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Review

Saponarin, a Di-glycosyl Flavone from Barley (*Hordeum vulgare* L.): An Effective Compound for Plant Defense and Therapeutic Application

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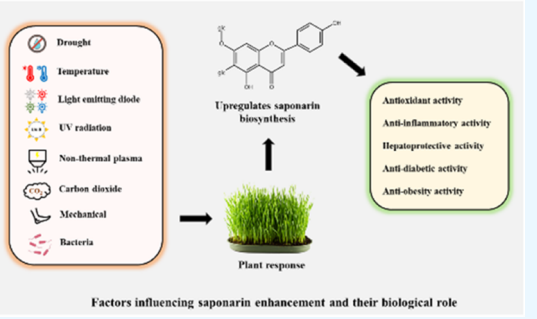
ABSTRACT: Saponarin (SA) is a major di-*C*-glycosyl-*O*-glycosyl flavone, which is predominantly accumulated in the young green leaves of barley (*Hordeum vulgare* L.), with numerous biological functions in plants, such as protection against environmental stresses. Generally, SA synthesis and its localization in the mesophyll vacuole or leaf epidermis are largely stimulated in response to biotic and abiotic stresses to participate in a plant's defense response. In addition, SA is also credited for its pharmacological properties, such as the regulation of signaling pathways associated with antioxidant and anti-inflammatory responses. In recent years, many researchers have shown the potential of SA to treat oxidative and inflammatory disorders, such as in protection against liver diseases, and reducing blood glucose, along with antiobesity effects. This review aims to highlight natural variations of SA in plants, biosynthesis pathway, and SA's role in response to environmental stress and implications in various therapeutic applications. In addition, we also discuss the challenges and knowledge gaps concerning SA use and commercialization.



Factors influencing saponarin enhancement and their biological role

INTRODUCTION

Barley (*Hordeum vulgare* L.) belongs to the family Poaceae (Graminaea), and is one of the leading global staple crops due to its wide range of nutrition and medicinal uses.[1,2] Although barley has been used as a food grain since the ancient times, until recently, the nutritional value of young barley leaves (YBL) was overlooked despite harboring significant metabolites, such as vitamins, minerals, chlorophyll, and flavone glycosides.[3,4] The green YBL also accumulates abundant saponarin (SA), which is a unique flavone with both *C*- and *O*-glycosides (apigenin-6-*C*-glucosyl-7-*O*-glucoside or isovitexin-7-*O*-glucoside) (Figure 1). The genetic variation of SA in plants and its biological implications have led to industrial demand for its extraction from YBL in Korea, Japan, and other parts of world.[5,6] However, the level of a single SA compound in YBL can greatly vary with environmental conditions and growth stages.[7−10] Numerous previous studies have shown the significant capacity of SA to counter the effects of severe environmental stresses, such as drought, heat, and erratic temperature fluctuation. Additionally, exposure to ultraviolet (UV) and light-emitting diode (LED) radiations resulted in elevated SA production in YBL, which was accompanied by increased and steady transcript levels of the corresponding SA biosynthetic genes.[11−14] Moreover, the application of non-thermal plasma and mechanical stresses, as well as elevated carbon dioxide (eCO_2) have been reported to upregulate the SA level and enhance its antioxidant activity.[15−17]

Flavonoid biosynthesis is one of the most extensively explored metabolic pathways in numerous plant species. However, the enzymes and several steps involved in the biosynthetic routes of *C*-glycosyl flavones still remain largely

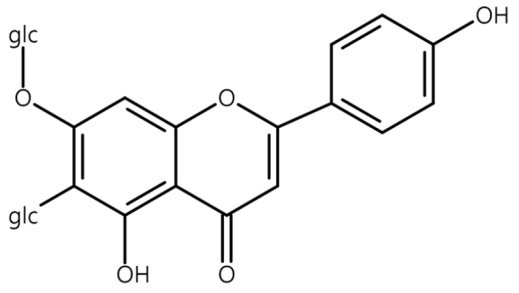


Figure 1. Chemical structure of saponarin ($C_{27}H_{30}O_{15}$).

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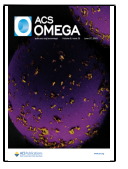

Table 1. Saponarin Accumulation and Natural Variation in Plants

plants source	tissue	content	references
barley (*Hordeum vulgare L.*)	leaves	1,143 mg 100 g^{-1}	Seo et al. (2014)[23]
Hibiscus syriacus	flower	0.76 mg cm^{-2}	Yoo et al. (1996)[6]
Phalaenopsis orchids	flower	5 g	Lam et al. (2019)[32]
Gypsophila trichotoma Wend.	aerial parts	2 g	Simeonova et al. (2011)[5]
Tinospora cordifolia Miers	leaves	45.5 mg g^{-1}	Sengupta et al. (2009)[33]
Gentiana piasezkii	aerial parts	53 mg g^{-1}	Wu et al. (2010)[34]

