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In-vitro propagation of *Senna alata* (linn) on WPM and MS media with varying PGR

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Abstract

Plants react differently to various media concentration and constituents. Growth of *Senna alata (*linn*)* inoculated on woody plant media (WPM) and Murashige & Skoog (MS) supplemented with growth regulators were investigated. Mature seeds were collected from the medicinal garden of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan; latitude $7^{0}22^{1}$N; longitude 3^{0} 55^{1}E. Seeds of the species were inoculated into WPM and MS media. After 3 weeks, proliferated shoots were excised and inoculated into WPM and MS supplemented with the following concentrations of growth regulators 0.05, 0.075, 0.10, 0.125, 0.15mg/L BAP; 0.05, 0.075, 0.10, 0.125, 0.15mg/L KIN and 0.01mg/L NAA. Within 14 weeks of culture, shoot elongation was observed. Well rooted plantlets were transplanted and acclimatized in the screen house where 75% plantlets survived and grew successfully. The highest mean values of 3.90cm±0.15, 1.37±0.08 and 1.79cm±0.17 for shoot length, shoot number and root length respectively were obtained in plantlets inoculated in WPM supplemented with BAP 0.075mg/L+ NAA 0.01mg/L. Highest mean values of 2.81±0.08 for number of nodes, 2.29±0.15 for root number and 12.78±0.24 for number of leaves were obtained in plantlets inoculated in WPM supplemented with BAP 0.10mg/L+ NAA 0.01mg/L; KIN 0.15mg/L+ NAA0.01mg/L and BAP 0.05mg/L+ NAA 0.01mg/L respectively. The lowest mean values of 0.24±0.04 and 0.46±0.08 were obtained for numbers of nodes and leaves respectively under pure MS (control). Propagation of *Senna alata* (linn) was most effective with WPM supplemented with BAP 0.05mg/L + NAA 0.01mg/L.

Keywords: *In-vitro* regeneration, Basal media with growth regulators, *Senna alata* (linn)

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1. Introduction

Medicinal plants are the most exclusive source of life saving drugs for majority of the world population (Ravikumar and Ved, 2002). In recent years, traditional system of medicine has become a topic of global importance. Although modern medicine may be available in developed countries; yet two-third of the world still depend on plants for their primary health care (Eloff, 1998).

One of these important medicinal plant species is *Senna alata* (linn). *S. alata* is naturally distributed in Americas, and widely cultivated in South America and Tropical Africa. It is a shrub named for its flower bud which grow in a column and look like fat yellow candle each complete with flame, the leaves fold together at night (Keay, 1989). Its leaves sap is used to treat fungal infection such as ringworms (Gbile, 1998). They contain a fungicide, chrysophanic acid (Tradekey et al., 2009). Because of its anti-fungal properties it is a common ingredient in soap, shampoos and lotion in the Philippines. Other chemicals contained in the plant include saponin which act as laxative and expels intestinal worms. In Africa, the boiled leaves are used to treat a wide range of ailments from stomach problem, fever, snake bite (poisonous bite) and venereal diseases such as syphilis, gonorrhea (Ivan, 2002).

These diverse ends uses often exert pressure on the available stands of these plants. In-vitro regeneration technique provide a good opportunity for conservation and multiplication of pharmaceutically important bioactive compounds found in naturally occurring species (Nalawade et at., 2003; Phatak and Hesle 2002). Genetically homogenous clones containing uniform amounts of secondary metabolites occasioned by somatic embryogenesis or shoot organogenesis are regular occurrences in in-vitro propagated plants (Lin et al., 2003; Chawla, 2009). Hence, the need to develop tissue culture protocols for medicinally important species such as Senna alata (Linn) using different explants for the conservation and mass production of this useful plant in Nigeria.

This investigation therefore, deals with the standardization of protocol for the in- vitro propagation of the species from shoot tips.

