International Journal of Innovation and Scientific Research
ISSN 2351-8014 Vol. 57 No. 2 Nov. 2021, pp. 223-228

http://www.ijisr.issr-journals.org/

Optimization trial of rooting conditions of *Moringa oleifera* Lam. *in vitro* culture

Justine Tshidibi Tshimbila[1-2], Hippolyte Nshimba Wa Malela[3], and Benoit Dhed'a Djailo[4]

[1]Visitor at Laboratory of Applied in Vitro Plant Biotechnology, Ghent Universit, Belgium

[2]PhD student and Researcher at Laboratory in vitro culture, Faculty of Sciences, University of Kisangani, RD Congo

[3]Professor, Laboratory of Botanic, Department of Botanic, Faculty of Sciences, University of Kisangani, RD Congo

[4]Professor and Director of Laboratory in vitro culture Plant, Department of Biotechnology, Faculty of Sciences, University of Kisangani, RD Congo

ABSTRACT: To optimize the in vitro rooting conditions of *M. oleifera*, a study was carried out to determine the effects of exogenous auxin types on the rhizogenesis of this species. The plant material consisted of the vitro plants obtained after shoot propagation. Nodal sections with induced axillary shoots were placed for two weeks in a root induction medium consisting of MS mod 3B basal medium without auxin (control) and with three different auxins including IAA, IBA and NAA at 0.1µM. The results obtained showed that shoots were better rooted in the absence of the exogenous auxins, yielding 4.76±0.51 roots per shoot with an average length of 9.21±0.93 cm. The least rooting was obtained with NAA, which produced 2.16±0.47 roots per shoot and 3.57±0.82 cm length. NAA induced the largest calli (0.22±0.02 g) in contrast to the control (0.087±0.01 g). Thus, the auxin-free MS mod 3B basal medium would be ideal for rooting *M. oleifera* shoots *in vitro*.

KEYWORDS: Exogenous auxins, rhizogenesis, *Moringa oleifera*, micropropagation, explants.

1 INTRODUCTION

The species *Moringa oleifera* Lam is one of the most useful and versatile plant species on earth. Due to its multiple uses, this species, a fast-growing tropical tree, has evolved in a decade from a marginal, if not unknown, plant to a new food and economic resource [1]. Besides, this miracle tree has received unprecedented attention, capable of providing healthy nutrition and food supplements [2]. It has a very rich profile in nutrients (vitamins, minerals and proteins) and exceptionally diverse metabolites, which is rare to be found in other plants [3]. Apart from its dietary virtues, this species is also known for its agronomic and medicinal virtues, and can be used for water purification [2]. According to Alhakmani and collaborators, *M. oleifera* is used to treat various diseases including diabetes, hypertension, skin infections, etc. It can also help rebuild brittle bones, produce abundant breast milk, combat anemia and malnutrition [4]. According to Foidl and colleagues and Gupta and collaborators, this species is also used as animal fodder, to produce high quality oil and for soil protection ([2], [5]). It should be noted that each organ of *M. oleifera* has enormous potential and beneficial properties for mankind. Processed or unprocessed, its leaves are not only a new agricultural production with high income and employment potential, but also a food with high nutritional value [1]. Thus, it constitutes an exceptional resource especially for developing countries that face multiple problems related to poverty, poor nutrition and health, especially during this period of global health crisis due to the Covid-19 pandemic.

However, its large-scale production requires the control of optimal conditions for its micropropagation. According to some authors, notably Demol and colleagues, the technique of micropropagation of plant species remains the best tool for obtaining healthy plants in mass and in a short time [6]. Although several studies including Förster and colleagues, Avila-Treviño and collaborators, Hassanein and colleagues, and Gupta and colleagues on the micropropagation of *M. oleifera*; so far the proposed

protocol does not provide optimal rooting conditions for this species. Among the difficulties observed, excessive callus production significantly inhibiting the root development process remains the main cause ([7], [8], [9], [10]). Some authors claim that rooting difficulties of young shoots remain a real obstacle limiting the success of the micropropagation technique. Besides, the rhizogenic properties of auxins and their interest in plant propagation were demonstrated a long time ago. Auxin plays a crucial role in the process of induction of root neoformation. This has justified the need to use exogenous auxin to induce rooting of cuttings in several species [11]. Thus, several literatures have reported on the effectiveness of exogenous auxins in rooting cuttings in several species. According to Mazinga and collaborators, each plant species may have its own requirements regarding the type and concentration of exogenous phytohormones [12].

