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Optimizing In Vitro Growth of Carob (*Ceratonia siliqua L.*).

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Abstract:

The carob tree (*Ceratonia siliqua L.*) is an important perennial leguminous species with high ecological and economic importance, particularly in arid and semi-arid environments. This research investigated the effects of plant growth regulators, namely indole-3-acetic acid (IAA) and benzyladenine (BA), applied both individually and in combination, on root development, biomass accumulation, mineral nutrient composition and carbohydrate metabolism of carob seedlings cultivated under in *vitro* conditions. The results revealed that IAA at a concentration of 1.5 mg/L significantly increased root length and dry root weight, thereby indicating an optimal auxin concentration for root growth. The application of BA at moderate concentration also improved the root/shoot ratio, however higher concentrations of either regulator caused inhibitory effects. The combined application of IAA (1.5 mg/L) and BA (0.5 mg/L) produced the most pronounced positive effects, resulting in balanced root and shoot growth, increased nutrient uptake, and improved carbohydrate distribution. This treatment resulted in a notable increase in the accumulation of soluble sugars and starch within both shoot and root tissues, signifying improved metabolic efficiency. An inverse relationship between sucrose and starch levels was observed, suggesting efficient conversion of sucrose into starch under combined hormone treatment. Overall, these results underscore the importance of a balanced application of auxins and cytokinins in optimizing initial growth, physiological efficacy and in *vitro* propagation efficiency of *Ceratonia siliqua L.*, providing a reliable strategy for sustainable cultivation and conservation of this valuable species.

Keywords: *Ceratonia siliqua L.*, auxin, cytokinin and tissue culture.

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تحسين النمو المخبري لنبات الخروب (*Ceratonia siliqua L.*)

الملخص:

تناول هذا البحث شجرة الخروب (*Ceratonia siliqua L.*) تعد نوعاً من البقوليات المعمره ذات الأهمية الاقتصادية وبيئية الكبيرة وينمو في الغالب في المناطق الجافة وشبه الجافة. هذه الدراسة استهدفت تقييم تأثيرات منظمات النمو النباتية وتحديداً حمض اندول 3-- خليك (IAA) والبنزيل أدينين (BA) سواء أضيفت منفردة أو مختلطة على تطور ونمو الجذور وتراكم الكتلة الحيوية ومحتوى العناصر المعدنية وأيض الكربوهيدرات في شتلات الخروب المزروعة مختبرياً. وأظهرت نتائج الدراسة إلى أن تركيز 1.5 ملغم/ لتر من حمض (IAA) أدى إلى تعزيز ملحوظ في طول الجذر والوزن الجاف مما يشير إلى أن تركيز الاوكسين هذا هو الأمثل لتعزيز نمو الجذور. بالإضافة لذلك ساهم التطبيق المعتدل لهرمون (BA) بشكل إيجابي في تحسين نسبة الجذر إلى الساق في حين أظهرت التراكيز المرتفعة لكل من منظمي النمو تأثيرات مثبطة. وقد أدى التطبيق المشترك لِ (IAA) بتركيز 1.5 مجم/ لتر و(BA) بِتركيز 0.5 مجم/ لتر أبرز النتائج الإيجابية وبلغت ذروتها ف النمو المتناغم للجذور والسيقان وزيادة في امتصاص العناصر الغذائية وتعزيز توزيع الكربوهيدرات. كما سجلت هذه المعاملة زيادة ملحوظة في تراكم السكريات القابلة للذوبان والنشا داخل الأنسجة للساق والجذر مما يدل على تحسين كفاءة التمثيل الغذائي. وقد لوحظ وجود علاقة عكسية بين محتوى السكروز والنشا مما يسهل التحويل الفعال للسكروز الى نشا تحت تاثير المعاملة الهرمونية المشتركة. وتؤكد نتائج هذه الدراسة على أهمية التوازن بين هرموني النمو الأوكسين والسايتوكاينين لتعزيز النمو المبكر والأداء الفسيولوجي وفعالية التكاثر المختبري لشجرة الخروب وبالتالي تقديم استراتيجية موثوقة قابلة للتطبيق للزراعة المستدامة والحفاظ على هذا النوع القيم الذي لا يقدّر بثمن.

الكلمات المفتاحية : شجرة الخروب (*Ceratonia siliqua L.*)، اوكسين, سايتوكاينين, زراعة الانسجة.

