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Tissue Culture and Prospects for Improvement of *Arachis hypogaea* and Other Oil Seed Crops

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ABSTRACT

Advances in tissue culture technology have improved our knowledge about the regeneration of plants from cultures of a wide range of cells, tissue and organs in a great diversity of plants, and have now provided a novel approach to crop improvement. The progress made in tissue cultures of major oil seed crops is reviewed. In **Arachis hypogaea**, tissue and organ cultures have been attempted in the past but with very limited success. The contraints to improving yields in **A. hypogaea** and the approaches made to overcome them using tissue culture techniques are discussed in detail.

INTRODUCTION

The science of crop improvement uses many breeding methods and techniques which are modified as necessary to suit particular crops or the aims of improved pests and disease resistance, yield, quality or adaptation to environment. The changes brought about range from single gene changes by induced mutation to creation of new species such as *Triticale*. Of late tissue culture technology has been proposed and recognised as a new tool for crop improvement (Vasil, 1976; Scowcroft, 1977; Reinert & Bajaj, 1977; Sneep & Hendriksen, 1979). This paper reviews briefly the progress made with tissue culture of oil crops and its possible applications to improvement of these crops.

PRODUCTION AND YIELD OF OIL SEEDS

The area under cultivation, yield and total production of some important oil seed crops are shown for 1979 in tables 1 & 2 (adapted from FAO, 1980). Developed countries produced most of the soybean, sunflower, olive, and rapeseed. Developing countries produced considerable amount of soybean and were major contributors to world production of groundnut and cotton. For some crops, yield per hectare in the developing countries is comparable to that in developed countries, but most crops yield far less in the developing countries. There are many reasons for this, and one of them is lack of suitable cultivars developed specially to suit local conditions in the developing countries.

For most crops, the areas grown, the yields, and therefore the total production have increased in the last decade (Table 2). This is particularly true for soybean. Total world area of castor has decreased slightly, but yield has increased in the developing world where most castor is grown, so total production has increased. In the case of linseeds, however, increases in yield have not been sufficient to offset the decrease in total area grown, and production has decreased. In groundnut, the increase in yield has more than offset the decrease in area, and production has increased slightly. There have also been reduction in yield in rapeseed in developed countries and in sesame in developing countries. Most of the area under oil seed crops is in the developing countries and in most cases yields are lower than those obtained in developed countries. Also, progress in improving yields in the developing countries has been slower than in the developed countries. This could be to a certain extent due to lack of inputs into plant breeding, or to lack of genetic variability in the crop, or to constraint on utilizing plant variability. These contraints and the means to overcome them will be elaborated for groundnut.

Area of crop grown depends on the farmer whose choice of crop is influenced by many factors. The plant breeder may increase yields of oilseed crops only to find that areas under cultivation have not increased; perhaps solely because of farmers' preference for cereals or pulses!

PROGRESS IN TISSUE CULTURES OF MAJOR OIL SEED CROPS

The Perspectives

Cell and tissue cultures are now established for in vitro regeneration of a large number of plant species. It is not

Table 1. Production of Oilseed in Developed and Developing Countries, 1979* – Production in 1000 MT. Figures in parentheses are yields in kg/ha.,

Crop	World Production	Total production by all developing countries	Total production by all developed countries	Developing countries with largest production	Developed countries with largest production	Countries with largest yields
I	II	III	IV	V	VI	VII
Castor	931	866	65	Argentina 370 (667) India 236 (528)	USSR 62 (283)	Philippines (3 364) Peru (2 000) Libya (1 833)
Coconut[1]	4 887	4 887	0	Malaysia 470 Nigeria 350 Brazil 239	0	–
Cotton[1]	26 366	14 577	11 819	China 4 414 India 2 440	USSR 5 924 USA 5 256	–
Groundnut	18 907	16 740	2 168	India 5 800 (806) China 2 812 (1 187)	USA 1 804 (2 922) Japan 67 (1 676)[2]	Israel (3 750) USA (2 922) Greece (2 461) Turkey (2 530)
Linseed	3 136	1 457	1 679	Argentina 751 (730) India 514 (264)	Canada 836 (802) USA 343 (832)	New Zealand (2 800) Mexico (1 260) Egypt (1 197)
Olive	8 085	1 976	6 109	Tunisia 900 Turkey 411 Morocco 380	Italy 2 400 Spain 2 270 Greece 1 080	
Rapeseed	10 824	4 629	5 996	China 2 464 (887) India 1 877 (628) Japan 5 (1 923)[2]	Canada 3 561 (1 035) France 480 (2 088) Sweden 313 (1 938)	Belgium Lux (3 088) UK (2 676) Switzerland (2 606) Netherland (2 600) Germany (2 532)
Sesame	2 691	2 688	3	India 600 (268) Sudan 210 (219) Burma 208 (215)		South Arabia (1 883) Iran, Yugoslavia (1 600) Guatemala (1 588)
Soybean	84 268	30 286	84 001	China 13 632 (904) Indonesia 674 (882) Iran 180 (2 143)	USA 61 715 (2 162) 3 769 (2 312)	Italy (2 500) Canada (2 371) Argentina (2 312) Yugoslavia (2 161)
Sunflower	15 089	2 833	12 435	Turkey 988 (1 341) China 380 (882) S Africa 315 (1 039) Afghanistan[2] 4 (1 833)	USA 3 314 (1 514) Romania 889 (1 710)	USA (2 162) Algeria (2 418) Yugoslavia (2 843) France (3 072)

* Source: FAO 1980. Preliminary figures for 1979
1 Yield data not available
2 Despite low total area, yields are above average

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Table 2. Area, Yield and Production of Oil Crops in ... and 1979 ... rld, Developing and ... ped Countries*

	Area (1000 ha)						Yield (Kg/ha)						Production (1000 MT)					
	Total World		D'ping**		D'ped**		Total World		D'ping		D'ped		Total World		D'ping		D'ped	
	1971	1979	1971	1979	1971	1979	1971	1979	1971	1979	1971	1979	1971	1979	1971	1979	1971	1979
Castor	1 434	1 420[1]	1 204	1 212	230	208	589	656	621	714	420	315	844	931	748	866	866	66
Coconut													3 838	4 412	3 838	4 412	0	0
Cotton													22 488	26 396	13 997	14 577	8 490	11 819
Groundnut	19 747	18 906	18 677	17 ^94	1 070	922	926	1 000	881	931	1 720	2 352	18 293	18 907	16 453	16 740	1 840	2 168
Linseed	6 769	6 228	2 979	3 88	3 79(	^9	514	504	420	430	488	591	3 481	3 136	1 251	1 457	2 230	1 679
Olive													7 651	8 085	1 799	1 976	5 852	6 109
Rapeseed	8 486	12 656	5 860	7 963	2 6	4 693	780	855	536	606	1 323	1 278	6 617	10 824	3 142	4 828	3 475	5 996
Safflower	Data Not Available																	
Sesame	6 241	6 967	6 218	6 960	23	7	322	300	322	300	358	369	2 012	2 091	2 004	2 088	8	3
Soybean	35 222	56 816	16 963	26 497	18 259	30 318	1 327	1 660	857	1 143	1 764	2 111	46 747	94 288	14 539	30 286	32 208	64 001
Sunflower	8 412	12 027	2 016	2 714	6 396	9 313	1 173	1 253	789	970	1 295	1 335	9 871	15 068	1 591	2 633	8 281	12 435

* Source FAO, 1980, **D'ping, Developing, D'ped, Developed

1 Figures double underlined are reductions over 1971 data

surprising that this tissue culture technique has now emerged as a powerful and promising tool for crop improvement, as is evidenced by the recent interest of plant geneticists and breeders. Serious application of this technology, while fairly recent, has already found rewarding application in *Citrus* (Button & Kochba, 1977; Spiegel-Roy & Kochba, 1977), coffee (Monaco *et al.*, 1977), maize (Green, 1978), sugarcane (Heinz *et al.*, 1977), rye and potato (Wenzel *et al.*, 1979; Wenzel, 1980) and *Brassica* (Keller & Armstrong, 1979; Hoffman, 1980; Wenzel, 1980). Considering the delay in application to crop improvement, the number of crops listed above reflects a significant achievement, but the status of technology with other crops is yet to show promise.

However, it is a fervent hope that tissue culture technique will have a great role in crop improvement through wide hybridization, and in induction of genetic variability, mutants and haploids, which can be achieved in far less time and in larger numbers than has been achieved so far by conventional methods, which it may supplement or replace. The embryo culture has been used in more than 30 interspecific and 10 intergeneric crosses which are normally incompatible, for overcoming seed dormancy (Raghavan, 1977), and for anther culture in over 100 species or hybrids (Vasil, 1980b). These coupled with the facility of large scale in vitro clonal propagation in about 400 species (Vasil & Vasil, 1980) has paved the way for production and multiplication of hybrids from incompatible crosses. Mutation and selection of cell or tissue variants (Maliga, 1980), genetic modification at cellular level by exogenous DNA (Soyfer, 1980) and organelle intake or fusion (Kao, 1980) are some of the more novel trends used in tissue culture technique.

Strikingly enough, in the list of plants cultured, many crop plants are conspicuously absent. The need for work on cereals and legumes has been repeatedly felt (Scowcroft, 1976; Vasil, 1977; Vasil *et al.*, 1979; Vasil & Vasil, 1980). While efforts are being made with cereal crops and certain legumes, oil crops have received little attention. A close look at the state of technology as applied to oil crops reveals that *Glycine max* and *Brassica* only have received considerable attention by plant tissue culturists though other important crops are being seriously considered lately.

