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Full Length Research Article

MICRO PROPAGATION OF WORMWOOD (Artemisia annua L.) USING LEAF PRIMORDIA.

.Tahir, S M[1], Usman I S[2], Katung, M D[2] & Ishiyaku, M F[2]

[1]Department of Biological Sciences, Kaduna State University, Kaduna.

[2]Plant Science Department, Ahmadu Bello University, Zaria.

Corresponding Author's Email: smtahir@kasu.edu.ng or stahir1990@gmail.com

ABSTRACT

A study was carried out to determine the effect of varying concentrations of some plant growth hormones on the in vitro propagation of Artemisia annua from leaf primordial in the Biotechnology Laboratory of Plant Science Department of Ahmadu Bello University, Zaria. Leaf primordial from a Chinyong variety were sterilized, excised and inoculated on a full and half strengths Murashige & Skoog basal media supplemented with different concentrations of GA3, BAP and NAA. Highest regeneration percent was observed using full strength MS media supplemented with a combination of 1.5µm/l GA3 and 0.5µm/l BAP. However, a combination of 0.5µm/l GA3 and 0.5µm/l NAA had the fewer days to regeneration. Highest height was observed at 1.0µm/l GA3 and 0.5µm/l BAP. Similarly, 2.0µm/l GA3 and 0.5µm/l BAP followed by 0.5µm/l GA3 and 0.1µm/l BAP produced the best vigor with no response from half strength MS media. Results of Analysis of Variance indicated significant difference among the treatments compared with the control which did not respond (P≤0.05). Regeneration of Artemisia through leaf primordial provides a biomass of leaf material needed for Artemisinin production. Therefore, this is a viable approach to the supply of the raw materials needed for the production of anti malaria drugs for the fight against malaria fever.

Key Words: Artemisia annua, Plant growth hormones, in vitro propagation, leaf primordial

INTRODUCTION

Artemisia annua L (Wormwood) is a member of the family Asteraceae, (Compositae). The family is characterized by extreme bitterness of all parts of the plant (Tripathi et al 2000; Tripathi et al., 2001; Ferreira and Janick 2009 Its cultivation has expanded from its centre of origin (China) to Nigeria in response to the call by the World Health Organization for the use of Artemisinin-Combination Therapies (ACT) for treating malaria fever. Ferreira et al., 2005; Brisibe 2006). Three common derivatives found in Artemisia include - Artesunate, Artemether and Artemisinin. (Bennett et al. 1982; El-haq et a.,l 1991; Jaime and Da silver 2003) Cytokinins such as BAP were observed to influence a diversity of responses when applied to plant tissues, or organs. Consequently, it has been used for the induction of organogenesis in several plants (Baskaran and Jayabalan 2005).

It was also reported to influence a multitude of morphological and physiological processes, including cell division and elongation, and the morphological appearance of cultured tissues. (Mannan, et al 2012). Similarly, the effect of BAP on stimulating shoot formation was reported for other species from the genus Artemisia (Holobiuc and Blindu, 2007; Zia et al., 2007) Cultivation of most Plants may be restricted due to geographical, conservative agricultural practices (Hamish 1998; Trigiano and Gray 2000). and devastating decline in the natural habitats which expose them to the threshold of extinction. Increased resistance to anti malarial drugs such as Chloroquine has spread rapidly, undermining malaria control measure (Rinaldi 2004; Mueller et a.,l 2004) Consequently, the only safe treatment for malaria to which the Plasmodium parasite is yet to develop resistance is artemisinin based combination therapy (ACT). These constraints may be overcome through the use of in vitro facilities using tissue culture technique (Trigiano and Gray; 2000). The aim of this project was to investigate the effect of different concentrations of some growth hormones on the micro regeneration of Artemisia using leaf primordial explants.

MATERIALS AND METHODS

Study Area.

The experiments were conducted in the Biotechnology Laboratory of Plant Science Department, Ahmadu Bello University, Zaria, latitude 11 º 11 N and 07º38 E, altitude 670 m above mean sea level, 640 km from the Atlantic shores of Nigeria in the south.

Materials

Leaf primordial of Chinyong variety were sourced, sterilized and inoculated into Murashige & Skoog (MS) basal media supplemented with varying combined concentrations of Gibberellic acid (GA3), Benzyle amino purine (BAP) and Naphtyle acetic acid (NAA) using the procedure of Hamish and Sue 1998. After inoculation, the in vitro cultures were kept under inflorescent light on the growth chamber.

