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RESPONSES OF *Andrographis paniculata* (BURM. F.) NEES SINGLE- NODE LEAFY STEM CUTTINGS TO DIFFERENT AUXINS AND AUXIN CONCENTRATIONS

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ABSTRACT

Conservation through vegetative propagation has been encountering setback due to difficulty in rooting stem cuttings as different plant species respond differently to auxins and auxins concentrations. *Andrographis paniculata* commonly known as "king of bitters" is an important medicinal plant which required its genetic base be preserved because of unsustainable harvesting and seed dormancy. So this study investigated auxin and auxin concentration that is appropriate for survival and rooting potential of its stem cuttings in order to produce sufficient seedlings for plantation establishment. Nine hundred single node leafy cuttings obtained from two positions of collection on mature plants were subjected to different treatments: Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and combination of IBA and NAA at different concentrations (0, 50, 100, 150 and 200mg/l) using quick dip method. The cuttings were planted in sterile washed river sand in 2x3x5 factorial design with 5replicates under high humidity propagator. The following parameters were assessed; cuttings survival, number of rooted cuttings, number of roots per cutting and length of longest root. Data collected were subjected to ANOVA and LSD. Auxin significantly affected the variables as IBA/NAA had the lowest cutting survival (95% survival) but highest mean rooted cutting (5.1± 1.3) and root length (13.8cm±3.1) while IBA had the highest mean root per cutting of 3.9±2.2. More so, concentration of auxins had significant effect on the variables as cuttings subjected to 200ml/L had 100% survival as 0mg/l (control) had 88.3%. More so, 100mg/l had the highest mean number of rooted cuttings (5.5±0.7) while 0mg/l had the least mean root length (11.8cm±2.3) and roots/cutting (2.1±1.1) In this experiment, cuttings from young shoot performed well with or without use of auxin but the use of NAA promoted massive root production while IBA had the highest mean root per cutting.

Keywords: Single node, leafy stem cuttings, Auxins, Cutting survival, *Andrographis paniculata*

Introduction

Andrographis paniculata (Burm. F.) Nees. commonly known as "king of bitters" is an important medicinal plant found in Nigeria though it is a native of India and Sri-lanka. It is an annual herb extremely bitter in taste (Niranjan *et al.*, 2010). It is of great importance and use because of the presence of phytochemicals needed in human health care. It has a broad range of pharmacological effects and can be used to treat many diseases. It also has low toxicity and side effect (Akbarsha *et al*., 1990). Different studies have been carried out on its effects on various diseases like cancer (Kumar *et al.*,2004), leukaemia (Matsuda *et al.*, 1994), Human Immunodeficiency (Holt and Comac, 1998), inflammations, cold and fever (Burgos and Caceres, 1994; Deng, 1978) and promising herb to explore for its potential for use against

COVID-19 as it showed to have potency against COVID-19 as its microscopic mechanism was evidenced through rational computational modeling (Natarajan *et al*., 2020; Sukanth *et al*., 2020) The wide tissue and organ distribution and the immune – stimulating and regulatory actions make it an ideal candidate in the prevention and treatment of many diseases and health conditions (Jean-Barilla, 1999).

In many countries medicinal plants are collected from the wild vegetation. But in response to the combined impacts of dwindling supplies due to over exploitation of natural resources and increasing demands due to population growth and growing global markets, conservation of medicinal plants has become important. The conservation of the wild medicinal plants or any other such threatened species can be tackled by scientific techniques such as use of vegetative propagation. Vegetative propagation which involves the use of other plant materials to produce seedlings can be seen as ideal way of multiplying plant species rapidly especially when desirable traits are to be maintained or the species has seed dormancy for plantation establishment. Plant growth regulators (auxin) have been used for root inducement in stem cuttings in various researches. Vegetative propagation has been carried out for different plant species; *Buchholzia coriacea* (Akinyele 2010), *Conosperum patens* and *Persoonia pinifolia* (Perry, 1997), *Cecropia obtusifolia* (LaPierre, 1999), *Irvingia gabonensis* (Shiembo *et al.*, 1996),*Milicia excelsa* (Ofori *et al.*, 1996),*Prosopis cineratia* (Arya *et.al*, 1994), *Parkia biglobosa* to mention few while different auxins and concentrations used and found to improve overall rooting percentages, hasten root initiation, increase the number and quality of roots (Blythe *et al*.,2007). Conservation of medicinal plants or other plant species through vegetative propagation may not be successful due to many factors especially cutting position from the shoot, age of the donor plant, growth regulating hormone and concentration used while responses to different auxins and concentrations vary among plant species (AbShukor and Liew 1994; Haissig, 1988; Al-Salem and Karam, 2001; Agbo and Obi 2007; Guo *et al*., 2009) so there is need to carry out this research to ascertain the appropriate auxin and concentration for survival and rootability of the cuttings of *Andrographis paniculata* collected from different positions on standing plant.

