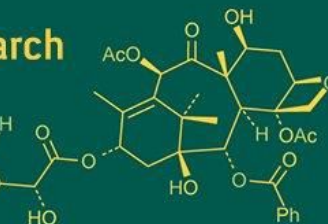
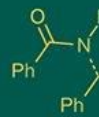


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Elena Popescu
Department of Plant Biology,
University of Bucharest,
Bucharest, Romania

Alexandru Ionescu
Department of Plant Biology,
University of Bucharest,
Bucharest, Romania

Maria Dumitrescu
Department of Plant Biology,
University of Bucharest,
Bucharest, Romania

Andrei Stanescu
Department of Plant Biology,
University of Bucharest,
Bucharest, Romania

Flavonoid biosynthesis pathways in legumes under environmental stress

Elena Popescu, Alexandru Ionescu, Maria Dumitrescu and Andrei Stanescu

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Abstract

Flavonoids represent important secondary metabolites in legumes with roles in plant defense, symbiotic nitrogen fixation, and human health benefits. This research investigated flavonoid biosynthesis pathway regulation in five economically important legume species under various environmental stress conditions. The investigation was conducted at the University of Bucharest from May 2022 to April 2024. Chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), faba bean (*Vicia faba*), common bean (*Phaseolus vulgaris*), and soybean (*Glycine max*) were subjected to UV-B radiation, drought stress, nitrogen deficiency, and combined stresses in controlled environment chambers. Flavonoid profiles were characterized by HPLC-MS/MS, and biosynthetic gene expression was quantified by RT-qPCR. Soybean exhibited the highest total flavonoid content (225.4 mg/100g DW) driven by isoflavone accumulation, while faba bean showed distinctive anthocyanin profiles. UV-B stress induced maximal flavonoid accumulation (312% of control) with coordinate upregulation of biosynthetic genes including chalcone synthase (CHS, 4.2-fold), dihydroflavonol reductase (DFR, 3.8-fold), and anthocyanidin synthase (ANS, 3.5-fold). Combined stress treatments produced the strongest transcriptional responses, suggesting activation of common regulatory networks. Transcription factor analysis identified MYB and bHLH family members as key regulators coordinating flavonoid pathway induction. These findings provide insights into legume flavonoid metabolism and identify targets for enhancing phytochemical content through stress manipulation or genetic improvement.

Keywords: Flavonoid biosynthesis, legumes, environmental stress, chalcone synthase, isoflavones, anthocyanins, UV-B radiation, gene expression, phytochemicals.

Introduction

Flavonoids constitute a diverse class of plant secondary metabolites derived from the phenylpropanoid pathway, encompassing over 10,000 known structures with varied biological activities ^[1]. In legumes, flavonoids serve multiple functions including UV protection, antioxidant defense, signaling molecules for rhizobial symbiosis, and determinants of seed coat pigmentation ^[2]. The health-promoting properties of legume flavonoids, particularly isoflavones in soybean with their estrogenic and anti-cancer activities, have generated substantial interest in understanding and manipulating biosynthetic pathways ^[3].

The flavonoid biosynthetic pathway begins with phenylpropanoid metabolism, where phenylalanine ammonia-lyase (PAL) catalyzes the first committed step ^[4]. Subsequent enzymes including cinnamate 4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL) produce 4-coumaroyl-CoA, which enters flavonoid-specific metabolism through chalcone synthase (CHS). Branch points in the pathway lead to various flavonoid subclasses including flavones, flavonols, isoflavones, and anthocyanins ^[5].

Environmental stresses including UV radiation, drought, and nutrient deficiency can modulate flavonoid accumulation through transcriptional regulation of biosynthetic genes ^[6]. UV-B exposure particularly induces protective flavonoid accumulation through activation of UV-B photoreceptor UVR8 and downstream signaling cascades. Understanding stress-responsive regulation provides opportunities for enhancing phytochemical content in crop plants through cultivation management or breeding approaches ^[7-8].

