



The impact of 6-benzyladenine concentrations on the in vitro clonal propagation of the medicinal plant *Allium kurrat* L

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ABSTRACT

Kurrat (*Allium kurrat* L.) is a widely consumed vegetable in the Eastern Mediterranean, particularly in Egypt, where it is valued for its fresh leaves, rich nutritional profile, and medicinal properties. In the current study, an in vitro propagation protocol was established using seed-derived cultures on Murashige and Skoog (MS) medium supplemented with varying concentrations of 6-benzyladenine (BA; 1–4 μ M). Shoot multiplication responded positively to BA supplementation, with the maximum number of shoots (19.0 ± 0.58 shoots/seed) recorded at 2.0 μ M BA, representing a significant increase compared with the control (1.0 ± 0.0 shoot/seed). However, shoot and root lengths decreased significantly with the addition of BA compared to the control. The shoot fresh weight was higher in the control (0.32 ± 0.01 g), whereas root fresh weight increased at 2.0 μ M BA (0.27 ± 0.01 g). Meanwhile, at 4.0 μ M BA, both shoot and root fresh weights declined compared with the control. The root-to-shoot ratio was also influenced by BA, reaching the highest value (1.74 ± 0.04) at 2.0 μ M BA, followed by 1.54 ± 0.02 at 1.0 μ M BA.

These findings indicate that 2.0 μ M BA is optimal for enhancing shoot proliferation and increasing root biomass while maintaining a favourable root-to-shoot balance in *A. kurrat* micropropagation. This study optimized a micropropagation system that provides an efficient and reproducible method for large-scale production of *A. kurrat* and serves as a valuable platform for future biotechnological and breeding applications.

1. Introduction

Medicinal plants are important because of their ability to provide continuous and valuable supplies of bioactive

metabolites. The genus *Allium* L. has gained widespread recognition globally due to its possessing significant nutritional and therapeutic metabolites [1]. It belongs to the family Amaryllidaceae, which encompasses approximately

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918 species, the majority of which are well known for their distinctive flavors and therapeutic properties [2]. The medicinal properties of *Allium* species have long been appreciated [3], with reported activities including anti-inflammatory, antitumor, antidiabetic, and antimicrobial properties [4]. In addition, *Allium* plants are recognized as potent natural anticancer and antioxidant agents [5].

Allium kurrat, commonly known as Egyptian leek or kurrat, is a widely used vegetable. Kurrat leaves possess a special taste, rendering them a flexible component in a multitude of culinary preparations [6]. Traditionally, this species has been used in folk medicine, particularly for the treatment of eye diseases such as night blindness [7]. Phytochemical analyses have revealed that *A. kurrat* contains flavonoids, alkaloids, terpenoids, tannins, and saponins [8]. The plant extracts showed antibacterial, anticancer, and antioxidant activities [2,8,9]. Furthermore, leek extract has a hepatoprotective effect, highlighting its potential in the prevention and treatment of liver damage [10]. It has also been used as a natural antioxidant and antibacterial agent to extend the shelf life of meat products, such as beef burgers, during cold storage [6].

In vitro plant propagation, an advanced methodology in the realm of plant biology, has emerged as an efficient approach for the large-scale production of medicinal plants. In previous studies, *Allium kurrat* was in vitro regenerated from different explants, including seedlings, basal plates of mature plants, and immature inflorescence [11–13]. Cytokinins, particularly 6-benzyladenine (BA), are widely used in plant tissue culture due to their ability to stimulate cell division, axillary bud proliferation, and shoot multiplication [14,15]. However, the response to BA is highly concentration-dependent; while low to moderate concentrations promote shoot proliferation, higher concentrations often inhibit shoot elongation and root development due to hormonal imbalances [16].

In addition to its horticultural value, optimizing in vitro regeneration protocols for *A. kurrat* has broader implications for crop improvement and biotechnology. Efficient micropropagation systems can serve as a platform for genetic transformation, mutagenesis, and secondary metabolite production, which are critical for enhancing stress tolerance and nutritional quality [17]. Moreover, standardized regeneration protocols are essential for germplasm conservation of *Allium* species, many of which face genetic erosion due to habitat loss and climate change [18,19].

The present study explores the micropropagation of *A. kurrat* from seed, which will likely play a significant role in the large-scale production and conservation of this medicinally valuable species.

