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*Full Length Research Paper*

# Effect of genotypes and culture medium on shoot regeneration and proliferation of *Gerbera jamesonii*

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***Gerbera jamesonii* is one of the most popular cut flowers in the world and micropropagation is the commercial way for its propagation. This method allows for obtaining large amounts of healthy homogenous plants. Thus, it is necessary to establish efficient micropropagation protocols. The objective of this study was to evaluate the organogenic response of *G. jamesonii*, orange and pink cultivars, under *in vitro* culture. Different levels of Gamborg's medium (BA) (2, 4 and 6 mg/l) and thidiazuron (TDZ) (0.2, 0.4 and 0.6 mg/l) in combination with indole-3-acetic acid (IAA) (0 and 0.1 mg/l) in MS medium were evaluated for shoot induction. For proliferation, regenerated shoots in TDZ were subcultured in medium supplemented with 0.2, 0.4 and 0.6 mg/l TDZ, 2 mg/l BA or 2 mg/l Kin and regenerated shoots were subcultured onto BA in induction medium. In the second phase, media of MS, $^1/_2$ MS, MS with $^1/_2$ $NH_4NO_3$ and $KNO_3$ concentration (MS-$^1/_2$N), MS with $^1/_2$ micro and iron elements (MS-$^1/_2$MI), $B_5$ and $^1/_2$ $B_5$ on shoot induction and proliferation, were evaluated. In order to induce shoot rooting, different levels of IAA (1, 2 and 3 mg/l) and 1-Naphthaleneacetic acid (NAA) (1, 2 and 3 mg/l) in combination with sucrose (30 and 40 g/l) were evaluated. Maximum shoot induction (88.8 and 44.4% for orange and pink cultivars, respectively) and multiplication rate per transplant (7.6 shoots/explant for orange cultivar and 1.33 shoots/explant for pink cultivar) were obtained in media with 4 mg/l BA and 0.1 mg/l IAA. The most efficient media for shoot induction and proliferation were MS-$^1/_2$N and MS, respectively. The best rate of shoot rooting in orange (4.6 roots/explants with 4.83 cm length) and pink cultivars (5.13 roots/explants with 6.2 cm length) was obtained in media with 3 mg/l IAA and 30 mg/l sucrose. The establishment of plantlets was done successfully with 92% of survival in the glasshouse.**

**Key words:** Micropropagation, organogenesis, *in vitro* culture, *Gerbera*, cut flower.

## INTRODUCTION

*Gerbera jamesonii* (Asteraceae family), native to south Africa and Asia, is one of the most famous cut flowers in the world (Parthasarathy and Nagaraju, 1999). In 2001, the production of *Gerbera* in US alone was approximately 220 million $ with 70 million stems (Broek et al., 2004).

*Gerbera* can be propagated by both sexual (seed) and asexual (clump division, cutting and micropropagation) methods. The multiplication through *in vivo* methods is too slow to be commercially valuable (Das and Singh, 1989). Therefore, micropropagation is commonly used for its rapid and large-scale multiplication (Kanwar and Kumar, 2008). Over the years, many researchers have studied micropropagation of *Gerbera* and different methods of *in vitro* multiplication and explants including callus induction and regeneration from leaves and ovules (Meynet and Sibi, 1984; Reynoird, 1993; Tyagi and Kothari, 2004), adventitious shoot regeneration from petiole (Orlikowska et al., 1999), direct adventious shoot formation from capitulum (Tyagi and Kothari, 2004; Chakrabarty and Datta, 2008) and direct shoot induction

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**Abbreviations: BA,** $N^6$-Benzyladenine; **$B_5$,** Gamborg's medium; **IAA,** indole-3-acetic acid; **MS,** Murashige and Skoog; **TDZ,** thidiazuron; **NAA,** 1-naphthaleneacetic acid.

in shoot tips (Aswath and Wazneen, 2004; Thakur et al., 2004).

