

IN VITRO CULTURE OF PRIMULA: A REVIEW

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ABSTRACT. Primula is the largest genus belongs to Primulaceae family. The majority of the genus is comprising short-lived perennial herbaceous plants. Members of the genus have attractive flowers and are cultivated as bedding plants and flowering potted plants. Additionally, some of the species among the genus are economically important for ornamental plant industry. In commercial production systems, hybrids are especially propagated by seed, while primroses can also be propagated by dividing clumps. However, some problems such as irregular or late germination and low germination rate of the seeds in many primula species prevent mass propagation. Therefore, tissue culture techniques are valuable tools in breeding program, enlargement of genetic pool, gene transformation, as well as in conservation plant genetic materials in primula. In this review, common plant tissue culture techniques used in *Primula* species were briefly presented.

Keywords: *primula*, *primrose*, *regeneration*, *tissue culture*

INTRODUCTION

Primula is the largest genus with approximately 500 species in the Primulaceae family. The first time the genus was described by Carl Linne in 1753 who named the genus as *Primula* derived from the word first in Latin, referring to the early blooming in spring [1]. Primula species are mostly originated from Northern Hemisphere and are naturally grown in Europe, South America, Asia, temperate zones of the Northern Africa [2]. Most of the native plants require humid and cool climate and grow in the forest belt, plain meadows, Alpine lawns, and meadow tundras [3]. The majority of the genus *Primula* is that it comprises short-lived perennial herbaceous plants with leaves arranged in basal rosettes. Leaves are revolute or involute when they are young, often with a white or yellow farinose coating. Leaf margins vary by species as entire, dentate or serrate [4]. Inflorescence of the species in the genus are single-flowered, or arranged in umbels, several superimposed whorls, heads, or spikes. The color of the flowers in genus varies by species as white, yellow, pink, red, purple, violet, or blue, but the mouth of the tube in a contrasting color [5]. *Primula* species are the most popular example of dimorphic floral development known as heterostyly in order to understand evolutionary, developmental biology, population genetics and recently molecular genetics of the system. There are two types flower morphology, named ‘pin’ and ‘thrum’, in dimorphic species. The major differences in heteromorph flowers are that the male and female organs are placed reciprocal position in flower tube which prevent self-pollination. Pin flower type has a long style and stigma can be seen easily at the mouth of the flower, whereas thrum flowers has a short style and anthers are located at the mouth of the flower [6] (Fig. 1).

Members of the genus have attractive flowers and are cultivated as bedding plants and flowering potted plants [1]. Some of the species among the genus are economically important for ornamental plant industry and they are classified as English primrose or

acaulis primula (*Primula vulgaris* synonym *P. acaulis*) ($2n=22$), polyanthus, polyantha primrose or hybrid primrose ($P.\times$ polyantha), fairy primrose or baby primrose (*P. malacoides*) ($2n= 18, 36, 72$), German primrose or poison primrose (*P. obconica*) ($2n= 24, 48$), cowslip (*P. veris*) ($2n= 22$) and Chinese primrose (*P. sinensis*) ($2n= 24, 36, 48$) [7].