2. Materials and method

The seeds of *Senna alata* (linn) were collected from the medicinal garden of National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan, Oyo State. The seeds were soaked with H_2SO_4 for 15 minutes, decanted, and rinsed thoroughly with sterile water; the seeds were later soaked in hot water (55^0C) for 19hours, washed first under running tap water for 15minutes and treated with 3 drops of tween 20 (polyxyethylene sorbitan monolanrate) for 15 minutes followed by repeated rinsing with autoclaved distill water. Further sterilization was done under aseptic condition in the laminar air flow hood. Explants were surface sterilized with 0.4% (W/V) $NaOCl_2$ for 15minutes, finally; the explants were washed thoroughly with autoclaved distilled water several times to remove traces of $NaOCl_2$. The seeds were inoculated into WPM and MS media. After 3 weeks, proliferated shoots were excised and inoculated in WPM and MS supplemented with the following concentration of growth regulators: 0.05, 0.075, 0.10, 0.125, 0.15mg/L BAP; 0.05, 0.075,

0.10, 0.125, 0.15mg/L KIN; and 0.01mg/L NAA. The WPM and MS media had 3% (W/V) sucrose and gelled with 0.8% (W/V) agar. The pH of all media was adjusted to 5.7 with 1N NaOH or HCl prior to autoclaving for 15minutes at 121^0C. The cultures were incubated in a culture room at 25 ± 2^0C with a photoperiod of 16hours at 1000-3000 lux light intensity provided by cool white fluorescent tubes. After 14weeks, well-rooted plantlets were obtained. Subsequently, the plantlets were removed from the culture vessel, washed gently under running tap-water and planted in plastic pots containing sterile soil, coconut fiber and stone-dust in the ratio 7:2:1 respectively, the potted plantlets were gradually exposed to the normal condition. Experiments were set up in a Completely Randomized Design (CRD) and each treatment had ten test-tube per treatment. Data were statistically analyzed (ANOVA) and mean comparison done by Duncan's multiple range test (Duncan, 1995) using the SAS 9.1 (SAS 1999).

3. Results

3.1. Shoot length

The effect of media and hormonal concentration was significant ($P \leq 0.05$) on shoot length of sprouted plantlets (Table 1). The highest mean value for shoot length of 3.90cm±0.15 was recorded in the WPM media supplemented with 0.075mg/L BAP + 0.01mg/L NAA, followed by mean values of 3.79cm±0.13 and 3.07cm±0.17 recorded in WPM media supplemented with 0.05mg/L BAP +0.01mg/LNAA and 0.125mg/L BAP + 0.01mg/L NAA respectively. The lowest mean value for shoot length of 0.11cm±0.02 was obtained in pure WPM media (control) (Table 2). There were significant differences in the mean shoot length values among the treatments (Table 3) (Plate 1 and 2).

Plate 1. In vitro regenerated Plantlets of *Senna alata* on WPM media supplemented with varying concentrations of growth hormones.

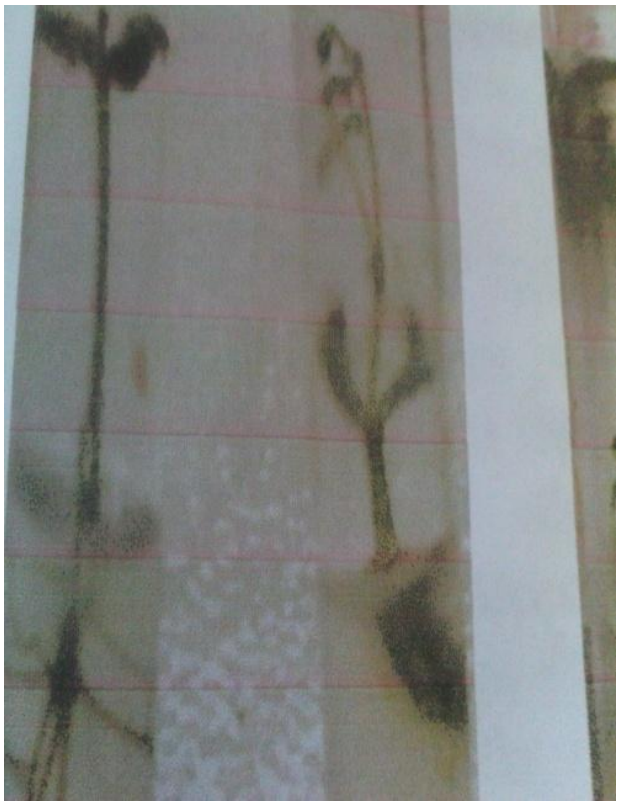

Plate 2. Initiation and elongation of shoots and roots of Senna alata on MS media supplemented with varying concentrations of growth hormones.

