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IN VITRO REGENERATION OF *MORINGA OLEIFERA* LAM. FROM LEAF EXPLANTS

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ABSTRACT: *Moringa oleifera* Lam. is the most important tree because every part of the plant has nutritional and medicinal use. As a tree plant, it is difficult to improve this plant by traditional breeding. Biotechnological approaches are useful for its genetic improvement. Therefore, the objective of this study was to develop *in vitro* regeneration protocol for *Moringa oleifera* by using leaf explants. Young leaves from *in vitro* multiplied shoots were excised, wounded and cultured on MS medium supplemented with different concentrations of BAP in combination with NAA for callus induction. For shoot regeneration, the calli were cultured on MS medium supplemented with BAP (0.0, 0.5, 1.5, 2.0mg/l) in combination with NAA (0.0, 0.5, 1.5, 2.0, 2.5, 3.0 mg/l). Multiplication of regenerated shoots was done on MS medium supplemented with BAP (0.0, 0.5, 1.0, 1.5, 2.0 mg/l) in combination with IBA (0.0, 0.5, 1.5, 2.5, 3.5 mg/l). For rooting, shoots were cultured on half strength MS medium containing 0.0, 0.5, 1.5, 2.0, 2.5, mg/l NAA or 0.0, 0.5, 1.0, 2.0, 3.0 mg/l IBA. The highest percentage of callus induction (73.3%) and shoot regeneration from callus (33.3%) were obtained on the MS medium supplemented with 0.5 mg/l BAP and 0.5 mg/l NAA, respectively. The highest mean shoot (3.13±0.73) and root (9.60±0.86) number per explant were obtained on medium containing1.0 mg/l BAP and 0.5 mg/l IBA, respectively. After acclimatization, 90% plants survived in greenhouse. This protocol can be used for genetic improvement of this tree species through genetic transformation and somaclonal selection.

Key words/phrases: Callus induction, *In vitro* regeneration, Miracle tree, *Moringa oleifera*, Shoot multiplication

INTRODUCTION

Moringa oleifera Lam. is the only genus in the family Moringaceae. It is the most widely cultivated species of the genus *Moringa.* It is commonly known as drumstick tree or horse-radish tree in most part of the world (Sabale *et al.*, 2008). *M. oleifera* is native to sub-Himalayan Mountains of northern India. Now it is cultivated for a variety of purposes in the whole tropical and subtropical regions of the world (Leone *et al.*, 2015). Its cultivation and usage has gained momentum in South East Asia, West Asia, Arabian Peninsula, East and West Africa, West Indies and Southern Florida, Central and South America from Mexico to Peru including Brazil and Paraguay at altitudes ranging from sea level to 2000 m a.s.l. (Ganatra *et al.*, 2012). In Ethiopia, it grows in southern part of the country along with *Moringa stenoptela* at low altitudes in dry and moist agro-climatic zones (Aberra Melesse, 2011). It is considered one of the most useful trees, as almost every part of *Moringa* tree can be used for food or has some other uses (Paliwal *et al.*, 2011). It has a high economic potential in Ethiopia. In addition to the use of its leaves as vegetable for human, animal feed and water purification, the tree produces a number of other useful products that are used for medicinal purposes so that it generates income for the growers.

M. oleifera is a drought tolerant fast-growing evergreen or deciduous tree that mostly grows up to 10 to 12m in height. The leaves are bipinnate or more commonly tripinnate, up to 45 cm long and are alternate and spirally arranged on the twigs (Roloff *et al.*, 2009). It is a multi-purpose tree including nutritional and medicinal uses and therefore described as a 'miracle tree' (Ashfaq *et al.*, 2012). The leaves are the most commonly used part of the plant to treat different diseases including hyperglycemia, flu, asthma, heart burn, dyslipidemia, syphilis, malaria, diarrhea, pneumonia, headaches, scurvy, bronchitis, skin diseases, and eye and ear infections. The leaves also reduce blood pressure and cholesterol level, act as an anticancer, antioxidant, antimicrobial, anti-atherosclerotic, anti-diabetic agents, and

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neuroprotectant (Ali *et al.,* 2015). The alcoholic extract of the leaves was reported to have analgesic activity (Sutar *et al.,* 2008). The roots and stem were used to cure diseases such as asthma, digestive disorders, gastritis, inflammation and skin disorders due to the presence of different chemicals like an immune enhancing polysaccharide (Mondal *et al*., 2004).

