PLANT TISSUE CULTURE GENETIC MANIPULATION AND SOMATIC HYBRIDISATION OF PLANT CELLS

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IN VITRO CULTURE OF ARACHIS OVARIES AND OVULES.

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INTRODUCTION

The cultivated groundnut (Arachis hypogaea L.) is susceptible to a number of diseases and pests. These cause a considerable loss in yield (1) and this justifies the urgent attention of groundnut biologists but not all the resistance genes have been found in A.hypogaea. Fortunately, in the wild collections of the genus a number of species are known to be resistant to some diseases (2). In the genus Arachis there are seven sections; Arachis hypogaea has been crossed only with species from its own section Arachis, though some other intersectional hybrids have been produced (3). Some intersectional crosses form pods, but mature seeds are not possible due to early degeneration of endosperm, as in A.hypogaea x A.diogoi (4), or of embryo or both. Members of the section Rhizomatosae are particularly valuable for disease and pest resistance, but cannot be crossed with A.hypogaea.

A project was started in the Groundnut Improvement Program at ICRISAT to investigate intersectional incompatibilities and to develop techniques to overcome them. Encouraged by the reports of successful culture of ovules at various stages of development in a number of taxa (Table 1; see also (5)) we started culture of Arachis monticola ovules in vitro. We have also attempted to simulate peg elongation and pod initiation in vitro. These twin objectives are aimed at obtaining a reliable method for crossing desirable but incompatible species of Arachis.

In this report we present out attempts to culture ovaries and ovules at different intervals after pollination, and also to pollinate ovules in vitro.

Table 1.

Stage of ovules cultured	Taxa	References
Fertilized ovules: zygotic stage	Gossypium arboreum x G.hirsutum	6
	G.hirsutum	7
	G.hirsutum 2n x 4n	8
	Zephyranthes sp.	9,10
Proembryo or globular embryo	Abelmoscus esculentus	11
	Arachis hypogaea	12
	Gossypium hirsutum	8,13,14,15
	Gynandropsis gynandra	16
	Hibiscus cannabinus x H.sabdariffa	17
	H.sabdariffa x H. asper	17
	Nicotiana sps	18
	Nicotiana rustica	19
	N.tabacum	19
	Papaver somniferum	20,21
	Petunia hybrida	22,23
	Solanum tuberosum	24
	Trifolium repens	25

MATERIALS AND METHODS

A.monticola [illegible] wild species [illegible]ly related [illegible]lly cross compatal[illegible] [illegible] flowers w[illegible]ated

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between 1500 and 1700 hrs and pollinated between 0600 and the following day. The flowers or developing ovaries were sterilised with chlorine water, and ovules and ovaries or the terminal 1cm of the developing peg were dissected under aseptic conditions and transferred to culture tubes at daily intervals from the day of pollination until 10 days after pollination (Table 2). Ovules were also pollinated in vitro.

Table 2. Rate of peg development after pollination

Days after pollination	Peg length	No. of cells in embryo (Approx.)	No. of ovules cultured
In vitro pollinated			134
Day of pollination			22
1 - 3 days after pollination	less than 1.0 cm	1-2	13
4 - 6 days after pollination	1-2 cm	3-7	10
7 - 10 days after pollination	3-4.5 cm	8-16	12
More than 10 days after pollination	7-10 cm (in soil)	more than 16	24
	7-10 cm (not in soil)		12
	5-7 cm (in soil)		33
	5-7 cm (not in soil)		11

For the initial experiments the media of Murashige and Skoog (26) White (27), Kao and Michayluk (28), Martin (12) and Ziv and Zamsky (29) with or without modifications were used.

RESULTS

Pegs and gynophores showed slight elongation on the plant after incompatible pollinations. This elongation was soon arrested; pegs longer than 5 cm were not observed as they ceased developing and dried from the tip before penetrating the soil.

Ovules

A maximum of 10% of the pollen germinated when ovules were pollinated in vitro. Many of the tubes grew long but very few were observed to gain entry into the ovule. The best growth of the ovules was when they were cultured and pollinated in vitro on Murashige and Skoog's medium supplemented with casein hydrolysate (500 mg/l). Within 2-3 weeks, the ovules grew to 5 times their original size, in 12 hrs light alternating with 12 hrs dark. Later they become brown and necrotic.