The plant tissue culture technology offers means of rapid clonal propagation, possibilities of wide hybridization by embryo, ovule or ovary culture, somatic fusion and in vitro pollination and fertilization, haploid production, creation of plants resistant to or free from diseases, mutation, and a number of other advantages. Most of these are reviewed in supplementary volumes 11a and 11b of the International Review of Cytology (Vasil, 1980a). The achievements in clonal propagation, embryo culture, haploid induction, protoplast isolation, culture and fusion and other miscellaneous applications are summarised in Tables 3–7. These are briefly discussed below for oil crops and in detail for groundnuts later.

Clonal Propagation

To be able to use this technique for crop improvement, it is necessary to recognize a suitable explant and develop a medium which can produce embryos, or shoots and subsequently complete plants. A modest beginning was made with embryo cultures of *Linum* (Laibach, 1929), *Arachis hypogaea* (Harvey & Shulz, 1943), *Cocos nucifera* (Cutter & Wilson, 1954), and of *Brassica napus* (Nishi *et al.*, 1959), but clonal propagation itself began only after 1970 when Staristky reported *in vitro* regeneration of oil palm.

From the available reports it is evident that to date only plants of seven oil seed crops viz., *Brassica* spp., *Cocos nucifera, Elaeis guineensis, Glycine max, Helianthus annuus* and *Linum usitatissimum* (Table 3) and *Arachis hypogaea* (present studies, see Table 9) have been regenerated *in vitro*. There are other minor oil crops for example, *Eucalyptus* sp. and *Santalum album* which can also be regenerated *in vitro* but have not been included in this review (see Mascarenhas, 1981; Sita, 1981, in these Proceedings). Of a range of organs and tissues cultured from all these crops, shoots or meristems are most frequently used, and several other explants have still to be tried. It is important to mention that haploid plants in *Brassica napus* (Thomas *et al.*, 1976)

Table 3. Attempts at Clonal Propagation of Oil Crops

Crop	Explant/tissue	Response	Reference
Castor *(Ricinus communis)*	endosperm	callus, roots	La Rue, 1944
	endosperm	callus, embryo like structures	Satsangi & Mohan Ram, 1965, Johri & Srivastava, 1972
Coconut *(Cocos nucifera)*	embryo	callus, shoots, roots	Guzman, 1971, Guzman *et al.*, 1976
	embryo	branched seedlings	Fisher & Tan, 1979
	apices, stem, petiole, rachillae	callus	Eeuwens, 1976, 1978
	stem	callus, embryoid	Apavatjrut & Blake, 1977
	hypocotyl	buds, roots, shoot like outgrowth	D'Souza, 1980, Kuruvinashetty & Iyer, 1980
	seedling apices, leaf, inflorescence	plants, callus, roots	Eeuwens & Blake, 1981
Cotton *(Gossypium arboreum)*	hypocotyl	rapidly growing callus	Smith *et al.*, 1977, Price *et al.*, 1977
(G. barbadense)	hypocotyl	rapidly growing callus	Smith *et al.*, 1977
(G. hirsutum)	stem	rapidly growing callus	Davis *et al.*, 1974
(G. klotzschianum)	hypocotyl	somatic embryogenesis	Price & Smith, 1979
Groundnut *(Arachis hypogaea)*	cotyledon	callus, roots	Guy *et al.*, 1978, 1980
	shoot tip	callus, elongation into shoot	Russo & Varnell, 1977, Bajaj, 1979a
	pericarp	callus, roots	Rangaswamy *et al.*, 1965
Linseed *(Linum usitatissimum)*		plants	Gamborg & Shyluk, 1976, Mathews & Narayanaswamy, 1976
	shoot	plants	Murray *et al.*, 1977, Chiyah *et al.*, 1980
Oil palm *(Elaeis guineensis)*	leaf	plants	Barret & Jones, 1974, Corley *et al.*, 1977, 1979
		clonal propagation	Jones, 1974
		callus	Martin & Cas, 1972
	embryo	plants	Ong, 1977
	leaf	plants	Rabechault & Martin, 1976
		clonal propagation	Staritsky, 1970, Wilson & Webb, 1974, Wooi *et al.*, 1981
Rapeseed *(Brassica campestris)*		plants	Bhattacharya & Sen, 1980
(B. juncea)	internode	shoots	Eimhauser *et al.*, 1978, Hinata *et al.*, 1978
	leaf of haploids	haploid plants	Thomas *et al.*, 1976
	hypocotyl	shoots	Hui & Zee, 1978
	cotyledon	plants	George & Rao, 1980
	internode and ovary	plants	Pareek & Chandra, 1980
(B. napus)	roots	callus & shoots	Drazdowska & Rapownska, 1975
	shoot tips	callus & shoots	Thomas *et al.*, 1976
		plants	Pereira, 1971
Soybean *(Glycine max)*	immature cotyledons	callus	Thomson *et al.*, 1977, Beversdorf & Bingham, 1977
	node	plants	Cheng *et al.*, 1980
	hypocotyl	adventitious buds	Kimball & Bingham, 1973
	cotyledonary node segments	multiple shoots	Saka *et al.*, 1980
	cotyledonary cells (mechanically isolated)	callus	Schwenk, 1980
Sunflower *(Helianthus annuus)*	stem pith	plants	Sadhu, 1974, Hildebrandt *et al.*, 1946, Trifi *et al.*, 1981

and *Linum usitatissimum* (Murray *et al.*, 1977) have been regenerated and multiplied *in vitro*. The tissue culture technique however is more advanced in *Brassica*, *Glycine*, *Elaeis guineensis*, and *Cocos nucifera* than in other oil crops and *in vitro* manipulation for several purposes with these crops is in full swing (see Table 7). In fact Unilever Co. Ltd., UK, has just launched a massive program for producing 9 million test tube plants of oil palm per year for planting in Malaysia where 20% greater productivity is expected (Webb, 1980). Having achieved this with oil palm they are now attempting coconut multiplication. Wooi *et al.*, (1981) and Eeuwens and Blake (1981) have provided greater details on oil palm and coconut respectively in these Proceedings.

The Culture of Embryos, Ovules, Ovaries and its Application to Wide Hybridization: The application of this technique for raising hybrids in oil crops is still in the initial stages when compared with other crops such as tobacco, potato, and maize. However, in cotton, oil palm, rapeseed and linseed embryos, ovules and ovaries have been cultured either for production of hybrids, or for overcoming dormancy. Particular attention has to be paid to Laibach (1925, 1929) who used the embryo culture technique to raise hybrids between *Linum perenne* and *L. austriacum* which do not otherwise produce viable seeds. This initiated a series of later investigations to surmount barriers to interspecific breeding. Embryos from some interspecific crosses have been successfully cultured in *Brassica* spp. (Table 4; Nishi *et al.*, 1959) and *Gossypium* spp. (Table 4; Skovsted, 1935; Beasley, 1940; Weaver, 1957; 1958; Joshi & Pundir, 1966).

Subsequently Guzman and his coworkers (1964 *et seq.*) have employed the embryo culture technique for raising plants from coconut embryos and have gone a step ahead in culturing embryos from the prized "makapuno" variety of coconut which otherwise germinate with difficulty. Rabechault and his colleagues (1967 *et seq.*) have employed this technique to investigate the various factors of dormancy in oil palm kernels. With the rest of the oil crops, embryo culture technique is yet to find an application. Ladizinsky *et al.*, (1979) were unsuccessful in their initial trials with embryo cultures of wide crosses in soybeans but felt this was due to the lack of a suitable medium.

Ovule Culture: Culturing embryos in fact becomes technically difficult when in an incompatible cross the embryo degenerates soon after fertilization. In such instances *in-ovulo* embryo cultures have been profitably employed. Beasley *et al.* (1971) cultured cotton ovules 2 days after anthesis and thus showed the possibility of successfully culturing embryos within the ovules from incompatible crosses (Beasley *et al.*, 1974). Eid *et al.*, (1973) obtained embryos at the cotyledonary stage when cotton ovules, 5 days post-anthesis, were cultured; these embryos germinated when transferred to another medium and developed into abnormal plantlets. Subsequently, Beasley and Ting (1973; 1974) found a satisfactory medium which was further modified by Stewart and Hsu (1977) to culture cotton ovules from zygote stage to maturity. Stewart and Hsu (1978) have succeeded in culturing ovules from interspecific crosses of *Gossypium* (see Table 4).

Encouraging results are seen in the work of Takeshita *et al.* (1980) who have successfully applied the ovule culture technique to interspecific crosses in *Brassica*. Although the production rate was not high, the hybrids produced could be multiplied by tissue culture methods (see Table 3). Ovule cultures from intergeneric crosses between *Brassica* x *Raphanus* were not useful (Takeshita *et al.*, 1980).

Ovary Culture: It has been possible to culture ovaries after pollination and to stimulate pod or fruit formation *in vitro*. Inomata's success with culture of excised *Brassica* ovaries is the best illustration. Initial experiments were to standardize conditions for *in vitro* development of excised ovaries (Inomata, 1976). Subsequently, the method was applied to raise ovaries from crosses between *Brassica campestris* and *B. oleracea* (Inomata, 1977; 1978a, 1978b; 1979) and other interspecific crosses in *Brassica* (Takeshita *et al.*, 1980).

