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GLUCOSINOLATES RELATED GENE REGULATION IN BRASSICA SPECIES

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Introduction

Plants provide mankind with diverse useful and important secondary metabolites and one of the prominent metabolite family is glucosinolates (GSLs) found in Brassicaceae (Cruciferae) family as a major defence component. Apart from being functionally important for plants, these are of considerable human interest being responsible for typical taste and various health benefits including cardiometabolic, neurological and musculo-skeleton conditions and certain cancers. Structurally GSLs have sulfonated oxime group linked to a thioglucose and an amino acid derived side chain. Hence according to the parent amino acid GSLs can be aliphatic, aromatic or indolic. Their concentrations define their nutritive and anti-nutritive value. They are relatively stable compounds but are activated by myrosinases as a response to any challenge to plant like tissue damage resulting in the formation of aglycones that spontaneously rearrange into toxic isothiocyanates (Fig. 1). GSLs are synthesised as a result of stress and as part of both pattern & effector triggered immunity. Different GSL profiles have differential effects on different herbivores and also the microbial pathogens.

Transcriptional regulators involved in glucosinolate biosynthesis

The well described transcriptional regulators known to regulate GSL biosynthesis belonging to R2R3 subfamily are MYB TFs. These are characterized by two N-terminal MYB repeats each containing three α-helices of which the third helix of each repeat co-ordinately mediates DNA binding to a specific sequence.

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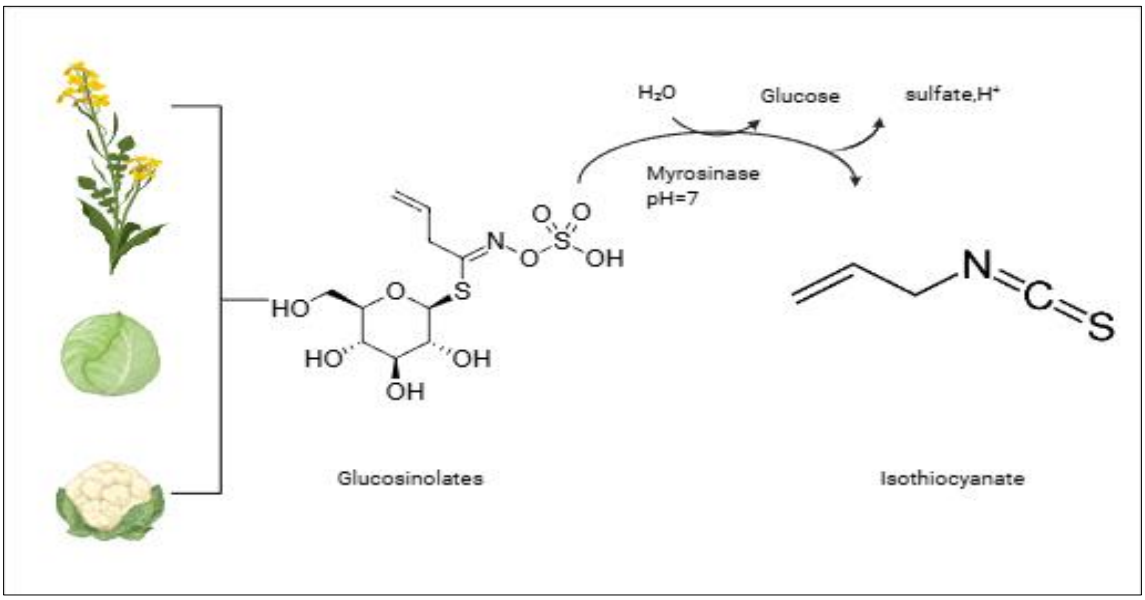

Fig. 1: Glucosinolates hydrolyzing mechanism

The R2R3 MYBs are further divided into subgroups according to sequence similarity that correspond with functional conservation. Six members of subgroup 12 are known to positively regulate genes responsible for GSL biosynthesis and connected reactions like amino acid synthesis and sulphur assimilation. There are three MYBs central to aliphatic and three to indolic GSLs. Aliphatic clad includes MYB28, MYB29, & MYB76 while indolic clade consists of MYB34, MYB51 & MYB122 (Fig. 2).

