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Review

The role of synthetic growth hormones in crop multiplication and improvement

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Crop improvement through conventional methods to provide food security for the ever growing population has several limitations. Modern plant biotechnology has held promise over the years to improve outputs from plants. The use of growth hormones as a way of improving plant yield through micro propagation and somatic embryogenesis is the focus of this paper. Improved and disease resistant crops could easily be made available to farmers if the use of synthetic growth hormones for plantlet regeneration is vigorously pursued. In this technique, hormones like auxins, cytokinines and gibberellins could be made available at reduced cost to users for rapid multiplication of cultivated crops.

Key words: Crop improvement, auxins, cytokinines, gibberellins.

INTRODUCTION

The technology enhancing output from living organism has been practiced for a long period of time. Modern biotechnology involves complex systems and equipment; it is often aimed at improving the output from both plants and animals. Biotechnological approaches like genetic engineering, haploid induction or somaclonal variation to improve traits of important crops, strongly depends on an efficient recovery of plants through *in vitro* systems. Previous studies had determined that immature cells and tissues are the best types of explants for plant regeneration, especially in recalcitrant crops like monocotyledonous species (Vasil and Vasil, 1994). Anther culture and genetic engineering has been used to improve plant genetic architecture and subsequent biodiversity of the target organism. Plant regeneration protocols have been developed mostly based on immature embryos (Vasil and Vasil, 1994; Repellin et al., 2001). The successful application of these methods is determined by the genotype (Lührs and Lörz, 1987; Popelka and Altpeter, 2001), donor plant quality (Maës et al., 1996; Dahleen, 1999), developmental stage of the explant (Thomas and Scott, 1985; Maës et al., 1996) and composition of the culture medium (Lührs and Lörz, 1987; Barro et al., 1999; Dahleen and Bregitzer, 2002).

In Nigeria, agriculture is growing in terms of government commitment to ensuring food security and the output gotten from the production fields. Conventional breeding methods are very slow and the results are attended with uncertainty, this has tied down the number of improve cultivars available to farmers. Traditional cultivars are low yielding and are photoperiod dependent. Rapid multiplication of cultivated plants to meet growing need of food demand is an urgent step that needs to be pursued. These cultivars will provide high yielding materials and immediate availability of cultivated crops to farmers and ensure food security. Therefore, synthetic growth hormones which can be easily purchased in chemical stores could be used to multiply plants which will improve yield and biodiversity. Under this arrangement, any part of the plant could be assessed for multiplication. Notably, disease free plants can be produced.

Abbreviations: PGRs, Plant growth regulators; **IAA,** indole butyric acid; **NAA,** naphthalene acetic acid; **BAP,** 6-benzylamino purine; **2,4-D,** 2,4-dichlorophenoxy acetic acid; **2,4,5-T,** 2,4,5-trichlorophenoxyacetic acid; **PBA,** pyranylbenzyladenine; **ABA,** abscisic acid.

AGRICULTURE BIOTECHNOLOGY AND MODERN CROP IMPROVEMENT

Agriculture biotechnology provides important tools for sustainable development of agriculture in recent times;

this can be of significant help in meeting the food needs of a growing and increasing urbanized population (FAO, 2001). Biotechnology could play a decisive role in agriculture because of its ability to directly modify plants, animals and agricultural processes in response to new needs (Thottappilly, 1992).

Plant tissue culture, an aspect of agriculture biotechnology provide a method for the mass clonal propagation of plants via *in vitro* regeneration, it is also a tool for their germplasm conservation as well as for reforestation and tree improvement (Raddy et al., 2001; Amoo and Ayisire, 2005). However, germinated embryo is a model system which has application in higher plant tissue, organ culture and genetic transformation, since the regeneration of specific organs may be effectively manipulated through the use of germinated embryos in conjunction with specifically controlled *in vitro* condition and exogenously applied plant growth regulators. Many problems hindering the improvement of *in vitro* plant systems are potentially removed (Jaime and Teixeira, 2003). Also, plants which are obtained through organogenesis or somatic or zygotic embryogenesis are frequently free of pathogens that might have systematically infected the mother plant (Thottapilly et al., 1992). According to Yu (1989), successful plantlet regeneration through the formation of multiple shoots from the plumule explant enabled rapid multiplication of virus free citrus varieties. These plantlets were stable and closely resembled the original parent. It was possible to multiply eight generations in a year. Each plantlet formed 8 to 10 shoots per generation. This approach can shorten the time needed to multiply a citrus variety as well as save manual labor and field space and a viable approach to the production of virus free plant. The natural occurrence of different types of variation in callus cultures has been known for a long time (Bajaj, 1989). The callus tissue on prolong culturing undergoes endomitosis, chromosome loss, polyploidy, aneuploidy, mutation and other genetic changes. Larkin and Scowcroft (1981) speculated that tissue culture may generate an environment for enhancing chromosome breakage and reunion events, thus a tissue culture cycle of the hybrid material may provide means for obtaining the genetic exchange needed between the genomes in the interspecific hybrids.

