In Vitro Differentiation of a Wild Peanut, *Arachis villosulicarpa* Hoehne[1,2]

R. N. Pittman*, B. B. Johnson, and D. J. Banks[3]

ABSTRACT

Immature leaflets 3 to 5 mm in length from *Arachis villosulicarpa* Hoehne seedlings were cultured *in vitro* on a medium consisting of the major and minor salts of Murashige and Skoog (MS), Gamborg's B5 vitamins, 20 g/L sucrose, 0.8% Difco agar, 4.44 μM N-6 benzyladenine (BA) and 5.37 μM napthaleneacetic acid (NAA). Hard callus with many shoot primordia formed on all leaflets within 4 to 6 weeks. Calli with primordia were further cultured for plant formation on 150 other media (MS major salts at 1/2 or 1/4 strength, full strength minor salts and vitamins, sucrose at 5 or 10 g/L, BA and NAA at 0 to 8 μM). Reduced concentrations of major salts with a high ratio of BA to NAA enhanced the formation of shoot primordia. Roots differentiated on media with 1/4 strength major salts, no BA, and 6 to 8 μM NAA and 5 g/L sucrose. Shoots differentiated from the branch points of roots after 2 to 4 months of culture. Plants were obtained via shoot primordia from callus and from the adventitious shoots from roots.

Key Words: Groundnut, Morphogenesis, Organogenesis, SEM, Tissue culture.

A number of *in vitro* techniques have been reported to succeed with *Papilionaceae* plants (5,6,8). Peanut, *Arachis hypogaea* L., suspension cultures have been established from isolated mesophyll cells (14,15,16) and parenchyma cells (17). Callus has formed from protoplasts isolated from mesophyll cells (24) also. Plants have differentiated from de-embryonated cotyledons (11,12), from immature leaflets (22,25,26) and from meristem culture (18). Also, whole organs, e.g., ovules (19,27) and gynophores (28) from *A. hypogaea* have been cultured.

There are fewer reports of successful *in vitro* manipulation of wild *Arachis* species than with cultivated peanuts. However, successes have been reported on callus production from cultured pollen (2,3) and plantlets have been achieved from anther cultures (20,21) and from "rescued" interspecific hybrids (4).

Some wild *Arachis* species are important because near immunity or very high resistance has been found for many economically important diseases, such as leafspot, rust, viruses and nematodes (10). However, interspecific hybrids are not always successful because of cross compatibility barriers (9). Johansen and Smith (13) attempted a hybrid between *A. hypogaea* L. and *A.* sp. (then erroneously referred to as *A. diogoi* Hoehne), but the hybrid failed to develop because of improper endosperm development. *A. villosulicarpa* could be a very valuable species because it has a high level of resistance to both leafspots (*Cercospora arachidicola* and *Cercosporidium personatum*) (1), but it does not hybridize successfully with *A. hypogaea* nor with any other species outside of its own taxonomic section (9).

This paper reports the *in vitro* performance of immature leaflets of a wild peanut, *Arachis villosulicarpa* and the development of shoots from roots.

Materials and Methods

Immature leaflets of *Arachis villosulicarpa* Hoehne, 3 to 5 mm long, from 6 to 10 day old seedlings were used as explants and cultured according to the procedures described by Mroginski et al. (22). The medium used contained the major and minor salts of Murashige and Skoog (MS) (23), B5 vitamins of Gamborg et al. (7), 20 g/L sucrose, 0.8 g/L agar, 4.44 μM N-6 benzyladenine (BA) and 5.37 μM napthaleneacetic acid (NAA). Forty cultures were maintained in a growth chamber with a 16/8 hour light/dark cycle at 27/21 C. General Electric F 20%12 CW fluorescent tubes provided 67 μmol m^{-2} sec^{-1} (400-700 nm range) at vial height.

The *in vitro* performance over a 50 day period was documented by notes, photographs and scanning electron microscopy (SEM). Leaflets were selected at random twice a week over the 50 day period and prepared by killing and fixing in glutaraldehyde, dehydrating in ethanol, drying to the critical point, mounting on aluminum stubs, and coating with gold/palladium.

Once calli with shoot primordia and shoots had formed on the leaflet cultures (37 to 41 days), they were tested on 150 different combinations of sucrose (0, 5 or 10 g/L), MS major salts (1/2 or 1/4 strength) with vitamins and MS minor salts at full strength, BA (0, 2, 4, 6, or 8 μM), and NAA (0,2,4,6, or 8 μM) to evaluate the effect of these media on further differentiation. Two replicates (each consisting of a tube of callus) were used for each treatment combination. In cases where favorable responses were achieved, an additional fifty replicates were cultured. All treatments were arranged completely at random among six trays with three trays per shelf on two shelves of the growth chamber.

