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In vitro Regeneration of Okra [*Abelmoschus esculentus* (L.) Moench] through Nodal and Shoot Apex Explants

Leila Belkhodja[1], Moulay Belkhodja[1], Samia Ghomari[1]

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ABSTRACT

Background: Okra is an economically important crop grown in tropical, subtropical and Mediterranean countries. It belongs to the Malvaceae family. This study aims to propagate this plant on a large scale by its *in vitro* regeneration and obtain healthy plants.

Methods: Okra is micropropagated from nodal and shoot apex explants excised from 21-day-old seedlings. Isolated explants were then cultured in Murashige and Skoog medium without plant growth regulators at a temperature of 27°C, a photoperiod of 16-h light/8-h dark and a light intensity of 3000 lux. Leaves formation occurred after one week of culture, followed after 10 days by rooting formation.

Result: The plantlets formed, grew and developed and after one month of cultivation under the same conditions, a difference in the length of the shoot was recorded, measuring 10.12 cm in the plantlets obtained from nodal segments and 7.37 cm in the plantlets from shoot apices. There was also a minimal difference in rooting, which was well observed in plantlets regenerated from nodal explants. Both types of explants developed into complete plantlets in only one month. Then, they were transferred to the greenhouse for acclimatisation. Using this technique, the *in vitro* production of plants is relatively simple, fast and less expensive.

Key words: Apex, Explants, *In vitro*, Micropropagation, Nodal, Okra, Regeneration.

INTRODUCTION

Okra or Bhindi [*Abelmoschus esculentus* (L.) Moench], commonly called *Gnaouia* in Algeria, is the most popular vegetable of the Malvaceae family. It is appreciated for its nutritional value and richness in vitamins, calcium, potassium and other minerals (Zodape *et al.*, 2008; Sharma and Parsad, 2015) and its medicinal properties (Persad and Sarma, 2012; Amba *et al.*, 2019). This plant is widely consumed in eastern and southern Algeria; only its production is very low compared to other countries because it's confronted by abiotic constraints such as saline stress and it is frequently infected by several systemic diseases caused by fungi, viruses, bacteria, mycoplasma and nematodes (Kabir *et al.*, 2016).

To preserve and protect this species, we were interested in analysing the behaviour of Okra at the micropropagation stage. Our study aims to propagate this plant on a large scale by its *in vitro* regeneration and obtain healthy plants. Little studies have been carried out on *in vitro* multiplication of Okra. It has been reported that regeneration of this species uses different explants such as hypocotyl, cotyledonary nodes, leaves, cotyledons, apical shoot (Mangat and Roy, 1986; Roy and Mangat, 1989; Anisuzzaman *et al.*, 2008a; Dhande *et al.*, 2012; Manickavasagam *et al.*, 2015; Daniel *et al.*, 2018) and requires the incorporation of plant growth regulators in the medium for shoot induction and root formation (Mangat and Roy, 1986; Ganesan *et al.*, 2007; Anisuzzaman *et al.,* 2008b; Dhande *et al.*, 2012; Kabir *et al.*, 2016).

However, the use of phytohormones is expensive and the cost of the medium varies depending on the ingredients such as sucrose, agar and plant growth regulators used. Our study focuses on the regeneration of this species through nodal and shoots apex explants in the absence of growth regulators to produce selected plants intended for subculture and reduce the cost of *in vitro* plant production. Moreover, according to Juturu *et al.,* (2015), direct organogenesis is a good alternative way of plant regeneration which takes less time, low somaclonal variability and a high-efficiency index compared to indirect regeneration.

[1]Department of Biology, Faculty of Natural and Life Sciences, University Oran 1, Ahmed Ben Bella, Oran, Algeria.

Corresponding Author: Leila Belkhodja, Department of Biology, Faculty of Natural and Life Sciences, University Oran1, Ahmed Ben Bella, Oran, Algeria. Email: belkhodjaleilaa@gmail.com

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MATERIALS AND METHODS

The experiment was conducted in 2019 at the Laboratory of Plant Physiology in Oran1 Ahmed Ben Bella University of Algeria.