The effect of medium basal salt, plant growth regulators and environmental conditions on shoot regeneration and propagation of *Gerbera* have been investigated (Kanwar and Kumar, 2008). The nutrient medium is important for successful micropropagation and different media such as Murashige and Skoog (MS), modified MS, Gamborg's medium ($B_5$), LS and DKW were used (Tyagi and Kothari, 2004; Kanwar and Kumar, 2008). Plant growth regulator composition in the medium is another important factor and generally high ratio of cytokinin/auxin was used (Kanwar and Kumar, 2008). Tyagi and Kothari (2004) used capitulum sections in the medium supplemented with 4 mg/l kinetin and 0.5 mg/l indole-3-acetic acid (IAA) for the rapid *in vitro* multiplication of *Gerbera*. Ray et al. (2005) proposed a protocol for rapid propagation of *Gerbera* with the capitulum explant in the medium containing 7 mg/l Kin and 0.1 mg/l IAA. Chakrabarty and Datta (2008) divided the capitulum explants into 4 to 16 pieces and cultured them in MS medium containing 8.8 µM BA and 2.8 µM IAA. For shoot multiplication, they cultured the apical meristem of regenerated shoots in different combination of cytokinins [$N^6$-Benzyladenine (BA), Kin and thidiazuron (TDZ)]. The optimum results were obtained with 8.8 µM BA.

In this work, the effect of genotype, cytokinin and medium on shoot regeneration and multiplication and also the effect of auxin and sucrose on rooting of regenerated shoots were studied.

## MATERIALS AND METHODS

### Plant material

Capitulum explants (0.5 to 1 cm in diameter) of two *Gerbera* cultivars (orange and pink) were washed under tap water for 20 min and soaked in 1.5% (w/v) solution of $Ca(ClO)_2$ for 10 min, then submerged in a 0.1% (w/v) solution of $HgCl_2$ for 10 min and rinsed three times in sterile distilled water. Sterilized capitulum were cut into 4 to 6 pieces and cultured in regeneration medium in 100 ml jars.

### Shoot induction and proliferation

In this experiment, the effect of different concentrations of BA (2, 4 and 6 mg/l) and TDZ (0.2, 0.4 and 0.6 mg/l) in combination with IAA (0 and 0.1 mg/l) on shoot regeneration were studied in factorial experiment by completely randomized design with 10 replications in each treatment. Capitulum of two *Gerbera* varieties were placed on MS medium containing 30 mg/l sucrose, 9 mg/l agar at pH 5.7 and different concentration of growth regulators. Cultures were placed under dark condition and 25°C for one month, then subcultured on the same medium and placed in 16 h light photoperiod with cool white fluorescent light at 5000 lux. Explants were subcultured in the same medium and condition until shoot regeneration. For shoot propagation, regenerated shoots on TDZ were subcultured on the MS medium that contained 0.2, 0.4, 0.6 mg/l TDZ, 2 mg/l BA or 2 mg/l Kin and regenerated shoots on BA were subcultured on the MS medium that contained 2, 4 and 6 mg/l BA in combination with IAA (0 and 0.1 mg/l). Then, the frequency of shoot regeneration and shoot numbers of both cultivars were determined after 8 weeks in light condition for all treatments.

In the second phase, the effect of basal medium including MS, $^1/_2$ MS, MS with $^1/_2$ $NH_4NO_3$ and $KNO_3$ concentration (MS-$^1/_2$N), MS with $^1/_2$ micro and iron elements (MS-$^1/_2$MI), $B_5$ and $^1/_2$ $B_5$ on shoot formation was examined only on orange cultivar by completely randomized design with 10 replications in each treatment. Capitulum of orange cultivar was cultured on these media supplemented with 30 mg/l sucrose, 9 mg/l agar, 4 mg/l BA and 0.1 mg/l IAA at pH 5.7. Cultures were placed in dark condition at 25°C. After one month, the explants were subcultured on the same medium under photoperiod (16-h light) and cool white fluorescent light at 5000 lux. Explants were subcultured at 30 days interval onto the same medium and condition until shoot regeneration. In the proliferation stage, regenerated shoots were subcultured on MS, MS-$^1/_2$N and $^1/_2$ $B_5$ media. Then, the average number of shoot proliferation, shoot number and the frequency of shoot regeneration were determined in the three subcultures.