The tree has maximum leaves at the end of the dry season when other foods are typically scarce. It is a promising food sources especially the leaves which are rich in nutrients and minerals (Fuglie, 1999). The absorption rate of carbon dioxide by the moringa tree is twenty times higher than that of general vegetation. In addition, it is also used for biogas production, food for animal, domestic cleaning agent, fertilizer, gum, manufacture of perfume and hair care products (Tsaknis *et al*, 1999). The preservation of the *Moringa* species is thus of great concern from biodiversity, ethnobotanical, dietary and pharmacological perspectives.

M. oleifera is not widely distributed in Ethiopia. Sexual propagation is tedious and not even possible without having enough individual plants for cross-pollination. Increasing its rooting efficiency through genetic improvement enables the plant to easily propagate asexually. Moreover, side effects due to consumption of moringa such as lower blood pressure and slow heart beat rate due to the alkaloids in the plant can be avoided through its genetic improvement. However, it is difficult to genetically improve through conventional breeding as it needs long generation time. Therefore, application of modern biotechnology is extremely important for genetic improvement of this plant. Establishment of an efficient in *vitro* regeneration protocol is an essential prerequisite for improving plants through different techniques such as *in vitro* screening, genetic engineering, genome editing, etc. Although there are some *in vitro* regeneration activities in other countries, different genotypes require different *in vitro* regeneration protocol (Islam *et al*., 2005; Riyathong *et al*., 2010; Saini *et al*., 2012). Therefore, the objective of the present study was to develop *in vitro* regeneration protocol of *M. oleifera* using leaf explants. This protocol could be used for genetic transformation of this plant in Ethiopia to improve the quality of the plant by removing toxic substances that create side effects during its consumption.

MATERIALS AND METHODS

Plant materials

Seeds of *Moringa oleifera* were collected from Karat, Konso, Southern Nations, Nationalities and People (SNNP), Ethiopia. The seed coats were removed and the de-coated seeds were stored at room temperature. Then, washed under running tap water for 5 min, surface sterilized with 70% ethanol for 9 min followed by sterilization with 10% calcium hypochlorite for 30 min and rinsed four times with sterile distilled water. The sterilized seeds were cultured on growth regulators free MS (Murashige and Skoog, 1962) medium containing 3% sucrose (w/v). The pH was adjusted to 5.8 before addition of 7.0 g/l agar and autoclaved at 121°C for 15 min. The medium (20 ml each) was then dispensed in 10 x 6 cm culture vessel. The cultures were maintained at 25 ± 2°C under 16 h photoperiod at light intensity of 22 µmol m^{-2} s^{-1} using cool white fluorescent.

The shoot tips of the seedlings from germinated seeds were excised and cultured on full strength MS medium containing 0.5 mg/l BAP and 3% sucrose for shoot initiation. The pH of the medium was adjusted to 5.8 and then 7 g/l agar was added and autoclaved. The explants were cultured in Magenta GA-7 culture vessels containing 50 ml medium. All cultures were maintained at 25±2°C under 16 h photoperiod at light intensity of 22 µmol $m^{-2}s^{-1}$ using cool white fluorescent light.

The initiated shoots were cultured on full strength MS medium containing 1.0 mg/l BAP in combination with 0.5 mg/l IBA in Magenta GA-7 culture vessels for shoot multiplication. The cultures were maintained under culture conditions as of that of shoot initiation.

Callus induction

Young leaves from *in vitro* multiplied shoots were excised and wounded perpendicular to the midrib. The wounded leaves were cultured upside down on Petri dish containing 25 ml MS medium supplemented with different concentrations of NAA (0.0, 2.0, 3.0, 4.0, 5.0, 6.0 mg/l) in combination with BAP (0.0, 0.5, 1.0, 2.0, 3.0 mg/l) and 3%sucrose. The pH was adjusted to 5.8 followed by addition of 7g/l agar and autoclaved at a temperature of 121°C for 15 min. Six explants were cultured in each Petri dish with 5 replications for each treatment. The cultures were maintained at 25

± 2°C under dark conditions until callus was induced. After four weeks, the number of leaves that induced callus was recorded.

