

EXPLOITING PLANT STRESS PATTERNS AS A WAY TO INCREASE SECONDARY METABOLITE PRODUCTION IN MEDICINAL PLANTS

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Abstract. With the growing popularity of phytotherapy, increasing the production of medicinal substances in plants has become more relevant. This two-stage review explores the hypothesis that growth bifurcations in stress-tolerant plants, induced by temperature or other stressors, drive the synthesis and accumulation of medicinal substances. Previous studies by the authors on *Poaceae* family plants (e.g., *Festuca pratensis*) indicate that at critical levels of stressors, a sudden blockage of meristematic growth occurs. This bifurcation mechanism may redirect carbon compounds in medicinal plants from biomass formation to the synthesis of protective compounds, including secondary metabolites. The review then focuses on stress-tolerant monocotyledonous medicinal plants commonly found in Lithuania, which are expected to exhibit growth bifurcations and increased secondary metabolite production. Stress tolerance as a criterion within ecological strategies is used to select and analyze these research subjects. Using Vavilov's law of homologous series and modern systematics, the study prioritizes monocotyledonous plant families and genera that are phylogenetically closer to the *Poaceae* family, such as *Festuca*, in which growth bifurcations are more likely to occur. Consequently, this approach could integrate various aspects of medicinal plant breeding, cultivation, harvesting, and the practical use of secondary metabolites in phytotherapy.

Keywords: *Poaceae, stress-tolerant plant, growth bifurcation, plant ecological strategies, homology*

Introduction

Recently, with the growing popularity of medicinal plants, it has become important to increase their production of medicinal substances. These substances are ligands that target human cells at the molecular level and are used to activate or inhibit biochemical processes as needed for treatment. Secondary metabolites can serve as highly effective antioxidants in aromatherapy and other applications (Punetha et al., 2022; Zagorskina and Nazarenko, 2019).

Medicinal substances extracted from plants are primarily secondary metabolites, including isoprenoids, phenolic compounds, and alkaloids (Olivoto et al., 2017). A recent review (Zhao et al., 2023a) provides a detailed analysis of the physicochemical properties of these metabolites, categorizing them into three major groups based on their biosynthetic pathways: phenolic compounds, terpenes, and nitrogen-containing compounds. These substances serve as biochemical tools that mediate interactions between plants and their environment (Nosov, 1994), ensuring the survival of sessile organisms. Traditionally, secondary metabolites function as herbivore deterrents, repellents, or attractants; however, recent studies have highlighted their significance in providing resistance to abiotic stressors (Punetha et al., 2022; He et al., 2023). For example, drought stress has been shown to induce the accumulation of phenolic compounds, such as flavonoids, in willow leaves and other plants (Larson, 1988). These

compounds play a protective role by acting as antioxidants that neutralize free radicals generated under environmental stress, such as drought or exposure to toxic metals like aluminum. Additionally, anthocyanins – water-soluble pigments derived from flavonoids via the shikimic acid pathway – have been found to accumulate under drought and low-temperature conditions (Akula and Ravishankar, 2011; He et al., 2023). Anthocyanins not only regulate osmotic potential and prevent dehydration but also shield plants from harmful levels or wavelengths of radiation (Chalker-Scott, 1999). Phenolic compounds accumulate at low temperatures and are integrated into the cell wall as suberin and lignin (Griffith, Yaish, 2004).

The role of certain isoprenoids in conferring resistance has been well-documented. For instance, abietic acid in pine acts not only as a diterpene repellent, protecting the plant, but also enhances its tolerance to low temperatures (Zagoskina and Nazarenko, 2019). Additionally, other studies (Nadeau et al., 1987) have reported a significant accumulation of polyamines, another group of secondary metabolites, in wheat, alfalfa, and other plants under abiotic stress conditions. These secondary metabolites are believed to function as antioxidants, contributing to the plant's resistance to stress.

Biotechnological approaches, such as cell cultures, genetic engineering, and other methodologies, have been widely employed to increase the production of secondary metabolites (Narayani and Srivastava, 2017; Zagoskina and Nazarenko, 2019). For instance, Zhao et al. (2023a) reviewed the genetic and epigenetic regulation of secondary metabolites in medicinal plants, addressing the challenges and potential solutions for improving their production. Tripathi et al. (2024) explored the role of ethylene and its inhibitors in manipulating secondary metabolite synthesis, offering insights into hormonal regulation. Advancements in gene-editing technologies, such as CRISPR/Cas9, have also been highlighted as promising tools for optimizing secondary metabolite production in plants (Mipeshwaree Devi, 2023).

This study presents a systematic two-stage review that differs from those previously mentioned by addressing the dynamics of plant growth processes and analyzing stress patterns observable only at higher organizational levels of plant study. Consequently, it encompasses a broader spectrum of concepts, ranging from tissue- and plant-level phenomena to ecosystem-level interactions, where plants adopt diverse ecological strategies when growing in unfavorable conditions.

The study investigates the hypothesis that the synthesis and accumulation of medicinal substances in plants can be influenced by growth bifurcations occurring in stress-tolerant species. In its second stage, the review focuses on identifying and selecting potential research targets, namely, common stress-tolerant medicinal plants native to Lithuania, a representative habitat within the middle latitude climate zone, where growth bifurcations can be expected.

The paper is structured as follows. The next section, entitled Stress-related concepts in plant growth, explores plant growth characteristics under exposure to stressors and provides an in-depth discussion of bifurcated growth mechanisms, which remain underexplored in existing literature. It also outlines principles and methods for selecting candidate medicinal plants from the extensive biodiversity available.

The following section, Results of the targeted review of medicinal plants, presents the findings of the systematic review, applying Grime's ecological strategies and Vavilov's law of homologous series to rank medicinal plants based on their stress tolerance, variability patterns, and potential for secondary metabolite production. Additionally, the therapeutic properties of these plants are summarized with appropriate references.