### Rooting and acclimatization

Single shoots derived from shoot clusters were rooted on MS medium that contained 1, 2 and 3 mg/l IAA or NAA with 30 and 40 mg/l sucrose. Data including the number of roots per shoot were recorded after 4 weeks of culture. Shoots with well-developed roots were transferred to autoclaved sand and perlite (1:1 ratio) and maintained in greenhouse at 25°C, 80 to 85% relative humidity and under natural light.

All data were analyzed by JMP software and the means were compared by Duncan test at 5% confidential level.

## RESULTS

### Shoot formation

The results showed that shoot regeneration started in the second week of subculture in the regeneration medium until the sixth week and maximum shoot regeneration per week in media and genotypes were different (Table 1).

### Effect of cytokinin and auxin on shoot regeneration

It was found that regeneration responses of capitulum explants were significantly influenced by the plant growth regulator compositions in the medium in terms of morphogenetic ability. Generally, the percentage and number of adventitious buds per explant was dependent on the type of cytokinin. In this experiment, BA favored more shoot regeneration than TDZ. The effect of auxin in the medium was correlated with cytokinin concentration. 0.1 mg/l IAA in combination with all level of BA increased shoot regeneration percentage and number, whereas in combination with TDZ, shoot regeneration percentage and number decreased (Table 1). For both genotypes, medium that contained 4 mg/l BA and 0.1 mg/l IAA was optimal for shoot induction (orange cultivar with 88% shoot regeneration and 3.1 shoots per explant and pink cultivar with 44.4% shoot regeneration and 1.7 shoots per

**Table 1.** The percentage of shoot regeneration in orange and pink cultivars during 8 weeks in light condition.

| Medium | | | Week after first subculture in regeneration medium | | | | | | | | Total regenerated shoot |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cytokinin | Cytokinin concentration (mg/L) | IAA concentration (mg/L) | First | Second | Third | Fourth | Fifth | Sixth | Seventh | Eighth | |
| BA | 2.0 | 0.0 | 0 (0) | 0 (0) | 11 (0) | 11 (0) | 11 (11) | 0 (0) | 0 (0) | 0 (0) | 33 (11) |
| BA | 2.0 | 0.1 | 0 (0) | 0 (0) | 11 (0) | 22 (11) | 0 (11) | 11 (0) | 0 (0) | 0 (0) | 44 (22) |
| BA | 4.0 | 0.0 | 0 (0) | 0 (0) | 11 (0) | 22 (11) | 11 (11) | 11 (0) | 0 (0) | 0 (0) | 55 (22) |
| BA | 4.0 | 0.1 | 0 (0) | 22(0) | 33 (0) | 22 (22) | 11 (11) | 0 (11) | 0 (0) | 0 (0) | 88 (44) |
| BA | 6.0 | 0.0 | 0 (0) | 0 (0) | 22 (0) | 22 (11) | 11 (0) | 0 (11) | 0 (0) | 0 (0) | 55 (22) |
| BA | 6.0 | 0.1 | 0 (0) | 0 (0) | 11 (0) | 22 (11) | 11 (11) | 11 (11) | 0 (0) | 0 (0) | 55 (33) |
| TDZ | 0.2 | 0.0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 11 (11) | 0 (0) | 0 (0) | 0 (0) | 11 (11) |
| TDZ | 0.2 | 0.1 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 11 (0) | 0 (0) | 0 (0) | 11 (0) |
| TDZ | 0.4 | 0.0 | 0 (0) | 0 (0) | 0 (0) | 0 (11) | 11 (0) | 22 (11) | 0 (0) | 0 (0) | 33 (22) |
| TDZ | 0.4 | 0.1 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| TDZ | 0.6 | 0.0 | 0 (0) | 0 (0) | 0 (0) | 11 (11) | 22 (11) | 11 (11) | 0 (0) | 0 (0) | 44 (33) |
| TDZ | 0.6 | 0.1 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

BA, $N^6$-Benzyladenine; TDZ, thidiazuron; IAA, indole -3-acetic acid.

