UDC 581.143:581.522.4:633.791 https://doi.org/10.7124/FEEO.v38.1813

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GROWTH INHIBITION AND RECOVERY OF HOP (*HUMULUS LUPULUS* L.) UNDER MANNITOL- AND SORBITOL-INDUCED OSMOTIC STRESS *IN VITRO*

Aim. To model water deficit in hop (*Humulus lupulus* L.) *in vitro* using mannitol and sorbitol and to evaluate genotype-dependent growth responses and recovery capacity. ***Methods.*** Six-week-old plants of two hop genotypes were cultured on modified Murashige and Skoog medium. Osmotic stress was induced by mannitol or sorbitol (0.01–1.0 M). Shoot height, callus formation, root number and length, and tissue water content were assessed during six weeks of cultivation. Recovery was evaluated after transfer to standard medium. ***Results.*** Both polyols induced concentration-dependent inhibition of shoot and root growth and reduced tissue water content. Mannitol caused stronger growth suppression than sorbitol at equivalent concentrations. Moderate sorbitol levels had limited effects on shoot growth and stimulated root development in some genotypes. Growth inhibition correlated with reduced tissue water content. Stress induced by moderate sorbitol concentrations was largely reversible, and transferred plants resumed active growth, in some cases exceeding control performance. ***Conclusions.*** Genotype-dependent differences in osmotic stress tolerance were revealed. Polyol-induced osmotic stress *in vitro* represents a practical approach for preliminary screening of hop genotypes for drought tolerance under climate change conditions.

Keywords: osmotic stress, polyols, drought tolerance, genotype response, stress recovery.

Climate change is intensifying the frequency and severity of drought events worldwide, posing a major threat to agricultural productivity and crop stability (Wu et al., 2022). Water deficit is one of the most detrimental abiotic stresses affecting plant growth and development, leading to reduced cell division and expansion, impaired photosynthesis, disruption of water relations, and metabolic imbalance. Prolonged drought stress ultimately decreases biomass accumulation and yield, making the development of drought-tolerant cultivars a priority in modern plant breeding (Wu et al., 2022).

Hop (*Humulus lupulus* L.) is a perennial crop of high economic importance for the brewing industry due to its unique secondary metabolites, including bitter acids and essential oils. However, hop plants are highly sensitive to water availability, and drought stress significantly affects their vegetative growth and cone production. Considering the increasing instability of climatic conditions in major hop-growing regions, the identification of stress-tolerant genotypes has become an urgent task (Marceddu et al., 2022).

In vitro culture systems provide a controlled and reproducible platform for modeling abiotic stresses at early developmental stages (Claeys et al., 2014). Osmotic stress can be simulated by adding osmotically active compounds to the culture medium, allowing evaluation of plant responses under precisely defined conditions. Among such compounds, sugar alcohols (polyols), particularly mannitol and sorbitol, are widely used as osmotic

agents (Linjikao et al., 2024). Despite having the same molecular formula, mannitol and sorbitol differ in their physiological roles and metabolic properties. Mannitol is frequently associated with osmoprotection and reactive oxygen species scavenging, whereas sorbitol often functions as a transport carbohydrate and carbon source in plants (Edmond et al., 2025). Polyol accumulation plays an important role in osmotic adjustment and interacts with phytohormonal signaling pathways under stress conditions (Singh et al., 2022).

Although polyol-induced osmotic stress has been studied in several plant species (Linjikao et al., 2024), comparative investigations of mannitol and sorbitol effects on hop growth, especially considering genotype-specific responses and post-stress recovery capacity, remain limited. Furthermore, the reversibility of growth inhibition under moderate osmotic stress is of particular interest for developing reliable *in vitro* screening approaches for drought tolerance (Claeys et al., 2014).