Results and Discussion

Within the first week of culture the leaflets in all vials began to expand and thicken (Fig. 1). By the third week, dense callus occurred which contained many meristematic regions (Fig. 2). By the seventh week, shoot primordia were prominent (Fig. 3) but no roots formed on the initial medium.

SEM examinations of primordia that formed on callus showed them to be structurally similar to shoot primordia dissected from seedlings (Fig. 4).

When calli with shoot primordia were tested on 150 different media, two interesting responses developed. Root formation was enhanced by a reduction of major salts to 1/4 strength with no BA, 6 to 8 μM NAA, and 5 g/L sucrose (Fig.. 5). When major salts were reduced to 1/2 or 1/4 strength, sucrose reduced to 5 g/L or omitted entirely, and a high ratio of BA to NAA (6μM BA/2μM NAA or 8 μM BA/4μM NAA) utilized, many small shoot primordia formed (Fig. 6). When 50 replicates of shoots

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[3]Former Research Associate, CSRS, Langston University, Langston, Oklahoma, presently, Research Geneticist, USDA-ARS, Dept. of Agronomy, Oklahoma Agricultural Experiment Station; Associate Professor of Botany and Biochemistry, Oklahoma State University; and Research Geneticist, USDA-ARS, Dept. of Agronomy, Oklahoma Agricultural Experiment Station, Stillwater, OK 74078.

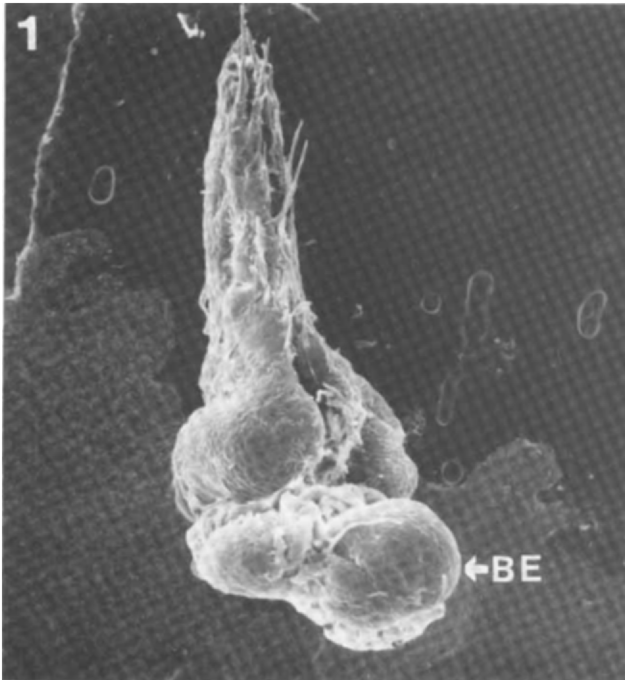

Fig. 1. Basal enlargement (BE) of leaflet after culturing for one week.

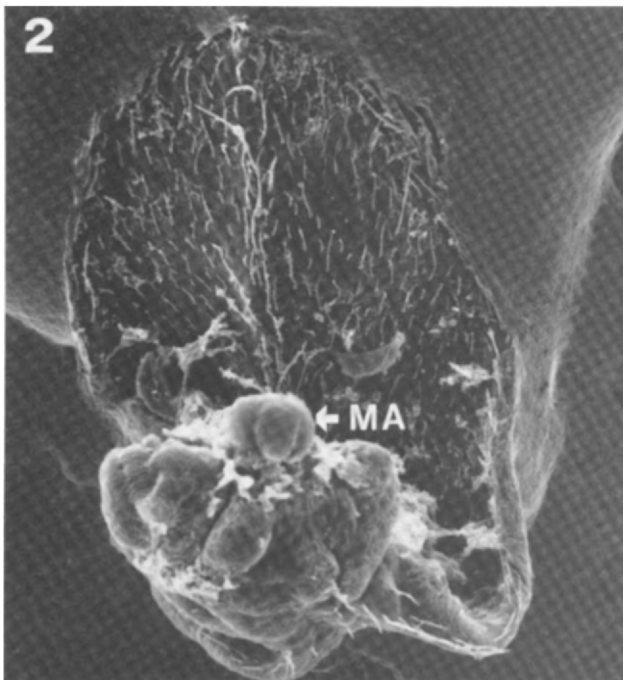

Fig. 2. After three weeks, meristematic areas (MA) are present.