**Table 2.** The effect of cultivar and plant growth regulators on the regenerated shoot number.

| Cultivar | BA concentration (mg/l) | | | | | | TDZ concentration (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | 4 | | 6 | | 0.2 | | 0.4 | | 0.6 | |
| | 0.0(IAA) | 0.1 (IAA) | 0.0 (IAA) | 0.1 (IAA) | 0.0 (IAA) | 0.1 (IAA) | 0.0 (IAA) | 0.1 (IAA) | 0.0 (IAA) | 0.1 (IAA) | 0.0 (IAA) | 0.1 (IAA) |
| Orange | $0.9^{b}$ | $1.3^{ab}$ | $1.3^{ab}$ | $3.1^{a}$ | $1.3^{ab}$ | $1.6^{ab}$ | $0.2^{b}$ | $0.2^{b}$ | $1.1^{b}$ | $0.0^{b}$ | $1.1^{b}$ | $0.0^{b}$ |
| Pink | $0.2^{b}$ | $0.9^{b}$ | $0.4^{b}$ | $1.7^{ab}$ | $0.4^{b}$ | $0.9^{b}$ | $0.2^{b}$ | 0.0 | $0.7^{b}$ | $0.0^{b}$ | $0.9^{b}$ | $0.0^{b}$ |

The means with the same letters have no significant differences by Duncan test ($p \geq 0.05$)

explants) (Tables 1 and 2).

## Effect of cytokinin and auxin on shoot proliferation

The results of this experiment showed better shoots proliferation on media with TDZ and 2 mg/l Kin (1.1 shoots per subculture) than other media. Also, shoots regenerated on BA showed better proliferation on medium that contained 4 mg/l BA and 0.1 mg/l IAA (1.5 shoots per subculture) (Tables 3 and 4). It was noticed that a positive correlation between subculture frequency and the rate of shoot proliferation, for both cultivars; 3.4 and 1.1 shoots in the third and second subcultures were obtained in the medium that contained 4 mg/l BA and 0.1 mg/l IAA and 2 mg/l BA alone. In addition, these media were the most effective for shoot proliferation of the two genotypes in all the subcultures (Tables 3 and 4).

## Effect of basal medium on shoot induction

It was found that the MS medium with $^{1}/_{2}$ $NH_4NO_3$ and $KNO_3$ concentration was the most effective

**Table 3.** The effect of cultivar, cytokinin and subculture on the average number of shoot proliferation (shoots were regenerated in medium with TDZ).

| Cultivar | Subculture | Cytokinin concentration (mg/l) | | | | |
|---|---|---|---|---|---|---|
| | | 0.2 (TDZ) | 0.4 (TDZ) | 0.6 (TDZ) | 2 (BA) | 2 (Kin) |
| **Orange** | First | $0.22^{de}$ | $0.33^{de}$ | $0.44^{de}$ | $0.66^{de}$ | $0.77^{de}$ |
| | Second | $0.33^{de}$ | $0.44^{de}$ | $0.33^{de}$ | $1.66^{b}$ | $1.55^{cb}$ |
| | Third | $0.44^{de}$ | $0.66^{de}$ | $0.66^{de}$ | $2.11^{ab}$ | $2.55^{a}$ |
| **Pink** | First | $0.11^{e}$ | $0.11^{e}$ | $0.22^{de}$ | $0.44^{de}$ | $0.44^{de}$ |
| | Second | $0.33^{ed}$ | $0.22^{de}$ | $0.22^{de}$ | $0.44^{de}$ | $0.66^{de}$ |
| | Third | $0.77^{cd}$ | $0.77^{cd}$ | $0.88^{cd}$ | $0.88^{cd}$ | $0.88^{cd}$ |

The means with the same letters have no significant differences by Duncan test ($p \geq 0.05$).

