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Characterization and *In Vitro* Investigation of the Puffy Stem Phenomenon in Capsicum annuum *Pfi* mutant plants

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1. INTRODUCTION AND OBJECTIVES

Capsicum annuum has been extensively valued across different cultures since ancient times for its characteristic flavor, color, and aroma. It is widely incorporated into culinary preparations and dietary formulations. Bell pepper has become an important high-value, low-volume cash crop cultivated under protected structures, providing immediate economic returns to stakeholders worldwide. Moreover, its pharmacological relevance is well established, as the fruit is traditionally employed for its circulatory stimulant properties (Khan *et al.*, 2014). Hungarian bell pepper holds a significant place in the nation's traditional cuisine and dietary practices (Horváth *et al.*, 2000). Peppers possess significant breeding potential for nutritional enhancement due to their richness in vitamins A and C, carotenoids, and capsaicin. In addition, they have recently gained prominence in the ornamental plant market (Batiha *et al.*, 2020).

Mutant traits represent important genetic resources for pepper breeding, offering wide phenotypic variation and novel alleles that contribute to improvements in plant architecture, fruit quality, and stress tolerance. Characterization of mutant traits requires detailed phenotypic, biochemical, and molecular evaluations, with studies reporting notable differences in capsaicinoid levels, antioxidant activity, and related biochemical attributes among mutant lines (Naegele, Mitchell and Hausbeck, 2016). Stem mutations were once regarded as deleterious with limited practical value; however, contemporary breeding approaches recognize their potential, utilizing mutant traits to enhance cultivars and develop innovative cultivation strategies (Shalaby and El-Banna, 2013). Traits such as leaf and branch growth angles can influence yield, making them potential targets for crop improvement (Waite and Dardick, 2021).

Gravitropism regulates plant posture and orientation through gravity perception and signal transduction, ensuring efficient growth and photosynthesis. Lignins, on the other hand, provide structural integrity, mechanical strength, and stress resistance. While lignin has been well-studied in several plant families, research within the *Solanaceae* especially in *Capsicum annuum* remains limited.

In *Capsicum annuum*, a mutant designated as *pfi* exhibits a pronounced laying growth habit and stem puffiness localized to the internodes, indicating possible impairments in structural support. This phenotype suggests underlying defects in mechanical rigidity, potentially resulting from altered water regulation, reduced lignin deposition, or other cell wall modifications. To investigate this, we propose studying in vitro germinated seedlings to assess their responses to light and gravity, including the speed of gravitropic reorientation and we aim to characterize the lignin content and biomechanical properties of a *pfi capsicum* mutant line. These experiments will clarify whether the laying phenotype is due to impaired tropistic signaling or compromised stem biomechanics.

Pfi mutants were found to have an unusual laying phenotype with puffiness in the stem and the exact reasons are unknown. Our main goal is to find the reasons behind this unusual phenotype. To achieve that goal we have set these objectives:

A. To record the gravitational and phototropic response by phenotypical analysis using *in vitro* method.
B. Characterize them based on their reaction to gravity and light.
C. To check if there are any differences in lignin content between the mutants and the control plants.

2. LITERATURE REVIEW

3.1. *Capsicum annuum*: Botany, Cultivation and Importance

The genus *Capsicum*, belonging to the *Solanaceae* family, comprises a diverse group of plants with significant economic and nutritional value. *Capsicum annuum* is an annual shrub reaching 0.75–1.8 m in height, characterized by angled, branched stems measuring 20–80 cm. The leaves are glabrous, long-petiolate, and ovate to lanceolate with an acuminate apex. Flowers are pedicellate, with a calyx bearing short teeth and a typically white corolla that may occasionally exhibit green or purple pigmentation. The fruit, a many-seeded berry, ranges from 1–25 cm in length and occurs in diverse shapes including cylindrical, oblong, obtuse, or ovoid forms. At maturity, the fruits turn either white, red or purple, displaying a smooth and glossy surface (Basu and De, 2003).

Capsicum includes over 30 species, with five domesticated ones: *C. annuum*, *C. chinense, C. frutescens, C. baccatum, and C. pubescens*. The genetic diversity within *Capsicum* species is substantial, particularly in regions like South America, which serve as primary centers of diversity. Studies using various genetic markers, such as MIG-seq and ISSR, have revealed distinct genetic groups and moderate genetic diversity among accessions. For instance, in Guam, *Capsicum* accessions were clustered into three distinct groups assignable to *C. frutescens, C. annuum, and C. chinense* . Similarly, in the Yucatan Peninsula, genetic diversity was distributed mainly among accessions, indicating valuable genetic resources for improvement programs (López Castilla *et al.*, 2019; Jaiswal *et al.*, 2021; Pavani *et al.*, 2024).

Capsicum species are rich in health-promoting compounds like carotenoids, ascorbic acid, capsinoids, and capsaicinoids (Jaiswal *et al.*, 2021). These compounds have various applications in food, spice, pharmaceuticals, and cosmetics industries (K *et al.*, 2024). Capsaicinoids, responsible for the pungency in peppers, have been studied for their health benefits, including antioxidant, antimicrobial, and potential therapeutic effects against cancer, diabetes, and gastrointestinal diseases. The bioactive compounds in *Capsicum* make them valuable for developing functional ingredients and natural preservatives (Salehi *et al.*, 2018).

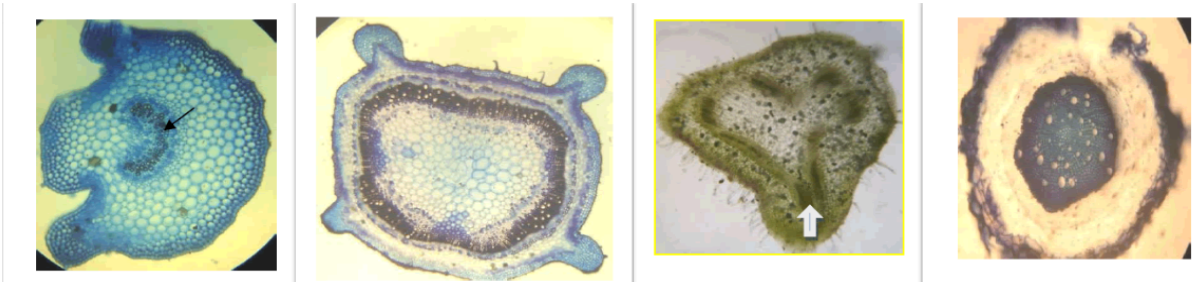
Capsicum annuum is an economically and nutritionally important crop with diverse applications in food, pharmaceutical, and industrial sectors. In food processing, peppers serve as natural colorants, flavor enhancers, and sources of pungency, with paprika and its oleoresins being particularly valuable for imparting red color, aroma, and texture to a wide range of products, including meats, cheeses, sauces, and snack foods (Govindarajan, 1986). Beyond their culinary role, peppers are widely acknowledged for their health benefits. They have been traditionally used to clear respiratory passages, stimulate digestion, and induce endorphin release, thereby providing natural pain relief. Additionally, the antioxidant compounds present in peppers contribute to disease prevention, including protection against certain cancers and oral health improvement (Andrews, 1995).

3.2. Stem morphology and anatomy in *Capsicum annuum*:

The stems of *Capsicum annuum* are generally characterized as angular and glabrous, commonly attaining heights of 60–65 cm or more. *Capsicum annuum* exhibits diverse growth habits, ranging from compact, determinate bushes to tall, indeterminate forms requiring staking, a variation largely governed by genetic factors and influenced through breeding (Bosland, Votava and Votava, 2012). The stem is initially herbaceous and erect, becoming semi-woody at the base with maturity, and follows a sympodial branching pattern in which monopodial growth terminates in a flower before continuing from axillary buds, thereby shaping plant architecture and fruit distribution (Singh, 2007).

Anatomically, the stem conforms to the dicotyledonous organization, consisting of an epidermis with a cuticle, a cortex containing chlorenchyma and collenchyma for flexible support, and a eustelic vascular system with collateral bundles. The vascular cambium facilitates secondary growth, enhancing mechanical strength at maturity, while the pith and medullary rays serve in storage and radial transport. Functionally, the integration of collenchyma and secondary tissues provides resilience against lodging and supports heavy fruit loads, while xylem vessel density is critical for hydraulic conductance and drought performance (Gniffke *et al.*, 2013). Moreover, the stem contributes to defense through structural barriers such as the cuticle, lignified tissues, and inducible responses like tyloses that limit pathogen spread (Agrios, 2005). Despite this understanding, research gaps remain, particularly in quantifying the relationship between specific anatomical traits (e.g., vessel density, collenchyma thickness) and agronomically relevant outcomes such as lodging resistance, stress adaptation, and the effects of modern breeding on stem plasticity. The petiole of *Capsicum annuum* consists of an epidermal cell layer, underlain by 2–4 layers of collenchyma in the hypodermis, while the cortex is largely composed of parenchymatous cells. During primary growth, two vascular traces and two rib bundle wings are evident at each wing position. The internode displays a four-sided or rectangular structure with swollen protuberances at each corner. Its hypodermis contains five layers of collenchyma, followed by three layers of thin-walled parenchyma in the cortex. The endodermis is formed by a single layer of barrel-shaped cells, beneath which lies a pericycle of 3–4 cell layers. The pith comprises large parenchymatous cells. The nodal pattern is unilacunar, with two vascular traces arising from one gap. Root anatomy shows a single-cell-thick piliferous layer, radially arranged vascular bundles with exarch xylem, and a pith region filled with parenchymatous cells (C, E and Edwin-Wosu, no date). See (Figure 01):

Figure 01. Petiole anatomy. Arrow point at vascular arc, Stem inter modal anatomy, Nodal; arrow reveals 2 vascular traces from 1 gap and Root anatomy with vascular system at radial symmetry

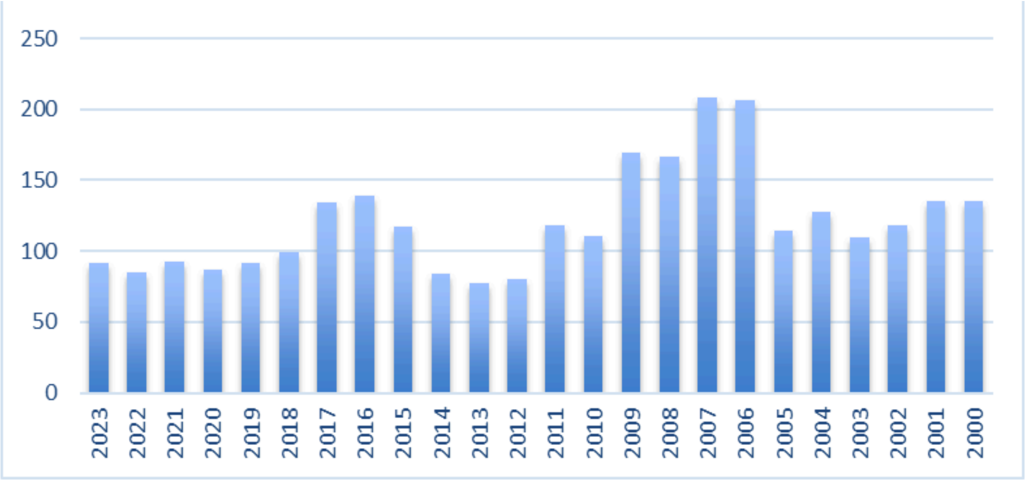


3.3. *Capsicum annuum* L. Production in Hungary

Hungarian pepper production spans open-field (notably the paprika belts of Szeged and Kalocsa) and extensive protected cultivation (walk-in plastic tunnels and heated greenhouses). In covered production, microclimate management is central: colored shade nets in Hungarian tunnels modify light spectra (–23–39% total radiation; –32–46% PAR), with yellow and red nets often improving yield and quality relative to green or black under local conditions (Darázsi, 2014; Ombódi *et al.*, 2015).

