CHAPTER IV

Discussion

Selection of seeds to be used as a source of explant in this work was based on the convenient in storage, germination and cultivation in a sterile environment. After surface sterilization, the seeds were germinated on a nutrient medium without growth regulator to produce contamination free seedlings which then were cut up under aseptic conditions and being used as explant material. Surface sterilization conditions were always necessary and may be carried out with any of several different reagents (Yeoman and Macleod 1977 cited by George and Sherrington 1984). Sometimes internal plant tissues of seed after sterilization were still contaminated with micro-organisms, so it was necessary to standardize the sterilization procedure for each lot of starting material being used as a source of explants. According to the present study as shown in Table 2, a suitable condition for sterilization of *Carthamus tinctorius* Linn. was by treating the seeds with 10% (v/v) of clorox containing with 0.05% (v/v) triton X-100 for 15 minutes before germination in the sterile agar medium. This condition differred from that reported by other groups which by using mercuric chloride solution (George and Rao 1982, Padmaja *et al.* 1990 and Singh 1991) for serface sterilization. Generally, mercuric chloride was more toxic to plant material than clorox.

Various responses on callus initiation among explants may be caused by the differences in their physiological status (Murashige 1974). Several reports supported the potential of cotyledons as suitable starting materials for callus ihduction and plant regeneration (Patton and Meinke 1988, Chaudhury and Signer 1989 Padmaja *et al.* 1990). The proper type of explants from germination seeds were tested and the

results from Table 5, 8 and Figure 9 and 12 indicated that cotyledon was more appropriate than hypocotyl to be used as a source for tissue culture of safflower. Maclean and Nowak (1989) reported that the percentages of callus induction and plant regeneration from cotyledon explants were higher than hypocotyl explants in all plants tested. The cotyledonary explants were more responsive to induction of multiple shoot bud regeneration than hypocotyl segments on MS medium containing BA in combination with NAA. Singh (1991) found that cotyledon explants could produce nodular structure on callus surface and develop into shoot proliferation while hypocotyl (longitudinally-exposed) segments produced only root initiation with no shoot formation.

Age of explant using as a source of tissue culture was one of the factors resulting in variation in the degree of regeneration of cultured tissue. Callus growth and shoot development were more rapid on *Solanum laciniatum* cut from mature leaves which had just completed full expansion. Young unexpanded leaves yielded explants which soon expanded and callused but produced shoots slowly, while older leaves took longer time for callus and shoot formation (Davies and Dale 1979). The regeneration efficiency was highly dependent on several possible factors such as material age (the period of time from its initiation), sequential age and phase of growth or physiological age which were explained in the term of young or old tissue derived from a part of plant. From the results indicated in Table 8, the excised cotyledon removed from 14 day-old seedlings was the only one giving the highest percentage of regeneration (76%) while seedlings at higher or lower age than 14 day-old were decreased in percentage of regeneration. This might be due to the 14 day-old seedlings produced the highest completed meristems and the totipotency of callus culture at this age was more appropriate to promote morphogenetic response than those of the other ages. Padmaja *et al.* (1990) reported that the excised

cotyledons of safflower from 2 to 3 day-old seedlings had a greater capacity for callus induction (90%) than that originated from older or younger seedlings.

When different portions of the explants were used for callus induction and plant regeneration, different degree of regeneration was observed. Pieces of explants in any specific area composing of different phases of growth, thus appeared to be the determination factors during tissue formation by cell division in a meristem and developing into a specific permanent tissue (Denton *et al.* 1977 cited by George and Sherrington 1984). Study of the direction of excision and polarity of regeneration of cotyledon and hypocotyl segments as shown in Table 7 and Fig. 9 indicated that the highest percentage of plant regeneration occurred from callus produced on the transversely cut - upper part of cotyledons. The results also showed that the longitudinal sections passing through the mid rib of cotyledon explants (Figure 2, explant a) producing high percentage of callus induction (82%) with satifactory regeneration (51%). The results reported here showed higher callus induction from cotyledons devoid of apical meristem, leaf and root primordia than those reported by others (Tejovathi and Anwar 1984 and Padmaja *et al.* 1990).

