African Journal of Biotechnology Vol. 10(50), pp. 10101-10107, 5 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.816
ISSN 1684-5315 © 2011 Academic Journals

Full Length Research Paper

Enhancement of callus induction and regeneration efficiency from embryo cultures of *Datura stramonium* by adjusting carbon sources and concentrations

S. Amiri[1]* and S. K. Kazemitabar[2]

[1]Department of Biotechnology and Plant Breeding, Tarbiat Modares University, Iran.
[2]Agricultural Sciences and Natural Resources, University of Sari, Iran.

Accepted 6 May, 2011

Carbon source in the medium is considered to be an essential component for the high production costs of callus and plantlets in tissue culture. We report here the establishment of an efficient tissue culture cycle (callus induction and plant regeneration) for *Datura stramonium* by adjusting carbon sources and concentrations. Embryo explants of *D. stramonium* L. were cultured *in vitro* with six different carbon sources (sucrose, glucose, fructose, galactose, maltose and lactose) and four concentrations (1, 2, 3 and 4%) for callus induction. After that, the best carbon sources for regeneration were investigated with four different carbon sources (sucrose, glucose, fructose and sorbitol) and four concentrations (1, 2, 3 and 4%). The best sugar for callus induction was 2% lactose. The medium containing 3% glucose shows the higher regeneration.

Key words: *Datura stramonium* L., carbon source, callus induction, plant regeneration and embryo culture.

INTRODUCTION

The genus *Datura* is comprised of 15 species of annuals, trees and shrubs distributed over warm and temperate regions of the world (Chittenden, 1965). Previous studies have demonstrated that *Datura* is amenable to tissue culture (Sharma et al., 1993). Plant cell, tissue or organ culture normally requires the incorporation of a carbon source to the culture medium (Karhu, 1997).

In living plant cells, carbohydrates are necessary as a source of energy and a carbon substrate for biosynthesis. Continuous supply of carbohydrates to plants cultured *in vitro* is essential, since photosynthetic activity of *in vitro* grown tissues is usually reduced. These compounds are also necessary in media as osmotic agents. For all these reasons, sugars have a great potential effect on the physiology, growth and differentiation of cells (Gibson, 2000). Therefore, the optimal carbon source for callus induction and plant regeneration needs to be considered (Mendoza and Kaeppler, 2002). The type of carbohydrate source in the regeneration medium was shown to influence the plants ability to regenerate shoots (Kim et al., 2003).

Although, sucrose has been the carbohydrate of choice in the vast majority of work on *in vitro* callus and shoots induction and development in various species, it is not always the most effective carbon source for these purposes (Thompson and Thorpe, 1987). The substitution of the culture medium carbon source by osmotically active solutes has shown that sugars act as a carbon source and as osmotic regulators (Neto and Otoni, 2003). The carbon source osmotic contribution has an inverse relationship with carbon source concentration and in general, causes an initial increase followed by a reduction in the values of the assessed parameter. This reduction can be caused by an excessive osmotic contribution or by toxicity of the carbohydrate (Slesak et al., 2004).

Species-specific carbon sources and carbon concentrations for optimal growth rates have been discovered for various culture systems. It was found that different patterns of morphogenesis were attributable to the type of carbohydrate and its concentration (Romano et al.,

*Corresponding author. E-mail: amiri_s_m@yahoo.com.

Abbreviations: 2,4-D, 2,4-Dichlorophenoxyacetic; **BAP,** 6-benzylaminopurine; **NAA,** a-naphthalene acetic acid.

1995). What happens to the absorbed carbon depends on the type of sugar in the medium. Blanc et al. (2002) reported that the morphological and biochemical changes reflected a specific physiological state as a consequence of the type of carbohydrate supplied in the medium.

This report, for the first time, details an assessment of the performance of embryos culture of *Datura stramonium* on a combination of media conditions, with varied carbon sources and concentrations. An efficient plant regeneration system allowing rapid callus induction and plant regeneration from mature *Datura* embryos by using carbohydrate is described.