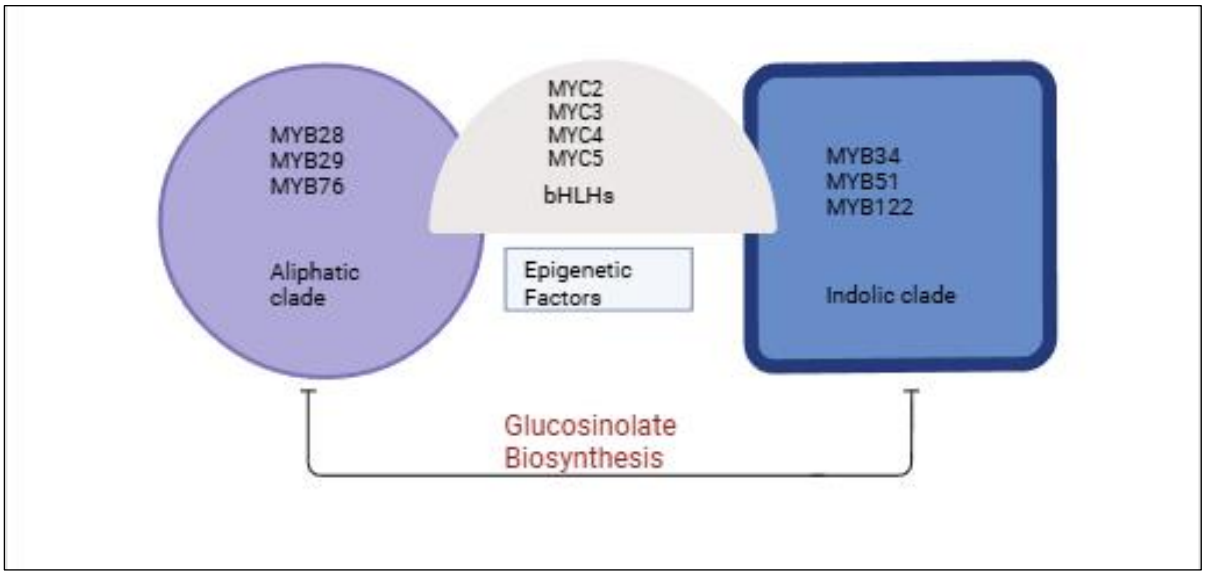

Fig. 2: Epigenetic factors involved in glucosinolates regulation

MYB28 & MYB29 are key aliphatic GSL regulators and MYB51 & MYB34 are key indolic regulators. Furthermore, the aliphatic and indolic clade work antagonistically towards each other to maintain homeostasis of GSL production. These clades display distinct tissue and elicitor expression, example MYB51 expressed in rosette leaves mediates PTI & ETI while

MYB34 & MYB122expressed in roots have minor contributions to PTI & ETI. MYB28 more strongly affects the production of long-chain GSLs than MYB29 & MYB76.RNA sequencing data study in *B. Napus* implied an ortholog of MYB29 in the regulation of benzenic GSLs. However, no specific regulators of benzenic and non-methionine aliphatic GSLs have yet been described.

Similar to subgroup 12, some members of the bHLH family are also important. The bHLH proteins are characterized by helix-loop-helix bHLH domain. These proteins can be categorized into 25-30 subgroups. Subgroup IIIe contains four TFs (MYC2/bHLH06, MYC3/bHLH05, MYC4/bHLH04 & MYC5/bHLH28) that interact with subgroup 12 MYBs by N-terminal MYB interacting region- JAZ interacting domain to regulate GSL biosynthesis (Table 1).

- MYB uncoupling can occur as a result of metabolic feedback.
- Brassinosteroid dependent factor BES1 competitively attenuates bHLH function.
- SLIM1 (EIL family) act as negative regulators under sulphur limitation; can downregulate MYB29 & MYB76and controls GSL suppressors SDI1(inhibits MYB28) & SDI2.

Table 1: Genes regulated in different Brassica species

SPECIES	CROP NAME	GENES COVERED
Brassica juncea	Indian mustard	MYB28,MYB29, MYB34,MYB51, MYB122
Brassica napus	Rapeseed	MYB28,MYB29, MYB34,MYB51, MYB122
Brassica nigra	Black mustard	MYB28
Sinapis alba	White mustard	MYB28,MYB29, MYB 34, MYB51

Hormonal and biotic influences

These three types of signaling generally behave antagonistically towards each other. Gibberellic acid (GA) enhances SA signaling and may have positive or negative effects on GSL accumulation (SA synergistic to Glu while antagonist to JA). Brassinosteroids (BR) antagonize

JA signaling and negatively affect GSL accumulation. IAA can also have an indirect negative effect on GSL accumulation due to mutated SUR1 and UGT74B1 genes. Apart from these abiotic factors like osmotic stress, high salinity, soil pH, temperature, photoperiod, light intensity & quality, heavy metals, O_2/CO_2 concentrations, glucose signaling and mineral nutrients influence GSL biosynthesis.

Epigenetic regulation

The priming of the defence mechanism makes responses more rapid & robust. Priming memory is thought to be stored epigenetically. Like, isothiocyanate GSL hydrolysis product sulforaphane is a priming compound. It is likely that GSL biosynthesis is subject to specific epigenetic regulation with GSLs themselves participating in this response however any mechanism still remains unknown.

Conclusion

In this constantly changing environment, plants develop survival strategies relying on gene regulation by TFs. GSLs have been model metabolites to study the plant responses. Although the GSL synthesis regulation is quite well understood but future research is awaited to understand GSL transport and accumulation in specific structures and how is it modulated. Also, additional regulatory proteins and epigenetic mechanisms require more focus for understanding of the assembly of regulatory complexes. Thus, providing a better insight of how plants engineer GSL biosynthesis in diverse environmental conditions.

Reference

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