The effects of light incubation period on tissue culture were studied with respect to callus morphogenesis, chlorophyll formation, development of chloroplast, shoot growth and proliferation, root formation, the biosynthesis and photomorphogenesis to induce development of structure of plants. *In vitro* organ formation from plant tissues normally occurred under optimal conditions. Light, sometimes, prevented initial cell divisions of explants and the growth of callus tissues (George and Sherrington 1984). There was a marked difference in this respect in the tissues of different plant species. The development of callus morphogenesis in many plant species was controlled by the relative lengths of light. Darkness sometimes promoted shoot morphogenesis. Murashige (1974) suggested

that the requirement for specific photoperiod for vegetative growth and development was very likely to manifest this need in *in vitro* culture that necessary for optimum regeneration of shoots and roots, or for shoot proliferation. Mazzeri *et al.* (1987) reported that although the light had no effect on photosynthesis of callus cell but light had effect to metabolic system in cell culture. Almost all researches on the effect of light incubation period were studied about somatic embryogenesis under a specific daily photoperiod or in continuous darkness. Few researches were examined the effect of alternating exposures to dark and light incubation conditions on shoot organogenesis (May and Trigiano 1991).

The effect of dark pre-incubation has never been reported before. From the experiments (Table 3 , 4 , 5 , 6 and Figure. 6 ,7, 8), it was found that the highest plant regeneration and shoot differentiation from cotyledon callus of *Carthamus tinctorius* Linn. occurred at 2 weeks of the initial dark incubation period, whereas other conditions showed a few responses.

The results showed that the effect of light incubation period could increase the percentage of regeneration especially the incubation for 2 weeks in darkness. This might be due to the correlation between auxin/cytokinin ratio and light incubation period to induce organ regeneration.

In general, the growth and morphogenesis of *in vitro* culture of plant cell are both regulated by the interaction and concentration of the exogeneous supply of auxins and/or cytokinins. The specific cells or tissues in natural plant produced endogeneous hormones which are systematically transported to their site of action for growth and developmental control (Shabde and Murashige 1977). The auxins and cytokinins are those of the greatest significance growth regulators in plant tissue culture. Skoog and Miller (1957) (cited by George and Sherrington 1984) found that shoot formation could be induced from tobacco pith callus using relatively low levels

of auxin and a high levels of cytokinin in the growth medium. At presence, many aspects of cellular differentiation and organogenesis in tissue or organ cultures have been found to be controlled by the interaction between cytokinin and auxin concentrations. It was observed in this studies (Table 3 , 4 and Figure. 6 , 7) that shoot and root formation from *Carthamus tinctorius* Linn. cotyledon calli appeared to be highly dependent on auxin/cytokinin ratio. Increasing BA concentration, shoot differentiation was observed but at high concentration of NAA, root formation was occurred on the callus surface. It was also shown that the auxin:cytokinin interaction leading to change in the external morphology in culture explants. Some of regenerated callus formed in the presence of 0.5 mg/l NAA combined with 1.0 and 2.0 mg/l BA usually developed abnormal shoots and roots with thickness and translucent leaves and similar airing roots. There was no regeneration response occurred in all levels of cytokinin used (0.2, 0.5 1.0 and 2.0 mg/l BA) but only a few callus formation and green spots differentiation at 0.5 mg/l BA level. The effects of hormonal balance between BA in combination with NAA were further studied (Figure. 15(a) and 15(b)). The result showed that cotyledon *Carthamus tinctorius* Linn. callus growth was observed on MS medium supplemented with every level of BA:NAA. A green meristematic spots was originated on callus at all treatments, and could be regenerated into different organ-like structures at different levels of BA to NAA. Different kinds of plant growth regulators were used to induce shoot formation and regeneration of callus cultures in order to find the appropriate kinds of auxins and cytokinins (Figure. 10 and Figure. 13). Data obtained from those experiments, indicated that different kinds of plant growth regulators were different in their mode of actions. Shoot formation occurred only on the media supplemented with BA and NAA. Similar results were previously reported (George and Rao 1982, Singh 1991, Prasad *et al.*1991 Ying and *et al.* 1992). 2,4-D was the only type of auxin which

causing limited regeneration and shoot formation but leading to the promotion of yellowish friable callus. Among the other auxins tested such as IAA and IBA, both promoted callus regeneration but failed to promote shoot formation. The data obtained was similar to those of Paterson and Rost (1981) which found that the callus formation and organogenesis from cultured leaf segments of *Crassula argentea*. were completely inhibited by IAA, NAA, IBA or 2,4-D even as little as 10^{-10} M. Cytokinin such as BA and Kn showed differences in induction of callus regeneration. The results showed that when Kn was added to the medium, shoot formation was completely suppressed, while BA in combination with some auxins present in the medium induce shoot formation of the regenerated callus. It was suggested that shoot differentiation or shoot formation highly dependent upon a function of different kind of cytokinins. Similar results were observed by Sangwan and Harada (1976), Vieitez and Vieitez (1980). The similar effect of interactions between auxins and cytokinins for organogenesis had been reported with other systems such as Begonia (Welander 1977), *Lotononis bainesii* (Bovo and Mroginski 1986), *Camellia sasanqua* (Torres and Carlisi 1986), *Arabidopsis thaliana* (Patton and Meinke 1988) and *Helianthus annuus* L. (Chraibi *et al.*1992).