It is in this context that the present study is aimed at determining the effects of auxin types on the rooting of *M. oleifera* to optimize its micropropagation.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

M. oleifera vitroplants obtained after shoot propagation were used as plant material.

2.2 GROWING MEDIUM AND CONDITIONS

For rooting of these vitroplants, nodal sections with induced axillary shoots were subjected to auxin treatments. These nodal explants were placed in a root induction medium consisting of MS mod 3B basal medium and three different auxins, namely, Naphthalene Acetic Acid (NAA), Indole Butyric Acid (IBA) and Indole Acetic Acid (IAA) at 0.1 µM. The MS mod 3B basal medium without auxin was considered as a control for this experiment. After two weeks of cultivation, root count, root length and callus weight were collected.

2.3 ANALYSIS OF THE DATA

The data obtained were processed using Graphpad Prism software (version 5.03). After verification of the homogeneity of the data variances by Bartlett's test, the analysis of variance (ANOVA) test was applied to compare the effects of different auxins used on the number and length of roots; in contrast, the Kruskal-Wallis test was used to compare the influence of auxins on callus production. Tukey's post-hoc test for ANOVA and Dunn's test for Kruskal-Wallis was subsequently used to compare the control and treatments in pairs. Differences were considered significant at the 5% threshold.

3 RESULTS

The results of the present study showed the non-influence of exogenous auxins on some parameters of *M. oleifera* rhizogenesis *in vitro* (figures 4 and 5). As regards the number of roots per shoot, it was high at the control level compared to treatments with auxins. It should be noted that among these auxins, IAA showed a slightly higher root count compared to the other two (IBA and NAA). The statistical test of Analysis of Variance (ANOVA) showed that there was a highly significant difference between the control and the treatments ($F=5.27$, p-value=0.002<0,005). In a two-by-two comparison, Tukey's post-hoc test showed that there is no significant difference between the control and the auxin IAA, however, there is a significant difference between the control and the IBA and a highly significant difference between the control and the NAA (figure 1). In terms of root length per shoot, control shoots had long roots compared to those of the auxin treatments. The IAA always had slightly longer roots than the auxins IBA and NAA. A very highly significant difference was found between the length of the control roots and that of the treatments ($F=7.06$, p-value=0.0002<0,001). When taken in pairs, the length of control roots differed significantly from those of auxins IAA and IBA and very highly significant from that of NAA (figure 2). As for callus weight per shoot, the highest weight was obtained at the NAA treatment, while the lowest was recorded at the control level. The Kruskal-Wallis non-parametric statistical test showed a very highly significant difference between the treatments and the control ($K=22.60$, p-value<0.001). Dunn's post-hoc test comparing them two by two showed that IAA differs significantly from control, IBA differs highly significantly from control and NAA differs highly significantly from control (figure 3). Note that the bars on the histograms are the standard deviations.

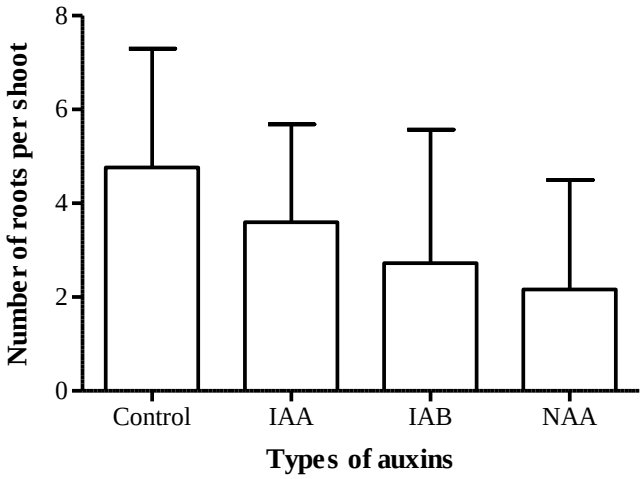

Fig. 1. Effect of auxin types on the number of roots per shoot in vitro of M. oleifera