1. **Introduction**

Ceratonia siliqua L., the carob tree known by its scientific name, is a long-cultivated perennial legume species that is a member of the Fabaceae family. Although hermaphrodite individuals are uncommon, they are typically dioecious. Despite its historical significance, carob production has been declining globally in recent years, raising questions regarding its sustainability and long-term use (Thomas *et al*., 2024). According to Benyaich *et al*. (2025), carob is an evergreen tree that thrives in semi-arid and desert climates and exhibits exceptional resilience to severe conditions such as salinity, poor soil, and drought.

The carob tree has a significant role in soil stabilisation, erosion management, and environmental sustainability, due to its deep root system and ecological adaptability. The species has been successfully introduced to many parts of the world. The species is native to the Med-

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iterranean basin and parts of the Middle East (Chlyah *et al*., 2024). The nutritional, ecological, and economic benefits of this food are largely why it is consumed in regions such as Africa, Australia, and the Americas (Menconi *et al.,* 2024).

The carob tree can grow up to 15 meters in height and has morphological characteristics that include dark green, leathery leaves with a smooth upper surface (Dahmani *et al*., 2023). The plant's different parts are used in a variety of ways, especially in traditional medicine, where carob has been used to treat digestive disorders, diabetes, and cardiovascular diseases. The popularity of carob has increased significantly in recent years because of its many uses and potential as a sustainable crop (Thomas *et al.,* 2024).

Carob pods are widely used as a natural alternative to sugar and cocoa in the food and pharmaceutical industries due to their low-fat content and high quantities of carbohydrates, dietary fiber, and protein, especially in seeds. Moreover, the species is regarded as a promising candidate for sustainable agricultural development in dry regions (Albanell *et al*., 2020), as it has a strong defense against drought and salinity stress (Kahkahi *et al*., 2024).

For carob to be successfully cultivated and propagated, suitable physiological and climatic conditions are necessary for the successful cultivation and propagation of carob, especially during the early phases of root and shoot development. Plant growth regulators are instrumental in regulating cell division, differentiation, and elongation in these processes. Plant morphogenesis is influenced by auxins and cytokinins, which are among the most important hormones involved in root and shoot formation (Daskalakis and Bertsouklis, 2021). Conventional field cultivation of carob and other medicinal plants is sometimes hindered by environmental variability, lengthy juvenile periods, and poor propagation efficiency (Moussa *et al.,* 2020).

An effective biotechnological technique for quickly and widely propagating plants under controlled circumstances is plant tissue culture (Pasternak and Steinmacher, 2025). By using this technique, plantlets can be produced uniformly and without pathogens throughout the year, and rooting and germination time are significantly reduced. *In vitro* culture techniques mostly rely on manipulating plant growth regulators in the culture medium to control root and shoot development.

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(Sánchez-Mendoza *et al.,* 2025).

Among the most popular growth regulators in tissue culture research are Indole-3-acetic acid (IAA), a key auxin involved in root initiation and cell elongation, and benzyladenine (BA), a cytokinin necessary for cell division and shoot proliferation, are among the most widely used growth regulators in tissue culture studies (Taiz *et al.,* 2014).

Therefore, investigating the effects of different concentrations of IAA and BA, applied separately or in combination, on root germination and early growth of carob plants under tissue culture conditions is essential for optimizing propagation protocols.

This study aims to evaluate how growth regulators affect root length, dry biomass accumulation, root-to-shoot ratio, and nutritional content of carob plantlets.

It is hypothesised that root development can be improved by an optimal balance of growth regulators without upsetting the plant's physiological equilibrium. Consequently, contributing to the development of an efficient in vitro propagation system for carob.

2. Materials and Methods

2.1. Preparation of culture media:

To make the culture media, we used a half-strength Murashige and Skoog (1962) MS salt solution, vitamins, glycine, 30 g L^{-1}, sucrose, and 0.1 g L^{-1}, myo-inositol. The media supplemented various concentrations of plant growth regulators, such as indole-3-acetic acid or 6-benzylaminopurine. 1 N potassium hydroxide or 1 N hydrochloric acid was used to adjust the pH to 5.8. The media were sterilized using an autoclave set to 121 °C and 1.2 kg/cm $^{-2}$ for 20 minutes with 7 g/L^{-1} agar incorporation. The sterilization process was followed by the pouring of 50 ml of the media into 350 ml culture jars for *in vitro* seed germination. To detect microbial contamination, all media were kept in complete darkness for three days post-preparation.

