ADDENDUM

RESPONSE TO THE QUERRIES RAISED BY THE FOREIGN REFEREE.*

Response to the general querries -

For a long time peanut is recognized globally as one of the protein-rich oilseed crop. But somatic embryogenesis in peanut was reported in recent past, for the first time from our laboratory (Hazra *et al.* 1989). However the explant used was immature zygotic embryo. Due to the limitation of obtaining immature embryo explants, studies on lipid status in the somatic embryos was not possible. Therefore prior to 1994 information on lipid status in somatic embryos of peanut was nil. After standardization of protocol to obtain large number of somatic embryos from mature zygotic embryos from mature zygotic embryo derived leaflets (Chengalrayan *et al.* 1994), the other studies towards synthesis and accumulation of lipid in peanut somatic embryos could be considered. Thus objectives of this research were defined to generate some background information on lipid status of the peanut somatic embryos. Following this the first report on lipid in somatic embryos of peanut (Mhaske and Hazra 1994) was published from this laboratory and the work was included in the thesis.

As mentioned in pp. 22-23 of the thesis the investigations were carried out to address the following questions:

1) Do somatic embryos of peanut produce triglycerides?
2) Can the triglyceride level be altered with altered media manipulations?
3) Does the increased accumulation of storage lipid in form of triglycerides support conversion of somatic embryos into plantlets?
4) What is the quality of the triglyceride accumulated in the somatic embryos. Is it influenced by altered media compositions?

Although the information generated is not a direct contribution to fields of lipid modification, genetic engineering or peanut biology, but it provides some background informations. On the basis of these, future studies can be extended to contribute directly to various fields mentioned.

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* - The literature referred to answer to the querries are enlisted at the end of this document. Most of these were also referred in the thesis.

The data generated on TG profile during somatic embryogenesis in peanut (described as Chapter III) is published in paper entitled "Appearance of storage lipid (triglycerides) in somatic embryos of peanut (*Arachis hypogaea* L.)" Authors: V.B. Mhaske and S. Hazra, which appeared in In Vitro Cell Dev. Biol. , vol. 30P : 113 - 116. A copy of the paper is attached at the end of the thesis.

A manuscript entitled "Influence of osmotica and abscisic acid on triglyceride accumulation in peanut somatic embryos" is accepted for publication in Plant Cell Reports after peer reviewing and revision. - Authors V.B. Mhaske, K. Chengalrayan and S. Hazra.

Both the above papers are enlisted in authors publications in p 122 of the thesis.

Responses to specific querries -

Q1) pV - The standard (SI unit) for micrograms and microliters are ug and uL. Unconventional abbreviations should not be used where an accepted SI unit is available.

Response - : As rightly suggested by the referee, more care will be taken to use accepted SI units in future documents. The abbreviations μg or μL are used in the thesis at some places but not consistently. However in the papers published / accepted in the international journals only these abbreviations were used.

Q2) p1 - et al. is an abbreviation of the Anglicized Latin words et alli meaning and others. Therefore only al. should be followed by a period.

Response - : This is an error and will be avoided in future.

Q3) p 31 - give more detail on the statistical analyses performed.

Response -: Statistical analyses - Estimations were carried out in several replicates as indicated in the thesis at appropriate places. Mean and standard deviations were determined using standard formulae as :

$$\text{mean } \bar{X} = \frac{\Sigma Xi \ (i = 1 \text{ to } n)}{n}$$

n = number of observations

$$\text{Standard deviation } s.d. = \sqrt{\frac{\Sigma(Xi-\bar{X})^2}{(n-1)}}$$

(Where Xi are the individual observations (i = 1 to n).

To compare the means of two sets, an F test was performed. This was as follows:

$$F = \frac{(s.d._1)^2}{(s.d._2)^2} \qquad (s.d._1 > sd._2)$$

where s.d.1 and s.d.2 are the standard deviations of the two sets having n_1 and n_2 observations respectively).

This calculated F value is compared with table value at (n_1-1) degrees of freedom for numerator and (n_2-1) degrees of freedom for denominator.