The paper subsequently examines the markers that enable the detection of growth bifurcations, highlighting their critical importance for the express selection of candidate plants and the precise identification of stress-tolerant medicinal plants in future stress-related studies. Finally, it outlines directions for further research to validate the hypothesis and establish quantitative parameters for metabolite production in medicinal plants across broader biodiversity.

Stress-related concepts in plant growth

Stress tolerance and blocking of shoot and root growth due to bifurcation

Previous studies by the authors have demonstrated that the linear growth rate of roots in *Lolium multiflorum*, *Lolium perenne* and $\times$ *Festulolium braunii* decreases exponentially as the temperature is lowered from 20°C to 0°C (Šimkūnas et al., 2011; Šimkūnas et al., 2018). This growth slowdown is attributed to the direct effect of temperature, consistent with the regularity of the Arrhenius law (see the upper lines at 0°C in Fig. 1). In contrast, the root growth rate of the more stress-tolerant *Festuca pratensis* exhibits a similar pattern to that of *Lolium* and *Festulolium* species when the temperature decreases from 20°C to 8°C, but declines sharply as the temperature drops from 8°C to 0°C (Fig. 1). This growth inhibition, or blockage, is not proportional to the direct impact of temperature but instead reflects qualitative changes. These changes signify a bifurcation in growth, representing a transition to a qualitatively new state characterized by altered metabolism and enhanced resistance to stressors (Šimkūnas et al., 2011). It has been demonstrated that the cause of this growth slowdown is localized in the meristem, where a drastic decrease in the cellular production rate (V) leads to growth blockage (Šimkūnas et al., 2007; Šimkūnas et al., 2018). As observed, bifurcation is linked to a marked reduction in the most energetically demanding component of growth, i.e., the meristemic growth. Consequently, when the environmental stressor reaches a critical threshold, bifurcation is manifested as a sudden and significant decrease in meristemic growth (Šimkūnas et al., 2018; Veselovskii et al., 1993).

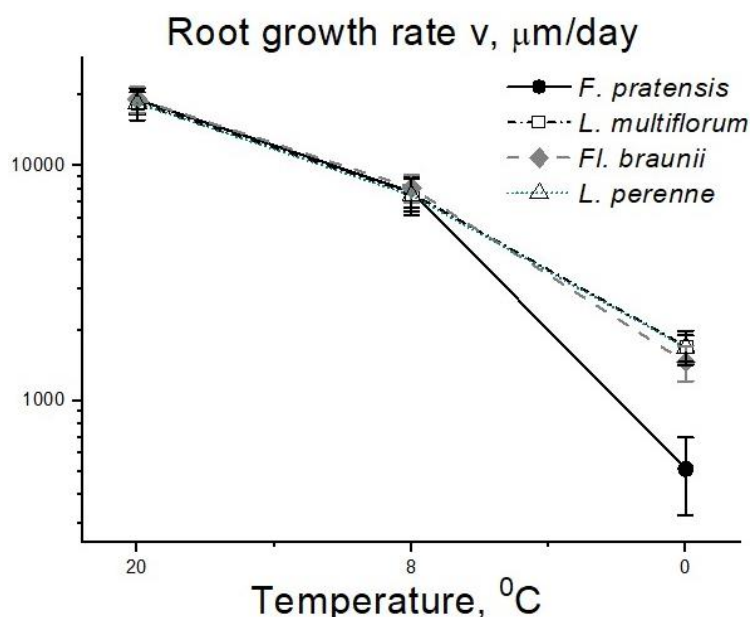


Figure 1. Linear root growth rate of *Festuca pratensis*, *Lolium multiflorum*, *Lolium perenne* and *Festulolium braunii* at 20°C, 8°C and 0°C

The conditions and results of these experiments are thoroughly detailed in the following publications (Šimkūnas et al., 2007; Šimkūnas et al., 2011; Šimkūnas et al., 2018). The graphical representation of this phenomenon, provided below (see *Fig. 1*), illustrates the key findings by visualizing the bifurcated dynamics of root growth rates on a logarithmic scale. The error bars represent the standard deviation (SD) relative to the mean values of root growth rates measured across different plant species. At 0°C, the error bars for the stress-tolerant *Festuca pratensis* and the non-stress-tolerant species do not overlap, and the difference between their means exceeds 5 SD, indicating a statistically significant difference. For instance, at 0°C, the root growth rate of the stress-tolerant *Festuca pratensis* is 530 ± 152 $\mu\text{m/day}$, while that of the non-stress-tolerant *Lolium multiflorum* is 1879 ± 221 $\mu\text{m/day}$.

It should be noted that the more stress-tolerant *Festuca pratensis* exhibits a two-stage growth blockage: a bifurcated shoot growth blockage at higher temperatures (8°C) and a root growth blockage at lower temperatures (4°C, 2°C, and 0°C) (Šimkūnas et al., 2018).

Increasing the production of secondary metabolites in medicinal stress-tolerant plants

How could growth blockage in stress-tolerant plants enhance the synthesis and accumulation of medicinal substances? It has been hypothesized (Bryant et al., 1983) that under stress conditions, carbon is redirected from biomass production towards the synthesis of defensive secondary compounds. This idea is indirectly supported by the source-sink “carbon-nutrient balance” hypothesis, which suggests that stress suppressing growth more than photosynthesis leads to the accumulation of secondary chemicals (Niinemets, 2016).

Therefore, if bifurcated growth inhibition does not occur in non-stress-tolerant plants under stressor exposure, materials and energy are used for shoot and root growth, resulting in less synthesis and accumulation of secondary metabolites (*Fig. 2a*). Conversely, in stress-tolerant plants, bifurcation-induced growth inhibition prevents the use of assimilates for growth processes. Instead, these assimilates are redirected toward the synthesis of compounds with protective functions, including secondary metabolites.

