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Full Length Research Paper

In *vitro* growth response of *Artemisia annua* seeds to different concentrations of plant growth regulators

Jamaleddine Z. O*. Lyam P., Fajimi O., Giwa A., Aina A., Lawyer E. F., Okere A. U. and Odofin W. T.

National Center for Genetic Resources and Biotechnology, Moor Plantation, Apata, Ibadan, Oyo State, Nigeria.

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***Artemisia annua* is a crop popularly known for its importance in the production of anti-malarial and possibly antibacterial agents and natural pesticides. *A. annua anamed* is a hybrid which is able to grow well in hot weather and still retain its anti-malarial characteristics. Seeds of *annua* were cultured on Murashige and Skoog (MS) full strength without plant growth regulators, and also on MS medium augmented with varied concentrations of kinetin (0.025 to 0.1 mg/L) and benzyl amino purine (BA) (0.025 to 0.1 mg/L), while the auxin used, naphthalene acetic acid (NAA 0.01 mg/L) was constant for all treatments. At 16 weeks, data was collected and results were analyzed. The mean shoot and root length of plantlets regenerated from seeds was optimum for elongation, on 0.1 mg/L kinetin combined with 0.01 mg/L NAA. Single shoots formed on auxin free MS fortified with 0.025 mg/L BA, gave the highest mean number of nodes. Callus formation was evident at concentrations above 0.05 mg/L BA in combination with 0.01 mg/L NAA.**

Key words: *Artemisia annua anamed*, plant regeneration, benzyl amino purine (BA), napthalene acetic acid (NAA).

INTRODUCTION

Artemisia annua, also known as Sweet Wormwood, Sweet Annie, Sweet Sagewort or Annual Wormwood, belonging to the family Asteraceae, is a common type of wormwood that grows throughout the world. It is a crop for the production of anti-malarial and possibly antibacterial agents and natural pesticides. It was originally collected by the Chinese as an herbal medicine and is currently processed by pharmaceutical firms for the production of artemisinin for artemisinin-based combination therapies (ACTs) in the treatment of malaria. ACTs have been shown to have rapid resolution to fever and parasitaemia, low toxicity and are well tolerated (Diemer and Griffee, 2005). Artemisinin has proved to be a dramatically effective anti-malarial against multi-drug resistant *Plasmodium* spp. (Brown, 1995; Duke, 1987). An infusion of the leaves and flowers is used internally to treat fevers, colds, diarrhea, etc. (Foster and Duke, 1990). Externally, the leaves are poulticed onto nose bleeds, boils and abscesses (Brown, 1995).

The plant has also been shown to have anti-cancer properties. It is said to have the ability to be selectively toxic to breast cancer cells (Cancer Research, 2005), and some form of prostate cancer. There have been exciting preclinical results against leukemia (Räth, 2004) and other cancer cells.

There is now a new cultivar called 'Anamed' that is apparently able to grow well in hot weather and still retain its anti-malarial characteristics. The special seeds are available from Europe but as they are not cheap, they need to be grown carefully, especially during the early stages of growth. This would be beyond the capabilities of the normal village gardeners, but if the early growing stage could be carried out by knowledgeable growers in controlled conditions, the young plants can be given to the villages to cultivate (Pageant, 2006). For this reason, *in vitro* propagation can serve as a method of cultivating

*Corresponding author. E-mail: glizzy00@yahoo.com. Tel: 08058567113.

Abbreviations: MS, Murashige and Skoog; **BA,** benzyl amino purine; **NAA,** naphthalene acetic acid.

and propagating the plant under controlled conditions.Vegetative propagation of *A. annua* has been developed. Tissue culture uses standard protocols with shoot tips of mature field grown plants (Simon et al., 1990). Shoot-tips and lateral buds of *A. annua* L. produced numerous shoots on MS medium and formed 100% roots on half strength Murashige minimal organic medium. The medicinal use of *A. annua anamed*, in the tropics, should be emphasized. There is therefore an urgent need for the conservation and rapid propagation of the seedlings using tissue culture techniques.This experiment was carried out to study growth response of *A. annua* seeds *in vitro,* under different combinations of plant growth regulators, to develop a protocol for production of clean healthy plantlets for subsequent multiplication.

MATERIALS AND METHODS

This experiment was carried out at NACGRABs Biotechnology Laboratory, Moor Plantation, Ibadan. Seeds *of A. annua* were purchased in Accra, Ghana. The seeds were kept in NACGRAB seed gene bank before use. Viable seeds were surface sterilized in a filter paper in a funnel using 70% ethyl alcohol for five minutes followed by disinfection with 10% (v/v) sodium hypochlorite for fifteen minutes. Seeds were then rinsed three times in sterile distilled water before inoculation on media.

