Somatic Embryogenesis in Saffron: Optimisation through Temporary Immersion and Polyamine Metabolism

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Abstract

Saffron embryogenic calli increased fresh weight four times when cultured in a temporary immersion system compared to those cultured in solid medium. Paclobutrazol was effective reducing hyperhydricity in the explants; the best results were obtained with 1mg/l. The development of somatic embryos on solid medium was significantly improved with the addition jasmonic acid (0,5 mg/l). Saffron somatic embryos contained more conjugated than free polyamines. This was true for all stages of embryogenesis except for initial stage. The establishment of polar embryos determined a significant increase in the level of polyamines mainly due to a drastic rise in the conjugation of the diamines diaminepropane and putrescine. This stage was also characterized by the depletion of free diaminepropane. In dipolar embryos a decrease in the concentration of conjugated polyamines was observed. The last stage of regeneration, in which corms are well formed at the base of each plantlet, coincided with further reduction in total polyamines; the almost complete depletion of most free polyamines (only traces of putrescine and spermine are detected), being conjugated spermidine the most predominant amine.

INTRODUCTION

Crocus sativus L. is cultivated for its red stigmatic lobes that constitute the spice saffron. The manual cultivation methods practised with the saffron crocus contribute greatly to the high price of the spice. Any attempt to modernize the cultivation of the saffron crocus will, therefore, require efficient mass production of pathogen-free corms, for which purpose tissue culture is ideal. Somatic embryogenesis is a very useful technique for plant regeneration and it has been used in saffron culture successfully (Ahuja et al., 1994). Temporary Immersion Systems (TIS) generally improve plant quality and production, increasing shoot vigour and the frequency of morphologically normal somatic embryos (Etienne and Berthouly, 2002). Polyamines have been suggested to play a significant role in plant morphogenic and developmental processes (Kakkar and Sawney, 2002). Studies on polyamine pattern and biosynthesis revealed that somatic embryo formation, development, and conversion, are affected by free polyamine levels (Faure et al., 1991; Singh et al., 1998, Shoeb et al., 2001). In the present work we study the polyamine metabolism in during somatic embryogenesis in saffron as a mean to improve the regenerative capacity of the system.

MATERIAL AND METHODS

Plant Tissue Culture

Embryogenic callus were initiated according to N medium described in Piqueras et al. (1999). We have established the culture sequence to optimise plant regeneration by means of somatic embryogenesis. Saffron callus were multiplied in TIS five min

immersions every three hours, on NAA1 medium (MS+1mg/l BAP+ 0.05mg/l NAA). 0.5, 1, 2 and 5mg/l of paclobutrazol (PAC) were added to reduce hyperhydricity (HH). 500mg/l of polivinilpirrolidone (PVP) were added to minimize oxidation and phenolic compounds. The effect of jasmonic acid (JA) on somatic embryos development was analysed. JA was combined with 3% and 4.5% of sucrose.

Polyamine Analysis

The polyamine content (free, conjugate and bound) of the different developmental stages of somatic embryogenesis in saffron was measured according to Sharma and Rajam (1995).

RESULTS AND DISCUSSION

After induction of somatic embryogenesis in N medium (MS+ 0.5mg/l BAP+ 0.1mg/l 2.4D), embryogenic calli were subcultured in NAA1 medium (MS+1mg/l BAP+0.05mg/l NAA) for multiplication in solid medium and TIS medium (RITA, Figure 2). A four-fold increase in the production of embryogenic calli (fresh weight increase) was observed in TIS culture when compared to solid medium (Figure 1, A). The best results were obtained when 1mg/l of PAC was added (Figure 1, B). Results comparable to ours in saffron embryogenic tissues have been reported in several plant species micropropagated in TIS with positive effects on shoot proliferation, microtuberization and somatic embryogenesis (Etienne and Berthouly, 2002).

The development of somatic embryos was improved in on solid medium supplemented with 0.5 mg/l JA (Figure 3, A). Plant regeneration via somatic embryogenesis was obtained after eight weeks of treatment with combination of JA and sucrose (Figure 3, B). The ideal conditions for maturation of somatic embryos (Figure 4) were as follow: first JA (0.5mg/l JA) weeks 0-4, then sucrose (4.5%) weeks 4-8 (Figure 3, B). As we have reported in saffron, jasmonates are involved and enhance the development of tubers, bulbs and corms of several geophytes because of its positive influence of carbohydrate accumulation (Koda, 1997; Ravnikar et al., 1993; Santos and Salema, 2000).

