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Effects of Benzylaminopurine on *in vitro* Proliferation and Shoot Growth of Okra [*Abelmoschus esculentus* (L.) Moench]

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ABSTRACT

Background: Okra is an important crop belonging to the *Malvaceae* family. This vegetable has significant nutritional, medicinal and economic importance. However, its consumption and production in Algeria remain very low due to limited seed availability and insufficient valorization in research programs. This study aimed to propagate okra *in vitro* through micropropagation at the proliferation stage. This method enables large-scale plant multiplication in a small space while ensuring protection and preservation of genotypes.

Methods: The Micropropagation of *Abelmoschus esculentus* through shoot proliferation was carried out on Murashige and Skoog medium and several benzylaminopurine concentrations were tested (0.0, 1.0, 2.0, 3.0 and 4.0 mgL^{-1}), the shoot explants were cultured for one month in a growth room under a temperature of 27°C±1 and a photoperiod of 16-h/8-h light/dark. The proliferated shoots were aseptically excised then cultured on Murashige and Skoog medium without plant growth regulator for root induction. After rooting, plantlets were acclimated and then transferred to the greenhouse.

Result: The best shoot proliferation was obtained in the medium supplemented with 2.0 mgL^{-1} of BAP. This medium provided the best results in terms of quality of shoots and multiplication rate. Frequent successive subcultures on the same medium allowed lthe elimination of phenolic compounds.

Key words: *Abelmoschus esculentus*, BAP, *In vitro*, Micropropagation, Okra, Shoot proliferation.

INTRODUCTION

The okra plant, *Abelmoschus esculentus* (L.) Moench, is a member of the botanical family *Malvaceae*. It is usually consumed for its immature fruits which are consumed as vegetables, this species has several local names around the world such as *Okra, Bhindi, Okra Fingers, Krajiab Kheaw, Ochro, Okoro, Quimgombo, Calou diab* and *Moullokhia*. However, in Algeria it is known as *Gnaouiya* in the north and *Bamia* in the South.

This vegetable has a very important nutritional and medicinal value. Its richness in biopolymers such as polysaccharides and bioactive compounds (Lengsfeld *et al*., 2004; Samavati, 2013) boosts immunity (Chen *et al*., 2016), reduces blood sugar (Liu *et al*., 2018), alleviates fatigue (Gao *et al*., 2018), in addition to its antioxidant properties (Xia, 2015) and antitumor effects (Zhu *et al*., 2020). Furthermore, Okra mucilage is used as a plasma replacement or blood volume expander. It also binds cholesterol and bile acid carrying toxins dumped into it by the liver (Gemede *et al*., 2015).

Also, the okra fruit is rich in calcium, iron, magnesium, vitamins A and C and amino acids (Hamon, 1988; Zodape *et al*., 2008; Adetuyi *et al*., 2012; Sharma and Parsad, 2015), therefore, it plays an important role in human food (Farinde *et al*., 2007).

Okra arrived in Algeria in the early 1950s (Algéria presse service, 2011), but despite the advantageous agroclimatic conditions for its cultivation in this country, its consumption in Algeria remains very low. Agricultural services estimated an average yield of 30 to 40 quintals in 2022 (Algéria presse service, 2022). This low production can be explained not only by the lack of valorization and marginalization of okra in development and research programs, but also by the lack of seeds and the harmful action of both biotic and abiotic constraints. Indeed, it is necessary to move towards strategies for the multiplication, safeguarding and protection of this species.

To achieve this goal, we propose an experiment on *in vitro* micropropagation of okra. This technique has many advantages such as the production of healthy plants on a large scale with more suitable quality in a small space

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(Singh, 2023), facilitating the exploitation of genetic resources and accelerating varietal creation (Moraes *et al*., 2021).

Very little work has been done on the micropropagation of okra in the proliferation stage. We mention the work of Daniel *et al*. (2023) and Rizwan *et al*. (2018). The proliferation step has a decisive role in the success of micropropagation. This method involves the use of plant growth regulators to guide the development of explants; in addition, Benzylaminopurine (BAP) is an efficient phytohormone that induces the proliferation of explants in several species. Therefore, this study aims to test the effect of different concentrations of BAP on the proliferation of okra shoots. This will allow to optimize the media to obtain the highest number of shoots.