Recent Hungarian figures indicate a large protected-cultivation footprint for pepper alongside a contracting open-field area. At present, around 24–25 million tons of pepper are harvested each year from approximately 2 million hectares globally, with Hungary contributing close to 1% of total world production (Figure 02). In 2023, a total of 90 thousand tons of peppers were harvested from 1,281 hectares in Hungary. The area dedicated to outdoor pepper cultivation has been steadily declining, with current production predominantly taking place in controlled plant-growing structures (Pepper | Land & Water | Food and Agriculture Organization of the United Nations | Land & Water | Food and Agriculture Organization of the United Nations, no date) . At the EU level, covered cultivation and geothermal heating underpin the competitiveness of Hungarian peppers in certain regions, even as total greenhouse hectares remain modest (Szathmáry, Szendrei and Fehér, 2025).

Figure 02: *Capsicum* production in Hungary (metric tonnes) from the year 2000 to 2023



In recent years, the economic position of pepper cultivation in Hungary has declined, leading many farmers to abandon production due to the numerous challenges involved. Consequently, the cultivated area has steadily decreased, while consumer demand has remained strong, resulting in increased reliance on imports, primarily from countries such as South Africa, China, and Spain. In some cases, imported red pepper powders from different origins have been blended, making authentic Hungarian pepper scarcely available in the market. Additionally, concerns regarding aflatoxin contamination have further undermined the reputation of Hungarian spice pepper. To address these issues, stakeholders across the sector—including researchers, breeders, farmers, and traders—must collaborate to mitigate risks in spice pepper cultivation and powder production, while enhancing the sector’s overall competitiveness. This requires the development of improved genotypes and innovative technologies by researchers and breeders, alongside the effective adoption of these advancements by other participants in the value chain (Lantos *et al.*, 2011).

3.4. Breeding strategies

Capsicum species display extensive diversity in their morphological traits, biochemical composition, and molecular characteristics, which account for the divergences observed among species (Sokona *et al.*, 2013). The extensive genetic diversity within *Capsicum* species serves as a critical foundation for the conservation of genetic resources, advancement of breeding strategies, understanding of evolutionary processes, and elucidation of mechanisms underlying adaptation to biotic and abiotic stresses, as well as broader ecological and environmental interactions (Caliskan, 2012; Meyer and Purugganan, 2013). Genetic diversity provides insight into the degree of differentiation within and between species or populations, facilitating the identification of relationships among species or cultivars and thereby enhancing our understanding of crop evolutionary history. Such variation is also essential for breeding programs, as it enables the selection of parental combinations that generate segregating progenies with maximum variability, while supporting the introgression of desirable traits from diverse germplasm into existing genetic backgrounds (Mohammadi and Prasanna, 2003). Consequently, the systematic assessment of genetic diversity represents a fundamental step in crop improvement, underpinning the development of superior cultivars through targeted breeding practices (Islam *et al.*, 2012).

Chilli germplasm demonstrates considerable genetic variability. Within gene bank accessions, novel sources of genetic diversity can be explored to identify desirable genotypes. However, accurate characterization of these accessions is essential to facilitate their effective utilization. Breeding efforts should focus on developing cultivars with elevated levels of bioactive compounds that contribute positively to consumer health (Hoffmann, Noga and Hunsche, 2015).

Peppers (*Capsicum spp.*) are predominantly self-pollinated, diploid species with perfect flowers containing both male and female reproductive organs, and are commonly bred using self-pollination, controlled crosses, and selection to develop improved cultivars. Despite significant variation in genome size across the family, members typically possess a relatively high chromosome number ($2n = 2x = 24$), although certain wild *Capsicum* species exhibit $2n =$

2x = 26 chromosomes. The genome of *C. annuum* (3.48 Gb) is approximately three times larger than that of the tomato. On average, exons and introns measure about 286.5 bp and 541.6 bp, respectively, encompassing roughly 34,900 genes with a total of 2.34 Gb (76.4%) of translated regions. Comparative genomic analyses have revealed that the hot pepper genome is organized into multiple syntenic blocks corresponding to those in the tomato genome, its closest relative within the *Solanaceae* family (Kim *et al.*, 2014).

In ancient times, indigenous peoples of tropical America effectively employed Mass Selection, saving seeds from superior plants for the next cultivation season, a technique still valuable for identifying genetically diverse populations with high heritability potential and improving genetic stocks for breeding programs (Mohan Rao and Anilkumar, 2020). Modern breeding methods such as the Pedigree Method involve controlled crossing, self-pollination, and meticulous record-keeping to select superior segregants with favorable heritable traits (Gosal *et al.*, 2020). The Single Seed Descent (SSD) Method accelerates the development of homozygous or recombinant inbred lines (RILs) by advancing generations without selection, often under controlled environments, and can be integrated with biotechnological approaches for developing lines resistant to biotic stresses (Bermejo, Gatti and Cointry, 2016). Recurrent Selection entails repeated cycles of selecting superior individuals from a base population, intercrossing them, and evaluating their progeny, thereby enhancing selection accuracy for desirable recombinants (Ridzuan *et al.*, 2018). Lastly, the Backcross Method is particularly useful for improving traits governed by a few genes, such as fruit quality or disease resistance, by repeatedly crossing selected individuals with a recurrent parent to introduce beneficial genes while minimizing genetic drag (Negi, Thakur and Sharma, 2018). Together, these conventional and contemporary approaches provide an integrated framework for enhancing efficiency and precision in chilli breeding.

In recent decades, DNA-based molecular marker technologies have become widely utilized in genetic studies owing to their efficiency, rapid application, cost-effectiveness, and high discriminatory power both within and between species or varieties (Azeem *et al.*, 2012). Molecular markers are considered more reliable for detecting genetic variation, as they are unaffected by environmental influences and enable the identification of differences at the genomic level, making them particularly suitable for assessing genetic diversity (Gupta *et al.*, 2019). In the present study, genetic variation among 54 Capsicum accessions was evaluated using ISSR markers to provide a comparative overview of genetic polymorphism, primer efficiency, cross-transferability, and structural plasticity. The ISSR marker system is widely recognized as a rapid, user-friendly, reliable, cost-effective, and highly informative tool for diverse genetic applications (Verma *et al.*, 2017). Owing to their reproducibility and ability to target microsatellites abundantly distributed across plant genomes, ISSR markers reveal a high degree of polymorphism. This is attributed to their use of longer primer sequences and higher annealing temperatures, while also offering the advantage of not requiring prior knowledge of flanking sequences, unlike SSR markers (Debnath, 2008). Biotechnological tools, such as tissue culture and genetic transformation, have been applied in Capsicum breeding to improve efficiency, leading to the development of genetically modified plants with enhanced traits (Jaiswal et al., 2021). More recently, the application of nanoparticles in seed treatments has demonstrated potential in promoting germination and improving seedling vigor (Henriquez-Alegría and Asmat-Campos, 2024).

Mutation breeding plays a vital role in developing new crop varieties for sustainable production. Mutations may occur spontaneously or be artificially induced, and they represent a key evolutionary force responsible for generating novel alleles and contributing to genetic diversity. Since spontaneous mutations occur at a relatively slow rate, induced mutagenesis is often employed to accelerate genetic variation, thereby enabling plant breeders to effectively exploit this diversity in breeding programs (Chaudhary *et al.*, 2019). Mutation breeding uses mutagenic agents, including radiation and chemical treatments for example- ethyl methanesulfonate (EMS), sodium azide (NaN_3), and gamma rays, to induce genetic alterations that generate novel variation from which superior and desirable mutants can be selected. The integration of radiation-induced mutagenesis with tissue culture techniques has significantly contributed to plant breeding by introducing innovative approaches that shorten breeding cycles and accelerate varietal development.

As part of breeding strategies aimed at developing disease-resistant cultivars, *Capsicum annuum* remains vulnerable to a wide range of pathogens, including fungi, viruses, and bacteria. Considerable research has therefore focused on identifying resistance sources and elucidating the molecular mechanisms underlying host–pathogen interactions. For example, the pvr2 gene from wild *C. annuum var. glabriusculum* has been extensively studied for its role in conferring resistance to potyviruses. Furthermore, the integration of pathogen population genomics with molecular plant pathology has advanced the development of cultivars with durable resistance (Poulicard *et al.*, 2016; Prokchorchik *et al.*, 2020).

3.5. Genetic mutations in *Capsicum annuum*

Genetic barriers have contributed to the reduction of genetic diversity in pepper, resulting in limited development of novel leaf-color cultivars in recent years. One effective strategy to overcome this constraint is the induction of mutations, as mutation breeding has been shown to provide plant breeders with valuable sources of variation for crop improvement. (Pathirana, 2011). Mutation breeding refers to the deliberate induction of mutations for use in crop improvement. It holds considerable potential for the domestication of underutilized wild species for agricultural and horticultural purposes, as well as for enhancing the adaptability of recently introduced crops to suboptimal environments. Mutagenesis has remained a widely adopted approach for nearly a century due to its methodological simplicity, cost-effectiveness, broad applicability across plant species, and suitability for implementation at both small and large scales (Konzak, 2001). To date, over 2,000 plant varieties developed through induced mutagenesis have been officially released for cultivation, either as direct commercial cultivars or as parental lines in breeding programs, without being subjected to the regulatory constraints imposed on genetically modified organisms (Maluszynski *et al.*, 2000).

According to the FAO/IAEA Mutant Varieties Database, a total of 1,847 mutant varieties have been documented, comprising 1,357 officially released cultivars of crop species and 490 ornamental or decorative plant varieties. The majority of crop mutants have been developed in seed-propagated species (1,284 entries), whereas vegetatively propagated crops account for only 73 varieties. Within cereals, which represent the largest group with 869 mutant varieties, rice dominates with 333 entries, followed by barley (261), bread wheat (147), maize (49), durum wheat (25), and other cereals (54). Mutants in *Capsicum* species provide valuable sources of agronomic traits such as disease resistance, stress tolerance, and improved yield. Structural stem mutations have also enabled innovative cultivation systems like on-wire and vertical farming, enhancing productivity under limited space (Vishvkarma *et al.*, 2025).

