Regeneration Studies on Plant Cells

Subjects: Agriculture, Dairy & Animal Science

Contributor: Jameel Al-Khayri, Praveen Nagella, EPSITA MUKHERJEE, Muthu Thiruvengadam, FATIMA MOHAMMED ALESSA, Muneera Al-Mssallem, Adel Rezk, Mahesh Attimarad

The in vitro regeneration approach allows us to conserve and proliferate rare and therapeutic plant species for the extraction of diverse bioactive compounds. Since *A. annua* is a medicinal herb with significant anti-malarial effects, it is critical to understand how to regenerate it, utilizing in vitro techniques to boost its bioactive compound content while also preserving its excellent quality germplasm.

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1. Regeneration Studies

The in vitro regeneration approach allows us to conserve and proliferate rare and therapeutic plant species for the extraction of diverse bioactive compounds. Since *A. annua* is a medicinal herb with significant anti-malarial effects, it is critical to understand how to regenerate it, utilizing in vitro techniques to boost its bioactive compound content while also preserving its excellent quality germplasm.

2. Direct Organogenesis

Direct organogenesis is associated with the formation of organs such as shoots and roots directly from cultured explants, avoiding the callusing phase of in vitro regeneration. Seeds, leaves, and shoot regions are some of the successful explants used for direct organogenesis in *A. annua*.

Multiple shoots were developed from seed explants collected from the European region when inoculated on an MS medium supplemented with 0.1 mg/L of benzyl adenine (BA) along with 1.0 mg/L of indole-3-acetic acid (IAA), and subsequently, when the shoots were sub-cultured on a Murashige and Skoog (MS) medium supplemented with 1.0 mg/L naphthalene acetic acid (NAA) along with 0.1 mg/L kinetin (KN), gave roots [1]. Optimal shoot length, root length, and the number of nodes were observed when seed explants were inoculated on MS media fortified with 0.1 mg/L KN along with 0.01 mg/L NAA. Similarly, media fortified with 0.1 mg/L BA along with 0.01 mg/L of NAA showed callus induction [2]. Surface sterilized seeds, when inoculated on an MS medium supplemented with 4.4 µM BA along with 0.35 µM NAA, showed good induction capacity; optimal multiplication of shoots was observed on an MS medium supplemented with 0.9 µM BA and 0.05 µM of NAA [3].

Induction of multiple shoots was observed with 100% efficiency (57 shoots per explant) when stem explants were inoculated on an MS medium supplemented with 0.1 mg/L thidiazuron (TDZ) and successive rooting was established on an MS basal medium [4]. Nodal explants, when inoculated on an MS medium supplemented with 0.2 mg/L benzyl amino purine (BAP) in combination with 0.2 mg/L NAA, showed the highest shoot multiplication capacity [5]. The MS medium supplemented with 4.44 µM BAP showed optimal induction capacity of shoots when nodal explants were inoculated. These shoots were elongated when sub-cultured on an MS medium supplemented with 1.44 µM gibberellic acid (GA_3) along with 10% coconut milk. These developed shoots were subjected to rooting when further transferred on a half-strength MS medium supplemented with indole-3-butyric acid (IBA) at 2.46 µM concentration [6]. Adventitious multiple shoots were developed from nodal explants when inoculated on an MS medium supplemented with 10 µM 2-iP and rooted on a medium supplemented with 5.0 µM NAA [7]. The MS medium supplemented with 0.8 mg/L BAP combined with 0.1 mg/L IBA showed optimal shoot induction (98.75 ± 2.50) and the highest multiplication capacity was recorded when nodal explants were inoculated on an MS medium supplemented with 1.0 mg/L BAP along with 0.1 mg/L IBA (8.05 ± 0.66 per explant). The best rooting with ideal root length and number was observed on a half-strength MS medium fortified with 0.5 mg/L IBA [8].

Explants, especially leaf and petiole obtained from seedlings, showed 100% regeneration efficiency when compared to greenhouse-grown plants. It is seen that leaves and petiole explants when inoculated on an MS medium supplemented

with 1.0 mg/L TDZ showed direct organogenesis with shooting and rooting [9]; especially, leaf explants, when inoculated on MS media along with 1.0 mg/L BAP and 0.05 mg/L NAA, induced shoots. However, the addition of silver nitrate at a 2.0 mg/L concentration helped in a significantly higher regeneration capacity [10]. MS media supplemented with 0.5 mg/L NAA in combination with 2.0 mg/L BA showed the highest frequency in shooting, and roots were developed when sub-cultured on a 0.1 mg/L IBA medium [11].

The nodal segments from the inflorescence of *A. annua* developed multiple shoots when inoculated on an MS medium supplemented with 1.0 mg/L BAP. Subsequent subculturing of shoots on MS media incorporated with 2.0 mg/L IBA developed roots [12]. **Table 1** depicts the data of comprehensive research on in vitro regeneration of *A. annua* using various concentrations and combinations of auxins and cytokinins, as well as their responses.

Table 1. In vitro regeneration of *Artemisia annua* through direct organogenesis from various explants.