The aim of this study was to evaluate genotype-dependent growth inhibition, water status changes, and recovery potential of hop plants cultured *in vitro* under osmotic stress induced by different concentrations of mannitol and sorbitol. The results obtained may contribute to the development of efficient screening strategies for drought tolerance in hop breeding programs under changing climatic conditions.

Materials and methods

Plant material and culture conditions

Six-week-old *in vitro*-grown plants of hop (*H. lupulus*) belonging to two genotypes, 12925 and 15249, were used in the experiment. Donor plants were maintained under sterile conditions prior to treatment.

Fully developed shoots were divided into single-node segments under aseptic conditions. Each explant was transferred into a 25 cm^3 culture tube containing 10 mL of prepared medium. For each treatment, ten biological replicates were established, each consisting of one explant per tube.

Cultures were maintained at 26 ± 1 °C under a 16 h light / 8 h dark photoperiod. The experiment lasted six weeks and was terminated when control plants reached the maximum height of the culture tubes.

Preparation of culture media and osmotic treatments

A modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was used as the basal medium. The medium contained a reduced nitrogen concentration and was free of plant growth regulators, following the protocol described by Faltus (Faltus et al., 2007, Faltus et al., 2012). The medium was supplemented with 20 g L^{-1} glucose and solidified with 6 g L^{-1} agar.

To simulate osmotic (water) stress, mannitol or sorbitol was added to the medium at the following concentrations: 0.01, 0.025, 0.05, 0.075, 0.1, and 1.0 M. Medium without added polyols served as the control treatment.

Growth assessment

Growth parameters were recorded weekly throughout the six-week cultivation period. The following traits were evaluated: callus formation (% of explants), number of roots per explant, root length (mm), shoot formation, height of the main shoot (mm).

Callus and root formation were assessed during the first three weeks of cultivation. Shoot height was measured weekly for the entire duration of the experiment.

Growth inhibition was calculated using the formula:

$$E = \frac{Lc - Lo}{Lc} * 100,$$

Where E – growth inhibition (%);
Lc – mean shoot height of control plants (mm);
Lo – mean shoot height of treated plants (mm).

Determination of plant water content

At the end of the six-week cultivation period, plant water content was determined gravimetrically. Fresh biomass of whole plants was recorded immediately after harvest. Samples were then dried in a drying oven at 105 °C for 72 hours to obtain dry weight.

Water content (WC) was expressed as a percentage of fresh mass:

$$WC = \frac{FW - DW}{FW} * 100,$$

where FW is fresh weight and DW is dry weight.

Post-stress recovery experiments

To evaluate the reversibility of osmotic stress effects, recovery capacity was assessed in two experimental approaches.

1. Recovery of whole plants: After six weeks of cultivation on sorbitol-containing media (0.05, 0.075, and 0.1 M), plants were transferred to standard basal MS medium without osmotic agents. Shoot growth increment was measured over an additional four-week period. The percentage of plants resuming active growth was recorded.

2. Recovery of explants derived from stressed plants: Single-node explants obtained from plants previously cultivated on 0.025 and 0.05 M sorbitol

were excised and transferred to standard basal medium. Their growth performance during four weeks was compared to explants derived from control plants.

Statistical analysis

All experiments were conducted using ten biological replicates per treatment. Data were analyzed using Statistica 14.0.0.15 (TIBCO Software Inc.). Results are presented as mean ± standard deviation (SD).

Results and discussion

Effects of mannitol-induced osmotic stress on hop growth

Mannitol supplementation resulted in a pronounced, concentration-dependent inhibition of hop growth in both genotypes (Table 1). Even the lowest concentration tested (0.01 M) reduced shoot height, particularly in genotype 12925, indicating higher sensitivity compared to 15249. Increasing mannitol concentration to 0.025 M caused severe growth inhibition in both genotypes, exceeding 89%, accompanied by a strong reduction in root formation and elongation.

Complete suppression of shoot and root development was observed at 1.0 M mannitol. The inhibitory effect of mannitol was consistently stronger than that of sorbitol at equivalent concentrations.

