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Evaluation of Optimum Concentration of Naphthalene Acetic Acid on *in Vitro* Rooting and Acclimatization of Tissue Culture Date Palm (*Phoenix dactylifera* L.) Plantlets

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ABSRACT: A major limitation in large scale application of micropropagation technology is high mortality experienced by *in vitro* raised plants during laboratory to land transfer. This study was aimed at investigating the best concentration of Naphthalene acetic acid (NAA) on *in vitro* rooting and also develop protocol for successful plant acclimatization with high survival rate. Date palm plantlets were rooted on Murashige and Skoog (MS) basal medium with different NAA concentrations (0.0, 0.1, 0.5 1.0, 1.5 and 2 mg/l) without activated charcoal. Result showed that all the NAA concentrations supported root formation. The optimal initiation and growth of roots of the plantlets were observed at NAA concentration of 1.0 mg/l (87 %) and least with Basal MS without NAA (47 %). Results also showed that root number and lengths increased with increasing NAA concentrations up to 1.0 mg/l and decreased thereafter. The culture mixture of peat moss + soil (2:1) gave the highest survival percentage of 80 % for plant acclimatization. This investigation had shown that accurate assessments of responses to medium at various stages should be considered as specific requirement for successful plant acclimatization.

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Micropropagation has been extensively used for rapid multiplication of many plant species. Its widespread use is restricted by the often high percentage of plant lost or damaged when transferred to *ex vitro* conditions (greenhouse or field). Micropropagation of many plants is achieved through the establishment of explants, their initial growth *in vitro* and followed by transplanting into the greenhouse or field. During *in vitro* culture, plantlets grow under very special conditions in relatively air-tight cultivation vessels (Pospisilova *et al.,* 1999). A major limitation in large scale application of micropropagation technology is high mortality experienced by *in vitro* raised plants during laboratory to land transfer. The plants on being transferred to *ex vitro* are exposed to (altered temperature, light intensity and water stress) conditions that need acclimatization for successful establishment and survival of plantlets (Kumar and Rao, 2012). Date palm is cultivated for its high productivity and high nutrient value of its fruit, for preservation of ecosystems threatened by desertification and creating appropriate microclimate for agriculture under arid conditions. Its cultivation generates considerable opportunities for rural employment, provides a major source of income for farmers and ensures livelihood and food security of the rural areas (Mazri and Meziani, 2015). As a monocot plant, the date palm trees produce fasciculate and mostly fibrous roots. Primary roots develop from seeds and secondary roots develop from primary root. Differentiation of root cortex is a key factor in the transport of compounds in and outside of the root, subsequently to other parts of the body. Also, the development of root system and vascular elements may significantly affect *ex vitro* acclimatization of tissue culture-derived date palm plantlets (Abul-Soad and Jatoi, 2014). Rooting is an important *in vitro* stage in the micropropagation of date palm. The transplanting of *in vitro* date palm plantlets depends mainly on *in vitro* good roots and trunk thickness. The well-rooted plantlets may be moved into the greenhouse through an *in vitro* hardening step (Sidky *et al.,* 2017). Successful acclimatization of tissue culture plantlets is a critical step in determining the efficiency and cost effectiveness of any tissue culture protocol. *Ex-vitro* survival and subsequent enhanced growth would shorten the marketing cycle and accelerate the establishment of propagules under field

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conditions (Idris *et al.,* 2015). In order to produce a good root-shoot system, some factors such as explants source, genotype, plant nutrient and tissue growth greatly enhance the regulation of shoot formation. It has been reported that the success of many *in vitro* techniques in higher plants depend on the success of plant regeneration which is usually controlled by auxin and cytokine concentration (Sidky *et al.,* 2017). The aim of this study is to investigate the best concentration of NAA (1-naphthalenacetic acid) on *in vitro* rooting and also develop protocol for successful plant acclimatization with high survival rate.