3.6. Plant response to environmental stimuli

Plants respond to a variety of external stimuli, including gravity, light, and touch. In response to gravitational forces, they adjust their orientation to a specific gravitropic set-point angle (Digby and Firn, 1995). This reorientation relies on differential cell elongation along the growth axis and is referred to as gravitropism. In seedlings, the process manifests as positive gravitropism in roots, directing them downward, and negative gravitropism in shoots, orienting them upward, with the overall response involving integrated mechanisms of perception, signal transduction, and growth regulation (Evans, Moore and Hasenstein, 1986). Plant stems generally display positive phototropism, growing toward light, whereas roots exhibit negative phototropism, growing away from light. This contrasting response is particularly critical during seed germination, ensuring that shoots access sunlight for photosynthesis while roots develop effectively, especially under drought conditions (Galen, Huddle and Liscum, 2004).

3.6.1. Gravitropism

Gravitropism is the physiological process through which plant growth is guided by gravitational forces, ensuring proper orientation of shoots, roots, and leaves. Maintaining correct posture is essential for efficient light capture and photosynthesis (Morita, 2010; Nakamura, Nishimura and Morita, 2019), while the capacity to sense gravity is equally critical for directing root growth downward into the soil, thereby facilitating access to water and essential minerals (Chen, Rosen and Masson, 1999). This process is highly complex and involves four sequential stages: gravity perception, initiation of signaling within specialized gravity-sensing cells, intra- and intercellular signal transduction, and finally, asymmetric cell elongation between the upper and lower sides of the responding organs (Cosgrove, 1997).

In roots, gravity perception primarily occurs in the root cap, a role demonstrated through experiments involving decapping or partial removal of the root cap. Following decapping, amyloplasts begin to form within the cells of the root apex, and it is proposed that as their development progresses, they acquire functionality as gravity-sensing organelles. Furthermore, it has been suggested that amyloplasts may serve as the source of an inhibitory substance that mediates the relationship between gravity perception and the subsequent geotropic response.

(Wilkins, Gibbons and Shaw, 1972; Shaw and Wilkins, 1973; Jackson and Barlow, 1981). Within this region, the columella cells function as the principal gravisensing units, with their sensitivity influenced by positional and developmental factors (Sack, 1991). These cells contain starch-filled amyloplasts (statoliths) that sediment and respond to internal forces, thereby serving as gravi-susceptors (Sack, Suyemoto and Leopold, 1984). The starch–statolith theory is strongly supported by several lines of evidence: reduced gravitropic responses in starch-deficient mutants (Sack, 1991 ; Kiss, Wright and Caspar, 1996) ,enhanced curvature in mutants with excess starch (Vitha *et al.*, 2007), and curvature induced by amyloplast-specific forces under high-gradient magnetic fields (Kuznetsov and Hasenstein, 1996, 1997). While the root cap is the dominant site of graviperception, it is not exclusive; gravitropic responses have also been observed outside this region (Poff and Martin, 1989), including curvature occurring when the root cap itself remained vertically aligned (Wolverton *et al.*, 2002). Collectively, these findings indicate that approximately 20% of overall gravisensing originates from tissues beyond the root cap (Wolverton, Ishikawa and Evans, 2002).

In plant stems, specialized gravity-sensing cells, known as statocytes, are found in the endodermis of structures such as hypocotyls, epicotyls, flower stalks, peduncles, gynophores, and leaf petioles (Vandenbrink and Kiss, 2019). When a plant senses gravity, the signal is first perceived as a mechanical force and then converted into biochemical messages through cell structures like membranes and the cytoskeleton. This process leads to an uneven distribution of the hormone auxin, which causes cells on one side of the organ to elongate more than those on the other, resulting in bending toward or against gravity (Blancaflor and Masson, 2003; Rakusová *et al.*, 2016; Nakamura, Nishimura and Morita, 2019a; Han *et al.*, 2021). If mutations interfere with this signaling pathway, plants may grow in the opposite direction of what is expected, a condition referred to here as the “anti-gravitropic” phenotype (Kawamoto *et al.*, 2020).

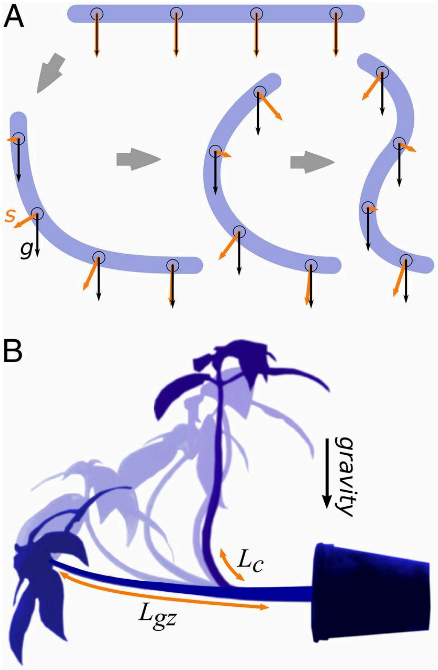
In a lot of gravitropic experiments, a 90° reorientation is used for the observation of the response that plants might have to the gravitational force (Stankovic, Volkmann and Sack, 1998; Ajala and Hasenstein, 2022; Barker *et al.*, 2022; Sharma, Pervaiz and Wysocka-Diller, 2022).

The classical model of gravitropism originates from the work of Julius Sachs, who proposed that the gravitational force component acting perpendicular to a plant organ’s axis (such as a stem or root) determines the intensity of the gravitropic stimulus (SACHS, 1882).

Based on this idea, the magnitude of a plant’s gravitropic response is proportional to the sine of the angle between the organ axis and the vertical. Consequently, a horizontally positioned stem would exhibit the strongest curvature response, which progressively diminishes as the stem reorients toward the vertical. Since its introduction, Sachs’ “sine law” has been repeatedly confirmed, sometimes with minor refinements (Iino, Tarui and Uematsu, 1996; Galland, 2002). Furthermore, the observation that statolith sedimentation within gravity-sensing cells follows an angular pattern consistent with a sinusoidal relationship (Audus, 1964; Larsen, 1969) provided a mechanistic basis for the law. Hence, despite its conceptual simplicity, the sine law effectively describes plant responses to gravity and maintains theoretical support through statolith behavior.

The limitations of the sine law become apparent when it is used as the basis for constructing a regulatory model of gravitropism. Developing such a model requires identifying the site of gravity perception and understanding how the sensory components respond to gravitational stimuli. In roots, gravity perception is confined to the columella cells within the root cap (Blancaflor, Fasano and Gilroy, 1998; Swarup *et al.*, 2005) and the resulting signal is conveyed to the elongation zone through a redistribution of auxin flow (Friml *et al.*, 2002; Ottenschläger *et al.*, 2003). In contrast, stems exhibit distinct behavior. Experimental findings demonstrate that gravity sensing in stems is distributed along their entire length, enabling local responses to gravitational cues (Fukaki, Fujisawa and Tasaka, 1996; Fukaki *et al.*, 1998). The apical region does not appear to have a unique role, as decapitated stems maintain normal gravitropic responses (Firn, Digby and Hall, 1981). As shown by (Bastien *et al.*, 2013), if each gravisensing region along the stem followed the sine law independently, the organ would oscillate around the vertical axis without ever stabilizing. This occurs because the lower portions of the stem continue to perceive and respond to gravity even after the upper regions have realigned vertically, causing repeated overshooting of the stem apex due to persistent curvature at the base (Figure 03).

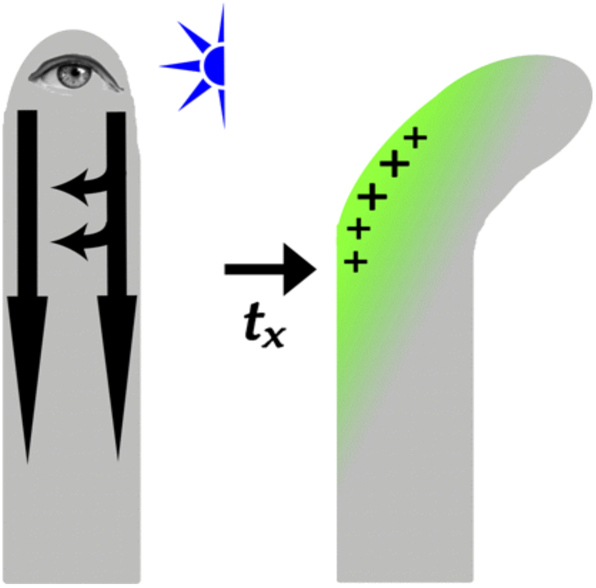
Figure 03: This figure illustrates the gravitropic behavior of plant stems following the sine law. (A) The stem's gravity response occurs along its entire length, with curvature induced by the gravity vector's effective component. As the basal region continues bending while the apex nears vertical, the stem temporarily overshoots the upright position. (B) In Impatiens glandulifera, a pronounced gravitropic response (bending number ≈ 9) leads to successive "C" and "S" shapes before the stem stabilizes upright. Initially, curvature develops throughout a large growth zone, later localizing to a shorter segment as the stem straightens. This final alignment demonstrates the role of autotrophic straightening in achieving stable vertical posture. Lgz = extensive growth zone , Lc = length scale. (Dumais, 2013)



3.6.2. Phototropism

The inherent sessility of plants necessitates a reliance on directed growth mechanisms to maximize photosynthetic efficiency. These tropic responses enable precise orientation in reaction to directional stimuli, a vital adaptive strategy for energy optimization. The mechanistic basis of these movements has captivated scientific thought since the era of Ancient Greece. In Charles Darwin's work, The Power of Movements in Plants, Darwin postulated several foundational principles that continue to underpin contemporary research on tropic responses. While not the first to investigate this phenomenon , Darwin first posited that directional growth in plants occurs through differential growth triggered by external stimuli like light or gravity. A second critical contribution was his demonstration that the perception of a stimulus and the subsequent growth response are localized in distinct anatomical regions of the plant. Using phototropism as a model, he illustrated that the apical portion of a young seedling perceives directional light. This signal is then transduced to more basal tissues, initiating a differential growth response that causes the shoot to bend toward the stimulus (Darwin, 2009) (Fig. 04). Finally, Darwin theorized that a mobile "influence" later identified as the hormone auxin is transmitted from the site of perception to the site of response to orchestrate this bending (Whippo and Hangarter, 2006).

Figure 04: This illustration shows phototropism in a dark-grown grass coleoptile. After exposure to one-sided blue light, the tip senses light and directs auxin to the shaded side. During the refractory period (t_x), auxin accumulates there, promoting localized cell elongation and causing the coleoptile to bend toward the light (Holland, Roberts and Liscum, 2009).



Plant stems generally display positive phototropism, growing toward light, whereas roots exhibit negative phototropism, growing away from light. This contrasting response is particularly critical during seed germination, ensuring that shoots access sunlight for photosynthesis while roots develop effectively, especially under drought conditions (Galen, Huddle and Liscum, 2004). Similar to gravitropism, the phototropic process is characterized by three sequential phases: the perception of a light signal, the transduction of this signal, and the resultant directional growth response. Research utilizing phototropism-deficient mutant plants has been instrumental in identifying several key proteins implicated in light perception. Specifically, studies have established that the primary photoreceptors mediating phototropism in angiosperms are blue light photoreceptors, which also exhibit sensitivity to UV-A and green light wavelengths (Christie and Briggs, 2001).