**Table 4.** The effect of cultivar, cytokinin and subculture on the average number of shoot proliferation (shoots were regenerated in medium with BA).

| Cultivar | Subculture | BA concentration (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | | 4 | | 6 | |
| | | 0 (IAA) | 0.1 (IAA) | 0 (IAA) | 0.1 (IAA) | 0 (IAA) | 0.1 (IAA) |
| Orange | First | $0.6^{f-h}$ | $0.9^{d-h}$ | $0.9^{d-h}$ | $1.6^{c-e}$ | $0.9^{d-h}$ | $1.1^{-g}$ |
| | Second | $1.1^{d-g}$ | $1.2^{c-g}$ | $1.9^{bc}$ | $2.6^{ab}$ | $1.1^{d-g}$ | $1.4^{c-f}$ |
| | Third | $1.2^{c-g}$ | $1.4^{c-f}$ | $2.6^{ab}$ | $3.4^{a}$ | $1.4^{c-f}$ | $1.6^{bc}$ |
| Pink | First | $1^{d-g}$ | $0.3^{gh}$ | $0.7^{-h}$ | $0.3^{gh}$ | $0.3^{gh}$ | $0.6^{f-h}$ |
| | Second | $1.1^{d-g}$ | $0.8^{d-h}$ | $0.7^{e-h}$ | $0.4^{gh}$ | $0.4^{gh}$ | $0.7^{e-h}$ |
| | Third | $1^{d-h}$ | $0.9^{d-h}$ | $0.8^{d-h}$ | $0.6^{f-h}$ | $0.6^{f-h}$ | $0.8^{d-h}$ |

The means with the same letters have no significant differences by Duncan test ($p \geq 0.05$).

for shoot induction (96.6%) and shoot number (2.6 regenerated shoots per explant) (Figure 1). In the proliferation stage, regenerated shoots had better shoot proliferation in MS medium and it reached as high as 2.5 shoots per subculture (Figure 2). In various studies, the basal medium utilized for *Gerbera* micropropagation varied differently.

### Effect of auxin and sucrose on rooting

All of the proliferated shoots were successfully rooted in all hormonal concentrations (100%); medium with 3 mg/l IAA been the most effective (4.1 roots per shoots) (Table 5). Increasing the sucrose in the medium from 30 to 40 mg/l decreased rooting of regenerated shoots (Table 5). Orange and pink cultivars in the medium supplemented with 3 mg/l IAA and 30 mg/l sucrose had optimal rooting and acclimatization (Table 5).

## DISCUSSION

Regeneration of *Gerbera* is dependent on cultivar and hormonal combination, so the procedure of regeneration and media should be optimized for each cultivar. In *Gerbera* micropropagation, cytokinin plays a significant role in the morphogenesis of capitulum explants. In this experiment, addition of BA to the medium increased the adventitious buds formation of all the cultivars at both induction and proliferation stages. The number of adventitious buds and regenerated shoots depends on plant genotype. Most researchers used combination of cytokinin (2 to 15 mg/l BA or Kin) and auxin (0.1 to 0.5 mg/l IAA) for shoot regeneration by capitulum explants as these results (Modh et al., 2002; Zheng et al., 2002; Tyagi and Kothari, 2004; Ray et al., 2008).

The decrease of nitrate in basal medium had positive effect on regeneration but it should be examined in other cultivars. Murashige et al. (1974) used modified MS medium, while Chu and Hung (1983) used medium containing MS macroelements ($^{1}/_{2}$ concentration), Heller's microelement, $Na_2FeEDTA$ (21.4 mg/l), MS organic compound and 0.8% Bacto-agar. Radice and Marconi (1998) had a good regeneration of *Gerbera* in $^{1}/_{2}$ MS salt medium with 80 mg/l adenine sulfate. Sato et al. (2001) recommended MS medium with 2.82 $NO_3^-/NH_4^+$ ratio for *Gerbera* micropropagation. Topoonyanont and Debergh (2001) utilized MS macro and microelements, 40 mg/l NaFeEDTA and 100 mg/l myoinositol. Mandal and Datta