Mannitol is widely used as an osmoticum in *in vitro* experiments because it is poorly metabolized by many plant species, thereby effectively reducing medium water potential (Linjikao et al., 2024). Its strong inhibitory effect observed in the present study confirms its high osmotic efficiency. Moreover, mannitol has been associated with osmoprotective and antioxidant roles in some plant species (Edmond et al., 2025); however, in hop tissues, its accumulation appears to predominantly impose osmotic stress rather than confer protection.

The greater sensitivity of genotype 12925 suggests genotype-specific differences in osmotic adjustment capacity, which is consistent with reports indicating that drought tolerance is highly genotype-dependent and linked to differential regulation of water balance and stress-related pathways (Wu et al., 2022).

Sorbitol also induced growth inhibition in a concentration-dependent manner, but its effect was more gradual compared to mannitol (Table 2). At moderate concentrations (0.025–0.05 M), partial inhibition of shoot growth and root elongation was observed, particularly in genotype 12925, while genotype 15249 maintained comparatively higher growth rates.

Table 1. Biometric growth parameters of hop (*H. lupulus*) explants cultured on medium supplemented with different concentrations of mannitol after six weeks of *in vitro* cultivation

Concentration	Callus, %	Number of roots	Root length, mm	Number of shoot	Shoot length, mm
Genotype 12925					
Control	100	3.13 ± 1.73	10.87 ± 7.64	2 ± 0.0	104.73 ± 1.94
0.01	100	3.67 ± 2.38	9.13 ± 6.13	2 ± 0.0	64.93 ± 47.51
0.025	100	2.5 ± 2.21	3.89 ± 3.52	2 ± 0.0	7.39 ± 12.09
0.05	100	0.47 ± 0,64	1.30 ± 1.71	2 ± 0.0	1.63 ± 1.19
0.075	100	0,33 ± 0.49	1.13 ± 1.68	1.80 ± 0.56	1.03 ± 1.45
0.1	100	0,47 ± 0.74	1.27 ± 2.02	1.73 ± 0.59	0.57 ± 0.42
1.0	0	0± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Genotype 15249					
Control	100	5.00 ± 1.51	22.67 ± 10.1	1.87 ± 0.35	111.3 ± 3.51
0.01	100	3.67 ± 1.15	24.67 ± 9.78	1.93 ± 0.27	91.13 ± 14.49
0.025	100	2.60 ± 1.18	5.43 ± 4.15	2 ± 0.0	11.53 ± 10.98
0.05	100	1.87 ± 1.19	2.70 ± 1.46	1.9 ± 30.26	1.50 ± 1.50
0.075	100	1.67 ± 0.98	2.77 ± 1.43	1.53 ± 0.74	1.07 ± 1.55
0.1	20	0.93 ± 0.88	1.80 ± 1.66	1.4 ± 0.91	0.47 ± 0.48
1.0	0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

Note. Data represent mean ± SD (n = 10).

Table 2. Biometric growth parameters of hop (*H. lupulus*) explants cultured on medium supplemented with different concentrations of sorbitol after six weeks of *in vitro* cultivation