The present work also showed that, root formation only observed on callus culturing in the medium supplemented with low levels of NAA (0.2 and 0.3 mg/l NAA). At high levels of BA (1.5, 2.0, 2.5 and 3.0 mg/l), profuse green spots on the regenerated callus were produced with no shoot formation. The shoot formation was only occurred on the same medium supplemented with 0.5 mg/l NAA and 0.5 mg/l BA (Figure. 16). However, organ-like structures developed on these medium failed to develop into normal plantlets, eventhough the good callus growth occurred. This results leading to the possible suggestion that genotype of the donor plant might be greatly influenced on the regeneration potential of the cotyledon explants. The

results were resembled to the previous results reported by George and Rao (1982), Tejovathi and Anwar (1984), Padmaja *et al.*(1990), Singh (1991), Prasad *et al.*(1991) and Ying *et al.*(1992).

Nutritional requirements for growth and development of plant tissue *in vitro* culture might be varied with certain species. Even the tissues from different parts of the same plant may differ in nutritional requirement for satisfactory growth (Murashige and Skoog 1962). Composition of basal culture medium was an important factor for the successful establishment of tissue culture in which each tissue required different formulation (Dixon 1987). This might be due to the difference of macronutrient and micronutrient compositions in each medium (George and Sherrington 1984). As evidence in Table. 9 and Figure. 12 , it was noticed that among the various media tested for cultivating of *Carthamus tinctorius* Linn. cotyledon segments, MS medium supplemented with 1.0 mg/l 2,4-D was the most effective both in the callus induction and growth. MS medium supported a good growth and gave satisfactory callus induction efficiency. Generally, MS macronutrients were developed and proved to be satisfactory for tissue culture of many plant species for micropropagation (Murashige and Skoog 1962). Most of other media were developed based on the composition of original MS macronutrients, such as of Hasegawa (1979), Jarret *et al.* (1980), Linsmaier and Skoog (1965), Rao *et al.* (1973), Sangwan and Harada (1975), Skirvin and Chu (1979) and Vieitez and Vieitez (1980). The other media which were tested for culturing of *Carthamus tinctorius* Linn. cotyledons in this study (B5, N6, LS and HM medium) yielded lower responsed for callus induction and growth development in comparison to the MS medium due to a wide diversity in the concentration of the nutrient components. The results were similar to the previous results of Padmaja *et al.* 1990

who obtained the highest callus induction of cotyledon on MS medium supplemented with 2.0 mg/l 2,4-D.

Nitrogen was one of the most important components for growth and development of natural plant cells. The amount and form of nitrogen presence in the *in vitro* culture medium had significant effects on the rate of cell growth, cell morphology and cell differentiation (Gamborg and Shyluk 1970, Veliky and Rose 1973, Rose and Martin 1975, David *et al.* 1984 and Zhang and Mackown 1992). The form and level of nitrogen had a profound effect on development of adventive embryogenesis *in vitro* (Thompson and Aderkas 1992). Plant cell was able to use reduced nitrogen from the ammonium ion and from compounds containing amide and amine groups. Results obtained from the culturing of *Carthamus tinctorius* Linn. cotyledons indicated that adding ammonium nitrate into the culture media caused a decrease in percentage of plant regeneration but shoot formation was occurred in the culture medium supplemented with 1.65 g/l of ammonium nitrate (Table.12,13 and Figure. 18).

Potassium nitrate was found to be the important form of nitrogen source for the growth and development of natural plant tissue. The utilization of potassium nitrate as a sole nitrogen source for culturing plant cells was also reported (Durzan 1968). Addition of even low concentration of ammonium could still stimulate both growth and embryogenesis. In the case of cotyledonary *Carthamus tinctorius* Linn. callus shown in Table. 11 and 12 , it was noticed that similarity to ammonium nitrate, the percentage of plant regeneration decreased when potassium nitrate was added to the culture media. This was in agreement with those studied previously by Lee (1983) (cited by George and Sherrington 1984).