Shoot regeneration

The calli induced on callus induction media were transferred to culture vessels containing 50 ml MS medium supplemented with different concentrations of NAA (0.0, 0.5, 1.5, 2.0, 2.5, 3.0 mg/l) in combination with BAP (0.0, 0.5, 1.5, 2.0 mg/l) and 30 g/l sucrose was added. The pH was adjusted to 5.8 before addition of 7g/l agar followed by autoclaving. The cultures were kept in dark condition at 25 ± 2°C. When shoots of about 0.5 cm appear from the callus, the cultures were transferred to light intensity of 22 µmol $m^{-2}s^{-1}$ at 16 h photoperiod with the same temperature as above. The number of calli in each culture vessel and replications that regenerated shoots per explant was recorded.

Shoot multiplication

Shoots regenerated from calli were excised aseptically and transferred to culture vessels containing 50 ml MS medium supplemented with different concentrations of BAP (0.0, 0.5, 1.0, 1.5, 2.0 mg/l) in combination with IBA (0.0, 0.5, 1.5, 2.5, 3.5 mg/l). Five explants were cultured in each culture vessel with 6 replications. The cultures were maintained under the same condition used for shoot initiation for four weeks. Number of shoots per explant was recorded after four weeks.

Rooting

The multiplied shoots were excised and cultured in culture vessel containing 50 ml half strength MS medium supplemented with different concentrations of NAA (0.0, 0.5 1.5, 2.0, 2.5 mg/l) or IBA (0.0, 0.5, 1.0, 2.0, 3.0 mg/l) and 15 g/l sucrose was added. Five explants were cultured in each jar with 6 replications. The cultures were maintained at the same culture conditions as of shoot initiation. Number of shoots that produced roots and number of roots per plantlet was recorded after four weeks.

Acclimatization

Well rooted plantlets were taken out of the medium gently and the roots were washed thoroughly using running tap water. Then the plantlets were planted in pots containing a sterile garden soil and covered with polyethylene bags. The pots were kept in the culture room for two weeks by watering every day. After two weeks, the plants were transferred to greenhouse. The polyethylene bags were gradually removed after two weeks. Finally, the plants were fully exposed to the normal growth conditions and the number of survived plants was recorded after a month.

Data analysis

A completely randomized design (CRD) was used for all experiments. Callus induction and regeneration percentage was calculated. For multiplication and rooting, data were subjected to one-way analysis of variance (ANOVA) to detect if there are significant differences among treatments. The means of treatments were compared by Tukey's test using statistical data analysis software SPSS 20.0 version at 5%probability level.

RESULTS

Callus induction

The results of the present study showed high percentage of callus induction was obtained on medium containing low concentration of BAP (0.5 mg/l) (73.3%) (Fig. 1A) followed by high percentage of NAA (6.0 mg/l) (70%) (Fig. 1B) and 5.0 mg/l NAA (66.6%). These three different concentrations of growth regulators provided better than the other concentrations and combinations of BAP and NAA for callus induction (Table 1).

Shoot regeneration

The highest percentage of shoot regeneration (33.3%) was obtained on MS medium supplemented with 0.5 mg/l NAA (Fig. 1C) followed by medium containing 0.5 mg/l BAP (30%) (Table 2). When combinations of NAA with BAP were considered, all treatments did not provide any shoot, except combinations of 2.0 mg/l NAA with 0.5 mg/l BAP.

Table 1. Effects of NAA and BAP on callus induction.

NAA (mg/l)	BAP (mg/l)	Callus induction (%)
0.0	0.0	23.3
2.0	0.0	40
2.0	0.5	10
2.0	1.0	0
2.0	2.0	0
2.0	3.0	0
3.0	0.0	16.16
3.0	0.5	30
3.0	1.0	0
3.0	2.0	6.6
3.0	3.0	0
4.0	0.0	60
4.0	0.5	20
4.0	1.0	0
4.0	2.0	0
4.0	3.0	33.3
5.0	0.0	66.6
5.0	0.5	3.3
5.0	1.0	0
5.0	2.0	0
5.0	3.0	16
6.0	0.0	70
6.0	0.5	23.3
6.0	1.0	3.3
6.0	2.0	30
6.0	3.0	40
0.0	0.5	73.3
0.0	1.0	6.6
0.0	2.0	43.3
0.0	3.0	25

Table 2. Effect of NAA and BAP on shoot regeneration from callus.