In the case of two-stage growth inhibition, which initially involves shoot growth blockage, unused assimilates are redirected toward the synthesis of secondary metabolites and other compounds (*Fig. 2b*). If root growth blockage subsequently occurs, an even greater accumulation of these metabolites can take place (*Fig. 2c*).

Finally, the targeted accumulation of these metabolites under stressors is supported by the fact that some secondary metabolites, such as phenolic compounds and polyamines, have antioxidant and osmotic adjustment functions that are crucial for developing resistance to stressors. Under various abiotic stressors, such as drought, salinity, or low temperatures, the causes of plant death are often associated with the formation of reactive oxygen species (ROS) and cell dehydration, which result in structural changes in molecules and membranes (Sung et al., 2003; Chinnusamy et al., 2010). As part of the adaptive response, antioxidants and osmotic adjusters are synthesized to enhance resistance to these stressors. Alongside synthesized osmolytes, such as sucrose and proline, and specific proteins, these functions are also carried out by plant secondary metabolites, including phenolic compounds, certain flavonoids, polyamines, and other related compounds (Nadeau et al., 1987; Zagoskina and Nazarenko, 2019; Punetha et al., 2022).

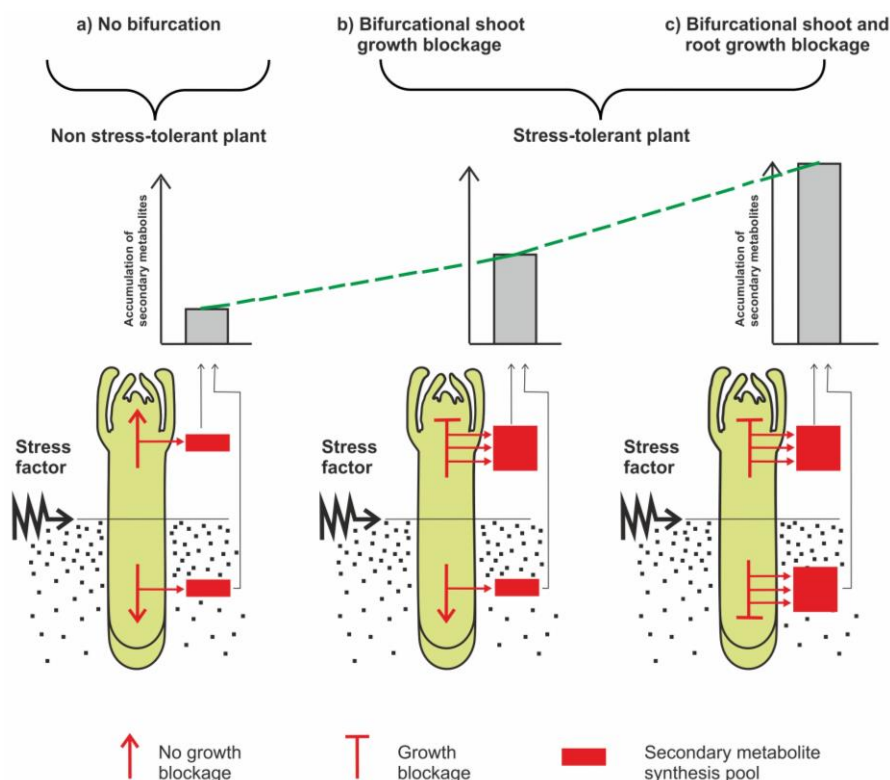


Figure 2. Growth states under stressor exposure and possible redistribution of carbon compounds between biomass formation and the synthesis of protective secondary metabolites. (a) Stress effect in non-stress-tolerant plants: no growth blockage. (b) First stage of growth blockage in stress-tolerant plants: shoot growth blockage. (c) Second stage of growth blockage in stress-tolerant plants: both shoot and root growth blockage

Diversity and homology as principles for determining plant candidates

It is essential to select promising medicinal plant candidates from a diverse range of species that can exhibit the described bifurcated growth response and, as a result, accumulate higher levels of secondary metabolites. To compile a table of such candidate plants, J. P. Grime's theory of ecological strategies will be utilized. This theory categorizes plants into competitors (C), stress tolerators (S), ruderals (R), and intermediate strategies (e.g., C-S-R, C-S, C-R, R-S, etc.), with intermediate strategies being the most prevalent (Grime et al., 2007). Stress-tolerant (S) plants and those with intermediate strategies expected to exhibit stress tolerance (e.g., C-S-R, C-S) will be included in the table as potential candidates for growth bifurcations. These intermediate strategy plants may be particularly noteworthy, as most of the subjects in our previous study were classified as C-S-R (Šimkūnas et al., 2011; Šimkūnas et al., 2018). Notably, *Festuca pratensis*, the most stress-tolerant species observed, demonstrated growth bifurcations.

To refine and expand the list of candidate plants, a targeted systematic literature review was conducted. A total of 27 publications meeting the established criteria were identified across electronic databases, including ScienceDirect, PubMed, EBSCO, and SpringerLink. The search specifically utilized the following keywords: stress resistance, monocotyledons, secondary metabolites, and medicinal properties. To maintain quality, duplicate articles across databases and non-English publications were excluded.

The second criterion for selecting plants is the similarity of variation (homology) among genetically closer-related plants. According to Vavilov's law of homologous series in variation, taxonomically related organisms (i.e., genetically similar species and genera) exhibit similar patterns of variability (Vavilov, 1922; Nanjundiah et al., 2022).

In this review, we will first investigate growth bifurcations among the more stress-tolerant members of the monocotyledonous class, as they are more closely related to previously studied subjects. Building on recent advancements in phylogenetic analysis and modern systematics, we will further prioritize the monocotyledonous study objects by identifying families that are phylogenetically closer to the *Poaceae* family and genera closely related to *Festuca*. These plants are expected to have the highest likelihood of exhibiting growth bifurcations. In plant systematics, closely related families are grouped within the same order, while closely related genera are grouped within the same tribe.