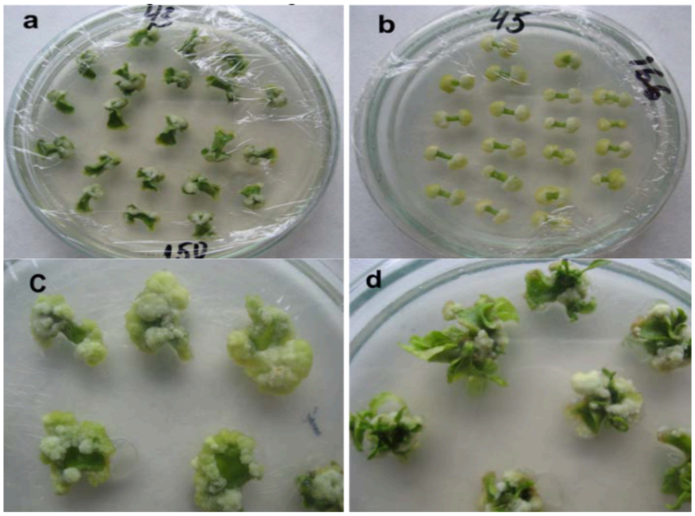
3.7. *In vitro* micropropagation

Micropropagation is a technique where small pieces of plant tissue, known as explants, are grown under sterile conditions in vessels with carefully prepared culture media and controlled environments. It is one of the most effective and widely used biotechnological methods today, as it allows for the rapid production of large numbers of identical plants that are often free from viruses and other pathogens. Beyond large-scale multiplication, micropropagation plays a vital role in developing transgenic plants, as it enables the regeneration of whole plants from cells or tissues that have incorporated new DNA through genetic transformation. Combined with other tissue culture and molecular biology tools, this approach also speeds up the testing and evaluation of new plant genotypes and field selections.(Loberant and Altman, 2010)

By importance, plant breeding can be made faster and more efficiently with a specific biotechnological method known as *in vitro* micropropagation. It helps conserve gene pools (Kumar and Reddy, 2011), allows selective cloning of plants (George, Hall and De Klerk, 2008), and makes it possible to produce large numbers of disease-free, uniform, and high-quality plants in a small space, regardless of the season (George, Hall and De Klerk, 2008; Abdalla *et al.*, 2022). However, the success of *in vitro* micropropagation depends on many factors, including the culture medium, growth environment, and the type of tissue used as explants (George, Hall and De Klerk, 2008; Zhao *et al.*, 2021). Moreover, different plant genotypes often respond differently under the same culture conditions, and in some cases, certain inherited traits can make plants unable to adapt to *in vitro* conditions, preventing them from forming new shoots (Hundleby *et al.*, 2004). Thus to face common issues like contaminations and phenotypic anomalies (Abdalla *et al.*, 2022), it is essential to consider genotype-specific in vitro micropropagation to achieve sustainable, disinfected and reproducible regeneration efficiency (Teixeira da Silva *et al.*, 2015).

By many experiments in *Capsicum annuum*, it has been noticed that, for establishing optimal conditions to induce organogenesis and regeneration to prove the influence of the genotype, explant type and culture medium composition has been used on the plants (Steinitz *et al.*, 1999; Peddaboina and Subhash, 2001; Kumar and Sape, 2010). In a few Bulgarian *Capsicum spp.* such as Hebar, Stryama, Kurtovska kapiya 1619 and Maritsa, cotyledon and hypocotyl explants exhibited callus formation across all tested culture media and genotypes. Callus induction appeared white or pale green tissue. The morphology of the callus varied by the genotypes and culture mediums. In some genotypes, callus was hard and compact with no shoot development, whereas in others it was friable, whitish, and produced leafy structures and elongated shoots. (Grozeva, Rodeva and Todorova, 2012) Referring to Figure no. 05:

Figure 05: a. Induction of callus formation in cotyledons. b. Induction of callus formation in hypocotyls. c. Callus without organogenesis. d. Callus with shoots. (Grozeva, Rodeva and Todorova, 2012)

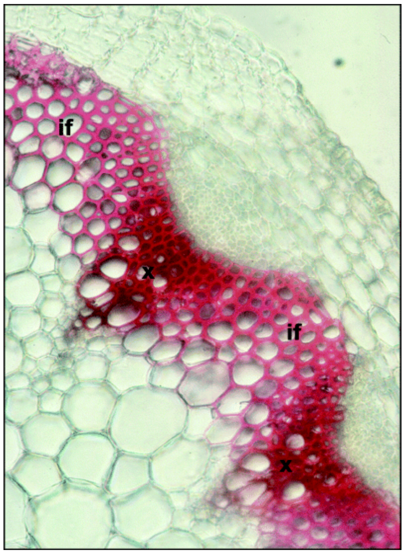


Plants are constantly exposed to various environmental stimuli, which highlights the need for systems that allow precise analysis of their gravitropic responses (Yaseen *et al.*, 2013). In vitro experiments provide such an opportunity by enabling the study of plant growth under controlled conditions, free from external biotic or abiotic influences, and with a defined culture medium (Kumar and Reddy, 2011). Beyond these advantages, in vitro methods ensure uniform plant material, allow work under sterile conditions, and make it possible to observe individual plant organs separately for more accurate analysis.

3.8. Lignin content in plants

Lignins are complex and heterogeneous polymers embedded within the cell walls, synthesized by nearly all terrestrial plants (Rogers and Campbell, 2004). In extant plant species, lignins are predominantly deposited in the cell walls of tracheids and vessel elements of the xylem, and are also present in sclerenchyma, phloem fibers, and periderm tissues (Esau, 1960) refer Figure 06.

Figure 06. Lignin deposition in a herbaceous angiosperm stem was visualized in a cross-section of an *Arabidopsis thaliana* inflorescence stem using phloroglucinol-HCl staining. Lignified tissues appear red to magenta, prominently marking the xylem cells (x) and interfascicular fibers (IF) (Esau, 1960).



Lignins confer mechanical support to the plant body and allow the xylem to transport water and minerals under negative pressure without tissue collapse (Jones, Ennos and Turner, 2001). The induction of lignin synthesis following pathogen attack or physical wounding serves a dual defensive function. Primarily, the deposition of lignins confers hydrophobic properties to cell walls, which reduces water loss and mitigates the risk of dehydration (Reina, Domínguez and Heredia, 2001). Furthermore, the complex and heterogeneous structure of lignin polymers makes them inherently recalcitrant to degradation. This characteristic presents a formidable physical and chemical barrier that impedes pathogen penetration and spread (Hammond-Kosack and Jones, 1996). Additionally, by reducing the digestibility of plant tissue, lignification diminishes the nutritive value and palatability for herbivores, thereby functioning as a deterrent (Moore and Jung, 2001). As such, lignin biosynthesis is a critical adaptation beyond its well-established roles in structural support and hydraulics, constituting a fundamental component of plant defense. It is consequently regarded as a pivotal evolutionary innovation that facilitated the colonization of terrestrial environments (Kubitzki, 1987).

The deposition of lignin is tightly coordinated with plant development, as spatial and temporal regulation of lignification is essential for structural support, efficient water transport, and disease resistance. Plants with impaired lignin synthesis are unable to adequately support their bodies (Zhong, Taylor and Ye, 1997). A productive strategy for elucidating the diverse roles of lignin involves the characterization of both naturally occurring and chemically induced lignin mutants. Such genetic variants provide valuable models for studying the consequences of altered lignin composition and quantity. For instance, the *cad-n1* mutant in loblolly pine (Pinus taeda) (MacKay *et al.*, 1997), which affects the cinnamyl alcohol dehydrogenase enzyme, and the fah1 mutant in Arabidopsis thaliana (Chapple *et al.*, 1992), which disrupts ferulate-5-hydroxylase activity, have been instrumental in linking specific genetic lesions to defined changes in lignin structure. The analysis of these and other mutants enables a direct investigation into the relationships between lignin biochemistry and its multifaceted functions in plant development and defense. Characterization of the irregular *xylem4 (irx4)* mutant in Arabidopsis thaliana, which exhibits a pronounced collapsed xylem phenotype, has been instrumental in elucidating the role of lignin in structural support. This mutant, impaired in the development of secondary cell walls, fails to withstand the negative pressures generated by transpirational pull within the xylem (Turner and Somerville, 1997). In contrast to cellulose-deficient mutants such as *irx3* (Taylor *et al.*, 1999), the *irx4* mutation specifically disrupts the lignin biosynthetic pathway. Subsequent molecular analysis demonstrated that the IRX4 gene encodes cinnamoyl-CoA reductase (CCR), a key enzyme acting in the monolignol-specific later stages of lignin production. The severe reduction in lignin content in *irx4* plants leads to profound alterations in cell wall ultrastructure and a significant reduction in the mechanical strength of the stem.

Recent research on the mechanical properties of plant stems has increasingly focused on their potential as sustainable biological resources for energy production and industrial applications. In particular, investigations have evaluated key mechanical attributes such as compressive strength and bending resistance in the stalks of crops like sorghum (Bakeer *et al.*, 2013), which serve as model systems for bioenergy feedstocks. These studies aim to optimize biomass quality and processing efficiency for use in biofuel and biomaterial production. Indeed, these findings collectively indicate that elevated deposition of secondary cell wall components, particularly lignin significantly enhances stem mechanical strength. Increased lignin content contributes directly to greater resistance to bending and structural failure under mechanical stress. However, despite the well-established role of lignin in stem robustness, experimental data specific to Solanaceous species remain limited. Most available studies in this family have focused on tomato stems (Zhang *et al.*, 2016), leaving a substantial gap in comparative understanding across economically and ecologically significant genera such as *Capsicum*, *Solanum,* and *Nicotiana*. Further research into the biomechanical role of lignin in Solanaceous stems could therefore provide valuable insights for both crop improvement and bioresource utilization.

3. MATERIALS AND METHODS

4.1. Plant materials

The breeding lines involved are *pfi* (puffy-structured stem). The *pfi* (puffy-structured stem) mutants originate from the collection of Bergh and Lippert (Bergh & Lippert, 1964). Plants grown from seeds of this collection have been maintained by self-pollination and were further selected by Gábor Csilléry for this mutant phenotype.

The phenotype is not easily detectable at early developmental stages, when the hypocotyl is particularly weak. Above the cotyledons, the plants begin to collapse, since the mutant-specific stem structure starts to develop only in this region, where the stem becomes considerably thicker. The swollen stem structure is striking and easily observable. It appears exclusively in the internodes, with no growth occurring at the nodes, which suggests that the mutation is responsible merely for volume expansion rather than for additional organogenesis (Figure 07). A commercially available cultivar ‘Garai Fehér’ was used as control.

Figure 07: *pfi* mutant plants at a mature growth stage showing puffiness in the stem

4.2. Preparation of MS media

For our experiments we used standard MS medium following the methodology of (Murashige and Skoog, 1962). Table 01 contains all the ingredients of the standard MS medium.