Plant material

The plant material used in this experiment was obtained from a farmer in the *Guelma* region (eastern Algeria).

Surface sterilisation, seed germination and culture condition

Okra seeds were sterilised in a laminar airflow hood with 80% ethanol for 1 min and then treated in 7% sodium hypochlorite for 20 min; then rinsed 3 times with sterile distilled water. Germination of the seeds was done under dark conditions at a temperature of 27°C in Murashige and Skoog Medium, (1962) with 30 g/l sucrose (Woldeyes *et al*., 2021) and solidified with 8 g/l of agar in glass bottles containing 50 ml of nutrient media. The pH was adjusted to 5.8 before autoclaving at 120°C for 20 min.

Plantlet regeneration and acclimatisation

After 2 to 3 days, the germinated seeds were transferred to a growth chamber maintained at 27°C with 16-h photoperiod and a light intensity of 3000 lux. After 21 days of culture, nodal and shoot apex segments of 1.5 to 2 cm were isolated from seedlings under sterile conditions in a laminar airflow hood and cultured at a rate of one explant per bottle containing 50 ml of Murashige and Skoog Medium, (1962) (Fig 1a; Fig 2j) without the addition of growth regulators and under the same culture conditions for 30 days.

After one month of cultivation (Fig 1g; Fig 2p), the number of nodes, leaves and shoots were counted and each plantlet's length was measured. Each measurement was made on 40 replicates of each explant. The obtained plantlets are carefully removed from the MS medium and the roots were washed with sterile distilled water then transferred to small pots containing sterile soil (Fig 1h; Fig 2q). During this phase, *in vivo* adaptation of these vitroplants was necessary by covering them with a plastic bag to maintain humidity. After 10 days of cultivation, the bag was removed and the plantlets were transplanted into big pots filled with sterile soil and transferred to a controlled greenhouse for two months until fruits and seeds formed (Fig 4).

Statistical analysis

The data were analysed statistically with STATISTICA 10. The analysis of variance for different parameters was performed. The difference among the means values was compared by least significant difference for all statistical analyses, except the leaves number was analysed by the Man-Whitney test because it doesn't have a normal distribution (p-value≤0.05).

RESULTS AND DISCUSSION

Regeneration and root formation

The results show that both nodal and shoot apex explants can be grown in MS0 medium to produce a whole plant but with a different growth rate between the two explants. For both themes, swelling is observed after 4 days, followed after one week of cultivation by the initiation of shoot formation and the appearance of leaves (Fig 1b; Fig 2k). After one month of cultivation, measurements on the vitroplants reveal that those obtained from the nodal explants reach an average length of 10.12±1.83 cm, with a number of nodes of 4.06±1.74 and a number of leaves of 4.80±1.51 and a better rooting. In contrast, in the plantlets obtained from the shoot apex explants, the average length reaches a value of 7.36±3.42 cm with a number of nodes of 2.62±1.58 and 4.37±1.51 leaves, *i.e.,* a minimal difference in rooting, which is less important than in the plantlets obtained from nodes; this difference is probably due to the effect of a high endogenous auxin level in Okra, so the addition of auxin to the media for rooting induction remains optional.

The ANOVA (Table 1) revealed a highly significant effect of the explant on shoot length and the number of leaves ($p<0.001$), the least significant difference test of these two parameters indicate that this difference is highly significant (Table 2). The normality test showed that data are not normally distributed; therefore, the non-parametric Mann-Whitney test is used. The results showed that there was no significant difference between the number of leaves in the plantlets obtained from nodal explants and those obtained from apex explants ($p>0.05$) (Table 2).