MATERIALS AND METHODS

Callus induction condition

Immature seeds of surface sterilized fruits were removed. The seed coat encasing the immature embryo was peeled away and the seed was squeezed hard using scalpel handle until the immature zygotic embryo was on the loose. Immature embryos were transferred to MS callus induction medium supplemented with 2 mgl^{-1} *2,4*-dichlorophenoxyacetic (2,4-D). To test the effect of sugar types, different types of carbon sources, including sucrose, glucose, fructose, galactose, maltose and lactose at 3% (w/v) were added before autoclaving. After determining the best sugar type, the best concentration of sugars was examined at 1 to 4% (w/v) concentration. The experiment was designed in complete randomized design with four replications. All the cultures were incubated in the dark at 25 ± 2°C.

Regeneration condition

To investigate the best type of sugars for regeneration, four types of carbon source including sucrose, glucose, fructose and sorbitol at 3% (w/v) were added to B5 medium supplemented with 2 mgl^{-1} BAP and 1 mgl^{-1} NAA. After identifying the best type of sugar, various concentrations (1, 2, 3 or 4% w/v) were also tested. The experiment was performed with 5 replications. After calli culturing in test tubes, they were incubated at 25± 2°C under cool white fluorescent light (35 µmol photons m-2 s-1) with 16 h photoperiod. The experiment was performed with 5 replications. PH of all media was regulated between 5.8 and 5.9 by using 1 N NaOH before autoclaving.

Statistical analysis

Analyses were carried out for these characters on transformed data (by using $\sqrt{(X+0.5)}$ transformation). The statistical analysis was carried out by using the SAS. Differences were determined by analysis of variance (ANOVA) and the differences between the means were compared by Duncan's multiple range tests using MSTAT-C computer program.

RESULTS AND DISCUSSION

Effects of carbohydrates on callus induction

Carbohydrate type and concentration have been found to play important roles in different stages of the tissue culture processes.

Determine the best type of carbon source

The results of variance analysis related to effects of different carbon sources on frequency of callus were shown in Table 1. Based on this table, the effect of carbon sources on callus induction from embryo explants were significantly different at $P < 0.01$. From Figure 1, the highest fresh weight of callus was obtained in lactose. The lowest callus formation amount was observed in medium containing galactose.

Based on Table 2, it was known that various concentrations of lactose can significantly affect callus fresh weight ($p < 0.01$). From Figure 2, 2% lactose produced the most callus fresh weight. The lowest callus fresh weight was related to 4% concentration.

Effects of carbohydrates on regeneration

Regeneration took the form of indirect organogenesis and its frequency was strongly dependent on sugar supply.

Determine the best type of carbon source

The results of Table 3 indicated that sugar types influenced regeneration from embryo-derived calli significantly. Among different carbon sources, the medium containing glucose produced the more shoot buds (Figure 3). It was observed that there is no significant difference between sucrose and glucose. The lowest frequency of regeneration was related to fructose.

Determine the best concentration of carbon source

Analysis of variance (Table 4) showed that different glucose concentrations play important role in producing shoot from embryo-derived calli, so that different concentrations of glucose have influence were significant ($p < 0.01$). 3% glucose allocated the highest regeneration to itself, while the lowest percentage of regeneration obtained at 1% glucose (Figure 4).

DISCUSSION

Experiments demonstrated that the addition of carbohydrates was essential, inducing callus and regeneration of embryo cultures of *D. stramonium* is affected both type and concentration of sugar in the culture medium (Figure 5). The best type and concentration of carbohydrate were 2% lactose and 3% glucose for callus induction and regeneration, respectively.

The disaccharide lactose has been detected in only a few plants. When added to tissue culture media, it has been found to induce the activity of β-galactosidase enzyme which can be secreted into the medium (George

Table 1. Analysis of variance of carbon source effect on callus fresh weight (gr).