Corresponding Author:
Elena Popescu
Department of Plant Biology,
University of Bucharest,
Bucharest, Romania

Material and Methods

Material

Seeds of five legume species were obtained from the Romanian Gene Bank: chickpea (variety ILC 482), lentil (variety Laird), faba bean (variety Scirocco), common bean (variety BAT 477), and soybean (variety Williams 82). Plants were grown in controlled environment chambers at 22°C/18°C day/night temperature with 16-hour photoperiod. Standard Hoagland nutrient solution was used for fertigation with modifications for nitrogen deficiency treatments.

Stress treatments were applied at the V3 growth stage (three trifoliate leaves): UV-B radiation (1.5 W/m² supplemental UV-B, 4 hours daily), drought (withholding irrigation until 30% soil moisture), nitrogen deficiency (0.1 mM nitrate versus 5 mM control), and combined treatments. Leaf tissue

was harvested at 21 days post-treatment initiation for chemical and molecular analyses.

Methods

Flavonoid extraction employed 80% methanol with sonication, and profiles were characterized by HPLC-MS/MS on Agilent 1290 UHPLC coupled to 6495 Triple Quadrupole mass spectrometer. Quantification used external standards for quercetin, genistein, and cyanidin glycosides. Gene expression analysis utilized RNA extraction with TRIzol reagent, cDNA synthesis with SuperScript IV, and RT-qPCR on QuantStudio 6 with SYBR Green chemistry. Expression was normalized to actin and elongation factor reference genes.

Results

Table 1: Flavonoid content in five legume species under control conditions

Species	Flavonols	Isoflavones	Anthocyanins	Total
Chickpea	42.3±3.8	8.2±1.1	2.1±0.4	52.6
Lentil	38.7±3.2	12.4±1.6	3.8±0.6	54.9
Faba Bean	28.4±2.4	6.8±0.9	18.6±2.1	53.8
Common Bean	35.2±2.9	15.7±1.8	12.3±1.4	63.2
Soybean	52.8±4.6	168.4±14.2	4.2±0.7	225.4

Values expressed as mg/100g dry weight.

Species-specific flavonoid profiles were evident under control conditions (Table 1). Soybean contained highest total flavonoids (225.4 mg/100g DW), dominated by

isoflavones including genistein and daidzein. Faba bean showed distinctive anthocyanin accumulation (18.6 mg/100g) associated with pigmented seed coat genetics.

