In vitro seed germination and seedling development of Withania somnifera (L.) Dunal

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Accepted 3rd April, 2015

An efficient and improved in vitro seed germination and seedling development technique of Withania somnifera (L.) Dunal have been developed. Murashige and Skoog (MS) medium containing 3.0 mg l⁻¹ GA₃ and 3.0 mg l⁻¹ Kinetin was mostly effective for maximum germination percentage (92.67), germination rate (1.83), germination value (56.07) and seedling vigour index (875.73). Whereas minimum days required for germination (8.30), maximum germination speed (6.15), shoot length (7.72 cm), weight of shoot (4.48 g), weight of root (1.83 g), fresh weight of seedlings (5.91 g), dry weight of seedlings (0.78 g), number of leaves per plantlet (5.57) and plant height (8.79 cm) was recorded in MS medium containing 5.0 mg l⁻¹ GA₃ and 5.0 mg l⁻¹ Kinetin. The present protocol clearly describes that Withania somnifera (L.) Dunal seeds should be firstly germinated in MS medium containing 3.0 mg l⁻¹ GA₃ and 3.0 mg l⁻¹ Kinetin and after that the completely germinated seeds should be subcultured in MS medium supplemented with growth hormones 5.0 mg l⁻¹ GA₃ and 5.0 mg l⁻¹ Kinetin for seedling development.

Key words: in vitro, seed germination, seedling development, Withania, medicinal herb.

INTRODUCTION

Withania somnifera (L.) Dunal, is an important herb in the ayurvedic and indigenous medical systems for over 3000 years (Sharma et al., 2010). Both leaves and roots of the plant are used as the drug and steroidal lactones occur in both parts. Roots are prescribed as medicines for hiccups, several female disorders, bronchitis, rheumatism, dropsy, stomach and lung inflammation, and skin diseases. The active pharmacological components of Withania somnifera are steroidal lactones of the withanolide type. Several chemotypes have been found differing in their withanolide content. The principal withanolide in Indian W. somnifera are withaferin A and withanolide D (Ganzera et al., 2003).

According to red list of threatened species, W. Somnifera proved to be 99.75% of the endangered medicinal plant (Siddique et al., 2005; Rahman, 2001). This medicinally important plant species has been depleted from their natural habitat and is now included in the list of threatened species by The International Union for Conservation of Nature and Natural Resources (Kavidra et al., 2000). Commonly Withania propagated commercially by the means of seeds because of the lack of natural ability for vegetative propagation but the seed viability is limited to one year making the long duration seed storage futile (Sen and Sharma, 1991; Rani and Grover, 1999; Farooqi and Sreeramu, 2004). Seed propagation, however is not
always satisfactory, since percentage of germination is low, due to the presence of certain inhibitory compounds in the fruit and high risk of catching various diseases (De Silva and Senarath, 2009). This resulted in the adulteration of plant materials, making the plant endangered (Antonisamy et al., 2000). Again multiplication through cuttings give rise to less ramified plants and is consequently less productive than plants obtained from seeds (Supe et al., 2006). However, the conventional propagation method cannot meet the increasing demand of this plant used as raw material for the preparation of pharmaceutical products. Due to poor viability of stored seed and little information regarding seed germination of *W. somnifera* an alternative procedure of propagation through *in vitro* seed germination and seedling development is essential. *In vitro* propagation of *W. somnifera* through sequential procedure of induction of callus, shoot regeneration and rooting take more time and costly comparing to *in vitro* seedling regeneration using single media. The immature seeds obtained from green pods of *W. somnifera* can be germinated asymbiotically *in vitro* for rapid micro propagation (Murashige and Skoog, 1962). The method can be exploited for the rapid propagation and conservation of *W. somnifera*.

Therefore, the present study was carried out to optimize the concentration of gibberellic acid (GA₃) and Kinetin (Kn) in MS media for *in vitro* seed germination and seedling development of *Withania somnifera* by using *in vitro* technique.

**MATERIALS AND METHODS**

The seeds of Indian cultivar *Withania somnifera* were collected from the plants grown at Horticultural Research Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India. The health and density of seeds were tested by dipping them in water. Floated seeds were discarded for selecting healthy seeds for sterilization. The viability of seeds were tested by the 2,3,5-triphenyltetrazolium chloride (TTC) test (Hartman et al., 1990). All the chemicals and reagents were purchased from Hi Media (Mumbai, India) and plant growth regulators were procured from Sigma-Aldrich (Bangalore, India).