S.O.V	df	MS	F-value
Carbon source	5	0.01022745	10.03**
Error	18	0.0010197	

** is significant at 0.01 probability level.

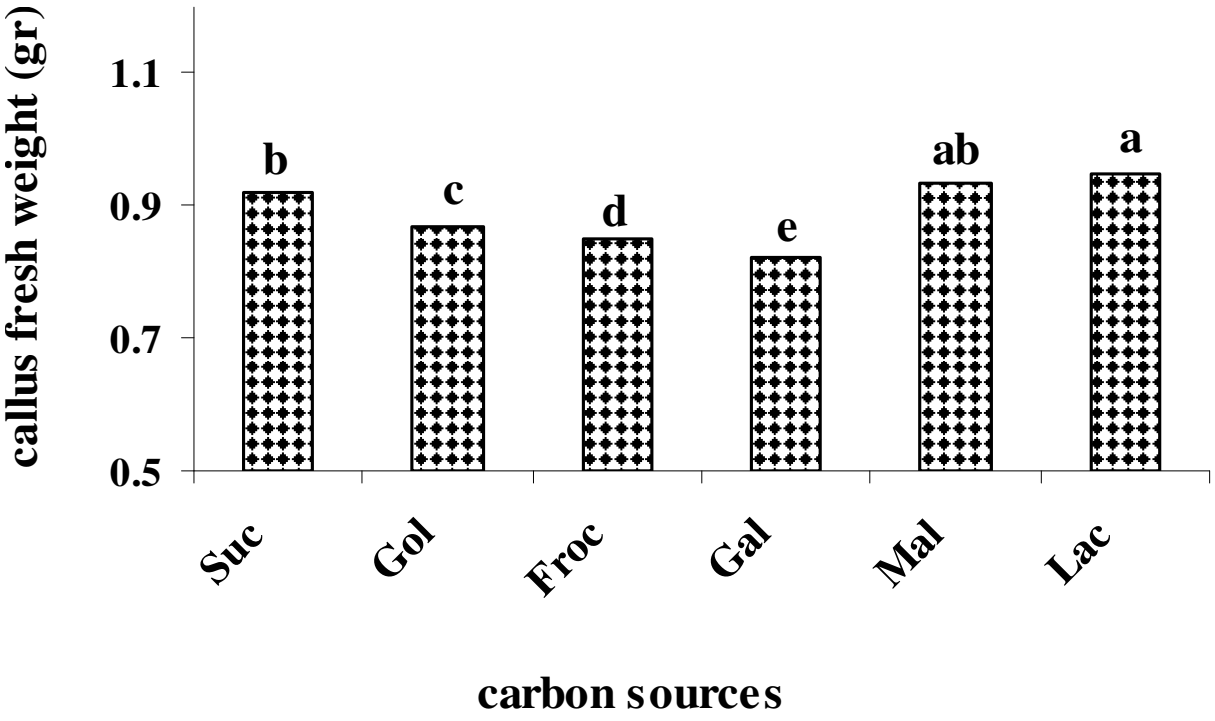

Figure 1. Effect of carbon source on callus fresh weight (g).

Table 2. Variance analysis of different lactose concentration influence on callus fresh weight (gr).

S.O.V	df	MS	F-value
Concentration	3	0.527258	67.16**
Error	12	0.007851	

** is significant at 0.01 probability level.

et al., 2008). Similar result were obtained by Chen and Dribnenki (2002) who reported that in comparison to sucrose, lactose was found to increase callus induction from *Linum usitatissimum* L. anthers for all three genotypes tested. In contrast, Jain et al. (1997) observed media with lactose, manitol or sorbitol supported limited growth or failed to induce any measurable increase in *indica* and *japonica* rice varieties callus fresh weight.

