academicJournals

Vol. 11(17), pp. 1552-1558, 28 April, 2016
DOI: 10.5897/AJAR2015.9880
Article Number: E53762558357
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

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

Sachin Sharma, Subhendu S. Gantait and Rocky Thokchom*

Department of Floriculture, Medicinal and Aromatic Plants, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar-736165, W.B., India.

Received 4 May, 2015; Accepted 25 September, 2015

An efficient and improved protocol for *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} gibberellic acid (GA_3) and 3.0 mg l^{-1} Kinetin (Kn) was found 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} GA_3 and 5.0 mg l^{-1} Kn. The present protocol clearly describes that *W. somnifera* (L.) Dunal seeds should be germinated first in MS medium containing 3.0 mg l^{-1} GA_3 and 3.0 mg l^{-1} Kn and after that the completely germinated seeds should be subcultured in MS medium supplemented with growth hormones 5.0 mg l^{-1} GA_3 and 5.0 mg l^{-1} Kn 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 *W. 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

*Corresponding author. E-mail: rockythokchomaau@gmail.com.

(Kavidra et al., 2000). Usually, *Withania* is propagated commercially by 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 (GA_3) and Kinetin (Kn) in MS media for *in vitro* seed germination and seedling development of *W. somnifera* by using *in vitro* technique.

MATERIALS AND METHODS

The seeds of Indian cultivar *W. 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. Seeds that float were discarded and healthy seeds were selected 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 thoroughly with running tap water for 5 to 10 min to remove surface dirty particles and then sterilized by immersing in 70% ethanol for 1 min 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 (Figure 1a).

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 GA_3 (0.5 to 5.0 mg/l) and Kn (0.5 to 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) MG_1K_1 {MS + GA_3 1.0 mg l^{-1} + Kn 1.0 mg l^{-1}}; (c) MG_2K_2 {MS + GA_3 2.0 mg l^{-1} + Kn 2.0 mg l^{-1}}; (d) MG_3K_3 {MS + GA_3 3.0 mg l^{-1} + Kn 3.0 mg l^{-1}}; (e) MG_4K_4 {MS + GA_3 4.0 mg l^{-1} + Kn 4.0 mg l^{-1}}; (f) MG_5K_5 {MS + GA_3 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, Phillips, 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 were counted as germinated seeds (Figure 1b). The final germination percentage (Gp) was calculated from the total seeds that germinated on the day of completion. The other germination parameters, such as 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) (Abdual Baki and Anderson, 1973) and growth value (GV) (Meredith, 1978) were calculated. Root length and shoot length of the seedlings were recorded (Figure 1e) 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) × 100

Plantlets with well developed shoots and roots (Figure 1c and d) were transferred to plastic cup containing autoclaved perlite (Figure 1. f) and maintained for four weeks in culture room. The plantlets were then transferred to poly cups containing garden soil and were maintained in a shade net house (Figure 1g).

The experiments were designed in Completely Randomized Design (CRD). In each treatment, 50 seeds were inoculated at 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 (O.P. Sheoran, 1968) and the mean 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 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