Results of the targeted review of medicinal plants

Selection of medicinal plant candidates with potential for growth bifurcation

As mentioned earlier, J. P. Grime's theory categorizes plants into distinct ecological strategies: competitors (C), stress tolerators (S), and ruderals (R) (Grime et al., 2007). Competitors are plants that excel in favorable conditions (productive communities); they are typically fast-growing and large, thriving due to their pronounced competitive traits. Stress-tolerant plants, on the other hand, adapt to unfavorable conditions by growing slowly to conserve resources and exhibiting specific morphological and physiological-biochemical adaptations. These traits enable their survival under various stressors, such as drought, nutrient or light deficiencies, and cold climates (Grime et al., 2007; P'yankov et al., 2000). Furthermore, a recent study investigating differences in grass species' strategies based on their lifespan revealed that stress tolerance values are higher in perennials compared to annuals (Wingler and Sandel, 2023).

Plants with a ruderal life strategy are characterized by rapid growth, short life cycles, and high reproductive capacity, such as prolific seed production or vegetative reproduction. These traits enable ruderals to colonize vulnerable soils and persist in environments subject to frequent community disturbances (Grime et al., 2007; P'yankov et al., 2000). An analysis of the differences in grass species' strategies regarding their naturalization, conducted by Wingler and Sandel (2023), revealed that introduced species exhibit more pronounced competitive-ruderal strategies compared to native species. Ruderals are the first to restore plant communities following disturbances and play a key role as the initial species in secondary succession (Mirkin et al., 2002). Beyond these fundamental strategies, J. P. Grime's classification of plant ecological strategies also encompasses various intermediate variants, such as C–S, R–S, C–R, and C–R–S, among others (Grime et al., 2007).

Previous studies by the authors examined several *Poaceae* species categorized under the intermediate C-S-R strategy. Among these, the more stress-tolerant *Festuca pratensis* was shown to undergo growth bifurcations when exposed to temperature stress (Šimkūnas et al., 2018). This raises the question of how widespread the phenomenon of growth slowdown is across biodiversity, particularly among medicinal plant species, and within which ecological strategies it should be investigated. The characteristics of plants with competitive and ruderal strategies suggest that growth bifurcation phenomena are unlikely to occur in these groups.

Competitors survive by leveraging their rapid growth and competitiveness, while ruderals are similarly characterized by fast growth and development, completing short life cycles. As such, these plants do not meet the above criterion. Slow growth or even growth blockage is more likely to be observed in stress-tolerant (S) plants or those with intermediate strategies that include a stress-tolerant component (e.g., C-S-R). These plants conserve resources by redirecting them toward the development of resistance mechanisms under adverse conditions, which is a defining characteristic of stress tolerance.

The primary focus of this phase of the review was the selection of medicinal plant candidates, the investigation of their therapeutic properties, and their systematic classification. At the outset, over 50 species of medicinal monocotyledonous plants found in Lithuania, representing 13 plant families, were analyzed based on an initial literature review and the recommendations of phytotherapy experts (Gudžinskas and Balvočiūtė, 2007).

Based on the criterion of stress tolerance and a systematic literature analysis, promising candidate plants were identified as having the potential to exhibit growth bifurcation and significant increases in secondary metabolite production. Perennial plants demonstrating a more pronounced stress tolerance strategy (e.g., S; C-S-R, etc.) were selected. The final set of carefully chosen monocotyledonous stress-tolerant medicinal plants includes 19 species across 10 families. The outcomes of this research phase are summarized in *Table 1*, which presents not only the identified ecological strategies of these plants but also their medicinal applications, secondary metabolites, and valuable therapeutic properties. Additionally, the table includes recommendations for phytotherapeutic use of the plants and their respective parts. The table also includes labels for the characteristics of plant life cycles: M represents monocarpic species, while P denotes perennial species.

The medicinal plants selected for the final collection belong to the following families within the monocotyledonous class: *Acoraceae*, *Butomaceae*, *Araceae*, *Colchicaceae*, *Amaryllidaceae*, *Iridaceae*, *Asparagaceae*, *Orchidaceae*, *Poaceae* and *Cyperaceae* (see *Table 1*, where plant species are italicized, while plant families appear in roman (regular) font). The candidate plants, identified as objects for further research, are closely related to those previously studied by the authors. These plants share key characteristics, being monocotyledonous, stress-tolerant, or intermediate strategy medicinal plants, and are expected to exhibit significant increases in secondary metabolite production through growth bifurcation.

Further prioritization of candidate plants

Another issue to be addressed is the prioritization of medicinal plants listed in the table with a stress tolerance component for examining their potential to exhibit growth bifurcations. Vavilov's law of homologous series states that genetically related species and genera display similar patterns of heritable variation. Consequently, understanding the variability in one species can aid in predicting parallel forms in other species and genera (Vavilov, 1922; Nanjundiah et al., 2022). The closer the genetic relationship between genera and species, the greater the overlap in their series of variation. Vavilov expressed his law in the formula (Vavilov, 1922):

$$G_1(a + b + c.....)$$

$$G_2(a + b + c.....)$$

$$G_3(a + b + c.....)$$

where G_1 , G_2 , G_3 represent species, and a , b , c denote distinct traits, such as growth bifurcations or structural, biochemical, and other characteristics of the organism. Thus, according to this law and its recent interpretations (Nanjundiah et al., 2022), the bifurcation phenomena described above are expected to occur in other genetically close, stress-tolerant species.

Subsequent genomic discoveries have revealed that the similarity in inherited variation (homology) among closely related species is connected to the homology of the underlying genes. Specifically, homologous traits or functions in different organisms are determined by genes with homologous nucleotide sequences.