Since the test yielded a non significant difference between the variances,

(F_{table} being more than $F_{calculated}$); t test was performed as given below :

$$t = \frac{Abs(\bar{X1} - \bar{X2})}{s\sqrt{\frac{1}{n_1} - \frac{1}{n_2}}}$$

$$s = \frac{n_1 sd_1^2 + n_2 sd_2^2}{n_1 + n_2}$$

(X1 and X2 are the means of set 1 and set 2 respectively).

The calculated value of t was compared with table value at $(n_1 + n_2) - 2$ degrees of freedom at 5% level of significance. If $t_{calculated}$ was more than t_{table}, null hypothesis was rejected and means of two samples were treated as significantly different from one another.

(Ref. Snedecor and Cochran 1967).

Q4) p 34 - it also should be stated that this investigation addressed the question as to the effect of media composition on triglyceride content and composition.

Response - - Refer last paragraph in p 23 of General Introduction. Chapter III (pp. 32 - 43) does not describe the effect of media composition. Therefore it is not

included in the objectives of the experiment described for chapter III in page 34. The four objectives given in page 34 are only for the studies included in chapter III. However the effect of media compositions referred in the querry, is studied in the next set of experiments described in chapter IV.

Q5) p. 36 - The procedure described for hydrolysis of triglycerides to fatty acids is a sort of saponification and should hydrolyze all glycerolipids and therefore is not specific for triglycerides? The data presented in this thesis are therefore for total glycerolipids not just triglycerides. What was the purpose of alumina?

Response - In plants the glycerolipids (acyl lipids with glycerol) include (Hitchcock and Nichols 1971):

a) triglycerides, diglycerides and monoglycerides.

b) phosphoglycerides - phosphotidic acid, phosphtidyl choline, phosphotidyl ethanol amine, phosphotidyl serine, phosphotidyl glycerol etc.

c) glycosyl diglycerides - monogalactosyl and digalactosyldiglycerides, trigalactosyl diglyceride, o - acetyl galactosyl diglyceride, sulphoquinovosyldiglyceride (sulpholipid).

d) other glycerides - containing long chain alkenyl and saturated ether residues rather than acyl ester groups.

Of these different glycerolipids, triglycerides are the neutral lipids.

To assay these triglycerides, the extracted lipids are passed over activated alumina which adsorbs the polar compounds (Robyt and White 1987). Thus the polar glycerolipids enlisted above gets adsorbed on alumina and are removed from the extract by centrifugation. Therefore the purified extract contains triglycerides which are hydrolyzed (alkali saponification) and quantified furthur as described on p.36 of the thesis.

The method followed in our research has been used repeatedly to quantify triglycerides in plant tissues. This method, originally used for quantification of triglycerides from serum (Sigma diagnostics) was adopted by Feirer *et al.* (1989) to determine triglycerides from conifer calli Subsequently the same method was applied to quantify triglycerides in somatic embryos of Brassica (Avjioglu and Knox 1989), white spruce (Joy IV *et al.*1991), pecan (Burns and Wetzstein 1994) and grapevine (Faure and Aarrouf 1994).

Q6) p 50 - There have been reports on effects of ABA on oilbody processes in embryos e.g. :

Holbrook L. A. , Van Rooijen G. J. H. , Wilen R. W. and Moloney, M. M. (1991) Oil body proteins in microspore -derived embryos of Brassica napus: Hormonal, osmotic and developmental regulation of synthesis. Plant Physiol. 97, 1051-1058.

Wilen R. W. , Van Rooijen, G. J. H. , Pearce D. W. , Pharis R. P. , Holbrook L. A. and Moloney M. M. (1991) Effects of jasmonic acid on embryo-specific processes in Brassica and Linum oilseeds. Plant Physiol. 95, 399-405.

