MICROPROPAGATION OF MULBERRY (MORUS ALBA L.) CV. AL-TAIFY

ATTIA O ATTIA1, ELDESSOKY SDESSOKY2, EHAB I EL-HALLOUS3 & HANAN F SHAABAN4

1,2Biotechnology and Genetic Engineering Unit, Deanship of Scientific Research, Taif University, Taif, Saudi Arabia
1,2Department of Plant Genetic Transformation, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Centre (ARC), Giza, Egypt
1,3Department of Biological Science, Faculty of Education, El-Arish, Suez Canal University, Egypt
4Department of Botany, Faculty of Science, Ain Sham University, Abasia, Egypt

ABSTRACT

A protocol for rapid multiplication of mulberry (Morus alba L.) cv. Al-Taify through in vitro culture was developed using axillary buds explants excised from mature plants. Shoot initiation was induced on Murashige and Skoog (MS) medium supplemented with different concentrations of 6-bezyl amino purine. Effect of different concentrations of 6-bezyl amino purine with a combination of 1mg/l kinetin on shoot multiplication was studied. To induce rooting, elongated shoots (3-4cm) were cultured on MS medium added with different concentrations and combinations of indole-3-butyric acid and indole-3-acetic acid. Higher percentage of shoot initiation (90%) was observed on MS medium supplemented with 2 mg/l 6-bezyl amino purine. Initiated shoots gave a higher average number of shoots (4 shoots/explant) when it were subcultured on MS medium supplemented with 2 mg/l 6-bezyl amino purine and 1mg/l kinetin. Higher average number of elongated shoots (19) was noticed on MS medium contains 1 mg/l 6-bezyl amino purine and 1mg/l kinetin. A maximum percentage of root formation (70%) was observed when elongated shoots were subcultured on MS medium containing 2 mg/l indole-3-butyric acid. Good rooted plantlets were transferred to pots containing sterile peat moss for acclimatization in greenhouse with (80%) survival rate.

KEYWORDS: Mulberry, Morus alba, Axillary Bud Explant, In vitro Propagation, Shoot Initiation, Multiplication, Rooting, Acclimatization

Abbreviations: BAP, 6-benzyl amino purine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; Kn, kinetin; MS, Murashige and Skoog medium; s.d.H2O, sterile distilled water.

INTRODUCTION

Mulberry (Morus sp.), typical woody plant of the Mediterranean basin, belongs to the Morus genus and to the Moraceae family. It’s cultivated for the tasteful fruits, for its potential pharmaceutical and cosmetic use and for its economic importance in silk industry for its foliage (Chiancone et al., 2007).

Mulberry is an invaluable tree of immense, which constitutes the chief food for the silkworm (Bombyx mori L). The improvement of productivity traits in mulberry plays a vital role in the progress of sericulture industry (Tewary et al., 1997). However, perennial nature of the plant coupled with prolonged juvenile period slows down the process of mulberry improvement (Kavyashree et al., 2001).
White mulberry (Morus alba L.) and black mulberry (Morus nigra L.) are of great relevance among the species belonging to the Morus genus. Both species originated in Central and Oriental Asia, from where they spread through Europe at the beginning of sixteenth century. In the past, Morus L. cultivation was widespread in Central Italy and in Sicily, because of the mulberry foliage use for rearing silkworms (Bombyx mori L.). Nowadays, its cultivation has greatly decreased and it is mainly used as ornamental plant in gardens. Particularly, black mulberry is cultivated for the tasteful fruits, but also for its potential pharmaceutical and cosmetic use (Chiancone et al., 2007).

Although propagation through stem cuttings is possible and being used, poor rooting ability of promising genotypes is a major problem for large scale multiplication, thus posing problems for mulberry breeders (Fotadar et al., 1990). Because it is hardly practicable to produce mulberry dwarf phytoplasma free mulberry plants by normal propagation techniques such as grafting and cuttings, other new propagation methods are necessary to control the disease. Stem culture in vitro was attempted for the elimination of mulberry dwarf phytoplasma (Dai et al., 1997).