Table 01: MS medium components*Ph -5.6-5.8

Content	**Ratio (mg/L or g/L)**
MACROELEMENTS	
KNO3	1900
NH4NO3	1650
CaCl2×2H2O	440
MgSO4	370
KH2PO4	170
MESOELEMENTS	
FeSO4×7H2O	27.80
Na–EDTA	37.30
MICROELEMENTS	
MnSO4×4H2O	22.30
ZnSO4×7H2O	8.60
H3BO3	6.20
KI	0.83
Na2MoO4×2H2O	0.25
CuSO4×5H2O	0.025
CoCl2×6H2O	0.025
VITAMINS	
Inositol	100.00
Thiamine (B1)	0.10
Nicotinic acid (B3)	0.50

Pyridoxine (B6)	0.10
Glycine	2.00
OTHERS	
Plant Agar	8.00
Sucrose	30.00

Initially, the macro-, micro-, and meso-nutrients were combined in a 500 mL flask, followed by the addition of solid components such as myo-inositol and sugar. The mixture was then transferred to a volumetric flask and made up to a final volume of 500 mL with distilled water. Subsequently, the solution was returned to the flask, and its pH was measured using a pH meter, ensuring it remained within the optimal range of 5.6–5.8. Agar was then weighed and incorporated into the solution, which was tightly sealed with aluminum foil and sterilized in an autoclave for 20 minutes. This step aimed to ensure complete sterilization of the culture medium. After autoclaving, the medium was cooled by swirling in cold water. Once cooled, it was transferred under a laminar flow hood, where it was dispensed into pre-sterilized jars and securely covered with aluminum foil. No hormone supplementation was included in this experiment.

4.3. Seed sterilization and *in vitro* conditions

4.3.1. Materials for sterilization

For preparing *in vitro* plant cultures the seeds of *pfi* were used. Materials for sterilization protocol are summarized on Table 02.

Table 02: Solutions and instruments for sterilization process

1.	1% Hypochlorite solution
2.	70% ethanol solution
3.	Sterile distilled water
4.	Sterile flasks

The number of seeds sterilized was determined by the quantity required for each experimental setup. For assessing the plants' responses to light and gravity, four seeds were placed in each jar in one row, whereas for evaluating the time required for plants to respond solely to gravity, a single seed was placed in each glass vessel. Distilled water was used for sterilization using an autoclave for 25 minutes after reaching the boiling point. Glassware sterilization was carried out through dry heat treatment in a hot air sterilizer at 240°C for 120 minutes.

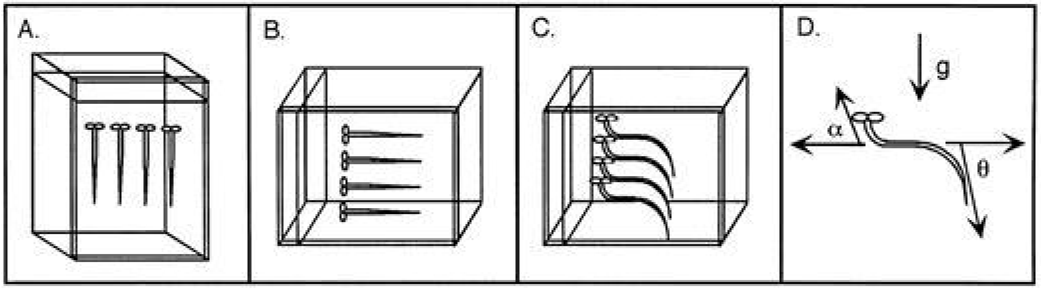
4.3.2. Sterilization of seeds

All procedures were conducted under sterile conditions within a laminar airflow chamber. The seeds were first placed in pre-sterilized flasks and treated with 70% ethanol, gently swirled for 45 seconds, and the ethanol was discarded. Subsequently, a 1% sodium hypochlorite solution was added, and the flasks were shaken for 20 minutes to ensure thorough surface sterilization. Following this, the hypochlorite solution was removed, and the seeds were rinsed three times with sterile distilled water to eliminate any remaining traces of disinfectant. The sterilized seeds were then transferred onto MS medium, positioned a few centimeters apart to prevent overcrowding during germination and growth. Finally, the cultures were tightly sealed with transparent foil to avoid contamination and placed in a phytotron chamber BINDER Model KBWF 240 (BINDER GmbH, Tuttlingen, Germany) with built in Osram BIOLUX T8 L 18W/965 G13 fluorescent lamps (ams-OSRAM AG, Premstaetten, Austria).

4.4. *In vitro* phototropic and gravitropic experiments

The samples were subsequently transferred to a growth chamber, where seed germination occurred at 25 ± 1 °C under a 16-hour light and 8-hour dark photoperiod, with a light intensity of 5000 lux. The experimental procedure followed the methodology described by Grube et al. (2003) (Grube, Brennan and Ryder, 2003). (Figure 08). To assess the plants' responses to light and gravity, one set of seeds was wrapped in foil to block light exposure, while the remaining seeds were placed uncovered in the growth chamber. Within the chamber, illumination was provided from one side only. Additional experiments were conducted two weeks after germination, during which the glass jars containing two-week-old plants were inclined at a 90° angle. Observations and documentation of plant responses commenced 24 hours after inclination.

Figure 08. Method used to evaluate gravitropic response in seedlings Straight seedlings were placed on solid plant media (A), which was then rotated 90° (B). Responding seedlings reoriented to resume vertical growth (C). Measurements taken included hypocotyl curvature. α, hypocotyl curvature; θ, root curvature; g, direction of the gravity vector. After 24 h, the hypocotyl curvatures were checked (°) (D) (Grube, Brennan and Ryder, 2003).



For assessing the time required for plants to respond to gravity, all *in vitro* plants were cultivated without any light-restrictive covering. This experiment followed the methodology described by (Ajala and Hasenstein, 2019). Two weeks after germination, the glass vessels containing the plants were inclined at a 90° angle. Observations were recorded hourly over an 8-hour period. The degree of gravitropic curvature in the stems was quantified using ImageJ, an image analysis software and the data were subsequently plotted in Microsoft Excel 365 to visualize the progression of the gravitropic response from 0 to 8 hours.

4.5. Measurement of lignin content

To determine the lignin content of the shoots, we applied the acetyl-bromide (C_2H_3BrO) method described by (Moreira-Vilar *et al.*, 2014).

Materials Required:

Lyophilized shoot tissue (mixed stem and leaf), Potassium phosphate buffer (50 mM, pH 7), Triton X-100 (1%, pH 7), Sodium chloride (1 M, pH 7), Bidistilled water, Acetone, Acetyl bromide (25% v/v, dissolved in glacial acetic acid), Glacial acetic acid, Sodium hydroxide (2 M), Hydroxylamine-hydrogen chloride (5 M, $NH_2OH \cdot HCl$), Alkali lignin (for standard curve; Sigma-Aldrich), Centrifuge tubes and centrifuge, Water bath (70 °C), Ice bath, WPA Biotech Photometer 1101 or equivalent spectrophotometer

Preparation of protein-free cell wall extract:

Two hundred milligrams of lyophilized shoot tissue were homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7) and centrifuged at 1400 g for 5 minutes. The supernatant was discarded, and the step repeated twice. The resulting pellet was treated sequentially with 1% Triton-X-100, 1 M NaCl, bidistilled water, and finally acetone—each time centrifuging at 1400 g for 5 minutes and discarding the supernatant. Each washing step was repeated twice to remove proteins and soluble impurities. The final acetone-washed pellet was dried overnight at 60 °C, yielding a protein-free cell wall extract.

Determination of lignin content using acetyl-bromide:

From the dried cell wall extract, 20 mg was mixed with 0.5 mL of 25% acetyl-bromide (in glacial acetic acid) and incubated at 70 °C for 30 minutes. The reaction was stopped by placing the tubes on ice, followed by the addition of 0.9 mL 2 M NaOH, 0.1 mL 5 M $NH_2OH \cdot HCl$, and 4 mL glacial acetic acid. The samples were centrifuged at 1400 g for 5 minutes, and absorbance was measured at 280 nm using a WPA Biotech Photometer 1101 (Cambridge, UK). For the preparation of the standard curve, alkali lignin (Sigma-Aldrich, Saint Louis, USA) was used.

4.6. Detection of starch accumulation in root tips by lugol staining

Materials required: Lugol solution (I_2/KI solution), Ethanol and distilled water

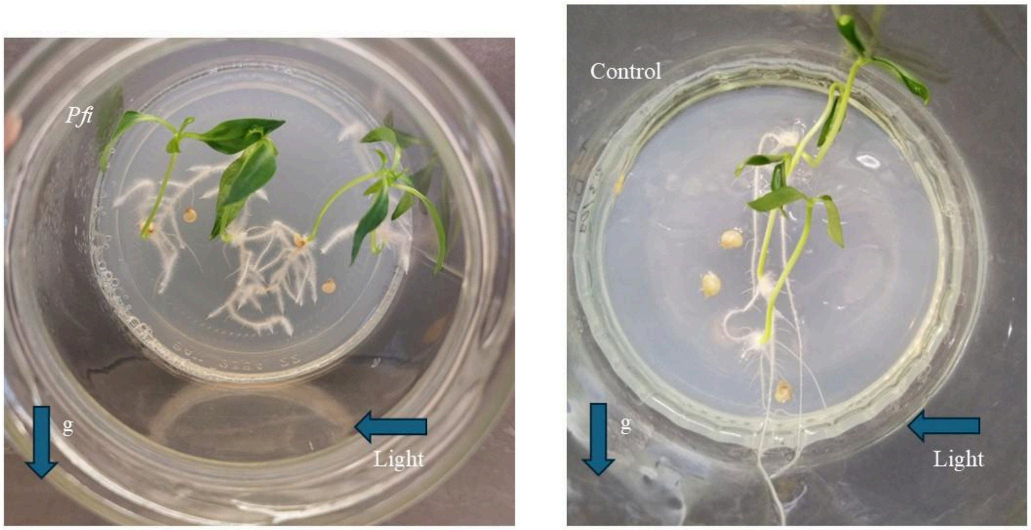
Root tips were collected from mature *pfi* mutant and control plants, thoroughly washed with distilled water, surface-sterilized using alcohol, and rinsed again with distilled water. The cleaned root tips from *pfi* plants were then immersed in a 3% Lugol solution and incubated in a Petri dish for 5–10 minutes to allow adequate staining and absorption.

