A One-Step Procedure for In Vitro Micropropagation of Stevia (*Stevia rebaudiana* Bertoni)

A. Peixe[1,2], J. Calado[1,2] and R. Carlos[2]
[1] ICAAM, Ap. 94, 7002-554 Évora, Portugal
[2] Universidade de Évora, Ap. 94, 7002-554 Évora, Portugal

Keywords: adventitious rooting, axillary shoots, FeEDDHA, FeEDTA, micropropagation, paclobutrazol, stevia

Abstract

Most studies about stevia micropropagation are based on a two phased procedure; in vitro shoot proliferation followed by root induction and expression. We propose a one-step procedure which allows obtaining simultaneously, in a thirty-days period, in vitro rooted plantlets and nodal shoot segments to start a new micropropagation cycle. The auxin indole-3-butyric-acid (IBA) was tested for root induction capacity and the anti-gibberellin paclobutrazol (PBZ) was tested for its capacity to control shoot elongation and internode length. All growth regulators were used in different concentrations aiming to achieve the optimal response. Data on shoot and internode number, shoot length, and rooting rates, were recorded. In vitro rooted plants were easily acclimatized in a plant growth chamber under 25/20°C day/night temperatures and 75% humidity, after being potted on a 3:1 (v:v) peat/perlite substrate moistened with Complesal®, a liquid 5:8:10 (N:P:K) commercial fertilizer. After one month, acclimatized plants were transferred into a water-cooling equipped greenhouse.

INTRODUCTION

Stevia rebaudiana (Bert.) is a perennial sweet herb growing up to 65-80 cm tall (Goyal et al., 2010); it belongs to the *Asteraceae* family with brittle stems and elliptic leaves. The leaves accumulate more biomass than the stems during the vegetative phase indicating that stevia plants favour the photosynthetic activity during this phase.

Due to its composition and its content of health-constituents, stevia is considered an interesting raw material for the extraction and production of functional food ingredients (Laribi et al., 2012). Leaves of stevia contain glycosides (Goyal et al., 2010; Aggarwal et al., 2013) and the most important are stevioside (3-10%), rebaudiside A (13%), and rebaudiside-B, C and D (Smitha and Umesha, 2012). According to Madan et al. (2010), stevia leaf extract is extremely sweet, calorie free and has therefore a great potential to replace sucrose sugar as a high-potency natural non-caloric bio-sweetener (Laribi et al., 2012).

As the glycosides synthesis is reduced during and after the flowering period, long days that delay flowering allow more time for glycoside accumulation. Thus stevia herbage production adapts best in a long day environment where the vegetative phase is longer and the consequent steviol glycoside yield higher (Madan et al., 2010).

The commercial value and medicinal properties of stevia lead to a high worldwide demand of this non-caloric sweetener plant (Laribi et al., 2012; Sairkar et al., 2009). The major problem is the difficulty of propagation by seed (Alhady, 2011; Smitha and Umesha, 2012), due to a low seed germination rate (Abdullateef et al., 2012). Seed viability is affected by growing conditions during pollination (Abdullateef et al., 2012) and, for example, excessive rainfall during pollination can affect both seed yield and germination (Madan et al., 2010). Deficiency in pollen production and performance could have direct effects on seed formation, seed viability and seed germination capacity (Abdullateef et al., 2012).

Stevia can be propagated by seed, tissue culture and by stem cuttings, but seed germination is notably very poor, due to infertile seed, small size of seed (1000 seeds weigh 0.3-1.0 g) and their self-incompatibility. According to Abdullateef et al. (2012), the