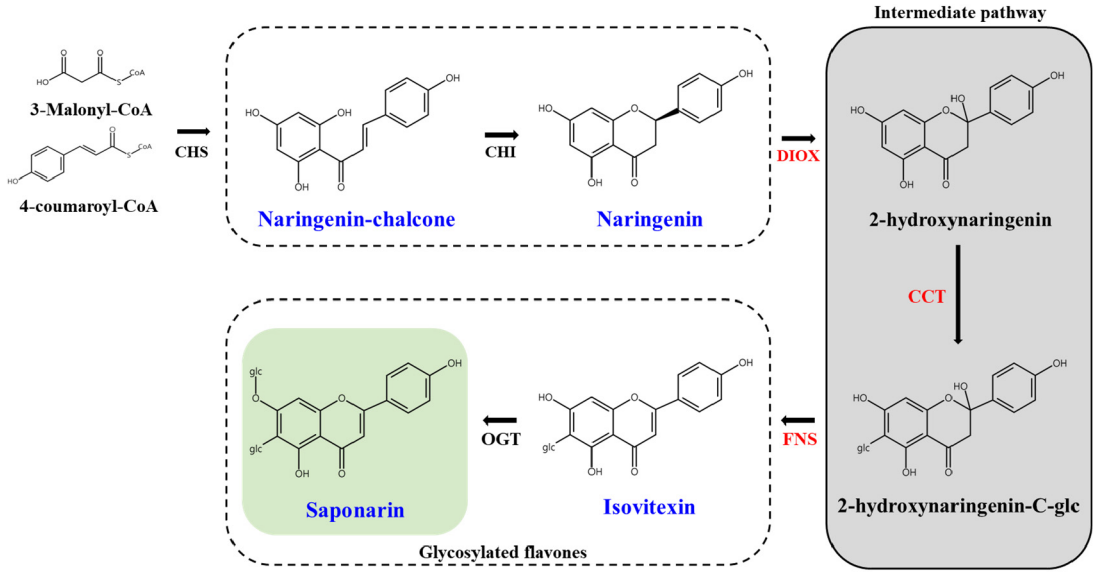

Figure 2. Schematic representation of saponarin biosynthesis pathway in barley. The malonyl-CoA and 4-coumaroyl-CoA are condensed to form naringenin via CHS, followed by a ring closure reaction by CHI. The further successive step involved in the formation of isovitexin flavone remains unelucidated up to now. OGT catalyzes competing reactions to generate saponarin by adding another sugar substituent to the 7-*O* position of the isovitexin backbone. Enzyme abbreviations: CHS, chalcone synthase; CHI, chalcone isomerase; CGT, DIOX, dioxygenase; UDP-Glc-dependent *C*-glucosyltransferase; FNS, flavone synthase (hypothetical), OGT, UDP-Glc:Flavone-7-*O*-glycosyltransferase.

unknown in plants.[8,18] The di-*C*-glycosides biosynthesis and *C*-glycosyltransferase (*CGTs*), which catalyze glycosylation were first reported in citrus fruits (*UGT708G1* and *UGT708G2*).[19] The role of a bifunctional *UGT708A6* *C*-/*O*-glycosyltransferase in catalyzing the synthesis of both *C*- and *O*-glycoside from 2-hydroxyflavanones and flavanones, respectively, was previously demonstrated in maize.[20] Despite knowledge on the activation of SA synthesis and activities of their key *O*-glycosyltransferases (*OGT*) enzymes in response to various environmental factors in YBL, the flavonone intermediate pathway at the internode for the SA percussor molecule, isovitexin synthesis and its enzymatic regulation are yet to be elucidated.[21,22] The bioactive polyphenol SA accumulated in the YBL has been reported to exhibit various pharmacological properties, such as anti-inflammatory response, by modulating cytokines expression,[23,24] lowering the blood sugar level, insulin resistance, postprandial blood glucose control in diabetics,[25,26] hepatoprotective activities against the *in vivo* and *in vitro* alcohol-induced liver damage,[27,28] and antiobesity by suppressing the adipocytes growth and differentiation.[29,30]

In this review, we first briefly discuss about the natural variation of SA in plants and SA biosynthesis, and subsequently explore the existing studies on SA roles, including responses to environmental stress and therapeutic benefits. Furthermore, we highlight the obstacles and research gaps that remain to overcome in SA use and commercialization.

NATURAL SAPONARIN VARIATION IN PLANTS

Availability of natural genetic variations is a key contributor of crop genetic improvement due to their significant effects to the metabolic properties of bioactive compounds, such as anthocyanins and flavonoids.[31] The increase in accumulation of plant metabolites can be achieved by agronomical strategies in a variety of crop species that are naturally unable to produce optimal levels of biochemical compounds with beneficial effects on plant fitness and human health. Of natural secondary metabolites, SA accumulation in the leaves, flowers, and other parts of plants depends on a series of complex processes, such as biomodification (by glycosyltransferase), transportation, and localization. Additionally, diverse environmental stresses can greatly accelerate SA synthesis. However, the currently available literature reveals only limited information about SA variation in plants. Cereal plants, such as maize, rice, wheat, barley, sorghum, and millets are major staples to millions of people worldwide. Among these staple crop species, barley has been reported to predominantly synthesize SA (ca. 1143 mg per 100 g), especially in the YBL.[23] This indicates that SA is not only a major biochemical compound in certain immature leaves, but also points to its roles in specific defense mechanism in the YBL. Other than the vegetative leaves, variation in SA accumulation in other barley plant tissues still remains unreported.

The distribution of SA in other plant species has also been documented. For example, in horticultural shrubs, *Hibiscus*

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syriacus Linnaeus (Mugunghwa) was shown to accumulate up to 1.528 mg cm^{-2} total flavonoids in flowers, and its major component (more than 50%) was identified as SA.[6] Similarly, a substantial 5 g of SA content and other secondary metabolites was isolated from the extracts of fresh flowers of *Phalaenopsis* (moth orchids) hybrids.[32] In perennial herbaceous plants, *Gypsophila trichotoma* Wend. (Caryophyllaceae) well-known for their medicinal and industrial application was reported to accumulate as high as ∼2 g of SA.[5] The newly emerged leaves of the predominantly tropical *Tinospora cordifolia* Miers from the Menispermaceae plant family showed a dramatically higher SA level of 45.5 mg g^{-1} than the mature and old yellow leaves.[33] Moreover, *Gentiana piasezkii* (Gentianaceae), which is used as a folk herbal medicine for various diseases, was shown to accumulate 53 mg of SA.[34] Additional information on saponarin variation in barley and other plants is summarized in Table 1.