4. RESULTS AND DISCUSSION

5.1. Phototropic and gravitropic response of *pfi* plants

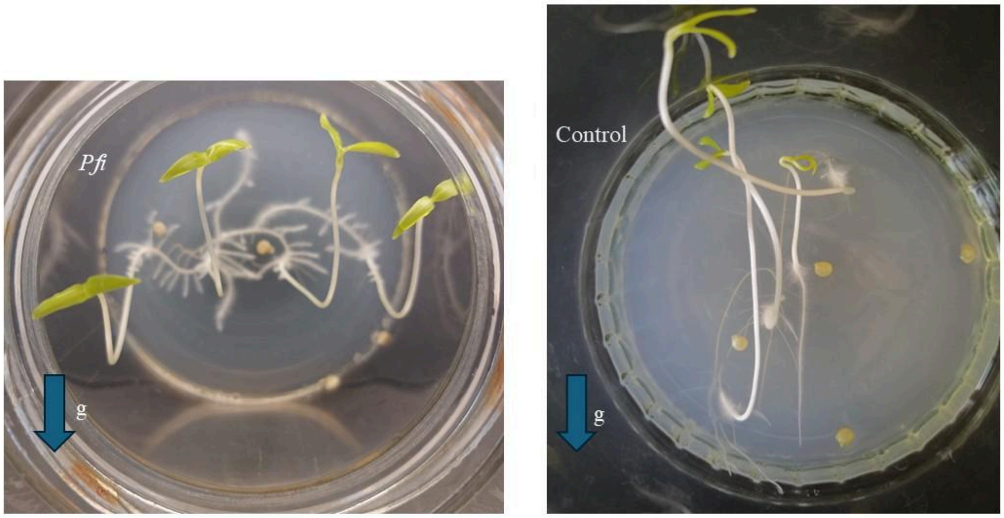
The objective of this experiment was to evaluate whether the *pfi* mutant breeding line exhibits the capacity to respond and adapt to varying environmental stimuli, including light and gravity. The corresponding results are presented in Figure 09 and Figure 10:

Figure 09: *pfi* and control plants 24 hours after bending, **g** - direction of the gravity vector, **light** - direction of light beaming from only one side.



Each jar contained four seeds, which were allowed to germinate and grow for two weeks. After this period, the plants were bent by 90° for 24 hours, and unilateral light was applied from the right side to both the *pfi* and control plants. In *pfi* plants, a notably dense root growth was observed. These plants exhibited positive phototropic responses, bending toward the light source, while also displaying gravitropic behavior by growing against the direction of gravity. In contrast, control plants similarly exhibited gravitropic growth; however, their response to light was more pronounced due to the elongated structure of their shoots, which grew vertically while orienting toward the light. In *pfi* plants, although an immediate attraction to light was evident, the degree of bending was limited, likely due to the structural characteristics of their shoots.

Figure 10: *Pfi* and control plants germinated in a completely dark environment, 24 hours after bending, **g -** direction of the gravity vector.



The jars containing *pfi* and control seeds were maintained in complete darkness until observation. After 24 hours, both *pfi* and control shoots exhibited gravitropic responses, growing directly upwards. In the absence of light, the plants did not display directional growth toward a specific stimulus but instead grew normally in the upward direction. *pfi* plants demonstrated higher root density, with roots appearing more scattered, while their gravitropic behavior was clearly evident. In control plants, shoots elongated more due to their structural characteristics and similarly displayed antigravitropic growth. As no external directional cue was present, both plant types grew primarily in the vertical upward direction without orientation toward a specific point.

5.2. Time course of gravitropic curvature in *pfi* hypocotyls

The primary objective of this subsequent experiment was not merely to assess whether the *pfi* mutant breeding line can respond to gravitational stimuli, but rather to determine the duration required for the plants to align with the gravitational pull, as this line exhibited a higher degree of growth randomization. It was of particular interest to investigate whether their capacity to orient along the gravitational vector occurs at a delayed stage or fails to develop entirely during the experiment. As only the *pfi* plants displayed no immediate response to gravitational force, further investigations were conducted specifically on this line.

Figure 11: Time course of gravitropic response in *pfi* plants (*pfi* 1)

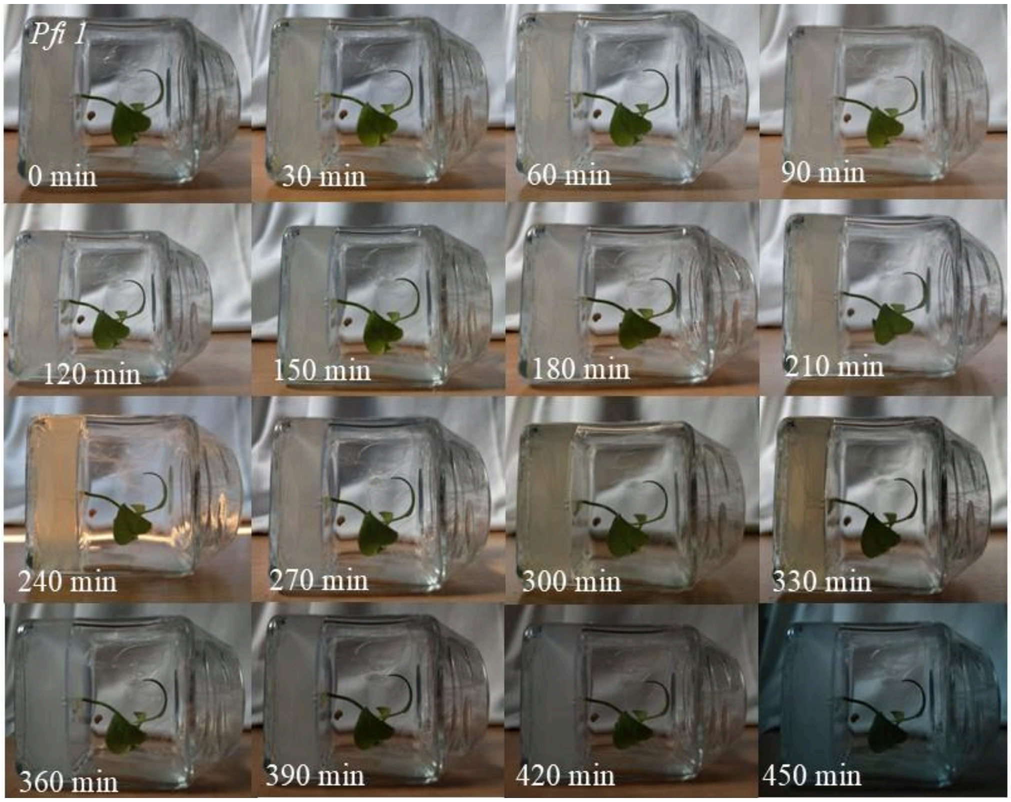


Figure 12: Time course of gravitropic response in *pfi* plants (*pfi* 2)

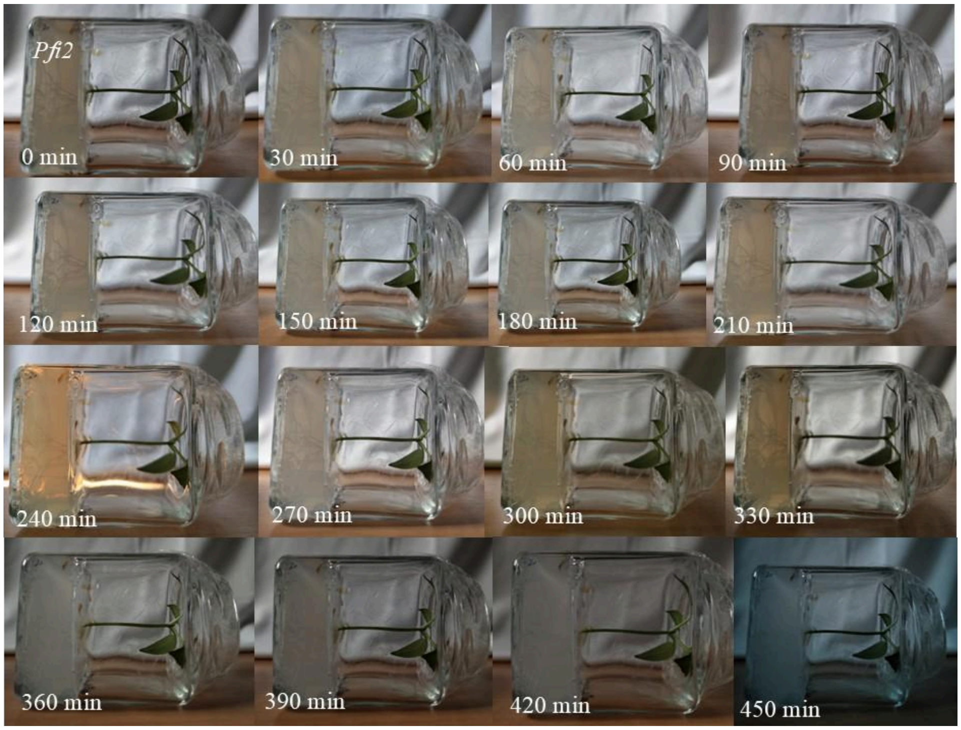
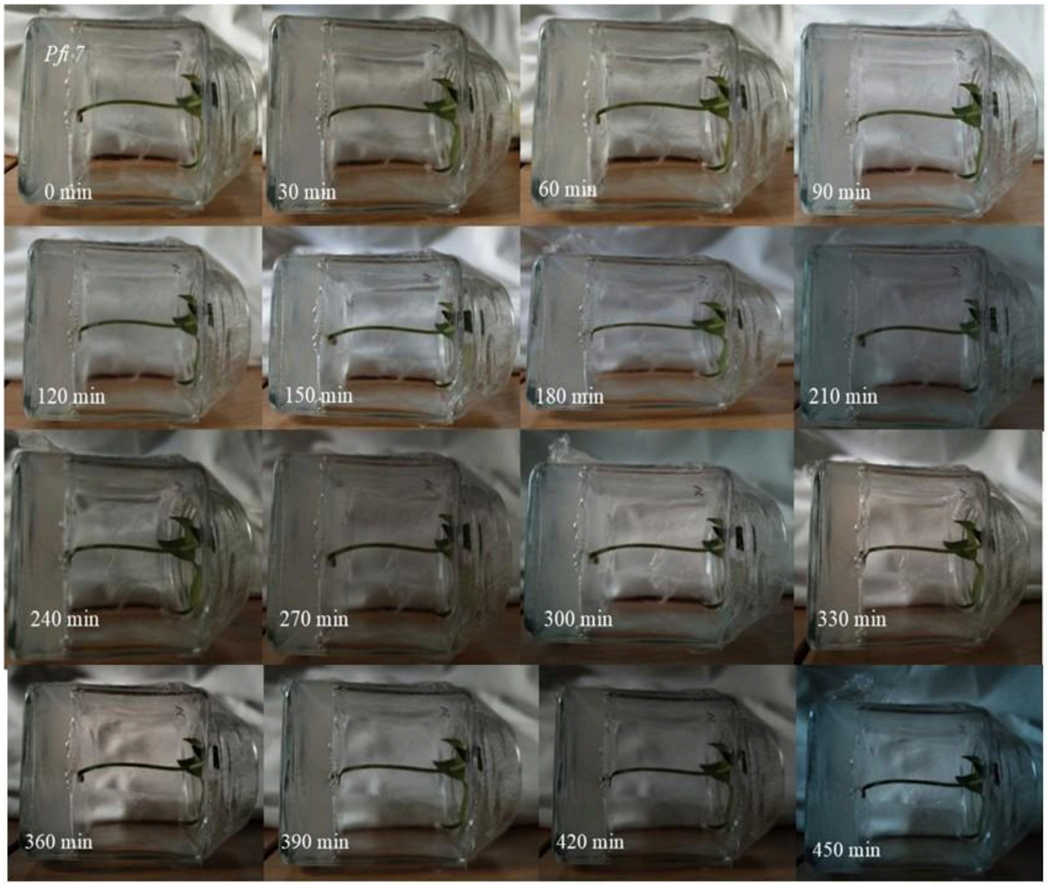


Figure 13: Time course of gravitropic response in *pfi* plants (*pfi* 7)



Two-week-old *pfi* seedlings, one per jar, were subjected to bending and monitored over a period of 450 minutes, with observations recorded at 30-minute intervals. Among the seedlings, *pfi1*, *pfi*3, *pfi4*, and *pfi6* exhibited relatively stronger gravitropic responses, aligning more effectively with the gravitational vector. In contrast, *pfi*2 showed minor deviations during the intermediate phase but remained largely unchanged by the end of the observation period. *pfi*5 initially remained near the neutral 0° position but gradually exhibited gravitropic curvature toward the conclusion of the experiment. *pfi*7 was an outlier, displaying a small degree of antigravitropic behavior. Due to their abnormal stem structure, most of the mutants could not grow upwards in this time course.