Changes in the polyamine (PA) levels and pattern have been observed in the different stages of saffron somatic embryogenesis. Increased levels of total polyamines were found during the embryo development (Figure 5, A). The most predominant fraction of PAs corresponded to conjugated PAs (Figure 5, A). Highest levels of PAs corresponded to putrescine (Put), free and conjugated, during all developmental stages of somatic embryogenesis, while diaminopropane (Dap) appeared principally conjugated; and cadaverine (Cad), spermidine (Spd) and spermine (Spm) were present in lower concentrations (Figure 5, B, C). In relation to changes observed in polyamine metabolism during saffron somatic embryogenesis, these are comparable to those previously reported by Li and Burrit (2003) who observed a significant increase in the level of PAs during the initial stages of somatic embryogenesis in *Dactilys glomerata.*

The qualitative analysis of PAs during the time course can be considered as an effective biomarker system of the morphogenic process (Shoeb et al. 2001)

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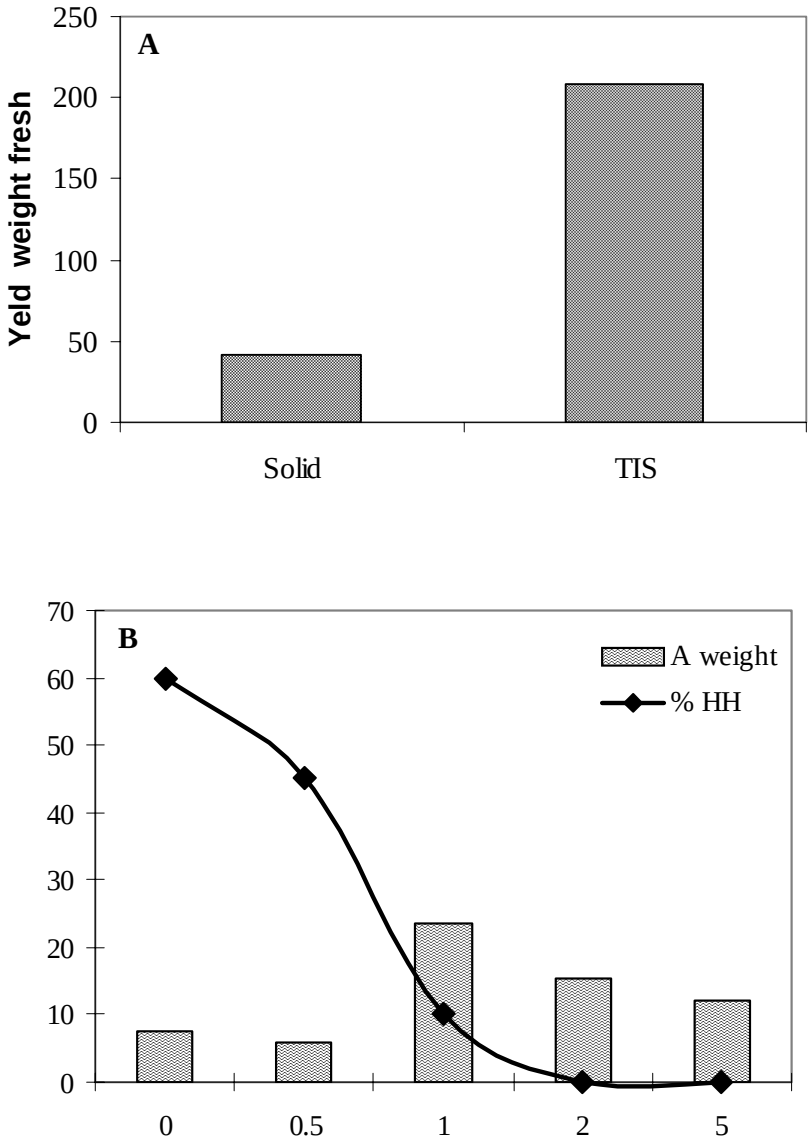
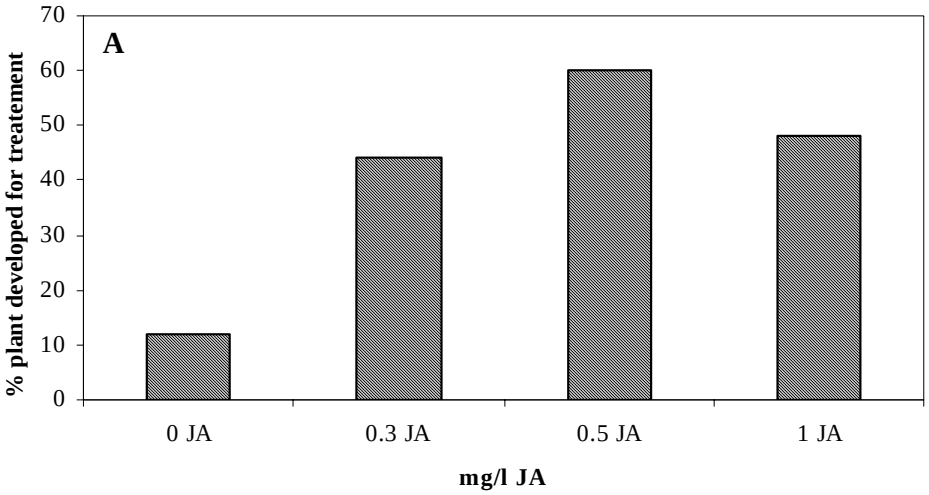