Plant cell, tissue or organ culture normally requires a carbohydrate supply in order to satisfy energy demands. Many studies have been conducted to define type and concentration of carbon source that allow culture establishment and development (Karhu, 1997). Al-Khateeb (2008) observed significant difference among the three different concentrations of carbohydrates for callus fresh weight and axillary shoot numbers. Our study indicated that the most callus fresh weight was observed at 2% lactose. This observation confirms data of Short and Warburton, (1987) who reported that, dry weight of plants are reduced due to high sugar concentration, while Al-

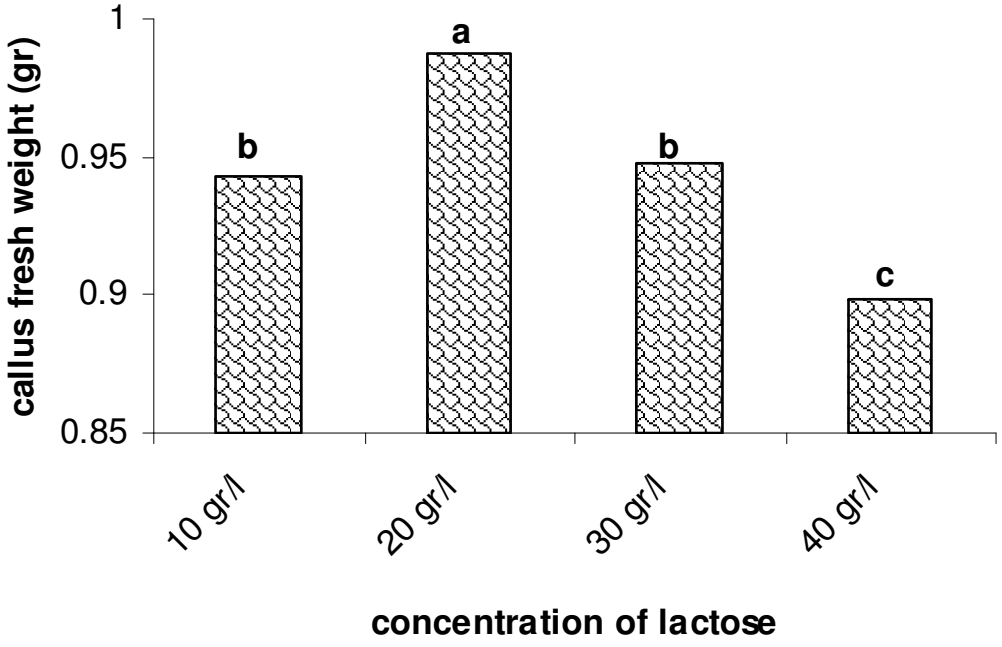

Figure 2. Effect of different lactose concentrations on callus fresh weight (g).

Table 3. Analysis of variance of carbon source influence on percentage of regeneration.