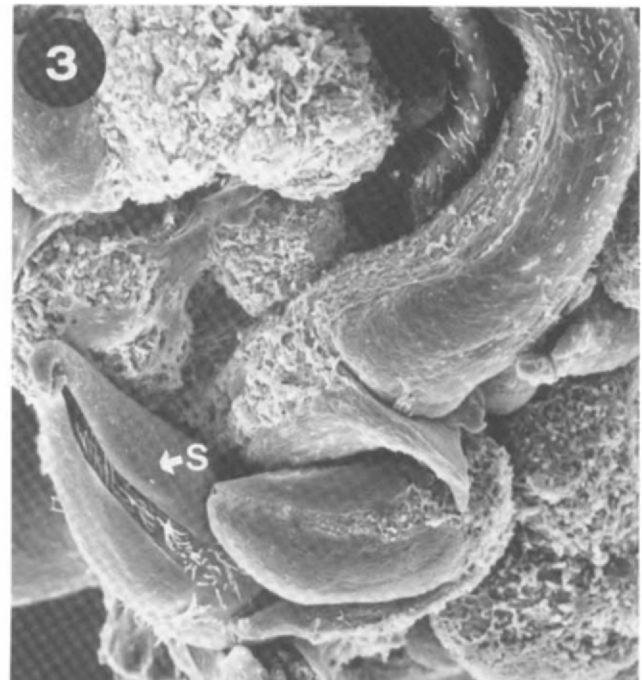

Fig. 3. Shoots (S) are prominent after seven weeks of culture.

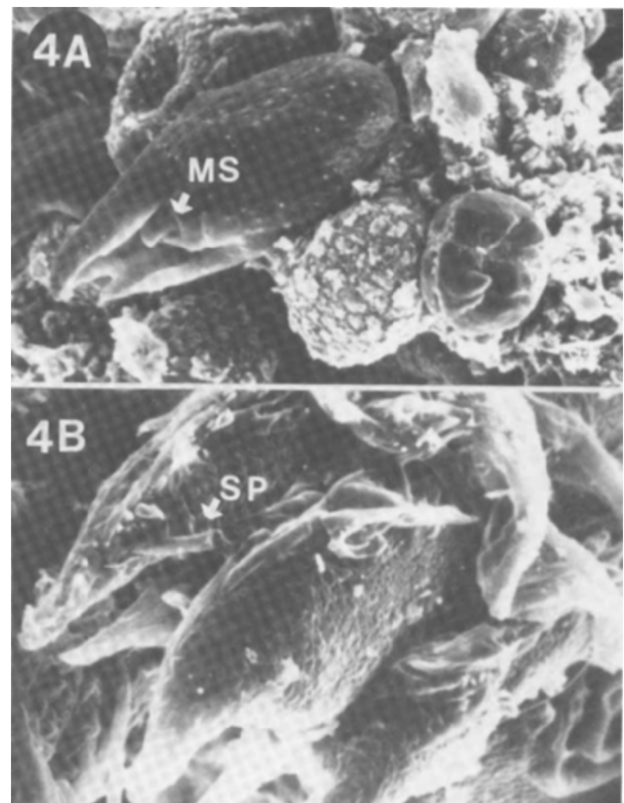

Fig. 4. Meristematic shoots (MS) via tissue culture (4A) appear to be very similar to shoot primordia (SP) from seedlings (4B).

from previous studies were placed on the root inducing medium, all produced roots. Similarly, when 50 pieces of callus with shoot primordia were transferred to 1/2 strength major minerals, 6 μM BA and 2 μM NAA with no sucrose, all exhibited proliferation of shoots and additional small shoot primordia.

A developmental pattern not previously reported for *Arachis* was observed after two to four months in culture. Shoots (3-4%) differentiated from the branch points of roots of aged cultures (Fig 7).

The ability of *Arachis villosulicarpa* to form plantlets *in vitro* is encouraging because such a manipulative technique may aid in maintaining and increasing germplasm in this species. The section *Extranervosae* contains several species that are difficult to maintain by traditional methods because of low seed yield. *In vitro* propagation from leaflets could accelerate replication of the wild