Fig. 1. TIS culture. Yielding (%) in fresh weight of saffron embryogenic calli analysed after four weeks in culture (A). Hyperhydricity percentage (% HH) and weight increase (A weight, gr) of embryogenic calli after four weeks in culture at different PAC concentrations (B).

Fig. 2. Detail of TIS (RITAs) at work



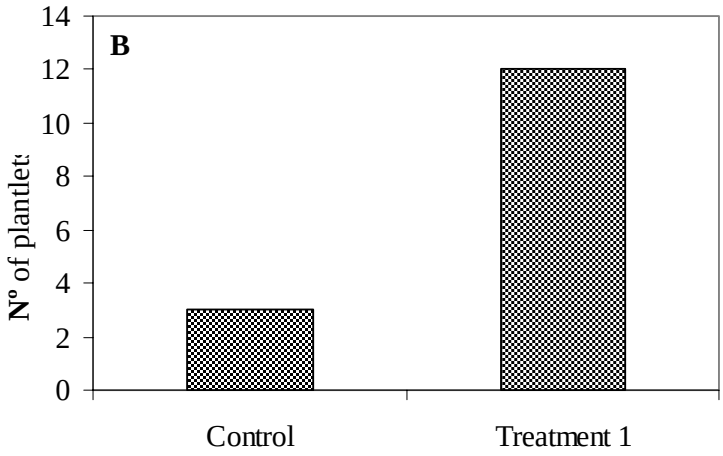


Fig. 3. JA and sucrose treatment. Percentage of plant developed with different levels of JA (A). Effect of treatment combined JA with 4.5% of sucrose on number of plantlets developed (B).

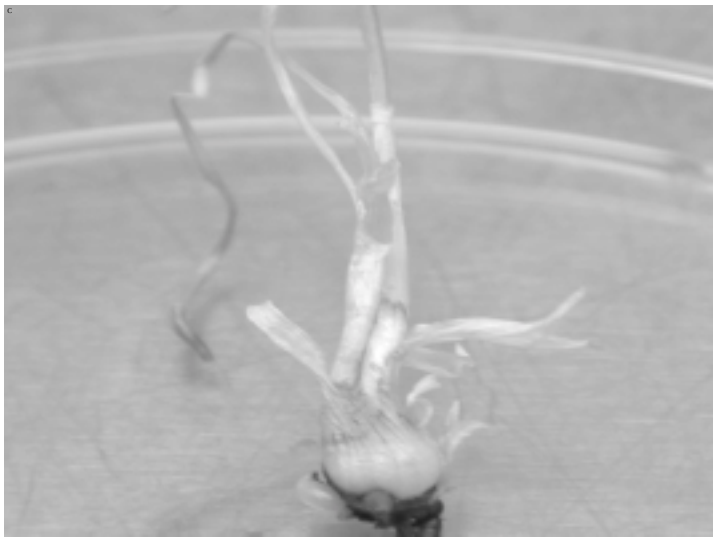


Fig. 4. Somatic embryo development to plantlet.

