Effect of adenine, sucrose and plant growth regulators on the indirect organogenesis and on in vitro flowering in Begonia x hiemalis Fotsch.

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Abstract

Efficient shoot bud formation (94.5 ± 7.59%), in vitro regeneration and production of flowers were obtained from sterile plants of Begonia x hiemalis Fotsch. An in vitro regeneration was attempted using immature reproductive organs, which were not commonly used before, such as young inflorescences, peduncles and petals of flowers collected in the field. The flower segments were cultured on solid Murashige and Skoog (MS) medium, supplemented with various concentrations and combinations of plant growth regulators (PGRs) and adenine. Within 8 weeks of the culture period, the highest frequency of reproductive shoot regeneration (red calyx, 8.50%) was obtained from explants of immature inflorescence cultured on the MS medium supplemented with 1.0 mg L⁻¹ benzyl amino purine (BA) and 1.0 mg L⁻¹ 1-naphthalene acetic acid (NAA), added with 40 mg L⁻¹ adenine and 3% sucrose. To attain further root growth and maturity, the clonal plantlets (with or without reproductive shoots) were excised and sub-cultured onto the MS medium fortified with 1.0 mg L⁻¹ Gibberellic acid (GA₃). 87% of the regenerated plantlets were successfully acclimatized on 2:1:1 ratio of peat: soil: sand, under greenhouse conditions. All of them were capable of producing true-to-type flowers. The in vitro developed reproductive shoots, also further generated complete, small flowers, which were morphologically similar to the in vivo types, following an ex vitro acclimatization.

This study provides an alternative approach to generate early flowering in Begonia without undergoing the full growth cycle and hence can help overcome problems associated with flower growth and development in this species. The present study revealed that floral parts (inflorescence, peduncle and petals) could also be used as a source of explants besides the commonly used tissues such as leaf, stem, shoot and root segments.

Keywords: Acclimatization; adenine; Begonia x hiemalis; indirect organogenesis; in vitro flowering.

Abbreviations: MS - Murashige and Skoog; KN/KN - Kinetin; NAA - 1-naphthalene acetic acid; BAP/BA - Benzyl adenine purine; 2,4-D - 2,4-dichlorophenoxy acetic acid; IAA - Indole acetic acid; GA₃ - Gibberellic acid.

Introduction

In vitro fruiting offers a novel method for research on the physiology of fruits and seeds developed under an in vitro environment. Under natural growth conditions, the transition from the vegetative to the reproductive stage (flowering) in plant occurs after attaining maturity (Virupakshi et al., 2002). Flowering is generally considered as a complex process regulated by a combination of environmental and genetic factors, such as plant growth regulators, carbohydrates, light and pH of the culture medium (Heylen and Vendrig, 1988). Deliberate flowering in culture can serve as a tool for studying flower induction and development as well as to control breeding programs involving species with a long juvenile period (Lin et al., 2003). In vitro flowering has been reported for many plant species (Chang et al., 2010), however, in vitro fruiting and seed set are not readily encountered (Saritha and Naidu, 2007). In order to achieve in vitro fruiting, the plants must be able to produce flowers under in vitro conditions. Flowering and fruiting are two related processes and it is ideally studied together. Flowering is a complex process regulated by both internal plant factors and environmental signals. In vitro flowering serves as an important tool in studying flower induction, initiation and the floral developmental process by utilizing plant growth regulators such as cytokinins, gibberellins and auxins (Ziv and Noar, 2006). In vitro flowering can also reduce the influence of environmental factors and can clarify the key influences affecting the flowering process by controlling environmental factors and the application of plant growth regulators (Zhang et al., 2008). Begonia L. is a genus exceeding 1500 wild species throughout the tropical and subtropical regions of Asia, Africa and America. Begonia includes species with a variety of natural foliar variegation patterns, providing diverse examples of this phenomenon. This genus is one of the largest among the flowering plants and has a large morphological variation (Kiew, 2005). In recent years, many field surveys on begonias had been carried out in the world and new species continues to be discovered and described (Peng et al., 2010). Begonias are versatile temperate plants that belong to the family Begoniaceae, which consists of 5 genera and 920 species (Hickey and King, 1981). They are gaining popularity and commercially available as flowerbeds, in hanging baskets, pot plants and as indoor houseplants. Begonia x hiemalis Fotsch, cv. “Schwabenland red” or Elatior Begonia is developed from crosses between B. socotrana Hook and B. tuberhybrida. The inflorescences consist of single and double