Concentration	Callus, %	Number of roots	Root length, mm	Number of shoots	Shoot length, mm
			12925		
Control	100	3.93 ± 1.58	28.13 ± 7.74	2 ± 0.0	114.47 ± 3.0
0.01	100	3.80 ± 2.13	24.07 ± 8.86	2 ± 0.0	96.63 ± 36.76
0.025	100	4.93 ± 1.62	12.53 ± 6.88	2 ± 0.0	88.27 ± 36.39
0.05	100	3.93 ± 2.37	6.40 ± 2.03	1.93 ± 0.26	13.77 ± 12.41
0.075	100	1.13 ± 1.36	2.93 ± 3.97	2 ± 0.0	3.77 ± 1.89
0.1	100	0.73 ± 0.96	1.87 ± 2.20	1.93 ± 0.26	2.10 ± 1.80
1.0	0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
			15249		
Control	100	5.13 ± 1.55	20.20 ± 10.26	2 ± 0.0	108.87 ± 9.66
0.01	100	4.67 ± 1.45	22.60 ± 10.17	1.8 ± 70.35	101.53 ± 11.45
0.025	93.3	5.60 ± 2.13	20.60 ± 9.30	2 ± 0.0	91.33 ± 28.51
0.05	100	3.07 ± 1.83	9.27 ± 5.28	1.73 ± 0.46	53.67 ± 21.12
0.075	100	0.53 ± 0.92	1.20 ± 2.21	1.73 ± 0.46	7.73 ± 3.90
0.1	93.3	1.00 ± 0.85	2.13 ± 1.85	1.53 ± 0.64	6.90 ± 6.35
1.0	0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

Notes. Data represent mean ± SD (n = 10).

Unlike mannitol, sorbitol may serve as a transport carbohydrate and can be partially metabolized in certain plant systems. Polyols such as sorbitol contribute to osmotic adjustment and cellular protection under water deficit conditions (Linjikao et al., 2024). The milder inhibitory effect of sorbitol observed here may reflect partial metabolic utilization or improved osmotic balance compared to mannitol.

Notably, growth inhibition closely correlated with reductions in plant water content. A decrease in tissue water content exceeding 10% resulted in approximately 90% shoot growth suppression, depending on genotype. This relationship supports the central role of cellular water status in determining growth performance under osmotic stress. Similar correlations between water potential decline and growth inhibition have been documented in drought-stressed plants (Wu et al., 2022).

Genotype 15249 demonstrated lower sensitivity to sorbitol-induced osmotic stress, suggesting more efficient water retention or osmotic adjustment mechanisms. Such genotype-dependent variation is critical for developing reliable screening systems for drought tolerance.

Relationship between water content and growth inhibition

The data indicate a strong association between shoot height reduction and decreased water content in plant tissues (Figure 1). Mannitol at 0.025 M caused severe shoot growth inhibition (> 90%) accompanied by significant water loss in both genotypes. In contrast, sorbitol at equivalent concentrations resulted in more moderate water loss and correspondingly lower growth inhibition in genotype 15249.

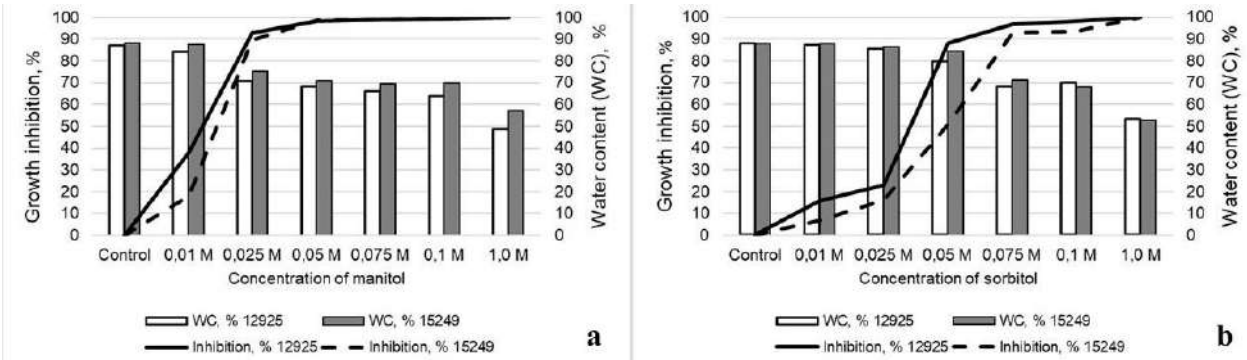

Fig. 1. Effects of mannitol and sorbitol on shoot height and tissue water content of hop (*H. lupulus*) plants under *in vitro* conditions. (a) Mannitol treatment; (b) sorbitol treatment. Values are presented as mean (n = 10).

