Regeneration of plants from callus tissue of *Aeschynomene spp.* (Leguminosae)

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Received 16 March 1995; accepted in revised form 5 March 1996

Key words: Adventitious shoots, forage species, organogenesis

Abstract

Plants were regenerated from leaflet-derived callus of *Aeschynomene sensitiva*, *A. americana* and *A. villosa*. Explants were induced to form callus when aseptically cultured on Murashige and Skoog medium solidified with 0.8 % agar and containing 0.5 or 0.05 µM naphthaleneacetic acid and 4.4 or 13.3 µM benzyladenine. Shoot regeneration was readily achieved. Roots were induced when shoots were transferred to medium devoid of growth regulators or with 0.05, 0.5 or 5.4 µM naphthaleneacetic acid. Plantlets were successfully transplanted to soil. Callus from *A. falcata* failed to regenerate shoots. Explants from leaflets of *A. fluminensis* did not produce callus when cultured in vitro.

Abbreviations: BA – benzyladenine; MS – Murashige and Skoog (1962) medium; NAA – naphthaleneacetic acid

Introduction

*Aeschynomene* (Leguminosae) is chiefly a tropical genus, with a few species growing in temperate areas of the world. It contains 350 species; most of them are hydrophytes and includes both herbaceous and shrubby plants, annuals and perennials (Rudd, 1955; 1981). The species are predominantly self-compatible (Arroyo, 1981; Fernández et al., 1988).

Although most of the species of *Aeschynomene* have relatively minor economic importance, cattle have been observed to graze various species of *Aeschynomene* (Rudd, 1955) and some of them (*A. falcata*, *A. americana* and *A. montevidensis*) have a great forage value in tropical and subtropical areas of the world (Fernández et al., 1988).

Plants have been regenerated from tissue, cell and protoplast cultures of several genera of forage legumes such as *Lotus*, *Medicago*, *Trifolium* and *Stylosanthes* (see reviews by Mroginski and Kartha, 1984 and Hammat et al., 1986). Plant regeneration from tissues cultured in vitro has also been accomplished in *Coronilla varia* (Arcioni et al., 1988; Gustine and Moyer, 1990), *Hedysarum coronarium* (Arcioni et al., 1985), *Galega officinalis* (Našinec and Newcová, 1990), *Lupinus spp* (Sator, 1990) and *Onobrychis viciifolia* (Arcioni et al., 1988). Other examples of plant regeneration in forage legumes - through organogenesis - include *Lotononis bainesii* (Bovo et al., 1986) and *Centrosema brasilianum* (Angeloni et al., 1992).

We studied the morphogenetic responses of in vitro - cultured explants from five species of *Aeschynomene* as influenced by a wide range of NAA and BA concentrations in the media. The present paper also describes the cultural conditions employed to induce plant regeneration in vitro from leaf – derived calli of *A. sensitiva*, *A. villosa*, and *A. americana*.

Materials and methods

Seeds of *Aeschynomene americana* L. (CIAT 1726), *A. falcata* D.C. (INTA Mercedes, Corrientes 4990), *A. fluminensis* Vill. (CIAT 2854), *A. sensitiva* SW. (INTA, Corrientes), *A. villosa* Poir. (CIAT 2927) were germinated in a potting mixture of soil and sand (1:1)
under greenhouse conditions. The first fully-expanded leaflet from 3 to 5-month old plants were employed as a source of explants.

The leaflets were surface sterilized by immersion in 70% ethanol for 1 min followed by immersion in a solution of commercial bleach (0.8 % sodium hypochlorite, final concentration) for 10 min and were thoroughly washed three times with autoclaved distilled water. The leaflets were transversally dissected into three pieces and both basal and apical thirds were discarded. The median portion was again transversally cut into rectangles of approximately 4 mm² and placed with the abaxial side down on 4 ml of nutrient medium, solidified with 0.8 % Sigma® agar, in a 10 ml glass tube. The tubes were covered with Resinite AF50® and incubated at a constant temperature of 27 ± 2°C and 14 - h photoperiod (40 µmol m⁻²s⁻¹ from fluorescent lamps). Each treatment consisted of 12-15 explants and each experiment was repeated 3 times.