NAA (mg/l)	BAP (mg/l)	Shoot regeneration (%)
0.0	0.0	3.3
0.5	0.0	33.3
0.5	0.5	3.3
0.5	1.5	0
0.5	2.0	0
1.5	0.0	20
1.5	0.5	0
1.5	1.5	0
1.5	2.0	0
2.0	0.0	3.3
2.0	0.5	6.6
2.0	1.5	0
2.0	2.0	0
2.5	0.0	0
2.5	0.5	0
2.5	1.5	0
2.5	2.0	0
3.0	0.0	0
3.0	0.5	0
3.0	1.5	0
3.0	2.0	0
0.0	0.5	30
0.0	1.5	26.6
0.0	2.0	0

Shoot multiplication

MS medium containing lower concentrations of BAP with low concentrations of IBA produced shoots that are not significantly different at $p < 0.05$ after 4 weeks of culture (Table 3). Among the treatments, medium containing 1.0 mg/l BAP resulted in the highest number (3.13 ± 0.73) of shoots per explant (Fig. 1D) although there was no significant difference with shoots obtained on medium containing 0.5 mg/l BAP alone (2.76 ± 0.91), 0.5 mg/l BAP in combination with 0.5 mg/l IBA (2.74 ± 0.67) and 0.5 mg/l BAP in combination with 1.5 mg/l IBA (2.54 ± 0.81). Regenerated shoots cultured on MS medium supplemented with 1.5 mg/l BAP in combination with 2.5 mg/l IBA and 1.5 mg/l BAP in combination with 3.5 mg/l IBA produced the lowest mean number of shoots per explant, 0.93±0.65 and 1.02± 0.55, respectively. Shoots cultured on 0.5 mg/l BAP resulted in the tallest shoot length (4.18 ± 0.86 cm) per explant and the shortest shoot length (1.32±0.95 cm) was obtained on MS medium supplemented with combination of 1.5mg/l BAP and 2.5mg/l IBA.

Table 3. Effect of BAP and IBA on shoot multiplication, values are given as Mean ±SE.

BAP (mg/l)	IBA (mg/l)	No. of shoots /explant	Shoot length (cm)
0.0	0.0	1.77 ± 0.60^{c}	2.58 ± 0.75^{bc}
0.5	0.0	2.76 ± 0.91^{a}	4.18 ± 0.86^{a}
0.5	0.5	2.74 ± 0.67^{a}	3.39 ± 0.72^{b}
0.5	1.5	2.54 ± 0.81^{a}	3.68 ± 0.39^{a}
0.5	2.5	1.43 ± 0.68^{cd}	2.86 ± 0.67^{d}
0.5	3.5	1.94 ± 0.72^{b}	1.81 ± 0.55^{e}
1.0	0.0	3.13 ± 0.73^{a}	3.26 ± 0.79^{ab}
1.0	0.5	1.71 ± 0.41^{bc}	1.41 ± 0.65^{fg}
1.0	1.5	1.69 ± 0.59^{bc}	1.53 ± 0.59^{f}
1.0	2.5	1.29 ± 0.50^{de}	1.72 ± 0.28^{e}
1.0	3.5	1.30 ± 0.36^{de}	1.40 ± 0.91^{fg}
1.5	0.0	3.01 ± 0.52^{a}	2.51 ± 0.74^{bc}
1.5	0.5	1.15 ± 0.57^{ef}	1.51 ± 0.75^{fg}
1.5	1.5	1.19 ± 0.47^{ef}	1.50 ± 0.69^{fg}
1.5	2.5	0.93 ± 0.65^{f}	1.32 ± 0.95^{g}
1.5	3.5	1.02 ± 0.55^{ef}	1.35 ± 0.39^{fg}
2.0	0.0	2.23 ± 0.73^{b}	2.57 ± 0.85^{bc}
2.0	0.5	1.33 ± 0.53^{de}	1.61 ± 0.73^{cd}
2.0	1.5	1.54 ± 0.44^{cd}	1.62 ± 0.75^{cd}
2.0	2.5	1.08 ± 0.18^{e}	1.19 ± 0.54^{d}
2.0	3.5	1.29 ± 0.67^{de}	2.05 ± 0.62^{bcd}

Means within each column connected by the same superscript are not significantly different at 5%probability level.