These findings support the concept that osmotic stress primarily restricts cell expansion due to reduced turgor pressure, which is a key determinant of plant growth under drought conditions (Wu et al., 2022). The stronger dehydration effect of mannitol likely explains its greater inhibitory impact on hop explants.

Recovery capacity after sorbitol-induced stress

A major finding of this study is the reversibility of growth inhibition following moderate sorbitol treatments (Figure 2). After transfer to standard medium, 70–90% of plants resumed active growth, depending on genotype and prior sorbitol concentration. Recovery was faster and more pronounced in genotype 15249.

Shoot elongation during the four-week recovery period demonstrated that plants previously exposed to 0.075 M sorbitol showed substantial regrowth, indicating that the stress effect was not lethal but primarily osmotic and reversible.

Interestingly, explants derived from plants previously cultured on 0.025–0.05 M sorbitol exhibited enhanced growth performance compared to control-derived explants. This suggests a possible priming effect induced by moderate osmotic stress. Stress priming has been described as a phenomenon where prior exposure to sublethal stress enhances subsequent growth or tolerance through physiological and metabolic adjustments (Edmond et al., 2025).

The improved post-stress growth may be associated with osmolyte accumulation or activation of stress-responsive pathways that persist after stress removal. Such responses may enhance adaptive capacity and could be exploited in breeding programs.

Implications for in vitro drought screening

The comparative evaluation of mannitol and sorbitol indicates that these osmotic agents differ substantially in their physiological impact on hop tissues. Mannitol provides strong and rapid growth suppression, making it suitable for high-stringency screening. Sorbitol, on the other hand, allows differentiation between genotypes under moderate stress conditions and enables assessment of recovery potential.

The clear genotype-dependent differences observed in both growth inhibition and recovery suggest that the described *in vitro* system may serve as an effective preliminary screening tool for drought tolerance in hop breeding programs.

The clear genotype-dependent differences observed in both growth inhibition and recovery suggest that the described *in vitro* system may serve as an effective preliminary screening tool for drought tolerance in hop breeding programs.

Given the increasing frequency of drought events under climate change scenarios, reliable and reproducible *in vitro* screening approaches are essential for accelerating the identification of tolerant genotypes. The results of this study demonstrate that polyol-induced osmotic stress can be effectively applied for this purpose in hop.

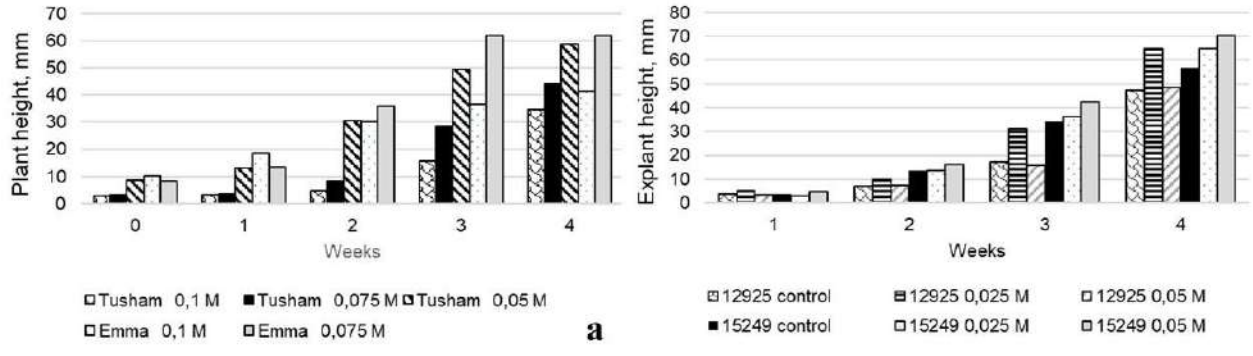

Fig. 2. Growth dynamics of whole hop plants after six weeks of cultivation on sorbitol-containing medium followed by transfer to standard basal medium (a) and growth dynamics of explants derived from hop plants previously cultured on sorbitol-supplemented medium after transfer to standard basal medium (b).