S.O.V	df	MS	F-value
Carbon source	3	0.02250712	72.59**
Error	12	0.00031007	

** is significant at 0.01 probability level.

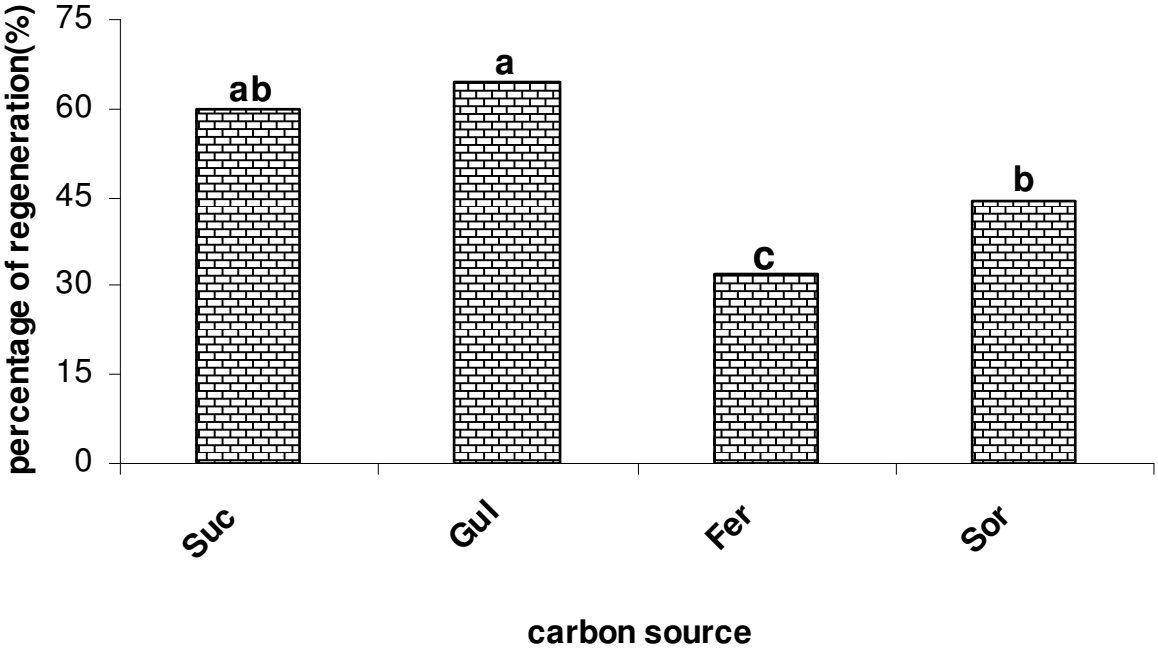

Figure 3. carbon source influence on percentage of regeneration.

Table 4. Variance analysis of different glucose concentration influence on percentage of regeneration.