Explant	Media	Response	References
Seed	MS + 0.1 ppm BA + 1.0 ppm NAA	Shooting	[1]
Inflorescence	MS + 1.0 mg/L BAP + 2.0 mg/L IBA	Multiple shooting	[12]
Stem	MS + 0.1 mg/L TDZ	Multiple shooting	[4]
Leaf, petiole	MS + 1.0 mg/L TDZ	Shooting and rooting	[9]
Seed	MS + 0.1 mg/L KN + 0.01 mg/L NAA	Shooting	[2]
Nodal stem explants	MS + 0.2 mg/L NAA + 0.2 mg/L BAP	Multiple shooting	[5]
Nodal stem explants	Shooting: MS + 4.44 µM BAP Rooting: ½ MS + 2.46 µM IBA	Multiple shooting and rooting	[6]
Nodal stem explants	Shooting: MS + 10.0 µM 2-iP; Rooting: 1/2 MS + 5.0 µM NAA	Multiple shooting and rooting	[7]
Nodal stem explants	Shooting: MS + 0.8 mg/L BAP + 0.1 mg/L IBA; Multiplication medium: MS + 1.0 mg/L BAP + 0.1 mg/L IBA; Rooting: 1/2 MS + 0.5 mg/L IBA	Multiple shooting and rooting	[8]
Leaf	Shooting: MS + 1.0 mg/L BAP + 0.05 mg/L NAA + 2.0 mg/L $AgNO_3$	Shoot regeneration	[10]
Leaf	Shooting: MS + 0.5 mg/L NAA + 2.0 mg/L BA; Rooting: MS + 0.1 mg/L IBA	Shooting and rooting	[11]
Seed	Shooting: MS + 4.4 µM BA + 0.35 µM IBA; Multiplication medium: MS + 0.9 µM BA + 0.05 µM NAA	Shooting and multiplication	[3]

3. Indirect Organogenesis

Indirect organogenesis refers to the formation of organs such as shoots and roots following the intrusion of the callusing stage. Callusing is a very important stage, which helps in the establishment of cell suspension cultures, usually used for metabolite production. Multiple shoots were developed from calluses induced on media supplemented with 5.4 µM NAA upon further sub-culturing on an MS medium supplemented with 13.32 µM BA and 1.08 µM of NAA [13]. Hypocotyl explants, when inoculated on an MS medium supplemented with a combination of 0.5 µM NAA along with 13.0 µM BAP and 0.3 µM GA_3, initiated the growth of calluses, and further sub-culturing on the same medium developed multiple shoots [14].

Leaf explants of *A. annua*, when inoculated on an MS medium supplemented with 0.1 mg/L BAP along with 0.05 mg/L NAA, helped in the induction of friable calluses. Further sub-culturing on an MS medium supplemented with 0.4 mg/L BAP along with 0.2 mg/L NAA exhibited multiple shoot regeneration [5]. Leaf explants, when inoculated on an MS medium supplemented with 1.0 mg/L BAP along with 0.05 mg/L NAA, induced calluses, and further organogenesis was established [15]. The MS medium supplemented with 0.5 mg/L NAA or 2,4-D along with 0.5 mg/L BAP showed callusing, and upon sub-culturing on media supplemented with 0.25 mg/L NAA in combination with 1.0 mg/L BAP, helped in optimal shooting and rooting. It was observed in 0.5 MS supplemented with 0.1 mg/L IBA [16]. **Table 2** depicts the data of comprehensive research on in vitro regeneration (in-direct organogenesis) of *A. annua* using various concentrations and combinations of auxins and cytokinins, as well as their responses.

Table 2. In vitro regeneration of *Artemisia annua* through indirect organogenesis from various explants.

Explant	Media	Response	References
Leaf, hypocotyl	Callus: MS + 5.4 µM NAA; Shooting: MS + 13.32 µM BA + 1.08 µM NAA	Callusing and organogenesis	[13]
Hypocotyl	Callusing and shooting: MS + 0.5 µM NAA + 13 µM BAP + 0.3 µM GA_3	Callusing and multiple shooting	[14]
Leaf	Callusing: MS + 0.1 mg/L BAP + 0.05 mg/L NAA; Shooting: 0.4 mg/L BAP + 0.2 mg/L NAA	Callusing and multiple shooting	[5]
Leaf	Callusing: MS + 1.0 mg/L BAP + 0.05 mg/L NAA	Callusing and organogenesis	[15]
Leaf	Callusing: MS + 0.5 mg/L NAA or 2,4-D + 0.5 mg/L BAP; Shooting: 0.25 mg/L NAA + 1.0 mg/L BAP; Rooting: ½ MS + 0.1 mg/L IBA	Callusing and organogenesis	[16]

4. Somatic Embryogenesis

Employing somatic tissues or cells for the development of the whole plant is the chief characteristic of somatic embryogenesis. Studies suggest that somatic embryogenesis is one of the most successful techniques which helps us to better understand the development of plants and is regarded as one of the best approaches to clonal propagation and disease-free varieties of plants [17]. Various embryos like globular, torpedo, heart and cotyledon embryos were developed from leaf explants when inoculated on an MS medium supplemented with 0.6 mg/L TDZ in combination with 0.1 mg/L IBA. Further rooting of the adventitious buds was seen on the MS medium fortified with 0.5 mg/L NAA [15].

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