Culture media and conditions

Basal medium used was full strength Murashige and Skoog (1962). The medium containing 3% (w/v) sucrose, B5 vitamins, 0.1 mg Inositol, was augmented with two different cytokinins and an auxin. Kinetin and BAP concentrations were varied between 0.025 and 0.1 mg/L, while concentrations of NAA (0.01 mg/L) were constant for treatments when used in combination with cytokinins. Hormone free MS medium served as control. The pH of the medium was adjusted to 5.8 prior to the addition of 0.7%w/v agar. 5 ml aliquots were dispensed into tubes and autoclaved at 121°C at 15 lb pressure for 15 min. Seeds were aseptically inoculated in tubes and the cultures were maintained at 25 ± 2°C using 14/10 light/dark period, under a light intensity of 3000 lux provided by cool-white fluorescent lamps and 50 to 55% relative humidity.

Statistical analysis

A minimum of ten tubes were raised for each treatment and the experiment were performed three times. After sixteen weeks of incubation, cultures were harvested and the shoot length, root length, number of nodes, number of leaves and callus rating were measured. Analysis of variance (ANOVA) and mean separations were carried out using Duncan's multiple range test.

RESULTS AND DISCUSSION

Micro propagation is an advanced technique for producing a large number of genetically uniform and pathogen free plants in limited time and space (Zobayed and Saxena, 2003). *In vitro* clonal propagation of species through tissue culture has been frequently based on the successful adjustment of the type and combinations of plant growth hormones (Murashige, 1990; Uranbey et al., 2005).

Germination of seeds of *A. annua anamed*, started at the 5th to 7th day of seed inoculation, and 80 to 90% mature seeds produced full plantlets. It was observed that addition of growth hormones to culture medium did not necessarily shorten the duration or increase percentage of germination of *A. annua anamed* as reports have shown that germination can occur at about 6th to 7th day when grown under field conditions (Info net biodivision, 2010). This does not conform with other studies like that of Nikolic et al. (2006), who reported that different hormones and hormonal concentrations on seeds of *Lotus coniculatus* stimulated the percentage of seed germination at least two fold in optimum concentrations. Results of this study however demonstrated that the growth response of germinating plantlets of *anamed* was apparently influenced by the combination of hormones used in the experiment; however, the variation in hormonal concentrations is small.

At 16 weeks, cultures were harvested and examined. Well developed single shoots were recorded for most treatments with the exception of BA (0.025 mg/L)/NAA (0.01 mg/L) and BA (0.10 mg/L)/NAA (0.01 mg/L) (Table 1). It was also observed that plantlet growth on hormone free MS was estimable; nevertheless, the addition of plant growth regulators (PGRs), still had significant effect on the growth of *in vitro* cultures. The mean shoot and root lengths of plantlets on MS fortified with kinetin increased as concentration level increased. Moreover, the combination of 0.1 mg/L kinetin and 0.0 1mg/L NAA was considered optimal for elongation (Table 1). Furthermore, on all kinetin treatments, stems appeared thicker and more elongated with lesser number of nodes.

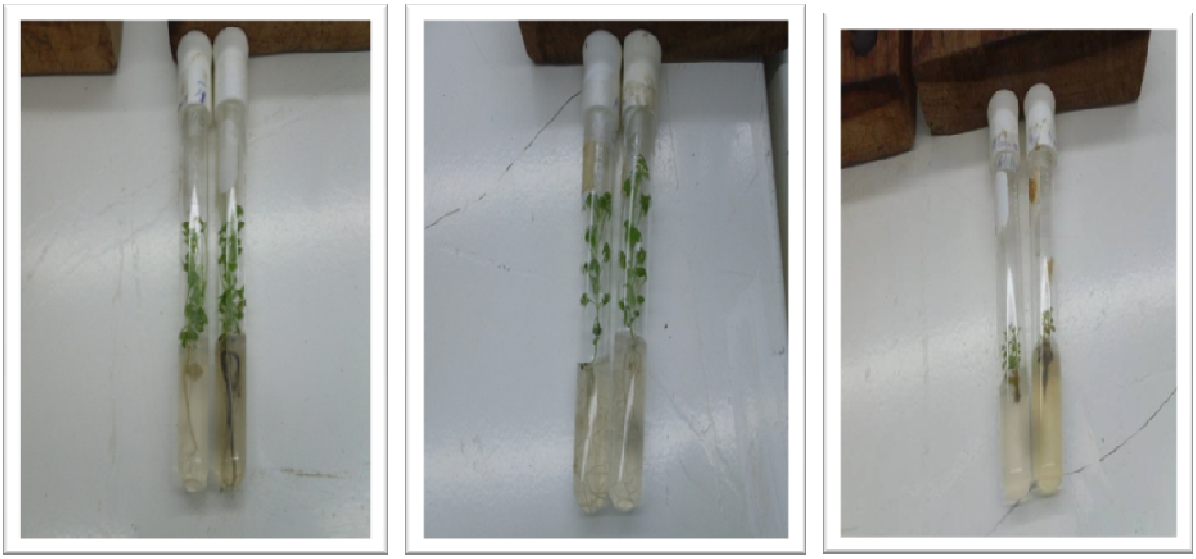
It was experiential that cultures on auxin free medium supplemented with smaller amounts of BA, showed better growth response with regards to number of nodes. Moreover, the highest node number in the whole experimental design was recorded for 0.025 mg/l BA without auxin, indicating that BA can be superior to kinetin in the provision of more nodals for subsequent mass propagation. Roots on this media also appeared thicker than all other treatments (Figure 1b).