SAPONARIN BIOSYNTHESIS

Flavonoid biosynthesis occurs through multiple and highly controlled transcriptional and enzymatic regulation mostly in the cytosolic face of the endoplasmic reticulum cell organelle.[35,36] The biosynthesis is derived from the general phenylalanine pathway through a series of complex reactions, including condensation, isomerization, oxidation, and reduction. In the initial step, chalcone synthase (CHS) enzyme catalyzes the sequential condensation of three acetate molecules of malonyl-CoA and one molecule of 4-coumaroyl-CoA to form the first flavonoid naringenin chalcone.[37] The substrate naringenin chalcone is then rapidly isomerized by chalcone isomerase (CHI) enzyme to naringenin, which is a central intermediate flavanone precursor compound of different classes of flavonoids, such as flavone, flavanone, flavonols, and anthocyanin.[38] Interestingly, the subsequent steps and *CGTs* enzyme involved in the formation of flavone mono-*C*-glycosides isovitexin (apigenin 6-*C*-glucoside) from the percussor flavanones in the barley leaf tissues have not been established to date. However, the complex formation of isovitexin from naringenin has only been adapted from buckwheat.[39,40] Only trace amounts of isovitexin accumulate in barley, which is immediately processed to SA di-*C*-glycosyl-*O*-glycosyl by the addition of another glycosyl substituent group to the 7-*O* position of isovitexin backbone through the UDP-Glc:Flavone-7-*O*-glycosyltransferase (*OGT*) enzyme (Figure 2). Since this last step is essential for further decoration of the sugar moieties and stable accumulation of the SA compound, *OGT* is regarded as a crucial enzyme in the pathway due to its role in enriching SA flavone. The barley genome contains two classes of *OGT* (*OGT1* and *OGT2*) genes, and changes in the expression levels of *OGT1* were shown to be strongly correlated with SA accumulation, which suggested its likely involvement in the SA biosynthesis.[13,22] The glycosylated SA and isovitexin can subsequently be transported across the tonoplast from the cytoplasm into the mesophyll vacuole by flavone glucoside/H^+- antiporter by the activity of H^+-ATPase and pyrophosphatase vacuolar proton pumps.

Previous studies have demonstrated a high association between increased naringenin accumulation due to CHI activity and SA production in barely plants. Analysis of the flavonoid biosynthetic pathway via the vacuolar transport system using a mutant barley *anthocyanin-less310* (*ant310*), which contains 5% less flavonoids relative to the wild type Ca33787, revealed no CHI activity in the *ant310* mutant, while the vacuolar SA transport was also greatly reduced in the primary leaves of the mutant plants.[12] In contrast, exogenous application of naringenin could rescue SA glucoside transport activity within 5−6 h in the leaves of *ant310*.[21] Moreover, a strong positive relationship was observed between the expression of the *chalcone synthase 1* (*CHS1*) gene and the SA content in "Kunalbori1" and "Heukdahyang" barely cultivars 3 days after germination.[41] This might suggest that a complete flavonoid pathway is necessary for SA synthesis and vacuolar flavonoid/H^+-antiport activity.

Despite extensive studies on YBL, the regulatory mechanism and candidate genes for *C*-glycosyl- and *O*-glycosyltransferases are yet to be reported. SA is highly associated with major physiological and biological processes in both plants and humans, therefore, understanding the potential mechanisms for its improved biosynthesis or response to various environmental conditions is necessary for the breeding of YBL with elevated SA content.

DEFENSIVE ROLE OF SAPONARIN PRODUCTION AGAINST ENVIRONMENTAL STRESSES

Plants have evolved extensive response mechanisms for protection against injury from deleterious effects. Increased biosynthesis of polyphenols and flavonoids occur in plants as defense molecules in response to extreme conditions.[42,43] As a high protective and nutritive biochemical compound, numerous efforts have been made to induce the accumulation of SA and other novel functional secondary metabolites under diverse environmental conditions (Figure 3).

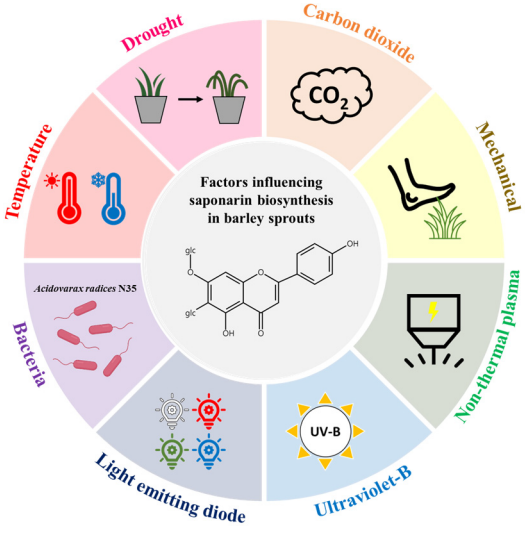

Figure 3. Effect of environmental factors on saponarin accumulation in young barley leaves.

