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Effect of genotype, gelling agent, and auxin on the induction of somatic embryogenesis in sweet potato (*Ipomoea batatas* Lam.)

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Abstract

Lateral buds of six cultivars of sweet potato were induced to form embryogenic callus in a culture medium solidified with two types of gelling agents, Agar or Gelrite, and supplemented with various concentrations of auxins, 2,4-D, 2,4,5-T and Picloram. Of the six cultivars screened, only three gave an embryogenic response. Best results with an average of 3.53% embryogenic response were obtained with the medium solidified with Agar, while in Gelrite only 0.45% of lateral buds gave rise to embryogenic callus. The interaction between the genotype and auxins was highly significant; particularly the optimal response was obtained with cv. Zho and 865 yielding 10.7 and 14.7% somatic embryogenesis, respectively, in the medium containing 2,4,5-T or Picloram. The plant conversion was dramatically improved by subculture of the embryogenic callus on the medium with the combination of 1 μM 2,4-D and 1 μM Kinetin or 5 μM ABA alone before transfer of mature embryos onto hormone-free medium. The embryogenic callus of sweet potato and its sustained ability to further regenerate plants have regularly been maintained for several years by frequent subculture in 5 μM 2,4,5-T or the combination of 10 μM 2,4-D and 1 μM BAP or kinetin. The embryo-derived plants seemed apparently genetically stable and similar to the hexaploid parental plants, based on morphological analysis and their ploidy level determined by using flow cytometry. ***To cite this article: Z. Triqui et al., C. R. Biologies 331 (2008).***

Résumé

Effet du génotype, de l'agent gélifiant et de l'auxine sur l'induction de l'embryogenèse somatique chez la patate douce (Ipomoea batatas Lam.). Des bourgeons axillaires de six cultivars de patate douce ont été ensemencés sur des milieux d'induction de cals embryogènes solidifiés par deux types d'agents gélifiants, qui sont l'agar et la gelrite, et additionnés de 2,4-D, 2,4,5-T ou Piclorame à différentes concentrations. Parmi les six cultivars testés, seuls trois ont donné une réponse embryogène. Les meilleurs résultats, avec une moyenne de 3,53%, ont été obtenus avec l'agar, alors qu'avec la gelrite, seuls 0,45% des bourgeons axillaires ensemencés ont donné une réponse embryogène. L'interaction entre auxine et génotype s'est montrée hautement significative.

Abbreviations: ANOVA, analysis of variance; ABA, abscisic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indol-3-acetic acid; MS, Murashige and Skoog basal medium; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid.

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L'optimum des réponses embryogènes a été obtenu avec les cv. Zho et 865, qui ont produit respectivement un pourcentage de cals embryogènes de 10,7 et 14,7%, en présence de 2,4,5T et Piclorame. La conversion des plantes a été fortement améliorée par le repiquage des cals embryogènes sur un milieu comprenant une combinaison de 2,4-D et de Kinétine ou de BAP à 1 μM chacune ou l'ABA seul à 5 μM avant de transférer les embryons mûrs sur un milieu sans régulateurs de croissance. La capacité embryogène des cals des variétés Zho et 865 a été maintenue pendant plusieurs années par repiquage régulier sur un milieu comprenant 5 μM de 2,4,5-T ou une combinaison de 10 μM 2,4-D et 1 μM de BAP. Les plants issus des embryons somatiques du cv. Zho se sont montrés génétiquement stables en se basant sur la morphologie et sur le niveau de ploïdie mesuré par la cytométrie en flux. ***Pour citer cet article : Z. Triqui et al., C. R. Biologies 331 (2008).***

Keywords: Auxin; Flow cytometry; Gelling agent; *Ipomoea batatas*; Somatic embryogenesis; Sweet potato

Mots-clés : Agent gélifiant ; Auxine ; Cytométrie en flux ; Embryogenèse somatique ; *Ipomoea batatas* ; Patate douce

1. Introduction

Sweet potato (*Ipomoea batatas* Lam.) is a tuber-bearing species and represents an economically important crop in tropical, subtropical and warm temperate regions [1]. The world production of sweet potato was estimated at 129.4 Mt in 2005, of which more than 88% were from Asian countries, particularly China, with 107.1 Mt [2]. The storage roots contain a high amount of starch, which is as high as 30% of fresh weight for some cultivars. They are used as staple food, raw material for alcohol production, and animal feed. Stems and foliage are also used as forage.

Although progress has been made in the improvement of sweet potato by using conventional breeding methods [1], the selection process is time-consuming and requires a high number of individuals and improved breeding systems, because of the hexaploid status of this species ($2n = 6x = 96$ chromosomes) [3]. Moreover, breeding efforts have been seriously limited by difficulties in sexual crosses, mainly due to incompatibility and sterility within species of sweet potato [4], as well as to specific physiological requirements for flowering [5]. Therefore, biotechnology has been developed to complement and supplement the classical methods in breeding programs for efficient improvement of this crop. Despite the economic importance of sweet potato, biotechnological applications are still in their infancy. Only little work has been achieved, particularly the exploitation of somaclonal variation [1], somatic hybridization [6] and genetic transformation [7]. One of the main difficulties is the control of plant regeneration, for which sweet potato is considered a recalcitrant species [8]. Among various systems of plant regeneration, somatic embryogenesis is highly desired, as the process regularly provides high multiplication rates and can effectively be maintained for a long time. Genotype has been shown to be a limiting factor in induction of somatic embryogenesis, as many cultivars of sweet potato gave low or no embryogenic responses at all [9–11]. Rapid and repetitive plant regeneration via somatic embryogenesis was demonstrated in only one genotype of sweet potato, PI318846-3 [12]. The type of auxin used was also found critical for successful induction of somatic embryogenesis [13,14]. Moreover, the gelling agent, which is an often-neglected parameter in the protocols for plant regeneration, can interfere with the ability of the medium to induce the desired response, such as induction of somatic embryogenesis or shoot formation [15,16]. The two gelling agents, which have so far most commonly been used to solidify the culture medium, are Agar or its purified derivative, Agarose, extracted from red algae such as *Gracilaria*, *Gelidium* or *Chondrus*, and gellan gum, also known as Gelrite or Phytagel, an extracellular polysaccharide produced by the bacterium *Sphingomonas elodea*. Although several factors have been shown to be involved in induction of somatic embryogenesis and plant regeneration, in most experiments their effects were examined separately, resulting in missing crucial information on the possible interaction between such parameters in embryogenic response. Besides, the occurrence of a callus phase during tissue culture, particularly the culture of sweet potato protoplasts, may result in the generation of variants with morphological and physiological abnormalities, reflecting the genetic instability [8,17].

In this study, the investigation was extended to examine the effect of genotype, gelling agent and auxin, as well as of their interaction, on the induction of somatic embryogenesis in sweet potato. We compared the effects of two gelling agents, Agar and Gelrite, combined with those of three auxins, 2,4,5-T, 2,4-D and Picloram, used at two concentrations on the induction of somatic embryogenesis in six cultivars of sweet potato. Moreover, the genetic stability of embryo-derived plants was