Conclusions

1. Mannitol and sorbitol effectively induced osmotic stress in hop (*H. lupulus*) under *in vitro* conditions, causing concentration-dependent inhibition of shoot and root growth as well as reductions in tissue water content.

2. Mannitol exerted a stronger inhibitory effect than sorbitol at equivalent concentrations, leading to more rapid and severe suppression of morphogenetic processes, indicating its higher osmotic efficiency in hop tissues.

3. A strong relationship was observed between decreased tissue water content and shoot growth inhibition, confirming the critical role of cellular water status in regulating growth under osmotic stress conditions.

4. Growth inhibition induced by moderate sorbitol concentrations (0.05–0.1 M) was largely reversible. After transfer to standard medium, the majority of plants resumed active growth, demonstrating the non-lethal and adaptive nature of moderate osmotic stress.

5. Explants derived from sorbitol-treated plants showed enhanced post-stress growth under standard conditions, indicating a possible stress-priming effect that may improve subsequent developmental performance.

6. The described *in vitro* system provides a reproducible and efficient tool for preliminary screening of hop genotypes for drought tolerance, which is particularly relevant under ongoing climate change.

Information about financial support, acknowledgements: The paper was prepared as part of the research project of the Ministry of Agriculture of the Czech Republic QL24010353 and the institutional project MZE – RO0423.

Conflict of interest: The author declares no conflict of interest.

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ПРИГНІЧЕННЯ РОСТУ ТА ВІДНОВЛЕННЯ РОСЛИН ХМЕЛЮ (*HUMULUS LUPULUS* L.) ЗА УМОВ ОСМОТИЧНОГО СТРЕСУ, ІНДУКОВАНОГО МАНІТОЛОМ І СОРБІТОЛОМ, *IN VITRO*

Мета. Змоделювати дефіцит води у хмелю (*Humulus lupulus* L.) *in vitro* за допомогою манітолу та сорбітолу й оцінити генотип-залежні реакції росту та здатність до відновлення. ***Методи.*** Шеститижневі рослини хмелю двох генотипів культивували на модифікованому середовищі Мурашіге та Скуга. Осмотичний стрес індукували манітолом або сорбітолом (0,01–1,0 М). Висоту пагонів, утворення калюсу, кількість і довжину коренів, а також вміст води у тканинах оцінювали протягом шести тижнів культивування. Відновлення оцінювали після

перенесення на стандартне середовище. ***Результати.*** Обидва поліоли індукували концентраційно-залежне пригнічення росту пагонів і коренів та знижували вміст води у тканинах. Манітол викликав сильніше пригнічення росту, ніж сорбітол в еквівалентних концентраціях. Помірні рівні сорбітолу мали обмежений вплив на ріст пагонів і стимулювали розвиток коренів у деяких генотипах. Пригнічення росту корелювало зі зниженим вмістом води у тканинах. Стрес, викликаний помірними концентраціями сорбітолу, був здебільшого оборотним і перенесені рослини відновили активний ріст, у деяких випадках перевищуючи контрольні показники. ***Висновки.*** Виявлено генотип-залежні відмінності у толерантності до осмотичного стресу. Осмотичний стрес *in vitro*, індукований поліолами, є практичним підходом до попереднього скринінгу генотипів хмелю на посухостійкість в умовах зміни клімату.
Ключові слова: осмотичний стрес, поліоли, посухостійкість, генотип-специфічна реакція, відновлення від стресу.

Надходження статті: 01 березня 2026 р.
Прийняття до друку після рецензування: 20 квітня 2026 р.
Публікація: 30 червня 2026 р.