2. Materials and Methods

2.1. Materials and Methods

2.1.1. Seed sterilization

Healthy seeds were obtained from the Agricultural Research Center in Giza, Egypt. Surface sterilization was carried out by stirring the seeds for 1 min in 95 % ethanol, followed by 20 min in 20 % Clorox solution. Surface-sterilized seeds were then washed 3 times, 5 min each, with sterile distilled water under aseptic conditions.

2.1.2. Micropropagation

Surface-sterilized seeds were cultured on Murashige and Skoog (MS) medium 20 with Gamborg's vitamins 21 supplemented with 0.0, 1.0, 2.0, 4.0 μ M benzyladenine (BA). The media was solidified with 0.2% phytagel (MSB5); pH was adjusted to 5.8 before solidification and autoclaving. After a month, the shoots separated into individual shoots and were transferred for rooting to MSB5 medium supplemented with 0.0 or 0.5 mg/L indole-3-acetic acid (IAA). After 4 months, the cultures were screened for the number of axillary shoots, shoot length, root length, shoot and root fresh weight, as well as root: shoot ratio of a fresh weight.

To calculate the root: shoot ratio of a fresh weight (g), the formula is:

Root: Shoot ratio = (Root fresh weight) / (Shoot fresh weight)

2.1.3. Culture Conditions

Cultures were incubated at 25 ± 2 °C under a 16/8 h (light/dark) photoperiod using white, fluorescent tubes (350-500 lux illumination).

2.1.4. Acclimatization

Plantlets were carefully removed from the tissue culture vessels, and any surplus medium was removed by rinsing in double-distilled water. The plantlets were then transferred to sterilized pots containing a (1:2, v/v) mixture of sterilized sand and peat moss and covered with polyethylene bags to maintain high humidity. The plantlets were irrigated daily with sterile water and placed in an incubator at 25 °C under a 16/8 hr (light/dark) photoperiod, using white fluorescent light for a duration of one week. The humidity gradually diminished. After a duration of eight weeks, the acclimatized plants were relocated to the greenhouse, where they were subjected to normal environmental conditions and received scheduled watering.

3. Statistical analysis

The data were analyzed statistically using Minitab® 17 software through one-way ANOVA. In instances where treatment differences are significant, treatment means were compared using Fisher's least significant difference (LSD) at a level of confidence of 95%.

4. Results

Figure 1 illustrates the in vitro propagation stages of *Allium kurrat* from seed culture on MS media supplemented with different BA concentrations. On the other hand, the effects of different concentrations of BA on shoot proliferation and growth parameters are presented in Table 1.

The number of shoots increased significantly with BA supplementation, reaching a maximum at 2.0 μM BA (19 ± 0.58 shoots/ seed), which was significantly higher than the control (1.0 ± 0.0 shoots/ explant) and other concentrations (Table 1 and Figure 2A, supplementary data).

Shoot and root length were strongly influenced by BA concentration (Table 1 and Figure 2B, supplementary data). The control (0.0 μM BA) produced the longest shoots (26.64 ± 1.29 cm) and roots (11.69 ± 1.28 cm). Increasing BA concentrations resulted in a progressive and significant reduction in root length, while shoot length followed a different pattern. At 1.0–4.0 μM BA, shoot length ranged from 6.37 to 13.20 cm, whereas root length decreased to 3.83–1.33 cm.

The addition of BA at specific concentrations enhanced root fresh weight, whereas increasing BA concentrations generally reduced shoot fresh weight (Table 1 and Figure 3C, supplementary data). Control explants exhibited the highest shoot fresh weight (0.32 ± 0.01 g), while the maximum root fresh weight (0.27 ± 0.01 g) was recorded at 2.0 μM BA. The minimum values of root fresh weight and total fresh weight were observed at 4.0 μM BA. The root-to-shoot ratio was also affected by BA concentration (Table 1 and Figure D, supplementary data), with the highest ratio (1.74 ± 0.04) recorded at 2.0 μM BA, followed by 1.54 ± 0.02 at 1.0 μM BA.

5. Discussion

The successful establishment of an in vitro propagation protocol for *kurrat* enables its mass production, an alternative and powerful biotechnology method that overcomes limitations in traditional techniques, providing new possibilities for agricultural, genetic conservation, and pharmaceutical applications.