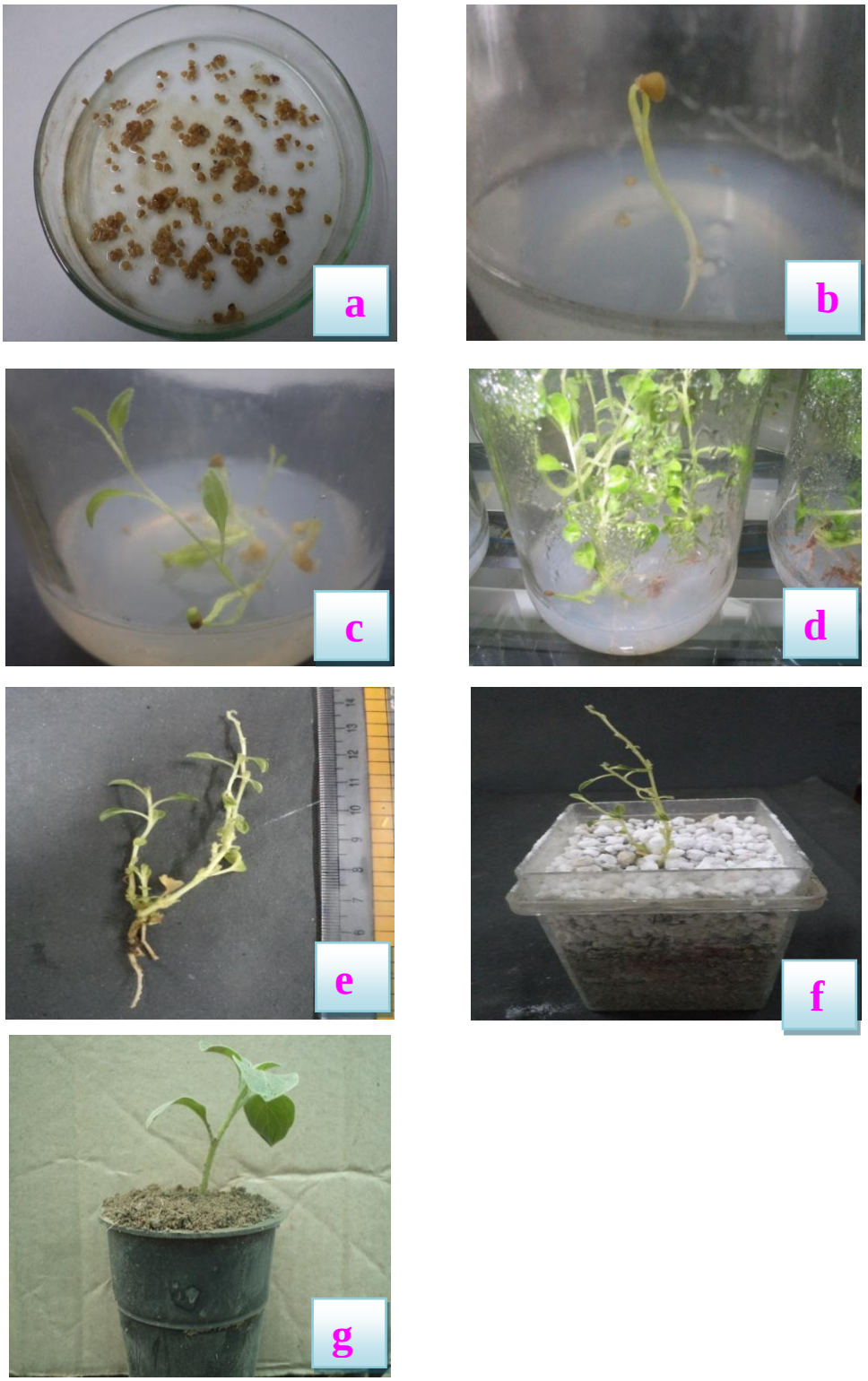

Figure 1. a. Treated seeds of Aswagandha for inoculation, b. Germinated seeds at growing stage, c. *In vitro* seedling at development stage, d. *In vitro* seedlings with profuse rooting, e. *In vitro* raised seedling, f. *In vitro* germinated seedling transferred to hardening media in plastic container, g. Two months old plantlets in hyco pot.

Table 1. Effect of growth regulator concentrations on *in vitro* seed germination characteristics of *Withania*.

Treatment	Treatment combinations	Days to germination	Germination percentage	Germination Speed (GS)	Germination rate (GR)	Germination value (GV)	Seedling vigour index (SVI)
M	MS	14.23	68.53	3.37	1.12	23.09	509.86
MG_1K_1	MS + GA_3 1.0 mg l^{-1} + Kn 1.0 mg l^{-1}	12.75	75.25	4.02	1.26	30.25	643.39
MG_2K_2	MS + GA_3 2.0 mg l^{-1} + Kn 2.0 mg l^{-1}	11.32	83.67	5.02	1.56	42.00	754.70
MG_3K_3	MS + GA_3 3.0 mg l^{-1} + Kn 3.0 mg l^{-1}	10.27	92.67	6.05	1.83	56.07	875.73
MG_4K_4	MS + GA_3 4.0 mg l^{-1} + Kn 4.0 mg l^{-1}	9.93	85.67	5.84	1.81	50.03	861.84
MG_5K_5	MS + GA_3 5.0 mg l^{-1} + Kn 5.0 mg l^{-1}	8.30	77.33	6.15	1.81	47.56	827.43
SEM±		0.53	1.285	0.199	0.130	0.904	25.38
CD at 5%		1.66	4.004	0.620	0.405	2.815	8.291

