South African Journal of Botany 93 (2014) 207–216

Contents lists available at ScienceDirect

South African Journal of Botany

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

Direct somatic embryogenesis with high frequency plantlet regeneration and successive cormlet production in saffron (*Crocus sativus* L.)

K. Devi [1], M. Sharma [1], P.S. Ahuja [1]

Division of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176 061 (H.P.), India

ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form 11 April 2014
Accepted 14 April 2014
Available online xxxx

Edited by TR Marks

Keywords:
Biochemical studies
Crocus sativus
Histology
Somatic embryogenesis

ABSTRACT

Saffron is a slowly propagating geophyte with fungal infestation of corms under field conditions. Moreover, genetic improvement through breeding is not possible due to male sterility. Therefore, tissue culture methods offer a great potential for mass propagation, and somatic embryogenesis is one of the efficient methods. In the present study, direct somatic embryogenesis from leaf base was obtained in thidiazuron (TDZ, 2.5 μM) and picloram (2.0 μM) supplemented Murashige and Skoog medium (Murashige and Skoog, 1962). Significantly higher secondary embryogenesis was observed in embryo proliferation medium (EPM; MS supplemented with TDZ 2.5 μM and picloram 1.0 μM). The origin and different developmental stages of somatic embryos were ascertained through histological and scanning electron microscopic studies. Stomata were also observed in some of the somatic embryos. An alluring observation was retention of embryogenic potential beyond 3 years of culture. Percent germination of secondary embryos in MS medium was 60.48 when matured in ½ EPM. Freshly initiated somatic embryos also germinated to form plantlets without undergoing secondary embryogenesis in MS medium containing TDZ (2.5 μM) and picloram (1.0 and 2.0 μM). Somatic embryo derived shoots were multiplied in 6-benzylaminopurine (BAP; 26.64 μM) and α-naphthaleneacetic acid (NAA; 1.0 μM). Growth performance of cormlets obtained from these shoots was evaluated under green house conditions. Somatic embryogenesis holds tremendous importance for mass propagation of saffron with formation of ultimate propagating material i.e., cormlets.

1. Introduction

Crocus sativus L. (family Iridaceae), a male sterile, self incompatible geophyte with no seed set, is propagated through corms. It produces one or two bisexual flowers in one growing season and only the orange red tripartite stigma with part of style produces the spice saffron. In conventional methods crop improvement is restricted to evaluation, selection and vegetative propagation of naturally occurring or artificially induced clonal variants. Moreover, large scale production of daughter corms is a long drawn process. The mother corm perishes after forming 4–5 replacement corms. Besides, the latent endogenous infection in mother corms infects daughter corms and thereby affects the productivity. In saffron, spice yield is dependent upon flowering which is directly related to corm size and health. Application of tissue culture methods offers great potential in overcoming these challenges for production of new varieties, mass multiplication of selected ecotypes or improved varieties and genetic improvement.

Somatic embryogenesis and subsequent regeneration into plantlets is a striking alternative for cormlet production and large scale propagation in saffron. The cormlets produced through tissue culture can be used as planting material in conventional method of propagation to increase productivity and decrease disease incidence. Few reports are available on in vitro cormlet formation through somatic embryo derived shoots but with an intermediate callus formation (Ahuja et al., 1994; Karamian, 2004; Raja et al., 2007; Sheibani et al., 2007) and viable protocol is unavailable. Moreover, in vitro produced cormlets were not evaluated for growth performance under in vivo conditions, which is imperative for success of a micro-propagation protocol.

Present study was aimed at direct somatic embryogenesis using leaf bases as explants, cormlets production and growth performance evaluation of in vitro cormlets under green house conditions. In order to ascertain the different developmental stages histological and scanning electron microscopic studies were carried out. The effect of reserve accumulation and amylase activity during different developmental stages was also studied.

2. Materials and methods

2.1. Induction of somatic embryos

Corms procured from natural habitat (Pampore, Srinagar, J&K, India, 34°1′12″ N and 74°55′5″ E at 1574 m amsl) were surface sterilized

Abbreviations: EPM, embryo proliferation medium; NRS, non-reducing sugars; PBZ, paclobutrazol; RS, reducing sugars; TSS, total soluble sugars.

E-mail addresses: madhusharma@ihbt.res.in, m_vsharma@yahoo.com (M. Sharma).
[1] Tel.: +91 1894 233339; fax: +91 1894 230433.

http://dx.doi.org/10.1016/j.sajb.2014.04.006
0254-6299/

following the standardized protocol (Devi et al., 2011) and inoculated on MS (Murashige and Skoog, 1962) basal medium. Once the buds sprouted (both apical and axillary), young true leaves (5.0–6.0 cm) which were still inside the leaf sheath (cataphylls), were carefully separated, cut into segments (2.0–2.5 cm, Fig. 1a) and inoculated on MS medium supplemented with TDZ (1.0, 2.5 and 5.0 μM) and picloram (0.5, 1.0 and 2.0 μM) in factorial combinations. Medium without PGRs served as control. Three leaves per replicate (Petriplate, 90 mm, Tarsons, India) and five replicates per treatment were used. Data in terms of percent response of swelling of leaf base and number of somatic embryos formed were recorded after 4, 8 and 12 wks of culture. Cultures were maintained at 25 ± 2 °C under 16/8 h of light and dark cycle with photosynthetic photon flux density (PPFD) of 70 ± 5 μmol m^{-2} s^{-1}. In all the experiments, culture conditions were the same unless otherwise specified.