S.O.V	df	MS	F-value
Concentration	3	0.03742159	61.41**
Error	12	0.0060939	

** is significant at 0.01 probability level.

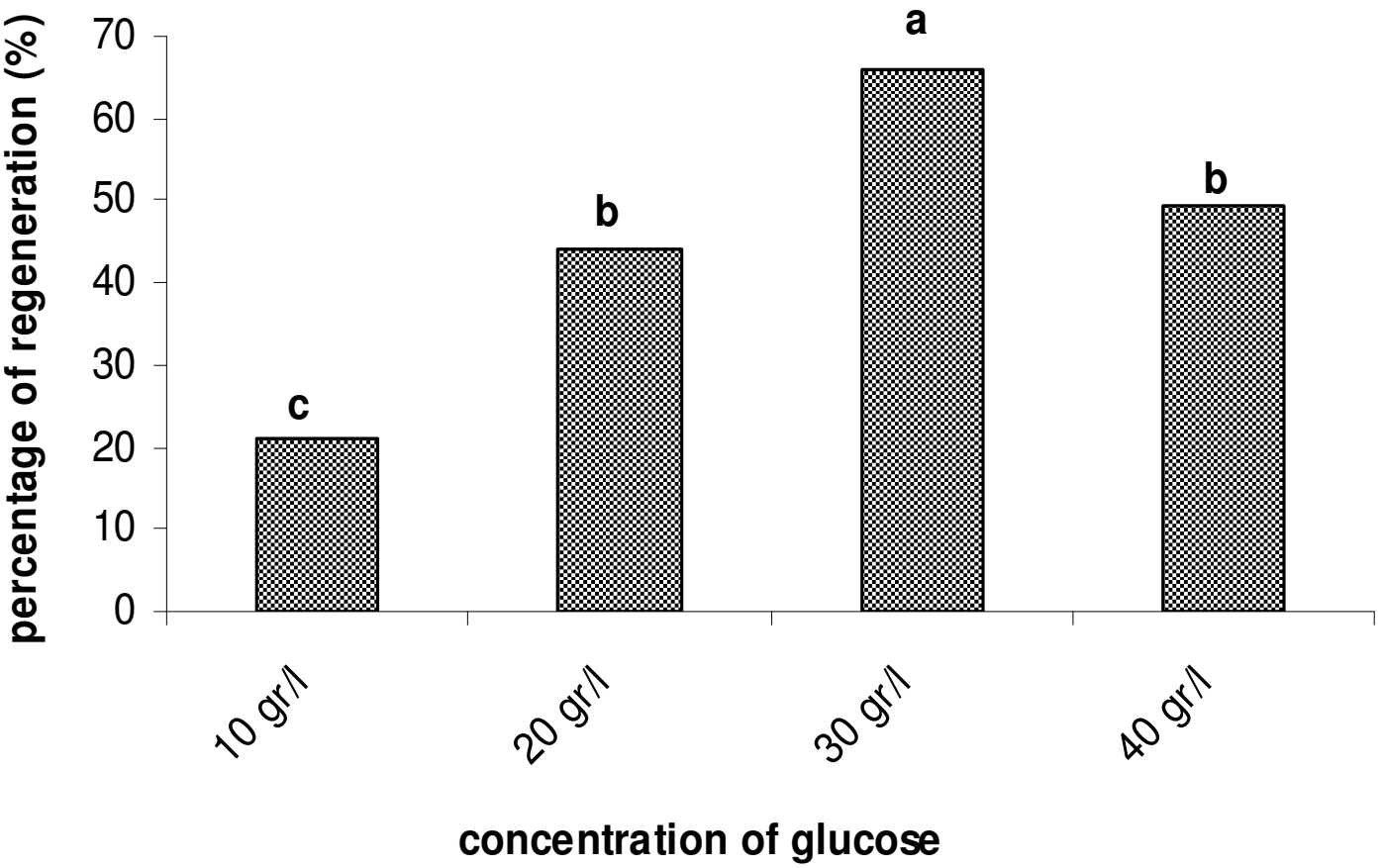

Figure 4. Effect of different glucose concentrations on percentage of regeneration.

Khateeb (2008) results were in contrast with the previous finding; he recorded an increase in dry weights of *Phoenix dactylifera* L. in response to high sugar concentration.

The results of the present study showed that the performance of shoot culture of *D. stramonium* is affected by both the type and concentration of sugar in the culture medium. In general, glucose was better for inducing shoot proliferation than other carbon sources. Similar results were obtained by Salvi et al. (2002) who reported that a significant increase in shoot length and number and length of roots was observed when glucose was added to the medium suggesting that glucose could be the best carbon source for *in vitro* multiplication of turmeric cv. 'elite'. Glucose was the most effective carbon source for both axillary branching and adventitious shoot regeneration in *in vitro* beech cultures (Cuenca and Vieitez, 2000).

In contrast our results, Baskaran and Jayabalan (2005) suggested among the three carbon sources, sucrose proved to be better than fructose or glucose for shoot regeneration of *Eclipta alba*. Amutha et al. (2003) reported that glucose, fructose and maltose led to very poor shoot differentiation. They observed sucrose evoked maximum regeneration frequency and increased the number of regenerating shoots. Nowak et al. (2004) results suggested that sucrose was a better carbon source than glucose for organogenesis of 'W¸egierka