Vegetative propagation of mulberry through grafting is not economically viable (Bhau, 1999). Mulberry tree improvement through conventional breeding is slow and also difficult due to its heterozygous nature (Song and Sink, 2006).

For targeted crop improvement through biotechnological approaches, attempts have been made to standardize in vitro regeneration protocols in different mulberry varieties (Sajeevan et al., 2011). Mulberry is a recalcitrant species in terms of tissue culture, and shoot regeneration is greatly dependent on the genotype, type of explant and combination of growth regulator used in the culture media (Feyissa et al., 2005). Using different explants such as stem (Narayan et al., 1989), shoot tip and nodal segment (Yadav et al., 1990; VijayaChitra and Padmaja, 1999), axillary bud (Vijayan et al., 2000), hypocotyl and cotyledon (Bhatnagar et al., 2001), leaf (Kapur et al., 2001; VijayaChitra and Padmaja, 2005). In vitro regeneration has been attempted with various degrees of success. Since there are variations in regeneration among mulberry varieties (Bhau and Wakhlu, 2003; Rao et al., 2010).

This study was designed to develop a protocol for rapid multiplication to produce micropropagated plants of mulberry (Morus sp.) cv. Al-Taify through in vitro culture using axillary buds explants which will be help to solve the poor rooting ability of stem cutting through conventional breeding.

MATERIALS AND METHODS

Plant Material

The nodal explants containing axillary buds were collected from juvenile branches of mulberry (Morus sp.) cv. Al-Taify plants grown in greenhouse. After that it were cut into pieces 1-2 cm long then it were surface sterilized by washed first under running tap water to remove the superficial dust followed by a detergent for 3 min and it were surface sterilized by dipping in 70 % ethanol for 1min, then incubated in 20% Clorox (sodium hypochlorite 5.25%) for 10 min and subsequently rinsed three to four times with sterile distilled water (s.d.H2O).

Culture Conditions

Axillary buds explants were excised and scale leaves were removed from the surface-disinfected explants. The explants were placed vertically on the culture medium containing full strength MS medium 4.4 g/l +30 g/l sucrose and supplemented with different concentrations and combinations of auxins and cytokinins. The pH of all media was adjusted...
to 5.7 using 1.0 N potassium hydroxide (KOH) and 1.0 N hydrochloric acid (HCl), before adding 7 g/l phytoagar. Media were autoclaved for 20 min at 121°C and 1.5 kilogram-force per square centimeter (kgf/cm²) pressure.

For shoot initiation, media containing MS mineral salts and vitamins added with 30 g/l sucrose with various concentrations of BAP (1, 2 and 3 mg/l). For 3-4 weeks. For multiplication and elongation stages, shoots were subcultured on the media containing MS salts and vitamins supplemented with different concentrations of BAP (1, 2 and 3 mg/l) in combination with 1 mg/l kin. Shoots obtained were subcultured every 3 weeks on the same media. To induce rooting, elongated shoots (3-4 cm) were subcultured on MS media supplemented with 30 g/l sucrose with different concentrations and combinations of indole acetic acid (IAA) (0.5, 1.0 and 2.0) and indole-3-butyric acid (IBA) (0.5, 1.0 and 2.0). MS media without growth regulators were used as a control treatment for all experiments. All the in vitro cultures were placed under 16/8 hour light/dark cycle and 3,000 lux light intensity provided by cool-white fluorescent light in a growth room maintained at 26 ± 2°C.

Acclimatization stage, good rooted plantlets (4-5 roots of length 3-5 cm) was carefully washed with S.d.H₂O to remove adhered agar and traces of medium; then they were transplanted to plastic pots (diameter: 10 cm) containing sterile soil, peat moss and sand (1:1:1). The top of the pots were covered with transparent plastic.

Experimental Design

All experiments were carried out in three replicates. Data were collected from different experiments four weeks after culture. For statistical analysis of data, analysis of variance and mean separation were carried out using ASSISAT Version 7.6 beta (2014).