MATERIALS AND METHODS

Plant material

The experiment was conducted at the Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario at la Alberca, Murcia, Spain. Shoot explants (≈ 2 cm in length) (Fig 1) were obtained from previous *in vitro* cultures of okra.

Shoot culture

The shoots explants were cultured in glass bottles containing 100 ml of MS medium with 30 g/L of lsucrose and solidified with 8 g/l of agar-agar, the medium was supplemented with different concentrations of BAP (0.0, 1.0, 2.0, 3.0 and 4.0 mgL^{-1}). The pH of the medium was adjusted to 5.8 and the medium was autoclaved at 20°C for 20 minutes.

Each treatment was carried out on 40 shoot explants which make up a set of 200 explants subdivided into 4 explants in each bottle (Fig 2).

Proliferation was evaluated after four weeks of cultivation by recording the number of shoots per explant (longer than 5 mm), their individual lengths and productivity (calculated as the average of the products of shoot number and shoot length per explant).

Rooting and acclimatization of shoots

Rooting and acclimatization of plantlets were carried out according to the method of Belkhodja *et al.* (2023).

Rooting was induced on MS free of plant growth regulators. Proliferated shoots were aseptically excised and then transferred into glass tubes containing 20 ml of MS medium (MS0). After 10 days, *in vitro* rooted plantlets were rinsed with distilled lwater land transplanted into pots containing sterile compost. Each pot was covered with a transparent plastic bag lto maintain humidity. After 10 days in lthe growth chamber, plantlets were transferred to the greenhouse in a larger pots until pod formation under a temperature of 30°C±3.

Statistical analysis

All statistical analyses were conducted using SPSS software (SPSS v.21). The dataset was initially examined to verify the homogeneity of variances and distribution normality. Statistical significance was assessed through analysis of variance (ANOVA) at a significance level of $p \leq 0.001$. When significant differences were detectedl, mean values were compared using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Effect of BAP on shoots number per explants

In order to stimulate the proliferation of *Abelmoschus esculentus* shoots and to optimize the proliferation medium, several concentrations of BAP were tested to select an optimal concentration allowing the highest number of shoots to be obtained.

The influence of BAP on proliferation was analyzed on MS medium. The results after 4 weeks of cultivation showed that the number of shoots depends significantly on the BAP concentration used in the proliferation medium ($p<0.001$) (Table 1).

The control MS medium produced only one shoot per explant and showed no proliferation in the absence of BAP, accompanied by the formation of large green leaves and a high density of roots, this was also observed by Belkhodja *et al*. (2023) in the same species. The medium supplemented with 1.0 mg L^{-1} of BAP produced an average of 2.05 shoots per explant, along with the emergence of a few roots on the proliferated shoots and the development of a midsized, whitish basal callus (Fig 4).

According to LSD comparison test (Table 2), this difference in the number of shoots under 1.0 mg L^{-1} of BAP and 0.0 BAP is highly significant. It should be noted that the number of shoots per explant increases significantly



Fig 1: Shoot explants used in the experiment.



Fig 2: Experimental design.

with increasing BAP concentrations, up to 2.0 mg L^{-1} (Table 2). Under this medium (2.0 mgL^{-1}), 3.07 shoots per explant were produced, characterized by their green coloration, a high number of leaves and the development of large calluses, compared to other media (Fig 3).