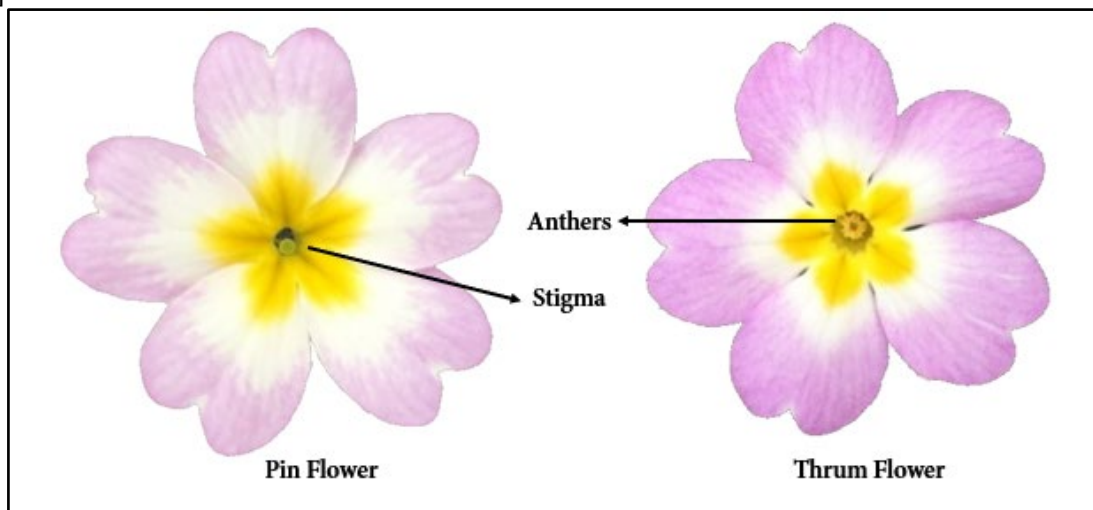


Fig. 1. Pin and Thrum flower types in *Primula vulgaris*

Primula or primrose breeding started in the early 1900s and many primula hybrids have been improved in different countries mainly through intraspecific crossings and partly through interspecific hybridization. In commercial production systems, hybrids are especially propagated by seed, while primroses can also be propagated by dividing clumps (Fig. 2). However, some problems such as irregular or late germination and low germination rate of the seeds in many primula species prevent mass propagation [8, 9, 10].

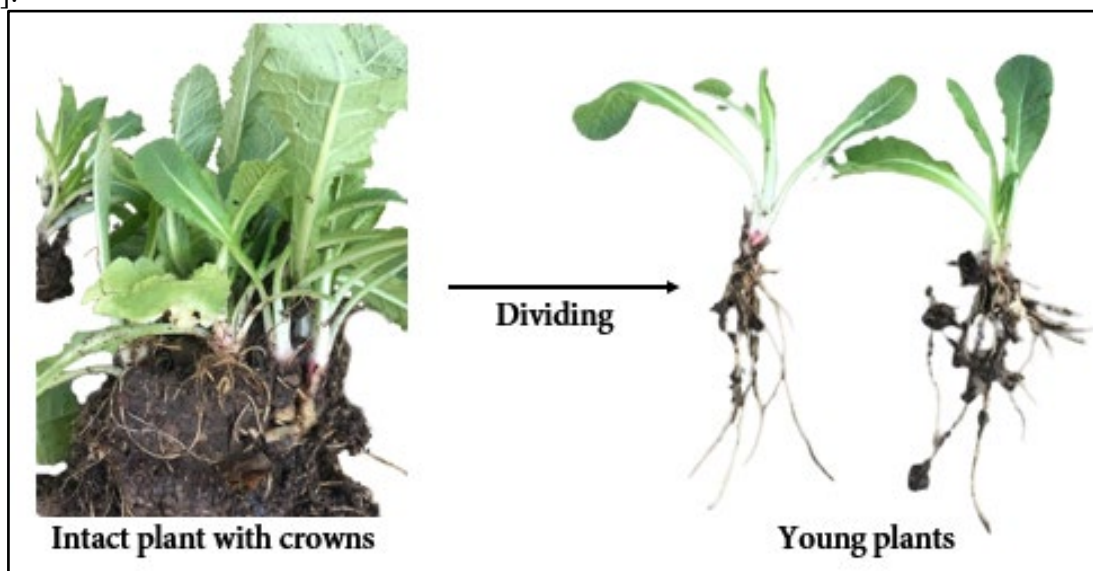


Fig. 2. Propagation of primroses by dividing clumps.

In addition, heteromorphic floral development and sporophytic incompatibility due to the S alleles are observed and these obstacles in reproductive biology of the plant cause self-incompatibility. Therefore, tissue culture techniques are valuable tools in breeding

program, enlargement of genetic pool, gene transformation, as well as in conservation plant genetic materials in primula.

SOME OF THE IN VITRO TECHNIQUES USED IN PRIMULA

Mass propagation of Primula

Primula is the rich genus with approximately 500 species and member of the genus naturally grown in many countries, however some of them are endangered or rare due to habitat losses or other anthropologic factors. Additionally, some of the species such as *Primula veris* [11], *P. denticulata* [12] and *P. macrophylla* [13] have valuable medicinal metabolites and tissue culture techniques are often used to propagate plant materials to obtain stable high-quality metabolites [14]. The optimization an *in vitro* propagation method is also beneficial for propagation important floral characteristics such as doubled flowers for plants which do not produce seed and parental lines of commercial cultivars [15].