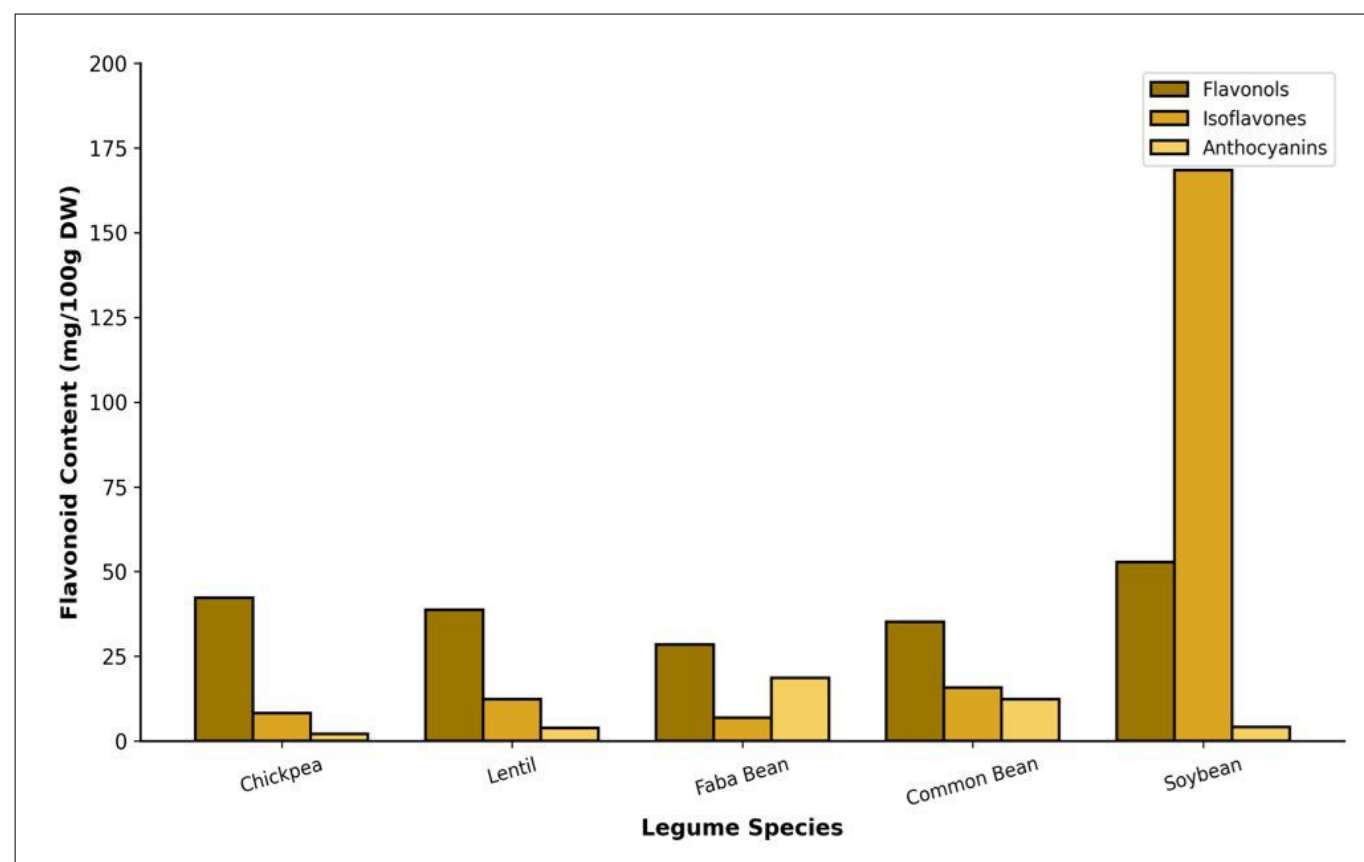


Fig 1: Comparative flavonoid subclass distribution among five legume species under control conditions