2.2. Sterilization and germination:

The seeds of carob tree were rinsed under running tap water for about 30 minutes. Seeds were moved to a laminar airflow cabinet, where they were sterilized by immersion in 70% (v/v) ethanol for 2 minutes, then rinsed with sterile distilled water. Following that, the seeds were disinfected with a commercial sodium hypochlorite solution with 5.25% chlorine, which was supplemented with two drops of 20 for 20 minutes. Three times with sterile distilled water were used to rinse

the seeds after disinfection. The culture medium was inoculated with three seeds per culture vessel under aseptic conditions using an equal number of sterilized seeds from both cultivars. The cultures were maintained under a 16 h light/8 h dark photoperiod with a light intensity of approximately 1500 lux provided by cool white, fluorescent lamps, and incubated in a controlled growth chamber at 26 ± 1 °C The plantlets were grown for four weeks before being harvested, taken out of the culture vessels, and washed with sterile distilled water. After careful separation, the plantlets were divided into shoots and roots. Liquid nitrogen was used to immerse each tissue for 5 minutes and then it was stored at -20 °C until further examination.

2.3. Morphological characteristics:

The morphological analysis examined variables that were related to seedling development, such as root length (cm) and the root-to-shoot ratio expressed as a percentage, as well as biomass characteristics, including dry weight of shoots and roots per jar. Leaf samples (0.5 g) were oven-dried at 70 °C, ground finely, and then digested with a solution of sulfuric acid and hydrogen peroxide (Lachica *et al*., 1973). The total nitrogen content was measured using the micro-Kjeldahl (Pregl, 1945) method, and the calcium content was measured using flame-emission photometry according to (Brown and Lillel, 1946) and colorimetric phosphorus determination (Jacckson's, 1958).

2.4. Extraction and estimation of soluble sugars, sucrose, and starch:

Control and treated plantlets had soluble carbohydrates extracted from them according to the procedure described by (Angelov *et al*.,1993). In boiling water bath, 20 ml of 80% (v/v) ethanol was used to homogenize and extract shoot and root tissues (0.5 g) repeatedly. The ethanol-soluble extracts were blended, evaporated to a fine powder, and then reconstituted with 15 ml of distilled water.

The anthrone method was used (Riazi *et al*., 1985) to determine the total soluble sugars colorimetrically. A mixture of 0.5 ml of extract and 3.0 ml of freshly prepared anthrone reagent was made (150 mg anthrone dissolved in 100 ml of 72% sulfuric acid) and heated in a boiling water bath until full color development. Absorbance was measured at 625 nm, and glucose solutions (0.1 mg ml^{-1}) treated under the same conditions were used to construct the calibration curve.

Sucrose content was quantified after alka-

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line hydrolysis of 2.0 ml of the alcoholic extract with 1.0 ml of 5.4 N potassium hydroxide at 97 °C for 5 minutes. After cooling, 3.0 ml of anthrone reagent was added (Clegg, 1956), and the mixture was heated in a boiling water bath until color formation. Absorbance was recorded at 620 nm, and sucrose standard solutions (0.2 mg ml^{-1}) were used for calibration.

Starch was estimated based on the method of (McCready *et al*.,1950) from the ethanol-insoluble residue. A 0.1 g portion of the dried residue was suspended in 2.5 ml of distilled water, followed by the addition of 3.5 ml of 52% (v/v) perchloric acid. The mixture was thoroughly stirred and centrifuged at 4,000 rpm for 15 minutes. The supernatant was collected, and the extraction was repeated twice. The combined supernatants were pooled, hydrolyzed, and adjusted to a final volume of 15 ml with distilled water. After filtration, a 1.0 ml aliquot was analyzed colorimetrically using the same anthrone procedure for total soluble sugars. Starch content was calculated as glucose equivalents and expressed as milligrams of glucose per gram of dry weight (mg glucose g^{-1} DW (Clegg, K.M. (1956).

3. **Results:**

The information in Table 1 shows the effects of applying indole-3-acetic acid (IAA) and benzyladenine (BA), both individually and in combination, on the root and vegetative growth characteristics, as well as the mineral nutrient content, of *Ceratonia siliqua* L. seedlings under controlled laboratory conditions.

In comparison to the control treatment, where these values were 10 cm and 3 g, respectively, the application of IAA at 1.5 mg/L considerably improved root development, with root length reaching 16 cm and dry root weight increasing to 6 g. This indicates the significant role of auxin in stimulating root growth and cell division.

However, root growth characteristics decreased when IAA concentration was increased to 2.0 mg/L, indicating that higher auxin levels may have negative effects on plant development.

Additionally, treatment with BA at 0.5 mg/L also improved root growth, showing increased dry weight and root length, as well as a higher root-to-shoot ratio (166.7%), which indicates increased root biomass compared to shoots (Kumar & Singh, 2019). However, increasing the BA concentration to 1.0 mg/L caused a

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decrease in growth features.