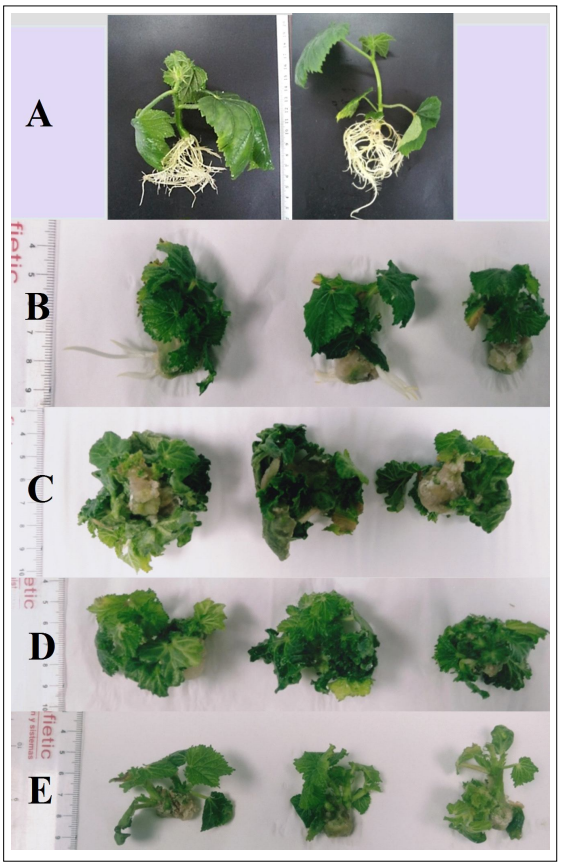


Fig 3: *Abelmoschus esculentus* explants cultured under 0.0 (A), 1.0 (B), 2.0 (C), 3.0 (D) and 4.0 (E) mgL^{-1} of BAP for 30 days in the proliferation experiment.



Fig 4: Shoots proliferated above 2.0 mgL^{-1} BAP (left) and below 4.0 mgL^{-1} BAP (right) at 4 weeks.

Above 2.0 mg L^{-1} of BAP, the number of shoots per explant decreases slightly, without a significant difference compared to media containing 2.0, 3.0, or 4.0 mg L^{-1} of BAP (Fig 3). The medium supplemented with 3.0 mg L^{-1} of BAP produced 2.82 shoots per explant, while the one with 4.0 mg L^{-1} produced 2.77 shoots per explant. It is also worth noting that the callus formed at 4.0 mg L^{-1} of BAP was less developed than that observed at 2.0 mg L^{-1}.

Depending on the number of shoots per explant, the medium enriched with 2.0 mg L^{-1} BAP is considered the most suitable for proliferation among those tested, followed by the medium containing 4.0 mgL^{-1} BAP. This medium (4.0 mg L^{-1}) appears to be effective for shoot proliferation and does not show a statistically significant difference compared to the medium supplemented with 2.0 mgL^{-1} of BAP.

In this experiment, However, the size of the calluses is not the key factor for assessing proliferation, but rather the number, length and productivity of the shoots. We observed that 2.0 mgL^{-1} BAP treatment induced enhanced shoot elongation and significantly increased callus biomass as compared to 4.0 mg L^{-1} BAP treatment as shown in Fig 3 and 4, respectively.

The observed finding aligns with previous studies showing that the increase in BAP concentration enhanced bud proliferation (Tanuwidjaja, 1998). Present results demonstrate that the efficiency of shoot proliferation varied according to the concentration of plant growth regulator, and the use of different concentrations of BAP significantly influenced the morphogenic responses in explant. Bensalem *et al*. (2023) further reported that combining TDZ with BAP effectively enhanced callus induction.

An increase in BAP concentration enhanced bud proliferation, as evidenced by an increase from 2.05 shoots per explant at 1.0 mgL^{-1} BAP to a maximum of 3.07 shoots per explant at 2.0 mgL^{-1}. The same result has already been reported with *Prosopis cineraria*, *Dendrocalamus strictus* and *Tinospora cordifolia* (Sangeetha and lenkatachalam, 2014; Venkatachalam *et al*., 2017; Goyal *et al*., 2015; Panwar *et al*., 2018). The number of shoots initially increased with BAP concentration, reaching a maximum at 2.0 mg L^{-1} and then gradually decreased at higher concentrations, with 2.82 shoots per explant at 3.0 mg L^{-1} and 2.77 shoots at 4.0 mg L^{-1}. The differences in shoot number were statistically significant, indicating that 2.0 mg L^{-1} BAP is the optimal concentration

Table 1: F-values resulting from the ANOVA illustrating the effect of different BAP concentrations on shoot number, shoot length and productivity of okra explants after 30 days of culture.

Source of variations	df	Mean square	F	Sig.
Shoot number	4	23,978	34,841	0.000***
Shoot length	4	57,292	129,432	0.000***
Productivity	4	11,550	6,701	0.000***

***: Significant at 0.1% of probability level.

for shoot proliferation.

Similar results were reported by Benmahioul *et al*. (2009); Barghchi and Aldersen (1983) and Abousalim *et al*. (1991) who found that the use of 2.0 to 4.0 mg L^{-1} of BAP increased significantly the number lof shoots produced by explant. In pistachio trees the low and high doses of BAP had a negative effect on bud productivity and their subsequent development. Tallón *et al*. (2009) demonstrated that shoot proliferation in *Citrus limon* is influenced by BAP and GA concentrations, with maximum shoot numbers observed at 2 mg L^{-1} BAP in combination with 1.0 or 2.0 mgL^{-1} GA. For *Curcuma zedoaria*, the best shoot multiplication rate was recorded in MS medium with 2.0 mgL^{-1} of BAP and 1.0 mgL^{-1} of TDZ yielding 5.3±0.24 shoots/explant (Hussain *et al*., 2023).