MATERIALS AND METHODS

Experimental location, Multiplication of Date palm shoots and maintenance of culture: This work was carried out at Nigerian Institute for Oil Palm Research (NIFOR), Tissue Culture Laboratory, Benin City, Nigeria in 2014-2015. In the study, the shoots were separated from proliferated clusters of shoots in the multiplication stage and recultured in ¾ Murashige and Skoog (MS) (1962) basal medium composed of vitamins, 0.1g/l glutamine, 50g/l sucrose, 3g/l agar and NAA at different concentrations (0.0mg/l, 0.1mg/l, 1.0mg/l and 2.0mg/l.). The pH of media was adjusted to 5.8, melted and dispensed into large tubes and then autoclaved at 121˚C for 15 min. Cultures were maintained at 25±2˚C and 80% relative humidity under low fluorescent light at 6000 lux with monthly subculture for three months. After which the number of leaves, leaf length, root length and number of roots were measured. The rooting experiment was repeated three times to justify the recorded results in Factorial Randomized Block Design.

Plant acclimatization: Healthy date palm plantlets with 3-4 leaves, 9-16cm in length and 3-4 roots were transferred from culture vessels, rinsed under a running tap water to remove nutrient medium and left for 15 min in a 2.5g/l fungicide (benlate) solution. Plants in plastic pots containing the following substrate: Soil and Peat-moss in ratio of 1:1, Peat-moss and Soil (1:2), and Peat-moss and Soil in ratio of 2:1. The relative humidity was maintained at 80% and temperature at 26˚C-27˚C by covering the plants with polyethylene bags for 6 weeks within the green house. Watering with 10% MS was done once a week with gradual opening of the polyethylene bags at 5 to 6 weeks. Plantlets were watered with 1g/l N-P-K (15-15-15) after one month. The survival percentage was recorded after 3months of transplanting into the nursery.

Data collection and analysis: At the end of the experiments, the following data were recorded: shoot length, root number and root length. After acclimatization, the percentage of survival plants was calculated. Result was subjected to analysis of variance. Separation of means among treatments was determined using LSD test at 5% according to Steel *et al.* (1997).

RESULTS AND DISCUSSION

The results of the *in vitro* rooting are shown in Table 1. Plantlets rooted on MS basal used with different NAA concentration (0-2mg/l) formed roots at all the NAA concentrations (Plates 1a, b, c and d). Roots were also seen to be initiated in the medium without NAA. Root number and lengths increased with increasing NAA concentrations up to 1.0mg/l and decreased thereafter. The optimal initiation and growth of roots in the plantlets were observed at NAA concentration of 1.0mg/l (87%) and least with Basal MS without NAA (47%). The highest mean leaf number was obtained in medium with 1.0mg/l NAA concentration.

Table 1: Effect of different concentrations of NAA (0 – 2mg/l) on *in vitro* rooting of plantlets

NAA Conc.(mg/l)	Mean Root number	Mean Root length (cm)	Rooting Percentage	Mean leaf number
0.0	1.40 ± 1.72	1.92 ± 2.17	47.0	1.53 ± 1.59
0.1	2.80 ± 2.39	2.67 ± 2.02	67.0	2.27 ± 1.79
0.5	3.04 ± 2.91	3.20 ± 1.57	70.0	2.80 ± 1.62
1.0	4.60 ± 2.06	3.65 ± 1.55	87.0	3.00 ± 1.51
1.5	3.68 ± 3.21	2.40 ± 1.76	72.0	2.20 ± 1.86
2.0	3.45 ± 3.16	2.20 ± 1.92	60.0	2.07 ± 1.94

Mean ± Standard error (SE), n = 15

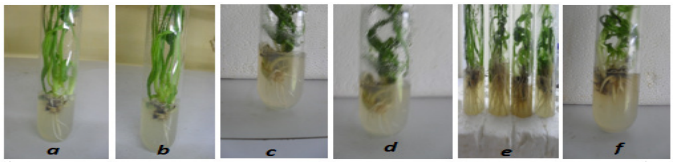

Plate 1: Plantlets rooting in different NAA concentrations with differences in their development a) 0.0mg/l NAA, b) 0. 1mg/l NAA, c) 0.5 mg/l NAA, d) 1.0mg/l NAA, e) 1.5 mg/l NAA, f) 2.0mg/l NAA

The plantlets from the rooting media supplemented with NAA (0-1mg/l) showing the best root formation and number of roots were transferred into *ex vitro* soil for acclimatization and their parameters recorded after 3 months (Plates 2a, b and Table 2). The plantlets looked different ($P<0.05$) depending on the medium they were cultured in. The efficiency of culture mixture was observed at the various survival percentages (Table 2). The culture mixture of peat moss (1) and soil (1) gave 20% and 60%, while peat moss + soil (1:1) and peat moss + soil (1.2) gave 40% and 60% respectively. The culture mixture of peat moss + soil (2:1) gave the highest survival percentage of 80% compared to the other culture mixture. From the result it was concluded that the use of soil and amount of peat moss mixture was the best for plants survival due to proper aeration (Plates 3a, and b).

