Scientia Horticulturae 237 (2018) 120–127

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

In vitro germination and growth protocols of the ornamental *Lophophora williamsii* (Lem.) Coult. as a tool for protecting endangered wild populations

Cortés-Olmos C., Gurrea-Ysasi G., Prohens J., Rodríguez-Burruezo A., Fita A.*

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València, Camino Vera s/n, CP 46022, Valencia, Spain

ARTICLE INFO

Keywords:
Peyote
Cactaceae
Ex-situ conservation
Threatened species
Plant growth regulator

ABSTRACT

Lophophora williamsii is an ornamental slow growth cactus highly appreciated by cacti growers and hobbyists. Its demand is often satisfied through illegal collection of wild plants and many populations are threatened with extinction. Thus, an efficient *in vitro* protocol without plant growth regulators will be of great interest for conservation purposes of this cactus. Eight different germination media, combining Murashige and Skoog medium (MS, full and half-strength), sucrose (20 and 30 g L^{-1}) and agar (8 and 10 g L^{-1}), were used to study germination rate, number of seedlings with areoles and initial seedling development. Germination rates among culture media only differed significantly in the first 14 days after sowing (DAS), reaching 67–75% at the end of the assay (49 DAS). Remarkable interactions among media components were detected, and 20 g L^{-1} sucrose and 8 g L^{-1} agar combination gave the highest performance for both size and number of areoles. Following germination assay, a growth assay was conducted during 105 days using three growth media (GrM) at different sucrose concentration (15, 30 and 45 g L^{-1}) to evaluate the increase in seedling size and number of areoles. Regardless of their initial size, 15 g L^{-1} sucrose provided the best results for both traits. Size increase was higher in the 4–5 mm seedling group, while increase in areoles was greater in 2-3 mm seedlings. It was possible to develop an *in vitro* protocol, in absence of plant growth regulators, which allows maximizing *L. williamsii* germination and growth during its first stages of development, which may increase the availability of plants in the market and avoid exhaustion of wild populations. Furthermore, plants grown *ex situ* could be reintroduced in endangered natural populations.

1. Introduction

Lophophora williamsii (Lem.) Coult., commonly known as 'peyote', is a small (5–12 cm in diameter) blue-green, button-like, spineless and slow growth cactus with napiform root (Fig. 1) whose wild populations are distributed in Mexican highlands and in the arid regions of Southwestern United States (Anderson, 1996). This plant has been used during centuries in several rituals and ceremonies by Indian Tribes (McLaughlin, 1973; Borchers et al., 2000; Halpern et al., 2005) due to its content in alkaloids (of which the major one is mescaline) with psychotropic activity (Casado et al., 2008). In addition to its ancestral ethnobotanical use, *L. williamsii* has always aroused a great interest among cacti lovers and collectors. In fact, its growing demand in the market (as in other Cactaceae) has been often satisfied through illegal collection of wild individuals (Anderson et al., 1994; Sajeva et al., 2013), in part due to the fact that its growth from seeds is very slow.

The plundering of wild plants, added to other problems related to human activity, such as agricultural and urban expansions, introduction of exotic grasses, use of herbicides and pesticides among others, has led to many cacti being threatened with extinction (Taylor 1997; Sanchez-Martinez et al., 2009), especially in those places close to urban areas where access to natural populations is easier. This high pressure on ecosystems compromises the viability of certain populations and could result in an irremediable loss of unique genetic pools. In this way, genus *Lophophora* is protected by Mexican laws and is included in the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) (Sajeva et al., 2013).

In vitro culture techniques may play a key role to accelerate the growth of *L. williamsii* plants, especially after sowing from seeds and during early stages of the development, when seedlings are more vulnerable. Furthermore, areoles are characteristic of Cactaceae, equivalent to the buds of other higher plants, and include two points of growth: one that leades to the thorns and another that originates the flowers and new buds (Ballester-Olmos, 1997). In this sense, as active growth organs, the number of areoles must considered for *in vitro* protocols as they represent the capacity of each plant for a higher

* Corresponding author.
E-mail address: anfifer@btc.upv.es (A. Fita).

https://doi.org/10.1016/j.scienta.2018.03.064
Received 6 February 2018; Received in revised form 27 March 2018; Accepted 29 March 2018
Available online 13 April 2018