The response to cytokinins in vitro is highly species-specific, with shoot proliferation rates being profoundly influenced by the type and concentration of cytokinin used. Among the various cytokinins, Benzyladenine (BA) is widely regarded as a highly effective choice for shoot multiplication across diverse plant species, with its efficacy in promoting rapid proliferation being well-documented [22]. Furthermore, BA is distinguished by its optimal balance of strong biological activity, chemical stability, and cost efficiency [23].

The present study shows that BA significantly affected both shoot multiplication and the subsequent growth and

development of the *kurrat* plantlets. BA dose-dependently affected shoot formation, shoot and root length, fresh weight, and root/shoot balance. While 2 μM BA significantly increased shoot number, while 4 μM BA led to a decrease in the above parameters. This decline in shoot multiplication at higher BA concentrations aligns with the findings in related *Allium* species, including garlic (*Allium sativum* L.) [24], wild garlic (*Allium victorialis* var. *platyphyllum*) [25], onion (*Allium cepa* L.) [26], and leek (*Allium ampeloprasum*) [27].

The decline in shoot number observed at 4.0 μM BA might result from a super-optimal cytokinin level, a phenomenon reported in other species that can inhibit meristem development through ethylene-mediated suppression of cell division [28] or by disrupting auxin-cytokinin homeostasis [29]. BA treatments significantly reduced shoot and root lengths, with hormone-free controls exhibiting the longest organs. This supports the established inhibitory effect of cytokinin on cell expansion [30] and root development [31,32], consistent with findings in *Allium* species [24,33]. Shoot multiplication and elongation are negatively related, likely due to resource limitation, as resources are distributed among a larger number of shoots, resulting in shorter shoots and roots, as stated previously by Arab et al. [34].

Control plants exhibited the highest shoot and root fresh weights, likely due to biomass accumulation in a single, elongated shoot. However, 2.0 μM BA resulted in the highest root fresh weight despite shorter root length, suggesting this concentration promotes a denser, more branched root system. This indicates that 2.0 μM BA optimizes shoot proliferation and enhances root biomass, which is essential for plantlet survival. This might be because the BA promotes root initials formation or a denser root system despite potentially reducing root length. Prior research indicates that cytokinins reduce root-meristem size by accelerating differentiation within the meristem [31,35]. Cytokinins also shift resource allocation from primary root elongation to secondary root formation and radial expansion, leading to a smaller but denser and massive root system. The decreased shoot and root fresh weights observed at 4.0 μM BA suggest that higher cytokinin concentrations may have growth-inhibitory or toxic effects. In monocots like rice, excessive cytokinin-mediated growth suppression is linked to cytokinin-induced ethylene production [28].

This indicates a trade-off where high cytokinin levels favor meristematic activity and proliferation but limit cell expansion and organ elongation. The observed pattern in *A. kurrat* is consistent with cytokinin-driven proliferation–elongation trade-offs described in other species.

