Benzyl adenine in plant tissue culture- succinct analysis of the overall influence in soybean [*Glycine max* (L.) Merrill.] seed and shoot culture establishment

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Received: December 27, 2019; accepted: February 3, 2020.

Cytokinins like benzyl adenine (BA) serve many principal functions in plant development and morphogenesis. BA is involved in the regulation of cell division, adventitious shoot formation, and the reduction of apical dominance, including root formation. It retards the senescence of leaves, promotes flowering and fruiting. Apart from the positive progress so far achieved, excessive amounts of BA have also proved detrimental, and caused irreversible changes on seedlings and explants grown through plant tissue culture. Therefore, this review paper analyzed literatures to evaluate the role of BA in plant tissue culture and its effects on soybean seed and shoot culture establishment. The analyses have clearly confirmed that BA continues to be a key role player on promoting the growth of stout seedlings during seed germination and explant preparation, and for the formation of desired adventitious multiple shoots during *in vitro* regeneration of soybean.

Keywords: Benzyl adenine; cytokinins; cotyledonary explants; seed germination; shoot regeneration; soybean; plant tissue culture.

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Introduction

Cytokinins are a group of plant growth hormones that stimulates cell division, cell differentiation, shoot initiation, and reproductive growth in many plants [1]. These hormones are also known as the classic plant growth regulators (PGRs) that constitute an adenine derivative carrying a variable side chain at the sixth nitrogen position of the purine nitrogenous base [1, 2]. Within this major group, there are different types of cytokinins that can influence the physiology, growth, and development of plants, which are the adenine-type represented by kinetin (KIN), benzyl adenine (BA), and zeatin (ZT), as well as the phenylurea-type represented by 1,3-diphenylurea (DPU) and Thidiazuron (TDZ). Other major groups of PGRs include auxins, abscisic acid (ABA), brassinosteroids (BR), ethylene (ETH), gibberellins (GA), jasmonates (JA), salicylic acid (SA), and strigolactones (SL) [2]. As key soluble glycoproteins, cytokinins are synthesized usually in the roots and transported to distant sites of function where they operate at low concentrations. Amongst these hormones, benzyl adenine (BA), also known as 6-benzyl aminopurine remains the most widely used PGR for the induction of cell division and shoot differentiation in plant tissue culture [3]. This is mainly by far, the only plant growth stimulator in which its biological functions have been intensively studied. The application of BA, sometimes in combination with other growth regulators, in many plant species have been reported. The growth characteristics of many plants, including a range of pulse leguminous

species like cowpea, chickpeas, dry bean, and soybean were also reported to be influenced by BA [4].

In soybean [*Glycine max* (L.) Merrill.], the role of BA on seed germination, callus and shoot regeneration has been broadly explored. Such investigations are necessary, because soybean offers high quality and quantity of proteins (41 %), oil (24 %), carbohydrates (35 %), unsaturated fats (81 %), has no cholesterol, and remains one of the most important leguminous crops grown in the tropical and subtropical regions all over the world [5, 6]. Soybean is considered the first most important oilseed crop amongst the entire group of legumes, and one of the best amidst the grain food crops [6]. Many researchers have reported the efficient use of BA in order to optimally establish soybean regeneration system, especially to serve as a prerequisite protocol for soybean genetic transformation. For example, Begum *et al*. [7] reported *in vitro* plant regeneration in soybean variety BARI-5 using both direct and indirect organogenesis from Murashige and Skoog (MS) [8] medium supplemented with different concentrations of BA. Raza *et al*. [9] also reported the production of adventitious shoots that ranged between 2.6-10.5 shoots per explant, with 50-100% regeneration rate, using half split hypocotyl and cotyledonary explants among all tested genotypes, in MS medium containing 1.67 mg/L BA in soybean. Other related studies that achieved key insights into the use of BA and other cytokinins in soybean regeneration include those of Paz *et al*. [10], Ma and Wu [11], Phat *et al*. [12], Liu *et al*. [13], and Mangena and Mokwala [14].