It was determined that constitutive overexpression of CBF genes (expressed at low temperatures) under the transcriptional control of the 35S promoter in transgenic plants resulted in severe growth retardation under normal growth conditions in diverse plant species, such as *Arabidopsis*, *B. napus*, tomato and rice (Chinnusamy et al., 2010). During cold stress, growth retardation appears to be regulated by CBF genes through nuclear-localized DELLA proteins, which repress growth in *Arabidopsis*. It can be suggested that homologues of CBF genes and their products may play a role in activating growth blockage under low-temperature stress in various plants (see Fig. 2).

As mentioned above, the plants from the families listed in Table 1 are proposed for further study due to their phylogenetic proximity to the *Poaceae* plants previously investigated. A comprehensive set of experiments must be designed to identify changes in the structural, biochemical, and other traits of these organisms. To optimize the scope of future experiments and prioritize study subjects, insights from modern systematics have been applied. Specifically, these insights help identify families closely related to *Poaceae* by categorizing them into orders (see Fig. 3a), thereby defining the order *Poales*, which encompasses *Poaceae* and its related families. Further analysis of these phylogenetic relationships (see Fig. 3b) subdivides the genera within the *Poaceae* family into tribes, ultimately defining the tribe *Poeae*, which includes *Festuca* and its allied genera.

As demonstrated in Figure 3a, among the selected plant families, *Cyperaceae* is phylogenetically closest to *Poaceae*, as both belong to the order *Poales*. Consequently, priority among monocotyledons is recommended for *Cyperaceae* (represented by one species – *Carex hirta*). To establish prioritization within the *Poales*, their systematics were further analysed (see Fig. 3b). All selected *Poaceae* plants are categorized within the subfamily *Pooideae* and distributed across the tribes *Stipeae*, *Triticeae*, and *Poeae*.

The genetic distances between the tribes *Stipeae*, *Triticeae*, and *Poeae* within the grass subfamily *Pooideae* have been extensively studied using phylogenetic analyses. These studies frequently employ molecular markers such as chloroplast DNA (e.g., *matK* gene) and nuclear DNA (e.g., ITS sequences) to estimate evolutionary relationships. Furthermore, mathematical statistical methods, including maximum likelihood (ML), neighbor-joining (NJ), principal component analysis (PCA), and Bayesian inference (BI), are utilized to visualize these relationships based on genetic data (Schneider et al., 2009; Zhang et al., 2022).

For instance, the study conducted by Zhang et al. (2022) examines the phylogenetic relationships and adaptive evolution within *Pooideae* using comprehensive

transcriptomic data. The findings indicate that these tribes diverged over the evolutionary history of *Pooideae*, with *Triticeae* and *Poeae* displaying closer genetic relationships compared to *Stipeae*. This is further supported by phylogenetic trees, which group *Triticeae* and *Poeae* into a closely related clade, while *Stipeae* forms a distinct lineage. Notably, the study by Gallaher et al. (2022) provides mean age values (Ma) for these tribes, with 95% confidence intervals (CI) presented in square brackets: *Triticeae* – 20.64 [18.01–25.38], *Poeae* (1) – 25.28 [22.26–27.83], and *Stipeae* – 30.37 [26–38.65].

Genera such as *Briza*, *Koeleria*, *Anthoxanthum*, *Phleum*, *Hierochloe*, along with our studied *Festuca*, are classified within the same tribe, *Poeae*. Therefore, future research on *Poaceae* should prioritize the following species: *Briza media*, *Koeleria glauca*, *Anthoxanthum odoratum*, *Phleum pratense*, *Hierochloe odorata*.