For other crops such methods of embryo, ovule or ovary cultures are yet to be developed and examined for their efficacy in the production of desirable wide crosses which otherwise may not be successful.

In Vitro Pollinations: *In vitro* manipulation of both the male and female gametophytes has been successfully tried in some plants (see Rangaswany, 1977) but has yet to be tried for oil crops, other than *Brassica* species in which *in*

Table 4. Embryo, Ovule and Ova[illegible]e for Hybridization and Other Purposes.

Castor	:	None
Coconut	:	Embryo culture (Cutter & Wilson, 1954; Abraham & Thomas, 1962); to raise plants in 'makapuno' variety (Guzman & Del Rosario, 1964; Guzman, 1971; Guzman *et al.*, 1978; Rosario & Guzman, 1976; Miniano & Guzman, 1978)
Cotton	:	Immature and mature embryos (Mauney, 1961; Mauney *et al.*, 1967; Dure & Jensen, 1957); *G. davidsonii* x *G. strutii* (Skovsted, 1935); *G. arboreum* x *G. hirsutum* (Joshi & Pundir, 1966; Beasley, 1940; Weaver, 1958) and reciprocal (Beasley, 1940; Weaver, 1957); Ovules of cotton: (*in vitro* fibre development; Joshi & Johri, 1972; Beasley & Ting, 1973; 1974; Eid *et al.*, 1973) Ovules of *G. arboreum* x *G. hirsutum* (Pundir, 1972): *G. hirsutum, G. hirsutum* x *G. arboreum; G. hirsutum* x *G. australe; G. barbadense* x *G. australe; G. arboreum* x *G. australe* (Stewart & Hsu, 1977; 1978).
Groundnut	:	Embryo (Harvey & Shulz, 1943; Nuchowiz, 1955); Ovule (Martin, 1970); Gynophore tips (Ziv & Zamsky, 1975)
Linseed	:	Immature and mature embryos (Erdelska *et al.*, 1971) Young embryos of *Linum perenne* x *L. austriacum* (Laibach, 1925; 1929)
Oil palm	:	Embryo (Pritchard, 1976) Embryo culture for overcoming dormancy (Rabechault 1967; Rabechault & Ahee, 1966; Rabechault *et al.*, 1968; 1969; 1970)
Olive	:	Embryo (Gilad & Lavee, 1974)
Rapeseed & mustard	:	Embryo culture from a cross between "c" and "a" genome spp. of *Brassica* (Nishi et al., 1959) Ovule culture for seed development for interspecific crosses (Takeshita *et al.*, 1980) Ovary culture and capsule formation in *Brassica* species and interspecific crosses (Inomata 1976; 1977; 1978a; 1978b; 1979; Matsuzawa, 1978). Test tube fertilization in *B. oleracea* (Kameya *et al.*, 1966) and in *B. pekinensis* (Kameya & Hinata, 1970b).
Safflower	:	Nine
Sesame	:	Embryos (Sastri, D. C., unpublished)
Soybean	:	Embryo (Braverman, 1975; Vagera & Hanackova, 1979) Immature seeds (Obendorf *et al.*, 1979)
Sunflower	:	None

*vi[illegible]*ion has been successf[illegible]ya & Hinata, 1970b, [illegible]uya *et al.*, 1966). Our preliminary observations with *Arachis* also indicate the possibility of success provided a good medium for ovule culture is developed. This would be particularly useful in crosses like *Sesamum indicum* x *S. mulayanum* where mentor pollen could only partially overcome incompatibility (Sastri & Shivanna, 1976). An essential prerequisite for these investigations is a thorough knowledge of the viability and the *in vitro* germinability of pollen grains and the nutrient requirements and conditions for the development of ovule or ovary.

PRODUCTION AND APPLICATIONS OF HAPLOIDS

Anthers and microspores from a number of plants have been cultured for haploid production, but the majority of oil seed crops do not figure in the list. However, progress has been made with *Brassica* species. Haploids obtained from *B. napus* cv. Oro by Thompson (1969) were responsible for a new cultivar called "Maris Haplona" which had a higher oil content and some better agronomic characters than Oro. Following the work of Stringam & Downey (1973) and Stringam (1974; 1980) it is now realised that haploid production in *Brassica* has advanced to a stage when it is possible to choose cultivars and define conditions of culture for high frequencies of haploids (Keller, 1980; Keller & Armstrong, 1977; 1978; Keller *et al.*, 1975) which can be extensively used for the production of commercially acceptable new cultivars (Keller & Armstrong, 1979; Wenzel, 1980; Hoffman, 1980). This is an achievement in rapeseed. Compared to this, results with several other oil crops are far from satisfactory, but the trends are improving (Table 5). Callus has been obtained in anther cultures of cotton (Barrow *et al.*, 1978), sesame (Sastri, D. C. unpublished), and *Arachis* species (Mroginski & Fernandez, 1979; 1980; Bajaj *et al.*, 1980; this report). Roots have been produced in cotton anther cultures (Barrow *et al.*, 1978) and plants (not haploids) in *Arachis* species (Mroginski & Fernandez, 1980). The reports are summarised in Table 5.

However, *Cocos nucifera, Gossypium barbadense, G. davidsonii, G. hirsutum, G. sturtii, Helianthus, Linum* are some oil yielding crops in which in vivo polyembryony has

Table 5. Haploid Production from Anther and Pollen Culture of Oil Crops

Crop	Response	Reference
Castor *Ricinus communis*	No report	
Coconut *Cocos nucifera*	No reports	
Cotton *Gossypium arboreum*		
G. barbadense	Haploid and diploid callus	Barrow *et al.*, 1978
G. barbadense	Callus; roots	Stanitsky, 1975
Groundnut *Arachis hypogaea*	Callus	Martin & Rabechault, 1976. Mroginski & Fernandez, 1979
A. villosa	Callus	Mroginski & Fernandez, 1980
A. correntina	Plantlets (not haploid)	Mroginski & Fernandez, 1980
A. ligneia	Plantlets (not haploid)	Mroginski & Fernandez, 1980
Arachis sp	Plantlets (not haploid)	Mroginski & Fernandez, 1980
A. glabrata	Callus, embryoid/shoots	Bajaj *et al.*, 1980
Linseed *Linum sertatissimum*	No reports	
Olive *Olea europea*	No reports	
Oil palm *Elaeis guineensis*	No reports	
Rapeseed *B. campestris*	Callus; plants	Keller *et al.*, 1975. Keller & Armstrong, 1977
	Haploids in high frequencies by higher temp treatment	Keller & Armstrong, 1978, 1979
B. napus	Differential haploid frequencies in different cultivars Embryogenesis	Stringam & Downey, 1973 Thomas & Wenzel, 1975
Sesame *Sesamum indicum*	callus	Sastri, D. C., unpublished

been reported in connection with haploidy (Riley, 1974). This suggests that haploidy in these crops is possible, and therefore it should be purposefully exploited.

PROTOPLASTS: ISOLATION, CULTURE AND FUSION

Isolation and culture of protoplasts is a fairly recent method which has been suggested as a new tool in crop improvement. Protoplasts have been isolated and cultured in cotton (Bhojwani *et al.*, 1977), linseed (Gamborg *et al.*, 1974; Gamborg and Shyluk, 1976), oil palm (Smith & Thomas, 1973), rapeseed (Kartha *et al.*, 1974b; Thomas *et al.*, 1976), sesame (Constabel, 1975) and soybean (Kao *et al.*, 1970; Oswalt *et al.*, 1977; Zeig & Outka, 1980). Protoplast isolation in safflower was attempted but was not satisfactory (Hughes *et al.*, 1978). In spite of successful isolation in these plants, regeneration has only been obtained in rapeseed (Kartha *et al.*, 1974c; Thomas *et al.*, 1976; Schenck & Hoffman, 1979).

Rapeseed protoplasts have been fused with those of soybean (Kartha *et al.*, 1974b; Gamborg, 1977) and with those of *Arabidopsis thaliana* (Gleba & Hoffman, 1979). In the latter fusion product, roots (Gleba *et al.*, 1978) and flowering plants called *"Arabidobrassica"* although sterile, have been generated (Gleba & Hoffman, 1980). Incidentally this is the first case of an intergeneric – intertribal hybrid. Soybean protoplasts have been fused with those of barley, corn, pea, sweet clover, alfalfa and tobacco (Gamborg, 1977) and heterokaryocytes in these somatic hybrids have been observed. In his review Bajaj (1977) has mentioned that aggregation of protoplasts was achieved mechanically in *Arachis hypogaea* and *Glycine max* (Michell, 1939; Schenk & Hildebrandt, 1971), in *Brassica chinensis* by some proteins (Kameya, 1973) and immunologically in *Glycine max* (Hartman *et al.*, 1973) (see Bajaj's paper in this proceedings).

These fusion products have been confirmed cytologically and by isoenzyme studies; for example in *Nicotiana – Glycine* hybrids (Wetter, 1977; Wetter & Kao, 1980). Wetter & Kao (1980) have gone a step ahead in 'backfusing' the *Glycine max – Nicotiana glauca* for stabilization of these hybrids. Table 6 gives an account of investigations on protoplasts of oil seed crops.