The best results were obtained when IAA and BA were at 1.5 and 0.5 mg/L, respectively, which balanced the growth of root and shoot. Along with higher concentrations of mineral nutrients, such as nitrogen and phosphorus. These results combined produced the highest dry weight and root length values. These findings suggest a synergistic effect between auxin and cytokinin, which work in concert to affect overall plant growth and nutrient uptake. On the other hand, the combination of IAA and BA at higher concentrations (2.0 + 1.0 mg/L) led to a decrease in all measured parameters, indicating potential toxic or inhibitory effects at elevated hormone levels.

In conclusion, carob seedlings' growth performance and nutrient accumulation are significantly improved by modest concentrations of IAA and BA, especially when combined. This emphasizes the importance of balanced hormonal control for ideal plant development.

The purpose of this study was to assess how plant growth regulators affected the carob plant's carbohydrate content. The amounts of carbohydrates, such as soluble sugars, sucrose, and starch, in the shoot and root under the effects of benzyl adenine (BA), indole-3-acetic acid (IAA), and their combination treatment (IAA + BA) are shown in Table 2.

The results showed that the combined treatment (IAA + BA) was the most successful treatment, leading to a significant increase in soluble sugar content in both the shoot (155 mg glucose/g dw) and root (142 mg glucose/g dw).

In contrast, the application of BA alone resulted in the lowest soluble sugar levels in the shoot (111) and root (109). IAA applied alone was the most effective treatment for increasing sucrose content, producing the highest values of 135 and 133mg glucose/g dw. For shoot and root, respectively. On the other hand, the combined treatment (IAA + BA) resulted in a significant decrease in sucrose levels, recording 117 mg glucose/g dw and 106 in the root.

The combined treatment (IAA + BA) demonstrated a pronounced stimulatory effect on starch accumulation, resulting in the highest starch content in both the shoot (176 mg glucose/g dw) and root (171 mg glucose/g dw). In contrast, IAA alone produced the lowest starch levels, with values of 112 mg glucose/g dw in the shoot and 110 in the root. An inverse relationship between sucrose

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and starch content was evident across the treatments. Under IAA treatment, sucrose levels were elevated while starch accumulation remained low, suggesting limited conversion of sucrose into starch. In contrast, the combined application of IAA and BA significantly enhanced starch accumulation while reducing sucrose levels, suggesting an efficient conversion of sucrose into starch for storage. These results are consistent with the physiological role of sucrose as a major energy source and precursor to the production of starch. Overall, the findings suggest that the combined application of auxin (IAA) and cytokinin (BA) at appropriate concentrations promotes optimal carbohydrate partitioning and starch storage, thereby supporting improved carob plant growth in the carob plant.

Table 1 summarizes the effects of IAA and BA, individually and combined, on root and vegetative traits and mineral content of *Ceratonia siliqua L.* seedlings grown under laboratory conditions.

Treatment	Root/Shoot Ratio (%)	Dry Root Weight (g)	Shoot Dry Weight (g)	Root Length (cm)	P (mg/g)	N (mg/g)	K (mg/g)	Ca^{2+} (mg/g)
Control	75	3	4	10	0.19	0.46	0.34	0.19
IAA 1.5 mg/L	150	6	4	16	0.23	0.52	0.44	0.30
IAA 2.0 mg/L	75	3	4	12	0.20	0.33	0.23	0.27
BA 0.5 mg/L	166.7	5	3	14	0.20	0.32	0.30	0.19
BA 1.0 mg/L	150	3	2	10	0.17	0.20	0.30	0.15
IAA + BA 1.5 + 0.5 mg/L	150	6	4	16	0.27	0.54	0.43	0.41
IAA + BA 2.0 + 1.0 mg/L	66.7	2	3	10	0.11	0.24	0.15	0.17

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I. Morphological Parameters and Growth

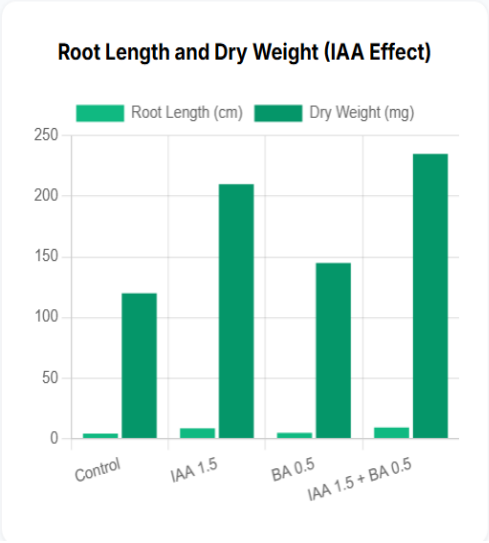


Figure 1. Effect of exogenous IAA, BA, and IAA+BA applications on root morphological parameters (length and biomass).