Drought. Barley is mainly cultivated under irrigated or rainfed conditions due to its susceptibility to drought, particularly during the grain filling stage. Maintenance of normal cellular physiological processes through osmotic adjustment is one of the primary adaptive mechanisms of plants to drought stress (DS).[44] Plants can alleviate drought stress by producing functional secondary metabolites, such as phenols and flavonoids, which are key reactive oxygen species (ROS) scavengers.[45,46] The changes in SA accumulation in YBL in response to DS at 5, 10, and 15 cm leaf length showed that DS

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Table 2. Effects of Abiotic Elicitors on Saponarin Production in Young Barley Leaves

no.	accession	type of stress	key finding	reference
1	Keunalbori No.1	Drought	Enhanced the principal compound SA accumulation about 10−19%	Yoon et al. (2021)[14]
2	Keunalbori No.1	Temperature 4 and 37 °C	Higher production of SA compound (10−14%), by maintaining the *HvOGT1* gene expression level than *HvCHS1* and *HvCHI*.	Lee et al. (2019)[22]
3	Haemi Keunalbori No.1 Bojos Atlas 46	LED-blue light (100−226 μmol m^{-2} s^{-1})	Provoked the photoprotection changes in YBLs, resulting in 2 to 3-fold elevated SA level through activation of enzymatic ROS scavenging machinery.	Chung et al. (2019)[56] Muthusamy et al. (2020)[13] Pech et al. (2022)[57] McClure and Wilson, (1970)[11]
4	Atlas 68 Hege Barke Express Ca 33787	UV-Blue light (280−320 nm)	Accelerated the SA accumulation about 40% in lower epidermis and mesophyll that involves in screening and serves as protectant against peroxidative damage under UV−B radiation.	Liu et al. (1995)[65] Reuber et al. (1996)[12] Kaspar et al. (2010)[70] Zancan et al. (2008)[72] Schmitz-Hoerner and Weissenböck, (2003)[71]
5	Barke Var	CO_2 (200−700 ppm)	Together with high and low light promoted the flavones (SA, LO, isovitexin and homoorientin) accumulations about 15−20% in the mesophyll cells than the epidermal cells.	Hunt et al. (2021)[17] Hong et al. (2019)[75]
6	Saechalssal	Nonthermal plasma (130 and 0.8 μL L^{-1}) for 6−12 min	Triggered the plant growth and target compound SA level by 1.5-fold, including GABA, and ploicosanols that stimulated the antioxidant defense system.	Song et al. (2020)[16]
7	Distichon	Mechanical stress (250 g)	High free radicals scavenging capacity mainly due to the increase in SA content from 10−24%, as well as glutamine, asparagine, and leucine composition.	Koga et al. (2013)[15]

application to the 10 cm length leaves for 1 or 2 days could considerably enhance SA content by 10 or 12%, respectively.[14] In addition, a more substantial increase in SA content (19%) was observed when DS was applied to the 5 cm leaves, which indicated that drought could provoke SA accumulation during sprouting rather than reproductive stage. The analysis of mRNA expression levels of SA biosynthetic pathway genes, including *HvCHS1*, *HvCHI*, and *HvOGT1* revealed a drastic decrease in the expression levels of *HvCHS1* and *HvCHI* in the 15-day-old barley seedlings after 1−2 days of DS exposure, while *HvOGT1* expression remained stable.[22] Similarly, numerous studies have demonstrated the increase in accumulation of functional biochemical compounds in plants due to DS. For example, a 6-day barley leaf sample exposed to DS exhibited a high ratio of three flavonoids with different glycosidic residues throughout the entire experimental duration.[47] The total phenolic contents and antioxidative flavonoids showed considerably elevated levels under DS treatment in nine accessions of "Ardhaoui" barley landraces compared to the control.[48] Moreover, LC-MS analysis of 100 barley recombinant inbred lines showed a significant accumulation of glycosylated flavonoids after drought treatment, which could serve as antioxidants and modulators of gene as well as protein expression.[49]

Temperature. Secondary metabolites, such as flavonoids play key roles in coping with oxidative stresses induced by abiotic stresses, and their production is significantly associated with temperature.[50] For example, significant sensitivity of SA production to temperature change has been reported in barley. The ultrahigh performance liquid-chromatography (UHPLC) analysis of SA content in barley seedling exposed to low (4 °C) and high temperature (37 °C) revealed that both temperature conditions could significantly improve SA content by 10% and 14% compared to the control condition.[22] Similarly, mRNA expression analysis of SA synthesis genes in barley revealed a stable expression of *HvOGT1*, which is a key gene involved in the catalysis of the first step of SA biosynthetic pathway. Higher accumulation of phenolics mainly consisting of SA was detected in barley seedlings at low temperature (5 °C), but seedling growth showed an opposite trend.[51] Moreover, this study elucidated that peroxidase and phenylalanine ammonia-lyase activated more at low temperature were responsible for SA accumulation in barley seedlings. On the other hand, adaptation of barley plants under high temperature has been observed by Mikkelsen et al.[52] In addition to SA, high temperature exposure also increased the accumulation of other flavonoid families, such as lutonarin in barley, which suggested their potential involvement in the plant response to unfavorable temperature conditions.[52]

LED. Light is one of the most important environmental factors that regulates plant growth and development by providing chemical energy and carbon gain via photosynthesis. It can also trigger plant defense systems by inducing secondary metabolite biosynthesis under unfavorable growth conditions.[53] Artificial lighting resources, such as LEDs, are simple, precise, and with comparable spectral quality as well as light intensity to the traditional lighting system, which has enabled their wide application in analyzing the effects of particular light on the concentration of secondary metabolites.[54,55] Exposure to LED light showed different effects on the quantity of SA in barley sprouts depending on the light quality. For example, UHPLC analysis revealed increased SA production in barley sprouts exposed to the blue LED light radiation for 20 min 1 day before harvest relative to the control plant grown in the dark.[11] This observation was attributed to possible change in the photoprotection system in YBL as a result of the blue LED light. A 2−3 fold increase in SA levels with elevated antioxidant potentials was observed in barley leaves after 8 days when exposed to the blue LED light (226 μmol m^{-2} s^{-1} with 450 nm), which was in contrast to other lighting conditions, including red, far-red, fluorescence, and dark (Table 2).[56] Similarly, SA production differed with the light quality and growth period, with the blue LED light illumination significantly improving SA production (57.7%) across all