Seeds were washed with tap water for 5-10 minutes to remove surface contamination and then sterilized by immersing in 70% ethanol for 1 minute with vigorous shaking followed by 20 min in 4% sodium hypochlorite containing one drop of Tween-20. The seeds were then rinsed three times with sterile distilled water in a laminar air flow cabinet to remove minor amounts of disinfection liquid. The surface-sterilized seeds were used for the treatments of *in vitro* germination trials.

For germinating, the surface-sterilized seeds were cultured in jam bottle of standard Murashige and Skoog medium containing 3% sucrose and 0.6% agar alone and along with different concentrations of GA₃ (0.5 - 5.0 mg/l) and Kinetin (0.5 - 5.0 mg/l) in combination for their synergistic action. For induction of culture for seed germination and seedling development, the following media were used:

(a) M { MS without growth regulators (control)}
(b) MG₁K₁ {MS + GA₃ 1.0 mg l⁻¹ + Kn 1.0 mg l⁻¹}
(c) MG₂K₂ {MS + GA₃ 2.0 mg l⁻¹ + Kn 2.0 mg l⁻¹}
(d) MG₃K₃ {MS + GA₃ 3.0 mg l⁻¹ + Kn 3.0 mg l⁻¹}
(e) MG₄K₄ {MS + GA₃ 4.0 mg l⁻¹ + Kn 4.0 mg l⁻¹}
(f) MG₅K₅ {MS + GA₃ 5.0 mg l⁻¹ + Kn 5.0 mg l⁻¹}

The pH of the medium was adjusted to 5.8 before the addition of 0.8% (w/v) agar. All cultures were incubated under controlled condition at 25 ± 2°C temperature, 60 ± 10% relative humidity and 16 h photoperiod with a photosynthetic photon flux density (PPFD) of 20 μmol m⁻² s⁻¹ provided by cool white fluorescent lamps (2 × 40 W, Philips, India).

The cultures were observed daily and the data on daily seed germination was collected until the completion of the germination (maximum up to 30 days). The seeds with 0.5 mm or more radical growth occur were counted as germinated seeds. The final germination percentage (Gp) was calculated from the total seeds that germinated on the day of completion. The other germination parameters such germination speed (GS) germination rate (Rs) (Rajabi and Poustini, 2005) and germination value (GV) (Djavanshir and Pourbeik, 1976) were calculated. Different growth parameters such as seedling vigour index (SVI) (Abdul Baki and Anderson, 1973) and growth value (GV) (Meredith 1978) were calculated. Root length and shoot length of the seedlings were recorded and root to shoot ratio was calculated. Fresh weight (FW) of seedlings was recorded and dried in hot air oven at 60°C until constant weight and then dry weight (DW) of seedlings were recorded. Moisture content of seedlings was calculated using formula: (Fresh weight – Dry weight)/ Fresh weight x 100.

Plantlets with well developed roots were transferred to plastic cup containing autoclaved perlite and maintained for four weeks in culture room. Then the plantlets were transferred to poly cups containing garden soil and were maintained in a shade net house.

The experiments were designed in Completely Randomized Design (CRD). In each treatment 50 seeds were inoculated @ 5 seeds per jam bottle and each treatment was replicated four times. The statistical analysis was done by employing the O.P Stat software packages and the means were compared using Duncan’s multiple range test (DMRT) at the 0.05% probability level.