Parameters studied.

The following parameters were studied:

i. Days to regeneration was determined by No of Days to germinate per seed
Total No of seedlings (Fatimah, 2003)

ii. % regeneration was calculated according to Wiese and Binning (1987) where Gr = (number germinating since n-1)/ n. Where: Gr = germination (regeneration) rate; n = the days of incubation.

iii. Explant's vigor was determined based on morphological appearance, seedling emergence and early percentage germination adopting the procedure of (Gibson,1980). A scale of 1-5 was used where 1= very high vigor and 5=very low vigor.

iv. Plant height was determined by spreading a thread against the length of a plantlet in the test tube after

which it was placed on a measuring tape to measure its height.

Treatments
Explants were inoculated on a full and half strengths Murashige and Skoog (MS) basal media supplemented with varying concentrations of Gibberellic acid (GA3), Benzyle amino purine (BAP) and Naphthyle acetic acid (NAA). A completely randomized design was used for the experiment. Fifteen explants were inoculated per treatment with two replications (Table I).

Table I: Hormone composition for the regeneration of Seed explants (Full & Half strengths MS)

Hormone	**Concentration** $(\mu m / l)$											
GA3/BAP												
GA3	0.5	1.0	1.5	2.0	2.5	3.0	0.5	1.0	1.5	2.0	2.5	3.0
BAP	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0
BAP/NAA												
BAP	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0
NAA	0.5	1.0	1.5	2.0	2.5	3.0	0.5	1.0	1.5	2.0	2.5	3.0
GA3/NAA												
GA3	0.5	1.0	1.5	2.0	2.5	3.0	0.5	1.0	1.5	2.0	2.5	3.0
NAA	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0
control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Analysis of Data.
The data was analyzed using Analysis of Variance (ANOVA), SAS (2002) statistical package. Least Significant difference(LSD) was also used to compare treatment means ($P < 0.05$).

RESULTS
Green, aromatic and deeply dissected leaf materials were regenerated using leaf primordial excised from 70 days old A. annua inoculated on both a full (FS) and half (HS) strengths MS media supplemented with varying concentrations of some plant growth regulators (Plates I-II). Plantlets were sub cultured twice and transferred into elongation media and monitored for growth and development.(Plate III)

Highest regeneration percent (88%) was observed using full strength MS media supplemented with a combination of 1.5µm/l GA3 and 0.5µm/l BAP (Fig.3). Fewer days to regeneration (3days) was produced by combination of 0.5µm/l GA3 and 0.5µm/l NAA (Fig.2).

Highest height (8.5cm) was observed at 1.0µm/l GA3 and 0.5µm/l BAP (Fig.4). Similarly, a qualitative morphology was obtained with 2.0µm/l GA3 and 0.5µm/l BAP followed by 0.5µm/l GA3 and 0.1µm/l BAP (Fig.1). Results of Analysis of Variance indicated significant difference among the treatments compared with the control and the half strength media which did not show any response ($P \leq 0.05$).

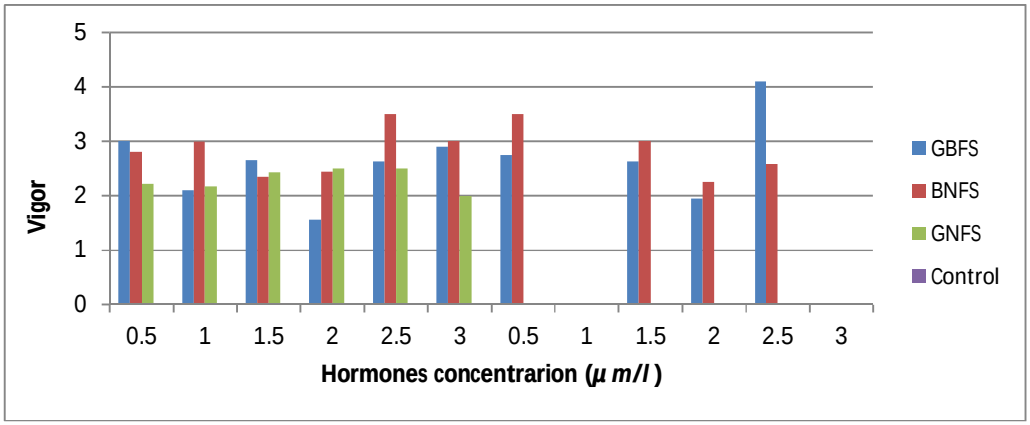


Figure 1 Response to treatment in relation to vigor.