3.2. Shoot number

The effect of media and hormonal concentration was significant (P≤ 0.05) on shoot number of sprouted plantlets (Table 1). The highest mean value for shoot number of 1.37±0.08 was obtained in WPM media supplemented with 0.075mg/L BAP +0.01mg/L NAA, followed by mean values of 1.32±0.09 and 1.23±0.09 obtained in WPM media supplemented with 0.075mg/L KIN + 0.01mg/L NAA and 0.05mg/LBAP +0.01mg/L NAA respectively (Table 2 and 3). The lowest mean value for shoot number of 0.80±0.06 was obtained in MS media supplemented with 0.05mg/L KIN + 0.01mg/L NAA. There were no shoot sprouts in pure MS and WPM media (Table 2). There were significant differences in mean shoot number values of sprouted plantlets (Table 3).

3.3. Root length

The effect of media and hormonal concentration was significant (P≤ 0.05) on root length of sprouted plantlets. The highest mean value for root length of 1.79cm±0.17 was observed in WPM media supplemented with 0.075mg/L BAP +0.01mg/L NAA; plantlets inoculated in WPM media supplemented with 0.10mg/L BAP + 0.01mg/L NAA followed with mean value of 1.58cm±0.15 while the lowest mean value of 0.02cm±0.01 was

obtained in MS media supplemented with 0.10mg/L BAP + 0.01mg/L NAA. There was no root response in pure MS and WPM media; MS media supplemented with 0.05mg/LBAP +0.01mg/LNAA, 0.075mg/LBAP+0.01 mg/LNAA, 0.125mg/LBAP+0.01mg/LNAA, 0.05mg/LKIN+0.01mg/LNAA, 0.10mg/LKIN+0.01mg/LNAA, 0. 125mg/LKIN+0.01mg/LNAA and 0.15mg/LKIN+0.01mg/LNAA (Table 2). There were significant differences in mean root length values of rooted plantlets (Table 3).

Table 1. Summary of analysis of variance for growth variables of *Senna alata* shoot tips in WPM and MS media with different concentration of hormones.

	Source	Sum of square	Df	Mean Square	F Value	Significant
Shoot Number	Model	359.478	21	17.118	22.800	0.00
	Error	2295.950	3058	0.751		
	Total	2655.428	3079			
Shoot Number	Model	1106.074	21	52.670	100.707	0.00
	Error	1599.343	3058	0.523		
	Total	2705.417	3079			
Root Length(cm)	Model	1040.591	21	49.552	61.385	0.00
	Error	2468.511	3058	0.807		
	Total	3509.102	3079			
Shoot Length(cm)	Model	3333.204	21	158.724	161.572	0.00
	Error	3004.101	3058	0.982		
	Total	6337.305	3079			
Root Number	Model	1705.542	21	81.216	61.510	0.00
	Error	4037.679	3058	1.320		
	Total	5743.220	3079			
Leaf Number	Model	28895.724	21	1375.987	99.018	0.00
	Error	42494.821	3058	13.896		
	Total	71390.545	3079			

3.4. Root number

The effect of media and hormonal concentration was significant (P≤ 0.05) on root number of sprouted plantlet (Table 1). The highest mean value for root number of 2.29±0.15 was observed in WPM media supplemented with 0.15mg/LKIN + 0.01mg/LNAA, followed by mean values of 1.81±0.11 and 1.66±0.15 obtained in WPM media supplemented with 0.125mg/L KIN + 0.01mg/L NAA and 0.075mg/L BAP + 0.01mg/L NAA respectively (Table 2 and 3). The lowest mean value root number of 0.01±0.01 was observed in MS media supplemented with 0.10mg/l BAP + 0.01mg/l NAA (Table 2). There were significant differences in mean root number values of rooted plantlets (Table 3).

Table 2. Means and standard errors of growth variables of *Senna alata* (linn) shoot tips inoculated in WPM and MS media supplemented with different concentrations of hormones.