Rooting and acclimatization

The shoots cultured on half strength MS medium supplemented with different concentrations of IBA and NAA resulted in different rooting responses. The highest mean number of roots per explant (9.60 ± 0.86) was obtained on half strength MS medium supplemented with 0.5 mg/l IBA (Fig. 1E) followed by 2.84 ± 0.48 mean number of roots per explant on growth regulators free half strength MS medium and 1.0 mg/l IBA that produced 2.65±0.91 mean root number. Shoot explants cultured on half strength MS medium supplemented with 2.5 mg/l NAA produced the lowest mean root number per explant (0.74± 0.77) (Table 4). The highest mean length of roots per explant (2.27±0.06 cm) was obtained on growth regulators free half strength MS medium. After one month of acclimatization, from the total of 30 plants, 27(90 %) survived in the greenhouse (Fig. 1F).

Table 4. Effects of different concentrations of NAA and IBA on rooting, values are given as Mean ±SE.

IBA (mg/l)	NAA (mg/ l)	No. of roots/ explants	Root length (cm)
0.0	0.0	2.84±0.48^{b}	2.27±0.06^{a}
0.5	0.0	9.60±0.86^{a}	1.41±0.09^{b}
1.0	0.0	2.65±0.91^{b}	2.06±0.09^{a}
2.0	0.0	1.24±0.52^{cd}	1.00±0.08^{c}
3.0	0.0	0.98±0.83 ^{cd}	1.06±0.09^{c}
0.0	0.5	2.58±0.81^{b}	2.12±0.08^{a}
0.0	1.5	2.09±0.76^{bc}	1.36±0.07^{b}
0.0	2.0	1.25±0.72^{cd}	0.76±0.04^{d}
0.0	2.5	0.74±0.77^{d}	0.53±.05^{e}

Means within each column connected by the same superscript are not significantly different at 5%probability level.