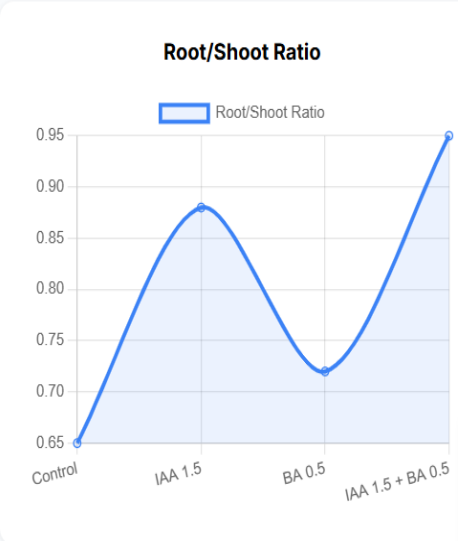


Figure 2. Response curve of the root/shoot ratio to individual and combined treatments of IAA and BA.

The application of plant growth regulators exerted a pronounced promotional effect on root development and overall growth biomass allocations compared to the untreated control group, as illustrated in Figure 1.

Root Length and Dry Weight Accumulation

Control Group exhibited baseline growth with the lowest recorded root length (5 cm) and a dry weight of approximately 120 mg (Figure 1).

Exogenous IAA (1.5 mg/L) significantly stimulated root development, increasing dry weight drastically to 210 mg and enhancing root length. This reflects the classic role of auxins in stimulating lateral and adventitious root initiation.

Exogenous BA (0.5 mg/L) showed a moderate increase in dry weight (145 mg) but had a negligible impact on root elongation compared to the control group

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(Figure 1).

Combined treatment (IAA 1.5 + BA 0.5) produced the most profound synergistic response, maximizing root dry weight accumulation at its highest peak (235 mg) and optimal root length extension.

Root / Shoot Ratio Allocation

The root-to-shoot (R/S) ratio tracked a distinct non-linear curve across treatments (Figure 2) highlighting differential biomass partitioning:

The Control maintained the lowest R/S ratio at 0.65.

Monotherapy of IAA 1.5 sharply increased the ratio to 0.88, indicating preferential biomass allocation toward the root system.

Under BA 0.5 application, the ratio experienced a localized drop to 0.72, pointing toward cytokinin mediated shoot allocation.

The combined formulation (IAA 1.5 + BA 0.5) achieved the highest value overall at 0.95 (Figure 2), demonstrating that the combined application profoundly shifts structural allocation in favor of root architecture development.

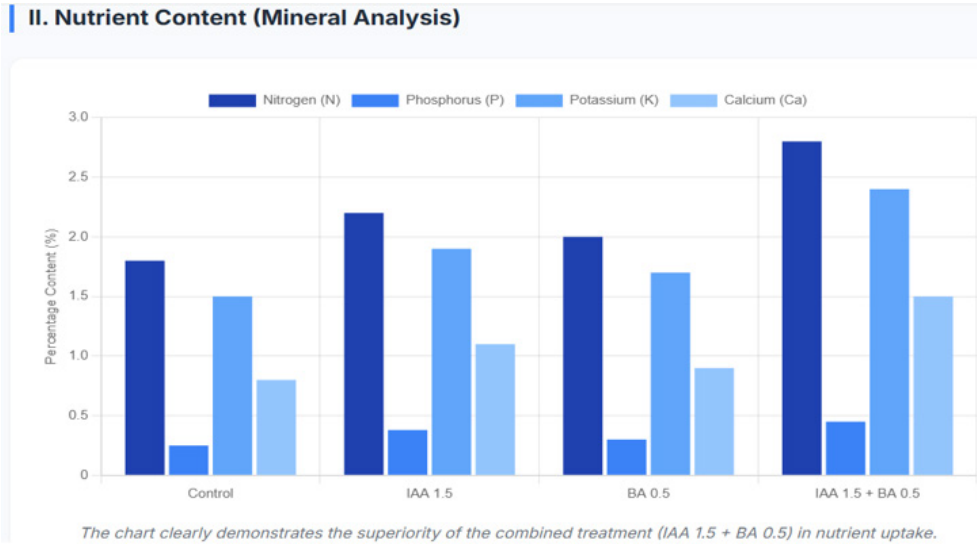


Figure 3: Quantitative profile of mineral nutrient uptake (N, P, K, and Ca percentage content) under individual and combined applications of Indole-3-Acetic Acid (IAA) and Benzyladenine (BA).