TREATMENTS	Shoot Number	Nodes Number	Root Length (cm)	Shoot Length (cm)	Root Number	Number of leaf
MS(BAP 0.05mg/l+NAA0.01mg/l)	1.02±0.08	2.08±0.07	0.00±0.00	1.89±0.07	0.00±0.00	11.76±0.45
WPM(BAP 0.05mg/l+NAA0.01mg/l)	1.23±0.09	2.61±0.09	0.94±0.09	3.79±0.13	1.39±0.15	12.78±0.24
MS(BAP 0.075mg/l+NAA0.01mg/l)	1.13±0.08	1.96±0.06	0.00±0.00	1.61±0.07	0.00±0.00	10.24±0.40
WPM(BAP0.075mg/l+NAA0.01mg/l)	1.37±0.08	2.51±0.06	1.79±0.17	3.90±0.15	1.66±0.15	12.34±0.21
MS(BAP 0.10mg/l+NAA0.01mg/l)	0.85±0.08	2.14±0.06	0.02±0.01	1.26±0.06	0.01±0.01	9.49±0.45
WPM(BAP 0.10mg/l+NAA0.01mg/l)	1.04±0.09	2.81±0.08	1.58±0.15	3.02±0.15	1.44±0.12	11.95±0.24
MS(BAP 0.125mg/l+NAA0.01mg/l)	0.89±0.07	2.03±0.05	0.00±0.00	1.29±0.05	0.01±0.01	9.65±0.38
WPM(BAP0.125mg/l+NAA0.01mg/l)	1.18±0.08	1.94±0.06	1.08±0.15	3.07±0.17	1.07±0.13	10.91±0.28
MS(BAP 0.15mg/l+NAA0.01mg/l)	1.09±0.08	1.71±0.05	0.23±0.04	1.48±0.06	0.64±0.10	10.72±0.41
WPM(BAP 0.15mg/l+NAA0.01mg/l)	1.18±0.08	2.02±0.07	0.66±0.52	1.71±0.04	0.94±0.12	8.60±0.29
MS(KIN 0.05mg/l+NAA0.01mg/l)	0.80±0.06	1.83±0.06	0.00±0.00	1.24±0.05	0.00±0.00	10.61±0.37
WPM(KIN 0.05mg/l+NAA0.01mg/l)	0.84±0.06	1.99±0.05	0.63±0.06	1.68±0.05	1.61±0.14	8.87±0.30
MS(KIN 0.075mg/l+NAA0.01mg/l)	0.82±0.06	1.89±0.04	0.25±0.05	1.12±0.04	0.79±0.11	10.58±0.37
WPM(KIN0.075mg/l+NAA0.01mg/l)	1.32±0.09	2.42±0.07	0.52±0.05	2.92±0.06	1.37±0.14	10.56±0.39
MS(KIN 0.10mg/l+NAA0.01mg/l)	1.06±0.08	1.79±0.05	0.00±0.00	1.18±0.04	0.00±0.00	9.50±0.34
WPM(KIN 0.10mg/l+NAA0.01mg/l)	1.17±0.08	2.04±0.06	0.75±0.08	2.33±0.08	1.11±0.12	10.32±0.18
MS(KIN 0.125mg/l+NAA0.01mg/l)	0.96±0.07	1.76±0.05	0.00±0.00	0.96±0.03	0.00±0.00	8.28±0.26
WPM(KIN0.125mg/l+NAA0.01mg/l)	1.21±0.09	2.40±0.08	1.20±0.10	2.81±0.07	1.81±0.11	9.48±0.27
MS(KIN 0.15mg/l+NAA0.01mg/l)	0.90±0.07	1.69±0.05	0.00±0.00	1.02±0.04	0.00±0.00	7.67±0.32
WPM(KIN 0.15mg/l+NAA0.01mg/l)	1.01±0.08	2.22±0.08	1.42±0.11	2.54±0.08	2.29±0.15	10.01±0.28
MS (CONTROL)	0.00±0.00	0.24±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.46±0.08
WPM (CONTROL)	0.00±0.00	0.30±0.04	0.00±0.00	0.11±0.02	0.00±0.00	0.51±0.07

Table 3. Duncan's Multiple Range Test for growth variables of Senna alata (linn) shoot tips inoculated in WPM and MS media supplemented with different concentrations of hormones