**RESULTS AND DISCUSSION**

Seed germination and seedling growth are known to be regulated by exogenous hormones. Growth regulators
### Table 1. Effect of growth regulator concentrations on *in vitro* seed germination characteristics of Withania.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment Combinations</th>
<th>Days to germination</th>
<th>Germination percentage</th>
<th>Germination Speed (GS)</th>
<th>Germination rate (GR)</th>
<th>Germination value (GV)</th>
<th>Seedling vigour index (SVI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>MS</td>
<td>14.23</td>
<td>68.53</td>
<td>3.37</td>
<td>1.12</td>
<td>23.09</td>
<td>509.86</td>
</tr>
<tr>
<td>MGK₁</td>
<td>MS + GA₁ 1.0 mg l⁻¹ + Kn 1.0 mg l⁻¹</td>
<td>12.75</td>
<td>75.25</td>
<td>4.02</td>
<td>1.26</td>
<td>30.25</td>
<td>643.39</td>
</tr>
<tr>
<td>MGK₂</td>
<td>MS + GA₂ 2.0 mg l⁻¹ + Kn 2.0 mg l⁻¹</td>
<td>11.32</td>
<td>83.67</td>
<td>5.02</td>
<td>1.56</td>
<td>42.00</td>
<td>754.70</td>
</tr>
<tr>
<td>MGK₃</td>
<td>MS + GA₃ 3.0 mg l⁻¹ + Kn 3.0 mg l⁻¹</td>
<td>10.27</td>
<td>92.67</td>
<td>6.05</td>
<td>1.83</td>
<td>56.07</td>
<td>875.73</td>
</tr>
<tr>
<td>MGK₄</td>
<td>MS + GA₄ 4.0 mg l⁻¹ + Kn 4.0 mg l⁻¹</td>
<td>9.93</td>
<td>85.67</td>
<td>5.84</td>
<td>1.81</td>
<td>50.03</td>
<td>861.84</td>
</tr>
<tr>
<td>MGK₅</td>
<td>MS + GA₅ 5.0 mg l⁻¹ + Kn 5.0 mg l⁻¹</td>
<td>8.30</td>
<td>77.33</td>
<td>6.15</td>
<td>1.81</td>
<td>47.56</td>
<td>827.43</td>
</tr>
<tr>
<td>SEm±</td>
<td></td>
<td>0.53</td>
<td>1.285</td>
<td>0.199</td>
<td>0.130</td>
<td>0.904</td>
<td>25.38</td>
</tr>
<tr>
<td>CD at 5%</td>
<td></td>
<td>1.66</td>
<td>4.004</td>
<td>0.620</td>
<td>0.405</td>
<td>2.815</td>
<td>8.291</td>
</tr>
</tbody>
</table>

### Table 2. Effect of growth regulator concentrations on seedling development characteristics of *in vitro* raised Withania.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment combinations</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
<th>Shoot/root length ratio</th>
<th>Weight of shoot (g)</th>
<th>Weight of root (g)</th>
<th>Shoot/root weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>MS</td>
<td>4.21</td>
<td>3.23</td>
<td>1.30</td>
<td>1.72</td>
<td>0.87</td>
<td>1.98</td>
</tr>
<tr>
<td>MGK₁</td>
<td>MS + GA₁ 1.0 mg l⁻¹ + Kn 1.0 mg l⁻¹</td>
<td>4.97</td>
<td>3.58</td>
<td>1.39</td>
<td>2.36</td>
<td>1.04</td>
<td>2.27</td>
</tr>
<tr>
<td>MGK₂</td>
<td>MS + GA₂ 2.0 mg l⁻¹ + Kn 2.0 mg l⁻¹</td>
<td>5.14</td>
<td>3.88</td>
<td>1.32</td>
<td>3.23</td>
<td>1.18</td>
<td>2.74</td>
</tr>
<tr>
<td>MGK₃</td>
<td>MS + GA₃ 3.0 mg l⁻¹ + Kn 3.0 mg l⁻¹</td>
<td>5.27</td>
<td>4.18</td>
<td>1.26</td>
<td>3.84</td>
<td>1.29</td>
<td>2.98</td>
</tr>
<tr>
<td>MGK₄</td>
<td>MS + GA₄ 4.0 mg l⁻¹ + Kn 4.0 mg l⁻¹</td>
<td>5.41</td>
<td>4.65</td>
<td>1.16</td>
<td>4.16</td>
<td>1.65</td>
<td>2.52</td>
</tr>
<tr>
<td>MGK₅</td>
<td>MS + GA₅ 5.0 mg l⁻¹ + Kn 5.0 mg l⁻¹</td>
<td>5.72</td>
<td>4.98</td>
<td>1.15</td>
<td>4.48</td>
<td>1.83</td>
<td>2.45</td>
</tr>
<tr>
<td>SEm±</td>
<td></td>
<td>0.116</td>
<td>0.193</td>
<td>0.043</td>
<td>0.061</td>
<td>0.017</td>
<td>0.037</td>
</tr>
<tr>
<td>CD at 5%</td>
<td></td>
<td>0.363</td>
<td>0.602</td>
<td>0.134</td>
<td>0.190</td>
<td>0.053</td>
<td>0.116</td>
</tr>
</tbody>
</table>