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Nutrient Content and Mineral Analysis (Figure 3) mineral uptake analysis demonstrated a clear, positive correlation between PGR treatments and macro-nutrient accumulation within plant tissues (Figure 3). Nitrogen (N) Accumulation: Nitrogen content expanded from 1.80% in the control group to 2.20 % with IAA and 2.00 % with BA. Crucially, the combined treatment triggered a maximum accumulation of 2.80 %, reflecting accelerated protein synthesis and metabolic activity (Figure 2). Phosphorus (P) remained low in control tissues (0.25 %) but experienced an upward shift under combined applications, reaching its apex at 0.45 % (Figure 3).

Potassium (K) and Calcium (Ca) profiles followed an identical regulatory pattern. Potassium reached its maximum threshold under the combined matrix at 2.40 % (up from 1.50 % in control). Concurrently, Calcium content nearly doubled from 0.80 % (Control) to 1.50 % (IAA + BA), supporting structural cell-wall integrity during rapid growth phase expansions (Figure 3). As stated in Figure 3, the multi-element mineral profiles clearly validate the systematic superiority of the combined treatment matrix (IAA1.5 + BA 0.5) over individual growth hormone variants in augmenting cellular nutrient assimilation capacity.

The purpose of this study was to assess how plant growth regulators affected the carob plant's carbohydrate content. The amounts of carbohydrates, including soluble sugars, sucrose, and starch, in the shoot and root under the effects of benzyl adenine (BA), indole-3-acetic acid (IAA), and their combination treatment (IAA + BA) are shown in Table 2.

The results showed that the most successful treatment was the combination of IAA and BA, which significantly increased the amount of soluble sugar in the shoot (155 mg glucose/g dw) and root (142 mg glucose/g dw).

However, the shoot (111) and root (109) had the lowest quantities of soluble sugar levels when BA was applied alone. With the highest values of 135 and 133 mg glucose/g dw. for shoot and root, respectively, IAA administered alone proved to be the most successful therapy for increasing sucrose content. On the other hand, sucrose levels were significantly lowered by the combination treatment (IAA + BA) with 117 mg glucose/g dw.

The shoot (176 mg glucose/g dw) and root (171 mg glucose/g dw) had the maximum starch content because of the com-

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bination treatment (IAA + BA) which showed a strong stimulatory effect on starch accumulation. With values of (112 mg glucose/g dw) in the shoot and (110) in the root, IAA alone, on the other hand, generated the lowest starch levels.

Across all treatments, there was a clear inverse association between the amounts of sucrose and starch. While starch accumulation stayed modest after IAA administration, sucrose levels increased, indicating a restricted conversion of sucrose into starch. However, when IAA and BA were applied together, starch accumulation was much increased while sucrose levels were decreased, suggesting an effective conversion of sucrose into starch for storage.

Table 2: summarizes the effects of IAA and BA, individually and combined, on soluble sugars, starch, and sucrose contents (mg g^{-1} DW) in shoots and roots of *Ceratonia siliqua L.* seedlings grown under laboratory conditions.

Con mg/l-1	soluble sugars, starch,mg glucose/g DW	sucrose mg /g DW		Starch,mg glucose/g DW	
Control	Shoot	Root	Shoot	Root	Shoot	Root
IAA 1.5 mg/L	122	121	135	133	112	110
BA 0.5 mg/L	111	109	130	127	110	118
IAA + BA 1.5 + 0.5 mg/L	155	142	117	106	176	171