2.2. Proliferation of somatic embryos

2.2.1. Effect of TDZ and picloram

TDZ (1.0, 2.5 and 5.0 μM) and picloram (0.5, 1.0 and 2.0 μM) were used in factorial combinations. In all the experiments uniform samples i.e., clumps of somatic embryos (1.0 g each) per flask (250 ml Erlenmeyer, Borosil, India) were inoculated on medium and five replicates per treatment were used. Data were recorded after 6 wks of incubation.

2.2.2. Effect of light conditions

Embryo proliferation medium (EPM; MS medium supplemented with TDZ 2.5 μM and picloram 1.0 μM) used was derived from the above experiment. Uniform sample per flask was used as initial explants. Activated charcoal (AC, 1.0%, w/v) and ascorbic acid (0.1%, w/v) was added to check phenolic exudations. Four replicates per treatment were taken in further experiments. Data were recorded after 6 wks of incubation. Cultures were incubated at 25 ± 2 °C in continuous dark and light/dark cycle of 16/8 h with PPFD 70 ± 5 μmol m^{-2} s^{-1}.

2.2.3. Effect of temperature

Clumps of somatic embryos on EPM were incubated in the dark at 10, 15, 20 and 25 ± 2 °C. Data were recorded after 6 and 10 wks of culture and the parameters studied were increase in biomass i.e., fresh weight of cultures and growth index. Growth index (GI) was calculated by using following formula

$$GI = \frac{FW_f - FW_i}{FW_i} \times 100$$

where

FW_i initial fresh weight
FW_f final fresh weight

2.3. Maturation and conversion of somatic embryos

2.3.1. Effect of ABA and GA_3

Clumps of somatic embryos were transferred to MS medium supplemented with ABA (5.67 and 11.34 μM) and GA_3 (28.9 and 57.8 μM) in factorial combinations. Three replicates per treatment were used and data recorded after 8 wks of culture.

In another set of experiments, EPM supplemented with ABA (1.89, 3.78, 7.56 and 15.12 μM) was used for maturation of somatic embryos. Medium without ABA served as control. Clumps containing ~150 somatic embryos per replicate were used. After 4 wks of culture on respective media with different ABA concentrations, somatic embryos were transferred to MS medium without PGRs or fortified with GA_3 (conversion medium) at concentrations of 14.95, 28.9 and 57.8 μM. Data were recorded after 8 wks of transfer of somatic embryos from maturation to conversion medium.

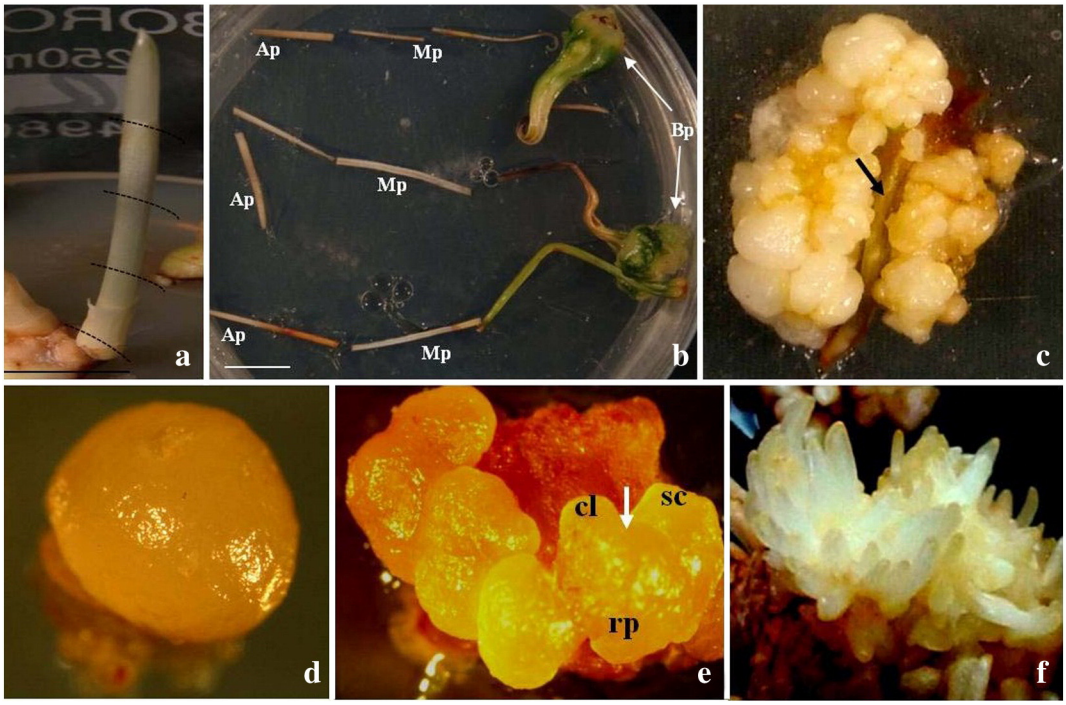

Fig. 1. Somatic embryo induction a) Bud sprouts used for somatic embryo induction (lines indicate the plane of cutting); b) swelling of basal portion of leaf (arrow) (Bp = basal, Mp = middle, Ap = apical portion of leaf); c) induction of somatic embryogenesis from swollen basal portion of leaf in picloram (2.0 μM) and TDZ (2.5 μM) (arrow indicates leaf explant); d) globular somatic embryo; e) somatic embryo with a notch (arrow) showing coleoptile (cl), scutellum (sc) and root pole (rp); bar line = 1.0 cm; f) somatic embryo derived multiple shoots.