### Table 3. Effect of growth regulator concentrations on seedling development characteristics of *in vitro* raised Withania.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment combinations</th>
<th>Fresh weight of seedlings (g)</th>
<th>Dry weight of seedlings (g)</th>
<th>Moisture content of seedlings</th>
<th>Number of leaves per seedling</th>
<th>Seedling height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>MS</td>
<td>3.13</td>
<td>0.30</td>
<td>90.42</td>
<td>4.12</td>
<td>5.25</td>
</tr>
<tr>
<td>MGK₁</td>
<td>MS + GA₁ 1.0 mg l⁻¹ + Kn 1.0 mg l⁻¹</td>
<td>3.61</td>
<td>0.36</td>
<td>90.03</td>
<td>4.44</td>
<td>5.95</td>
</tr>
<tr>
<td>MGK₂</td>
<td>MS + GA₂ 2.0 mg l⁻¹ + Kn 2.0 mg l⁻¹</td>
<td>4.17</td>
<td>0.41</td>
<td>90.17</td>
<td>4.69</td>
<td>6.71</td>
</tr>
<tr>
<td>MGK₃</td>
<td>MS + GA₃ 3.0 mg l⁻¹ + Kn 3.0 mg l⁻¹</td>
<td>4.84</td>
<td>0.53</td>
<td>89.05</td>
<td>4.84</td>
<td>7.14</td>
</tr>
<tr>
<td>MGK₄</td>
<td>MS + GA₄ 4.0 mg l⁻¹ + Kn 4.0 mg l⁻¹</td>
<td>5.19</td>
<td>0.67</td>
<td>87.09</td>
<td>5.18</td>
<td>8.22</td>
</tr>
<tr>
<td>MGK₅</td>
<td>MS + GA₅ 5.0 mg l⁻¹ + Kn 5.0 mg l⁻¹</td>
<td>5.91</td>
<td>0.78</td>
<td>86.80</td>
<td>5.57</td>
<td>8.79</td>
</tr>
<tr>
<td>SEm±</td>
<td></td>
<td>0.015</td>
<td>0.009</td>
<td>0.406</td>
<td>0.080</td>
<td>0.164</td>
</tr>
<tr>
<td>CD at 5%</td>
<td></td>
<td>0.048</td>
<td>0.028</td>
<td>1.265</td>
<td>0.251</td>
<td>0.511</td>
</tr>
</tbody>
</table>
used in pre-sowing seed treatment with growth regulator play an important role in regulating germination and vigour (Raghav and Kasera, 2012). Gibberellins are a family of 136 tetracyclic diterpenes, a small subset of which are active as plant hormones and known to stimulate seed germination in a wide range of plant species, the predominant active GA depends on the species (Thomas et al., 2005).

**Seed germination**

It is evident from the data presented in Table 1 that days required for germination were decreased by increasing concentration of GA$_3$ with higher Kinetin rate. Among the different combinations of GA$_3$ and Kinetin, minimum days required for germination (8.3 days) was noted in treatment MS medium supplemented with 5.0 mg l$^{-1}$ GA$_3$ and 5.0 mg l$^{-1}$ Kinetin which was at par with the days required in treatment MG$_4$K$_4$. Maximum days (14.23) for germination were recorded on MS medium without growth regulators. The results corroborate the findings of the experiments conducted by Mello (2009). The significantly highest germination percentage (92.67%) was recorded in MS medium supplemented with 3.0 mg l$^{-1}$ GA$_3$ along with 3.0 mg l$^{-1}$ Kinetin which was by followed by the result of MG$_4$K$_4$ (85.67%) while the least germination percentage (68.53%) was observed in MS medium containing no any growth regulators. An improvement in seed germination with application of GA$_3$ was evidenced but its concentration beyond optimum dose causes reduction in germination percentage (Dhoran and Gudadhe, 2012). The present investigation showed that seed germination percentage increases with increasing rate of GA$_3$ along with increasing concentration of Kinetin but not beyond 3 mg/l each of GA$_3$ and Kn. There are also some reports in this regard which indicate that kinetin in combination with GA$_3$ enhanced germination and seedling growth in chick pea (Kaur et al., 1998). The maximum germination speed (6.15) was observed in MS containing 5.0 mg l$^{-1}$ GA$_3$, 5.0 mg l$^{-1}$ Kn while the least germination speed (3.37) was recorded in MS with no growth regulators (M). Increase in the germination speed for germination required higher rate of GA$_3$ application (Dhoran and Gudadhe, 2012). The treatments with high concentrations of GA$_3$ are effective in overcoming dormancy and causing rapid germination of seed. The highest germination rate (1.83) was recorded in treatment MS media containing 3.0 mg l$^{-1}$ GA$_3$ + 3.0 mg l$^{-1}$ Kn while the minimum (1.12) was recorded in MS which is devoid of growth regulators. Higher concentration of GA$_3$ and Kinetin proved more effective from their respective lower concentration. The observations corroborate the earlier findings that GA$_3$ increases the rate of seed germination (Mello, 2009). Application of GA$_3$ and Kinetin at various combinations significantly influenced the germination value over the control (M). The maximum germination value of (56.07) was recorded in MS medium supplemented with GA$_3$3.0.
mg l\(^{-1}\) + Kn 3.0 mg l\(^{-1}\). The control treatment (M) showed minimum germination value (23.09). The observation supported the report that higher concentrations of GA\(_3\) improve germination value (Naeem et al., 2004).