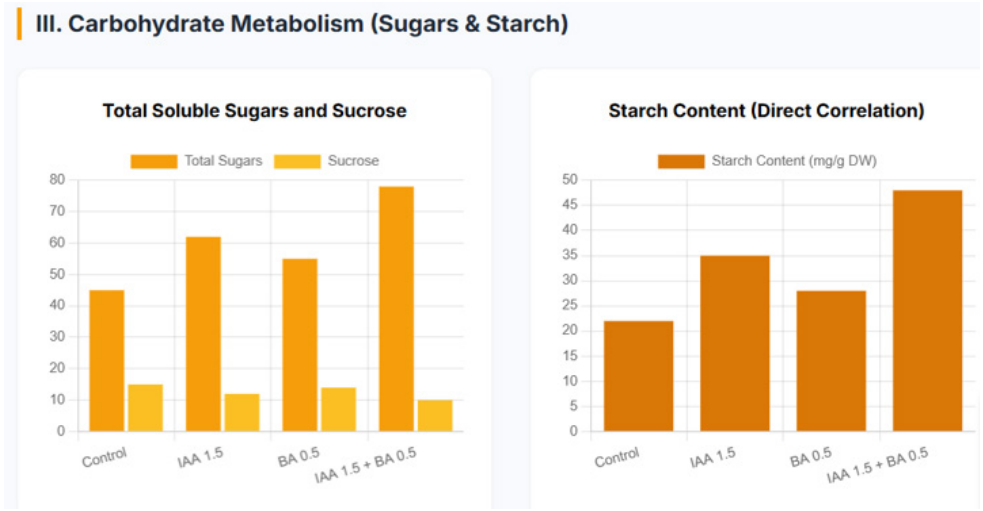


Figure 4. Carbohydrate metabolism dynamics under different growth regulator treatments: (A).Total soluble sugars and sucrose content; (B) Starch accumulation profile.

These results are consistent with sucrose's physiological role as a major energy source and a precursor to the production of starch. Overall, the results suggest that applying auxin (IAA) and cytokinin (BA) together at the right doses encourages ideal starch storage and carbohydrate partitioning, allowing for increased carob plant growth. Total Soluble Sugars vs. Sucrose Variations an inverse correlation was observed between total soluble sugars and sucrose accumulation (Figure 4) total soluble sugars steadily expanded across all treatment tiers compared to the control group (45 mg/g DW). The single hormone groups recorded 62 mg/g DW (IAA) and 55 mg/g DW (BA) while the combination group climbed exponentially to peak at 78 mg/g DW (Figure 4).

Sucrose levels, conversely, sucrose content entered a systemic decline. It moved downward from its highest level in the control (15 mg/g DW) to 12 mg/g DW (IAA), 14 mg/g DW (BA) and bottomed out at 10 mg/g DW under the combined interaction. This negative slope strongly suggests an accelerated rate of sucrose cleavage into hexose units to feed cellular respiration. Starch content strongly synchronized with the pattern observed in total soluble sugars (Figure The control baseline was limited to 22 mg/g DW. Individual setups triggered steady increments, yielding 35 mg/g DW (IAA) and 28 mg/g DW (BA). The dual application (IAA 1.5 + BA 0.5) induced a massive accumulation spike up to 48 mg/g DW (Figure 4) establishing a robust sink source capacity and long term energy storage facilitation within the root framework.

4. Discussion:

Exogenous application of plant growth regulators significantly modulates root architecture, vegetative biomass allocation, and nutrient and carbohydrate dynamics in *Ceratonia siliqua* L. Seedlings were cultivated under controlled laboratory conditions, as demonstrated by the current findings. Among the different treatments, IAA at 1.5 mg/L showed a strong stimulatory effect on dry root biomass and root length in comparison to the control, suggesting an ideal auxin concentration for meristematic activity and root cell elongation.

Notably, the combined application of IAA and BA at 1.5 + 0.5 mg/L produced the most consistent and robust improvements across root growth parameters, root/shoot ratio, and mineral nutrient accumulation, specifically nitrogen, phosphorus, and calcium. This response strongly suggests

a synergistic interaction between auxin and cytokinin signaling pathways, resulting in increased root sink strength and nutrient uptake efficiency.

Conversely, higher hormonal doses, particularly the IAA + BA 2.0 + 1.0 mg/L treatment, led to suppressed root development, reduced dry matter accumulation, and lower root/shoot ratios. Such inhibitory effects are indicative of hormonal imbalance and are consistent with the concept that supra-optimal growth regulator concentrations can disrupt cellular homeostasis and restrict biomass partitioning toward root systems.

With respect to carbohydrate metabolism, the observed increase in soluble sugars and sucrose contents under the combination of IAA and BA treatment reflects improved photosynthetic assimilate production along with efficient translocation toward root tissues. The concurrent changes in starch levels under individual hormone applications further highlight the regulatory role of auxins and cytokines in carbon allocation and storage processes within seedling organs. Together, these results show that using moderate and balanced application of IAA alone or in combination with BA represents an effective method for optimizing early seedling development, root system architecture, nutrient uptake, and metabolic performance in *Ceratonia siliqua* L. These insights are highly relevant for developing efficient propagation and establishment methods for this environmentally and economically significant species in controlled cultivation systems.

References:

1. Albanell, E., Tonti-Filippini, S., and ánchez, M. (2020). Carob (*Ceratonia siliqua* L.): A sustainable Mediterranean crop with multiple uses. *Journal of Mediterranean Agriculture*, 32(4), 245–259.

