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Specific nutrient uptake during initiation of somatic embryogenesis in coconut calluses

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Abstract

Changes in the concentration of major nutrients and sugars in the media were measured for two embryogenic strains of coconut calluses, L1 and L7, after 0, 14, 28, 42 and 56 days of culturing on multiplication medium and embryogenesis induction medium. A histological study [illegible]

pears that sucrose is the most effective carbohydrate source for somatic embryogenesis in the species examined so far [5] and that NH_4^+ is strictly required for the initiation of embryogenesis in *Daucus carota* [6,7], *Atropa belladona* [8], *Solanum melongena* [9] and *Medicago sativa* [10]. To our knowledge, a specific requirement for the

Sunnyvale, USA). Anion concentrations were measured using an isocratic method (eluent: 3.9 mM $NaHCO_3$, 3.1 mM Na_2CO_3; guard column IONPAC AGSA; analytic column IONPAC ASSA). For cations, a step-gradient was applied by changing the eluent 3 min after injection (eluent 1: 12 mM HCl and 0.5 mM 2,3 diaminopropionic

categorial means [17,18]. On the graphs, at each time, points within brackets are not significantly different at the 0.05 probability level as determined by the Newman and Keuls' test.

3. Results

matic zone was organized in a cambium-like structure, whereas, for strain L7, it corresponded to 4 or 5 non-organized layers of homogeneous cells. In the internal area of the L7 calluses, cells were differentiated into parenchymatous tissue. In the in-

Table 1
Results of two-way analysis-of-variance for strain L1

	2,4-D concentration effect F	Time effect F	2,4-D concentration-time effect F
Dry weight	4.322 *	15.53 ***	0.793 NS
Protein content	6.125 *	8.156 **	0.079 NS
Sucrose uptake	4.561 *	4.346 *	0.591 NS
Glucose uptake	0.326 NS	5.632 *	1.002 NS
Fructose uptake	0.569 NS	7.875 **	0.463 NS
Cl^- uptake	0.239 NS	3.246 *	0.452 NS
$H_2PO_4^-$ uptake	0.819 NS	3.345 *	0.938 NS
NO_3^- uptake	0.975 NS	3.917 *	0.784 NS
SO_4^{2-} uptake	3.068 NS	7.643 **	1.487 NS
Ca^{2+} uptake	5.120 **	6.416 **	0.055 NS
K^+ uptake	2.854 NS	1.549 NS	0.738 NS
Mg^{2+} uptake	4.832 *	6.213 **	0.651 NS
NH_4^+ uptake	10.658 **	3.567 *	0.921 NS

For each effect tested (rows) and trait studied (columns), F value is given.
NS, not significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$.

cells became isolated from the peripheral meristematic zone and developed into proembryos (Fig. 1b). These cells displayed numerous histological characteristics typical of embryogenic cells, as

Thus, in strain L7, the transfer from 2.7×10^{-4} M to 5×10^{-4} M 2,4-D resulted in the initiation of somatic embryogenesis from a unicellular origin.