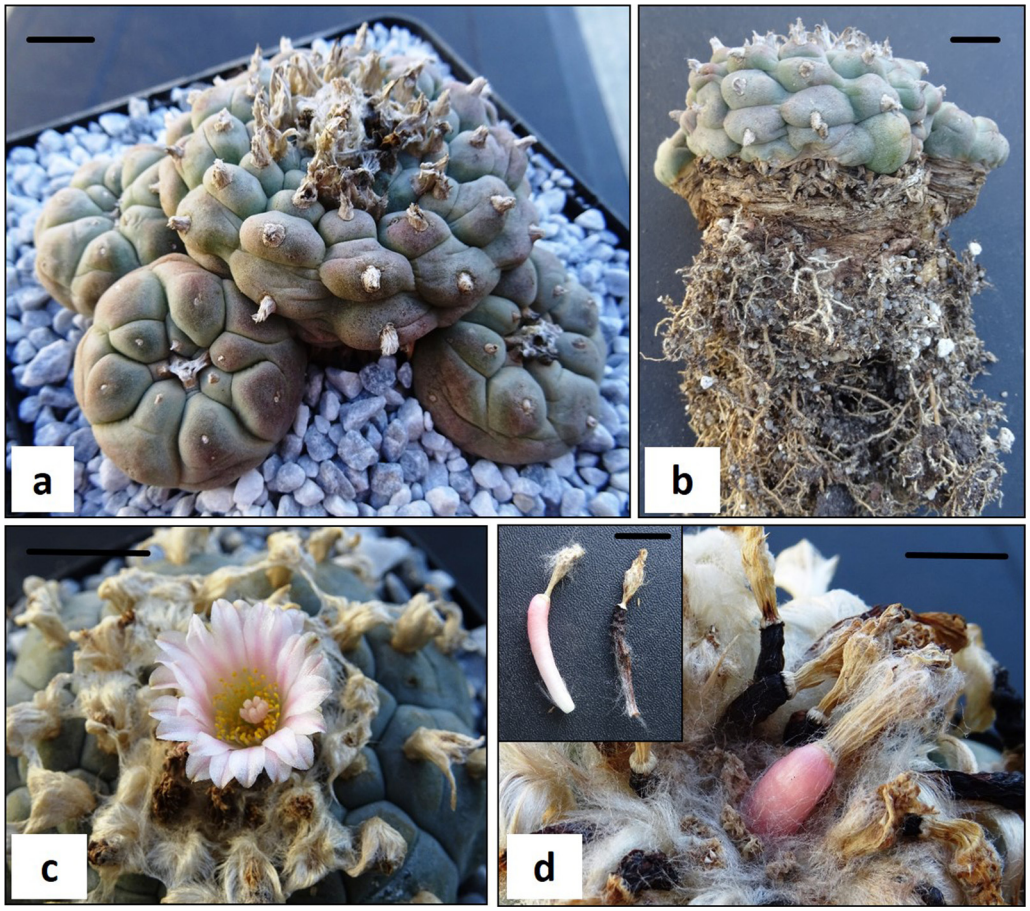

Fig. 1. *Lophophora williamsii* morphology: a) adult plant, b) napiform root, c) flowering and d) inmature fruit and mature dry fruit containing viable seeds. Black bar indicates 10 mm.

Table 1
Composition of*in vitro* media studied in both the germination and plant growth assays of *L. williamsii*.

Media	Murashige & Skoog	Sucrose (g L^{-1})	Agar (g L^{-1})
Germination Assay			
M1	full-strength	20	8
M2	full-strength	20	10
M3	full-strength	30	8
M4	full-strength	30	10
M5	half-strength	20	8
M6	half-strength	20	10
M7	half-strength	30	8
M8	half-strength	30	10
Growth Assay			
GrM-1	full-strength	15	8
GrM-2	full-strength	30	8
GrM-3	full-strength	45	8

multiplication. *In vitro* culture could help to obtain larger and flowering plants faster than by conventional seed reproduction, satisfying the demand of the market and thus reducing consequently the need to plunder wild plants. Also, *in vitro* culture could contribute to the *ex situ* conservation of plants and populations with the aim to reintroduce them in their habitat for restoring extinct or critically endangered natural populations.

In vitro plants propagation and micropropagation for conservation purposes requires efficient methods. In some cases, it has been reported that medium composition and its supplementation with plant growth regulators (PGRs) may alter morphological and physiological characters, even genetic stability, in the obtained plants (Lema-Ruminska and Kulus, 2014). Therefore, media without PGRs would be desirable as they are less prone to induct of somaclonal variations on material collected from the wild. In addition, media containing no PGRs are cheaper and easier to prepare than those formulations including PGR. Thus, in this study we compared different *in vitro* culture media in absence of PGRs, in order to detect those that maximize the development and growth of *L. williamsii* seedlings during the early stages after sowing. This would also be the first step to establish a *Lophophora* micropropagation protocol in absence of PGRs.

2. Material and methods

2.1. Seed disinfection

Seeds of a population of *L. williamsii* were kindly donated by Cactusloft (Valencia, Spain). Three hundred and twenty seeds were disinfected for 1 min in 70% etanol (v/v), followed by 25 min in 15% domestic bleach solution (v/v; 4% sodium hypochlorite), supplemented with 0.08% Tween-20 (v/v) and rinsed 3 times in distilled sterilized water under aseptic conditions under laminar flow cabinet conditions (model AH-100, Telstar, Terrassa, Spain).

2.2. Germination assay

Disinfected seeds were sown on different germination media (Table 1) in Petri dishes (10 seeds per dish, 4 dishes per medium, n = 4). A total of eight *in vitro* formulations were evaluated in this assay, which included all combinations of Murashige and Skoog medium (MS, at full-strength or half-strength, 1 × MS or 1/2 × MS, *i.e.*