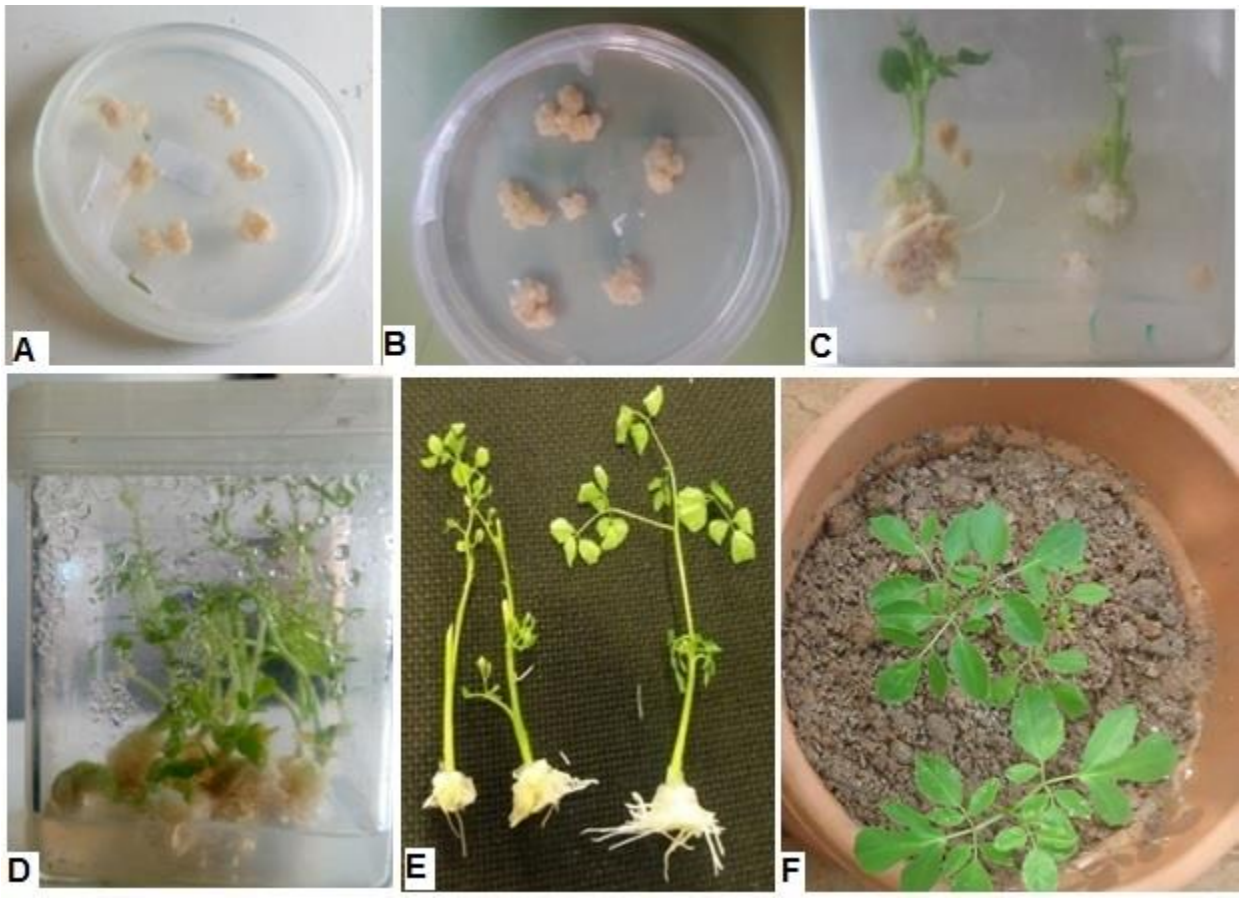

Figure 1. ***In vitro*** **regeneration of** ***Moringa oleifera*****. A) Callus induction on MS medium containing 0.5 mg/l BAP, B) Callus induction on MS medium containing 6.0 mg/l NAA C) Shoot regeneration from callus on MS medium containing 0.5 mg/l NAA D) Shoot multiplication on MS medium containing 1.0 mg/l BAP E) Rooting of shoots on half strength MS medium containing 0.5 mg/l NAA F) Plants after four weeks of acclimatization in greenhouse.**

DISCUSSION

Callus induction

In callus induction experiment, BAP, NAA and the combination of both were compared. NAA or BAP alone resulted in higher callus induction rate as compared to the combination of NAA and BAP. The maximum callus induction rate (73.3%) was obtained on MS medium supplemented with low concentration of BAP (0.5 mg/l). Riyathong *et al*. (2010) reported MS medium supplemented with 0.5 mg/l BAP resulted in 100% callus induction from the shoot of *Moringa oleifera*. The difference between the report of Riyathong *et al*. (2010) and the present study may be mainly due to the genotype differences. However, there could also be the difference in the juvenility of the explants. More juvenile explants are more responsive to callus induction than matured explants. When explants are taken from different parts of the mother plant, there could be difference in response in culture due difference in juvenility indicating physiological state of the explant is very important (Han *et al*., 1997; Tileye Feyissa *et al*., 2005). Reports show that in most cases 0.5 to 1.0 mg/l BAP concentrations alone or in combination with other auxins promote callus induction and differentiation (Riyathong *et al*., 2010; Seyyedyousefi *et al*., 2013). Callus formation increased when the concentration of NAA increased from 2.0 mg/l (40%) to 6.0 mg/l (70%), showing that the higher concentration of NAA resulted in the highest callus induction. Devendra *et al*. (2012), however, reported that the frequency of callus formation increased when the concentration of NAA increased from 0.2 mg/l to 2.0 mg/l but further increase of NAA decreased the rate of callus induction in *Moringa oleifera*. Low callus induction (0% to 40%) was recorded in combinations of NAA with BAP. No callus induction was obtained from leaf explants cultured on MS medium supplemented with most of the combinations of NAA with BAP. Generally, the present study showed the explants responded positively with callus induction when low BAP or higher NAA alone were used. However, callus induction is not a guarantee for shoot regeneration as not all calli are necessarily morphogenic.