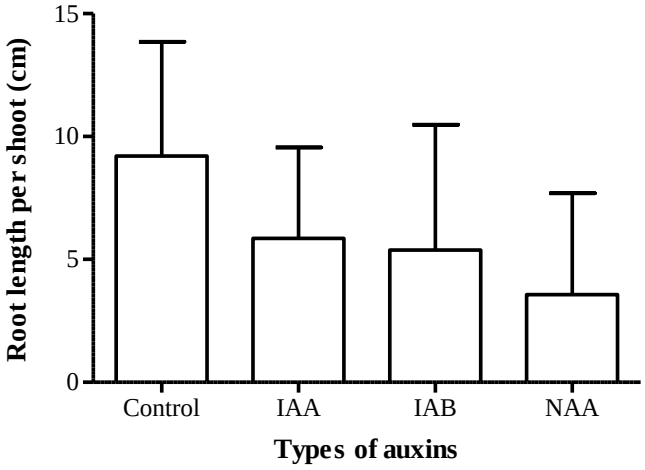

Fig. 2. Influence of auxin types on M. oleifera root length in vitro

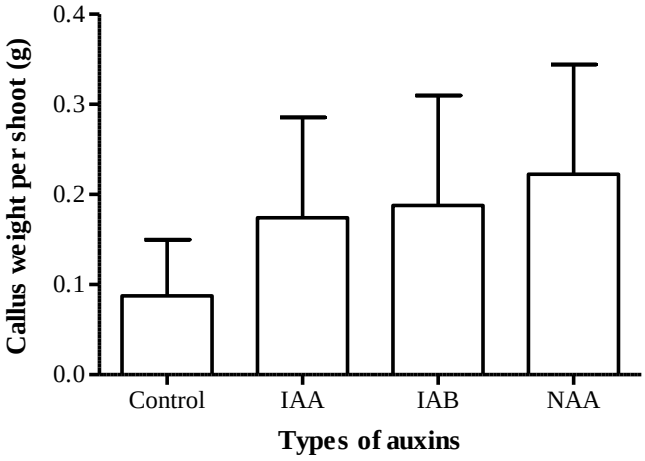

Fig. 3. Effect of auxin types on callus weight by in vitro growth of M. oleifera

Fig. 4. In vitro rooting of M. oleifera without auxin treatment (control)

Fig. 5. In vitro rooting of M. oleifera with different auxins: treatment IAA (left), treatment IAB (medium) and treatment NAA (right)

4 DISCUSSION

Within the framework of optimization of in vitro rooting conditions of *M. oleifera*, an experiment was carried out to determine the effects of exogenous auxin types on rhizogenesis of this species. The results obtained showed that the shoots were better rooted in the absence of the exogenous auxins tested. According to Gaspar et *al*. in Qaddoury and Amssa, in some plant species, cuttings root easily without auxin treatment; however, in others, a suitable supply of exogenous auxin is necessary for successful rooting [11].

Regarding the number of roots obtained, our results diverge with those obtained by other researchers on *M. oleifera* such as Stephenson and Fahey, Saini et *al*., Alkhateeb et *al*., Jemal ([13], [14], [15], [16]). For Stephenson and Fahey and Jemal, the highest average number of roots per shoot was recorded at NAA (4.7 and 7.96 roots respectively) ([13], [16]). In addition, Jemal obtained fewer roots at AIB (2.94 ± 0.44 roots per shoot). According to him, the number of roots produced per shoot increased with the increase of NAA concentration in MS/2 basal medium [16]. Adugna et *al*. also observed that NAA exhibited more roots compared to other auxins in *Moringa stenopetala* species [17]. This discrepancy could be explained by the nature of the basal medium used on the one hand as well as the auxin concentration on the other. As for our experiment, the modified MS medium with half concentration of nitrogen was used with an auxin concentration (AIA, AIB and NAA) of 0.1µM in contrast to Stephenson and Fahey, Jemal and Adugna et *al*. who used MS/2 medium with an auxin concentration of 0.5, 0.25 and 1 mg/l respectively ([13], [16], [17]). According to Herrbach, the action of auxin depends on both its concentration and the tissue on which it acts. Depending on the plant, the same concentration on the same organ can lead to different consequences. This implies a very fine regulation upstream and downstream of auxin. The action of auxin is dependent on its presence and concentration in the cell, its transport, its perception, and the regulation of target genes [18].