Response As mentioned in this page of the thesis , ABA is known to influence protein synthesis. Oleosin is a protein associated with formation of lipid bodies in the cells. The additional references provided by the referee (Wilen *et al.* 1991, Holbrook *et al.* 1991) shows that ABA and Jasmonic acid enhance the synthesis of oilbody protein - oleosin, in microspore derived embryos of Brassica and cultured zygotic embryos of flax. Therefore one of the possible mechanisms involved in increasing the triglycerides in the peanut somatic embryos grown in presence of ABA could be via increased synthesis of oleosins.

Q7) p 57 and elsewhere - was the triglyceride content per dry weight determined or was the water content of the fresh samples determined? If not the difference in triglyceride content per fresh weight observed may simply reflect a difference in water content and therefore are not very meaningful.

Response - This point was considered during the course of experiments included in the thesis and triglyceride was determined on dry weight basis by (an indirect method).

There is accepted literature on triglyceride estimation from fresh tissues eg. pecan (Burns and Wetzstein 1994) , conifer calli (Feirer *et al.* 1989). However keeping in view the possibility that difference in triglyceride content mg per g of fresh weight observed may simply reflect a difference in water content, triglyceride mg per g of dry weight (TG dw) was determined.

The approach used for determination of TG per g of dry weight is elaborated in page 54 and 55 of the thesis. Initially triglyceride content per g fresh weight (TG fw). was estimated. Since a destructive method was followed for this

estimation, dry weight per g of fresh weight (dw/fw) of the peanut somatic embryos was determined in embryo explants grown in identical conditions in parallel experment The TG dw was determined from these two sets of data (TG/fw and dw/fw).

Consequently, as per our assumption the triglyceride values did show a difference when calculated per gram of fresh and dry weight due to difference in water content (discussed on pp. 71 - 73 and figure 4.9).

Q8) p.66 and elsewhere - there was germination without conversion? Is germination without conversion to whole plants really very useful? Did you try working with peanut genotypes in which conversion to whole plants has been reported to work well?

Response - Refer p 21 para 2, 3 and 4. It is stated that germination without conversion is not really useful and conversion of peanut somatic embryos has always been a constraint.(Hazra *et al.* 1989, Ozias Akins 1989, McKently 1991, Baker and Wetzstein 1992, Chengalrayan *et al.* 1994). So with the assumption that somatic embryos with increased triglycerides may have improved potential for conversion, the embryos with or without increased triglyceride, were transferred to germination medium with charcoal. (Section 4.2.1, p. 53).

However even with increased triglycerides there was no improvement in conversion indicating that the failure of the embryos to develop into plantlets could probably be due to insufficient desiccation or abnormal shoot development (discussed in p 69 of the thesis). Incidently high frequency plant development could be achieved from these abnormal embryos in presence of cytokinins or thidiazuron.(Chengalrayan et al. 1997).

Q9) p.68 - the ABA concentration in the control media was 6 uM? Not 0?

Response - In Table 4.7 the ABA concentration in the control media was nil. This is typographical error. This error has also occurred in osmotica concentration column. The corrected table should read as follows :

TABLE 4.7 Influence of various treatments on germination behaviour of somatic embryos.

Sucrose		Osmotica		ABA	
Conc.%	**Percentage Rooting in Germination medium**	**Conc. moles / L**	**Percentage Rooting in Germination medium**	**Conc. µmoles / L**	**Percentage Rooting in Germination medium**
6 (control)	89.5	0 (control)	89.5	0 (control)	89.5
10	86.8	Mannitol		1	72.1
15	79.5	0.2	94.2	10	90.2
20	77.3	0.6	76.9	20	48.8
25	57.8	Sorbitol		50	63.3
		0.2	94.9	100	60.5
		0.6	70.3		

Q10) p.72 - Samples could have been weighed, freeze dried weighed again and the TG content then estimated. If freeze-drying is not available, pieces could be used for TG determinations and others for dry weight estimations. This should be done.