The average was 1.34±0.47 shoot per nodal explant and 1±0 shoot per apex explants for shoots number. For this trait, the ANOVA test revealed that the effect of the

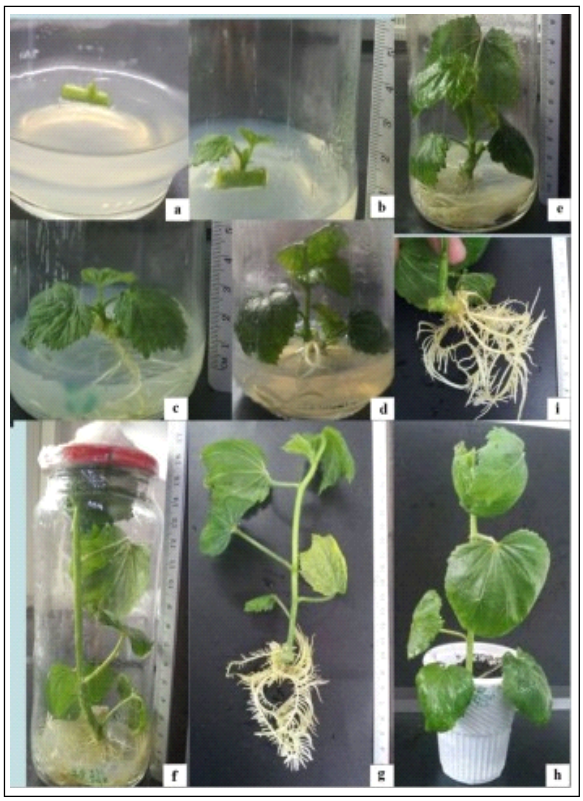


Fig 1: *In vitro* regeneration of Okra through nodal explant; a: Nodal explant, b: Shoot initiation in MS0 from nodal explant (7 days old), c: Rooting shoot after 10 days of culture, d: 15 days old vitroplants, e: 21 days old vitroplants, f: Elongated vitro plant after 30 days, g: Regenerated plantlet of 1 month, h: Acclimatisation of plantlet in soil, i: Rooting after 30 days old.

explant on the number of shoots is significant, but no significant difference between the two explants is expressed.

Acclimatisation

Acclimatisation of the plants under the experimental conditions already mentioned resulted in a 100% survival rate (Fig 3). Indeed, the seedlings obtained from the two explants produced a crop with a length of about 9 cm and a size of about 3.5 cm (Fig 4a), which corresponds to the characteristics of a crop product for consumption. After 90 days of cultivation in the greenhouse, the pods produce fertile seeds able to reproduce (Fig 4b).

The methodology developed in this study allowed rapid and simple *in vitro* regeneration of Okra from shoot apex and nodal explants isolated from 21-old-day seedlings. Acogo *et al.,* (1996) obtained a very high number of identical and healthy plants in a short time by subculture using this method. Much work on *in vitro* propagation of Okra has involved plant growth regulators. Dahande *et al.,* (2012) were able to regenerate new Okra plantlets from apical shoot culture in media containing different phytohormones; compared to this study. Our results indicate that the shoot length obtained from both explants remains higher than those obtained by Dahande *et al.,* (2012) in the presence of plant growth regulators.