In the experiment on *pcx* (procumbent) plants, a laying phenotype was observed during the time course, with some showing rapid antigravitropic bending within 120 minutes, while others remained unchanged even after 420 minutes of reorientation. The control plants exhibited a gradual but slow antigravitropic response beginning at 120 minutes. Compared to the *pfi* mutants, the *pcx* plants displayed fast or absolute still reaction rates, indicating that different *Capsicum annuum* mutants respond to gravity at varying speeds (Pápai *et al.*, 2023).

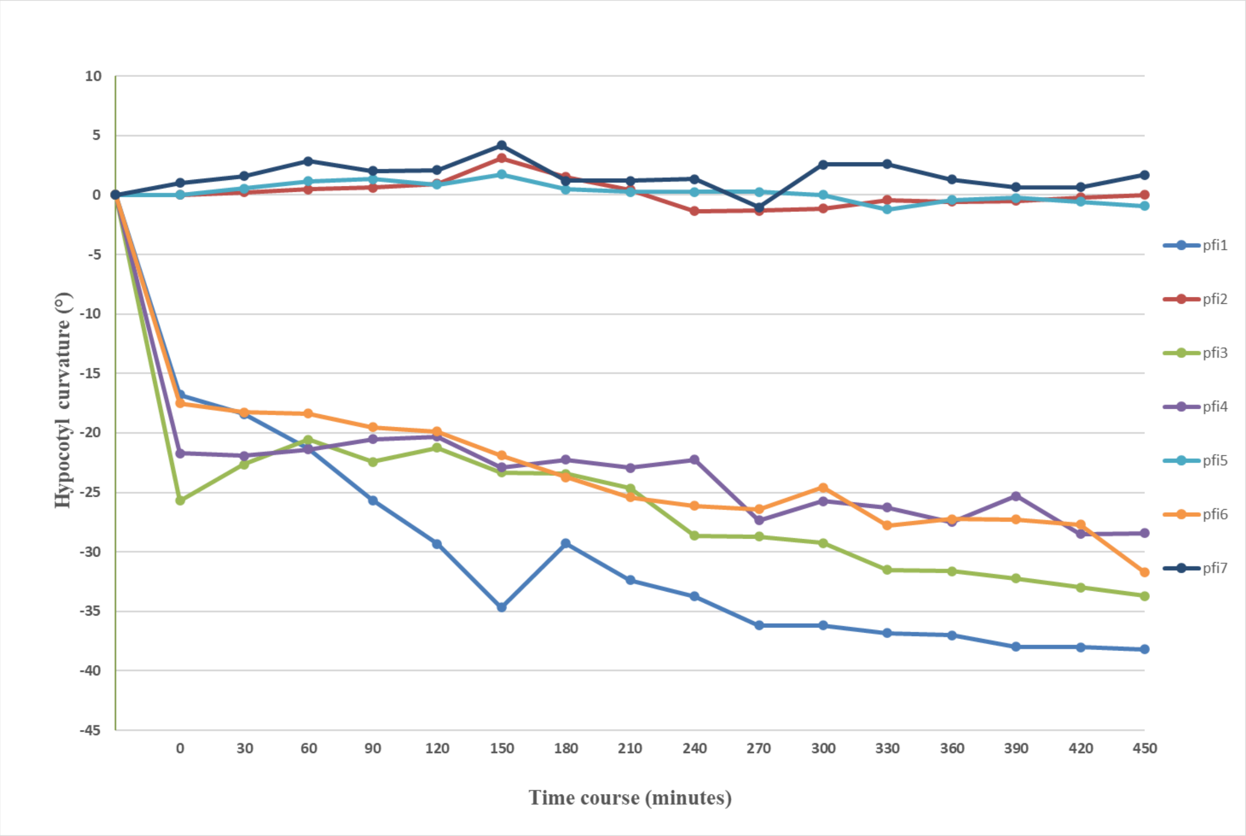
Overall, these observations indicate that the growth rate of *pfi* hypocotyls is slow, limiting their capacity to reorient cotyledons and fully express antigravitropic movements within the experimental timeframe. Despite some variability in gravitropic responses, the generally reduced gravitropic response resulted in only modest deviations in most *pfi* seedlings.

In order to also better visualize this data, a graph was constructed using Microsoft Excel after the images were analysed using the ImageJ analysis software after which the data was generated (Table 03), and graph plotted (Figure 14).

Table 03: Hypocotyl curvature (°) in *pfi* plants in the time course of 0 to 450 minutes

	Hypocotyl curvature (°)						
Time (minutes)	***pfi 1***	***pfi 2***	***pfi 3***	***pfi 4***	***pfi 5***	***pfi 6***	***pfi 7***
	0	**0**	**0**	**0**	**0**	**0**	**0**
0	**-16.8**	**0**	**-25.7**	**-21.7**	**0**	**-17.5**	**1**
30	**-18.4**	**0.24**	**-22.64**	**-21.93**	**0.54**	**-18.25**	**1.58**
60	**-21.31**	**0.46**	**-20.56**	**-21.38**	**1.17**	**-18.37**	**2.86**
90	**-25.65**	**0.62**	**-22.43**	**-20.53**	**1.33**	**-19.54**	**2.03**
120	**-29.32**	**0.95**	**-21.23**	**-20.32**	**0.86**	**-19.9**	**2.09**
150	**-34.66**	**3.1**	**-23.32**	**-22.91**	**1.73**	**-21.88**	**4.18**
180	**-29.3**	**1.51**	**-23.45**	**-22.26**	**0.48**	**-23.71**	**1.19**
210	**-32.36**	**0.43**	**-24.64**	**-22.92**	**0.25**	**-25.41**	**1.19**
240	**-33.73**	**-1.36**	**-28.66**	**-22.27**	**0.25**	**-26.14**	**1.32**
270	**-36.17**	**-1.31**	**-28.71**	**-27.35**	**0.27**	**-26.43**	**-1.03**
300	**-36.17**	**-1.15**	**-29.27**	**-25.73**	**0**	**-24.58**	**2.56**
330	**-36.84**	**-0.43**	**-31.5**	**-26.29**	**-1.21**	**-27.79**	**2.6**
360	**-37**	**-0.58**	**-31.64**	**-27.49**	**-0.44**	**-27.26**	**1.3**
390	**-37.97**	**-0.49**	**-32.25**	**-25.32**	**-0.24**	**-27.28**	**0.64**
420	**-38**	**-0.2**	**-33**	**-28.49**	**-0.56**	**-27.7**	**0.66**
450	**-38.2**	**0**	**-33.69**	**-28.42**	**-0.93**	**-31.73**	**1.71**

Figure 14: Time course of gravitropic responses in *pfi* mutant hypocotyls



5.3. Lignin content of the hypocotyls

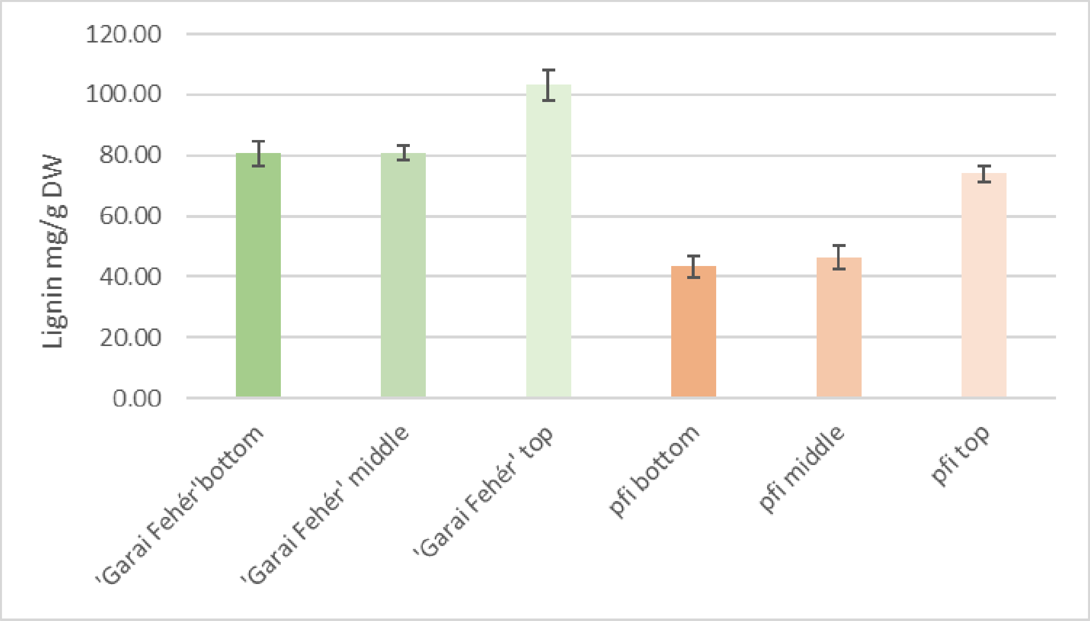
Lignin content in *pfi* plants is consistently and substantially lower than in the control (Garai Fehér) plants across all stem positions (bottom, middle, top). This suggests a broad, systemic reduction in lignification in *pfi* mutants.

Table 04: Average values of lignin content in *pfi* and control plants

Lignin mg/g DW		
	Average value (N)	Deviation
'Garai Fehér'bottom	80.60	3.98
'Garai Fehér' middle	80.76	2.33
'Garai Fehér' top	103.09	5.15
pfi bottom	43.29	3.68
pfi middle	46.32	3.74
pfi top	73.91	2.60

In both genotypes, lignin content increases from bottom to top, but this gradient is flatter in *pfi*. While both show an upward increase, the baseline lignin content is lower in *pfi*, particularly in basal stem regions where structural lignification is normally strongest.