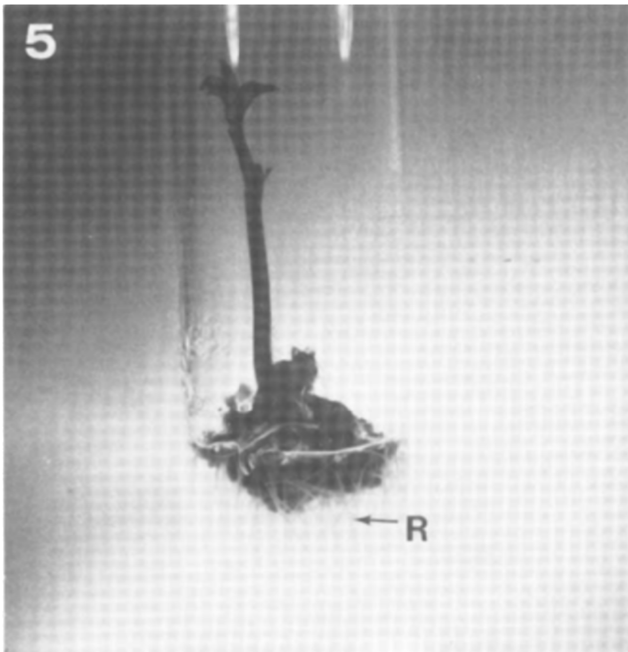

Fig. 5. Enhanced root (R) formation with 1/4 strength major salts, no BA, 6 to 8 μM NAA and 5 g/L sucrose.

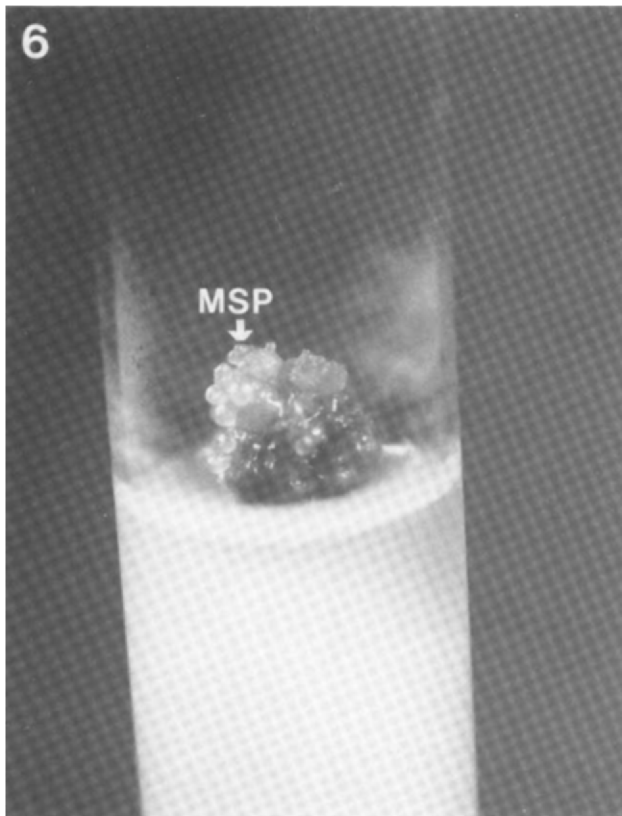

Fig. 6. Small meristematic shoot primordia (MSP) formed with 1/4 or 1/2 strength major salts, 0.0 g/L or 5 g/L sucrose, and a high ratio of BA:NAA (6:2 or 8:4 μM).

germplasm which could then be maintained and distributed. Studies are now being conducted in our laboratory to regenerate *A. villosulicarpa* plants from single cells.

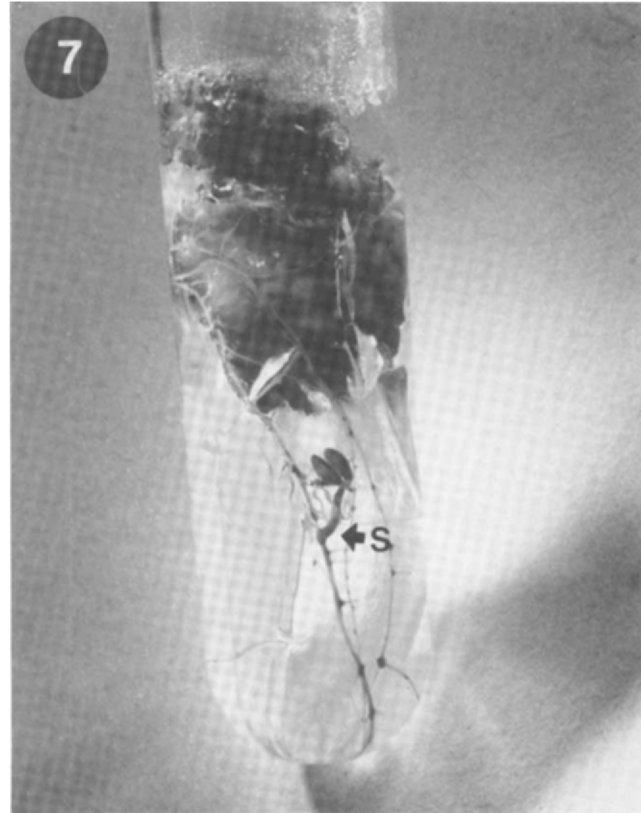

Fig. 7. Shoot (S) forming at the branch points of roots.

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