Introduction of Gerbera Cultivation in Lucknow Agro-climate through Tissue Culture of Young Flower Head

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Gerbera (Gerbera jamesonii H. Bolus ex Hook. f.) a temperate crop, distributed in the temperate Himalayas from Kashmir to Nepal at altitudes of 13,00 to 32,00 m. It is very difficult to grow this temperate crop in sub-tropical climate of Lucknow. Organogenic cultures were established from immature flower heads on modified MS medium supplemented with IAA and BA. Regenerated shoots were rooted on growth regulator-free medium. Rooted shoots were hardened during July-August and transferred to field conditions, where they grew well and produced flowers within 6-8 months.

Keywords: Gerbera introduction, shoot regeneration, sub-tropical climate

India has a long tradition of floriculture. During the last 2-3 decades, commercial exploitation in floriculture has resulted in extension of this trade. Despite many remarkable achievements, development of suitable cultivars or introduction of existing superior cultivars in non-traditional areas having slightly unsuitable agro-climatic conditions are the major constraints, which need to be circumvented to reduce the production cost. In the process of commercializing floriculture, biotechnology is playing a vital role. Floriculture Laboratory of NBRI, Lucknow, has recently tried to introduce different exotic temperate ornamentals in the sub-tropical climate of Lucknow through tissue culture.

Gerbera jamesonii H. Bolus ex Hook. f. (Family; Asteraceae: Compositae), commonly known as Transvaal Daisy or Berberton Diasy, is one of the 10 most important commercial flowers grown throughout the world. In India, it is distributed in the temperate Himalayas from Kashmir to Nepal at the altitudes of 13,00 to 32,00 m. In Lucknow, where temperature during extreme winter drops to c. 2°C and shoots up to 45-46°C during extreme summer, the gerbera plants after flowering do not perfume easily. Therefore, an attempt has been made to develop a tissue culture protocol, where gerbera plantlets are multiplied and maintained in vitro to overcome the adverse summer climate and then brought to the field conditions to produce flowers in a programmed manner and the results are presented in this communication.

For in vitro establishment of cultures, flower heads of different diam., e.g. 0.5 cm (H1), 1.0 cm (H2), 1.5 cm (H3) and 2.0 cm (H4) were used as an explant source. Flower heads, collected from greenhouse-grown plants, were washed in 5% Teepol (a liquid detergent) and running tap-water for 15 min and 30 min, respectively. After removing bracts, flower heads were pre-treated with 70% ethanol for 1 min followed by surface sterilization with HgCl2 solution (0.1%, w/v) for 2 min and finally washed 3 times thoroughly with sterile distilled water. Each flower head was segmented into 4 to 16 pieces (H1 head into 4, H2 head into 8, H3 head into 12 and H4 head into 16) and used as explants. Explants were cultured on semi-solid medium in culture tubes (25 x 150 mm). Two basal media were used in the experiments, namely, MS medium (Murashige & Skoog, 1962) and modified MS [MMS, MS major salts and iron-half strength, MS vitamins and minor salts of Heller's (1953) medium excluding FeCl3]. Two auxins, 1-naphthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) at 0.5, 1.0 and 2.0 mg l-1, and two cytokinins, 6-benzylaminopurine (BA) and kinetin (KN) at 1.0, 2.0 and 5.0 mg l-1 concentrations, were supplemented into the basal medium in different combinations (auxin and cytokinin). The media contained 30 g l-1 sucrose and solidified with 800 mg l-1 agar. The pH at all media was adjusted to 5.6 before adding agar and autoclaving at 121°C for 15 min. Cultures were incubated at 25 ± 1°C under 56.8 μmol m-2 s-1 cool white light for 16 hrs daily and at 55-60% relative humidity. Shoots (2-3 cm long) were subcultured on growth regulator-free MMS medium for root induction. Well-rooted plantlets were transferred to sand and soil (1:1) mixture and kept covered to maintain high humidity during the first one week. After 3 weeks, plants were finally transplanted to soil under field conditions.
All the explants cultured in MS medium turned brown within one month of incubation. In case of MMS medium also, browning of explants was observed but with low frequency and it was dependent on the type of flower head used as explant. In this medium, all the explants taken from H1 flower head turned brown within one month, while in case of H2, H3 and H4 flower heads, browning was from 45 to 75%. The minimum browning was observed with H3 and H4 flower heads. All the surviving (non-brown) explants in MMS medium supplemented with different concentrations of IAA and BA formed meristematic tissue within one month of incubation. None of the explants cultured on MMS medium supplemented with NAA and BA, NAA and KN or IAA and KN formed meristematic tissue even after sub-culture on the same medium. The meristematic tissue explants were regularly sub-cultured on the same medium at one month intervals. Shoot bud initiation was observed after 3 months of culture initiation in all the IAA and BA combinations tested. Frequency of shoot regeneration varied among the type of flower heads used as explants source and concentration of IAA and BA. Among the different types of flower heads, H3 was found highly regenerative while H4 was the least. This indicates the gradual loss of organogenic potential with age of the flower head. Such variation in regeneration potential at different developmental stages is well documented (Chakrabarty et al, 1999; Croes et al, 1985). In the present experiment, immature flower heads were used as explants, which are easy to decontaminate. On the contrary, the vegetative parts like shoot apices are reported to be difficult to decontaminate (Murashige et al, 1974; Orlikowska et al, 1999; Reynoid et al, 1993).

A combination of 0.5 mg l⁻¹ IAA and 2.0 mg l⁻¹ BA was found to be the best, where 72.7% organogenic meristematic tissue with 4.5 number of shoots per explant was recorded in case of H3 flower head (Table 1). The organogenic frequency decreased with the increase of IAA concentration in the medium. This frequency in other combinations varied from 18.2 to 50%.

Since the combination of 0.5 mg l⁻¹ IAA and 2 mg l⁻¹ BA was found the best suitable medium for shoot regeneration, all the meristematic tissue explants were further sub-cultured only on this medium at one-month-intervals for shoot proliferation. During every subculture, 8-10 shoots were harvested from each culture and rate was consistent for last 2 years (Fig. 1 A). All the harvested shoots (2 to 3 cm long) when transferred to growth regulator-free MMS medium, produced roots within 10 days (Fig. 1 B). Well-rooted shoots were transferred to soil, where survival
of plantlets was 100% in the field conditions and all plants grew to maturity producing flower true-to-type within 6-8 months (Fig. 1 C). Following this regeneration protocol, gerbera plantlets, which were transplanted to the field during July-August produced quality flowers during December-March in succession. Such a programmed blooming of gerbera with the in vitro strategy holds promise to commercialize cultivation of exotic flowers at competitive price in non-traditional areas.

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References