required for germination were decreased by increasing concentration of GA_3 with higher Kn rate. Among the different combinations of GA_3 and Kn, 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} Kn which was at par with the days required in treatment MG_4K_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} Kn followed by MG_4K_4 (85.67%). The least germination percentage (68.53%) was observed in MS medium containing no 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 Kn, but not beyond 3 mg l^{-1} each of GA_3 and Kn. The finding is in line with that of Kaur et al. (1998) which reported the enhanced germination and seedling growth of chick pea when seeds were treated with Kn in combination with GA_3. The maximum germination speed (6.15) was observed in MS medium supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn, while the least germination speed (3.37) was recorded under control (M). This may be due to the fact that germination speed is greatly enhanced by higher rate of GA_3 application (Dhoran and Gudadhe, 2012). The highest germination rate (1.83) was recorded in MS medium supplemented with 3.0 mg l^{-1} GA_3+ 3.0 mg l^{-1} Kn, while the minimum (1.12) was recorded in MS medium which is devoid of growth regulators. The result is in agreement with the findings of Mello (2009) who reported that GA_3 increases the rate of seed germination. This might be due to effectiveness of GA_3, which at higher concentrations overcome dormancy, causing rapid germination of seed. Higher concentration of GA_3 and Kn proved more effective from their respective lower concentration. Application of GA_3 and Kn 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).). MS medium when supplemented with 3.0 mg l-1 GA3 + 3.0 mg l-1 Kn produced maximum seedling vigour index (875.73) followed by the treatment MG4K4; while the minimum seedling vigour (509.86) was noticed in control treatment. The result is in agreement with the finding of Mello et al. (2009) which reported that the GA3 treated seedlings of Penstemon digitalis cv Husker Red showed highest vigour index during light period.

Seedling growth

The data presented in Table 2 shows that MS medium when supplemented with 3.0 mg l^{-1} GA_3 + 3.0 mg l^{-1} Kn produced maximum seedling vigour index (875.73) followed by the treatment MG_4K_4; while the minimum seedling vigour (509.86) was noticed in control treatment. The result is in agreement with the finding of Mello et al. (2009)

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

Treatment	Treatment combinations	Shoot length (cm)	Root length (cm)	Shoot/root length ratio	Weight of shoot (g)	Weight of root (g)	Shoot/root weight ratio
M	MS	4.21	3.23	1.30	1.72	0.87	1.98
MG_1K_1	MS + GA_3 1.0 mg l^{-1} + Kn 1.0 mg l^{-1}	4.97	3.58	1.39	2.36	1.04	2.27
MG_2K_2	MS + GA_3 2.0 mg l^{-1} + Kn 2.0 mg l^{-1}	5.14	3.88	1.32	3.23	1.18	2.74
MG_3K_3	MS + GA_3 3.0 mg l^{-1} + Kn 3.0 mg l^{-1}	5.27	4.18	1.26	3.84	1.29	2.98
MG_4K_4	MS + GA_3 4.0 mg l^{-1} + Kn 4.0 mg l^{-1}	5.41	4.65	1.16	4.16	1.65	2.52
MG_5K_5	MS + GA_3 5.0 mg l^{-1} + Kn 5.0 mg l^{-1}	5.72	4.98	1.15	4.48	1.83	2.45
SEM±		0.116	0.193	0.043	0.061	0.017	0.037
CD at 5%		0.363	0.602	0.134	0.190	0.053	0.116

which reported that the GA_3 treated seedlings of *Penstemon digitalis* cv Husker Red showed highest vigour index during light period. Maximum shoot length (5.72 cm) was recorded to be significantly higher than the rest of the treatment combinations when the germinated seeds were transferred to the 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_4K_4. The minimum shoot length (4.21 cm) was observed in MS containing no growth regulators. Similar result was observed by Tolera et al. (2009) in *Saccharum officinarum* where application of GA3 and Kn showed significant higher value for shoot length as compared to control.