2. Angelov, M. N., Sung, S. S., Doong, R. L., and Black, C. C. (1993). *Extraction and isolation of soluble carbohydrates*. [Publisher information not in text].

3. Benyaich, A., Nouayti, A., Mekki, O. E., Errafia, R., Bahtat, F., and Aksissou, M. (2025). Phytochemical and pharmacological properties of *Ceratonia siliqua* L.: A comparative review of Moroccan and Mediterranean varieties. *Journal of Applied Pharmaceutical Scienc.* 15(10), 022-042.

4. Brown, J. G., and Lillel, O. (1946). Rapid determination of calcium and magnesium in plant tissue by hydrogen electrode titration. *Proceedings of the American Society for Horticultural Science*.

5. Chlyah, O., Mdarhi, M. A., El Ghoubali, D., Kieras, M., Pirro, S., and Ghazal, H. (2024). The complete genome sequence of *Ceratonia siliqua* L. (Fabaceae, Fabales), carob. *Biodiversity Genomes*.

6. Clegg, K. M. (1956). The application of the anthrone reagent to the estimation of starch in cereals. *Journal of the Science of Food and Agriculture*, 7(1), 40-44. https://doi.org/10.1002/jsfa.2740070108.

7. Dahmani, W., Elaouni, N., Abousalim, A., Akissi, Z. L., Legssyer, A., Ziyyat, A., and Sahpaz, S. (2023). Exploring carob (*Ceratonia siliqua* L.): A comprehensive assessment of its characteristics, ethnomedicinal uses, phytochemical aspects, and pharmacological activities. *Plants*, 12 (18), 3303.

8. Daskalakis, I., & Bertsouklis, K. (2021). Seed germination improvement and propagation challenges of *Ceratonia siliqua* L. *Forest Science Review*, 9(2), 77–89.

9. Jackson, M. L. (1958). *Soil chemical analysis*. Prentice-Hall, Inc.

10. Kahkahi, R. E., Moustaine, M., & Zouhair, R. (2024). Botanical, chemical, and pharmacological characteristics of carob tree (*Ceratonia siliqua* L.). *Med Discov,* 3(6), 1168.

11. Kumar, R., and Singh, A. (2019). Interaction of auxin and cytokinin in plant morphogenesis. *Plant Growth Regulation*, 87(2), 211–220.

12. Lachica, M., Aguilar, A., and Yanez, J. (1973). Acid digestion of plant samples. *Analyst*.

13. McCready, R. M., Guggolz, J., Silviera, V., and Owens, H. S. (1950). Determination of starch and amylose in vegetables. *Analytical Chemistry*, 22(9), 1156–1158.

14. Menconi, M. E., Abbate, R., and Grohmann, D. (2024). Exploring the uses and landscape value of *Ceratonia siliqua* L.: A systematic review. *Forests, Trees and Livelihoods*, 33, 231–247.

15. Moussa, H., El-Hassan, S., and Khalil, R. (2020). Effects of auxin–cytokinin balance on root induction in woody plant species grown in vitro. *Journal of Plant Biotechnology*, 19(4), 233–241.

16. Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–497.

17. Pasternak, T., and Steinmacher, D. (2025). Plant tissue culture in vitro: A long journey with lingering challenges. *International Journal of Plant Biology*.

ISSUE May Tenth 2026 DOI : https://doi.org/ 10.37376/asj.vi10

18. Pregl, F. (1945). *Quantitative organic microanalysis*. Philadelphia:Blakiston.
Riazi, A., Matsuda, K., and Arslan, A. (1985). Water stress induced changes in concentrations of proline and other solutes in growing regions of young barley leaves. *Journal of Experimental Botany*, 36(11), 1716–1725.
19. Sánchez-Mendoza, E. A., Pérez-Molphe-Balch, E., Guzmán-Mendoza, R., Ruiz-Aguilar, G., García-Munguía, A. M., Costilla-Salazar, R., and Núñez-Palenius, H. G. (2025). Plant growth regulators use in the in vitro culture of Agave species. *Plants*, 14(21), 3402.
Taiz, L., Zeiger, E., Møller, I. M., and Murphy, A. (2014). *Plant Physiology and Development*. Sinauer Associates. Inc., 6th edition edition.
20. Thomas, P. A., Garcia-Martí, X., Mukassabi, T. A., and Tous, J. (2024). International biological flora: *Ceratonia siliqua*. *Journal of Ecology*, 112, 1885–1922.