Shoot regeneration

The percentages of shoot regeneration from callus ranged from 0.0 to 33.3%. The highest percentage (33.3%) was obtained on MS medium supplemented with 0.5 mg/l NAA. Riyathong *et al*. (2010) reported the highest (80%) regeneration of shoots on MS medium supplemented with the same NAA concentration (0.5 mg/l) in *Moringa oleifera*. In the present study, the regeneration rate for shoot from callus is relatively low and more than half of the treatments did not respond probably due to the used genotypes are recalcitrant types indicating the need for using other growth regulators to optimize the regeneration. Chand *et al*. (2019) reported variation in response of *M. oleifera* regeneration with respect to the form of callus developing on the culture medium due to changes in concentration of plant growth regulators. Except medium containing 0.5 mg/l NAA in combination with 0.5 mg/l BAP and 2.0 mg/l NAA in combination with 0.5 mg/l BAP, all MS media supplemented with a combination of NAA and BAP did not result in shoot regeneration. This shows the effect of genotype in culture is extremely strong.

Shoot multiplication

In the present study, MS medium containing different concentrations of BAP and IBA were used to multiply the regenerated shoots of *Moringa oleifera*. Among the treatments, the highest mean shoot number per explant (3.13) was obtained on medium containing 1.0 mg/l BAP. Many authors had reported the proliferation of multiple shoots of different tree plants on medium containing different growth regulators (Islam *et al*., 2005; Aloufa *et al*., 2010; Riyathong *et al*., 2010). Islam *et al*. (2005) obtained 4±0.29 mean shoot number per explant on MS medium supplemented with 1.0 mg/l BAP in *Moringa oleifera*. In agreement with this report, 3.13 ± 0.73 was obtained on MS medium supplemented with 1.0 mg/l BAP in the present study. In contrast, Shahzad *et al*. (2014) obtained 2.18±0.31cm mean shoot length in *Moringa oleifera* on MS medium containing 0.1, 0.5, 1.0, 1.5 and 2.0 mg/l BAP. In the present study, high concentration of BAP (2.0 mg/l) relatively reduced the shoot number as well as shoot length. This is due to inhibitory effect of supra-optimal concentrations of BAP on shoot multiplication (Bhojwani, 1996). In contrast, Saini *et al*. (2013) reported MS medium containing 2.0 mg/l BAP produced the highest

(10.8) mean shoot number per explant. This variation among these reports could be most likely due to genotype differences as different genotypes respond differently to the same culture conditions (Tileye Feyissa *et al*., 2005).

Rooting and acclimatization

The analysis of variance revealed that root number and length varied significantly on half strength MS medium supplemented with NAA and IBA. Application of IBA alone (0.5 mg/l) exhibited the highest mean root number per shoot, 9.60 .60 ± 0.86, as compared to MS medium supplemented with NAA alone. In contrast, Saini *et al*. (2012) reported application of NAA alone exhibited higher mean root number per shoot as compared to IBA alone in *Moringa oleifera*. These contrasting results could be due to differences in the used genotypes. The number of roots per shoots declined as the concentration of auxin (IBA or NAA) was increased. This is in agreement with the results reported by Aloufa *et al*. (2010), where increase in the concentration of IBA from 0.5 mg/l to 2.0 mg/l showed a reduction in the mean root number per shoot. Weiler (1984) also reported the inhibition of root elongation by higher concentration of growth regulators and stated ethylene deposition as the reason. This shows that using lower concentrations of IBA or NAA is better than higher concentrations for root induction and elongation. After acclimatization, 90% plants survived and established. No aberrant plants were observed. This result was better than previous report of Saini *et al*. (2012) where 80% of the plants survived.

CONCLUSIONS

Low concentration of BAP and high concentration of NAA is preferable for callus induction. BAP alone and NAA alone resulted in better shoot regeneration than different concentrations of BAP in combination with NAA. The highest percentage of shoot regeneration was obtained on MS medium supplemented with 0.5 mg/l NAA. The highest mean number of shoots per explant (3.13±0.73) was obtained on full strength MS medium supplemented with 1.0 mg/l BAP alone and half strength MS medium supplemented with 0.5 mg/l IBA resulted in the highest mean number of roots per shoot (9.60±0.86). *In vitro* regeneration through callus phase is the prerequisite for genetic improvement of the plant through biotechnological approaches. The present result contributes to the genetic improvement of *M. oleifera*. However, further research especially with regard to optimization of this protocol using other growth regulators and other approach such as somatic embryogenesis is recommended.

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