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growth stages in all illumination periods.[13] The highest SA content in barley sprouts was detected 3 days after blue LED light illumination, while sprouts exposed to red light displayed a considerable reduction in SA content compared to the controls after 3, 5, 7, and 9 days of illumination. In contrast, an inverse correlation was observed between SA content and increased growth periods, and the high SA content in sprouts started to decline 3 days after sprouting in all the LED light conditions. The study also found an association between increased SA levels at each growth condition and the changes in expression levels of the *HvOGT1* gene, while the blue LED light irradiation could increase SA accumulation in barley sprouts by upregulating the expression levels of the *OGT1* gene. Compared to ultraviolet A (UV-A) and other light sources, the blue LED light was demonstrated as the most effective light in promoting SA accumulation, and accounted for approximately 78% of the total phenolic content, which enhanced the activities of antioxidant enzyme, such as superoxide dismutase (SOD) and ascorbate peroxidase (APX).[57] In addition, the LED blue illumination supplemented with 0.1% boric acid could significantly upregulate the SA level in the YBL, and the *in vitro* application of the SA obtained from this treatment displayed a potential inhibition of total lipid content in the 3T3-L1 adipocytes and HepG2 hepatocytes cells, which are associated with fat accumulation.[58] Moreover, barley leaves treated with the blue LED light showed an increasing trend in total phenolic contents with greater ROS scavenging capacity compared with other LED light treatments.[59,60]

Ultraviolet-B Light Irradiation. A high level of Ultraviolet-B (UV−B) light has a strong negative impact on plant growth and development, mainly due to high accumulation of ROS leading to DNA damage.[61−64] Interestingly, numerous previous reports have demonstrated that plants have evolved different tolerance mechanisms to UV−B irradiation via several defense responses, such as changes in plant architecture. However, the most crucial tolerance factor is the accumulation of flavonoids, which can directly block excessive UV−B radiation and alleviate ROS toxicity to protect the plant mesophyll cells.[65−68] A previous study revealed that ca. 50% of flavonoids in barley leaves occurred in both the mesophyll and epidermis cells, with SA and lutonarin flavonoids showing marked increase in the barely primary leaves under UV−B light (280−320 nm) treatment for 5 to 25 days compared to control or UV-A light treatments.[69] Similarly, significant accumulation of SA in the barley leaf epidermis tissue but not the mesophyll was observed after UV−B light exposure.[70]

Comparative evaluation of the effect of UV−B irradiation on SA production using transgenic plants revealed that UV−B illumination could increase SA content by 26% on the primary leaves of the barley mother variety (Hege 550/75) compared to the control, whereas the flavonoid-deficient mutant (*Ant 287*) showed a significant decline in SA level and photosynthetic rate.[12] Similarly, the contribution of SA in the screening of YBL with DNA damage and repair under elevated UV−B irradiations was demonstrated using two barley lines, mutant (*ant30−310*) and its parent line (Ca 33787),[71] and the results showed that UV-induced accumulation of SA, which is a flavone glucoside, could protect the epidermal and mesophyll tissues by improving antioxidant activities and DNA repair mechanisms after UV−B irradiation damage (Figure 4).

UV−B exposure in combination with other treatments has been reported to increase the accumulation of saponarin

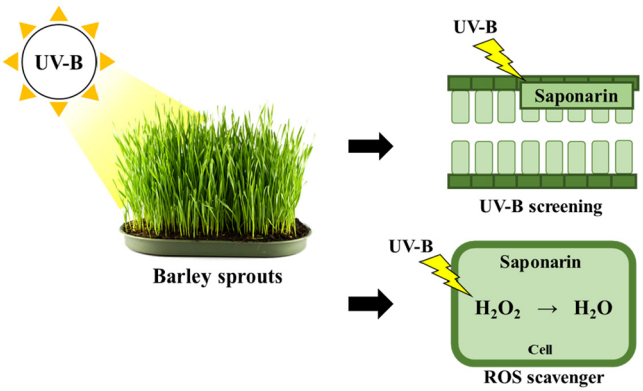

Figure 4. Screening and antioxidant roles of saponarin in young barley leaves under UV−B radiation.

flavonoids. For example, significantly increased SA accumulation was observed in the methanol extract of 8-day-old barley plants grown under UV−B radiation and supplemented with iron, while the photosynthetic pigments, ascorbate, and antioxidant enzyme levels, such as APX and catalase (CAT) were not altered.[72] In addition, UV−B light, particularly in combination with photosynthetically active radiation could increase flavones, such as SA and isovitexin, which are also closely associated with balanced carbon (C) and nitrogen (N) levels in the leaves.[73]

Carbon Dioxide (CO_2). Elevated concentrations of CO_2 (eCO_2) affects photosynthetic processes, and the growth of C3 plant species, which primarily occur due to the shift in the turnover of water and nutrients.[74] A previous study demonstrated the influence of atmospheric CO_2 concentration on phenolic compound accumulation and localization.[17] Barley plants grown under eCO_2 conditions could accumulate higher contents of phenolic compounds, especially SA, than the control plants, with predominant localization in the barley mesophyll rather than in the epidermis. Hong et al.[75] reported that pure supercritical carbon dioxide used for drying the methanolic extract solution of YBL could result in a 75% decrease and a 30% increase in chlorophyll and SA content, respectively, in comparison to the conventional evaporation method. eCO_2 treatment was also shown to mitigate oxidative stress induced by indium oxide nanoparticles (In_2O_3−NPs) in both young and old barley leaves, by enhancing production of antioxidants, such as polyphenols and flavonoids, which improved the antioxidant defense system and caused considerable increase in ROS scavenging enzymes, such as CAT, peroxidase (POD), and SOD.[76]