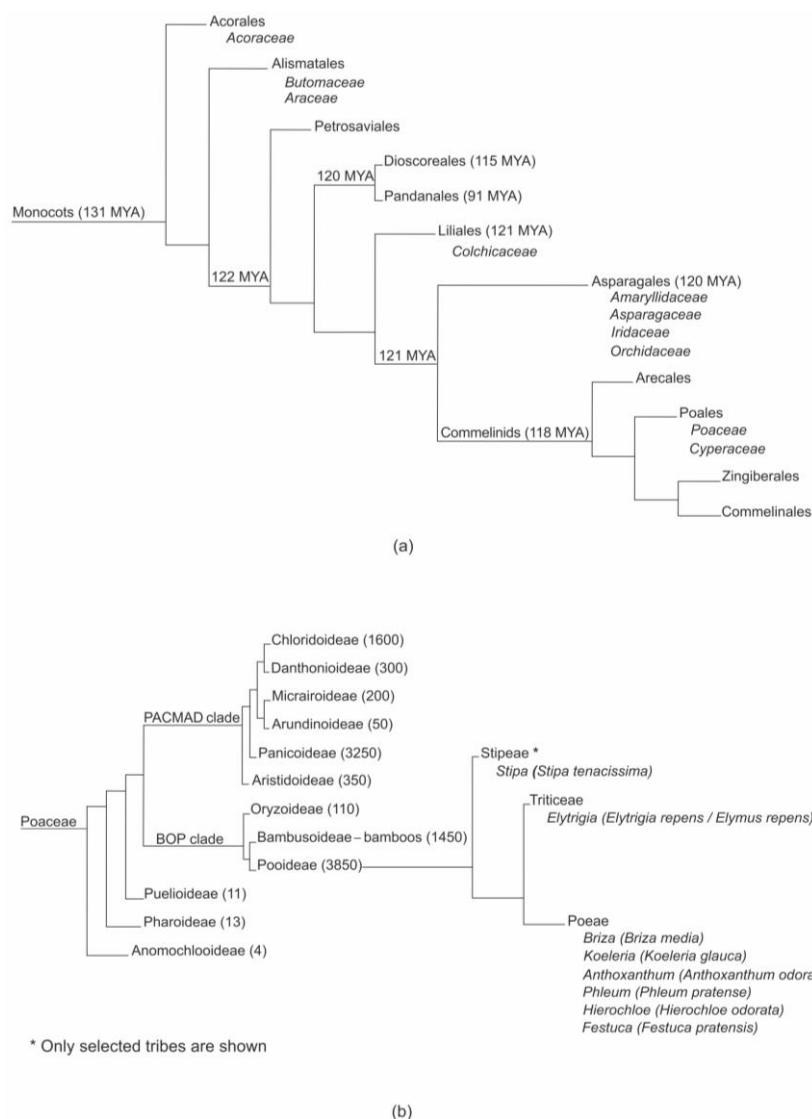


Figure 3. The phylogenetic composition of monocots and their *Poaceae* plant family (prepared according to (The Angiosperm Phylogeny Group, 2016; Givnish et al., 2018; Grass Phylogeny Working Group II, 2012)). (a) Cladogram of the phylogenetic composition of monocots. (b) Cladogram of *Poaceae* plant family: subfamilies with approximate species numbers, tribes, and genera

Table 1. Systemized list of selected stress-tolerant monocots medicinal plants

Family/scientific name	Established strategies	Life cycle	Part used	Secondary metabolites	Therapeutic property
Butomaceae Mirb. <i>Butomus umbellatus</i> L.	S/SRC	P	Rhizomes, roots, leaves and flowers	Flavonoids, triterpenoids, phenolic compounds, essential oils (Saxena et al., 2021)	Antioxidant, anti-inflammatory, and antimicrobial properties, anti-inflammatory and anticancer activities, potential diuretic properties
Araceae Juss. <i>Spirodela polyrhiza</i> (L.) Schleid.	R/RSC	M	Whole plant	Bioactive metabolites (phenolic acids, flavonoids, and terpenoids) (Kim, 2023)	Anti-inflammatory and antimicrobial, anti-allergic therapies (epidemic fever, dysuria, and various skin ailments, such as measles eruptions, eczema, and pruritus), detoxification and nutritional supplementation (Kim, 2023)
Colchicaceae DC. <i>Colchicum autumnale</i> L.	S	M	Corms and seeds	Alkaloids (colchicine, colchicoside, demecolcine), flavonoid (luteolin, quercetin), phenolic compounds (Dhanarasu and Rao, 2020)	Anti-inflammatory: colchicine is widely used in the treatment of gout due to its ability to reduce the inflammatory response triggered by urate crystals. Muscle relaxant: colchicoside is used to treat muscle spasms and related musculoskeletal disorders. Luteolin and quercetin have a role in preventing chronic diseases like cardiovascular disorders and cancer (Dhanarasu and Rao, 2020)
Iridaceae Juss. <i>Iris pseudacorus</i> L.	SC	P	Rhizomes, leaves, seeds and flowers	Flavonoids (kaempferol and quercetin), polyphenolic compounds (tannins), glycoside (iridin), xanthones, flavonoids (Gilmore, 2019)	Antioxidant properties: They scavenge free radicals and protect cells from oxidative stress. Anti-inflammatory properties: They may reduce inflammation, making them potentially useful for treating various inflammatory conditions. Tannins have been used to treat diarrhea and other gastrointestinal issues due to their ability to contract tissues and they inhibit the growth of certain microorganisms. Iridin have been used as a laxative due to its ability to stimulate the intestines. The xanthones and flavonoids have shown potential in anticancer research (Gilmore, 2019)
Amaryllidaceae J.St.-Hil. <i>Allium ursinum</i> L.	CR/CSR	P	Leaves, flowers, stems, bulbs	Organosulfur compounds (allicin), diallyl disulfide (DADS) and diallyl trisulfide (DATS), flavonoids (quercetin, kaempferol), phenolic compounds (caffeic acid and ferulic acid), saponins, essential oils (Krivokapic et al., 2021)	Allicin, essential oils and saponins has antimicrobial properties, making it effective against various bacteria, fungi, and viruses. Allicin and phenolic compounds scavenges free radicals, offering protection against oxidative stress. It is believed to lower blood pressure, improve cholesterol levels, and reduce the risk of cardiovascular diseases. DADS, DATS and flavonoids possess potent anticancer, anti-inflammatory properties. Saponins can help lower blood cholesterol levels and have heart-protective qualities (Krivokapic et al., 2021)