However, as indicated by Savelieva *et al*. [3], PGRs including BA, are not perfect plant growth stimulators, since they also exert several negative effects upon plant materials subcultured on culture medium containing these hormones for direct and indirect establishment of *in vitro* seed and shoot cultures. Therefore, the aim of this review was to provide a succinct assessment of the effects that BA has on *in vitro* seed germination and shoot regeneration in soybean. Primarily, the objective of this study was to evaluate the literatures and various data sources in order to highlight the plant tissue culture barriers and benefits of using BA. Furthermore, emphasizing the lack of attention that still need to be given to the application of this plant growth regulator in developing fertile plants that show tolerance or resistance to biotic and abiotic stress. Although, this hormone remains one of the most successfully used and reported PGR, either alone or in combination with other growth regulators (cytokinins, gibberellins or auxins), its role on soybean growth and culture establishment still need to be interrogated, especially during seed germination and shoot induction.

Cytokinin structure and benzyl adenine (BA)

Many types of *in vitro* cultures have become popular since 1904 following Hannig's developed protocol for embryo culture establishment using species of *Brassicaceae*, also known as Cruciferae. Then, a German plant physiologist, Haberlandt, presumed the presence of plant hormones from potato tubers in 1913. Later, between 1940s and 1950s, various plant tissue culture techniques were invented using a variety of supplements like coconut milk and yeast extract. These substances were also given as supplements to establish callus culture of carrot and tobacco roots. Subsequently, this led to the discovery of a potent diffusible substance that was named kinetin. This diffusible potent substance was observed to promote cell division of tobacco pith explants [1, 15]. A number of naturally occurring and synthetic growth stimulators were then later discovered. These metabolites, currently known as cytokinins, are structurally similar and are derived from N^6-substitued adenine derivatives belonging to a class of hormones capable of influencing plant growth and development. Some examples of such compounds, including BA cytokinin, are illustrated on Figure 1. They originate from the interconvertible pool of free bases, nucleotides, nucleoside, glycosides, and other reduced forms

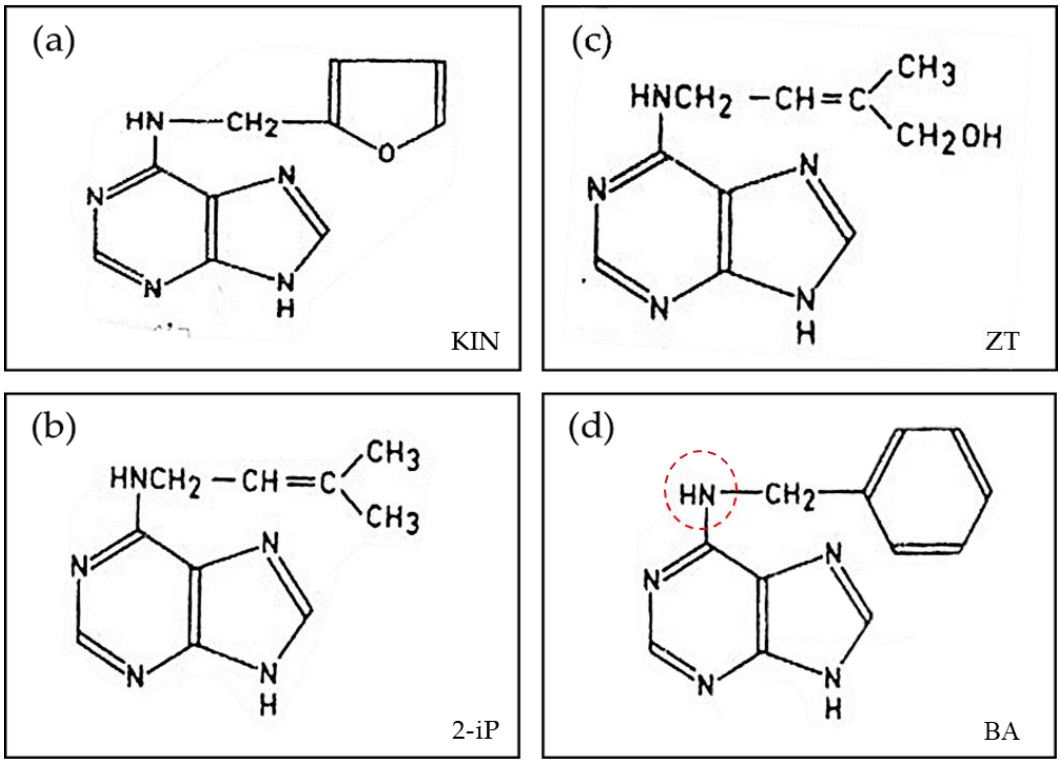

Figure 1. Structural formulae of the commonly used cytokinins: (a) kinetin; (b) Zeatin; (c) 2-isopentenyladenine, and (d) benzyl adenine. Designated abbreviations KIN (kinetin), ZT (zeatin), 2-iP (2-isopentenyladenine), and BA (benzyl adenine) are provided as described by Kaminek *et al*. [18] with slight modification for Zeatin. The N^6 position on BA purine ring is indicated with a dashed circle.

