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Gerbera micropropagation

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ABSTRACT

Gerbera jamesonii (gerbera) is an important cut-flower in the global floricultural industry. Micropropagation is the main system used to clonally propagate gerbera *in vitro* resulting in the production of millions of plantlets each year. Numerous types of explants and protocols for micropropagation have been established and used for gerbera. Shoot tips are the commonly used explant while adventitious shoot induction from the capitulum is also a popular method. Most papers in the literature have focused on testing the influence of different types and combinations of plant growth regulators with the aim of improving the regeneration and multiplication stage of one or few cultivars. Genotype is one of the most influential factors on the response of gerbera *in vitro*. Despite this, no successful universal protocol has yet been developed for multiple cultivars, limiting the usefulness of current protocols for commercial biotechnology labs. Slow-growing endogenous bacteria are one of the most important problems in gerbera micropropagation but require more studies on control and prevention. Individual shoots are normally easy to root, usually in excess of 90% of plantlets, but the acclimatization stage requires improvements and new technologies to increase the survival of plants. Epigenetic variations in micropropagated gerbera are frequently observed only with high concentrations of cytokinins in the culture medium but somaclonal variation is rare.

Contents

Abbreviations: ADS, adenine sulphate; Aux, auxin; BA, 6-benzyladenine; B5, Gamborg et al. 1968; Ck, cytokinin; DKW, Driver and Kiniyuki 1984; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; 2-ip, 2-isopentenyladenine; ISSR, inter-simple sequence repeat; Kin, 6-furfurylaminopurine (kinetin); LS, Linsmaier and Skoog 1965; MS, Murashige and Skoog 1962; ½ MS, half-strength Murashige and Skoog (1962); NAA, 1-naphthaleneacetic acid; N6, Chu 1978; PGR, plant growth regulator; Phot, photoperiod; PPFD, photosynthetic photon flux density; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SE, somatic embryogenesis; TDZ, thidiazuron; tTCL, transverse thin cell layer; Zea, Zeatin.

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1. Introduction and history

The *Gerbera* complex belongs to the Mutisiinae (tribe Mutisieae) and includes around 100 species distributed in seven genera, of which *Gerbera* has approximately 29 (Katinas, 2004) to 37 (Gao and Hind, 2011) species, spanning from Africa to Asia (Hansen, 1988; Katinas, 2004), although there is heated discussion about the number of species in this genus complex (Hansen, 2006; Katinas, 2004; Nesom, 2004). *Gerbera jamesonii*, better known by its popular name, gerbera, belongs to the Asteraceae (Compositae).

The genus was named *Gerbera* after a German naturalist, Traugott Gerber although it was the Scottsman Robert Jameson who discovered the species in about 1880 near Barberton in the former Transvaal, in South Africa. At that time, he donated the plant to the Durban Botanical Garden where John Medley Wood, the curator there, sent the plant to Harry Bolus for identification of the species. Harry Bolus then sent the species to the Royal Botanic Gardens in Kew, UK, suggesting the scientific name as *G. jamesonii*. As the first official description of the species was made by J. D. Hooker and published in 1889 by Curtis Botanical Magazine, it became officially registered thereafter as *G. jamesonii* Bolus ex. Hook f. (University of Helsinki, 2012).

A programme of genetic improvement of gerbera was initiated about ten years after its discovery, most likely by the Englishman Richard Irwin Lynch, who started an inter- and intraspecific hybridization programme (*G. jamesonii* × *Gerbera viridifolia*) (Seifert, 2011), eventually developing the actual current cultivars that are used for pot plants and cut flowers.

In fact, it is the dedicated focus to genetic improvement to obtain new cultivars, particularly by commercial companies based in the Netherlands, as well as the fact that this ornamental grows very well in greenhouse culture in most countries around the world, that rapidly led to the development of new cultivars, making it one of the ornamental market leaders (Bhatia et al., 2011; Chakabrarty and Datta, 2008). These breeding companies introduced many new cultivars with different inflorescence colors every year into the world market, but failed to develop resistant cultivars to important pests and diseases, which is actually one of the major problems in gerbera culture. Increasing pressure to reduce the use of agrochemicals in agriculture and horticulture, including in ornamental plant culture, is a priority, but to achieve this goal, needs resistant cultivars.

The inflorescences of commercial gerbera varieties are renowned for their array of bright colors that serve a small, but distinct slice of the global ornamental trade, either as potted flowering plants, or as cut flowers. It is not easy to obtain market data. In the USA, in 2010, the wholesale value of the cut flower industry was U$375 million, of which gerbera daisies accounted for 32.7 million, or 8.7% (NASS-USDA, 2011).