RESULTS AND DISCUSSIONS

Axillary buds explants started to break after one week of culture on shoot initiation media and were kept for 4 weeks. Percentage of induced shoots varied from 26 to 90% when axillary buds were culture on MS medium without and with different concentrations of BAP (Table 1, Figure 1 A). MS medium supplemented with 2mg/l BAP showed a maximum percentage (90%) of induced shoots, this treatment was more significant (0.01 ≤ p > 0.05) than other treatments. Same results were obtained from nodal explants of Morus nigra L. (Zaki et al., 2011). But the achieved results were shoot induction by culturing nodal segments explants on MS medium supplemented with BAP (5mg/l). BAP was observed to be more effective than Kin in inducing shoot induction from both, shoot tip and nodal explants in the three different mulberry species (Pattnaik and Chand, 1997; VijayaChitra and Padmaja, 1999; Anis et al., 2003).

The effect of combination of Plant growth regulators on shoot multiplication was noticed when induced shoots were subculture on MS medium supplemented with different concentrations of BAP in combination with 1 mg/l Kin. Production of multiple shoots was increased when nodal explants were cultured on MS medium supplemented with plant growth regulators as compared to hormone free medium. Initiated shoots gave a higher average number of shoots (4 shoots/explant) when it were subcultured on MS medium with 2 mg/l BAP+1 mg/l Kin as shown in Table 2 and Figure 1B. It was reported that the combination of Plant growth regulators was found to be most effective in inducing higher percentage of multiple shoots than individual ones (Sajeevan et al., 2011). Results showed a decrease in the average number from 4 to 3 shoots/explants with the increase of BAP concentration (2-3 mg/l). This result was similar to others work (Lobna et al., 2008; Abdulla et al., 2010). They noticed that, the use of high cytokinin levels was one of the most effective methods to reduce shoot and leaf growth and promote the formation of meristematic clusters. The analysis of
variance showed significant differences (0.01 ≤ p > 0.05) between treatments for average number of shoots/Explant and the percentage of elongated shoots.

Higher average number of elongated shoots (19) was noticed on elongated medium contains 1 mg/l BAP and 1 mg/l kin. The elongated multiple shoots (3-4 cm) formed best root (70%) within four weeks from subculture on rotting medium supplemented with 2 mg/l IBA (Table 3, Figure 1C). On the contrast to has been reported before that single healthy elongated shoots of *Morus alba* L. variety V1 subcultured on rooting media (MS + 0.5 mg/l IBA) with or without activated charcoal (1% w/v) showed 100% initiations of roots (Sajeevan et al., 2011). Rooting capacity of micro-cuttings was reported to be improved from 42% to 64% when MS media supplemented with 0.5 mg/l IBA were repeated for the micro-cuttings that initially failed to root (Mng’omba et al., 2007). It was concluded that IBA is a potential auxin that induces rooting in *in vitro* regenerated shoots (Rajore and Batra, 2005). On the other hand, NAA (0.1-0.5 mg/l) was found to be the optimum concentration for the production of roots in the *in vitro* raised shoots of *Morus nigra* L. (Zaki et al., 2011). Contrary, it was reported that 2, 4-D to be more effective than NAA as a rooting agent (VijayaChitra and Padmaja, 1999). The analysis of variance showed significant differences (0.01 ≤ p > 0.05) between treatments for average number of shoots/Explant and the percentage of elongated shoots.

CONCLUSIONS

An effective *in vitro* micropropagation system was established using axillary buds explants of mulberry plant (*Morus sp.*) cv. Al-Taify, which consists of shoot initiation of nodal cuttings containing axillary bud explants in the presence of 2 mg/l BAP, shoot multiplication with 2 mg/l BAP and 1 mg/l Kn and finally a rooting stage with 2 mg/l IBA. This protocol will help for mass propagation for horticulture, pharmaceutical industries and *in vitro* germplasm conservation of mulberry (*Morus sp.*) cv. Al-Taify.