of organic compounds found in the cell protoplasts [16]. These compounds were further discovered because they proved to have a definite role to play in plant tissue culture and are continuously used to achieve desired *in vivo* or *in vitro* culture results of enormous importance for forestry, agriculture, and horticulture.

BA is one of the well-known cytokinins, which serves as a natural or synthetic plant hormone that induces cell division and often adventitious shoot buds. It achieves these meanwhile causing inhibition of adventitious root formation and reduction in apical dominance. However, the reduction in apical dominance usually promotes axillary shoot formation [17]. This hormone and its associate conjugates have long showed commitment to shoot organogenesis. Auer and Cohen [16] investigated the patterns of BA uptake and metabolism during shoot organogenesis in relation to the developmental commitment for shoot production. The study showed that BA is rapidly converted to a variety of conjugates including benzyladenosine-5-monophasphate, bezyladenosine-5-diphosphate, and benzyladenosine-5-triphosphate that contribute to a pool of BA metabolites required for shoot organogenesis and cell growths. Two *Petunia* lines MD1 and St40 contained 25% and 39% of these compounds, respectively, which were associated with early commitment to 100% shoot formation (between 6 and 10 d of exposure to BA) [16]. The biological role of BA is attributed to the presence of a ribose sugar attached to the 9^{th} nitrogen of the purine ring (riboside) or ribose moiety containing a phosphate group (ribotide). However, recent reports indicated that, the existing structural differences in their forms affect the biological activity and functions of the growth regulators. This case remains the same for the *cis* or *trans* structural forms of zeatin, which is another type of cytokinin, although the

precise role of the various existing forms remains unclear [18, 19].

Biological functions and potency of BA in plant tissue culture

The use of BA in plant tissue culture was first reported more than five decades ago. Amongst the reported studies, Sachs and Thimann [20] reported the stimulatory effect of this plant hormone on axillary bud formation in peas. In another study, Black and Osborne [21] reported that BA, especially in combination with indole-3-acetic acid (IAA), has caused increased upward movement of nutrients towards the stimulation of axillary shoot growths, also in peas. Recently, Han *et al*. [22] achieved a regeneration efficiency of about 80.6% from cotyledonary explants of *Lagenaria siceraria* using culture medium containing BA. Aygun and Dumanoglu [23] achieved over 80% of shoot proliferation per explant on MS medium containing 9.0 µM BA and 0.5 µM IAA. This was established from shoot-tip culture of *Pyrus elaeagrifolia* Pallas. In addition, the shoot tips of *Gardenia jasminoides* Ellis were also successfully propagated using B5 agar medium supplemented with the various concentrations of BA (0, 2.5, 5, 7.5, and 10 mg/L). These outcomes clearly display how BA has been and continues to be used in eliciting plant growth and developmental responses in major and economic plant species, including findings that were also made in legumes.

Both naturally and synthetic occurring BA hormones have been discovered as very influential compounds, owing their effective biological activity and potency to the widely abundant and free N^6-substituted adenine conjugates. The metabolic and proliferative effects of free cellular endogenous and exogenously applied conjugates have been well established. Conjugates' role in plant tissue culture involving tissue proliferation and cellular expansion activities have been established, particularly on how BA regulates the ratio between shoot and root growths, how it retards tissue senescence as well as chlorophyll degradation. Commonly, a myriad of studies has showed that BA, especially in combination with any other cytokinins or auxins, can be used to achieve routine regeneration of fertile plants even in recalcitrant legumes, like soybean. Somatic embryogenesis, adventitious bud formation, callus induction, shoot induction, plant regeneration etc. under various culture systems involving BA were reported in Table 1. For example, somatic embryos were derived from immature cotyledonary explants using soybean cultivar BRAGG, IAS-5, and RS-7 [24]. *In vitro* seed germination and multiple shoot bud developments were also reported using a basal culture medium conditioned with BA. This report showed that 5-50 µM BA was used for seed germination and seedling production for use as a source of cotyledonary explants. Meanwhile, to stimulate shoot bud proliferation the medium was supplemented with 10-50 µM BA [25]. These studies showed successful recovery of whole fertile plants. However, organogenic variations in terms of the number of shoots emanated from the different concentrations of BA used, which were sometimes combined with other plant hormones.