Nonthermal Plasma. Plasma is a partially, or fully ionized gas, with a mixture of electrons, protons, atoms, radicals, and several excited and nonexcited molecules produced at room temperature under atmospheric pressure.[77] Plasma treatment has been used in agriculture and food industries as a viable alternative to the conventional presowing seed treatments. In addition, activation of plant vitality and microbial inactivation are typically observed when strong electric discharge is applied to air or aqueous solution.[78,79] A single or double plasma exposure to fully hydrated barley seeds for 6 min during seed germination was shown to enhance the fresh weight of barley sprouts by 37% and 15.1%, respectively, relative to the untreated plants.[16] Under single exposure, primary metabolites, such as soluble sugars and amino acids increased by 3.5% and 31%, respectively, compared to the control. Moreover, total

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Table 3. Saponarin Immunomodulatory Activities

no.	biological effect	cell line/animal model	dosage range	key finding	reference
1	anti-inflammatory	RAW 264.7/RBL-2H3, HaCaT	40−100 μM	SA attenuated the NF-κB activation, MAPK ERK as well as p38 phosphorylation, decreased the *TNF-α*, *IL-1β*, *IL-4*, *IL-5*, *IL-6*, *IL-13*, *COX-2*, and *FcεRIα* expression.	Seo et al. (2014)[23] Min et al. (2021)[24]
2	hepatoprotective	Hep G2 (mouse and mice)	50−200 μM	Ameliorated the hepatic steatosis and hepatic triglyceride levels by activating AMPK and MTP, and suppressed the activities of AST, and ALT.	Kim et al. (2017)[27] Kim et al. (2020)[58]
3	antidiabetic	Hep G2 and TE671	100 μM	Modulated the gluconeogenesis and glucose uptake via activation of AMPK in a calcium-dependent manner.	Seo et al. (2015)[26]
4	antiobesity	3T3-L1/Hep G2	50−100 μM	Reduction of lipid and fat accumulation decreased TAG synthesis-related gene *DGAT1*, *GPAT*, and *AGPAT2* expression.	Kim et al. (2020)[58] Kim et al. (2022)[30]

polyphenols contents increased by approximately 9% under single exposure treatment, while two secondary metabolites, saponarin and policosanols, also showed a 1.5- and 1.9-fold increase, respectively, compared to the untreated control. However, triple plasma exposure resulted in negative effects on the growth of barley seedlings, soluble sugar content, and SA accumulation. Notably, γ-aminobutyric acid (GABA) content increased in all plasma treatments regardless of the exposure frequency. The above findings showed that plasma exposure could enhance the metabolic processes of barley sprouts by upregulating the bioactive phytochemicals such as polyphenols, SA, GABA, and policosanols, leading to an improved antioxidant system.

Mechanical stress. Barley stepping or treading is a common field farming practice in South Korea and Japan during winter season to improve growth and prevent freezing or excessive growth due to warmer weather. Stepping can induce mechanical stress or injuries to barley stems and leaves, suppress the above-ground growth of plant parts before winter, and promote tillering.[80] In addition, stress and wound caused by stepping can increase water transpiration and subsequent concentration of cell fluids, leading to improved cold tolerance. Barley stepping at 10 days after germination using a 250 g stainless-steel instrument as a model for examination was reported to increase SA (161 mg g^{-1}) and lutonarin (38 mg g^{-1}) contents in YBL,[15] and could significantly increase the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, indicating that increased accumulation of phenolic compounds, and several amino acids (e.g., glutamine, asparagine, proline, cystine, methionine) could activate the antioxidant system against ROS generated by mechanical stress.

Biotic Stresses. In the natural ecosystem, plants are constantly exposed to an array of pathogenic fungi, bacteria, and herbivorous pests, thus they have evolved defense mechanisms against these biotic agents. *C*-glycosyl flavones are key plant defense factors against abiotic stresses, and are also effective in imparting resistance against insects and fungal diseases, including *Verticillium alboatrum, Phytophthora parasitica*, and *Fusarium graminearum* to plants, such as maize and rice.[81−83] Phytochemical analysis of tolerance to powdery mildew in cucumber confirmed that *C*-glycosyl flavonoids act as phytoalexins in plants.[84] Likewise, using *Acidovarax radicis* N35 induced *N*-acyl homoserine lactone (AHL) rhizosphere bacterium wild type and the AHL *araI* negative mutant strain on root colonization, and the perception in the barley plant was demonstrated by Han et al.[85] The experimental outcome from this study revealed that the *A. radicis* N35 *araI* mutant strain significantly enhanced the accumulation of two-glycoside flavones SA and LO up to 100% in the barley leaves resulting from the root colonization as compared to wild type and uninoculated control plants. The study also detected increased expression levels of flavonoid biosynthesis pathway genes, such as *4-coumarate CoA ligase* (*4-CL*), *chalcone-flavonone isomerase*, *chalcone synthase* (*CHS*), *UDP-glycosyltransferase-like protein* (*UGT*), and *chaperone protein* (*DnaJ*) in the AHL deficient *araI* barley mutant. These findings revealed that biotic stress induced by AHL deficiency could trigger the synthesis of SA and LO glycoside flavones in the barley leaves, which demonstrate a classical example of a defense response activation mechanism. Combined transcriptomic and metabolomic analyses of the defense responses of Tibetan hulless barley against the powdery mildew infection showed that activated defenses could alter the accumulation of secondary metabolites, such as phenylpropanoids, phenolamides, flavone, and flavonoids.[86]

BIOLOGICAL ROLES OF YBL DERIVED SAPONARIN

SA is a natural antioxidant with several positive effects on biological systems (Table 3). As a therapeutic activator especially against various ROS-induced oxidative damages to cell lines, and animals, increasing efforts have been recently made to isolate SA from young green barley leaves (Figure 5). In the following sections we discuss the biological functions of SA derived from YBL.