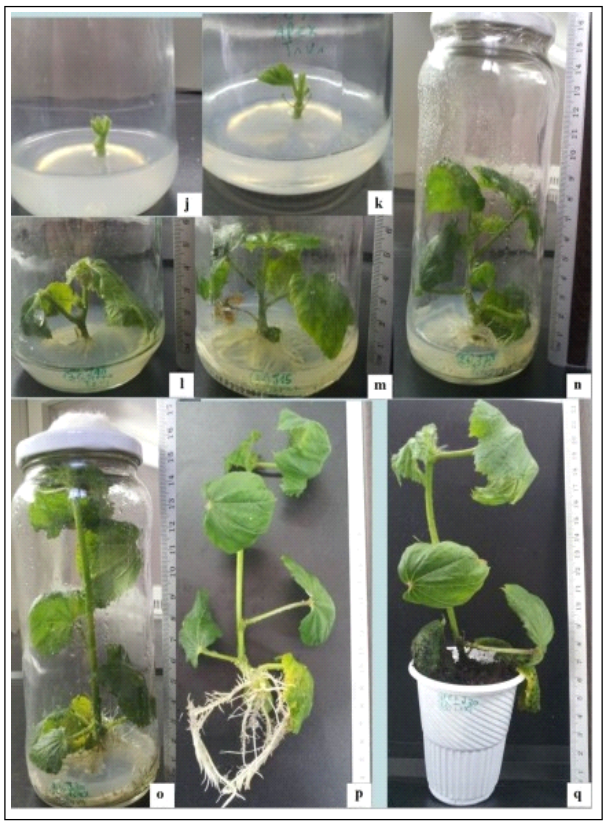


Fig 2: *In vitro* regeneration of Okra through apex explant; j: Apex explant, k: Shoot initiation in MS0 from apex explant (7 days old), l: Rooting plantlet after 10 days of culture, m: 15 days old vitroplants, n: 21 days old vitroplants, o: Elongated vitroplant after 30 days, p: Regenerated plantlet of 1 month, q: Acclimatisation of plantlet in soil.

Table 1: F-values obtained from ANOVA showing the effect of explant types (Node/Apex) cultured *in vitro* on the length and the number of nodes and shoots per explant after 30 days of culture.

Source of variation	Shoot length	Number of shoots	Number of nodes
Effect of explant types	21.37***	16.62***	13.88***

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

Table 2: Average shoot length, number of shoots, number of nodes and number of leaves per explant recorded from nodal and shoot apices of Okra cultured on MS0.

Mean values of the studied parameters	Explanatory notes	
	Nodes	Shoot apex
Mean length (cm) of shoots/explant	10.12^{a}±1.83	7.36^{b}±3.42
Mean number of shoots	1.34^{a}±0.48	1.00^{a}±0.00
Average number of nodes/explant	4.06^{a}±1.74	2.62^{b}±1.58
Average number of leaves/explant	4.80^{a}±1.51	4.37^{a}±1.51

Values are means±standard error (SE) from forty replicated experiments. Those followed by a different letter within a column are significantly different at the 0.05 level according to an LSD test and Mann-Whitney for leaves number.

Acogo *et al.,* (1996) indicated that Okra's tissue culture in MS0 is still possible and mother plantlets of *Abelmoschus cannabinus* could give about four thousand vegetative copies after one year of culture. However, contradictory results were obtained by Kabir *et al.,* (2016); Rizwan *et al.,* (2018) and Irshad *et al.,* (2018), who reported that the culture of cotyledonary nodes in MS0 gave no response with 0% of shoot proliferation. Anisuzzaman *et al.,* (2008b) revealed that the micropropagation of Okra through meristems culture in MS0 medium did not develop, although explants remained alive. Furthermore, Rizwan *et al.,* (2018) showed that shoot induction is not observed in a medium without plant growth regulators. This difference in results may be due to several factors such as genotype and cultural conditions (Kabir *et al*., 2016; Anbukkarasi and Sadasakthi, 2017). The regeneration frequency of Okra to a large extent depends on its genetic structure and recalcitricity (Rizwan *et al*., 2018), explant type, growth regulators, culture conditions and secretions of phenolic compounds that cause browning of explants and affect the *in vitro* regeneration of Okra (Narendran *et al*., 2013; Irshad *et al*., 2017; Irshad *et al*., 2018).