Response - Since freeze drying was not available embryo clusters were used for TG determinations (pg. 54) and parallel experiments were conducted to determine dry weight under identical experimental conditions. This strategy was adopted to overcome a practical difficulty encountered during the estimations. In some of the experimental conditions eg.- somatic embryos from control medium, as well as those from lower concentrations of sucrose and other osmotica, the triglyceride content was low and could not be estimated accurately from the pieces of embryos. Hence the whole tissue had to be used for each estimation. Therefore to have uniformity in the experiment the dry weights were determined from embryos cultured under identical condition.

Possibly similar situation was encountered by Attree *et al.* (1992) while working with white spruce somatic embryos. This group also adopted the similar strategy.White spruce somatic embryos from various treatments such as

polythylene glycol were blotted dry, their fresh weight was determined , the samples were placed in an oven at 80°C for 24 h and dry weights were recorded. Lipids were extracted from fresh somatic embryos and quantified. Data was expressed as TAG dry weight %.

Q11) Figure 4.9B - the sorbitol concentration is not .2 and .6M?

Response - Sorbitol concentrations in the figure are 0.2 M and 0.6 M. Due to a typographical error, both concentrations appeared as 0.2 M.

Q12) p. 81 :which peanut cultivars provided the basis of the data in Table 5.3.

Response - As mentioned in materials and methods of the thesis all the experiments were conducted with JL - 24 cultivar of peanut. This cultivar was released in 1978. It is a spanish bunch type, high yielding (1800 kg/ha) variety adopted all over India. It is early (90 days) variety with large dark green leaves and compact bearing of pods (Reddy 1988).

The fatty acid composition included in Table 5.3 was taken from a reference (Downey and McGregor 1976). The range presented indicates variation in fatty acid composition observed in peanut crops grown in various states / regions of India (genotypes not specified in the original literature).

Q13) p.82 : why was acetone used for extractions? This is a poor choice of solvents for extraction of TG.

Response - Various solvents used in extraction of lipids include petroleum ether , carbon tetrachloride, chloroform, acetone, ethyl acetate. Usually oil is extracted from oilseeds on industrial scale either by expelling or by solvent extraction using hexane. In the present investigation fresh somatic embryos of peanut, used for lipid extraction, contained about 50 to 70% water. Hence hexane was not suitable. Similarly chloroform and ethyl ether being immiscible with water, do not achieve good penetration of tissues and extraction is incomplete (Ruiz - Gutierrez and Barron 1995). Therefore acetone being completely miscible with water, was chosen as solvent for extraction. It has been used for recovery of oil from wet materials such as fish liver (Norris 1982 and the ref. therein).

Q14) p. 85 check the literature; there many better ways to methylate glycerolipids for routine analysis. Most are one step transesterifications rather than two step reactions such as used here.

Response - There are different ways of methylating glycerolipids such as use of sulphuric acid - methanol (Dahmer *et al.* 1989), HCl and methanol (Browse *et al.* 1986), which achieves hydrolysis and methylation in one step. The method followed in the present studies is derivitization of fatty acids by diazomethane. This is also an accepted method (Drozd 1981, Berezkin 1983). The experiment on fatty acid composition was carried out in collaboration with an organic chemist who is using the diazomethane method routinely for methyl esterification of fatty acids for analysis of oils and finds it quite reliable.

Q15)p 87 a standard technique for total lipid extraction is: Bligh and Dyer, 1959. Can. J. Biochem. Physiol. 37 : 911.

Response - The technique suggested by the referee for lipid extraction employs chloroform and methanol as lipid extracting solvents (Bligh and Dyer 1959). The wet tissue is homogenized with the mixture of these two solvents which form a miscible system with water in the tissue. The homogenate is separated in two phases by diluting with chloroform and water i.e. by solvent partition method.

We achieved similar results by using acetone as a solvent for extraction in two steps (mentioned on pp. 82-83)- 1st for extraction of total lipid (repeated extraction three times) and 2nd time for separation of lipids from other water soluble substances. Moreover traditionally used chloroform and methanol although made miscible, do not penetrate the tissue (Ruiz Gutierrez and Barron 1995).