Materials and Methods

Nine hundred single node leafy cuttings were obtained from mature plants and subjected to different treatments: two points of collection (young shoot from upper position and old shoot from lower position on standing plant), three different hormones and five concentrations. Four hundred and fifty were collected from tender shoot while other four hundred and fifty were collected from old shoot. These cuttings had their leaf size reduced to half of their original size. The cuttings were treated with different hormones: Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and combination of IBA and NAA at different concentrations (0, 50, 100, 150 and 200mg/l) using quick dip method (Oni, 1987). These cuttings were then planted in sterile washed river sand and arranged in 2x3x5 factorial design with 5 replicates under high humidity propagator. Watering was done twice a day with knapsack sprayer while the following parameters were assessed; percentage survival, number of roots per cutting, length of longest root and total root length. Percentage survival was obtained by counting the cuttings that

survived and the percentage calculated. Number of roots per cuttings was obtained by uprooting each survived cutting and the roots counted. Length of longest root was measured with meter rule and recorded for each treatment while with meter rule, the length of all the roots on survived cuttings per treatment were measured. Data collected was analysed using ANOVA while LSD was used as post hoc test.

Results

Effect of points of collection, different auxins and concentrations on percentage survival of *A. paniculata* stem cuttings

Stem cuttings collected from young shoot had mean value of 5.9±0.6 cuttings while old shoot had mean value of 5.7±0.7. Also, cuttings from young shoot had 98.3 percent survival while cuttings from old shoot had 95 percent survival (Table 1). Likewise, there was no significant difference among different auxins used. NAA and IBA had the same mean value of 5.8±0.7 cuttings while combination of NAA and IBA had the mean value of 5.7±0.6 cuttings. Concentration of auxin positively affected the cutting survival as 200mg/l had 100% of the cuttings survived while 0mg/l had 88.3% of its cuttings survived.

Concentration of auxin (C) was observed to affect the survival of cuttings while point of collection (P), auxin used (A) and their interactions did not significantly affect cuttings survival at P=0.05 (Table 2). There was no significant difference between cutting survival of the two points of collections.

Table 1: Effects of Point of Collection, Hormones and Concentrations on parameters measured on *A. paniculata* stem cuttings

Treatments	Parameters					
	Percentage survival (%)	Mean cutting survival	Mean rooted cuttings	Longest root length (cm)	Total root length (cm)	Mean root per cutting
Point of collection						
Young shoot	98.3	5.9^{ns}±0.6	5.7^{a}±0.8	13.8^{a}±2.2	58.9^{a}±27.3	3.9^{a}±1.1
Old shoot	95	5.7^{ns}±0.7	4.3^{b}±1.3	11.6^{b}±2.8	55.6^{b}±25.9	2.3^{b}±0.9
Auxin						
NAA	96.7	5.8^{ns}±0.7	4.9^{ns}±1.3	12.6^{a}±3.1	75.3^{ns}±33.4	2.9^{b}±1.3
IBA	96.7	5.8^{ns}±0.7	5.0^{ns}±1.3	12.6^{a}±3.1	71.3^{ns}±32.1	3.9^{a}±2.2
NAA + IBA	95	5.7^{ns}±0.7	5.1^{ns}±1.3	13.8^{b}±2.7	70.1^{ns}±28.9	3.2^{ab}±1.3
Concentrations						
0mg/l	88.3	5.3^{b}±1.2	4.4^{a}±1.6	11.8^{b}±2.3	46.0^{b}±25.6	2.1^{c}±1.1
50mg/L	98.3	5.9^{a}±0.5	4.7^{a}±1.4	13.4^{a}±3.1	76.6^{a}±27.2	4.0^{a}±2.5
100mg/L	96.7	5.8^{a}±0.4	5.5^{b}±0.7	13.2^{a}±3.1	78.9^{a}±24.6	3.3^{b}±1.1
150mg/L	98.3	5.9a±0.4	5.2^{b}±1.2	13.4^{a}±3.3	80.0^{a}±29.3	3.4±1.2^{b}
200mg/L	100	6.0^{a}±0.0	5.3^{b}±1.3	13.2^{a}±3.1	80.1^{a}±36.2	3.3±1.4^{b}

Means followed by different letter(s) within a treatment group are significantly different at 0.05

level of probability. ns = not significant

Table 2: Analysis of Variance for the Effects of Point of Collection, Hormones and Concentrations on Percentage Survival