Anti-inflammatory. Inflammation is a positive host response mechanism to a variety of diseases, foreign agents, or tissue injury, and it is a critical factor in tumor initiation, growth, invasion, and metastasis.[87−89] The anti-inflammatory capacity of SA has been extensively reported. A previous *in vitro* study showed that SA is a potential natural anti-

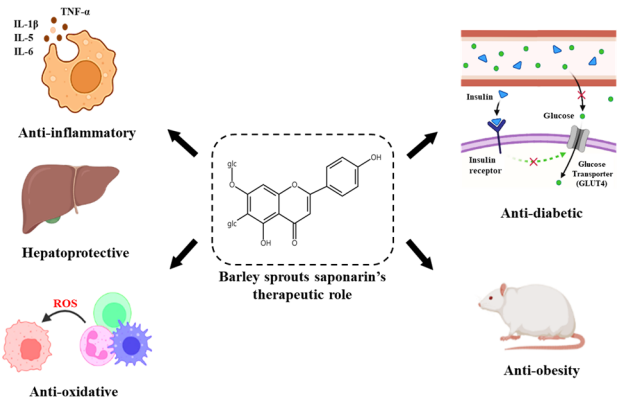

Figure 5. Therapeutic potential of saponarin against inflammatory disorders.

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inflammatory agent through blockage of the nuclear factor κB (NF-κB) activation and reduction of the mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) and p38 phosphorylation.[23] The anti-inflammatory and allergic responses of SA extracted from barley sprouts was recently demonstrated using RAW264.7, RBL-2H3, and HaCaT cells.[24] The result showed that SA treatment efficiently downregulated the mRNA expression level of important pro-inflammatory mediators genes, such as *TNF-α*, *IL-1β*, *IL-4*, *IL-5*, *IL-6*, *IL-13*, *COX-2*, and *FcεRIα /γ*, and also phosphorylation inhibition of ERK and p38 in the MAPK pathway in LPS-induced RAW264.7 and RBL-2H3 cells. At the same time upregulating the expression level of *hyaluronan synthase-3*, *aquaporin 3*, and cathelicidin in the HaCaT cells demonstrated the role of SA as a potential target in the prevention and mitigation of immune-linked skin disorders, such as atopic dermatitis.

Hepatoprotective Activity. Liver diseases, such as steatosis, hepatitis, and fibrosis/cirrhosis are mainly caused by alcohol intake, and they present serious health problems worldwide. Currently, there are still no food and drug administration approved drugs for the treatment of alcohol-induced liver diseases. The SA-rich barley sprout extract has been demonstrated to prevent inflammatory responses and suppress liver injury induced by chronic alcohol intake in cell lines and animal studies. For example, the low or high levels of SA were shown to modulate hepatic steatosis in mice with alcoholic fatty liver disease through multiple mechanisms.[27] First, SA application could enhance the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) that regulates the fatty acid oxidation, and inhibits fatty acid synthesis by phosphorylating target protein involved in energy metabolism. Second, SA could increase autophagic activity by AMP-activated protein kinase (AMPK) activation leading to the breakdown of intracellular lipid droplets. Third, SA could activate the microsomal transfer triglyceride protein that inhibits intracellular lipid accumulation. Similarly, the anti-inflammatory effects of SA-rich barley sprouts extract on experimental mice fed with alcohol for 4 weeks revealed that all the alcohol-induced changes, such as increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and tumor necrosis factor (TNF)-*α* levels were potentially suppressed by SA supplementation.[28] Moreover, SA could decrease the alcohol-induced nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX)-2 expression levels in LPS-stimulated RAW 264.7 cells. An *in vitro* study also showed that SA-rich barley sprouts extract could downregulate the total intracellular and free cholesterol concentrations in the HepG2 cells by activating the AMPK pathway and subsequently inhibiting the activities of hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) and sterol regulatory element-binding protein-2 (SREBP2).[90] Additionally, elevated SA levels in barley sprout extracts after blue LED light treatment during germination resulted in strong inhibition of total lipid accumulation in the 3T3-L1 adipocytes and HepG2 hepatocytes cell lines.[55] The first clinical study to determine the protective potential of YBL extract against alcohol-induced oxidative stress in habitual alcohol drinkers showed that in a randomized placebo-controlled group, the administration of 480 mg/SA-rich barley sprouts extract for 12 weeks could remarkably reduce liver enzyme levels by increasing glutathione-s-transferase activity in habitual drinkers.[91] Moreover, a plasma metabolomics study was used to investigate the association between ROS and lipid peroxidation inhibition and the improvement in hepatic cell damage and steatosis in habitual alcohol drinkers associated with fatty liver.[92] The above *in vitro* and *in vivo* findings showed that SA is a potential alternative primary or adjuvant therapeutic agent for reducing alcohol-induced liver injury or diseases.

Antidiabetic Activity. The potential capacity of *C*-glycosyl flavonoids to modulate glucose resistance is likely associated with its hypothesized *in vivo* preservation of glycosyl moiety due to its stable carbon−carbon glycosyl bonding.[93] Despite intriguing evidence of efficacy, the antidiabetic effects of SA have been rarely studied. Generally, YBL are known to be a rich source of magnesium (Mg) that acts as a cofactor for many enzymes involved in glucose metabolism and insulin secretion. In addition, the antidiabetic properties of YBL SA flavones have been determined through *in vitro* and *in vivo* studies. For example, the role of SA-rich barley leaves powder (BLP) on the postprandial blood glucose was *in vivo* analyzed in rats and healthy Japanese volunteers, and the results showed that BLP could significantly suppress the postprandial blood glucose increment in rats and human subjects by increasing digesta viscosity.[25] In addition, *in vitro* evidence showed that unlike in the untreated control, 100 μM SA could cause a substantial 56% decrease in glucose production in HepG2 cells, increase phosphorylation of both the cAMP response element-binding protein (CREB) regulated transcription coactivator-2 (CRTC2) and histone deacetylase-5 (HDAC5), and decrease the phosphorylation of nuclear CRTC2 as well as HDAC5, leading to suppressed gluconeogenesis.[26] In contrast, the mRNA expression level of glucose transporter-4 (*GLUT4*) gene associated with glucose uptake was significantly enhanced by SA in the TE671 cells. This is likely due to the fact that SA can activate the expression of *GLUT4* gene and regulate the hypoglycemic effects through activation of the CAMKK*β*-AMPK-CREB signaling complex in hepatocytes. Consistently, an *in vivo* pharmacological study also indicated that a 12-week SA administration to mice model could significantly improve their glucose tolerance and insulin sensitivity by suppressing the plasma IL-6 concentrations of the pro-inflammatory cytokine, without significantly altering the expression level of gluconeogenesis related genes, such as *fructose-1,6-bisphosphatase*, *pyruvate carboxylase*, *phosphoenolpyruvate carboxyl kinase*, and *glucose-6 phosphatase*, which suggested that glucose metabolism and insulin sensitivity could be regulated by other alternative mechanisms in the mice fed with SA.[87]