Table 6. Protoplast Isolation and Fusion Studies

Crop	Protoplasts isolated and cultured	Plants regenerated	Protoplasts fused with
Castor	None		
Coconut	None		
Cotton	Bhojwani *et al.*, 1977		
Groundnut	None		
Linseed	Cheerano, 1988; Gamborg *et al.*, 1974; Gamborg & Shyluk, 1976		Sphaero *et al.*, 1980
Olive	None		
Oil palm	Smith & Thomas, 1973		
Rapeseed	Kartha *et al.*, 1974b; Thomas *et al.*, 1976	Kartha *et al.*, 1974c; Schenk & Hoffman, 1979; Thomas *et al.*, 1976	Soybean (Kartha *et al.* 1974b; Gamborg, 1977); *Arabidopsis thaliana* (Gleba & Hoffman, 1978, 1980)
Sesame	Constabel, 1975		
Safflower	Hughes *et al.*, 1978		
Soybean	Kao *et al.*, 1970; Reid & Gelman, 1975; Oswalt *et al.*, 1977; Zeig & Outka, 1980		Barley, corn, pea, sweet clover alfalfa, rapeseed, tobacco and others (Gamborg, 1977) *Nicotiana* glauca; chromosome and isoenzyme behaviour of hybrids (Wetter, 1977; Wetter & Kao, 1980)
Sunflower	None		

Table 7. Miscellaneous Applications of Tissue Culture of Oil Crops

	Organ/tissue	Application	Reference
Castor	endosperm	to study growth and metabolism of isolated tissue	Bhojwani *et al.*, 1970; Jalan & Srivastava, 1972; Banerjee & Mishra Rao, 1986
	callus	to study pesticide metabolism	Sanderman *et al.*, 1977
Cotton	callus suspension	to study pesticide metabolism	Sanderman *et al.* 1977
	nucellus	to investigate the absence of nucellar polyembryony	Batra & Rangaswamy, 1980
Linseed	protoplasts	freeze preservation	Chatterjee, 1988
	callus	interaction and association of obligate pathogens	Ingram, 1977
		growth of free cells on agar plates	Mishra, 1972
Groundnut	mesophyll cell suspension	requirements for culture	Bell & Joshi, 1986; Joshi & Nagpal, 1967; Joshi & Bell, 1968; Verma & van Huystee, 1971b; Kumar, 1974 a, b, c
	mesophyll cell suspension	activities of catalase and peroxidase in cell groups of different sizes	Verma & van Huystee, 1970; Mastrandrea & van Huystee, 1980; Kossatz & van Huystee, 1976
	mesophyll cell suspension	immunological identification of enzymes in media	van Huystee, 1976; Srivastava & van Huystee, 1973
	mesophyll cell suspension	inhibition of cell division and altered protein synthesis due to ionizing radiation	van Huystee & Turcan, 1973
	mesophyll cell suspension	giant cells after irradiation with ionizing rays	Verma & van Huystee, 1971a
	mesophyll cell suspension	polyphenol accumulation	Rao & Mishra, 1980
Oil palm	embryo	investigate factors for dormancy	Rabechault, 1967; Rabechault & Ahee, 1986; Rabechault *et al.*, 1968, 1969
Rapeseed	callus	demonstrate association with rhizobium and nitrogenase activity	Child, 1976
		interaction and association of obligate parasites	Ingram, 1977; Sacvan & Hoffman, 1979
		effect of age, light and aeration on lipid synthesis in suspension culture	Mongold, 1977
Safflower	suspension culture	pesticide metabolism	Sanderman *et al.*, 1977
Sesame	callus from seed	isolation of a glycoside, pedalin	Khanna & Jain, 1973; Jain & Jain, 1980
Soybean	callus	freeze preservation	Bajaj, 1976
		demonstrate association with Rhizobium and nitrogenase activity	Child & Le Rue, 1974; Dowry, 1977
		assay of 6-benzylamino purine and its ribosyde and ribotide derivatives	van Staden, 1973
	embryo	disease free plants	Braverman, 1975
	suspension culture	herbicide metabolism	Dave & Shivnabhusuna, 1980; Dave *et al.*, 1977
		pesticide metabolism	Sanderman *et al.*, 1977
		Lindane (insecticide) metabolism	Stockigt & Rietz, 1977
		lipid synthesis	Mongold, 1977
		direct and indirect effect of gamma rays	Bajaj, 1971
		analysis of macromolecular complex in medium during culture	Moore, 1973
		DNA replication in isolated nuclei	Roman *et al.*, 1980
		requirements for culture	Blaydon, 1966; Gamborg *et al.*, 1968; Paskert & Short, 1973
Sunflower	callus	interaction and association with obligate pathogens	Ingram, 1977
	crown gall infected and non-infected tissue cultures	biochemical changes of infection *in vitro* and *in vivo*	Reddy & Stahman, 1973

MISCELLANEOUS APPLICATIONS

Plant cell cultures have often been used for mutation studies and isolation of desirable mutants. But so far such investigations have not had much impact on applied biology, and have been largely of interest only to fundamental biologists. However, a few reports do exist for oil seed crops (Table 7). From soybean suspension cultures, 5-bromodeoxyuridine resistant lines (Ohyama, 1974a; 1974b) and 8-azaguanine resistant lines (Weber & Lark, 1979) have been reported. Weber and Lark (1979) have even described an efficient plating system for rapid isolation of mutants. Cytochimeras of *Brassica* spp. (Horak, 1972; Horak *et al.*, 1975) have been produced. Coconut tissue cultures have been used for induced mutation studies (Guzman *et al.*, 1976).

Reddy and Stahman (1973) compared the isozyme patterns of crown gall and bacteria free gall tissue cultures with infected and non-infected stems and non-infected tissue cultures of sunflower to study methods of infection *in vitro* and *in vivo*.

Cell suspensions have also been used by biochemists to study metabolism of pesticides in cotton, safflower, sunflower (Sanderman et al., 1977), of insecticides (Stockigt

& Riess, 1977) and of herbicides (Davis & Shimabukuro, 1980; Davis *et al.*, 1980), and synthesis of lipids in soybean and rapeseed (Mangold, 1977) or isolation of pedalin, a glycoside in *Sesamum indicum* tissue cultures (Khanna & Jain, 1973; Jain & Jain, 1980; see also Butcher, 1977). Interaction and association of certain *Rhizobium* strains with cell suspension of soybean, a legume (Child & La Rue, 1974) were compared with those for *Brassica napus*, a non-legume (Child, 1975; see Giles & Vasil, 1980). Similarly, interaction of certain obligate pathogens with cultures of sunflower, rape and linseed have been reported (Ingram, 1977; Sacristan & Hoffman, 1979).

Quatrano (1968) and Bajaj (1976) based on their studies on flax protoplasts and soybean cells respectively, have suggested the freeze-preservation and use as gene banks. Various aspects of cryopreservation and its applications have been reviewed by Bajaj & Reinert (1977) and Baja (1979b). In recent papers, Withers (1980) and Kartha (1981) review meristem with no reference to oil seed crops indicating the lack of attention to these crops.

TISSUE CULTURE IN *ARACHIS HYPOGAEA*

Review of Literature: From a survey of the literature on groundnut tissue cultures it is apparent that progress has been limited to optimizing the conditions for callus growth and root induction, though some rare plants from ovule cultures *in vitro*, pod formation in gynophore tip cultures and a few non-haploid plants from anther cultures have been produced. These few attempts and reports to date are summarised below.

Tissue and Organ Cultures: Harvey & Schulz (1943) and Nuchowiz (1955) were probably the first to initiate such studies by using embryos or embryo segments. A decade after that, the next report of root formation in groundnut pericarp cultures appeared (Rangaswamy *et al.*, 1965). Following this was the report of formation of some viable plants from culture of *A. hypogaea* ovules measuring 0.3 mm (Martin, 1970). This opened up the possibilities of ovule culture from incompatible crosses but no further attempts were made although interspecific incompatibilities in the genus were known. Ziv & Zamski (1975) showed that cultured gynophore tips could exhibit three major kinds of responses viz., callus formation at the cut surfaces, gynophore elongation and its geotropic curvature, and swelling of the ovary followed by pod formation. The responses largely depended upon the availability or non-availability of light, concentrations and ratios of kinetin to naphthalene acetic acid, and the orientation of the explant with respect to surface of the medium and the gravitational force. Kumar (1974a) studied growth and chlorophyll development during callus formation from mesophyll tissues cultured on a sugar-free medium. Using hypocotyl and cotyledon segments, Kumar (1974b; 1974c) investigated different concentrations of some vitamins, iron salts and magnesium sulphate for optimum callus growth.

In 1975, Braverman suggested aseptic embryo culture as a means of getting virus free plants and thus improving the phytosanitation of plant introductions.

Mroginski & Fernandez (1979) cultured anthers of *A. villosa* and *A. correntina*, both wild diploid species and *A. hypogaea*, at various stages of microsporogenesis on media with different combinations of naphthalene acetic acid and benzyl aminopurine and found MS BAP 0.5 NAA 2 inositol 100 was best for maximum callus production, mostly from sporophytic tissue. In a later report they found plants (with 2n=20) when calli from anther cultures of *A. lignosa* and *A.* sp. (both diploid) were transferred from MS NAA 2 BAP 0.5 to MS BAP 0.01. Bajaj *et al.*, (1980) found callus formation and early pollen embryogenesis and multicellular pollen grains from anthers of *A. hypogaea* and *A. glabrata* (both tetraploid) cultured on MS IAA 4 Kn 2.