In vitro culture studies of *Primula* species started in late 1970s and it is focused on mass propagation due to irregular or delayed seed germination of *P. obconica* which is economically important pot plant worldwide. At present, several of the *Primula* species are successfully propagated by *in vitro* techniques, but the studies are still limited. The determination explant types, culture medium and plant growth regulators are essential prior to the *in vitro* culture. Coumans et al. [10] reported that inflorescence tip explants of *P. obconica* gave best vegetative proliferation when cultured on MS medium supplemented with 1 mg/L benzyladenine (BA) and 1 mg/L naphthaleneacetic acid (NAA). Further subculture of explants allowed proliferation of vegetative buds and fivefold propagation ratio per month. Shimada et al. [16] reported that Ohashi and Mii [17], successfully obtained plantlets regenerated from leaf explants cultured on MS medium supplemented with 1 mg/L IAA (Indole-3-acetic acid) and 5 mg/L Zeatin in *P. obconica* and *P. malacoides*. Yamamoto et al. [18] cultured explants prepared from distal and proximal half of the young leaves of *P. sieboldii* on MS medium supplemented with different concentrations of BA (6-Benzylaminopurine) and NAA (1-Naphthaleneacetic acid). They determined best propagation results for shoot formation in MS medium supplemented with 1 mg/L BA and 0.1 mg/L NAA. Additionally, shoot regeneration was not obtained from the medium without NAA and distal part of the leaves of *in vitro* grown plants gave better results than explants taken from *in vivo* donor plants. Morozowska et al. [9] used shoot tip explants of *P. vulgaris* for micropropagation and MS supplemented with 4.44 μ M BA and 1.13 μ M 2,4-D (2,4-dichlorophenoxyacetic acid) was found to be optimal regeneration medium.

Mass propagation studies of *Primula* species conducted by many researchers and they were successful on inducing adventitious shoots from different explants cultured on medium supplemented with various concentrations of auxin and cytokinin. In our lab, wild *P. vulgaris* plants cultured ex situ and seeds were harvested after flowering period. Surface sterilized seeds were cultured on MS medium supplemented with 1.0-1.5 mg/L BA, 3% sucrose and a few seeds were germinated and turn into plantlets. Then each clump was divided and plantlets were sub-cultured on same medium. Further subculture was performed approximately every two months. We observed 4-8 fold propagation ratio in each subculture and some of the plantlets were rooted during *in vitro* culture (Fig 3a).

Additionally, some of the plants were died, when the subculture period extended (data not published) (Fig 3b).

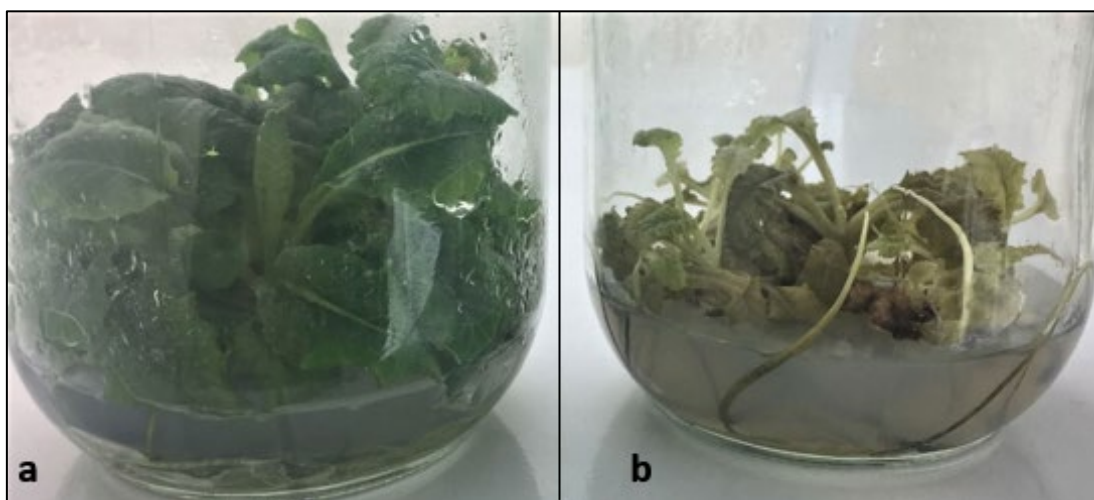


Fig. 3. *In vitro* culture of wild *P. vulgaris* genotypes (a: rooted plantlets during culture, b: dead plants in extended subculture period)