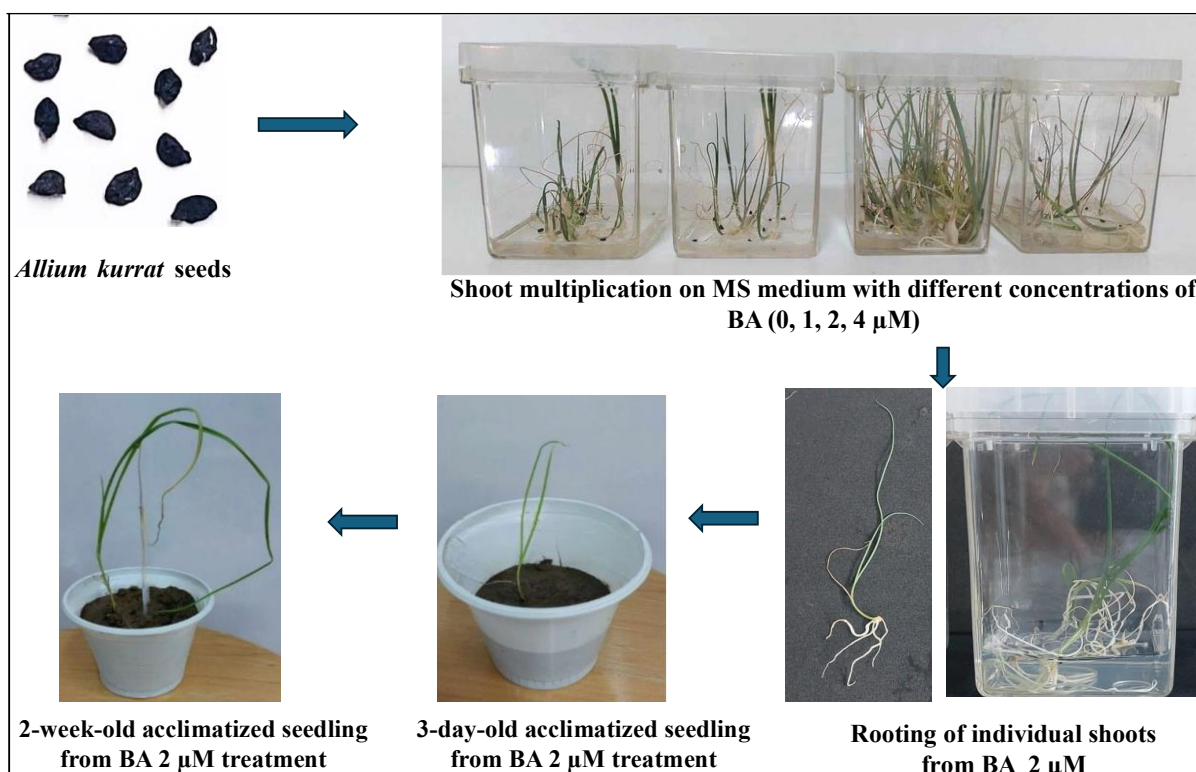


Figure 1. Photographs illustrating the stages of the *in vitro* regeneration and acclimatization of *Allium kurrat*.

Table 1. The effect of different concentrations of BA (0, 1, 2, 4 μ M) supplemented to MS medium on micropropagation of *Allium kurrat* L. from seed culture. Results are represented as mean $\pm$ SE of 10 replicates; different letters indicate significant differences at $P = 0.05$.

BA concentration	Shoot number/ explant	Shoot length (cm) / micro-shoot	Root length (cm) / micro-shoot	Shoot fresh weight (g) / micro-shoot	Root fresh weight (g) / micro-shoot	Total fresh weight (g)	Root: Shoot FW ratio (g)
0.0 μ M	1 $\pm$ 0.0 c	26.64 $\pm$ 1.29 a	11.69 $\pm$ 1.28 a	0.32 $\pm$ 0.01 a	0.20 $\pm$ 0.01 b	0.52 $\pm$ 0.01 a	0.61 $\pm$ 0.01 c
1.0 μ M	3 $\pm$ 0.58 b	6.37 $\pm$ 1.32 c	3.83 $\pm$ 0.17 b	0.12 $\pm$ 0.00 d	0.18 $\pm$ 0.01 c	0.30 $\pm$ 0.01 c	1.54 $\pm$ 0.02 b
2.0 μ M	19 $\pm$ 0.58 a	9.57 $\pm$ 0.35 bc	2.10 $\pm$ 0.49 bc	0.16 $\pm$ 0.00 c	0.27 $\pm$ 0.00 a	0.43 $\pm$ 0.001b	1.74 $\pm$ 0.00 a
4.0 μ M	3.33 $\pm$ 0.88 b	13.22 $\pm$ 1.79 b	1.33 $\pm$ 0.33 c	0.18 $\pm$ 0.01 b	0.05 $\pm$ 0.00 d	0.23 $\pm$ 0.01 d	0.29 $\pm$ 0.01 d
LSD 0.05	2	6.86	2.50	0.04	0.02	0.07	0.32

Conclusion

This study successfully developed an efficient *in vitro* micropropagation system for *Allium kurrat* (Egyptian leek) from seed-derived cultures using Murashige and Skoog medium. Low benzyl adenine concentration of 2.0 μ M optimized shoot proliferation and root biomass with a balanced root-to-shoot ratio. Higher BA concentrations (4.0 μ M) reduced shoot and root growth, suggesting a cytokinin-driven trade-off between proliferation and elongation. These findings inform large-scale propagation, acclimatization, and biotechnological applications. Future study required to investigate the metabolite enhancement and nutritional changes of *Allium* plants propagated via this *in vitro* protocol. Subsequent research should focus on analyzing secondary metabolites and nutritional compounds *in vitro*

-derived plants to validate their equivalence or superiority to conventional counterparts.

Conflict of interest

The authors confirm that there is no conflict of interest.

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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