MICROPROPAGATION

Micro-propagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods (Eneobong, 2003). Micropropagation has become a reliable and routine approach for large-scale rapid plant multiplication, which is based on plant cell culture, tissue and organ culture on well-defined tissue culture media under aseptic conditions (Jain, 2007). The basic and main principal approaches are:

1. Auxiliary budding: This is the induction of adventitious buds on non-meristematic tissue.
2. Somatic embryogenesis: This is a situation where individual cultured cells or small group of cells undergo development resembling that of the zygotic embryo. The embryoids produced can be used to produce whole plants (Eneobong, 2003).

The attraction of micropropagation as an alternative to other propagation methods lies in its ability to multiply elite clonal materials very rapidly. According to Food Agriculture Organization (2001), more than 1000 plant species have been micropropagated including more than 100 forest tree species. Work done with some crop species indicated the probability of encapsulating somatic embryos to form artificial seeds, which can then be handled like conventional seeds (Clyawetal, 2003).

Micropropagation exploits the “totipotency” nature of plant cells and tissues. The explants are made to form callus under appropriate nutrient conditions and environments. Numerous clonal plants can be obtained from sub-cultured callus, which forms embryoids. These are then transferred to potted soil in nurseries where they harden and acclimatized. In this way, plant propagules can be provided for rare or threatened plant species as well as for plants with inviable or difficult to germinate seeds (Eneobong, 2003).

Tissue culture techniques can also serve as an enhancing tool in plant breeding for the rescue of defective hybrid embryo, caused by post zygotic incompatibility during crossing (Eneobong and Okonkwo, 1994). Embryo culture also adopts the nutritious and physical requirements for embryonic development to bypass seed dormancy, thus shortening the breeding cycle of seed sterility and produce micro cloning material (Hu and Wang, 1986).

MICRO PROPAGATION PROTOCOLS

Micro propagation provides an attractive alternative for large-scale propagation of plants without environmental problem. Research so far has led to the development of protocols for mass production of propagules and plants. *In vitro* regeneration via somatic embryogenesis has been demonstrated in several woody plants Tautorus et al. (1991). Esan (1997) achieved somatic embryogenesis using embryo axes of matured seeds and cotyledons of immature embryo of cocoa. Amoo and Ayisire (2005) also successfully developed somatic embryogenesis and callus induction from cotyledon explants of *Pakia biglobosa* (African locust beans). Eke et al. (2005) have successfully attempted somatic embryogenesis in date palm from the apical meristem tissues. Previous reports on date palm micro propagation through callus somatic embryo pathway have been reported (Tisserat, 1997; Letowze et al., 2000). Aboel-Nil (2002) and Puchooan (2005) have also

reported *in vitro* regeneration of lychee (*Litchi chinensis*) through the leaves by somatic embryogenesis. Balogun et al. (2004) investigated the formation of callus leading to shoot and root formation in fluted pumpkin (*Telfaria occidentalis*). Ikrein-ul-Haq (2005), equally reported callus proliferation and somatic embryogenesis in cotton (*Gossypium hirsutum*). Somatic embryogenesis has been reported on many medicinal plants (Fennel et al., 2001). Other successful protocols developed for selection over exploited medicinal plants are reported by McCarten and Vantadon (1999), McCarten and Vanstaclen (2003), Thomas and Jacob (2004) Gopi and Vastsala (2006). Food crops like maize, soybean, cassava, yam, plantain and banana have protocols that are well documented.

PLANT GROWTH REGULATORS (HORMONES)

Plant hormones are chemicals that regulate plant growth. Plant hormones are signal molecules produced at specific location in the plant and in extremely low concentrations. Hormones are naturally produced within plants, though very similar chemicals are produced by fungi and bacteria that can affect plant growth (Srivastava, 2002). Also, a large number of related chemical compounds synthesized in the laboratory that function as hormones are called plant growth regulators (PGRs).