Our results show that the number of shoots obtained in MS0 medium is only one shoot per explant for both types of nodal and apices explants. These results are similar to the work of Acogo *et al.,* (1996) and Irshad *et al.,* (2018). According to the findings of other studies, shoot proliferation

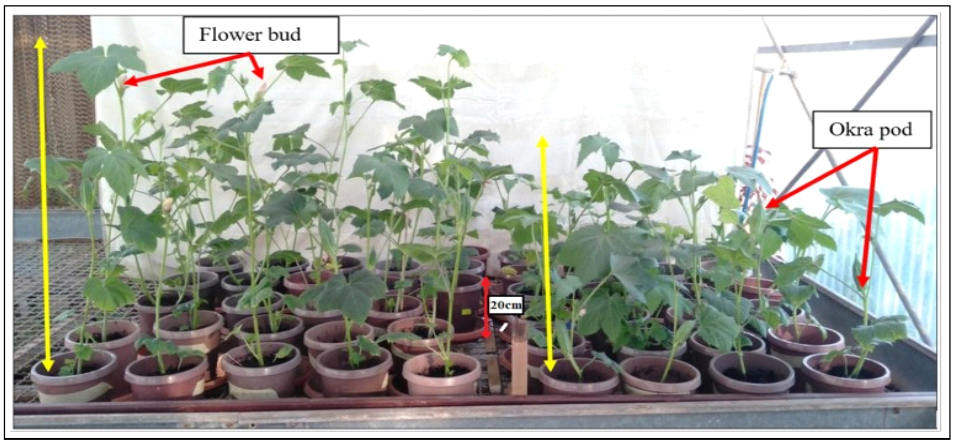


Fig 3: Three-month-old acclimatised plants; left from nodal micropropagation, right from shoot apex micropropagation (the yellow arrow represents the length scale).

appears only under hormonal treatments (Mellela *et al.,* 2009; Kabir *et al.,* 2016; Rizwan *et al.,* 2018; Irshad *et al.,* 2018). Irshad *et al.,* (2018) obtained a browning on the cotyledonary nodes explants of Okra *in vitro* cultured due to phenolic secretion leading to explants death. Our results show that this phenomenon did not occur during the development of our explants in the absence of plant growth regulators. According to Koné *et al.,* (2010), the rate of necrosis due to phenolic oxidation products in small explants is high; these compounds rapidly invade the small explant, which suffocates and necroses. On the other hand, large explants will not have all their cells invaded and may nevertheless develop mechanisms that allow them to resist the presence of these undesirable products.

Rooting is the most important step for plant regeneration. Explants of nodal origin seem to have a better rooting than those of shoot apex origin, probably because of the migration of endogenous auxins such as IAA from the shoot apical meristem to the other parts of the plant. In addition, it was observed a flower bud formation on the plants characterising this species that has a determined growth. Our results show that Okra root formation is early in both explants; this was also observed by Acogo *et al.,* (1996) in the same species during the first two weeks when cultured on MS0. Anissuzzaman *et al.,* (2008b) reported that rooting starts after 2 weeks under MS medium supplemented with 1 mg/l IBA. The authors also reveal that shoots exposed to higher NAA or IBA concentrations (2 mg/l or more) are affected by necrosis.

Our results show that the time for rooting all explants varies between 10 to 15 days. The study by Agrawal *et al.,* (1997) on cotton shows that the initiation of root formation took place after 12-15 days on MS ½ medium without phytohormones. On the other hand, Kabir *et al.,* (2016) obtained rooting on Okra up to 9.7 days under MS medium supplemented with 2.46 µM IBA while in MS½ medium without any growth regulator rooting extended up to 16.7 days. Mellela *et al.,* (2009) report that root induction in rooting medium containing 1mg/L NAA evolves between 18 and 24 days.



Fig 4: Okra pod, (a) before seed formation; (b) after seed germination.

CONCLUSION

This study reported that *in vitro* regeneration of Okra without the addition of plants growth regulators is still very efficient from nodal and apices and can be used in direct micropropagation of this species for rapid and healthy multiplication. Also, shoot regeneration and elongation are achievable from nodal and shoot apex explants in Murashige and Skoog medium without plant growth regulators. The nodal segment responds better to the plantlet's length than shoot apices. Rooting formation is induced in the MS medium. So it can be concluded that the addition of plant growth regulators to the media does not seem to be a mandatory factor in this propagation phase. This regeneration system from nodal and shoot apices can be used for *in vitro* selection of this species.

Conflict of interest: None.

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