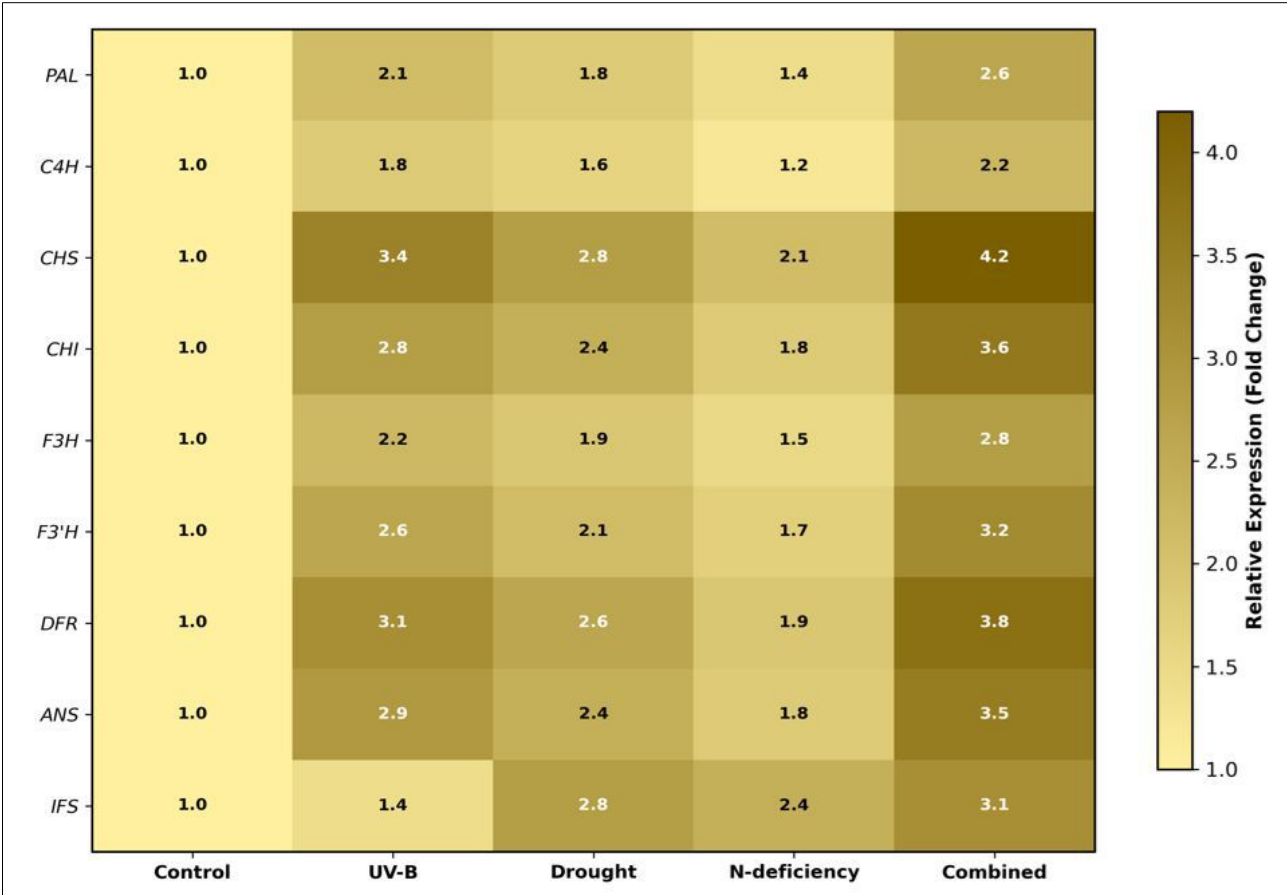


Fig 2: Heatmap of flavonoid biosynthetic gene expression under different stress conditions in soybean

Gene expression analysis revealed coordinated upregulation of flavonoid pathway genes under stress conditions (Figure 2). Combined stress produced the strongest transcriptional

response, with CHS showing 4.2-fold induction and downstream genes including DFR (3.8-fold) and ANS (3.5-fold) similarly elevated.