Plate 2: Plantlets in different mixture of Peat Moss and soil under acclimatization

Table 2: Effect of culture mixture on plants survival during acclimatization

Culture Mixture	Survival (%)
Peat Moss (1)	20
Soil (1)	60
Peat Moss + Soil (1:1)	40
Peat Moss + Soil (1:2)	60
Peat Moss + Soil (2:1)	80

Plate 3: Date palms at different stages after successful plant acclimatization a) an acclimatized date palm in the nursery, b) an established hardened date palm in the field

Plantlets production with profuse rooting *in vitro* is important for successful establishment of regenerated plants in soil *ex vitro* (Idris *et al.,* 2015). Most plants derived from *in vitro* conditions require an acclimatization process to ensure the survival of sufficient number and vigorous growth of plantlets when transferred to soil under ambient conditions (Al-Khalifah *et al* 2010). The success of plant acclimatization is hidden in the nature of the substrate which is high in organic matter, must possess optimum water holding capacity and proper aeration. In this study, media with different NAA concentrations were tested to know the best quality roots for acclimatization of tissue cultured raised date palm plantlets. Root formation during the elongation phase depended on plant growth regulators in culture medium. The results (Table 1) showed that media supplemented with 1.0mg/l NAA (Plate 1d) showed the highest number of roots per explants (4.6±2.06), indicating the effect of NAA concentrations on root formation and confirming the findings of Bekheet *et al* (2013) who suggested 1mg/l NAA induces better and optimum rooting at the same concentration as IAA and IBA. Saifullah and Tabassum (2012) in their findings mentioned that among the auxins, NAA is the most frequently exploited exogenous hormone for roots development of *in vitro* date palm shoots. It was also observed from the result (Table 1) that root number and root lengths increased with increasing NAA concentrations and decreased thereafter. This result is also in agreement with El-Hammady (1999) and Bekheet and Saker (1998) who used NAA. They noticed that average root length decreased with the increasing auxin concentration and found that 1mg/l NAA had positive effect on root formation rather than 6-Benzylaminopurine (BAP) and IAA at the same concentrations. Tisserat (1984), Eke *et al*. (2005) and Eke *et al*. (2013) on the other hand, reported profuse rooting on generated shoots on media supplemented with 0.1mg/l NAA but however, only about 20% survived hardening through nursery until when transferred to the soil or field. The process between rooting and acclimatization is a very important step to complete propagation process. According to the report of Pospisilova *et al*. (1999) in their study acclimatization of micropropagation plants to *ex vitro* condition stated that acclimatization can be improved by hormonal stimulation of root development and also the concentration of sucrose and agar in the medium can affect subsequent acclimatization to *ex vitro* conditions. Acclimatization, hardening-off, or conditioning plantlets from the *in vitro* to the ambient environment can be a challenge that may result in death or damage to a large percentage of micropropagated plants according to the report of Darwesh (2015). The rooted plantlets from media having NAA (0-1mg/l) were tested for plant acclimatization. The highest survival of acclimatized plants was observed in the mixture of a ratio 2: 1 peat moss and soil which gave 80% (Table 2) compared to others. This value indicated that the mixture has great effect on the acclimatization process because plantlets grow in an optimum condition in media. Khierallah and Bader (2007) got a similar result using 2 peat moss and 1 perlite as best for acclimatized plants. This investigation had shown that accurate assessments of

responses to medium manipulation should be considered as specific requirement at various culture stages.

*Conclusion***:** The development of *in vitro* roots for successful plant acclimatization is an essential stage in every tissue culture raised plantlets. The protocol developed in this study is very safe for rooting, acclimatization and hardening of date palm plantlets for successful *ex-vitro* condition. The results on rooting obtained show that low hormonal concentration is best for rooting tissue culture date palm plantlets. Well acclimatized plants were produced and transferred to the field in NIFOR date palm experimental station in order to study their agronomic behaviour.

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