Q16) Figure 5.1 - state the solvent used and plate type fpr the TLC. Why is the resolution so poor? Why are three or more major bands seen with tri-olein?

Response - Refer to p 83 of the thesis .Solvent used for TLC is 5% acetone in petroleum ether. Volume of this mobile phase was 50 ml. The TLC plates were prepared by applying water slurry of silica gel (TLC grade - with 13% gypsum) to glass plates. The plates were then air dried and subsequently activated at 110°C for 1 hour. Triloein used for spotting was stored for a long time The three bands seen in the TLC plate could be due to partial decomposition of triolein. This

wasn't purified furthur before spotting. The least polar band was Triolein. This was later confirmed by spotting it with fresh triolein (photo of TLC not shown).

Q17) Table 5.6 - Give an indication of statistical variance associated with the numbers presented.

Response - Refer p 84 . As seen from the Table 5.5, 171 to 992 mg of tissue from various sources were used for extraction of lipids, transterification (in 2 steps) and analysis of fatty acids. To collect the amount of tissue for each fatty acid analysis several explants were pooled. Thus it may be assumed that the individual variatons were averaged. However standard deviations were →

calculated from the data which were available in duplicates and triplicates. The standard deviations calculated from these values are now incoporated in the table below. Incidently the standard deviations were quite less. The experiments are now being continued to accumulate enough data for appropriate statistical analysis and confirmation.

Table 5.6 Fatty Acid Composition of Somatic Embryos Cultured in *Modified Media.*

Tissue / Somatic embryos	Relative Percentile of Fatty acids (FAME)						
	Palmitic	Stearic	Oleic	Linol-eic	Linolenic +Arachidic	Behe-nic	Ligno-ceric
Cotyledons	14.6	2.6	41.4	37.6	0.4	2.5	0.9
Immature Leaflets	20.2	1.8	37.1	36.0	1.2	2.1	1.0
Somatic embryos from EDM	17.1 ±0.07	1.2 ± 0.3	40.3 ± 7.9	26.7 ± 6.7	8.4 ± 2.5	2.7 ± 0.6	2.6 ± 0.5
Somatic embryos in 20% sucrose	17.1	1.5	50.5	19.0	6.1	2.4	2.7
Somatic embryos in 20 µmoles/L ABA	17.2 ± 1.9	1.1 ± 0.18	49.4 ± 3.4	20.8 ± 4.5	6.9 ±1.4	2.1 ± 0.3	2.1 ± 0.3
Somatic embryos in 0.6 moles/L sorbitol	17.2 ± 1.9	1.8 ± 0.09	42.6 ±2.6	24.4 ± 5.3	10.4 ± 1.4	2.0 ± 0.1	1.2 ± 0
Somatic embryos in 0.6 moles/L mannitol	14.7	1.6	37.1	27.4	13.9	2.7	2.3

Q18) p95 elaborate on the pharmaceutical applications of linolenic acid.

Response - Linolenic acid being a poly unsaturated fatty acid, is nutritionally desirable. Linoleic and linolenic acids are the essential fatty acids in human nutrition (Lehninger 1987). They act as precursors of fatty acids such as arachidonic acid and docosahexaenoic acid. These acids are important in formation of eicosanoids and membrane phospholipids . The process of

arachidonic and docosahexaenoic acid synthesis require an initial Δ6 desaturation, plus furthur chain elongation and desaturation steps from original precursors. In most countries, nowadays, there is an adequate supply of essential fatty acids in the regular diet and linoleic acid is usually available in sufficient quantity. However there is an additional problem caued by the fact that Δ6-desaturase deficiency is found in a small percentage of the general population, particularly in old age and under conditions of diabetes or stress. This can lead to a secondary induced essential fatty acid deficiency because desaturation of linoleic acid to linolenic acid at position Δ6 does not occur or is restricted.