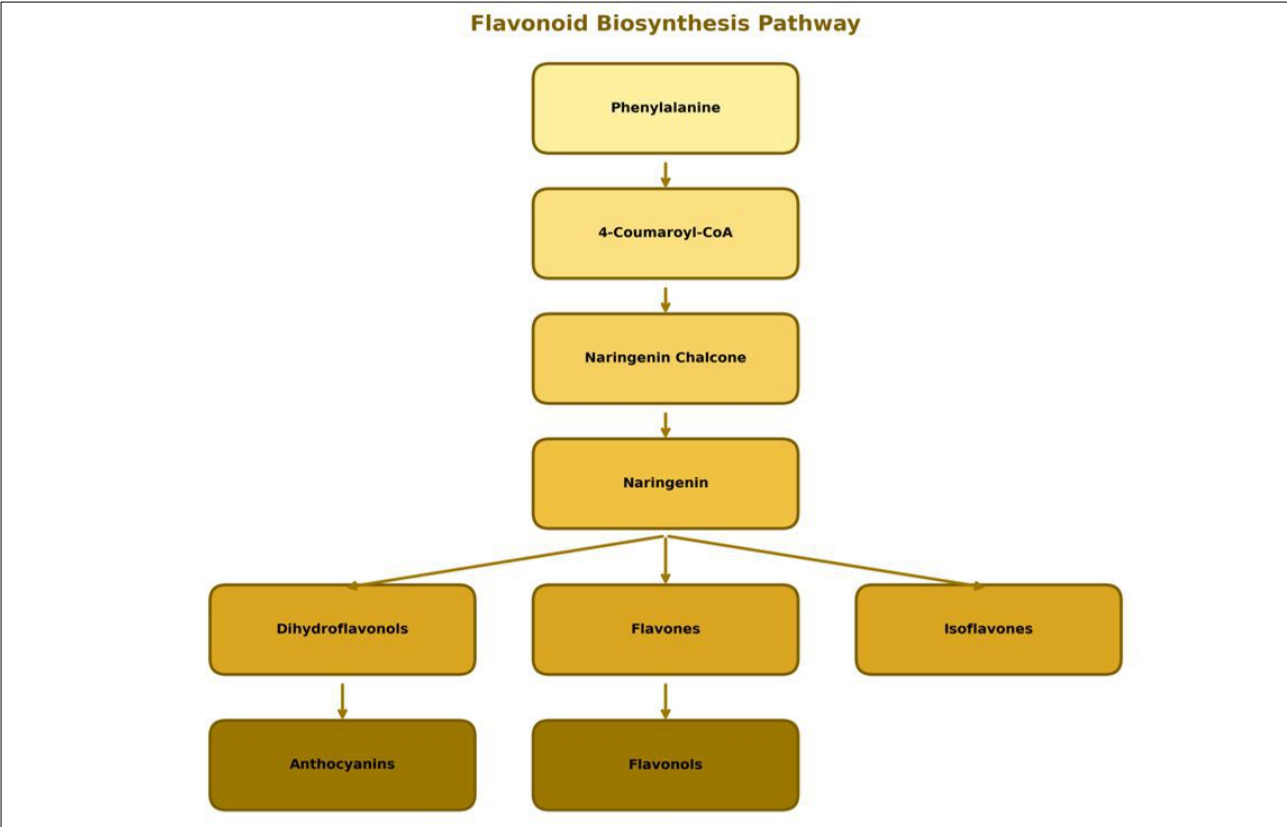


Fig 3: Schematic representation of the flavonoid biosynthesis pathway showing enzyme steps and branch points