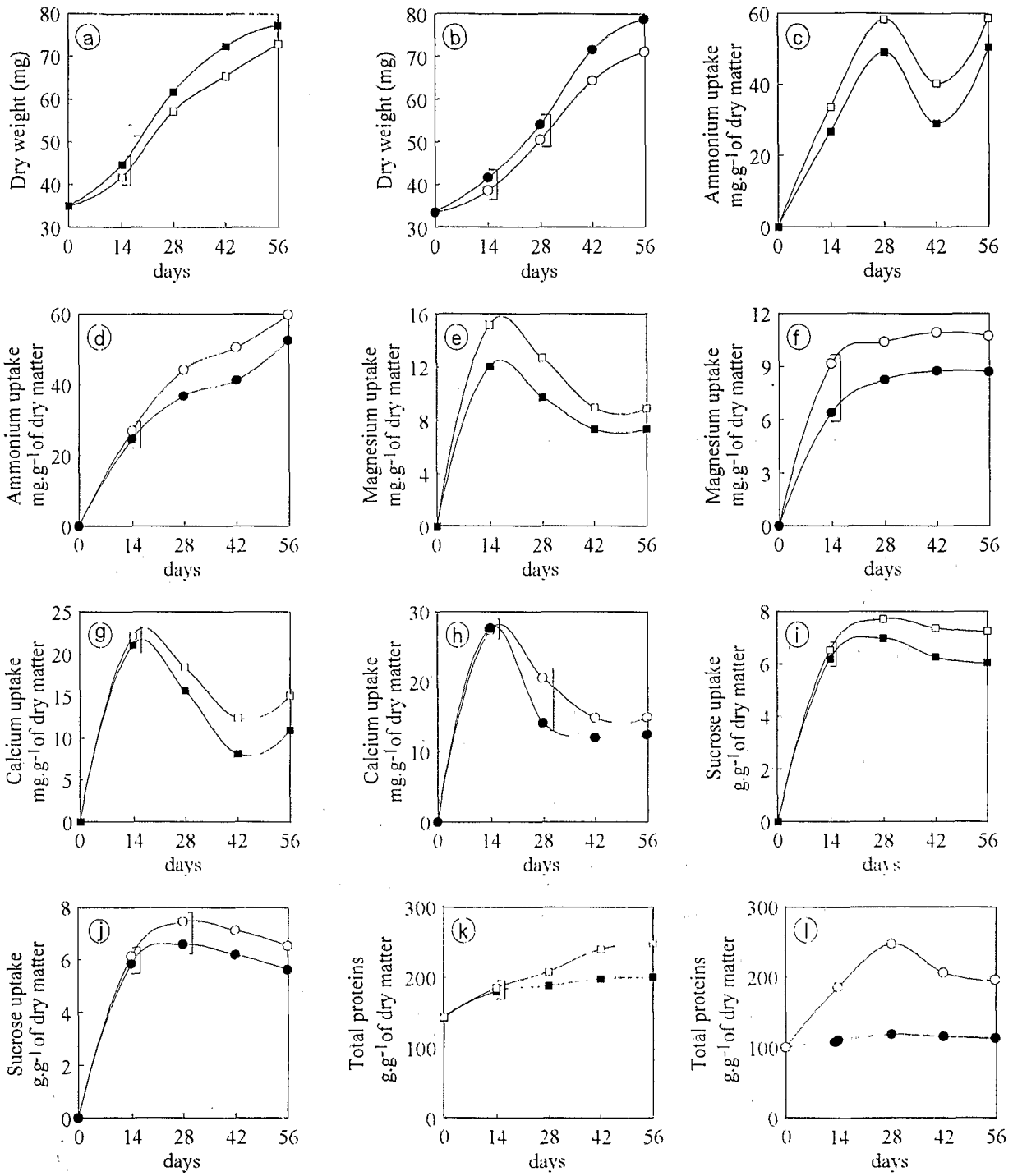

Fig. 2. Changes in the dry weight of calluses (a, b), the uptake of NH_4^+ (c, d), Mg^{2+} (e, f), Ca^{2+} (g, h) and sucrose (i, j) per g of dry matter and the total protein content per g of dry matter (k, l) of the strain L1 (a, c, e, g, i, k), on embryogenesis initiation medium (□) (M1) and multiplication medium (■) (M3), and of the strain L7 (b, d, f, h, j, l), on embryogenesis initiation medium (○) (M5) and multiplication medium (●) (M2). At each time, points within brackets are not significantly different at the 0.05 probability level

bules, which corresponded to embryogenic structures, were composed of highly meristematic cells containing more soluble proteins than the cells cultured on the multiplication medium. These embryogenic structures were very similar to those observed in oil palm [19] and could be oriented to

calluses cultured on M5 medium and for L7 calluses cultured on M1 medium.

3.5. Total protein content

The total protein content per g of dry matter was significantly higher on embryogenesis initia-

NH_4^+. This preferential requirement for NH_4^+ can be attributed to the increase of protein synthesis during the initiation of somatic embryogenesis. Indeed, it has been established that *Rosa* cells accumulate twice more protein reserves on a medium containing both NH_4^+ and NO_3^- than on a medium containing only NO_3^- [24]. In rice suspension cultures, it was clearly shown that NH_4^+ had a higher influence on protein content in cells when compared to NO_3^- [25]. It has also been proven that, in *Daucus carota*, the initiation of somatic embryogenesis cannot be started if the intracellular concentration in ammonium does not reach the minimal value of 5 mmol/g of fresh matter [26]. This minimum content cannot be reached without NH_4^+ in the medium.

Implication of Ca^{2+} in the initiation of somatic embryogenesis has been the subject of quite recent studies. To our knowledge, this study is the first one that provides evidence for the importance of Mg^{2+} in the initiation of somatic embryogenesis. The activation of protein synthesis must imply an increase of the enzymatic activity. With the multiple functions of Ca^{2+} and Mg^{2+} in enzymatic systems, it is conceivable that the initiation of embryogenesis is associated with specific requirement for these two cations. The modulation of intracellular concentration of Ca^{2+} is involved in the initiation of many processes of cellular differentiation [27]. In *Daucus carota*, it has been shown that the initiation of somatic embryogenesis is controlled by an increase in the concentration of Ca^{2+} [28]. Therefore, the specific uptake of Ca^{2+} during the initiation of somatic embryogenesis in *Cocos nucifera* could be attributed to an increase of the intracellular concentration.

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References

[2] J. Buffard-Morel, J.L. Verdeil and C. Pannetier, Embryogenèse somatique du Cocotier (*Cocos nucifera* L.) à partir d'explants foliaires: étude histologique. Can. J. Bot., 70 (1992) 735–741.

[3] J.L. Verdeil, C. Huet, F. Grosdemange and J. Buffard-Morel, Plant regeneration from cultured immature inflorescences of coconut (*Cocos nucifera* L.): evidence for somatic embryogenesis. Plant Cell Rep., 13 (1994) 218–221.