Deficiency of gamma linolenic acid (GLA) observed in patients with atopic diseases (eczema, asthma, allergies) is attributed to the deficiency of Δ6 desaturase resulting in insufficient conversion of linoleic acid to GLA. Potential of externally provided GLA in counteracting this deficiency is demonstrated. The patients receiving Efamol (primrose oil which contains 9% GLA) showed *increased levels of GLA and also improvements in the skin disorders (Horrobin et al.* 1983). Therefore it is suggestd that (Aitzetmuller *et al.* 1993) it can be used by persons suffereing from D6D deficiency to by pass the rate limiting step of D6D supported desaturation because GLA already has a Δ6 double bond.

The importance of GLA has been recognized in treatment of skin diseases, diabetes, reproductive disorders, inflammatory and auto-immune disorders, cardio vascular disorders, cancer etc(Horrobin 1992). Alpha linolenic acid (ALA) has also been implicated in immunity (Johnston and Marshall 1983).

With an ultimate objective to introduce GLA producing oilseed crops cyanobacterial Δ6 desaturase gene was cloned and was expressed in transgenic tobacco which resulted in accumulation of GLA (Reddy and Thomas 1996).
Some of the biomedical and nutreaceutical applications of linolenic acids are listed below (Gill and Velivety 1997 and ref. therein). Most of the biomedical effects ascribed to the above products have as yet to be fully verified in clinical trials.

Linolenic acid product	Application
GLA	Antiaging and anti-inflammatory agents for smokers Therapeutics for drug or alcohol induced liver damage.
GLA triglycerides	Parenternal and enteral therapeutics and nutritional.
ALA	Thearapeutics for diorrhoea

Q19) Table 6.1 - Are the first three entries the same?

Response - Yes. It is an error.

Q20) Fig. 6.1 - What was the concentration of 2,4-D used?

Response - Fig. 6.1 A and B - Concentration of 2,4-D used was 5 ppm.

Q21) Fig. 6.2 -Give an indication of the statistical variances associated with the numbers presented.

Response The statistical variances associated with the numbers used for plotting the graph of figure 6.2, are included in the following table.

Comparison of growth ratios during nine passages of repetitive embryogenesis in liquid medium supplemented with 5 ppm and 7 ppm 2,4 - D.

Subculture	Avg. Growth Ratio ± s.d.	
	5 ppm 2,4-D	7 ppm 2.4-D
1	1.3 ± 0.2 (10)	1.0 ± 0.2 (7)
2	1.2 ± 0.4 (9)	2.0 ± 0.3 (5)
3	1.3 ± 0.4 (17)	1.6 ± 1.0 (9)
4	1.2 ± 0.5 (17)	0.9 ± 0.4 (17)
5	0.9 ± 0.5 (15)	0.8 ± 0.3 (18)
6	1.2 ± 0.6 (17)	0.7 ± 0.6 (5)
7	1.1 ± 0.4 (15)	0.9 ± 0.4 (4)
8	1.55 ± 0.5 (15)	1.2 ± 0.4 (3)
9	1.2 ± 0.4 (19)	0.73 ± 0.4 (4)

The figures in the parentheses indicate number of flasks; each flask considered as a replicate.

Q22) p 105 - give examples of the advantages leaflet-derived embryos have over immature embryo-derived explants.

Response - Immature cotyledon derived primary embryos of peanut have been used by other researchers for induction of secondary embryogenesis in peanut (Durham and Parrott 1992) whereas in the present studies for secondary embryogenesis the primary embryos were developed from mature embryo derived leaflets. Using immature embryo derived explants of peanut has several disadvantages including : seasonal availability of explants, need to maintain plants in greenhouse, laborious harvesting of underground explants, tideous selection of appropriate phase of development and difficulty in isolating sterile cultures because of its underground growing habit. The mature embryo derived leaflet explants of peanut can overcome several of these disadvantages, due to the fact that the explants are obtained directly from the dry seeds throughout the year.

Q23) p 107 What is the evidence that the method presented can be used effectively for genetic transformation? What sort of metabolites might be produced in vitro with this system?