During the last five years the technique of cotyledon cultures has been used in three cultivars (Guy *et al.*, 1978) largely for optimizing callus growth and for the identification of biochemical parameters. This was the first report on cultivar specific differences in aseptic cultures. Guy *et al.*, (1980) reported partial morphogenesis in "sterile" cotyledon cultures (authors' inverted commas). They induced root formation in callus by modifying the MS medium. Russo & Varnell (1977) obtained elongation of shoot meristem and callus formation depending upon the hormones. Bajaj's (1979a) successful report on culture of freeze-preserved shoot meristems opened up the possibility of germplasm preservation and minimizing quarantine problems.

Cell Culture: Groundnut cell cultures were initiated by Ball & Joshi (1965) using mesophyll tissue. By modification of the medium Joshi & Noggle (1967) suggested that the mesophyll cells could synthesize their own metabolites from mineral nutrients among which ammonia seems to be of crucial importance. Subsequently, cell cultures were obtained from cotyledons and used for biochemical and morphological investigations. Jullian (1970) obtained a preparation of free cells by mechanical means as a first step to protoplast isolation. In these suspension cultures the cell sizes and the colony sizes were highly variable. Cell groups of different sizes differed qualitatively and quantitatively in peroxidase and catalase activities (Verma & van Huystee, 1970), so a balanced growth of these cell-derived populations in cultures was doubtful (Verma & van Huystee, 1971) and is yet to be achieved. Van Huystee and his coworkers later observed that cell suspension cultures derived from cotyledonary cells released certain macromolecular complexes, in which peroxidase isozymes have been identified immunologically (van Huystee, 1976; Srivastava & van Huystee, 1973). Maldonado & van Huystee (1980) have found that a medium supporting the growth of groundnut cells in suspension was a rich source of peroxidases which could be used for studies on species relationships. High doses of ionizing radiations (500 kr) to these cultures were found to degrade these complexes, inhibit cell divisions, alter protein synthesis (van Huystee & Turcon, 1973) and induce formation of giant cells (Verma & van Huystee, 1971a). Biochemical investigations (Guy *et al.*, 1978) on growing calli from cotyledon cultures of three groundnut cultivars revealed that though most cultivar specific differences in the cotyledons were maintained by the respective callus tissues, the concentrations of high molecular weight proteins and neutral aromatic amino acids were less in calli than in cotyledons while basic amino acids were higher in calli. Recently Rao & Mehta (1980) have shown how the polyphenols accumulate in groundnut suspension cultures. Callus and roots were reported from single cell cultures of *A. hypogaea* (Yung-ru & Yu-hung, 1978).

The Genus Arachis and its Wild Species: Several expeditions into Brazil and neighbouring countries have led to the discovery of a large number of wild species related to *Arachis hypogaea*. Preliminary screening has indicated the potential of these wild species as sources of pest and disease resistance. The fungal, viral and insect pests cause a significant reduction in yields of *A. hypogaea*. Chemical control of some of these pests has been practised with good returns, but it is not a permanent solution nor is it the best approach socially and economically, particularly for the small farmer in developing countries of the semi-arid tropical regions of the world. A promising alternative, therefore, is the incorporation of genetic resistance, but with *A. hypogaea* the necessary resistances are not always available to breeders.

However, a number of wild relatives have been found to be resistant to one or more pests. The availability, cross-ability with *A. hypogaea*, ploidy differences, evolution and means of utilization of this germplasm have been recently reviewed (Gregory & Gregory, 1979; Moss, 1980; Singh *et al.*, 1980). Stalker (1980) has illustrated how wild germplasm has been profitably used in a few important crops, and has shown how tissue culture technique has helped in some cases.

A. hypogaea has many serious disease and pest problems. Immediate attention has to be paid to those listed in Table 8. The species of the genus *Arachis* are grouped into seven sections (Gregory & Gregory, 1979) and not all species have been screened against the major pathogens. Among those screened several wild species of great potential have been identified (see Table 8). Some of the compatible species which belong to the section *Arachis* have been utilized despite ploidy differences, and resistances are being transferred to *A. hypogaea* (Moss, 1980; Singh *et al.*, 1980). A number of species in other sections are highly resistant or immune to a wide range of pests (Table 8) but have not been successfully crossed with *A. hypogaea*.

Table 8. Important Pests and Diseases of *A. hypogaea* and some Sources of Resistance

Causal Organism	Disease	Source of Resistance	Section
FUNGAL			
Cercospora arachidicola	Early leafspot	*A. cardenasii*	*Arachis*
		A. chacoense	*Arachis*
		A. glabrata	*Rhizomatosae*
Cercosporidium personatum	Late leafspot	*A. cardenasii*	*Arachis*
Puccinia arachidis	Rust	*A. glabrata*	*Rhizomatosae*
		A. sp. PI No. 276233	*Rhizomatosae*
		A. sp. PI No. 262848	*Rhizomatosae*
		A. pusilla	*Trierenuleis*
		A. correntina	*Arachis*
		A. chacoense	*Arachis*
		A. cardenasii	*Arachis*
		A. duranensis	*Arachis*
VIRAL			
Tomato Spotted Wilt Virus	Bud necrosis	*A. glabrata*	*Rhizomatosae*
		A. sp. PI No. 276233	*Rhizomatosae*
		A. sp. PI No. 262848	*Rhizomatosae*
		A. pusilla	*Trierenuleis*
		A. correntina	*Arachis*
INSECT & NEMATODE			
Meloidogyne hapla (Nematode)	Root knot	*A. sp. PI No. 262786*	*Rhizomatosae*
		A. sp. PI No. 262841	*Rhizomatosae*
		A. sp. PI No. 262614	*Rhizomatosae*
		A. sp. PI No. 262844	*Rhizomatosae*
Tetranychus urticae (Spider mite)		*A. sp. PI No. 262142*	*Erectoides*
		A. pusilla	*Trierenuleis*
		A. sp. PI No. 262840	*Rhizomatosae*
		A. sp. PI No. 262786	*Rhizomatosae*
		A. sp. PI No. 262827	*Rhizomatosae*
		A. glabrata	*Rhizomatosae*
		A. sp. PI No. 262233	*Rhizomatosae*
Aphis craccivora (Aphid)		*A. chacoense*	*Arachis*
		A. glabrata	*Rhizomatosae*
		A. pusilla	*Trierenuleis*

This paper reviews initial investigations of the barriers to hybridization between the tetraploid species of the section *Arachis* and those of the section *Rhizomatosae*. Two species of the section *Arachis* used were, *A. hypogaea* cv. Robut 33-1 (2n=4x=40) and *A. monticola* (2n=4x=40), a wild species closely related to *A. hypogaea*. An unnamed collection, *A.* species P. I. No. 276233, also a tetraploid (2n=4x=40), collected from Paraguay, South America by Gregory & Krapovickas in 1961 and resistant or immune to major pathogens was crossed with the above two species which are normally incompatible with it. The studies are aimed at the use of tissue culture for large scale production of hybrids for utilisation in a breeding program.

Fruit Development in Arachis: Before attempting to produce hybrids by culture of embryos, ovules, ovaries, gynophore tips or other means, it is essential to know the *in vivo* morphogenesis and the important factors regulating the same. *Arachis* is one of the few genera showing geocarpy. It undergoes a two step process: a) gynophore elongation which continues for about two weeks; b) pod development and maturation after the ovary at the tip of the gynophore is pushed into the soil. Throughout the first step the zygote remains dormant after the initial 3 or 4 divisions, and it is only in the soil that it resumes development concomitant with pod formation.

Interspecific incompatibility: The first report of an attempted but unsuccessful cross between *A. hypogaea* and a wild species was by Hull and Carver (1938); one of the species used was *A. glabrata* Benth., a member of the section *Rhizomatosae*. Subsequently although several unsuccessful attempts were made in crossing *A. hypogaea* with species of *Rhizomatosae*, no effort was made to study the mechanism of incompatibility, though Johansen and Smith (1956) attributed the failure of seed set in *A. hypogaea* x *A. diogoi* to retarded growth and degeneration of the embryo accompanied by hypertrophy of integuments, thus destroying the fusion product. They suggested a similar reason for failure of the *A. hypogaea* x *A. glabrata* cross (Johansen & Smith, 1956). From this information it is possible that embryos from such ovules could be grown to maturity by dissecting them out and providing a suitable environment *in vitro*. This was not attempted until recently for interspecific crosses in *Arachis* although employed in a number of other crops.

Pegs were rarely produced in incompatible crosses. Initial investigations by fluorescent microscopy on the behaviour of *Rhizomatosae* pollen on *A. hypogaea* pistils, revealed that pollen germination on the stigma was normal and the pollen tubes penetrated into the stigma. However, the callose deposits were larger and more frequent than in the compatible pollen tubes, and growth of incompatible tubes was mostly inhibited in the style. Pollen tubes rarely reached the ovary, when fertilization can occur followed by a slight elongation of the gynophore. These gynophores soon dried up and degenerated (Singh *et al.*, 1980). Several techniques to overcome incompatibility were tried among which application of growth hormones to the bases of incompatibly pollinated flowers was encouraging (Singh *et al.*, 1980). This substantially increased the percentage of gynophores which developed slowly and rarely set mature pods. Some of them do develop to a stage when the immature embryos could be conveniently excised and cultured for raising hybrid plants.