Cut-flowers in general, such as roses and gerberas, actually represent an important social improvement to many developed and developing countries around the world, increasing and improving the quality of life. According to Dolan et al. (2002) and Muhammad et al. (2010), the cut flower production for export provides jobs, increases trade, provides education, health and child care services, and social development. At the same time, the problems of labor conditions in the cut flower industry and trade in developing countries persists, but with international pressure and code implementation for the sector, there are signs of some improvements in these conditions. As an example, Hale and Opondo (2005) studied the case of the Kenya–UK cut flower supply chain. Kenya is an important example of floriculture representing an important tool to social and economic development in developing countries (Hale and Opondo, 2005).

2. Propagation and micropropagation of gerbera

Gerbera can be traditionally propagated by seed (sexual reproduction) or by vegetative propagation using stem cuttings, or division of the rhizome *in vivo* (Leffring, 1971; Osiecki, 1988; Son et al., 2011), or by *in vitro* micropropagation (Shabanpour et al., 2011), although it is only the latter method that provides a reliable method for the clonal propagation or elite germplasm with apparent relative genetic fidelity (Kanwar and Kumar, 2008).

The propagation of gerbera by seed is possible in some gerbera cultivars, although the greatest problem with such a method is the high level of heterozygosity, which can be problematic for the cut flower trade that requires coordinated flowering of uniform size and color (Harding et al., 1991). Despite these risks, the commercialized gerbera for use as pot plants has primarily relied on seed (Ludwig et al., 2008, 2010). Moreover, in some countries, seeds of superior cultivars are used to initiate *in vitro* cultures for micropropagation purposes (Altaf et al., 2009; Budi, 2000; Feng et al., 2009), primarily due to the ease of this technique and not necessarily intimidated by payment of royalties to breeders, even though the high genetic variability of plantlets derived from seeds remains the greatest limitation of this technique.

One of the greatest weaknesses of using traditional vegetative propagation by splitting or division of rhizomes or clumps is the low rate of propagation (Kanwar and Kumar, 2008; Son et al., 2011), the long period of time required to obtain commercial quantities of new plants, around five plants from one per year (Kumar and Kanwar, 2007) and the increase in the frequency of plantlets with phytosanitary problems (Das and Singh, 1989), mainly due to the use of non-sterile tools at the time of cutting and division of parts of rhizomes or clumps.

In fact, micropropagation using terminal buds/apices or through organogenesis of somatic tissue is considered to be the only possible viable method for the rapid mass propagation of elite gerbera germplasm (Bhatia et al., 2009) while maintaining the genetic fidelity (i.e., reducing genetic and epigenetic variation) of these cultivars (Bhatia et al., 2009, 2011; Cardoso and Teixeira da Silva, 2012; Reynoird et al., 1993). Moreover, plant tissue culture methods such as shoot tip culture result in disease-free plantlets (Dobránzki and Teixeira da Silva, 2010), including floricultural and ornamental plants such as chrysanthemum (Teixeira da Silva, 2003) and others (Rout et al., 2006). Such micropropagation protocols should be developed within a wider genetic improvement programme aimed at creating disease-free germplasm.

The induction and regeneration of micropropagated gerbera plantlets has been extensively studied (Table 1). Plantlets have been obtained from several kinds of tissues, including the culture of shoot tips (Cardoso and Teixeira da Silva, 2012; Huang and Chu, 1985; Murashige et al., 1974), axillary buds (Murashige et al., 1974), leaves (Aswath and Choudhary, 2002a; Palai et al., 1998; Radojević et al. 1987), petioles (Orlikowska et al., 1999), flower buds (Chakabrarty and Datta, 2008; Pierik et al., 1973, 1975; Posada et al., 1999; Son et al., 2011), capitulum (Pierik et al., 1975; Shabanpour et al., 2011; Topoonyanont and Dillen, 1988) and ovules (Meynet and Sibi, 1984; Miyoshi and Asakura, 1996; Tosca et al., 1999).

Pierik et al. (1979) concluded that shoot tip culture is more suitable for gerbera mass propagation than capitulum organogenesis, but Murashige et al. (1974) reported advantages and disadvantages of both techniques and observed that shoot tip culture is more rapid, but needs a high number of explants for the establishment stage, because of the high contamination of explants (around 80%). Capitulum as an explant results in fewer shoots per explant, but the contamination rate is very low (10%). Shailaja (2002) agreed that bud flowers and inflorescences were the best explants to initiate gerbera micropropagation, but observed that each cultivar needed a different protocol to improve shoot induction and regeneration. Tyagi and Kothari (2004) compared organogenesis from leaves and capitulum *in vitro* and obtained shoot regeneration in both, although the highest number of shoots/explant was obtained from the capitulum (10/explant) rather than from leaf explants (6.8).

Another *in vitro* system of propagation was successfully developed for gerbera using 0.2–0.5 mm thick receptacle explants as transverse

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