Effect of BA on in vitro regeneration of soybean

Legumes like soybean remain recalcitrant and difficult to regenerate *in vitro*. As such, researchers continue to explore a range of potential totipotent tissue explants (mature/immature embryos, whole cotyledons, epicotyls, nodal, leaf tissues, etc.) in addition to the varied hormonal combinations and basal media in order to establish a routine protocol required for efficient *in vitro* regeneration of plants. The already existing regeneration protocols involve laborious multi-step manipulations [11] and are time consuming. Although some protocols appear highly rapid, with simplified clearly outlined procedures for *in vitro* and *ex vitro* shoot developments using diverse types of explants (Table 1), challenges still exist, particularly attributed to the

Table 1. Examples of citations on the use of BA during *in vitro* plant tissue culture in soybean [*Glycine max* (L.) Merr.] from earliest to current developments.

Established culture	Explant tissue type	Medium type	Reference
Induction of shoot buds	Cotyledonary nodes	MS	Cheng *et al*. [25]
Callus culture via embryo organogenesis	Seed embryo	MS	Barwale *et al*. [26]
Plant regeneration	Leaf tissues	MS, SH, and B_5	Wright *et al*. [27]
Shoot initiation and proliferation	Epicotyl tissues	MS	Wright *et al*. [28]
Adventitious shoot development	Cotyledons	MS	Mante *et al*. [29]
Plant regeneration	Half-seed	MS	Paz *et al*. [10]
Plant regeneration	Whole cotyledonary node explant	MSB_5	Ma and Wu [11]
Plant regeneration	Cotyledons, cotyledonary node, hypocotyls, and roots	MS	Phat *et al*. [12]
Multiple shoot induction	Single and double coty-nodes	MS	Mangena *et al*. [6]
Embryogenic regeneration	Hypocotyls	MS	Liu *et al*. [13]
Callus and embryo culture	Shoot tips and cotyledonary nodal segments	MS	Islam *et al*. [30]
Plant regeneration	Half split hypocotyls, complete hypocotyl, and cotyledonary node	B_5	Raza *et al*. [9]
In vitro micropropagation	Cotyledonary nodal segments	MS and half strength MS	Begum *et al*. [7]

Note: Basal culture media compositions as indicated on the table are Murashige and Skoog (MS), Schenk and Hildebrandt (SH), Gamborg's B_5 (B_5).

inefficiencies caused by the chosen amounts of PGRs. Pierik [17] highlighted that using too high or too low concentrations of PGRs could result in complete failure of the targeted culture establishment. Meanwhile, average amounts of hormones supplemented onto the culture media may not achieve the desired results, like vigorous embryogenesis, callus induction, shoot initiation or root growths. Thus, the sections below examine the inhibitory effects of BA on seed germination and plant regeneration during *in vitro* culture of soybean.

(1) Effect of BA on soybean seed culture establishment

In previous researches, it was indicated that there are abundant unidentified endogenous cytokinin conjugates observed in tissue extracts from many plant spp., including *Pertunia* leaf explants as previously discussed by Auer and Cohen [16]. Despite these observations, many studies have consistently reported on the positive influences that exogenously applied BA has on germination and shoot culture development. Almost all reports rarely establish the effects of endogenous BA levels or its negative consequences on germination. However, BA is known to stimulate shoot growth while inhibiting root development. These are some of the negative consequences that this hormone has on seed culture establishment. Mangena *et al*. [6] demonstrated this when soybean seeds were germinated on MS medium containing varied concentrations of BA (Figure 2). Findings indicated that BA inhibited radicle and lateral root development as expected (Figure 2a-2d). When these outcomes were analyzed, this study further showed that soybean seeds were not compatible with higher amounts of BA in a culture with or without being in combination with other growth regulators like kinetin or NAA [6, 30].