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Pegs were rarely produced in incompatible crosses. Initial investigations by fluorescent microscopy on the behaviour of *Rhizomatosae* pollen on *A. hypogaea* pistils, revealed that pollen germination on the stigma was normal and the pollen tubes penetrated into the stigma. However, the callose deposits were larger and more frequent than in the compatible pollen tubes, and growth of incompatible tubes was mostly inhibited in the style. Pollen tubes rarely reached the ovary, when fertilization can occur followed by a slight elongation of the gynophore. These gynophores soon dried up and degenerated (Singh *et al.*, 1980). Several techniques to overcome incompatibility were tried among which application of growth hormones to the bases of incompatibly pollinated flowers was encouraging (Singh *et al.*, 1980). This substantially increased the percentage of gynophores which developed slowly and rarely set mature pods. Some of them do develop to a stage when the immature embryos could be conveniently excised and cultured for raising hybrid plants.

& Riess, 1977) and of herbicides (Davis & Shimabukuro, 1980; Davis *et al.*, 1980), and synthesis of lipids in soybean and rapeseed (Mangold, 1977) or isolation of pedalin, a glycoside in *Sesamum indicum* tissue cultures (Khanna & Jain, 1973; Jain & Jain, 1980; see also Butcher, 1977). Interaction and association of certain *Rhizobium* strains with cell suspension of soybean, a legume (Child & La Rue, 1974) were compared with those for *Brassica napus*, a non-legume (Child, 1975; see Giles & Vasil, 1980). Similarly, interaction of certain obligate pathogens with cultures of sunflower, rape and linseed have been reported (Ingram, 1977; Sacristan & Hoffman, 1979).

Quatrano (1968) and Bajaj (1976) based on their studies on flax protoplasts and soybean cells respectively, have suggested the freeze-preservation and use as gene banks. Various aspects of cryopreservation and its applications have been reviewed by Bajaj & Reinert (1977) and Baja (1979b). In recent papers, Withers (1980) and Kartha (1981) review meristem with no reference to oil seed crops indicating the lack of attention to these crops.

TISSUE CULTURE IN *ARACHIS HYPOGAEA*

Review of Literature: From a survey of the literature on groundnut tissue cultures it is apparent that progress has been limited to optimizing the conditions for callus growth and root induction, though some rare plants from ovule cultures *in vitro*, pod formation in gynophore tip cultures and a few non-haploid plants from anther cultures have been produced. These few attempts and reports to date are summarised below.

Tissue and Organ Cultures: Harvey & Schulz (1943) and Nuchowiz (1955) were probably the first to initiate such studies by using embryos or embryo segments. A decade after that, the next report of root formation in groundnut pericarp cultures appeared (Rangaswamy *et al.*, 1965). Following this was the report of formation of some viable plants from culture of *A. hypogaea* ovules measuring 0.3 mm (Martin, 1970). This opened up the possibilities of ovule culture from incompatible crosses but no further attempts were made although interspecific incompatibilities in the genus were known. Ziv & Zamski (1975) showed that cultured gynophore tips could exhibit three major kinds of responses viz., callus formation at the cut surfaces, gynophore elongation and its geotropic curvature, and swelling of the ovary followed by pod formation. The responses largely depended upon the availability or non-availability of light, concentrations and ratios of kinetin to naphthalene acetic acid, and the orientation of the explant with respect to surface of the medium and the gravitational force. Kumar (1974a) studied growth and chlorophyll development during callus formation from mesophyll tissues cultured on a sugar-free medium. Using hypocotyl and cotyledon segments, Kumar (1974b; 1974c) investigated different concentrations of some vitamins, iron salts and magnesium sulphate for optimum callus growth.

In 1975, Braverman suggested aseptic embryo culture as a means of getting virus free plants and thus improving the phytosanitation of plant introductions.

Mroginski & Fernandez (1979) cultured anthers of *A. villosa* and *A. correntina*, both wild diploid species and *A. hypogaea*, at various stages of microsporogenesis on media with different combinations of naphthalene acetic acid and benzyl aminopurine and found MS BAP 0.5 NAA 2 inositol 100 was best for maximum callus production, mostly from sporophytic tissue. In a later report they found plants (with 2n=20) when calli from anther cultures of *A. lignosa* and $A_{.}$ sp. (both diploid) were transferred from MS NAA 2 BAP 0.5 to MS BAP 0.01. Bajaj *et al.*, (1980) found callus formation and early pollen embryogenesis and multicellular pollen grains from anthers of *A. hypogaea* and *A. glabrata* (both tetraploid) cultured on MS IAA 4 Kn 2.

During the last five years the technique of cotyledon cultures has been used in three cultivars (Guy *et al.*, 1978) largely for optimizing callus growth and for the identification of biochemical parameters. This was the first report on cultivar specific differences in aseptic cultures. Guy *et al.*, (1980) reported partial morphogenesis in "sterile" cotyledon cultures (authors' inverted commas). They induced root formation in callus by modifying the MS medium. Russo & Varnell (1977) obtained elongation of shoot meristem and callus formation depending upon the hormones. Bajaj's (1979a) successful report on culture of freeze-preserved shoot meristems opened up the possibility of germplasm preservation and minimizing quarantine problems.

Cell Culture: Groundnut cell cultures were initiated by Ball & Joshi (1965) using mesophyll tissue. By modification of the medium Joshi & Noggle (1967) suggested that the mesophyll cells could synthesize their own metabolites from mineral nutrients among which ammonia seems to be of crucial importance. Subsequently, cell cultures were obtained from cotyledons and used for biochemical and morphological investigations. Jullian (1970) obtained a preparation of free cells by mechanical means as a first step to protoplast isolation. In these suspension cultures the cell sizes and the colony sizes were highly variable. Cell groups of different sizes differed qualitatively and quantitatively in peroxidase and catalase activities (Verma & van Huystee, 1970), so a balanced growth of these cell-derived populations in cultures was doubtful (Verma & van Huystee, 1971) and is yet to be achieved. Van Huystee and his coworkers later observed that cell suspension cultures derived from cotyledonary cells released certain macromolecular complexes, in which peroxidase isozymes have been identified immunologically (van Huystee, 1976; Srivastava & van Huystee, 1973). Maldonado & van Huystee (1980) have found that a medium supporting the growth of groundnut cells in suspension was a rich source of peroxidases which could be used for studies on species relationships. High doses of ionizing radiations (500 kr) to these cultures were found to degrade these complexes, inhibit cell divisions, alter protein synthesis (van Huystee & Turcon, 1973) and induce formation of giant cells (Verma & van Huystee, 1971a). Biochemical investigations (Guy *et al.*, 1978) on growing calli from cotyledon cultures of three groundnut cultivars revealed that though most cultivar specific differences in the cotyledons were maintained by the respective callus tissues, the concentrations of high molecular weight proteins and neutral aromatic amino acids were less in calli than in cotyledons while basic amino acids were higher in calli. Recently Rao & Mehta (1980) have shown how the polyphenols accumulate in groundnut suspension cultures. Callus and roots were reported from single cell cultures of *A. hypogaea* (Yung-ru & Yu-hung, 1978).

The Genus Arachis and its Wild Species Several expeditions into Brazil and neighbouring countries have led to the discovery of a large number of wild species related to *Arachis hypogaea*. Preliminary screening has indicated the potential of these wild species as sources of pest and disease resistance. The fungal, viral and insect pests cause a significant reduction in yields of *A hypogaea* Chemical control of some of these pests has been practised with good returns, but it is not a permanent solution nor is it the best approach socially and economically, particularly for the small farmer in developing countries of the semi-arid tropical regions of the world A promising alternative, therefore, is the incorporation of genetic resistance, but with *A hypogaea* the necessary resistances are not always available to breeders

However, a number of wild relatives have been found to be resistant to one or more pests The availability, cross-ability with *A. hypogaea*, ploidy differences, evolution and means of utilization of this germplasm have been recently reviewed (Gregory & Gregory, 1979, Moss, 1980, Singh *et al*, 1980) Stalker (1980) has illustrated how wild germplasm has been profitably used in a few important crops. and has shown how tissue culture technique has helped in some cases

A. hypogaea has many serious disease and pest problems Immediate attention has to be paid to those listed in Table 8 The species of the genus *Arachis* are grouped into seven sections (Gregory & Gregory, 1979) and not all species have been screened against the major pathogens. Among those screened several wild species of great potential have been identified (see Table 8) Some of the compatible species which belong to the section *Arachis* have been utilized despite ploidy differences, and resistances are being transferred to *A. hypogaea* (Moss, 1980, Singh *et al.*, 1980) A number of species in other sections are highly resistant or immune to a wide range of pests (Table 8) but have not been successfully crossed with *A. hypogaea*.