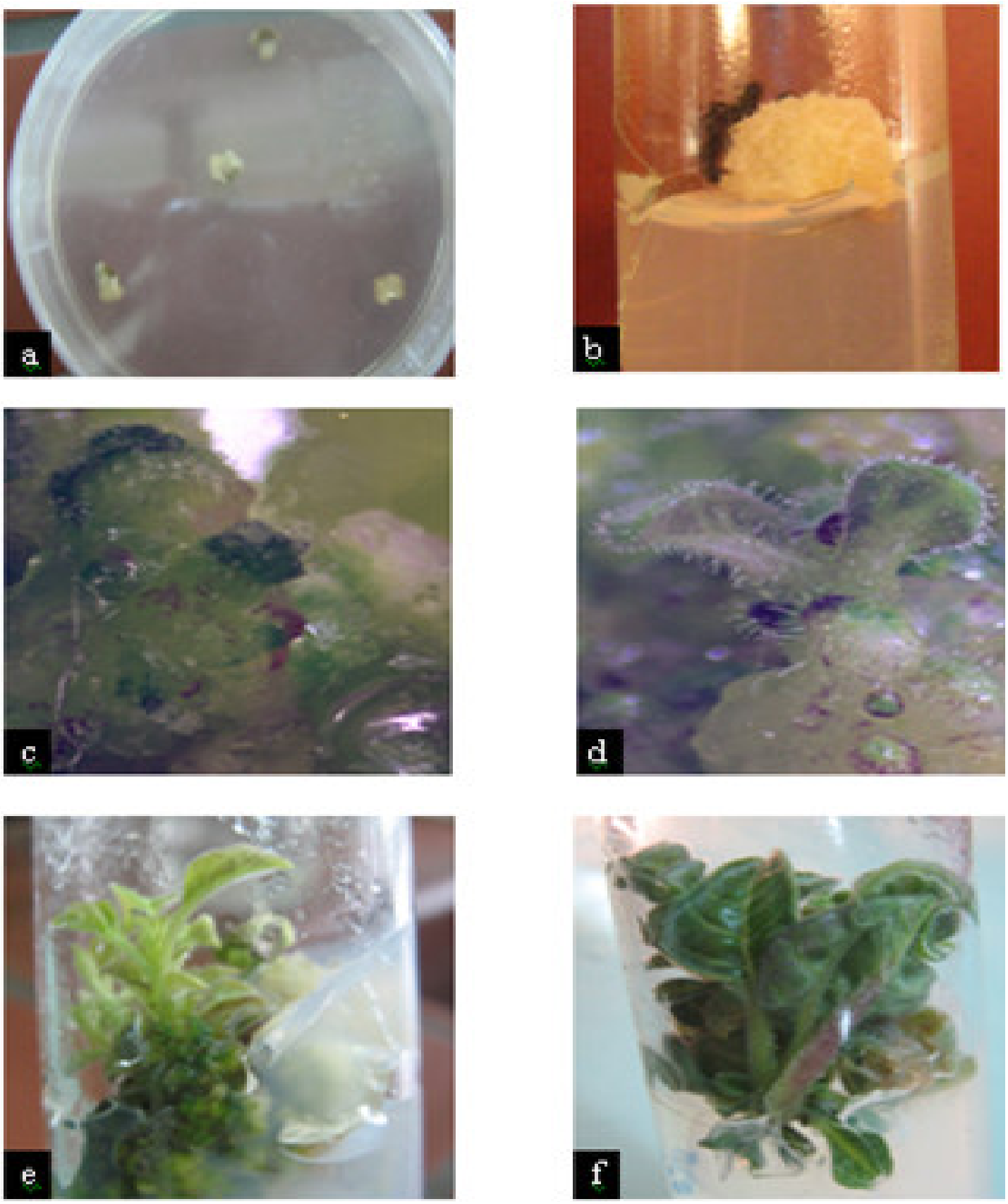

Figure 5. a-b: Embryo-derived calli; c-f: Regeneration of embryo-derived calli.

Zwykła', even though at lower concentrations the efficiency of the sugars was comparable.

REFERENCES

Al-Khateeb AA (2008). Regulation of in vitro bud formation of date palm (Phoenix dactylifera L.) cv. Khanezi by different carbon sources. Bioresour. Technol., 99: 6550-6555.

Amutha S, Ganapathi A, Muruganantham M (2003). *In vitro* organogenesis and plant formation in *Vigna radiata* (L.) Wilczek. Plant. Cell. Tissue Organ. Culture, 72: 203-207.