Figure 15: Lignin content (mg/g DW) of *pfi* and control plants

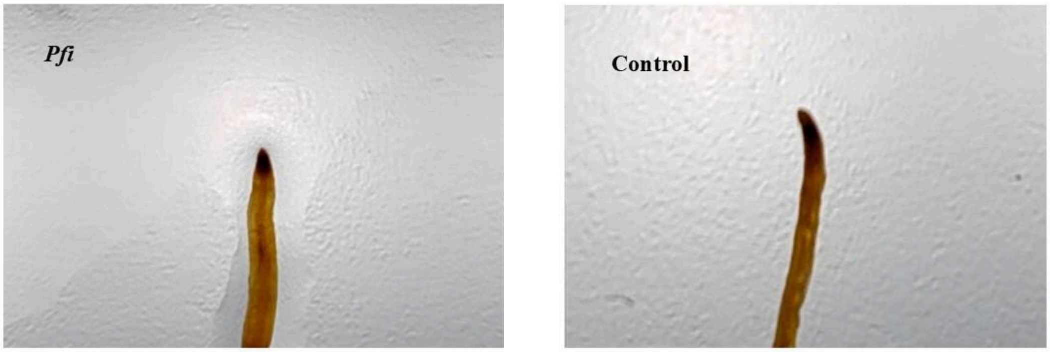


In conclusion, these results suggest that *pfi* mutants show a marked reduction in lignin content across all stem regions compared to the control (Garai Fehér), indicating a defect in secondary cell wall biosynthesis. This decreased lignification weakens mechanical strength, leading to the less rigid stem structure, consistent with lignin-deficient mutant phenotypes which explains the mutant's inability to remain upright and its laying tendency to bend readily towards gravity.

5.4. Detection of Starch in root tips of *pfi* mutant plants

We examined the presence of starch-filled amyloplasts (statoliths) in the root tips using Lugol's iodine staining solution, which produces a characteristic blue-black coloration in starch granules (Figure 16). Our observations revealed that the root tips of *pfi* plants contained abundant statoliths, indicating that starch accumulation in amyloplasts was not impaired. This suggests that the observed "laying" phenotype is not due to defects in starch-mediated gravity perception and that other factors are likely responsible for the antigravitropic behavior in these plants.

Figure 16: Starch (blue-black coloration) observed in *pfi* and control root tip using lens



5. CONCLUSION

The comprehensive analyses conducted in this study provide insight into the physiological and structural characteristics underlying the unique growth behavior of the *pfi* mutant line of *Capsicum annuum*. Our phototropic and gravitropic experiments demonstrated that *pfi* plants retain the capacity to respond to environmental stimuli such as light and gravity, although their responses are modulated by structural features. In the presence of unilateral light, *pfi* shoots displayed immediate phototropic bending, but the degree of curvature was limited compared to control plants, likely due to differences in shoot architecture. Similarly, in darkness, both *pfi* and control plants exhibited antigravitropic growth, indicating that directional growth toward light is stimulus-dependent, while vertical upward growth remains largely intact. Time-course analyses of gravitropic curvature revealed variability among individual *pfi* seedlings, with some displaying delayed or partial alignment with the gravity vector, suggesting a heterogeneous response in hypocotyls that may contribute to the overall “laying” phenotype observed in this line.

Structural analysis revealed a marked reduction in lignin content across all stem regions of *pfi* plants relative to the control, particularly in basal regions, which are typically associated with mechanical support. The flattened lignin gradient and overall lower lignification in *pfi* stems likely compromise rigidity, providing a mechanistic explanation for their inability to maintain upright growth and their tendency to bend under gravitational force. Notably, the detection of starch-filled amyloplasts (statoliths) in root tips indicated that gravity sensing per se is not impaired in *pfi* plants, ruling out defects in starch-mediated perception as the cause of the altered gravitropic response. The increased root density observed in *pfi* further supports the notion that the mutant’s phenotype is influenced more by structural and mechanical factors rather than by deficiencies in gravity sensing.

Collectively, these findings suggest that the laying phenotype of *pfi* plants is primarily associated with reduced secondary cell wall lignification rather than impaired phototropic or gravitropic perception. While *pfi* plants maintain normal sensory and signaling responses to environmental cues, their structural limitations constrain the visible extent of these responses, leading to altered shoot orientation and reduced mechanical stability. These results highlight the intricate interplay between structural integrity and environmental responsiveness in plant development and provide a foundation for future studies aimed at dissecting the genetic and molecular determinants of lignification and mechanical strength in *Capsicum* mutants. Understanding these relationships may also inform targeted breeding strategies for enhancing stem strength and overall plant architecture in cultivated pepper lines.

6. SUMMARY

This study investigates the physiological and structural basis of the unique “laying” phenotype, *pfi* mutant of *Capsicum annuum*, characterized by stem puffiness and reduced upright growth. Bell pepper is an economically and nutritionally important crop with significant breeding potential, and mutant traits provide valuable genetic resources for improving plant architecture, fruit quality, and stress tolerance. The *pfi* mutant presents a distinct opportunity to study the interplay between structural integrity, tropistic responses, and mechanical strength.

Phototropic and gravitropic experiments revealed that *pfi* plants retain the capacity to sense and respond to environmental stimuli. Under unilateral light, *pfi* shoots displayed immediate but limited phototropic bending, while in darkness, shoots grew vertically upward, indicating that light-independent antigravitropic responses are largely intact. Time-course analyses of hypocotyl curvature demonstrated variability in gravitropic reorientation among individual seedlings, suggesting partial delays rather than complete loss of gravity perception. Lugol’s iodine staining confirmed the presence of starch-filled amyloplasts in root tips, ruling out defects in statolith-mediated gravity sensing as the cause of the altered growth habit.

Structural analysis revealed a substantial reduction in lignin content across all stem regions in *pfi* plants compared to the control, particularly in basal internodes where mechanical support is critical. This decreased lignification likely compromises stem rigidity, explaining the laying phenotype and puffed stem morphology. Collectively, the findings indicate that the *pfi* phenotype arises primarily from reduced secondary cell wall deposition rather than impaired tropistic signaling, highlighting the critical role of lignin in maintaining mechanical stability and upright growth in *Capsicum annuum.* These insights provide a foundation for future breeding strategies targeting stem strength and architectural traits in bell pepper.

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9. LIST OF FIGURES AND TABLES

MATE Organizational and Operational Regulations
III. Requirements for Students
III.1. Study and Examination Regulations
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Annex 4.2: Declaration of public access and authenticity of the thesis/thesis/dissertation/portfolio

DECLARATION

the public access and authenticity of the thesis/dissertation/portfolio[1]

Student's name: SYEDA MAHJABIN TABASSSUM
Student's Neptun code: Y1XVNB
Title of thesis: Characterization and In Vitro Investigation of Puffy Stem phenomenon in Capsicum annuum Pfi mutant plants.
Year of publication: 2025
Name of the consultant's institute: Institute of Genetics and Biotechnology
Name of consultant's deparment: Department of Genetics and Genomics

I declare that the final thesis/thesis/dissertation/portfolio[2] submitted by me is an individual, original work of my own intellectual creation. I have clearly indicated the parts of my thesis or dissertation which I have taken from other authors' work and have included them in the bibliography. Furthermore, I declare that the artificial intelligence tools (e.g. text generation, linguistic correction, translation, data analysis) used during the preparation of the thesis did not substitute my own research and creative work; their use was indicated either in the list of sources or in the methodology section, and I acted in accordance with professional and ethical expectations.

If the above statement is untrue, I understand that I will be disqualified from the final examination by the final examination board and that I will have to take the final examination after writing a new thesis.

I do not allow editing of the submitted thesis, but I allow the viewing and printing, which is a PDF document.

I acknowledge that the use and exploitation of my thesis as an intellectual work is governed by the intellectual property management regulations of the Hungarian University of Agricultural and Life Sciences.

I acknowledge that the electronic version of my thesis will be uploaded to the library repository of the Hungarian University of Agricultural and Life Sciences. I acknowledge that the defended and

- not confidential thesis after the defence
- confidential thesis 5 years after the submission

will be available publicly and can be searched in the repository system of the University.

Date: 2025 year November month 03rd day

Mahjabin
Student's signature

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[1] While keeping the appropriate thesis type, all other types are to be removed.

[2] While keeping the appropriate thesis type, all other types are to be removed.0

DECLARATION

Syeda Mahjabin Tabasssum (name) (student Neptun code: Y1XVNB)
as a consultant, I declare that I have reviewed the final thesis/thesis/dissertation/portfolio[1] and that I have informed the student of the requirements, legal and ethical rules for the correct handling of literary sources.

[✓ I recommend] / do not recommend[2] the [✓ final thesis] / dissertation / portfolio to be defended in the final examination.

The thesis contains a state or official secret: yes [✓ no]*[3]

Date: 2025 year november month 03 day

insider consultant

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[1] The other types should be deleted while retaining the corresponding thesis type.

[2] The appropriate one should be underlined.

[3] The appropriate one should be underlined.

Declaration of Students and Doctoral Candidates on the Use of Artificial Intelligence (AI)”

1. general information:

Name of the student:	SYEDA MAHJABIN TABASSSUM
Neptun ID:	Y1XVNB
Level of program (mark with X):	☒ BSc/BA ☐ MSc/MA ☐ Doctoral School (PhD) ☐ Other: …………………………
Name and code of the subject*:	Thesis Work
Title of the work:	Characterization and In Vitro Investigation of Puffy stem phenomenon in Capsicum annuum Pfi mutant plants.

* Not required to be completed in the case of a doctoral dissertation.

2. Declaration on the Use of AI

I, the undersigned, fully aware of my ethical responsibility, make the following declaration:

(Please choose one of the options below!)

☒ A) I have not used any artificial intelligence system or service.

(If you selected this option, completing the subsequent tables is not required.)

☐ B) I have used an artificial intelligence system or service.

(Please fill in the relevant tables!)

3. Details of Artificial Intelligence Usage

TABLE I: Assistant or Minor Usage (e.g., translation, language proofreading, brainstorming, etc.)

(For these uses, attaching the specific prompts and responses is not required.)

Purpose of Use	Name and Version of the AI Tool Used	Affected Section (if not applicable to the entire text)

TABLE II: Significant Content Contribution (e.g., generating an entire figure or a longer text section)

(In these cases, documenting the key prompts used and the raw responses provided by the AI, and attaching them as an appendix to the work, is required.)

Purpose of Use	Name, Version, and Access Information of the AI Tool Used	Exact Number of the Affected Chapter / Figure / Table	Entry Number of the Appendix Containing the Prompt Log

3/A. Additional Rules Prescribed by the Lecturer (if any)

If the instructor or supervisor of the course has established specific rules or expectations regarding the use of AI tools, please summarize them in the field below:

For example: prohibition of AI use for certain types of tasks; only specific tools are permitted; different citation requirements; documentation format, etc.

Rules Prescribed by the Lecturer or Supervisor

……………………………………………………………………………………………

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4. Declaration Applicable to All Students:

I declare that I have critically reviewed, edited, and incorporated any content potentially generated by AI in all cases. I take full responsibility for every element of the submitted work, including its originality and scientific validity. I acknowledge that the Hungarian University of Agriculture and Life Sciences may check the submitted work with an artificial intelligence detector and may initiate proceedings if my declaration is found to be false or incomplete.

Place and Date: Gödöllő, ……………………………………, 2025. November….. month 03rd…. day

Mahjabin

……………………………………………………………

Signature of the Student

……………………………………………………………

Signature of the Advisor/Supervisor