Response of cultures to the different media protocols agrees with the work of Mahmoodzadeh et al. (2010), who investigated the effect of hormones and sucrose level on *Zinnia elegans* plants *in vitro*, proving that it is possible to improve the production of plantlets from seeds using different cytokinins and carbon sources. It also confirms the study conducted by Rashmi et al. (2010) in which concentration of various PGRs increased the root and shoot length of plantlets germinating from bamboo seeds as compared to control. If properly planned, growth regulator can bring in very quick, rapid and distinctive changes in the target plants, and show

Table 1. Growth response of *A. annua* seeds to different PGRs.

MS media	Shoot length (cm)	Root length (cm)	Number of node	Callus rating
Kin (0.025)	5.26 ± 0.15^{cd}	3.24 ± 0.34^{b}	8.80 ± 1.11^{fe}	-
Kin (0.05)	5.48 ± 0.41^{bc}	3.44 ± 0.17^{b}	9.40 ± 0.24^{fe}	-
Kin (0.025) + NAA (0.01)	5.60 ± 0.041^{bc}	3.48 ± 0.3^{b}	9.60 ± 0.4^{fe}	-
Kin (0.05) + NAA (0.01)	5.96 ± 0.5^{ab}	4.58 ± 0.24^{a}	10.60 ± 0.73^{e}	-
Kin (0.075) + NAA (0.01)	6.02 ± 0.24^{ab}	4.69 ± 0.32^{a}	12.20 ± 0.58^{dc}	-
Kin (0.10) + NAA (0.01)	6.60 ± 0.36^{a}	4.80 ± 0.45^{a}	15.60 ± 0.51^{ab}	-
BA (0.025)	5.02 ± 0.11^{de}	3.26 ± 0.37^{b}	16.20 ± 0.8^{a}	-
BA (0.05)	4.78 ± 0.12^{f}	3.10 ± 0.45^{b}	14.40 ± 0.87^{ab}	-
BA (0.025) + NAA (0.01)	2.26 ± 0.48^{f}	3.02 ± 0.23^{b}	6.60 ± 0.4^{g}	-
BA (0.05) + NAA (0.01)	4.32 ± 0.18^{e}	3.10 ± 0.1^{b}	13.80 ± 0.66^{bc}	-
BA (0.075) + NAA (0.01)	3.18 ± 0.24^{f}	2.74 ± 0.13^{b}	8.40 ± 0.51^{gf}	+
BA (0.10) + NAA (0.01)	2.50 ± 0.29^{f}	1.22 ± 0.07^{c}	$6.40 \pm 0..51^{g}$	++
No hormone	5.06 ± 0.33^{cd}	3.32 ± 0.6^{b}	10.0 ± 1.26^{f}	-

In each column, the mean values with different superscripts are significantly different ($P < 0.05$) according to DMRT test.
The value of each concentration consisted of mean± S.E of 5 replications.



a **b** **c**

Figure 1. Plantlets growing on MS supplemented with 0.025 mg/L BA (a), 0.1 mg/L kinetin and 0.01 mg/L NAA (b) and 0.1 mg/L BA and 0.01 mg/L NAA (c).

appreciable improvement with high commercial and aesthetic value; which no other technology can offer in such a short span of time (Dorsen, 2009).

High concentrations were not suitable for germination of plantlets from seeds *in vitro* for *A. annua anamed*. From seeds, multiple shoots were not produced within the range of experiment, Moreover, an increase in BA to 0.075 mg/L led to the formation of callus on roots and at 0.1 mg/L BA/0.01mg/L NAA, shoots had become stunted, and roots were almost nonexistent. Compact basal callus became more prominent without emergence of any new shoots. In another study on *A. annua*, callus was induced on medium containing Murashige and Skoog inorganic salts and Gamborg B5 vitamins, as a basal medium and was maximal at 1.0 mg/L 2,4-D and 0.1 mg/L kinetin (Ehlag et al.,1991).

Conclusion

It has been proven in several studies including this present one that plant growth regulators exerts far reaching effects on plant growth, the precise action depends on the concentrations of the substances present

and the sensitivity of the concerned organ. *In vitro* seed culture is a method for producing improved regenerants under controlled condition, especially under conditions where seeds are scarce and expensive to purchase. Conservation and multiplication can however be more easily carried out on plantlets grown *in vitro* through seed culture by circumventing problems of exposure to disease and contaminants usually associated with plants grown under field conditions.

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