TREATMENTS	Shoot Number	Nodes Number	Root Length (cm)	Shoot Length (cm)	Root Number	Leave Number
MS(BAP0.05mg/l+NAA0.01mg/l)	1.02^{bcdefgh}	2.08^{ghi}	0.00^{a}	1.89^{g}	0.00^{a}	11.76^{ij}
WPM(BAP0.05mg/l+NAA0.01mg/l)	1.23^{hik}	2.61^{k}	0.94^{de}	3.79^{k}	1.39^{ef}	12.78^{k}
MS(BAP0.075mg/l+NAA0.01mg/l)	1.13^{fghi}	1.96^{defgh}	0.00^{a}	1.61^{f}	0.00^{a}	10.24^{fgh}
WPM(BAP0.075mg/l+NAA0.01mg/l)	1.37^{k}	2.51^{ik}	1.79^{h}	3.90^{k}	1.66^{fg}	12.34^{jk}
MS(BAP0.10mg/l+NAA0.01mg/l)	0.85^{bcd}	2.14^{hi}	0.02^{a}	1.26^{cde}	0.01^{a}	9.49^{def}
WPM(BAP0.10mg/l+NAA0.01mg/l)	1.04^{cdefgh}	2.81^{i}	1.58^{g}	3.02^{ij}	1.44^{f}	11.95^{jk}
MS(BAP0.125mg/l+NAA0.01mg/l)	0.89^{bcde}	2.03^{gh}	0.00^{a}	1.29^{de}	0.01^{a}	9.65^{efg}
WPM(BAP0.125mg/l+NAA0.01mg/l)	1.18^{ghik}	1.94^{defgh}	1.08^{ef}	3.07^{j}	1.07^{d}	10.91^{hi}
MS(BAP0.15mg/l+NAA0.01mg/l)	1.09^{efghi}	1.71^{bc}	0.23^{a}	1.48^{ef}	0.64^{b}	10.72^{h}
WPM(BAP0.15mg/l+NAA0.01mg/l)	1.18^{ghik}	2.02^{gh}	0.66^{bc}	1.71^{fg}	0.94^{cd}	8.60^{cd}
MS(KIN0.05mg/l+NAA0.01mg/l)	0.80^{b}	1.83^{bcdef}	0.00^{a}	1.24^{cde}	0.00^{a}	10.61^{gh}
WPM(KIN0.05mg/l+NAA0.01mg/l)	0.84^{bcd}	1.99^{fgh}	0.63^{bc}	1.68^{fg}	1.61^{fg}	8.87^{cde}
MS(KIN0.075mg/l+NAA0.01mg/l)	0.82^{bc}	1.89^{cdefg}	0.25^{a}	1.12^{bcd}	0.79^{bc}	10.58^{gh}
WPM(KIN0.075mg/l+NAA0. 01mg/l)	1.32^{ik}	2.42^{j}	0.52^{b}	2.92^{ij}	1.37^{ef}	10.56^{gh}
MS(KIN0.10mg/l+NAA0.01mg/l)	1.06^{defgh}	1.79^{bcde}	0.00^{a}	1.18^{bcd}	0.00^{a}	9.50^{def}
WPM(KIN0.10mg/l+NAA0.01mg/l)	1.17^{ghik}	2.04^{ghi}	0.75^{cd}	2.33^{h}	1.11^{de}	10.32^{fgh}
MS(KIN0.125mg/l+NAA0.01mg/l)	0.96^{bcdefg}	1.76^{bcd}	0.00^{a}	0.96^{b}	0.00^{a}	8.28^{bc}
WPM(KIN0.125mg/l+NAA0.01mg/l)	1.21^{hik}	2.40^{j}	1.20^{f}	2.81^{j}	1.81^{g}	9.48^{def}
MS(KIN0.15mg/l+NAA0.01mg/l)	0.90^{bcdef}	1.69^{b}	0.00^{a}	1.02^{bc}	0.00^{a}	7.67^{b}
WPM(KIN0.15mg/l+NAA0.01mg/l)	1.01^{bcdefgh}	2.22^{i}	1.42^{g}	2.54^{h}	2.29^{h}	10.01^{fgh}
MS(CONTROL)	0.00^{a}	0.24^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.46^{a}
WPM(CONTROL)	0.00^{a}	0.30^{a}	0.00^{a}	0.11^{a}	0.00^{a}	0.51^{a}

3.5. Node number

The effect of media and hormonal concentration was significant (P≤ 0.05) on node number of sprouted plantlets (Table 1). The highest mean value for node number of 2.81±0.08 was obtained in WPM media supplemented with 0.10mg/L BAP +0.01mg/L NAA; followed by mean value of 2.61±0.09 and 2.51±0.06 obtained in WPM media supplemented with 0.05mg/L BAP + 0.01mg/L NAA and 0.075mg/L BAP +0.01mg/L NAA respectively. The lowest mean value for node number of 0.24±0.04 was obtained in pure MS media (Table 2).The mean node number of plantlets under supplemented media were significantly different from those under pure MS and WPM (Table 3).