Family/scientific name	Established strategies	Life cycle	Part used	Secondary metabolites	Therapeutic property
<i>Galanthus nivalis</i> L.	C/CS	P	Bulb, leaves, flowers	Alkaloids (galantamine, isoquinoline, caranine, narciclasine, tazettine, narwedine and montanine), antioxidants (Kong et al., 2021)	Galantamine is used in the treatment of Alzheimer's disease and other forms of dementia due to its ability to increase levels of acetylcholine, a neurotransmitter important for memory and learning. Other alkaloids exhibit acetylcholinesterase inhibitory potential, antibacterial, antifungal, antiparasitic (malaria), antiviral, antioxidant, anticancer, anti-inflammatory activities (Kong et al., 2021)
Asparagaceae Juss. <i>Convallaria majalis</i> L.	C/CSR	P	Rhizome, leaves, flowers, berries	Cardiac glycosides (convallatoxin, convallaside, lokunjoside) (Cortinovis and Caloni, 2013)	The glycosides can increase the force of heart contractions and may be used in treating certain heart conditions, diuretic properties (Cortinovis and Caloni, 2013)
Orchidaceae Juss. <i>Platanthera bifolia</i> (L.) Rich.	S	P	Tuber, leaves	Stilbenes, dihydrostilbenoids, phenanthrenes, alkaloids, terpenes, flavonoids, anthocyanins, and phenolic acids (Bazzicalupo et al., 2023)	Tuber used to treat cough, cold, flu, body warmers (Teoh, 2016); leaves were used against rheumatism and as antineuralgic, by putting swell dry leaves in water–vinegar and by applying swollen leaves to the affected area (Cappelletti et al., 1982)
<i>Orchis mascula</i> (L.) L.	S/SR	P	Tuber, rhizomes, leaves, flowering stem	9,10-Dihydrophenanthrenes (orchinol and hircinol); quercetin, kaempferol; loroglossin, p-hydroxybenzylalcohol, orchinol; high content of mucilage (Teoh, 2016)	Tuber as demulcent, astringent, tonic and nutrient, used to treat diarrhea (Teoh, 2016); as aphrodisiac, antipyretic, anti-consumption and for anti-diarrheal effects (Pant, 2013)
<i>Dactylorhiza incarnata</i> (L.) Soo	S	P	Tuber	4-hydroxyphenylacetic acid methyl ester, 4-hydroxybenzyl alcohol, 4-hydroxybenzaldehyde and two unidentified compounds as predominant (Naczki et al., 2018); Acids (mainly palmitic acid, nonanoic acid, lactic acid, caproic acid); benzoids (mainly p-hydroxybenzyl alcohol, p-coumaric acid, hydroquinone, 3,4-dihydroxybenzyl alcohol) (Wróblewska et al., 2019)	Tuber used to produce Salep (mixture) and taken as tonic, aphrodisiac (Mincheva et al., 2018)
Poaceae Barnhart <i>Elytrigia repens</i> (L.) Desv./ <i>Elymus repens</i> (L.) Gould	R/RSC	P	Rhizomes	Tannins, saponins, flavonoids, alkaloids, coumarins, anthocyanins, phenols, quinines, terpenes, triterpenoids, phenols, cardiac glycosides, and steroids (Prakash et al., 2024)	Tea is used in cases of urinary (cystitis and urethritis) incompetence and as a worm expellant. Externally it is applied as a wash to swollen limbs (European Medicines Agency, 2021)

Family/scientific name	Established strategies	Life cycle	Part used	Secondary metabolites	Therapeutic property
<i>Stipa tenacissima</i> L.	S/SCR	P	Leaves	Heneicosyl formate, gamma-sitosterol, lupeol, loliolide, pentoxone etc. (Mehdi et al., 2021)	Chronic scalp ulcers, diabetes (Makhlouf et al., 2024); colorectal adenocarcinoma and human breast cancer (Mehdi et al., 2021)
<i>Hierochloe odorata</i> (L.) P. Beauv.	S/SCR	P	Leaves, rhizomes	Coumarins, flavonoids and polyphenolic acids (Baczek et al., 2015)	Antioxidant, antifungal, antibacterial, and anti-inflammatory (White, 2002)
<i>Anthoxanthum odoratum</i> L.	SR/CSR	P	Leaves, flowers	Curarine, essential oil (Tava, 2007)	Antifungal (Tava, 2007)
<i>Briza media</i> L.	S	P	Leaves	Flavonoids, phenolic acids, saponins, tannins, alkaloids, terpenoids (Williams and Harborne, 1988)	Antimicrobial, antioxidant, antifungal and anti-inflammatory (Williams and Harborne, 1988)
<i>Phleum pratense</i> L.	CSR	P	Pollen, stems and leaves	Phenolic compounds (flavonoids - apigenin, luteolin; phenolic acids - ferulic acid, p-coumaric acid), carbohydrates (fructans - levan), fatty acids and terpenoids (Shi et al., 2023)	Desensitization therapy to treat allergic rhinitis and asthma, ublingual and subcutaneous immunotherapy to build tolerance of allergens (Bauer, Rank, 2014), prebiotics effects, reducing the risk of colon cancer, obesity and diabetes (Shi et al., 2023)
<i>Koeleria glauca</i> (Spreng.) DC.	S	P	Leaves and stems	Flavonoids, phenolic acids, saponins, tannins, terpenoids (Wink, 2015)	Anti-inflammatory, antioxidant, antifungal and antimicrobial properties, anticancer activities, cardioprotective and cholesterol-lowering effects (Wink, 2015)
Cyperacea Juss. <i>Carex hirta</i> L.	C/CSR	P	Stems, leaves, roots and rhizomes	Phenolic compounds (flavonoids, phenolic acids and tannins), saponins, alkaloids, terpenoids (Gajbhiye, 2024)	Antioxidant, anti-inflammatory, antifungal, immune-boosting and antimicrobial properties, anticancer effects (Gajbhiye, 2024)
Acoraceae Martinov <i>Acorus calamus</i> L.	S/SCR	P	Rhizome	Essential oils (β -asarone, α -asarone, shyobunone, cadinene, calamendiol, camphor, and isoelemicin), phenolic compounds (flavonoids, tannins), alkaloids (acolamidine) (Zhao et al., 2023b; Chandra and Prasad, 2017)	Cognitive enhancer and neuroprotective agent (to improve memory and cognition, mood stabilizers, with antidepressant and anxiolytic properties, antimicrobial and antifungal activity, gastroprotective and antispasmodic properties, anti-inflammatory and analgesic effects (arthritis and chronic pain), anticancer properties (Zhao et al., 2023b)

Identification of growth bifurcations with morphological markers

Meristemic growth bifurcations can be efficiently identified using morphological markers. One such marker of meristemic growth blockage is the shortened root growth zone, which, in *F. pratensis* plants under stress conditions, measures approximately 1000 µm—3 to 3.5 times shorter than in plants where growth bifurcations do not occur (Šimkūnas et al., 2007; Šimkūnas and Denisov, 2021). A significant reduction in the cell production rate (*V*) leads to a decrease in the number of growing cells, resulting in a substantially shorter growth zone.