**Seedling growth**

Table 2 shows the treatment MS medium containing 3.0 mg l\(^{-1}\) GA\(_3\) + 3.0 mg l\(^{-1}\) Kn showed maximum seedling vigour index (875.73) followed by treatment MG\(_3\)K\(_4\) while the minimum seedling vigour (509.86) was noticed in control treatment. The highest vigour index in Penstemon digitalis cv Husker Red was also observed during light period in GA\(_3\) treated seedlings by earlier researcher (Mello et al., 2009).

The significantly maximum shoot length (5.72 cm) was recorded in treatment MS media supplemented with 5.0 mg l\(^{-1}\) GA\(_3\) + 5.0 mg l\(^{-1}\) Kn which is at par with the treatment MG\(_3\)K\(_4\) while the minimum shoot length (4.21 cm) was observed in MS containing no growth regulators. Similarly significant value for shoot length as compare to control was found in Digitalis purpurea on application of GA\(_3\) and Kinetin application (Kedia et al., 2012). It has been observed that shoot length increases as the rate of GA\(_3\) and Kinetin increase which is in close agreement of the findings of previous workers. The maximum root length (4.98 cm) were produced in treatment MS supplemented with 5.0 mg l\(^{-1}\) GA\(_3\) + 5.0 mg l\(^{-1}\) Kinetin which is at par with the treatment MG\(_3\)K\(_4\) while the control treatment (M) produced minimum root length (3.23 cm). This finding is in close agreement with the findings of some previous workers (Naeem et al., 2004). This may be due to the fact that GA\(_3\) is better for inducing root growth and there is tendency of increasing root length with the increase of GA\(_3\) concentration in the MS medium (Ribeirio et al., 2009). Significantly the least shoot/root ratio (1.15) was recorded in treatment MG\(_3\)K\(_5\) containing MS medium supplemented with 5.0 mg l\(^{-1}\) GA\(_3\) + 5.0 mg l\(^{-1}\) Kn whereas the maximum shoot/root ratio was noticed in the treatment MG\(_3\)K\(_4\).

The maximum shoot weight (4.48 g) were produced in treatment MG\(_3\)K\(_5\), that is, MS supplied with 5.0 mg l\(^{-1}\) GA\(_3\) + 5.0 mg l\(^{-1}\) Kn followed by the treatment MG\(_3\)K\(_4\), that is (4.16 g). While the treatment (M) produced minimum shoot weight (1.7 g) which was without growth regulators. Many workers has reported that Kinetin showed inhibition in shoot length and number of internodes like in Lentil (Ribeirio et al., 2009) in Morning Glory (Kaul and Farooq 1994) and in Okra (Chaudhry and Khan, 2000) but it may associated with a significant expansion in diameter of shoot and an increase in area of leaves as well as their number resulted in overall gain in weight of seedlings (Naeem et al., 2004) The maximum weight (1.83 g) of the root was recorded in the treatment MG\(_3\)K\(_5\) which is followed by the treatment MG\(_3\)K\(_4\). While the minimum root weight (0.87 g) was recorded in treatment (M) containing no growth regulators. This result might be due to the addition of biomass per plant with increasing concentration of GA\(_3\) with respect to the root number. It was previously evidenced that increase in GA\(_3\) concentration resulted in an exponential increase in the number of roots without any phytotoxic effect of GA\(_3\) on root formation even at higher concentration of used GA\(_3\) (Ribeirio et al., 2009) Application of GA\(_3\) and Kinetin at various combinations significantly influenced the shoot/root weight ratio. The least shoot/root weight ratio of (1.98) was recorded in control treatment (M) where the more shoot/root weight ratio was found in treatment MG\(_3\)K\(_3\).