Addition of $AgNO_3$ to inhibit activity of ethylene resulted in increasing the regeneration of shoot from callus tissues (Purnhauser *et al.* 1987, Songstad *et al.*

1988). There has been reported on effect of $AgNO_3$ on the physiological or morphological characteristics of safflower tissues. $CoCl_2$ inhibited ethylene formation by blocking the conversion of 1-aminocyclopropane carboxylic acid to ethylene (Yang and Hoffman 1984 cited by George and Sherrington 1984). $CoCl_2$ was known to promote elongation, leaf expansion and hook opening in excised plant parts by inhibiting ethylene formation. Lau and Yang (1976)(cited by George and Sherrington 1984) and Chraibi *et al.* (1991) suggested that $CoCl_2$ effectively inhibited ethylene production of Sunflower callus, thus, resulting in increasing shoot regeneration. The results from Table. 15 and Figure. 20 showed that stimulation of shoot regeneration by silver nitrate and cobalt chloride was first observed in cotyledonary callus cultures of *Carthamus tinctorius* Linn. This might be due to auxin-induced ethylene production by tissue culture suppressed shoot regeneration. Additional data also pointed out that $AgNO_3$ itself was not responsible for increasing the number of adventitious shoots in Safflower. However, it tended to produce an elongated shoot with large well developed stem and leaves similar to normal seedlings. At high concentration of $AgNO_3$ (5.0 and 10 mg/l), it was noticed that the base of callus exhibiting necrosis due to the inhibition of cell growth. These results also observed in carrot cell suspension cultures (Rousten *et al.* 1990).

The present work indicated that $CoCl_2$ effectively stimulated shoot regeneration from cotyledon callus of Safflower. In the presence of this inhibitor, the percentage of shoot formation was increased at the low concentration of $CoCl_2$ (Table. 15 and 21). At higher concentration of $CoCl_2$ (5 and 7 mg/l), the toxicity of this enzyme inhibitor could be observed indicating by callus necrosis.

Various developmental stages of explants required different concentration of sucrose (Thorpe and Meier 1973). Sucrose levels in culture media showing good growth of callus might not be optimal for morphogenesis (George and Sherrington

1984). Carceller *et al.* (1971) reported that at high concentration of sucrose lead to a decrease in the specific growth rate and callus growth of *Acer pseudoplalatanus*. The effect of sucrose concentration on callus induction efficiency of *Carthamus tinctorius* Linn. cotyledons was also studied (Table. 19 and Figure. 24). The results showed that percentage of callus induction was decreased when increasing sucrose concentration, and 20 g/l of sucrose proved to be the best level for callus induction efficiency. At higher concentration of sucrose (40 and 50 g/l), the growth index of cotyledon callus of *Carthamus tinctorius* Linn. was decreased. This might be due to the effect of osmotic potential balance. Similar observation was found in tobacco callus (Schank and Hildebrandt 1972). There have been reported that high sucrose concentration suppressed chlorophyll synthesis such as carrot (Edelman and Hanson 1971), tobacco (Kauf and Sabhawal 1971). which were grown on the culture medium containing more than 50 g/l of sucrose. The present study observed that cotyledon calli of *Carthamus tinctorius* Linn. culturing on the medium containing 40 and 50 g/l of sucrose were yellowish, with no differentiated organs as early as 2 weeks of culture.

Determination of fatty acid compositions of total lipids in the cotyledon at different stages of seedling development, excised cotyledon at different stages of callus initiation and in callus tissue developing from subculturing of *Carthamus tinctorius* Linn. have never been reported elsewhere. The results shown in Table 21 indicated that total lipid contents in soaked seeds (nature cotyledons tissue) were highest due to the natural storage forms of lipids. Cotyledons developed from seedling germination still contained fairly high level of lipids and started to decrease in the higher maturity of cotyledons. The stored lipids were remarkably higher in comparison of their calli. This might be due to the cotyledon as a stored lipids at different stages of seedling development comprised uniform undifferentiated cells representing high level of differentiated parenchymal cells in callus (Pandey *et al* 1986), and the mobilization of stored lipid was essential to provide energy and carbon skeletons for the developing embryonic axis (Harwood and Stumpf 1970 and Shewry and Pinfield 1972). In this study, the excised cotyledon segments which were not in the process of proliferation but showing visible callus were taken for callus formation. No shoot axis was observed on any part of the callus. Although total lipid contents of excised cotyledon of 14 day-culture was higher than that of excised cotyledon of 28 day-culture, both cultures showed almost similarity in fatty acid compositions (C16:0, C16:1, C18:0, C18:1, C18:2 and C22:0) These results suggested that the cell of excised cotyledons of 14 day-culture were still consisted of uniform undifferentiated cells while the cells of excised cotyledons of 28 day-culturing were not only undergone through all division but the newly formed meristmatic cells started to dominate and component of fatty acids synthesize were altered in the fatty acid compositions. This results were in agreement with the works of Harwood and Stumpf (1970) and Shewry and Pinfield (1972). For the callus stages, there was almost no change in total lipid quality. The concentrations of

myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), arachidic acid (C20:0) and behenic acid (C22:0) seemed to be constant in each stage of subculturing. Palmitoleic acid (C16:1) was not detected in all callus stages. The absence of this acid in the callus of *Carthamus tinctorius* Linn. might be explained on the basis that callus cultures compose of a continuously proliferating system where accumulation of some fatty acids were not occurred, possibly because of quick turnover which is in contrast to the cotyledon, a storage organ, where accumulation of some fatty acids occurs during seed maturation (Radwan *et al.* 975 cited by Halder and Gadgil 1983, Ichihara and Noda 1980, 1981, 1982). The amounts of saturated fatty acids found in callus growth stages were higher than unsaturated fatty acids. This might be due to the inoculum callus which was excised from the cotyledon consisting of only newly formed cells which were able to synthesize large amounts of saturated fatty acids (Halder and Gadgil 1983).

The calli induced from cotyledon of *Carthamus tinctorius* Linn. were separately cultivated in the dark and light period. The dark grown callus was creamy white and friable while the light grown callus was pale green and compact showing groups of meristematic cells. The total lipid content and the growth rate of dark grown callus were higher than that of light grown callus but the contents of major fatty acids in light grown callus such as palmitic acid (C16:0), stearic acid (C18:0) and linoleic acid (C18:2) were higher than that of dark grown callus. It may be explained that dark grown callus contained only proplastids or etioplasts which were only part of location of fatty acid biosynthesis (Gemmrich 1982) while light grown callus consisted of the green part of chloroplasts which a completed complex series of enzymes for fatty acid biosynthesis were localized (Heemskerk and Wintermans 1987). However, distribution patterns of fatty acids and total lipids of *Carthamus tinctorius* Linn. cotyledon calli both cultivated in dark and light conditions showed little variation in

the values of some individual components. This might be because of each cell was under the direct control of genes in a greater diversity and little variation observed here was probably due to the use of homozygous seeds as an experimental materials. Similar results was observed by Knowles (1972), his results suggested that the lack of variation in the species or varieties of *Carthamus tinctorius* Linn. calli would be reflected constantly on major fatty acid contents.

Cultural conditions such as temperature (Skoczowski and Filek 1994), light (Gemmrich 1982), carbon source (Pence *et al* 1981) have been reported to affect fatty acid compositions of callus cells. The variation in lipid patterns might be resulting from a direct effect of these factors on enzyme activities. It was also suggested that these factors may directly affect fatty acid composition by influencing the types and amounts of fatty acid during the growth of callus. Essential constituents of the culture medium also influenced in fatty acid composition of callus cells (Manoharan *et al* 1988). Pandey and Gadgil (1984) demonstrated that growth factors were not only induced meristematic activity to form new cells of *Cucumis melo.*,but also brought about a change in fatty acid metabolism in such cells. From the result, it was shown that addition of Kn in combination with 2,4-D could increase the level of linoleic acid (C18:2) to the highest. The presence of BA in combination with auxins (NAA, IAA, IBA and 2,4-D) produced myristic acid (C14:0) while replacing BA with of Kn showed opposite effects. These results might be explained that the growth regulators might directly or indirectly affect specific steps in the biosynthesis of fatty acids (Halder and Gadgil 1983). Particular combinations of growth regulators might favour cell division and increase cell population with genetic ability to synthesize particular lipid patterns, since callus cultures were normally composed of genetically heterogeneous cell populations (Manoharan *et al.* 1988).