Another marker of meristemic growth blockage is the high dynamics of root hair length, or in other words, an increased length of root hairs in the beginning of the differentiation zone (Šimkūnas and Denisov, 2021). The drastic decrease in *V* caused by bifurcation results in a reduced frequency of cells exiting the growth zone. Consequently, earlier root hairs (each being a single cell with tip growth) have sufficient time to elongate, leading to a sudden increase in root hair length.

In the absence of bifurcation and under conditions of a high cell production rate (*V*), cells exit the growth zone frequently, preventing root hairs from having sufficient time to grow. As a result, a more gradual, rather than abrupt, increase in root hair length is observed. These proposed markers can effectively facilitate the rapid (express) selection of medicinal plants exhibiting growth bifurcations.

Markers can also be established at levels beyond morphological, including cytological, subcellular structures or molecular markers. The primary alternatives to morphological markers are various types of molecular markers, including various DNA markers, such as RFLP, SSR, AFLP and SNPs, as well as functional markers. Functional markers, in particular, are distinguished by the absence of recombination and complete linkage to the desired allele, what prevent information loss and false selection. The review by Amom and Nongdam (2017) provides an in-depth description of various molecular markers, detailing their methodologies, strengths, limitations, and applications in plant breeding and genetic research. In studies of growth bifurcation, such markers could be employed at a later, final selection stage, as their use typically requires more advanced laboratory equipment and is more time-consuming compared to morphological markers.

Some technology-based solutions can also be highlighted, including advanced imaging techniques such as confocal microscopy and fluorescence imaging. These methods are instrumental in visualizing stress responses at the cellular level (Jing et al., 2024). Additionally, the authors observed another potential cytological marker of growth bifurcation: a higher number of two-nucleolus cells in a meristem compared to when bifurcation is absent. Further studies are needed to explore this effect, which could be the focus of another publication.

Future directions and practical implications

As it has been demonstrated above, the growth bifurcations in medicinal plants can be detected using morphological markers. These markers include features such as a short growth zone and rapidly growing root hairs. Finding these markers across broader biodiversity and applying them in marker-assisted selection represents a promising direction for future research.

Once the stress tolerance traits (e.g., growth bifurcations) of specific plants are identified, subsequent studies could focus on the accumulation of secondary

metabolites, their types, quality, and the quantification of metabolite production parameters. Such experiments should be conducted under both natural and controlled conditions to ensure comprehensive analysis.

A prospective direction for further investigation involves studying medicinal plants in their natural environments while monitoring stress events of varying durations (e.g., low-temperature hardening lasting 5–7 days) and employing standard meteorological data to evaluate stress severity.

For plants that produce particularly valuable secondary metabolites, cultivation in controlled environments – such as climate chambers or hydroponic systems – presents a viable solution. The climatic hydroponics system (CHS) developed by the authors offers a suitable platform, as it allows precise regulation of nutrition, temperature, photoperiod, and light conditions (Šimkūnas et al., 2018).

As previously mentioned, growth bifurcation is linked to a significant reduction in the energetically intensive process of meristemic growth. The underlying cause of root growth bifurcation is localized at the tissue level, specifically within the meristem. A promising research approach, therefore, involves the systemic investigation of medicinal plants at multiple hierarchical levels. At the sub-cellular level, this could include analyzing meristemic growth components associated with stress tolerance (e.g., cell cycle duration, lifespan of meristematic cells) alongside their structural characteristics. This analysis could then be integrated with targeted molecular studies, such as the identification of proteins and genes functionally involved in the growth blockage mechanism.

Conclusion

This paper presents a systematic two-stage review based on findings from various sources, summarizing and discussing stress patterns that drive the synthesis and accumulation of secondary metabolites in medicinal plants.

The first stage focuses on stress-related plant growth phenomena that can only be observed through higher-level studies. It delves into detailed reviews and discussions of phenomena such as growth blockage and growth bifurcation, incorporating findings from previous studies by the authors and other sources. This section concludes with the hypothesis that the increased synthesis and accumulation of medicinal compounds in stress-tolerant plants may stem from growth bifurcations triggered by temperature or other stress conditions. Evidence from prior studies within the *Poaceae* family suggests that growth bifurcations – marked by a significant decline in meristematic growth – redirect carbon compounds from biomass production toward the synthesis of protective compounds, including secondary metabolites. This targeted accumulation is further supported by the source-sink “carbon-nutrient balance” hypothesis, which posits that stress suppressing growth more than photosynthesis results in secondary metabolite buildup. Moreover, the role of key secondary metabolites, such as phenolic compounds and polyamines, in providing antioxidant and osmoprotective functions critical for stress resistance, reinforces the hypothesis.

The second stage integrates literature analysis with the principles of diversity and homology to guide plant selection. This process has enabled the compilation of a prioritized list of research candidates, focusing on monocotyledonous perennial medicinal plants exhibiting stress tolerance and intermediate growth strategies. These species primarily belong to the *Poales* order and, within its *Poaceae* family, the *Poeae*

tribe – common in Lithuania, a typical middle-latitude climate zone habitat. These plants are considered the most likely candidates for experiencing growth bifurcations and consequently enhanced secondary metabolite production. While the list itself is inherently valuable for identifying medicinal plants with potentially high production of medicinal substances, it also serves as a tool for selecting promising subjects for future stress-related studies.

To simplify the identification of growth bifurcations in medicinal plants, morphological markers such as short growth zone and rapidly growing root hairs are proposed as preferable alternatives to molecular markers. These morphological indicators offer a more efficient method for detecting growth bifurcations without the need for complex laboratory equipment, making them less time-consuming and more accessible.

The paper outlines key directions for further research, including the confirmation of the hypothesis and the establishment of numerical parameters for metabolite production across a broader range of medicinal plants. This approach is expected to facilitate the integration of various aspects of medicinal plant breeding, harvesting, and the practical application of secondary metabolites.

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