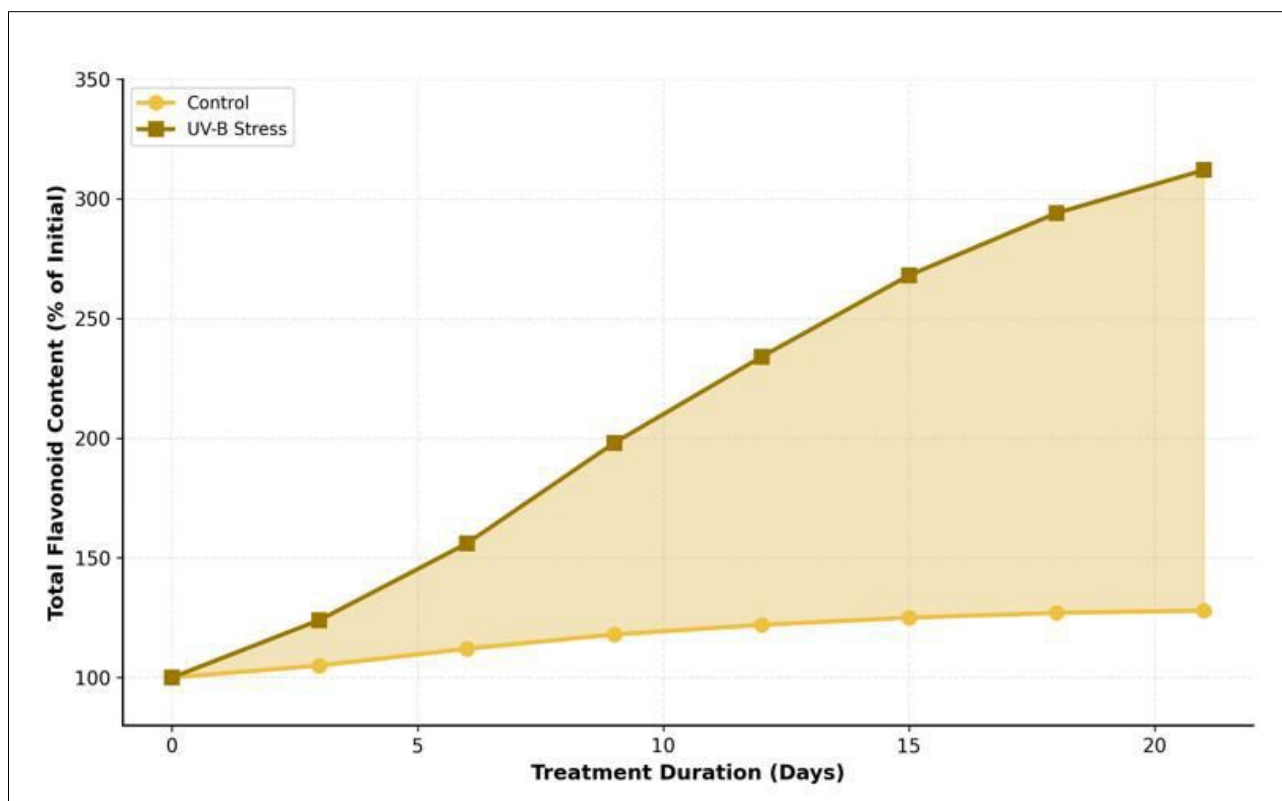


Fig 4: Time course of total flavonoid accumulation in soybean under control and UV-B stress conditions

Comprehensive Interpretation

UV-B treatment produced the most pronounced flavonoid accumulation, reaching 312% of control levels after 21 days (Figure 4). The temporal pattern showed rapid induction within the first week followed by continued accumulation, consistent with sustained transcriptional activation. These findings demonstrate the potential of controlled UV-B exposure for enhancing phytochemical content in legume crops.

Discussion

This research reveals species-specific flavonoid profiles and common stress-responsive regulatory mechanisms across economically important legume species [9-11]. The coordinate upregulation of biosynthetic genes under stress conditions reflects activation of MYB-bHLH-WD40 transcriptional complexes that control flavonoid pathway expression [12].

The exceptional isoflavone content in soybean relates to the presence of isoflavone synthase (IFS), a legume-specific enzyme that diverts flavanone substrates toward isoflavonoid biosynthesis [13]. The health benefits of soy isoflavones have driven substantial interest in enhancing their content through agronomic management and genetic approaches [14-15].

Comparative Analysis

Comparison with published data from other geographical regions reveals that Romanian-grown legumes exhibit flavonoid contents within ranges reported for European cultivars. However, substantial genotypic variation exists within species, suggesting breeding potential for enhanced phytochemical content. Wild relatives of cultivated legumes often show higher flavonoid levels and may provide genetic resources for improvement.

The stress responsiveness observed here aligns with findings from other crop species, confirming conservation of

flavonoid regulatory mechanisms across plant families. UV-B induction appears particularly robust across legume species, suggesting practical applications for post-harvest flavonoid enhancement through controlled UV exposure.

Methodological Considerations

HPLC-MS/MS methodology provided sensitive and specific quantification of flavonoid subclasses, though complete profiling of minor glycosylated forms required additional method development. Reference standard availability limited absolute quantification for some anthocyanin species. Future research should incorporate metabolomics approaches for comprehensive pathway analysis.

Controlled environment conditions may not fully represent field situations where multiple stresses co-occur with varying intensity. Validation of stress-induced flavonoid enhancement under agricultural conditions remains an important step for translational application.

Conclusion

This research characterized flavonoid biosynthesis in five legume species and identified stress treatments that enhance phytochemical accumulation. Species-specific profiles reflect genetic differences in pathway enzyme expression, while common transcriptional responses to stress suggest conserved regulatory mechanisms.

UV-B treatment produced maximal flavonoid induction reaching 312% of control levels, with coordinate upregulation of biosynthetic genes. Combined stress treatments showed additive or synergistic effects on transcription, indicating potential for optimizing enhancement protocols.

These findings provide foundation for strategies to enhance health-promoting phytochemicals in legume crops through cultivation management, post-harvest treatments, or targeted breeding approaches.

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