The nutrient medium consisted of major and minor salts as well as vitamins according to Murashige and Skoog (1962), and 3 % sucrose. NAA and BA were added in various combinations and concentrations. The pH of the medium was adjusted to 5.7 with KOH or HCl prior to adding the agar. After 30 days culture, small pieces of callus were transferred to fresh medium in an attempt to induce and/or improve shoot regeneration. These media were also based on MS with various combinations and concentrations of NAA and BA.

The regenerated shoots were transferred to medium composed of MS and various concentrations of NAA in order to induce root formation.

### Results and discussion

After 12 days of incubation, small compact, white yellowish calluses were observed to grow on the explants. After 45 days, the callus had grown into a compact brown mass of cells. In some cases, structures resembling shoot bud primordia (Fig. 1a) and roots were detected. The growth pattern as well as the browning of the calli appeared to be similar to that reported in other legumes in which plant regeneration has been accomplished; for example, *Stylosanthes guianensis* (Mroginski and Kartha, 1981), *Arachis spp.* (Mroginski and Fernández, 1980), *Centrosema brasilianum* (Angeloni et al., 1992). The effects of 30 combinations of NAA and BA on the morphogenetic responses of leaflet explants of *Aeschynomene sensitiva* are presented in Table 1. No callus induction was observed with MS media lacking growth regulators, as well as with MS media supplemented with NAA alone at the lowest concentrations, or in combinations of NAA at 0.05 or 0.5 µM with BA at 0.04 or 0.4 µM. Callus induction was not observed when 0.04, 0.4 and 26.6 µM BA was the only growth regulator present in the regeneration medium. In contrast, various combinations of NAA and BA resulted in callus formation. Both 5.4 and 16.1 µM NAA, in combinations with the lowest BA concentrations, produced calluses with roots. In contrast, an increase in the concentration of BA resulted in a decrease of calluses with roots and an increase of calluses with shoots and/or bud primordia.

The requirement of adequate combinations of NAA and BA for shoot regeneration (Fig. 1b) is in agreement with the results obtained with other legumes (see reviews by Mroginski and Kartha, 1984; Hammat et al., 1986).

Shoot regeneration from leaflet-derived callus was not restricted to *Aeschynomene sensitiva*. It was also found to occur in 2 out of 4 other *Aeschynomene* species tested. The callus proliferation ability of explants of *A. americana*, *A. villosa*, *A. falcata* and *A. fluminensis*, was tested by culture on media containing various combinations of NAA and BA and the results are presented in Table 2. The results show that interspecific differences do exist since shoot regeneration was observed only in one half of the plant species tested. Similar differences have been described in other legumes (Mroginski and Kartha, 1984; Hammat et al., 1986). Shoot regeneration was readily achieved in both *A. americana* and *A. villosa* (Fig. 1c,d) in the various media tested. However, leaflet cultures of *A. falcata* only produced callus and no callus with shoots and/or buds were detected. It is interesting to note that no callus induction was observed when pieces of leaflets of *A. fluminensis* were cultured on four different media. These morphogenetic responses are not common when herbaceous plant material are cultured (Litz and Jarret, 1991; Flick et al., 1983).

The frequency of shoot regeneration was greatly affected by the medium employed for callus induction from leaflets. Table 3 summarizes the results obtained when callus of *Aeschynomene sensitiva*, *A. americana*, and *A. villosa* induced with twelve combinations of NAA and BA were subcultured onto shoot regenerating medium containing MS supplemented with 0.05 µM NAA and 13.3 µM BA. It is interesting to note that shoot regeneration - independent of the plant species - was highest when the initial callus induction medium contained relatively low concentrations of NAA.