Source	df	Sum of Squares	Mean Square	F	Sig.
Point of Collection (P)	1	.807	.807	1.862	.175
Hormones (H)	2	.360	.180	.415	.661
Concentrations (C)	4	9.107	2.277	5.254	.001
P x H	2	.093	.047	.108	.898
P x C	4	.360	.090	.208	.934
H x C	8	.773	.097	.223	.986
P x H x C	8	.240	.030	.069	1.000
Error	120	52.000	.433		
Total	149	63.740			

Effect of points of collection, different auxins and concentrations on Number of Rooted Cuttings of *A. paniculata*

There was significant difference between the mean values of the two points of collections (young shoot and old shoot) as cuttings collected from young shoot had mean value of 5.7±0.8 cuttings while those from old shoot had mean value of 4.3±1.3 cuttings. There was no significant difference among different auxins used, but combination of NAA and IBA had the highest mean value of 5.1±1.3 cuttings. Concentration of auxin used significantly affected the number of rooted cuttings, but there was no significant difference among the means of concentrations 100mg\L, 150mg\L and 200mg\L but 100mg\L had the highest mean value of 5.5±0.7 cuttings followed by 200mg/l (5.3±1.3 cuttings) while 0 mg\L had the lowest mean value of 4.4±1.6 cuttings (Table 1).

Point of collection (P), Concentration of auxin(C) and P x C interaction were observed to affect the rooting of the cuttings while hormone used and their interactions did not significantly affect cuttings rooting ability (Table 3).

Table 3: Analysis of Variance for the Effects of Point of Collection, Hormones and Concentrations on Number Rooted

Source	Sum of Squares	df	Mean Square	F	Sig.
Point of Collection (P)	76.327	1	76.327	78.150	.000
Hormones (H)	1.080	2	.540	.553	.577
Concentrations (C)	23.173	4	5.793	5.932	.000
P x H	3.453	2	1.727	1.768	.175
P x C	10.107	4	2.527	2.587	.040
H x C	11.987	8	1.498	1.534	.152

P x H x C	7.613	8	.952	.974	.459
Error	117.200	120	.977		
Total	250.940	149			

Effect of points of collection, different auxins and concentrations on Root Length of *A. paniculata* stem cuttings

Cuttings from young shoot had the higher mean value of 13.8±2.2 cm while old shoot had 11.6±2.8 cm. Combination of NAA and IBA had the highest mean value of 13.8±2.7 cm while the mean values of NAA and IBA were not different from each other (12.6±3.1 cm). The mean values of different concentration used were not significantly different from one another but different from control (0mg\L) which had the least root length (11.8±2.3 cm) (Table 1).

Point of collection, auxin and concentration had significant effect on root development as well as interactions between auxin and concentration and among point of collection, auxin and concentration had significant effect of root length (Table 4).

Table 4: Analysis of Variance for the Effects of Point of Collection, Hormones and Concentrations on Longest Root

Source	df	Sum of Squares	Mean Square	F	Sig.
Point of Collection (P)	1	306.735	306.735	53.098	.000
Hormones (H)	2	46.178	23.089	3.997	.021
Concentrations (C)	4	54.360	13.590	2.353	.058
P x H	2	17.424	8.712	1.508	.225
P x C	4	7.016	1.754	.304	.875
H x C	8	131.152	16.394	2.838	.006
P x H x C	8	87.456	10.932	1.892	.067
Error	120	693.208	5.777		
Total	149	1343.528			

Effect of points of collection, different auxins and concentrations on Total Root Length

of *A. paniculata* stem cuttings

Cuttings collected from young shoot had the higher mean value of 58.9±27.3 cm while those from old shoot had 55.6±25.9cm. There was no significant difference among the means of NAA, IBA and Combination of NAA and IBA. But NAA had the highest mean value of 75.3±33.4cm, while IBA and combination of NAA and IBA had 71.3±32.1cm and 70.1±28.9cm respectively. The mean values of different concentration used were not significantly different from one another but different from control which had the least mean total root length (46.0±25.6cm) (Table 1).

Total root length of the cuttings was significantly affected by point of collection, and concentration of the hormone used. More

so, the interactions between point of collection and concentration of the hormone, hormone and concentration and among point of collection, hormone and concentration of the hormone (Table 5)

Table 5: Analysis of Variance for the Effects of Point of Collection, Hormones and Concentrations on Total Root Length

Source	df	Sum of Squares	Mean Square	F	Sig.
Point of Collection (P)	1	41686.669	41686.669	89.638	.000
Hormones (H)	2	743.664	371.832	.800	.452
Concentrations (C)	4	25968.019	6492.005	13.960	.000
P x H	2	1032.402	516.201	1.110	.333
P x C	4	5087.241	1271.810	2.735	.032
H x C	8	8677.287	1084.661	2.332	.023
P x H x C	8	7873.410	984.176	2.116	.039
Error	120	55806.912	465.058		
Total	149	146875.604			

Effect of point of collection, different auxins and concentrations on Root per Cuttings

of *A. paniculata* stem cuttings

As shown in Table 6, cuttings from young shoot had the higher mean number of root per cutting (3.9±1.1 root/cutting) while those from old shoot had 2.3±0.9 root per cutting. There was significant difference among different auxins used. IBA had the highest mean root per cutting (3.9±2.2 roots/cutting), followed by combination of NAA and IBA with 3.2±1.3 mean root per cuttings while NAA had the least mean number of root per cutting (2.9±1.1 root/ cuttings).