The biosynthesis of plant hormones within plant tissues is often diffused and not always localized, and unlike animals which have two or more hearts that move fluids around the body, plants utilize simple chemical hormones that move more easily through the plants tissues. The concentration of hormones required for plant responses are very low (10^{-6} to 10^{-5} mol/l). Because of these low concentrations, it has been very difficult to study plant hormones (Srivastava, 2002). Most of the early work of plant hormones involved studying plants that were genetically deficient in one or involved the use of tissue cultured plants grown *in vitro* that were subjected to different ratios of hormones and the resultant growth are compared.

Plant hormones affect gene expression and transcription levels, cellular division and growth. It is generally accepted that there are five major classes of plant hormones, some of which are made up of different chemicals that can vary in structure from one plant to the other. Each class has positive and inhibitory functions, and they often work in tandem with each other, with varying ratios of one or more interplaying to affect growth regulators (Rost and Eliot, 1979). Hormones like cytokimins and auxins are chemicals that regulate plant growth. As such, they shape the plant and affect seed growth, time of flowering, sex of flowers and the senescence of leaves and fruits. Also, they affect the tissues that grow upward and downward, the formation of the leaf and the growth of the stem (Helgi opik and Stephen, 2005). Indole butyric acid (IAA) and naphthalene acetic acid (NAA) which is auxins are compounds that positively influence root initiation and in conjunction with cytokinins, they control growth of stems, roots, flowers and fruits (Helgi opik and Stephen, 2005). Cytokinins which include 6-benzylamino purine (BAP) and zeatin are group of chemicals that influence cell division and shoot formation. Plants need hormones at very specific times during plant growth and at specific locations; they also need to disengage the effects hormones have when they are no longer needed (Helgi opik and Stephen, 2005).

Cytokinins and auxins are very significant *in vitro* culture of higher plants. It is important to note that an auxin and or a cytokinin have to be added to a nutrient medium to obtain cell extension and/or cell division. This is completely dependent on the type of explants and the plant species. For example, explants which produce enough auxin do not need extra auxin for cell extension and/or cell division. There are also explants which produce sufficient cytokinins and also need no extra cytokinins to be added to the media. Responses of genotype to auxins and cytokinins have been reported to be genotype dependant and to some extent on medium composition (Henk et al., 1978; Fukui, 1980). The following divisions can be made with respect to growth of cells, tissues and organs:

1. Cultures that need only auxin.
2. Cultures that need only cytokinin.
3. Cultures that need both auxin and cytokinin.

Plant growth regulators are added using stock solutions. For example, a stock solution of 100 mg/ml equals 10^{-4} gml^{-1}; other lower concentrations are obtained by dilution (1 ml of this stock solution per litre gives a final concentration of 10 gml^{-1}). Sometimes, there are problems encountered when dissolving different growth regulators in water, it is recommended that auxins: IAA, IBA and NAA are obtained in the more soluble form than in k-salt form. It is therefore possible to dissolve them as acids with the help of 0.1 M KOH or NaOH. Stock solutions of IAA and kinetin should be stored in the dark since they are unstable in the light; consequently, these two growth regulators are broken down by light in the nutrient media. It has been observed that IBA, NAA and 2, 4-dichlorophenoxy acetic acid (auxins) and BAP (cytokinin) are more stable in light (Pierik, 1987).

ROLES OF SOME SPECIFIC GROWTH HORMONE

There are five main classes of plant growth regulator used in plant cell culture, namely: (1) Auxins, (2) cytokinins, (3) gibberellins, (4) abscisic acid and (5) ethylene.

Auxins

Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in

conjunction with cytokinins, they control the growth of stems, roots, flowers and fruits (Daphne et al., 2005). IAA, IBA and 2,4-dichlorophenoxy acetic acid (2,4-D) as well as picloram are often added to nutrient media. The naturally occurring auxin, IAA, is added in a concentration of 0.001 to 10 mg/ml. Pierik (1987) showed the influence of high concentration of a weak auxin compared to that of a low concentration of a strong auxin. Results showed more adventitious root formation of NAA (1 mg/l) and IAA (5 mg/l) in *Gerbera jamesonii* shoot cutting.

Auxins generally cause cell elongation and swelling of tissues. Use of 2,4-dichlorophenoxy acetic acid should be limited as much as possible since it can induce mutation. Although a wide variety of auxin such as 2,4-D, (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), NAA, IAA pichloram and decamba have been used in cereal tissue culture, 2,4-D at a concentration of 1 to 2.0 mg/l have been found to be most satisfactory for the production of calli capable of subsequent morphogenesis (Wang and Zapata, 1987). Heyser et al. (1983), however, reported the use of high concentration of auxin (20 mg/l, 2,4-D) for embryogenic callus production.