The effect of hormonal concentration on shoot length per explant

The analysis of variance reveals that the BAP concentration in the media has a highly significant effect on the shoots length ($p<0.001$) (Table 1).

As per LSD resultl (Table 2), Shoot length was significantly reduced with increasing concentrations of the plant growth regulator. Shoot length obtained in the MS0 was significantly better compared to the media supplemented with 1.0, 2.0, 3.0 and 4.0 mg L^{-1} of BAP. Specifically, shoots in the control medium reached an average length of 4.26 cm, which was significantly higher than those grown in the BAP-supplemented media (Table 2). Although a decreasing trend in shoot length was observed with increasing BAP concentrations, but this reduction was not statistically significant.

From our study, it was observed that the presence of BAP in the culture media had reduced the length of proliferated shoots. Moreover, the length of the shoots obtained was short in the presence of BAP compared to the control without PGR. Similar results were reported by Tallón *et al*. (2009) and de Jesus *et al*. (2022) working respectively on *Citrus macrophylla* and *Guazuma ulmifolia Lam*.

Arab *et al*. (2014) reported that hormone concentration affects significantly shoot length, so increasing hormone level triggers a significant decrease in shoot length. Their findings indicated that the longest shoots were obtained from media without PGRs. This could be explained by the fact that media containing cytokinins promote the formation of a higher number of shoots, which consume more nutrients. As a result, the limited availability of nutrients per shoot may reduce their capacity for elongation.

It has been reported that in *Guazuma ulmifolia* Lamp, the reduction in shoot ength may be related to the antagonistic interaction between the cytokinins and gibberellins of the shoot meristems. Cytokinin stimulates cytokinesis by promoting the proliferation of cells with meristematic characteristics, while inhibiting the activity of gibberellins, which are responsible for cell elongation (Maekawa *et al*., 2009; Shi and Vernoux, 2022).

Effect of hormonal concentration on explant productivity

Productivity is a valuable parameter as it reflects the combined effect of the treatment on both shoot number and length and gives a general idea of the *in vitro* behaviour of shoots (Pérez-Tornero and Burgos, 2000).

The analysis of variance reveals that productivity varies very significantly according to the hormonal concentration in the media ($p<0.001$) (Table 1).

Statistical analysis of shoot productivity across the different culture media, performed using the LSD test (Table 2), showed that the MS0 medium was the most productive, with an average of 4.26 shoots per explant. This productivity was significantly higher than that observed in media supplemented with 1.0, 3.0 and 4.0 mg L^{-1} of BAP. The medium supplemented with 2.0 mg L^{-1} of BAP showed intermediate productivity (3.91 shoots per explant), with no significant difference compared to either MS0 or the medium containing 4.0 mg L^{-1} of BAP. However, MS0 recorded the lowest number of shoots.

The shoot productivity observed in the 4.0 mg L^{-1} BAP medium was close to that of the 2.0 mg L^{-1} BAP medium which proved to be the most efficient for shoot proliferation (Table 2). The LSD test indicated no significant difference between the 2.0 mg L^{-1} and 4.0 mg L^{-1} BAP treatments. In contrast, the media supplemented with 1.0 and 3.0 mg L^{-1} of BAP exhibited the lowest productivity, with mean values of 2.84 and 3.13 shoots per explant, respectively. These values were significantly lower than those obtained in the control (MS0) and the media containing 2.0 and 4.0 mg L^{-1} of BAP.

From our work, the best productivity results were obtained in the control and at 2.0 mg L^{-1} BAP. It has also been observed by Tallón *et al*. (2009) that Productivity peaks below 2.0 mgL^{-1} BAP and 2.0 mgL^{-1} Ga.The results obtained in this work conclude that the concentration of BAP in the media has a highly significant effect on productivity ($p<0.001$). Similar results are reported by Pérez-Tornero and Burgos (2000). In apricots, they found that productivity is significantly affected by the cultivar and by the interaction between the genotype and the media.