The regeneration capacity of *Primula* species varies depending on culture conditions, explant types, subculture interval, concentrations of plant growth regulator. As a result, lower concentrations of cytokinin or combination with auxine in tissue culture media gave successful results for mass propagation. On the other hand, concentrations of surface sterilization agents should be lower, when the young tissues such as young leaves and shoot tips will be used as explants. Moreover, endangered or rare plants can be propagated *ex situ* before tissue culture studies in order to prevent losing plant material or seeds can be chosen as an explant resource.

Organogenesis and somatic embryogenesis

One of the important key points in tissue culture studies is planning which explants will be cultured and which developmental stages will be followed. The explants cultured on medium follows two regeneration pathways, organogenesis and somatic embryogenesis. In the somatic embryogenesis pathway, bipolar structures regenerate from a single cell and they have no vascular connection [19]. Unlike somatic embryogenesis, unipolar structures occur in the organogenesis pathway and vascular connection is formed between the formed structures and the donor plant. Structurally, the main difference of both pathways from each other is the formation of bipolar or unipolar structures as a result of the development phase [20].

According to previous studies of direct and indirect organogenesis, leaf explants commonly were used to induce regeneration in *Primula*. The regeneration was also obtained from root and pedicle explants in limited number of the studies. This is probably due to higher regeneration capacity of leaf explants or to overcome taking explants depended vegetation period of the donor plants. Additionally, leaves can be superior taken from *in vitro* grown seedling are preferred to overcome contamination problem and seasonal limitations.

The explant cultured on tissue culture media follow two organogenic pathways in organogenesis. In indirect organogenesis, a callus phase develops from explant cultured on medium, while shoots or roots are regenerated in direct organogenesis. The callus

phase can be obtained from explants cultured on media containing higher auxin than cytokinin plant growth regulators. Different concentrations of 2,4-D and TDZ are major growth regulators to induce callus in *Primula* studies in order to obtain adventitious shoots through indirect organogenesis technique (Table 1). Hayta et al. [21] reported that TDZ and auxin concentrations were crucial for promoting dedifferentiation and regeneration in *P. vulgaris*. They observed that the media supplemented with 4.0 mgL⁻¹ 2,4-D+2.0 mgL⁻¹ TDZ, and 2.0 mgL⁻¹ TDZ+0.5 mgL⁻¹ NAA and 3.0 mgL⁻¹ TDZ+ 0.3 mgL⁻¹ NAA had the highest callus induction rates (100%). Additionally, they indicated that TDZ alone was detrimental to the plant material and failed to initiate callus. However, Shimada et al. [16] reported that using media supplemented with 1.0 mg/L TDZ alone induced somatic embryogenesis from leaf explants of *P. cuneifolia* var. *hakusanensis*. Takihira et al. [22] successfully obtained adventitious shoots from explants cultured on LS + 0.2 TDZ mg/L +2.0 mg/L NAA in *P. x pubescens* cv. 'Borders mixed', while Hamidoghli et al. [23] reported that adventitious shoots regenerated from explants cultured on MS + 2.0 mg/L TDZ + 1.0 mg/L NAA (Table 1). This differences in regeneration response occurred in explants cultured on TDZ containing media could be reasoned chemical behavior of TDZ which auxin and cytokinin like affects due to its ability to modulate endogenous growth regulators [21]. Additionally, Schween and Schwenkel [24] observed genotypic effects on regeneration of six cultivars of *Primula*. The callus regeneration rates ranged from 25% to 96% and shoots regeneration rates were 0.0-11.6% depending on genotypes, the duration of plant growth regulator treatment.