This might be due to the fact that increase in both the concentrations of GA_3 and Kn triggers the cell elongation and faster multiplication of the cells that results in rapid growth and development of the seedlings as compared to the treatments with lower hormonal concentrations. The maximum root length (4.98 cm) was observed in MS medium supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn which is at par with the treatment MG_4K_4, while the control treatment (M) produced minimum root length (3.23 cm). This finding is in close affirmation with the findings of Naeem et al. (2004). This may be due to the fact that GA_3 is better for inducing root growth and has the 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 MS medium supplemented with 5.0 mg l^{-1} GA_3+ 5.0 mg l^{-1} Kn (MG_5K_5) whereas the maximum shoot/root ratio was recorded in the treatment MG_1K_1.

The maximum shoot weight (4.48 g) was produced in treatment MG_5K_5, that is, MS supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn followed by the treatment MG_4K_4 (4.16 g). Least shoot weight (1.7 g) was observed in control. The result is in conformity with the findings of Ribeirio et al. (2009) in Lentil; Kaul and Farooq (1994) in Morning Glory; and Chaudhary and Khan (2000) in Okra where they concluded that the hormone Kn show inhibition in shoot length and number of internodes. It may also be 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 of the root (1.83 g) was recorded in the treatment MG_5K_5 followed by the treatment MG_4K_4. While the minimum root weight (0.87 g) was recorded in control (M). This might be due to the addition of biomass per plant with increasing concentration of GA_3 with respect to the root number. Increase in GA_3 concentration might also result 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 Kn 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_3K_3.

The data presented in Table 3 showed that the maximum fresh weight of seedling (5.91 g) was observed in the treatment MG5K5, that is, MS + 5.0 mg l-1 GA3 + 5.0 mg l-1 Kn followed by the treatment MG4K4 (5.19 g). While the minimum seedling fresh weight (3.13 g) was recorded in control (M). Significantly, the maximum dry weight of the seedling (0.78 g) was recorded in MS medium supplemented with 5.0 mg l^{-1} GA_3 + 5.0 mg l^{-1} Kn followed by MG_4K_4

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

Treatment	Treatment combinations	Fresh weight of Seedlings (g)	Dry weight of seedlings (g)	Moisture content of seedling	Number of leaves per seedling	Seedling height (cm)
M	MS	3.13	0.30	90.42	4.12	5.25
MG_1K_1	MS + GA_3 1.0 mg l^{-1} + Kn 1.0 mg l^{-1}	3.61	0.36	90.03	4.44	5.95
MG_2K_2	MS + GA_3 2.0 mg l^{-1} + Kn 2.0 mg l^{-1}	4.17	0.41	90.17	4.69	6.71
MG_3K_3	MS + GA_3 3.0 mg l^{-1} + Kn 3.0 mg l^{-1}	4.84	0.53	89.05	4.84	7.14
MG_4K_4	MS + GA_3 4.0 mg l^{-1} + Kn 4.0 mg l^{-1}	5.19	0.67	87.09	5.18	8.22
MG_5K_5	MS + GA_3 5.0 mg l^{-1} + Kn 5.0 mg l^{-1}	5.91	0.78	86.80	5.57	8.79
SEM±		0.015	0.009	0.406	0.080	0.164
CD at 5%		0.048	0.028	1.265	0.251	0.511

(0.67 g) and the least was observed in control (0.30 g). The minimum moisture content in seedling (86.80%) was found in MS medium supplemented with GA_3 5.0 mg l^{-1} and Kn 5.0 mg l^{-1} whereas maximum (90.42%) was observed in control treatment. The result is in line with the finding of Stojicic et al. (2012) where the highest seedling dry weight of *Pinus peuce* was obtained by application of higher concentration of GA_3. The reason might be due to the addition of biomass per plant with increasing concentration of GA_3 with respect to the root number.