3.6. Number of leaves

The effect of media and hormonal concentration was significant (P≤ 0.05) on number of leaves of sprouted plantlets (Table 1). The highest mean value for number of leaves (12.78±0.24) was obtained in WPM media supplemented with 0.05mg/L BAP + 0.01mg/L NAA, followed by mean value of 12.34±0.21 and 11.95±0.24 observed in WPM media supplemented with 0.075mg/L BAP + 0.01mg/L NAA and 0.10mg/L BAP + 0.01mg/L NAA respectively. The lowest mean number of leaves of 0.46±0.08 was obtained in pure MS media (Table 2). There were significant differences in the number of leaves of sprouted plantlets (Table 3).

4. Discussion

The regeneration of high value medicinal plants through rapid and mass multiplication using in-vitro culture technique is a *sine qua non* to their domestication and conservation. The present study deals with in-vitro propagation of *Senna alata* (linn) on WPM and MS media supplemented with different concentrations of growth regulators. Overall observations suggest that WPM media supplemented with BAP, KIN and NAA in low concentrations of 0.01 – 0.15mg/L supports the somatic embryogenesis as well as root and shoot development in sprouted plantlets. This agreed with the findings of Baksha et al. (2002) on *Saccharum officinarum*.

The presence of BAP, KIN and NAA in WPM and MS media which underscores the synergistic effects of cytokinins and auxin was very effective in stimulating growth unlike pure WPM and MS media which gave a dismal result; this is similar to the result obtained by Tonon et al. (2001) in *F. angustifolia*. However, in all concentrations tested in the present study, WPM supplemented media performed better than MS for every growth variable assessed; Palla and Pijut (2010) reported the favourable effect of WPM supplemented with auxins and cytokinins on the micropropagation of *Fraxinus spp.* However, Van Sambeek and Preece (2007) indicated a contrary result.

Type of cytokinin and their concentration influenced growth performance of *S. alata* plantlets; Faheem et al. (2011) made similar observation in *Catharanthus roseus*. BAP favoured shoot proliferation while KIN proved to be more efficient in stimulating rooting; studies have revealed that media supplemented with NAA and BAP promoted shoot development (Borman and Janson, 1980; Mcpheeters and Skirvin, 1980). Infact,

increasing BAP concentration led to decreased rooting in *Ocimum basilicum* (Asghari et al., 2012). Gaj (2004) observed that the concentration of plant growth regulators especially the balance between auxins and cytokinins is a key factor in callus induction and somatic embryogenesis initiation.

Auxins usually enhance root induction and seedling growth in many species (Jain and Ochatt, 2010); since the concentration of auxin is the same for the media in the present study, the better result with KIN might have revealed a better synergy between KIN and NAA for root induction in *S. alata*; Beck (1983) reported that specific concentration of NAA must be present with specific concentration of KIN for maximal root production. Kaviani (2011) similarly reported a positive effect of KIN on root induction and root length in *Matthiola incana*; Gomes et al. (2010) for *Arbutus unedo*. The role of growth regulators in regular media seems to be specific to each species; Beck (1983) reported that KIN is the major factor involved in maximal shoot production in *Nephrolepis falcata*, on the other hand, Nayak et al. (2010) submitted that highest shoot multiplication in *Bambusa arundinacea* was obtained in medium without KIN. Hence, the need for ongoing research in the in vitro regeneration of useful plant species.

5. Conclusion

Morphogenetic processes like somatic embryogenesis and organogenesis are complex phenomena characterized by different phases each with specific nutritional requirements in different species; in the present study, WPM medium supplement with cytokinins (BAP and KIN) in combination with auxin (NAA) conclusively proved effective. Therefore, WPM media supplemented with 0.05mg/l. BAP and 0.01mg/l. NAA is recommended for the in vitro regeneration of *Senna alata* (linn).

Abbreviations

BAP: 6 Benzylaminopurine; NAA: naphthalene acetic acid; KIN: kinetine; MS: Murashige and Skoog medium; WPM: Woody Plant Medium

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