Maji et al. (2002) reported that seedlings that varied in 2,4-D medium had their length and weight reduced. As the concentration of 2,4-D increases, values of seedlings length and weight raised in them decreased. But seeds in contact with 2,4-D containing medium levels of the solution gradually increased from 0.0 to 2.0 mg/l, but declined gradually at higher levels from 5.0 to 0 mg/l. Commonly used auxins include: 2,4-Dichlorophenoxyacetic acid, 2-methoxy-3,6-dichlorobenzoic acid (dicamba), indole-3-acetic acid (IAA); indole-3-butyric acid; 2-methyl-4-chlorophenoxyacetic acid (MCPA), 1-naphthylacetic acid (NAA), 2-naphthyloxyacetic acid (NOA), 4-amino-2,5,6-trichloropicolinic acid (picloram).

Cytokinins

Cytokinins are often used to stimulate growth and development, zeatin, kinetin, BAP, 2, P and pyranylbenzyladenine (PBA) being common. They usually promote cell division, especially if added together with an auxin. In high concentration (1 to 10 mg/ml), they can induce adventitious shoot formation, but root formation is generally inhibited. They promote auxiliary shoot formation by decreasing apical dormancy (Slater et al., 2005). Commonly used cytokinins include 6-benzylaminopurine (BAP*a*), *b* [N6-(2-isopentyl) adenine] (2iP (IPA)), 6-furfurylaminopurine (Kinetin*a*), 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea (Thidiazuron*c*); *b* 4-hydroxy-3-methyl-trans-2-butenylaminopurine (Zeatin). Where, *as* is synthetic analogues, *b* is naturally occurring cytokinins and *c* a substituted phenylurea-type cytokinin.

Gibberellins

Gibberellins are group of compounds that are not generally used in the *in vitro* culture of higher plants. They appear in most cases to be non essential for *in vitro* culture. Gibberellic acid is mostly used, but it must be borne in mind that it is very heat sensitive, after autoclaving, 90% of the biological activity is lost (Pierik, 1987). In general, gibberellins induce elongation of internodes and the growth of plants or buds *in vitro*. They also break dormancy of isolated embryos or seeds. Gibberellins usually inhibit adventitious root formation as well as adventitious shoot formation (Slater et al., 2005).

Abscisic acid

Abscisic acid (ABA) inhibits cell division. It is most commonly used in plant tissue culture to promote distinct developmental pathways such as somatic embryogenesis. This class of PGR is composed of one chemical compound normally produced in the leaves of plants, originating from chloroplast, especially when plants are under stress (Tsai et al., 1997). In general, it acts as an inhibitory chemical compound that affects bud growth, seed and bud dormancy. It mediates changes within the apical meristem causing bud dormancy and the alteration of the last set of leaves into protective bud covers (Else et al., 2001). Since it was found in freshly-adscissed leaves, it was thought to play a role in the processes of natural leaf drop but further research has disproven this. In plant species from temperate parts of the world, it plays a role in leaf and seed dormancy by inhibiting growth. Without ABA, buds and seeds would start to grow during warm periods in winter and be killed when it freeze again. Since ABA dissipates slowly from the tissues and its effects take time to be offset by other plant hormones, there is a delay in physiological pathways that provide some protection from premature growth. It accumulates within seeds during fruit maturation, preventing seed germination within the fruit, or seed germination before winter.

Ethylene

Ethylene is a gaseous, naturally occurring, plant growth regulator most commonly associated with controlling fruit ripening in climacteric fruits, and its use in plant tissue culture is not widespread. It does, though, present a particular problem for plant tissue culture. Some plant cell cultures produce ethylene, which, if it builds up sufficiently, can inhibit the growth and development of the culture. The type of culture vessel used and its means of closure affect the gaseous exchange between the culture vessel and the outside atmosphere and thus, the levels of ethylene present in the culture.

CONCLUSION

One of the practical approaches to enhance the multiplication of plants through the use of growth horm-

ones is for the government to make the products available to many institutions and setting a simple laboratory where somatic embryogenesis and anther culture activities will be carried out. Crops like bananas, plantain, ornamentals and tree crops can be multiplied and these will be made available for farmers. Synthetic plant hormones or PGRs are commonly used in a number of different techniques involving plant propagation from cuttings, grafting, micro-propagation and tissue culture.

The propagation of plants by cuttings of fully-developed leaves, stems or roots is performed by gardeners utilizing auxin as a rooting compound applied to the cut surface; the auxins are taken into the plant, and promote root initiation. In grafting, auxin promotes callus tissue formation, which joins the surfaces of the graft together. In micropropagation, different PGRs are used to promote multiplication and then rooting of new plantlets.

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