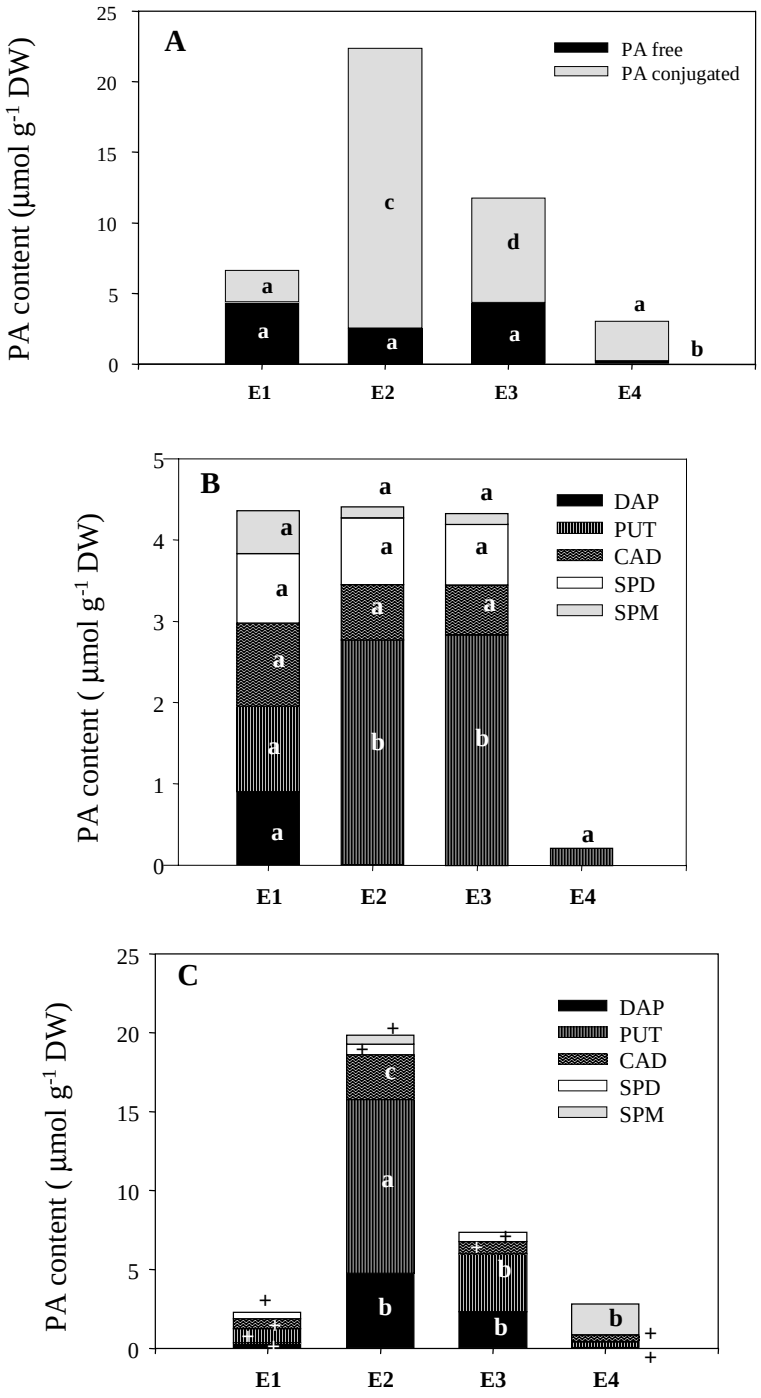


Fig. 5. PA pattern of diferent stages in somatic embryos. Concentration of free and conjugated PA in somatic embryos of saffron (A). Free PA (b). Conjugated PA (C). E1, nodular embryos. E2, monopolar embryos. E3, dipolar embryos. E4, plantlets. Bars with the same letter (or ++) in each fraction are not significantly different (α=0.05).