[4] J.L. Verdeil and J. Buffard-Morel, Somatic embryogenesis in coconut (*Cocos nucifera* L.), in: Y.P.S. Bajaj (Ed.), Biotechnology in Agriculture and Forestry, Vol. 30, Somatic Embryogenesis and Synthetic Seeds I, Springler-Verlag, Berlin, 1995, pp. 299–317.

[5] P.V. Ammirato, Embryogenesis, in: D.A. Evans., W.R. Sharp, P.V. Ammirato and Y. Yamada (Eds.), Handbook of Plant Cell Culture, Vol. 1, Macmillan Press, New York, 1983, pp. 82–123.

[6] W. Halperin and D.F. Wetherell, Ammonium requirement for somatic embryogenesis in vitro. Nature, 205 (1965) 519–520.

[7] D.F. Wettherell and D.K. Dougall, Source of nitrogen supporting growth and embryogenesis in cultured wild carrot tissue. Physiol. Plant, 37 (1976) 97–103.

[8] E. Thomas and H.E. Street, Factors influencing morphogenesis in excised roots and suspension culture of *Atropa belladona*. Ann. Bot., 74 (1972) 77–81.

[9] S. Gleddie, W. Keller and G. Setterfield, Somatic embryogenesis and plant regeneration from leaf explants and cell suspensions of *Solanum melongena* L. (eggplant). Can. J. Bot., 61 (1983) 656–666.

[10] E.G.M. Meijer and D.C.W. Brown, Role of exogenous reduced nitrogen and sucrose in rapid high frequency somatic embryogenesis in *Medicago sativa*. Plant Cell Tissue Organ Culture, 10 (1987) 11–19.

[11] J. Reinert, M. Tazawa and S. Semenoff, Nitrogen compounds as factors of embryogenesis in vitro. Nature, 216 (1967) 1215–1216.

[12] J.L. Verdeil, J. Buffard-Morel and C. Pannetier, Embryogenèse somatique du cocotier (*Cocos nucifera* L.) à partir de tissus foliaires et inflorescenciels. Bilan des recherches et perspectives. Oléagineux, 44 (1989) 403–411.

[13] T. Murashige and F. Skoog, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15 (1962) 473–497.

[14] G. Morel and R.M. Wetmore, Fern callus tissue culture. Am. J. Bot., 38 (1951) 141–143.

[15] H.U. Bergmeyer and E. Bernt, in: H.U. Bergmeyer (Ed.), Methods of Enzymatic Analysis, Academic Press, New York, 1974, pp. 1176–1179.

[16] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem.

a normal population expressed in terms of an independent estimate of standard deviation. Biometrika, 31 (1939) 20–30.

[19] J. Schwendiman, C. Pannetier and N. Michaux-Ferrière, Histology of embryonic formations during in vitro culture of oil palm *Elaeis guineensis* Jacq. Oléagineux, 45 (1990) 403–411.

[20] T.A. Thorpe, Organogenesis in vitro: structural, physiological and biochemical aspects, in: I.K. Vasil (Ed.), Perspectives in Plant Cell and Tissue Culture, Academic Press, New York, 1980, pp. 71–111.

[21] T. Fujimura and A. Komamine, Molecular aspects of somatic embryogenesis in a synchronous system, in: A. Fujiwara (Ed.), Plant Tissue Culture, Abe Photo Printing, Tokyo, 1982, pp. 105–106.

[22] H. Kamada and H. Harada, Studies on nitrogen metabolism during somatic embryogenesis in carrot, in: A. Fujiwara (Ed.), Plant Tissue Culture, Abe Photo Printing, Tokyo, 1982, pp. 115–116.

[23] E.F. George and P.D. Sherington, Plant Propagation by Tissue Culture, Eastern Press, Hants, 1982, pp. 709.

[24] B. Mohanty and J.S. Fletcher, Ammonium influence on the growth and nitrate reductase activity of Paul's Scarlet Rose suspension cultures. Plant Physiol., 58 (1976) 152–155.

[25] U. Schmitz and H. Lörz, Nutrient uptake in suspension cultures of *Gramineae*. II. Suspension cultures of rice (*Oryza sativa* L.). Plant Sci., 66 (1990) 95–111.

[26] M. Tazawa and J. Reinert, Extracellular and intracellular chemical environments in relation to somatic embryogenesis in vitro. Protoplasma, 68 (1969) 157–173.

[27] A.C.J. Timmers and J.N.H. Schel, Immunocytochemical localization of calmodulin during carrot embryogenesis, in: H.J.J. Nijkamp, L.H.W. Van Der Plas and J. Van Aartrijk (Eds.), Progress in Plant Cellular and Molecular Biology, Kluwer Academic Publishers, Dordrecht, 1990, pp. 443–448.

[28] A.C.J. Timmers and J.N.H. Schel, Carrot somatic embryogenesis coincides with a rise in free cytosolic calcium level, in: Proceedings 14th International Congress on Plant Growth Substances, Amsterdam, 1991, pp. 73.