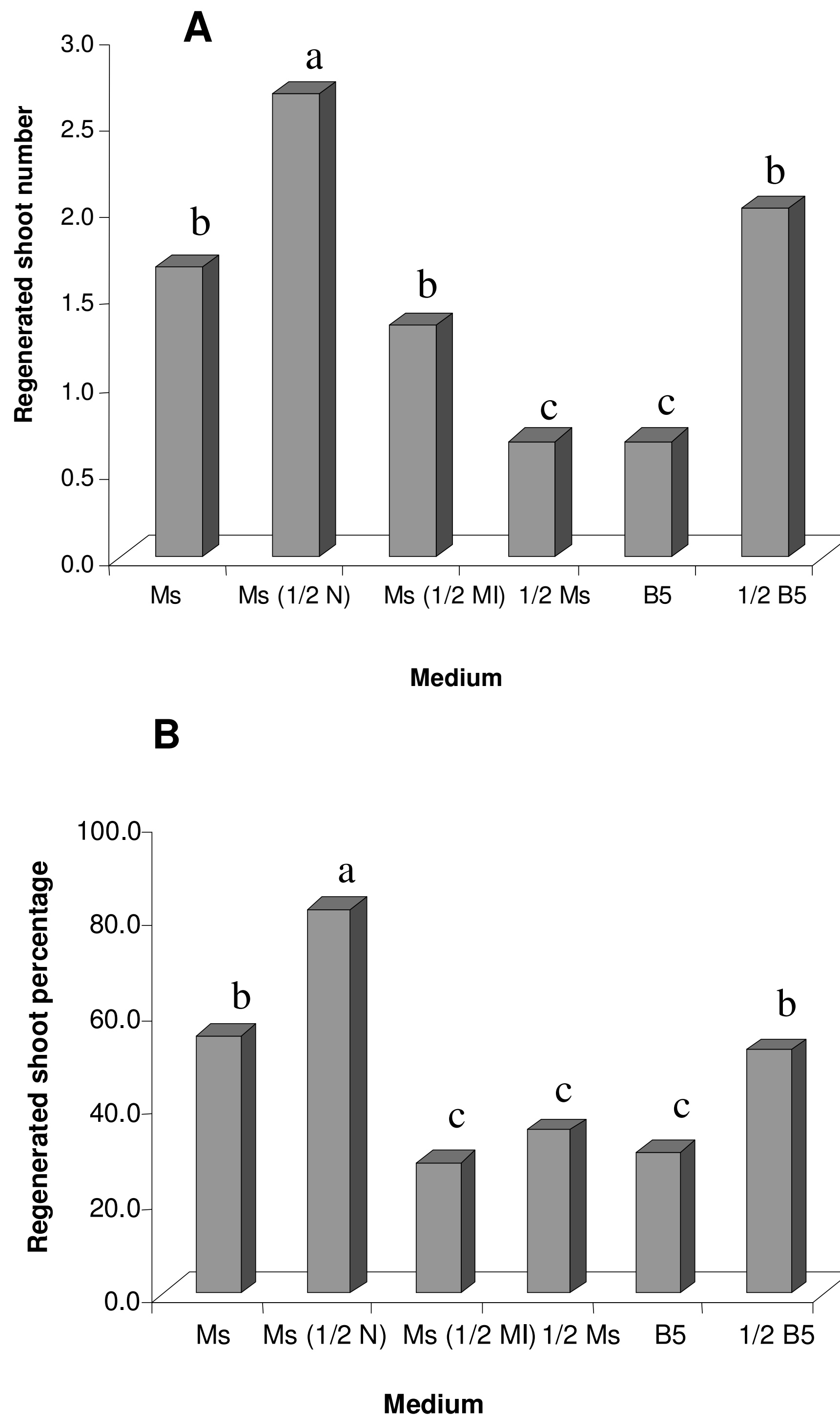


**Figure 1.** The effect of basal medium on the number (A) and percentage of shoot regeneration (B).

(2002) also used MS and Heller's mineral salts. Tyagi and Kothari (2004) utilized MS salts with altered concentrations of $MnSO_4$, $MgSO_4$, $ZnSO_4$ and thiamin HCl, pyridoxine HCl, nicotinic acid with addition of tyrosine, adenine sulfate and $NaH_2PO_4$. So, modification of nitrogen source by using other materials can improved the regeneration of *Gerbera*. In this experiment, MS medium with half nitrate concentration was the most