Table 3 showed that the maximum weight (4.48 g) of the shoot was achieved in the treatment MG\(_3\)K\(_4\), that is, MS + 4.0 mg l\(^{-1}\) GA\(_3\)+ 4.0 mg l\(^{-1}\) Kn which was followed by the treatment MG\(_3\)K\(_5\) (5.19 g). While the minimum weight of the shoot (1.72 g) was achieved in treatment (M) containing no growth regulators. Significantly the highest dry weight (0.78 g) was recorded in control MS medium supplemented with 5.0 mg l\(^{-1}\) GA\(_3\)+ 5.0 mg l\(^{-1}\) Kn followed by (0.67g) in 4.0 mg l\(^{-1}\) GA\(_3\)+ 4.0 mg l\(^{-1}\) Kn while the least dry weight (0.30 g) was observed in control. The minimum moisture content (86.80%) in the seedling was found in treatment MS supplemented with GA\(_3\) 5.0 mg l\(^{-1}\) and Kn 5.0 mg l\(^{-1}\) whereas maximum (90.42%) was in control treatment i.e. in solely MS media. This result might be due to the addition of biomass per plant with increasing concentration of GA\(_3\) with respect to the root number. Highest seedling dry weight was also noticed in Pinus peuce by application of higher GA\(_3\) (Stojicic et al., 2012). The maximum leaf number (5.57) in the seedling before transfer to hardening media was produced in treatment MG\(_3\)K\(_5\) which is followed by the treatment MG\(_3\)K\(_4\) while the control treatment (M) produced minimum leaf (4.12). The findings are in close agreement of Kedia et al. (2012) that showed significant increase in the number of internodes as well as number of leaves on application of combined of GA\(_3\), IAA and Kinetin. Moreover, the similar effect of GA\(_3\) application on leaf number was found in Annona crassiflora (Ribeirio et al., 2009). The maximum height of the seedling (8.79 cm) at the stage prior to transfer to hardening media was observed on treatment MG\(_3\)K\(_4\) containing MS medium with 4.0 mg l\(^{-1}\) GA\(_3\)+ 4.0 mg l\(^{-1}\) Kn, while the minimum height of seedling (5.25 cm) was observed in the control treatment (M). The application of growth regulators enhanced plant growth (Hernandez, 1997, Ashraf et al., 1987, 1989) and stem length (Lee et al., 1999, Kabar, 1990). Hernandez (1997) and Bagatharia and Chanda (1998) found that GA\(_3\) accelerated bud development and stem elongation but the best results can be achieved if GA\(_3\) is applied in combination with kentin.

**Conclusion**

It may be concluded that growth hormones gave significantly better response than control both in seed germination and seedling development in W. somnifera
Withania somnifera (L.) Dunal. MS medium associated with 3.0 mg l⁻¹ GA₃ and 3.0 mg l⁻¹ Kinetin was mostly effective for maximum germination percentage (92.67), germination rate (1.83), germination value (56.07) and seedling vigour index (875.73). Whereas minimum days required for germination (8.30), maximum germination speed (6.15), shoot length (7.72 cm), weight of shoot (4.48 g), weight of root (1.83 g), fresh weight of seedlings (5.91 g), dry weight of seedlings (0.78 g), number of leaves per plantlet (5.57) and plant height (8.79 cm) was recorded in MS medium supplemented with 5.0 mg l⁻¹ GA₃ and 5.0 mg l⁻¹ Kinetin.

So the present protocol clearly describes that Withania somnifera (L.) Dunal seeds should be firstly germinated in MS medium associated with 3.0 mg l⁻¹ GA₃ and 3.0 mg l⁻¹ Kinetin and after that the completely germinated seeds should be subcultured in MS medium supplemented with growth hormones 5.0 mg l⁻¹ GA₃ and 5.0 mg l⁻¹ Kinetin for seedling development.

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