The ovules did not respond well to other media (Table 3). There was only a marginal increase in the size of the ovules which became brown within three weeks and ceased developing irrespective of the photoconditions. More than 200 ovules of A.monticola were cultured at various stages, but no seedlings could be raised. Eightyfour of these ovules were transferred to culture more than 7 days after pollination, a stage at which Martin (12) reported he could recover "some" seedlings from 74 ovules. A reliable medium, technique and treatment still has to be developed before we can routinely culture ovules from incompatible crosses.

Table 3. Responses of A.monticola ovules to some media

Ovules	MS	MS with Kn (4 mg/l)	MS with IAA (4 mg/l)	MS with CH (500 mg/l)	White with IAA(3 mg/l)	KM	Martin
From pollinated flowers	NC	Slight swelling; remain green for 6 weeks then become brown	No change, but ovules when transferred from KM show swelling	Under trial	NT	NC	NC in ovules from pegs up to 10 cm long
From unpollinated flowers	NC after 9 weeks	Brown in 4 weeks	NC	NC	NT		NC
Pollinated *in vitro*	Slight Swelling in 8 weeks	Initial swelling then no change, brown in 4 weeks	Slight swelling then no change	Swelling & occasionally callus in 3 weeks	Very slow callus formation	Some ovules form callus	

Abbreviations:

CH	Casein hydrolysate	MS	Murashige and Skoog's medium
IAA	Indole acetic acid	NC	No change
KM	Kao & Michayluk's medium	NT	Not tried
KN	Kinetin	ZZ	Ziv & Zamsky's medium

Ovules	Kn	Bn (4 mg/l)	IAA (4 mg/l)	CM (500 mg/l)			
Pollinated ovaries	NC	Slight swelling; remain green for 6 weeks then become brown	No change, but ovules when transferred from KM show swelling	Under trial	NT	NC	NC in ovules from pegs up to 10 cm long
Non pollinated flowers	NC after 9 weeks	Brown in 4 weeks	NC	NC	NT		NC
Pollinated in vitro	Slight Swelling in 8 weeks	Initial swelling then no change, brown in 4 weeks	Slight swelling then no change	Swelling & occasionally callus in 3 weeks	Very slow callus formation	Some ovules form callus	

Abbreviations:

CH	Casein hydrolysate	MS	Murashige and Skoog's medium
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varies

Ziv and Zamsky could culture pegs and induce pod formation in rachis hypogaea cultured at various stages of development (29). Un he same conditions of culture, A.monticola pegs did not respond. On lurashige and Skoog's medium with kinetin (4mg/l) the ovaries constantly howed prolific callus initiation within a week after culture. The varies which responded best were from flowers 2 and 3 days after polli- ation, particularly when they were cold treated at 4°C for 4 or more ours. In three weeks the callus was profuse and a few organogenetic reas could be observed in the form of bulbils. Hand and microtome ections revealed that xylem had been formed and that there were regions hich were differentiating into shoots. Xylogenesis was more frequent hen such callus was transferred to White's medium with IAA (4 mg/l). ome of these protuberances elongated to form pale green stalk like tructures which remained organized but did not differentiate further.

When the pegs were cultured on White's medium with IAA (4mg/l), ne cultured peg showed an initial callus at a region just below the vules and later the tip showed geotropism, the latter being the normal esponse of the peg in vivo.

DISCUSSION

A few of the formulations tried so far have supported growth or allus formation when used to culture ovules, so our efforts are now irected at testing the efficacy of various components in the media and ther factors.

Arachis ovules do respond better on a medium supplemented with casein hydrolysate. Casein hydrolysate seems essential for an initial growth as has been shown for ovules in *Abelmoschus* (11). *Gossypium* (2n x 4n) (30), *Nicotiana rustica* and *N.tabacum* (19). However, on a medium composed of MS macroelements, Heller's microelements, Dulieu's vitamins with kinetin and gibberellin, Martin (12) was able to rear some seeds and seedlings from *A.hypogaea* ovules. However, the best growth of selfed *A.monticola* ovules occurred when the tip of the young gynophore was cultured, without excising the ovules, on MS with casein hydrolysate (500 mg/l).

Kinetin regularly induced profuse callus formation within three weeks when young gynophores 2 and 3 days after pollination were cultured on MS with kinetin (4mg/l). A few differentiating zones could be detected but these did not form shoots or roots. Only shoot like structures were seen. These cultures may need to be transferred to other media to continue differentiation. To our knowledge, callus initiation from ovaries and subsequent regeneration of plants in legumes has been successful in only one instance, with *Trifolium pratense* (31).

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