The maximum leaf number (5.57) of the seedling prior to transfer to the hardening media was observed in the treatment MG_5K_5 which is followed by the treatment MG_4K_4, while the least (4.12) was observed in control. The finding is in close concurrence with that of Kedia et al. (2012) which reported the significant increase in the number of internodes as well as number of leaves on application of combined of GA_3, IAA and Kn. Moreover, the similar effect of GA_3 application on leaf number was found in *Annona crassiflora* (Ribeirio et al., 2009). The maximum seedling height (8.79 cm) at the stage prior to transfer to hardening media was observed in the treatment MG_4K_4 containing MS medium with 4.0 mg l^{-1} GA_3 + 4.0 mg l^{-1} Kn, while the minimum seedling height (5.25 cm) was observed in the control treatment (M). The present observation is in harmony with the result obtained by various workers and they reported that application of growth regulators enhance plant growth (Hernandez, 1997; Ashraf et al., 1987; Ashraf et al., 1989) and stem length (Lee et al., 1999; Kabar, 1990). Application of GA_3 accelerates bud development and stem elongation, but the best results can be achieved if GA_3 is applied in combination with kn (Hernandez, 1997; Bagatharia and Chanda, 1998).

Conclusion

From the present investigation, it may be concluded that growth hormones gave significantly better response than control both in seed germination and seedling development in *W. somnifera* (L.) Dunal. MS medium when supplemented with 3.0 mg l^{-1} GA_3 and 3.0 mg l^{-1} Kn was found more 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) were recorded in MS medium supplemented with 5.0 mg l^{-1} GA_3 and 5.0 mg l^{-1} Kn.

So, the present protocol clearly describes that for *in vitro* seed germination of *W. somnifera* (L.) Dunal, MS basal medium supplemented with 3.0 mg l^{-1} GA_3 and 3.0 mg l^{-1} Kn is recommended best while subculture of the germinated seedlings in MS basal medium supplemented with 5.0 mg l^{-1} GA_3 and 5.0 mg l^{-1} Kn is recommended best for *in vitro* seedling development. Since the germination percentage of *Withania* seeds in the natural environment is very poor, the present protocol will be helpful to produce quality seedlings in large quantities and hence, conserve the rare species from natural collection.

Conflict of Interests

The authors have not declared any conflict of

interests.

REFERENCES

Abdual baki AA, Anderson JD (1973). Vigour determination of soybean seeds by multiple criteria. Crop sci. 130:630-63.

Antonisamy R, Manickam VS, Mathavan RE (2000). Regeneration of Indian ginseng plantlets from stem callus. Plant Cell Tissue Organ. Cult. 62:181-185.

Ashraf MY, Baig NA, Alam SM (1987). The influence of chlormequat on growth of raya (*Brassica juncea*). Pak. J. Bot. 19(2):259-262.

Ashraf MY, Baig NA, Baig F (1989). Response of wheat (*Triticum aestivum* L.). Treated with cycocel under water stress conditions. Acta Agron. Hung. 38(3-4):265-269.

Bagatharia SB, Chanda SV (1998). Modification of cell wall polysaccharides during cell elongation in *Phasealus vulgaris* hypocotyls. Acta Physiol. Plant. 20(1):15-18.

Chaudhary NY, Khan A (2000). Effect of growth hormones i.e., GA_3, IAA and kinetin on shoot of *Cicer arietinum* L. Pak J. Biol. Sci. 3(8):1263-1266.

De Silva MAN, Senarth WTPSK (2009). *In vitro* mass propagation and green house establishment of *Withania somnifera* (L) Dunal (solanaceae) and comparison of growth and chemical compounds of tissue cultured and seed raised plants. J. Natl. Sci. found. Srilanka 37(4):249-255.

Dhoran VS, Gudadhe SP (2012). Effect of Plant Growth Regulators on Seed Germination and Seedling Vigour in *Asparagus sprengeri* Regelin. Int. Res. J. Biol. Sci. 1(7):6-10.

Djavanshir K, Pourbeik H (1976). Germination value: A new formula. Silvae Genet. 25:79-83.

Farooqi AA, Sreeramu BS (2004). Cultivation of medicinal and aromatic plants. University Pess, India.

Ganzera M, Choudhary MI, Khan IA (2003). Quantitative HPLC analysis of withanolides in Withania somnifera. Fitoterapia.

Hartman HT, Kester DE, Davies DE (1990). Plant Propagation, principles and practices. 5th Edn., Prentice Hall Inc., Englewood, Cliffs, NJ., USA.

Hernandez P (1997). Morphogenesis in sunflower as affected by exogenous application of plant growth regulators. Agriscientia 13:3-11.