Present study indicates that 2.0 mgL^{-1} of BAP treatment

Table 2: Effect of different BAP concentrations on shoot number, shoot length and productivity of okra explants in the proliferation experiment.

BAP mgL^{-1}	Shoot number	Shoot length (cm)	Productivity
0.0	1^{a}±0	4.26 ^{a}±0.20	4.26 ^{a}±0.20
1.0	2.05^{b}±0.94	1.42 ^{b}±0.64	2.84^{c}±0.16
2.0	3.07^{c}±0.17	1.38 ^{b}±0.10	3.91^{ab}±0.23
3.0	2.82^{c}±0.16	1.15 ^{b}±0.78	3.13^{c}±0.23
4.0	2.77^{c}±0.16	1.26 ^{b}±0.69	3.40^{bc}±0.23

The values are expressed as means ± standard error (SE) based on forty replicates. Means followed by different letters within the same column indicate statistically significant differences at the 0.05 probability level, according to the LSD test.



Fig 5: Left: Rooted shoot after 10 days of culture on MS0 medium. In the middle: Plantlets cultivated for 10 days in sterile potting soil. Right: Okra plant cultivated for 30 days in a greenhouse.

significantly increased shoot proliferation. It has been found that the MS medium with the addition of 6-Benzylaminopurine promotes the proliferation of shoots. Our results corroborate several studies, such as those by Almeida *et al*. (1995) and Adel *et al*. (2011), which reported that BAP at 2.0 mgL^{-1} significantly affected shoot proliferation, length and weight in *Ananas comosus* L.

Rooting and acclimatization of plantlets

Okra shoots were rooted in MS0 culture medium in glass test tubes containing 10 ml of medium. Within one week, the elongated shoots formed 2 to 3 nodes developed roots (Fig 5). The resulting plantlets were lthen ltransferred lto lsmall lpots containing sterile compost and covered with transparent lplastic bags to maintain humidity and facilitate acclimatization. After 10 days (Fig 5), lthe lacclimatized lplants were transferred to llarger pots lin a controlled greenhouse at a temperature of 30°C±3. After 30 days of greenhouse cultivation, the plants showed robust development with lmature leaves land lvisible lflower bud initiation (Fig 5). The survival rate lof the lplantlets llwas 100%. These results are lsimilar lto llthe work of Belkhodja *et al*. (2023).

CONCLUSION

The composition of media in phytohormones plays a crucial role in the differentiation of plant tissues under *in vitro* conditions. The highest rates of shoot and apex proliferation were obtained on MS medium supplemented with 2.0 mgL^{-1} of BAP, which produced the maximum number of shoots per explant (3.07), promoted shoot elongation and enhanced overall productivity compared to other tested concentrations. Although higher BAP concentrations (3.0 - 4.0 mgL^{-1}) induced shoot proliferation, the increase was not statistically significant compared to 2.0 mgL^{-1} and callus development was reduced. Conversely, decreasing the concentration of BAP in the culture medium promoted root formation in regenerated shoots, with MS0 medium yielding the longest shoots and facilitating rooting. It is also noteworthy that successive subcultures on the same medium helped to reduce phenolic compound accumulation and limited the browning of apex and shoot explants, thus improving culture health and sustainability. The rooting of proliferated shoots on hormone-free MS medium resulted in a 100% survival rate during acclimatization, demonstrating the robustness and applicability of the developed micropropagation protocol. Overall, this study clearly demonstrates that shoot number, shoot length and productivity are significantly affected by benzylaminopurine concentration, with 2.0 mg L^{-1} identified as the optimal level for *in vitro* shoot proliferation of okra (*Abelmoschus esculentus* L.).

Further optimization of the micropropagation protocol could include, testing different combinations of cytokinins and auxins to enhance both shoot proliferation and rooting efficiency. Assessing the genetic stability of regenerated plants using molecular markers to ensure clonal fidelity for commercial propagation. Evaluating the performance of acclimatized plantlets under diverse agro-climatic conditions in Algeria to confirm field adaptability and practical application. Extending the protocol to other okra genotypes and local cultivars, which could support the conservation, improvement and large-scale propagation of this economically and nutritionally important crop. Investigating the physiological and biochemical quality of *in vitro*-derived plants, such as pod nutrient content and flowering potential, to ensure the agricultural and nutritional value of propagated plants.

Conflict of interest

The authors declare no conflict of interest.

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