Point of collection, different auxins and concentrations used significantly affected root per cutting while their interactions also had significant effect of the rooting ability of each cutting (Table 6).

Table 6: Analysis of Variance for the Effects of Point of Collection, Hormones and Concentrations on Number of Root per Cutting

Source	df	Sum of Squares	Mean Square	F	Sig.
Point of Collection (P)	1	131.977	131.977	117.658	.000
Hormones (H)	2	7.344	3.672	3.274	.041
Concentrations (C)	4	54.690	13.673	12.189	.000
P x H	2	10.040	5.020	4.475	.013
P x C	4	21.714	5.428	4.839	.001
H x C	8	20.307	2.538	2.263	.027
P x H x C	8	25.247	3.156	2.813	.007

Error	120	134.604	1.122
Total	149	405.922	

Discussions

In this experiment, the cuttings of this plant had no difficulty surviving especially under the high humidity but the point of collection had significant influence on its survival and rooting ability. Cuttings from young shoot performed well with any hormone and concentration used while cuttings from young shoot treated with no hormone also gave encouraging result. It was observed that young shoot had higher number of survived and rooted cuttings with massive and long root. Ab Shukor and Liew (1994) findings ran contrary to the result of this study as basal cuttings gave highest rooting percentage (100%) while the report of Nor Aini *et al.*, (2010) corroborated the finding of this study as cuttings from top position recorded highest survival percentage of 90.7% while bottom had 74.7% in the study of cutting positions and growth regulators on rooting ability of *Gonystylus bancanus.* More so, Singh *et al*,. (2012) reported significant effect of position of shoot from which stem cuttings of *Dalbergia sisso* on survival and rooting potential as cuttings from lower portion of stem recorded higher value irrespective of auxin treatments. In this study, use of NAA supported production of long roots while IBA had encouraged production more root per cutting. It was also observed that combination of NAA and IBA gave highest mean value for number of cuttings rooted and total root length. Preliminary investigations were made of the effects of auxin treatment and air temperature on the rooting and death of *Conospermum patens* and *Persoonia pinifolia*. Indole-butyric acid (IBA) by Perry, (1997) was found to be the most effective auxin in stimulating rooting of cuttings of all three species, while naphthalene acetic acid (NAA) had an adverse effect on cutting survival of the two *Conospermum* species. Azamal and Mohinder (2007) also observed the highest mean number of roots and length in cuttings taken from the branch emerging at the upper position of teak followed by branches of middle and lower position, this agrees to the findings of this study as cuttings collected from young shoots at the top position of standing plants performed better than those collected from old shoot. He also found out that treatment with 4000 ppm IBA increased percent rooting and percent sprouting whereas NAA suppressed it. This was contrary to the finding of this study as NAA also gave encouraging result. The effect of mixture of two auxins, NAA and IBA on adventitious root production in cuttings of *Cecropia obtusifolia* (Bertol.) by La Pierre, (1999) showed that the cuttings survived to root with intermediate values. This also corroborated the findings of this study where 95% of the cuttings treated with mixture of IBA and NAA irrespective of other factors considered survived and rooted. Ability of the stem cuttings of *A. paniculata* to survive and root with the influence of auxin is a big step towards its regeneration through macropropagation. Furthermore, highest concentration of auxins used supported massive rooting system. Root growth is critical to seedling establishment and gives opportunity of overcoming environmental stress when introduced to the field. Success of plantation establishment of this plant species may depend on the ability of the root to ensure seedling establishment on the field as Waters *et al.*, (1991) opined that inability of seedling roots to cope with environmental stress caused greatest mortality recorded on some sites leading to poor plantation

establishment.

Conclusion

In this experiment, stem cuttings were collected from apical and basal parts of standing *A. paniculata* and treated with IBA, NAA and combination of both auxins in 0, 50,100, 150 and 200 mglL . Auxins considered did not have any significant influence on survival of stem cuttings. Cuttings from young shoot performed well with any hormone and concentration used but the use of NAA promoted massive root production (Total root length) while the highest mean root per cutting was recorded with the use of IBA. In conclusion, collection of cuttings from young shoot at apical part of the plant promoted higher survival while the use of auxin at higher concentration supported rooting ability of the cuttings.

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