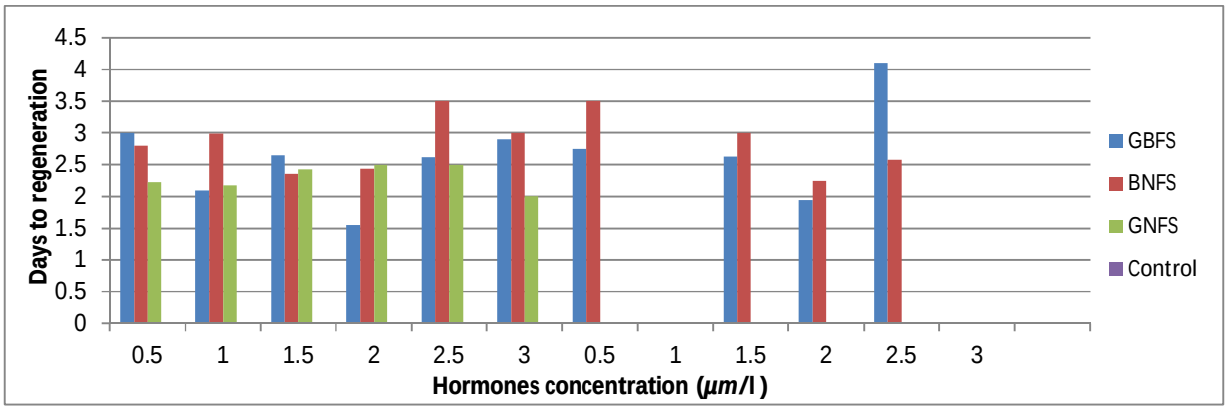


Figure 2 Response to treatment with respect to days to regeneration

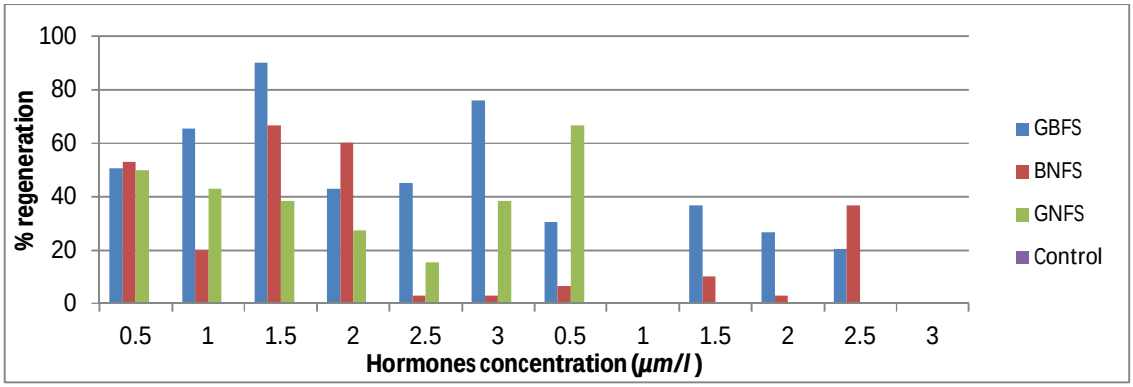


Figure 3 Response to treatment with respect to regeneration percent.