Table 8. Important Pests and Diseases of *A. hypogaea* and some Sources of Resistance

Causal Organism	Disease	Source of Resistance	Section
FUNGAL			
Cercospora arachidicola	Early leafspot	*A cardenasii*	*Arachis*
		A chacoense	*Arachis*
		A glabrata	*Rhizomatosae*
Cercosporidium personatum	Late leafspot	*A cardenasii*	*Arachis*
Puccinia arachidis	Rust	*A glabrata*	*Rhizomatosae*
		A sp P I No 276233	*Rhizomatosae*
		A sp P I No 262848	*Rhizomatosae*
		A pusilla	*Trierectoides*
		A correntina	*Arachis*
		A chacoense	*Arachis*
		A cardenasii	*Arachis*
		A duranensis	*Arachis*
VIRAL			
Tomato Spotted Wilt Virus	Bud necrosis	*A glabrata*	*Rhizomatosae*
		A sp P I No 276233	*Rhizomatosae*
		A sp P I No 262848	*Rhizomatosae*
		A pusilla	*Trierectoides*
		A correntina	*Arachis*
INSECT & NEMATODE			
Meloidogyne hapla (Nematode)	Root knot	*A* sp P I No 262786	*Rhizomatosae*
		A sp P I No 262841	*Rhizomatosae*
		A sp P I No 262814	*Rhizomatosae*
		A sp P I No 262844	*Rhizomatosae*
		A sp P I No 262142	*Erectoides*
Tetranychus urticae (Spider mite		*A pusilla*	*Trierectoides*
		A sp P I No 262840	*Rhizomatosae*
		A sp P I No 262786	*Rhizomatosae*
		A sp P I No 262827	*Rhizomatosae*
		A glabrata	*Rhizomatosae*
		A sp P I No 267233	*Rhizomatosae*
Aphis craccivora (Aphid)		*A chacoense*	*Arachis*
		A glabrata	*Rhizomatosae*
		A pusilla	*Trierectoides*

This paper reviews initial investigations of the barriers to hybridization between the tetraploid species of the section *Arachis* and those of the section *Rhizomatosae*. Two species of the section *Arachis* used were, *A. hypogaea* cv. Robut 33-1 (2n=4x=40) and *A. monticola* (2n=4x=40), a wild species closely related to *A. hypogaea*. An unnamed collection, *A.* species P. I. No. 276233, also a tetraploid (2n=4x=40), collected from Paraguay, South America by Gregory & Krapovickas in 1961 and resistant or immune to major pathogens was crossed with the above two species which are normally incompatible with it The studies are aimed at the use of tissue culture for large scale production of hybrids for utilisation in a breeding program

Fruit Development in Arachis Before attempting to produce hybrids by culture of embryos, ovules, ovaries, gynophore tips or other means, it is essential to know the *in vivo* morphogenesis and the important factors regulating the same. *Arachis* is one of the few genera showing geocarpy. It undergoes a two step process a) gynophore elongation which continues for about two weeks, b) pod development and maturation after the ovary at the tip of the gynophore is pushed into the soil Throughout the first step the zygote remains dormant after the initial 3 or 4 divisions, and it is only in the soil that it resumes development concomitant with pod formation

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Pegs were rarely produced in incompatible crosses Initial investigations by fluorescent microscopy on the behaviour of *Rhizomatosae* pollen on *A. hypogaea* pistils, revealed that pollen germination on the stigma was normal and the pollen tubes penetrated into the stigma However, the callose deposits were larger and more frequent than in the compatible pollen tubes, and growth of incompatible tubes was mostly inhibited in the style Pollen tubes rarely reached the ovary, when fertilization can occur followed by a slight elongation of the gynophore These gynophores soon dried up and degenerated (Singh *et al*, 1980) Several techniques to overcome incompatibility were tried among which application of growth hormones to the bases of incompatibly pollinated flowers was encouraging (Singh *et al.*, 1980). This substantially increased the percentage of gynophores which developed slowly and rarely set mature pods Some of them do develop to a stage when the immature embryos could be conveniently excised and cultured for raising hybrid plants.

TISSUE CULTURE AND HYBRIDS FROM INCOMPATIBLE CROSSES

Embryo Culture: Dissection and culture of immature embryos from undeveloped pods has been routinely done and hybrid plants obtained. These embryos germinated precociously on a range of media from simple, Knop's agar medium or White's (1943) medium, to complex Murashige and Skoog's (1962) medium. The cultures were kept in 8h day length at 25+3°C. The rate of germination of these embryos depended largely upon the stage of development at the time of culture. Very young embryos, heart shaped or early cotyledonary stages formed a little callus before producing shoots. The most developed among the cultured embryos were from physiologically mature pods; embryos from these germinated readily and formed healthy plants in 3 to 4 weeks (Fig. 1a). Optimum conditions for culture of embryos at various stages of development are being worked out.

Ovule Culture: We have been interested in culturing ovules excised from gynophores before they penetrate the soil and hence before the start of pod formation. Martin (1970) reported the possibility of obtaining plants from ovule cultures. Sastri *et al.*, (1980) found that Martin's medium was not all that satisfactory; only little callus, which soon became necrotic, was observed, and no plants were formed. Since then other media, varying with respect to auxin, kinetin, gibberellin and casein hydrolysate concentrations, have been tried but with no significant improvement in response.

Ovary Culture: Another possibility of obtaining hybrids could be to culture whole ovaries with ovules containing the gametic fusion product. In our attempts with cultures of ovaries 2–4 days after pollination (by which time fertilization had occurred) we found that the explants cultured on MS Kn 2 IAA 2 formed massive calli which occasionally produced a few shoot like structures and/or roots (Sastri *et al.*, 1980).

In fact Ziv and Zamski (1975) reported pod formation *in vitro* from culture of gynophore tips which included ovaries. Our efforts to repeat this work using *A. monticola* and *A. hypogaea* gynophores have not been very satisfactory. We have observed, in a small percentage of cultures, elongation and geotropic curving of the gynophore and callus formation at the injured parts of the gynophores. On WM Kn 3 there was no growth in pericarp tissues but ovules increased in size and emerged from the pericarp tissues. Subsequently, at the injured regions of the pericarp tissues, callus initiation was seen. Our observations thus differ from those of Ziv & Zamski (1975) in that we found swelling of the ovules only, while the latter reported an overall growth of ovary and concomitant ovule development thus showing an overall pod development as might happen under natural conditions.

CLONAL PROPAGATION

Plantlet formation in groundnut tissue cultures has been a rare and an inconsistent observation in the literature. From the account that follows it will be clear that organogenesis, shoot formation in particular, can be freely induced and complete plants obtained from cultures of a wide range of explants.

Cotyledon Culture: Cotyledon has been the most frequently used organ for establishment of callus and cell suspension cultures which have been used often for biochemical investigations. These aspects have been summarised earlier. Regarding differentiation from cotyledon cultures, only rhizogenesis has been reported recently (Guy *et al.*, 1980). From our experiments with cotyledon cultures, it emerges that this is the best explant for obtaining a large number of plants.

From mature dry seeds, cotyledons were severed from the embryos and each cotyledon was then implanted in one test tube. Murashige and Skoog's medium was tried, with various supplements of auxin and kinetin. Most of the combinations induced callus formation, which is generally localized at the cut ends i.e. the nodal ends of the cotyledons. Some media inducing the formation of shoot buds and shoots on the callus (in decreasing order of responsiveness) are: MS Z4; MS Kn 4 (Fig. 1d); MS Kn 2 IAA 1 GA 1. Roots or plants have been obtained on different compositions of the media.

Callus formation and subsequent caulogenesis occurs within 10 days after culture. Shoots are initiated in large numbers very rapidly and they can be rooted to obtain plants. Although this is a two step process, it has the advantage of a larger number of plants per explant than single step induction of plants from cotyledon cultures. Whether the cotyledon-derived plants are identical to the embryo-derived plants or not, is yet to be determined, but because of the rapidity with which the shoots are given out, plants from cotyledon culture are expected to be identical to embryo or seed-derived plants. Of the explants tried, cotyledons are suggested as the best source for rapid clonal propagation. Cotyledons have been cultured from immature seeds formed in an incompatible cross, viz., *A. monticola* x *A.* sp. P. I. No. 276233. These too have given rise to plants, which indicates that cotyledons can indeed be cultured for rapid clonal propagation of hybrids.

Leaf Culture: Leaflet segments at various developmental stages from groundnut plants of different ages were used as explants. Using mature leaflet segments from plants of any age, it was possible to induce calli in both MS liquid and agar media. The callus appeared faster at the cut ends than on the lamina itself. The liquid media were better suited for faster callus production than semi-solid media. Leaflets at earlier stages of development tended to respond faster than mature leaflets. However, within 7 days, cultures of young leaflet segments from germinating seeds and mature plants developed calli randomly on the surface, subsequently masking the original tissue with further growth of callus within about 2 weeks of culture on MS NAA 2 BAP 0.5 (Fig. 1b, e). These calli gave rise to a large number of shoot buds, which grew at different rates, some becoming considerably larger than others (Fig. 1b), but even the smallest grew satisfactorily when they were individually subcultured onto fresh media. A similar observation has been recently made by Mroginski *et al* (1981)

The kinds of auxin and kinetin and their concentrations required for optimum results are being further investigated. Temporal separation of the callus formation from subsequent caulogenesis or embryogenesis would facilitate a variety of experiments such as cell and protoplast

cultures, differentiation from them, somatic fusion and mutation.

Flower Bud Culture: In our attempts to give hormone pre treatment to flower buds for anther culture and haploid production, one medium, MS Kn 1 NAA 0.2 without agar, stimulated callusing from *A. monticola* flower bud surfaces, including the cut bases. Foliar structures developed from these cultures.