In vitro androgenesis and gynogenesis

Haploid and double haploid plants are one of the key factors in plant breeding program. It is superior to conventional techniques due to facilitate obtaining complete homozygosity in short time. The gynogenesis comprises culture of unfertilized ovary, ovule, or flower bud, while anther and microspore cultures are used in androgenesis. In both pathways, not only haploid plants can be regenerated from explants but also the plant with same chromosome level as donor plant can be regenerated on culture media due to undesired regeneration from somatic tissues.

In a number of studies androgenesis and gynogenesis conducted on *Primula* species. Ovule or ovary culture studies mainly conducted for embryo rescue processes in interspecific hybridization of *Primula* species, while anther culture preferred to induce haploidisation. Bajaj [26] reported that plantlets regenerated from anther culture of *P. obconica* frozen in liquid nitrogen aiming possibility of long-term preservation of haploid germplasm. The young anthers at the uninucleate pollen stage frozen in liquid nitrogen according to cryopreservation protocol of the author, then some of the explants cultured on MS medium supplemented with 0.5 mg/L IAA + 0.5 mg/L 2,4-D and 2.0 mg/L zeatin. Plantlets obtained directly from pollen-embryos, and indirectly through the differentiation of callus. Additionally, Jia et al. [27] demonstrated that lengths of 4.0–5.0 mm flower buds were at the appropriate microspore developmental stage and the highest callus induction was obtained from explants cultured on MS + 1.0 mg/L BAP + 0.5 mg/L 2,4-D. The highest induction and proliferation of indefinite buds (55.2%) was produced on MS + 0.2 mg/L BAP + 0.01 mg/L NAA. As a result of their results, totally 516 plantlets were obtained and 2% of the plantlets were haploid according to cytological analysis and flow cytometry.

Table 1. Studies on somatic embryogenesis and organogenesis in *Primula* spp.

Species	Path	Exp.	Media	Resp.	References
<i>P. cuneifolia</i>	SE	L.	LS + 1.0 mg/L TDZ or 5.0-10.0 mg/L Zeatin	Emb.	[16]
<i>Primula</i> spp.	IDO	P.	Callus induction media: ½ MS + 4.0 mg/L 2,4-D + 2.0 mg/L TDZ, shoots regenerated on hormone free media	ASh.	[25]
<i>P. vulgaris</i>	IDO	P.	Callus induction media: ½ MS 4.0 mg/L 2,4-D + 2.0 mg/L TDZ, shoots regenerated on hormone free media	ASh.	[24]
<i>P. x pubescens</i>	DO	L.	LS + 0.2 TDZ mg/L 2.0 mg/L NAA	ASh.	[22]
<i>P. heterochroma</i>	DO	L.	MS + 2.0 mg/L TDZ + 1.0 mg/L NAA	ASh.	[23]
<i>P. veris</i>	IDO	R.	Callus induction media: MS + 0.1 mg/L BA + 5.0 mg/L PIC, Shoot regeneration: 0.5 mg/L TDZ + 0.1 mg/L NAA	ASh.	[14]
<i>P. vulgaris</i>	IDO	L.	Callus induction media: B5 macro salts + ½ MS micro salts (basal media) + 4.0 mg/L 2,4-D + 2.0 mg/L TDZ, shoots regenerated on basal medium +3.0 mg/L TDZ + 0.3 mg/L NAA	ASh.	[21]

ASh.: Adventitious shoot, DO: Direct organogenesis, Emb.: Embryo, Exp.: Explant, IDO: Indirect organogenesis, L.: Leaf, P.: Pedicle, Resp. Response, R.: Root, SE: Somatic embryo.

CONCLUSIONS

The genus *Primula* is the largest genus in Primulaceae family and it has valuable species for ornamental plant industry. The member of the genus has various reproductive barriers such as heteromorph flower or self-incompatibility. Additionally, some of the species are under threat due to habitat loses or other anthropological affects. Modern biotechnological methods including plant tissue culture have been taken part in breeding strategies of plant. Several biotechnological methods could be applied to plant to have better ones in the process of plant breeding. Plant tissue culture techniques serve essential

tools for conservation of genetic diversity or development new cultivar through breeding [28, 29]. In different *Primula* species, *in vitro* culture techniques were successfully performed. It has been seen that *Primula* species can be easily propagated using appropriate explants, plant growth regulators and tissue culture media. However, these factors should be optimized individually for each species to obtain high efficiency in *in vitro* culture studies.

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