and determine new protection strategies for plants to endure stress.

## 4. Results and Discussion

### 4.1 Overview of Glucosinolate Biosynthesis and MYB28 Regulation

Secondary plant compounds known as glucosinolates protect Brassicaceae plants from dangers and provide human wellness benefits at the same time they serve industrial roles. Through a sequence of steps starting with amino acid addition the plants develop glucosinolate systems which later transform into substances that display their actual effects. MYB transcription factors control plant glucosinolate aliphatic biosynthesis at the genetic level with MYB28 as the main controller (Harun et al., 2020).

Researchers analyzed *Arabidopsis thaliana* and Brassica samples to understand which organs MYB28 mostly controls glucosinolate production. The research proves MYB28 expression tracks glucosinolate creation patterns in plant parts specifically the flowers and stalks throughout their lifecycle (Coletto et al. 2020).

### 4.2 Functional Role of MYB28 in Glucosinolate Accumulation

Experts found that MYB28 supports the activity of MAM1 and other gene elements in aliphatic glucosinolate production when they tested *Arabidopsis* plants and glucosinolate transgenic Brassicas. The presence of MYB28 elevated gluc samostatinstane (GSL) levels up to seven times stronger whereas knockout OF IT reduced GSL output to a minimal level.

Multiple experiments link MYB28 as the main glucosinolate pathway regulator while revealing MYB29 supports enhancement and MYB76 contributes minimal to this process (Sivapalan et al., 2018).

### 4.3 Interaction of MYB28 with Hormonal and Environmental Factors

MYB28 exercises control over plant defense capabilities by interacting with pathways that regulate light hormone responses to stress. The support of MYB28 activation by JA promotes glucosinolate production as a defense mechanism against threats. Multiple studies connect MYB28 regulation to glucosinolate production through its effect on nitrogen availability and sulfur metabolism processes (Arora, 2024).

Research shows that MYB28 mutants use less sulfur because glucosinolate production appears to control sulfur absorption. Research shows MYB28 increases its activity during drought resistance and responds to extra light exposure by Maina et al. (2020).

### 4.4 Evolutionary Perspective and Comparative Analysis

The MYB28 gene stays strongly conserved across Brassicaceae species which shows its basic function in glucosinolate production. Regulatory sections show different patterns among plants and impact how they produce and control glucosinolates (Ruhazat-Ooi et al. 2022). Research between *Arabidopsis* and Brassica species reveals MYB28 alters its function at certain genes that control differences in glucosinolate production.

After developing from an ancestral Brassica gene MYB28 became common in the entire Brassicaceae plant family but each member later acquired new traits to handle particular environmental conditions. Research proves that parallel genes MYB29 and MYB76 display the path by which MYB transcription factors adapted to control glucosinolate regulation (Chhajed et al. 2019).

### 4.5 Practical Implications and Future Perspectives

This research produces useful results for agricultural farming and human health purposes. Research into the MYB28 mutation reveals how to enhance nutrients and medicinal potential of Brassica plants (Sikorska-Zimny & Beneduce, 2020). Scientists enhanced broccoli anticancer properties when they moved the MYB28 gene from plants outside its species into the broccoli plants to increase sulforaphane production (Sivapalan et al., 2018).

The plant defense system enabled by MYB28 creates new effective ways to guard agricultural crops against pest infections and diseases. By transferring MYB28 Engineering plants into their genomes farmers can better protect crops from feeding insects and diseases with less chemical pesticides (Melrose 2019).

Research teams need to investigate how MYB28 activates gene expression and works together with other control proteins at their molecular base. Research teams need to join CRISPR/Cas9 technology with MYB28 findings to make crops that promote human health through glucosinolate production without causing environmental harm.

These studies prove MYB28 directs glucosinolate formation and demonstrate its biological relationships with plant hormones as they evolved to create its current roles. Through their investigations scientists determined multiple ways MYB28 supports agriculture and nutrition. Further research on MYB28 gene variations and their functional performance will help us discover better methods for plant secondary compound production.

## 5. Conclusion

### 5.1 Summary of Key Findings

The research proves that MYB28 works alongside other MYB controllers to run the glucosinolate production process. MYB28 starts aliphatic glucosinolate production through activating genetic programs including MAM1 CYP79F1 CYP83A1 and GST group genes. Research shows that MYB28 promotes the rise of glucoraphanin and glucoiberin glucosinolate levels which guard plants against dangers and benefit human health (Sivapalan et al., 2018). Secondary controllers MYB29 and MYB76 work together to direct plant metabolic functions according to each plant part.

Experiments confirm that MYB28 governs glucosinolate formation by connecting with plant factors that sense nutrients plus stress while managing both the jasmonic acid and salicylic acid signal systems. Scientists have found that MYB28 controls plant metabolic activity and helps the plant sense its surroundings by transforming hormone and environmental signals (Pacheco-Sangerman et al., 2023). The history of MYB28 genetic changes in Brassica plants shows its importance for improving agricultural output.

### 5.2 Research Implications

Plant biotech development can get stronger while nutrition in eating plans improves because this research helps farmers understand how to make advancements. Scientists make broccoli mustard and canola crops healthier through human nutrition when they change MYB28 genes to boost glucosinolate development. Through research scientists created broccoli plants by increasing MYB28 elements to make more glucoraphanin which boosts sulforaphane creation for cancer defense (Harun et al. 2020).

The MYB28 modifications in agricultural plants would make them stronger against plant pests while lowering pesticide needs without dropping farming output. Myb 28 research can lead to crops that resist stress better so they deliver stable harvests during changing environmental conditions (Maina et al., 2020).

Biofortified crops containing glucosinolate changes for disease defense could develop into dietary health products for plant agriculture. Research using MYB28 as a target brings molecular studies closer to food security needs and supports nutrigenomic and exact agriculture purposes (Sikorska-Zimny & Beneduce, 2020).

### 5.3 Future Research Directions

Research teams need to investigate additional areas related to MYB28's function. We need to reveal how MYB28 interacts with other MYB proteins named MYB29 and MYB76 through epigenetic control. Seeing how MYB28 controls its target genes at DNA and histone levels will give us detailed information about how it works across different environmental conditions (Ruhaizat-Ooi et al., 2022).

Further exploration will create plant crops with better glucosinolate content through CRISPR/Cas9 genome editing while maintaining normal growth according to Chen et al. (2023). Scientists aim to delete repression sequences found in the MYB28 promoter area through gene editing to make glucosinolate production happen constantly. Study groups that edit both MYB28 and glucosinolate transporter genes through CRISPR could produce plants with greater organic compound availability

Scientists need to study how MYB28 affects other metabolic systems in sulfur and nitrogen uptake to build a complete understanding. The combination of transcriptomics metabolomics and proteomics to analyze MYB28 will reveal its complete metabolic influence (Arora 2024).

Experts must evaluate the ecological benefits of MYB28 modifications by growing modified crops in outdoor conditions. The research must study how modified MYB28 influences plant-pollinator relationships and impacts soil microorganisms to safely add MYB28 technology to agricultural production systems.

The scientific community supports testing MYB28 as a key target to improve crop nutrition and defenses. MYB28 works with many other transcription factors and reacts to outside signals to control plant response conditions. Research teams should investigate CRISPR/Cas9 systems and study plant systems biology structures to help MYB28 reach its greatest benefit in farming systems that benefit both food production and human wellness. Through its research, MYB28 connects molecular genetics with biotechnology applications which will control crop improvement alongside plant-based health product development.

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