Baskaran P, Jayabalan N (2005). Role of basal media, carbon sources and growth regulators in micropropagation of *Eclipta alba* – a valuable medicinal herb. Kmitl. Sci. J. 5(2): 469-482.

Blanc G, Lardet L, Martin A, Jacob JL, Carron MP (2002). Differential carbohydrate metabolism conducts morphogenesis in embryogenic callus of *Hevea brasiliensis* (Müll. Arg.). J. Exp. Bot., 53: 1453–1462.

Chen Y, Dribnenk P (2002). Effect of genotype and medium composition on flax Linum usitatissimum L. anther culture. Plant Cell Rep. 21(3): 204-207.

Chittenden FJ (1965). The Royal Horticultural Society dictionary of gardening: a practical and scientific encyclopaedia of horticulture, vol. II, 2nd edn. Clarendon, Oxford, pp. 640–641.

Cuenca B, Vieitez AM (2000). Influence of carbon source on shoot multiplication and adventitious bud regeneration in *in vitro* beech cultures. Plant Growth Regul., 32: 1-12.

George EF, Hall MA, Klerk GJD (2008). Plant Propagation by Tissue Culture. 3rd Edition. Springer.

Gibson SI (2000). Plant sugar-response pathways. Part of a complex regulatory web. Plant Physiol., 124: 1532–1539.

Jain RK, Davey MR, Cocking EC, Wu1 R (1997). Carbohydrate and osmotic requirements for high frequency plant regeneration from protoplast-derived colonies of *indica* and *japonica* rice varieties. J. Exp. Bot., 48(308): 751-758.

Karhu, ST (1997). Sugar use in relation to shoot induction by sorbitol and cytokinin in apple. J. Am. Soc. Hort. Sci., 122: 476–480.

Kim SS, Guo DD, Jung DC, Kwon ST (2003). Multiple shoots regeneration and in vitro bulblet formation from Garlic callus. J. Plant Biotechnol., 5: 95–99.

Mendoza MG, Kaeppler HF (2002). Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of wheat (*Triticum aestivum* L.). *Invitro* Cell. Dev. Biol. Plant., 38: 39-45.

Neto VBP, Otoni WC (2003). Carbon sources and their osmotic potential in plant tissue culture: does it matter? Sci. Hortic. 97: 193–202.

Nowak B, Miczy´nski K, Hudy L (2004). Sugar uptake and utilisation during adventitious bud differentiation on *in vitro* leaf explants of 'W¸egierka Zwykła' plum (*Prunus domestica*). Plant Cell Tissue Organ. Culture, 76: 255–260.

Romano A, Noronha C, Martins-Louçao MA (1995). Role of carbohydrates in micropropagation of cork oak. Plant Cell. Tissue Org. Cult ., 40: 159–167.

Salvi ND, George L, Eapen S (2002). Micropropagation and field evaluation of micropropagated plants of turmeric. Plant. Cell. Tiss. Org. Cult., 68: 143–151.

Sharma VK, Jethwani V, Kothari SL (1993). Embryogenesis in suspension cultures of *Datura innoxia* Mill. Plant. Cell. Rep., 12: 581–584.

Short KC, Warburton J (1987). *In vitro* hardening of cultured cauliflower and chrysanthemum plantlets to humidity. Acta Hortic. 212: 329–334.

Slesak H, Skoczowski A, Przywara L (2004). Exogenous carbohydrate utilisation by explants of *Brassica napus* cultured *in vitro*. Plant Cell. Tissue Org. Cult., 79: 45–51.

Thompson MR, Thorpe TA (1987). Metabolic and nonmetabolic roles of carbohydrates. In: Bonga JM and Durzan DJ (eds) Cell and Tissue Culture in Forestry, Vol 1, General Principles and Biotechnology, Dordrecht: Martinus Nijhoff Publishers, pp. 89–112.