Shoot Meristem Culture: During the last few years there has been a rapidly growing interest in culture of shoot meristems. The attraction of this work is that they offer a wider range of benefits including rapid multiplication, and the plants thus obtained can be disease free thereby minimising quarantine problems during plant introductions across various countries. Once regeneration from these is achieved for any species, they could be lyophilized and used for germplasm preservation, as has been suggested and done for a wide range of plants (Withers, 1980). Initial results show that regeneration from shoot meristems even as small as 2 mm pose no problem. Callus and then multiple shoot buds were formed on MS BAP 1 NAA 2 (Fig. 1c) and MS IAA 1 Kn 2 GA 1. Experiments to subculture and obtain complete regeneration from each of these buds are in progress. Once established, this technique could indeed be of great advantage to all Genetic Resources Units, but we must know for what length of time the shoot meristems or calli can be preserved without any appreciable loss in their regenerative capacity. Bajaj (1979a) has already shown that groundnut shoot tips can be preserved for at least 27 days in liquid nitrogen, and successfully cultured subsequently.

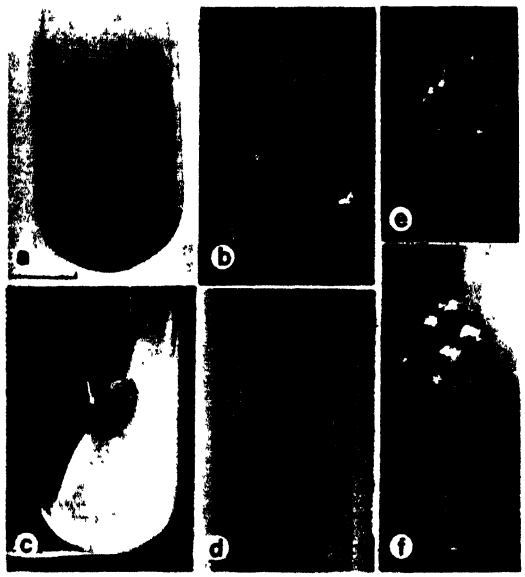

Fig. 1a–f: Embryo and tissue cultures in Arachis. a) Arachis monticola x A. glabrata, immature embryo culture; 2 weeks on White's medium. Plantlet. b) A. hypogaea, young leaflet culture of mature plant; 7 weeks on MS NAA 2.0 BAP 0.5. c) A. hypogaea, shoot tip culture; 9 weeks on MS NAA 2.0 BAP 1.0. d) A. hypogaea, de-embryonated cotyledon culture; 9 weeks on MS Kn 4.0. Developed shoots. e) A. pusilla, culture from young leaflet of young seedling; 2 weeks of MS NAA 2.0 BAP 0.5. Shoot primordia. f) A. pusilla, root disc culture; 2 weeks after culture on MS NAA 2.0 BAP 0.5. Developing shoots. (Scales: all 1 cm)

Root Disc Culture. Root discs from upto 5 day old germinating seeds have been used and the results have been very encouraging. As with the other explants several hormonal additives in Murashige and Skoog's medium were tried. The MS BAP 0.5 NAA 2 has given the best results so far with *Arachis hypogaea* and *A. pusilla* (a wild diploid species). Callus was formed on the cut surface of the disc in *A. pusilla,* microscopic examination of these calli revealed proembryos and several shoot primordia (Fig. 1f). Several green nodular structures were observed within 2 weeks; each of these developed into a shoot. Roots were rarely observed. The shoots were transferred to a rooting medium. *A. hypogaea* root discs formed callus and roots.

Anther Culture: Attempts have been made to culture anthers using MS as the basal medium. Mroginski & Fernandez (1979, 1980) used MS NAA 2 BAP 0.5 and obtained callus from gametophytic and sporophytic tissues in four diploid wild species and cultivated species; they found plantlet formation in *A. lignosa* and another wild species (both diploid) when calli were transferred to MS BAP 0.01 (Mroginski and Fernandez, 1980), Bajaj *et al.* (1980) cultured *A. hypogaea* and *A. glabrata* anthers, and found MS Kn 2 IAA 4 the best; but they observed only callus formation and pollen at early stages of embryogenesis. On MS NAA 2 BAP 0.5 and a few other hormone combinations we have observed callus formation and early stages of pollen embryogenesis.

CHALLENGES AND OPPORTUNITIES IN IMPROVEMENT OF OIL CROPS IMPACT OF TISSUE CULTURE TECHNOLOGY

The above account shows that tissue culturists have accepted the challenges of crop improvement and hopefully are on the verge of extending their successes to a wide range of crops. Opportunities and the methods to meet the requirements of a particular crop vary, and techniques that have been successful in one species have not been applied to all the crops, and the requirement for each may vary also.

The contribution could be far greater if currently available technology is tailored to the needs of a particular crop. In rapeseed for example great success has been achieved during the last decade. At the beginning of the last decade the importance of haploid plants was realised; now it is possible to produce a large number of haploids which have caught the attention of the breeders. There appear to be no limits to the extent of exploitation which is indicated by the production of an intergenetic hybrid, *"Arabidobrassica"* by the fusion of protoplasts of *Arabidopsis* and *Brassica*, genera of two taxonomic tribes! Test tube fertilization for desired crosses in *Brassica* can be achieved and all these new genetic resources can be propagated *in vitro* much more rapidly than can be done in the field. Because of the aseptic conditions of the test tube plants, breeders all over the world have an easier access to

Table 5. *Arachis hypogaea* Tissue & Organ Cultures; Explants, Media, and Responses

Explant and species	Medium for best response	Best response observed to date
ANTHERS		
A. hypogaea	MS BAP 0.5 NAA 2	callus from pollen and anther tissue, rarely roots, rarely embryogenesis
A. monticola		
COTYLEDONS		
A. hypogaea	MS Z 4	multiple shoots
and from incompatible	MS Kn 4	multiple shoots
crosses	MS Kn 2 IAA 1 GA 1	multiple shoots; occasionally plants
EMBRYOS*	WM, MS	plants (Fig. 1a)
A. hypogaea,		
A. hypogaea × *A. sp.* 276233		
A. monticola × *A. sp.* 276233		
A. marrisoli × *A. glabrata*,		
A. monticola × *A. pusilla*		
FLOWER BUDS		
A. monticola	MS Kn 1 NAA 0.2 (without agar)	shoots
GYNOPHORE TIPS	WM Kn 3	swelling of ovules, gastroplasm, callus at cut surfaces
A. hypogaea		
A. monticola		
IMMATURE LEAFLET SEGMENTS		
seedlings and mature plants of	MS BAP 0.5 NAA 2	callus multiple shoots (Fig. 1b)
A. hypogaea and *A. pusilla*	MS BAP 0.5 NAA 2	callus, multiple shoots (Fig. 1c)
ROOT DISCS		
A. pusilla	MS BAP 0.5 NAA 2	callus, multiple shoots (Fig. 1f)
SHOOT MERISTEM		
A. hypogaea	MS BAP 0.5 NAA 2	callus multiple shoots (Fig. 1e)
OVARIES		
A. hypogaea	MS BAP 0.5 NAA 2	callus, shoots roots
A. monticola	MS Kn 2 IAA 2	callus, shoot like structures & occasionally roots

* Hormone was applied to flowers after cross-pollination, but not after self-pollination

most of the material generated and propagated by tissue culture technique.

However, many breeders of the other oil crops are still not convinced that tissue culture technology can be of much benefit. There are certain instances where tissue culturists can claim an undisputed role in crop improvement, such as germination and propagation of the "makapuno" variety of coconut; large scale clonal propagation of oil palm *in vitro*, seed development from culture of cotton ovules at zygote stages and subsequent realization of several hitherto impossible interspecific hybrids and successful embryo cultures. We at ICRISAT have started to apply tissue culture technology to groundnut improvement, concentrating on areas of top priority decided after considering the pest and disease problems in different areas where groundnut is grown. Initial results have been encouraging for interspecific incompatible hybridization, clonal propagation, and shoot meristem culture. Induction of haploid plants is being attempted. From tables 3–7 it appears that olive, safflower, and sunflower have received very little attention and therefore tissue culturists and breeders could cooperate to assess the feasibility of improving yield in these. Possibly a few underexploited oil seed crops such as babassu palm *(Orbignya martiana)*, buffalo gourd *(Cucurbita foetidissima)*, *Caryocar* sp., *Jessenia polycarpa* and particularly jojoba should be investigated through a concerted effort and which will be justified by increased edible oil production especially in developing countries. Jojoba, *Simmondsia chinensis* has already been regenerated *in vitro* (Gladstone, 1976).

In addition to several applied aspects of tissue culture which are of immediate importance to breeders, some fundamental studies useful to breeders must be considered. Priorities are difficult to assign, but the vast losses due to pests and diseases justify the in vitro studies on details of host-pest interactions which may itself result in pest resistant genotypes, or may provide clues to induction and selection of such genotypes. Physical stresses such as drought and salinity are also being provided to tissue culture; although nothing significant has emerged so far it seems an i[illegible]t tool for selection o[illegible]ght, salinity and other physical stress tolerant lines. Similarly the *in vitro* system could be ideal for selection of *Rhizobium* strain – legume genotype associations.

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Abbreviations:

BAP, 6 Benzylaminopurine, CH, Casein hydrolysate, 2,4-D, 2,4-Dichlorophenoxy acetic acid, GA, Gibberellic acid, IAA, Indole acetic acid, Kn, Kinetin, MS, Murashige & Skooge's medium, NAA, 1-Naphthylacetic acid, WM, White's medium, Z, Zeatin Numeral suffixes with these abbreviations in the text refer to concentrations in mg per liter