The initial substrate for fatty acid synthesis in maturing of *Carthamus tinctorius* Linn. seeds is acetyl CoA. This important compound is formed by the glycolytic breakdown of sugar phosphates which are formed from sucrose (Stumpf 1975). Sucrose is normally synthesized in the cytosolic compartment of the leaf cell from triose phosphates which are originally synthesized in the chloroplast and then transported out into the cell cytosol (Heber 1974). Singh and Chatterjee (1991) reported that increasing in oil content in the presence of precursors or by adding extra sucrose was obviously due to the fact that sucrose would generate acetyl CoA through TCA cycle which would then be used for fatty acid chain elongation. Jalal and Collin 1979 and Pence *et al.* 1981 also reported on the effect of sucrose on growth and fatty acid composition in cacao. Their results suggested that high sucrose concentration in the medium decreased in length of embryo growth and in percentage of linoleic acid (C18:2) and linolenic acid (C18:3) in asexual embryo.

From the present study, it was found that increasing sucrose concentrations between 0-2% (w/v), total lipid content in cotyledon callus would be increased. However, higher sucrose concentration (3-5% w/v) decreased total lipid content which may be because of osmotic effect (Smith 1973). At high sucrose concentration (3-5% w/v), the growth index of callus was decreased continuously. Decreasing of growth index also resulted in changing in fatty acid composition. This effect might be due to variation in initial cells involving fatty acid biosynthesis.

CONCLUSION

The present work could be concluded as follows:

1. The optimal conditions selected for seed surface sterilization prior to use as the clean source of explants were 15 minutes of immersion in 10% (v/v) clorox solution containing 0.05% (v/v) triton X-100.
2. The best source of explants to yield a high callus induction efficiency and plant regeneration was the excised cotyledons.
3. The cotyledons taken from 14 day-old seedlings were the best source of explant which yielded highest in callus induction and plant regeneration.
4. To determine the appropriate direction of excised explants:
   - transversely cut - upper part of cotyledons yielded the highest percentage of callus induction and plant regeneration.
   - the hypocotyl segments with the outer surface placed horizontally onto the culture medium exhibited higher callus induction and plant regeneration
5. Among the plant growth regulators tested, the combination of cytokinin (BA) and auxin (NAA) at the same concentration (0.5 mg/l) had been selected as the optimal callus propagation medium.
6. The effective media for cotyledon callus growth and propagation of *Carthamus tinctorius* Linn. was MS basal medium.
7. Addition of 1.90 g/l KNO_3 or 1.65 g/l NH_4NO_3 or both inorganic salts to MS medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA could induce callus and shoot formation from safflower cotyledon. However,the best condition for shoot formation from cotyledon callus could be achieved on MS basal medium supplemented with 0.5 mg/l NAA, 0.5 mg/l BA and 1.65 g/l NH_4NO_3.

8. The presence of 3.0 mg/l $AgNO_3$ or 5.0 mg/l $CoCl_2$ in MS culture medium supplemented with optimal plant growth regulators increased the yield of shoot formation from the regenerated cotyledon callus.
9. Additions of 20 g/l of sucrose to the MS culture medium was optimal for growth, callus induction and plant regeneration.
10. The root regeneration condition could be established successfully within 4 days in full-strength MS medium without plant growth regulators.
11. Total lipid content and fatty acid compositions of the explants were rapidly decreased during growth stages. Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) were detected in the developmental explants and callus as the main fatty acid components. The average level of the main unsaturated fatty acids (C18:1 and C18:2) was higher than that of saturated fatty acids (C16:0 and C18:0).
12. The growth rate of dark grown cotyledon callus of *Carthamus tinctorius* Linn. was higher than that of light grown callus. Dark grown callus had higher total lipid content than that of light grown callus. The highest content of fatty acid was found in 2 week-old callus. The main fatty acids found in both type of callus were palmitic acid (C16:0) and stearic acid (C18:0) which were saturated fatty acids.
13. The presence of fixed concentration (0.5 mg/l) of 2,4-D or NAA as the auxins in combination with BA or Kn as the cytokinins in MS medium gave higher content of total lipid than the other combinations. Over 8 kinds of fatty acids were detected with palmitic acid (C16:0) and linoleic acid (C18:2) as the major components. The highest level of palmitic acid (C16:0) was detected in callus grown in the medium containing NAA plus BA while the highest level of linoleic acid (C18:2) was detected in the presence of 2,4-D plus kinetin.

14. *Carthamus tinctorius* Linn. cotyledon callus cultured on the medium containing 20 g/l sucrose as a carbon source synthesized the highest content of total lipids. The presence of sucrose at higher concentration (30-50 g/l) reduced the total lipid content of cultured calli.The major saturated fatty acid component observed in cotyledon callus was palmitic acid (C16:0) whereas linoleic acid (C18:2) was the only unsaturated fatty acid with low content.