With respect to root length, our results are in agreement with those obtained by Jemal, Riyathong et *al* Islam et *al*. ([16], [19], [20]). They observed that *M. Oleifera* shoots not treated with auxin exhibited long roots compared to those treated with

auxin. Jemal recorded an average root length of 2.44 ± 0.04 cm at the control [16]. In 2005, Islam et *al*. estimated that the untreated culture medium with phytohormones would be better for rooting of *M. oleifera* [19]. For other species such as *Ceratonia silique* (Carob tree), it was also found that the best result on rooting of this species was obtained when the shoots were not treated with auxin [21].

Regarding callus weight, Saidi et *al*. claimed that the increase in phytohormone concentration is responsible for callus production and vitrification, thus inhibiting the shoot rooting process [21]. According to Sané et *al*., auxins do not have the same action on the morphology of the root system [22]. For these authors, the application of growth regulators was essential for the root induction of Acacia tortilis microcuttings, but the actual root development only started after the transfer of the explants to the hormone-free medium [22]. Chalak et *al*. believe that the rooting ability of some species depends on the variety. Therefore, they demonstrated that short duration auxin shocks were sufficient to induce the development of the root system of *Prunus dulcis* (Almond, Lebanese varieties) in contrast to the use of auxins in the culture medium which inhibited its rooting [23].

5 CONCLUSION

In conclusion, the species *M. oleifera* Lam. (miracle tree) is renowned for its multiple dietetic, medicinal and agronomic virtues. In the space of a decade, it has gone from being a marginal or even unknown plant to becoming a new food and economic resource. So far, the existing protocol does not provide optimal conditions for the rooting of this species. This study aimed to determine the influence of auxin types on the rooting *of M. oleifera* to optimize its micropropagation. The vitro plants of this species after shoot propagation were used as plant material. The nodal explants of the Vitro plants were placed for two weeks in a root induction medium consisting of MS mod 3B auxin-free basal medium (control) and three different auxins including Naphthalene Acetic Acid (NAA), Indole Butyric Acid (IBA) and Indole Acetic Acid (IAA) at 0.1 µM. Statistical tests of ANOVA and Kruskal-Wallis were applied to the data to determine whether or not there was a significant difference between the control and the three treatments for each parameter measured at the 5% significance level. In contrast, Tukey's post-hoc statistical tests for ANOVA and Dunn's for Kruskal-Wallis were used to compare the data in pairs. At the end of this experiment, the results showed the non-improvement of measured rhizogenesis parameters (number of roots, root length and callus weight) by the use of exogenous auxins in vitro culture of *M. oleifera.* Among the auxins tested, the IAA showed a slightly higher root count and root length per shoot, and a reduced callus weight compared to IBA and NAA respectively. The ANOVA test showed that there was a highly significant difference in the root number between the control and treatments (F=5.27, p-value=0.002<0,005). In a two-by-two comparison, Tukey's post-hoc test showed that there is no significant difference between the control and auxin IAA, however, there is a significant difference between the control and IBA, and a highly significant difference between the control and NAA. Besides, there was a highly significant difference between the control and treatment root lengths (F=7.06, p-value=0.0002<0,001). When taken in pairs, the length of the control roots differed significantly from those of auxins IAA and IBA, and very highly significant from that of NAA. In addition, the Kruskal-Wallis non-parametric statistical test showed that there is a very highly significant difference in callus weight between the treatments and the control (K=22.60, p-value<0.001). Dunn's post-hoc test comparing them in pairs showed that the control differed significantly, highly significant and very highly significant from IAA, IBA and NAA respectively.

In short, the use of the auxin-free MS mod 3B basal medium would be ideal for the optimization of *in vitro* rooting conditions of *M. oleifera*.

ACKNOWLEDGMENTS

Sincere thanks to the VLIR-UOS project of the European Union for funding this research and to Professor Stefaan Werbrouck for hosting me in the Laboratory of Applied *in vitro* Plant Biotechnology of the University of Ghent to carry out this experiment.

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