![Figure 1: Micro Propagation Stages of Mulberry Plant (*Morus sp.*) cv. Al-Taify](image)

**Figure 1:** Micro Propagation Stages of Mulberry Plant (*Morus sp.*) cv. Al-Taify (A) Shoot Induction of Axillary Buds Explants on MS Medium Supplemented with BAP 2 Mg/L (B) Multiplication and Elongation of Induced Shoots on MS Medium Containing 2 Mg/L BAP + 1 Mg/L Kn (C) Rooted Plantlets on MS Medium with 2 Mg/L IBA (D, E) Good Rooted Plantlets on Towel Paper after Washing with S.D.H2O to Remove Adhered Agar (F) Acclimatization Stage in Greenhouse (G) Micropropagated Mulberry Plants (*Morus sp.*) cv. Al-Taify after Three Months from Acclimatization
Table 1: Effect of Different Concentrations of BAP on Shoot Initiation from Axillary Buds Explants of Mulberry Plant (Morus sp.) cv. Al-Taify

<table>
<thead>
<tr>
<th>Growth Regulators BAP (Mg/l)</th>
<th>No. of Explants (Axillary Buds)</th>
<th>Average No. of Shoot Initiation</th>
<th>% of Shoot Initiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>8d</td>
<td>26</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>20b</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>27a</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>22c</td>
<td>73</td>
</tr>
</tbody>
</table>

The T test at a level of 5% of probability (0.01 ≤ p > 0.05) was applied. The averages followed by the same letter are not significantly different.

Table 2: Effect of Different Concentrations and Combinations of BAP and Kin on Multiplication and Elongation Shoots of Mulberry Plant (Morus sp.) cv. Al-Taify

<table>
<thead>
<tr>
<th>Growth Regulators (Mg/l)</th>
<th>No. of Explants with Shoots</th>
<th>Average No. of Shoots /Explant</th>
<th>Average No. of Elongated Shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP 0.0 0.0</td>
<td>20</td>
<td>0.0d</td>
<td>0.0d</td>
</tr>
<tr>
<td>1.0 1.0</td>
<td>20</td>
<td>1.6c</td>
<td>19a</td>
</tr>
<tr>
<td>2.0 1.0</td>
<td>20</td>
<td>4a</td>
<td>13b</td>
</tr>
<tr>
<td>3.0 1.0</td>
<td>20</td>
<td>3b</td>
<td>11c</td>
</tr>
</tbody>
</table>

The T test at a level of 5% of probability (0.01 ≤ p > 0.05) was applied. The averages followed by the same letter are not significantly different.

Table 3: Effect of Different Concentrations and Combinations of IAA and on Root Formation from Shoots of Mulberry Plant (Morus sp.) cv. Al-Taify

<table>
<thead>
<tr>
<th>Growth Regulators (Mg/l)</th>
<th>No. of Shoots</th>
<th>Average No. of Rooted Shoots</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA 0.0 0.0</td>
<td>20</td>
<td>0.0f</td>
<td>0.0</td>
</tr>
<tr>
<td>0.5 0.0</td>
<td>20</td>
<td>0.6e</td>
<td>30</td>
</tr>
<tr>
<td>0.5 0.5</td>
<td>20</td>
<td>1.2b</td>
<td>60</td>
</tr>
<tr>
<td>1.0 0.0</td>
<td>20</td>
<td>1.0c</td>
<td>50</td>
</tr>
<tr>
<td>0.0 1.0</td>
<td>20</td>
<td>0.8d</td>
<td>40</td>
</tr>
<tr>
<td>0.0 2.0</td>
<td>20</td>
<td>1.4a</td>
<td>70</td>
</tr>
<tr>
<td>2.0 0.0</td>
<td>20</td>
<td>1.1bc</td>
<td>60</td>
</tr>
</tbody>
</table>

The T test at a level of 5% of probability (0.01 ≤ p > 0.05) was applied. The averages followed by the same letter are not significantly different.

REFERENCES