Kabar K (1990). Comparison of kinetin and gibberellic acid effects on seed germination under saline conditions. Phyton 30:291-298.

Kaul K, Farooq S (1994). Kinetin induced changes in extension growth activity of some enzymes in morning glory hypocotyls segments. Indian J. Plant Physiol. 4:214-216.

Kaur S, Gupta AK, Kaur N (1998). Gibberellic acids and kinetin partially reverse the effect of water stress on germination and seedling growth in chick pea. Plant Growth Regul. 25(1):29-33.

Kavidra NT, Neelesh CS, Vaibhav T, Brahma D (2000). Micropropagation of *Centella asiatica* a valuable medicinal herb. Plant Cell Tissue Organ Cult. 62:175-179.

Lee J, Joung KT, Hayain KH, Hee LS (1999). Effect of chilling and growth regulators in seedling stage on flowering of *Lilium formolongi*. Hangut Wanye Hakcheochi 40(2):248-252.

Mello AM (2009). Vernalisation and Gibberlic acid in the germination and development of Penstemon digitalis cv Husker Red. PhD Diss.fideral University of Santa Maria, Brazil.

Meredith CP (1978). Response of cultured tomato cells to aluminium. Plant Sci. Lett. 12:17-24.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio-assys with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Naeem M, Bhatti I, Ahmad RH, Ashraf MY (2004). Effect of some growth hormones (GA_3, IAA and Kinetin) on the morphology and early or delayed initiation of bud of lentil *(Lens culinaris* Medik*).* Pak. J. Bot. 36(4):801-809.

Raghav A, Kasera PK (2012). Seed germination behaviour of *Asparagus racemosus* (Shatavari) under *in-vivo* and *in-vitro* conditions. Asian J. Plant Sci. Res. 2(4):409-413.

Rahman M (2001). Red data Book of Vascular plants. Bangladesh National Herbarium. Dhaka, Bangladesh.

Rajabi R, Poustini K (2005). Effects of NaCl salinity on seed germination of 30 Wheat (*Triticum aestivum* L.) cultivars. Sci. J. Agric. 28:29-44.

Rani G, Grover IS (1999). *In vitro* callus induction and regeneration studies in *Withania somnifera*. Plant Cell Tissue Organ Cult. 57(1):23-27.

Ribeirio MN, Pasqual O, Villa FM, Pio SAL, Hilhorst MWH (2009). Invitro seed germination and seedling development of *Annona crassiflora* Mart. Sci. Agricola 66(3):410-413.

Sen J, Sharma AK (1991). Micropropagation of *Withania somnifera* from germinating seeds and shoots tips. Plant Cell Tissue Organ Cult. 26:71-73.

Sharma M, Ali MD, Batra A (2010). Plant regeneration through *in vitro* somatic embryogenesis in Ashwagandha (*Withania somnifera* L. Dunal). Researcher 2:1-6.

Sheoran OP (1968). Programmer, Computer Section CCS HAU, Hisar. Available at: http://14.139.232.166/opstat/default.asp

Siddique NA, Bari MA, Pervin MM, Nahar NLA (2005). Screening of Endangered Medicinal Plants Species by Questionnaire Survey in Barind Tract in Bangladesh. Pak. J. Biol. Sci. 8(12):1783-1793.

Stojicic D, Janosevic D, Uzelac B, Cokesa V, Budimir S (2012). *In-vitro* zygotic embryo culture of *Pinus peuce Gris* : Optimization of culture conditions affecting germination and early seedling growth. Arch. Biol. Sci. 64:503-509.

Supe U, Dhote F, Roymon MG (2006). *In vitro* plant regeneration of *Withania somnifera*. Plant Tissue Cult. Biotechnol. 16(2):111-115.

Thomas SG, Rieu I, Steber CM (2005). Gibberellin metabolism and signaling. Vitam. Horm. 72:289-338.

Tolera B, Diro M, Belew D (2014). Effects of Gibberellic acid and Kinetin on in vitro aseptic shoot tip culture establishment of sugarcane (*Saccharum officinarum* L.) varieties grown in Ethiopian sugar estates. Int. J. Sci. Basic Appl. Res. 16(1):496-504.