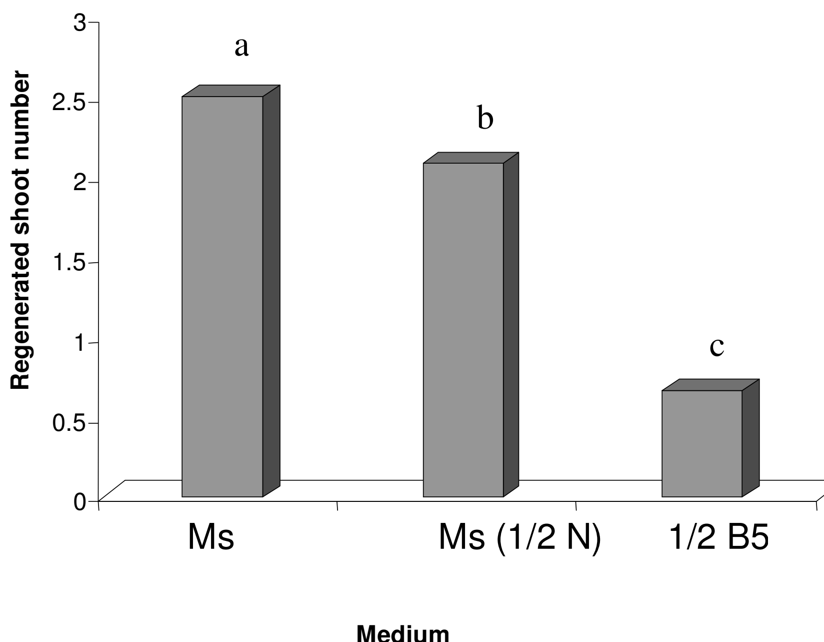


**Figure 2.** The effect of basal medium on shoot proliferation.

**Table 5.** Effect of cultivars, auxin and sucrose on the average number of roots per shoot.

| Cultivars | Auxin and sucrose concentration (mg/l) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Free auxin | | IAA | | | | | | NAA | | | | | |
| | 0.0 | | 1 | | 2 | | 3 | | 1 | | 2 | | 3 | |
| | 30 | 40 | 30 | 40 | 30 | 40 | 30 | 40 | 30 | 40 | 30 | 40 | 30 | 40 |
| Orange | 2.2ij | 2j | 3.5c-f | 2.8e-j | 3.6c-e | 2.9 d-i | 4.6ab | 3.4d-g | 2j | 2j | 2.5h-j | 2.3ij | 3.3d-h | 3 -i |
| Pink | 2.7f-j | 2j | 3.6cd | 2.3ij | 4.2bc | 2.8d-j | 5.2a | 3.2d-h | 2.5h-j | 2.7g-j | 3d-i | 3.2 -h | 3.4 -g | 35 c-f |

The means with the same letters have no significant differences by Duncan test (p≥0.05).

efficient for shoot induction and MS medium was the best for shoot proliferation.

Within *Gerbera* genus, rooting and acclimatization is not difficult to achieve. Different auxins (IAA, NAA and IBA) were reported as efficient for rooting of *in vitro* regenerated shoots as follows: IAA (Soczek and Hampel, 1986), 2.5 mg/l IAA (Maia et al. 1983), 0.5 mg/l NAA (Reynoird et al., 1993), 0.5 mg/l IAA (Tyagi and Kothari, 2004) and 1 mg/l IBA (Nhut et al., 2007). The results suggest that for the proposed cultivars, the most efficient way was the MS medium with 3 mg/l IAA and 30 mg/l sucrose.

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