Antiobesity Activity. Obesity is widely recognized as a fundamental cause of various human diseases, such as high blood pressure, fatty liver, diabetes, and digestive disorders, and in particular, and it is a potential predisposing factor to cancer.[94−97] Various therapies and supplements for controlling obesity are currently available globally. The *in vitro* and *in vivo* analysis of the antiobesity effect of SA in YBL extract using 3T3-L1 preadipocytes cell and mice model, respectively, showed that SA could not only suppress adipocyte growth and differentiation, but also mitigate the effects of obesity-related metabolic diseases.[29] Consistently, a recent study using 3T3-L1 preadipocyte cells and mice model revealed that SA administration could significantly block the accumulation of triacylglycerol (TAG) by downregulating the fatty acid synthase (FAS) and decrease the expression level of TAG (*DGAT1*, *GPAT*, and *AGPAT2*) and FAS (*PPARγ*, *FAS*, *C/EBPa*, and *FABP4*) related genes in 3T3-L1 adipocytes cells and diet-induced obese mice.[30]

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Antioxidant Activity. SA is widely known for its potent antioxidant activity, which can protect vital cell components, such as DNA, lipids, and proteins and prevent chronic disease. Nishiyama et al.[98] reported antioxidative capacity of SA as α-tocopherol, a natural antioxidant, to inhibit the lipid peroxidation. In addition, a significant inhibitory effect of a flavonoids extract, comprising SA and LO in a 9:2 ratio, from YBL on MDA formation from cod liver oil, ω-3 fatty acids, phospholipids, and blood plasma was illustrated by Benedet et al.,[99] and the antioxidant effect of the YBL extract containing SA was far superior to that of other natural antioxidants, such as α-tocopherol and ethyl butylated hydroxy toluene (BHT). Moreover, assessment of the effectiveness of antioxidant activities of SA as well as antioxidant stimulants, such as α-tocopherol and BHT by squalene oxidation upon UV radiation revealed that 2 μmol mL^{-1} SA supplementation exhibited strong antioxidant activity by 100% inhibition of MDA generation from squalene, whereas 16 μmol mL^{-1} SA was needed for BHT to obtain a 75% inhibition effect. In contrast, α-tocopherol showed no appreciable antioxidant activity in the assay.[100]

CONCLUSIONS AND FUTURE PERSPECTIVE

Saponarin is emerging as one of the most important functional metabolites with multiple protective effects to plants and human health. The goal of this review was to compile the most recent accessible information on the beneficial effects of SA as well as a detailed assessment based on the available data. Modern agriculture is faced with increased pressure from the booming population and changing consumer market demand for nutrient-rich foods. SA can be an interesting target for breeding programs, and biotechnological applications that can provide improved nutrition and health is of great interest. From the available literature knowledge, it is clear that, among the food crops, barley is a predominant source of SA. So far, no large-scale germplasm screening was conducted to find the SA genetic variation in barley. Identification of SA-rich germplasm would serve as potential genetic resources to the further development of SA-rich barley genotypes and could provide genetic stock for use in molecular breeding programs. In addition, SA is regarded as an interesting target for barley biofortification programs; however, intermediate pathways and genes controlling their biosynthesis remain unexplored. Therefore, increased efforts to prioritize and rigorously explore the SA biosynthesis in barley using recent advances in genomics, proteomics, and metabolomics is necessary. Results from these studies would enable reprogramming of SA pathway using genetic engineering technologies, such as zinc finger nucleases, TALENs, and CRISPR/Cas9.

SA regulates diverse metabolic functions, including protection against biotic and abiotic stresses for barley plant fitness. Despite the abundance of evidence pointing the SA response to withstand abiotic stresses, little is known about SA response to biotic stresses in barley. In addition, the precise mechanism underlying their apparent plant protection against extreme environmental conditions remains unclear and warrants future investigations.

Numerous recent *in vitro* and *in vivo* studies have demonstrated the therapeutic evidence of SA activity against a wide spectrum of diseases, such as in liver toxicity, diabetes, obesity, and other chronic diseases caused by free radicals, largely based on cell-based assays and animal models. To date, there are not enough human trials to understand the potential effects of SA on human health, thus warranting more *in vivo* assays and clinical trials based on the current knowledge and advanced molecular techniques to identify the exact mechanisms behind its beneficial activity. Therefore, additional studies including *in vivo* assays and clinical trials based on advanced molecular techniques should be conducted to uncover precise mechanisms underlying SA activities. Moreover, due to continuous increase in the commercial demand for SA, studies on its safety as a dietary supplement, including toxicity, dose–response relationship, efficacy, and bioavailability are needed. Further investigations are necessary to uncover the knowledge gaps and drawbacks to the implementation of plant-derived SA flavone as an alternative regimen to conventional synthetic drugs.

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Notes

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