Response - Till date there is no direct evidence that the method of repetitive embryogenesis of peanut in liquid, described in this thesis can be used effectively for genetic transformation of peanut. Keeping in view the literature accumulated for other species and also the ability of peanut somatic embryo to produce lipid (and may be protein also) it appears that the system offers several putative advantages. The primary somatic embryos are initiated from immature leaflets which are excised from zygotic embryo axis of dry seed. Since wounding is involved, the method might prove useful for *Agrobacterium* mediated transformation .Once transformed, primary embryos can be cycled and propagated via a series of repetitive embryogenesis. Such possibility has been demonstrated in soybean after achieving microprojectile aided transformation (Liu *et al.* 1996). The transformation system based on somatic embryogenesis may be suitable for establishing transgenic lines within a short period. Repetitive embryogenic cultures were used for transformation in alfalfa (Ninkovic *et al.* 1995). Isolated somatic embryos of alfalfa were released from repetitively embryogenic culture, and were transformed via *Agrobacterium*. Some clones

were selected and proliferated by repetitive embryogenesis on media with kanamycin. This way the time taken to establish the transformed line was reduced. The relatively low frequency of embryo transformation gets amplified by abundant proliferation in secondary somatic embryogenesis.

Repetitive embryogenesis in liquid medium has additional advantage. The medium being liquid, selection of transformants is more rigorous. Moreover often the growth of the tissues are faster than tissues cultured on solid media.

Regarding second part of the querry on production of metabolites, the culture of somatic embryos can be an alternative to cell culture ; it being an organ system with cell - cell co-ordination. For example, celery flavor compounds, which are absent in undifferentiated callus, are produced by somatic embryos (Al-Abta *et al.* 1979). Presence of triglycerides has been demonstrated recently by us in peanut somatic embryos (Mhaske and Hazra 1994). A great deal of optimization will be required to unveil the complete potential of this system to produce triglycerides and other cotyledon specific metabolites. This may also include peanut oil with increased oleic/linoleic ratio and subsequently with better shelf life. Production of selective fatty acids is also possible using somatic embryos. Accumulation of metabolites such as anthocyanins, lipids, alkaloid and proteins in cultures of somatic and zygotic embryos; especially with incorporation of high sucrose strengthen the possibility of production of these metabolites in vitro (Janick *et al.* 1991). Alkaloids such as caffeine and theobromine are produced in cacao somatic embryos (Paiva and Janick 1983). Thus primary as well as secondary metabolites can be produced *in vitro*.

Q24) p108 - Is it economically feasible or practical to produce vegetable oil using in vitro culture?

Response - Vegetable oil/wax from in vitro culture of somatic embryos appear to be a promising approach when compared to cell culture (Janick 1991). Somatic embryos being organized structures, can effectively produce metabolites which might require cell-cell coordination. Secondly these embryos show similarities with zygotic embryos therefore it should be possible to obtain cotyledonary metabolites from these embryos. Thirdly due to phenomenon of repetitive embryogenesis, the metabolites can be produced in bioreactors too.

As a rule of thumb, metabolites produced in vitro should have values of 1,000 to 10, 000 $ per kg or more (Janick 1993). This restriction limits products to drugs, and , perhaps flavoring compounds. For economic feasibility it must be demonstrated that in vitro systems would have an economic advantage over simple extraction from zygotic seed.

Lipids are rather inexpensive materials and presently there is no evidence that production by in vitro culture is economically feasible The economic feasibility of such production would depend upon several factors such as growth kinetics of embryo proliferation, efficiency of somatic embryos to produce metabolites (which can be altered by media composition), and the cost of the metabolite. For example, high value product such as cocoa butter, jojoba oil can be produced cost effectively after optimization. In case of peanut, since oil is comparatively cheap, the production of high value oil with specific fatty acid composition (subsequent to transformation) would be economically feasible. If altered culture conditions or incorporation of precursors favour formation of specific fatty acids , the somatic embryos can be made to produce triacylglycerols with composition which could have uses other than edible purpose. For instance, gamma linolenic acid for thearapeutic use. Only in such conditions, it is likely to be feasible economically.

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Research Guide

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