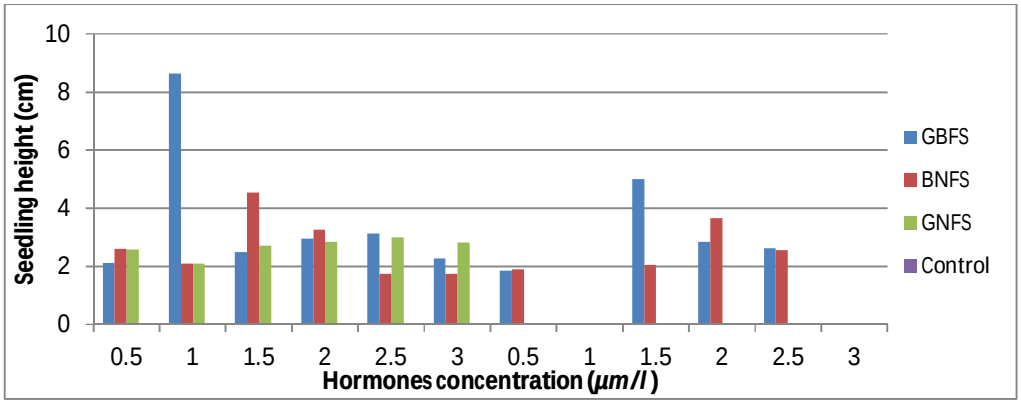
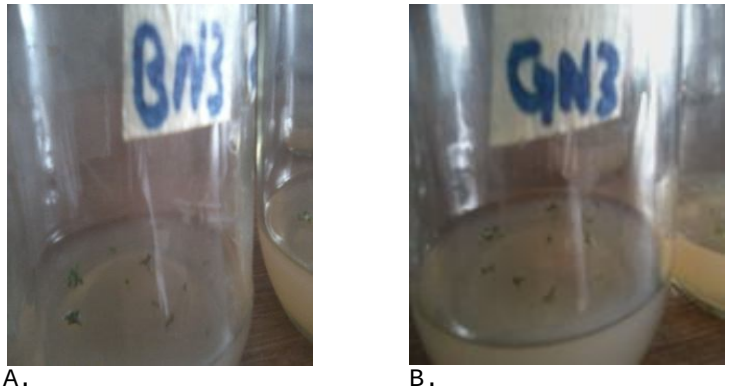


Figure 4 Response to treatment with respect to height.



A. B.

Plate I: Fresh inoculation of explants in two different media.
A= BAP + NAA Hormones media. B = GA3 + NAA Hormone media.

Plate II: Shoot formation began with several leaves in initiatiion media.

A . **B.**

Plate III. Mass of leaf materials formed in elongation media.

A= Matured leaves formed and ready for sub culture. B=Sub cultured plantlet with stem emerging.

DISCUSSION

The concentration of growth substances in an in vitro media has been manipulated by several workers to induce shoot regeneration (Razdan 2002). Leaves were found to be aromatic, deeply dissected and range from 3.0 to 8.5cm in length (Ferreira & Janick 2009). Similarly, explants isolated from younger parts of a plant were reported to be more regenerative than those from older regions and regeneration potentials of an explant in a tissue culture diminish with maturation (Razdan 2002). Apical dominance has been demonstrated to be under control of various growth regulators, therefore early regeneration within 3 days achieved at a balance between GA3 and NAA, revealed strong evidence that Gibberellic acid and Auxins balance is important in organ differentiation and its further development. It is also an indication of the role of GA3 in stimulating early growth and development of plants by promoting cell division in epical meristem and cambium tissues. (Taylor, et al. 1997). Mark, (2003) had also suggested that GA3, BAP, and NAA hormones would stimulate growth of A. annua.

The best vigor at GA3 & BAP combination is a reflection of the function of GA3 in causing the breakdown of stored starch and proteins into sugars and amino acids useful for seedlings growth (Graham et al , 2006; Taylor, et al. 1997). Also BAP was reported to enhance lateral bud growth by promoting cell division in shoot meristems, influences the development of Vascular tissues and promotes the development of shoots from undifferentiated tissues of cultured tissues (Graham, et al., 2006).

Highest regeneration percent produced by the combination of GA3 and BAP can also be attributed to the fact that shoot grown on culture media containing cytokinins develops precociously proliferating to form clusters of secondary and tertiary shoots (Razdan 2002). Likewise, GA3 has been used in the shoot proliferation media to improve shoot elongation, rate of multiplication, growth and quality of shoots (Brand and Lineberger 1992).

The in vitro cultures were kept under inflorescent light. This is therefore evident that the photosynthetic pigment in cultured tissues absorbed light thereby inducing the morphogenesis of these tissues as reported by Kato 1978 that bud induction in excised leaf segments of lily Heloniopsis orientalis was controlled by the photosynthetic system in its cells hence controlling the organogenic differentiation and growth of shoots in tissue cultures.

Conclusion

Combining GA3 with cytokinins and auxins at varying concentration is an efficient protocol for the regeneration of Artemisia annua using the leaf primordial. This pave the way to the production of Artemisinin as a raw material needed for the manufacturing of antimalarial drugs.

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