Such incompatibility was sustained and exacerbated when seeds were maintained subcultured on culture medium containing high BA levels for longer periods. As exemplified on Figure 2, prolonged subculture negatively affected the morphological and physiological quality of germinated seedlings. Soybean seedlings developed on medium containing 4.0 mg/L BA showed highly reduced or stunted epicotyls, and no developed primary root and lateral roots. Meanwhile, formation of callus cells

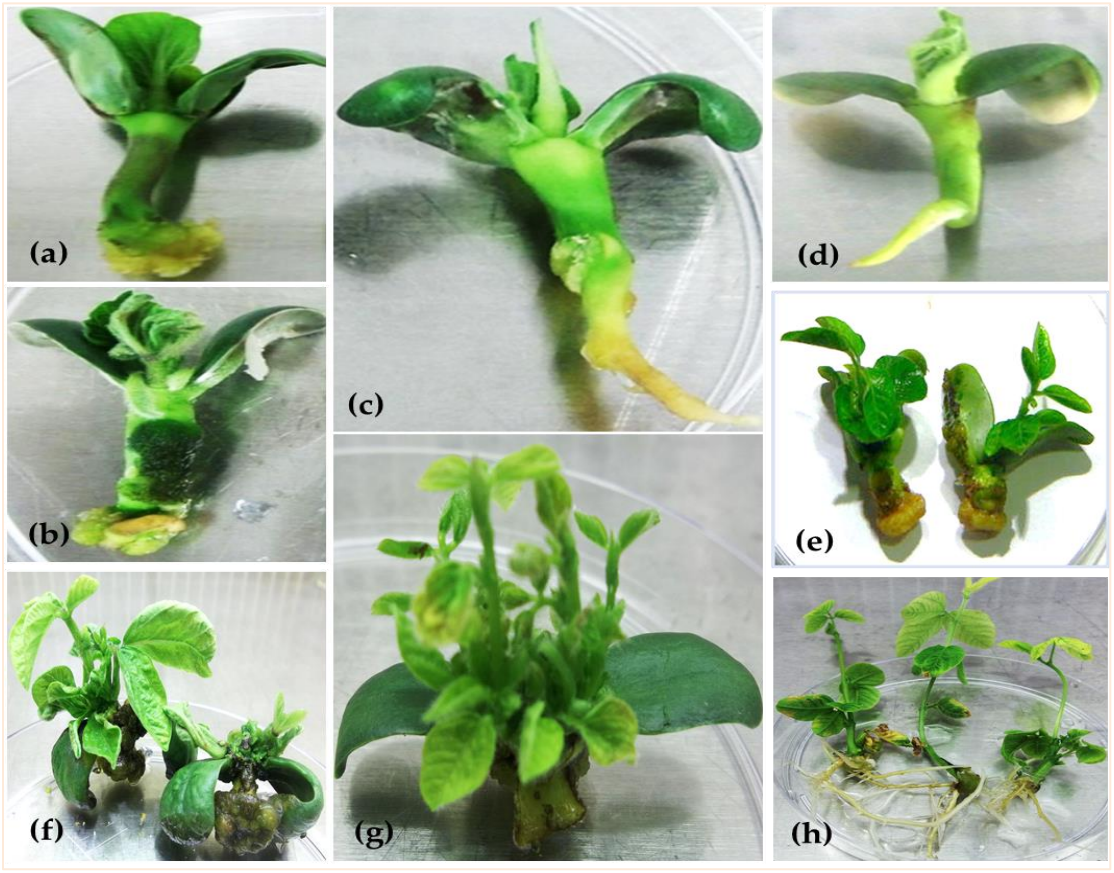

Figure 2. Morphological responses of soybean seedlings and shoots developed from MS medium supplemented with various levels of BA [6]. Seedling established from seeds germinated on medium containing increased amounts of BA (a: 3.0 mg/L, b: 4.0 mg/L) and decreased levels (c: 2.5 mg/L, d: 2.0 mg/L). Examples of adventitious shoots developed from cotyledonary node explants pre-inoculated on MS medium containing 3.0 mg/L (e), 4.0 mg/L (f), and 2.0 mg/L BA (g). Plantlets regenerated from BA modified shoot culture (h).

was rather formed at these hypocotyl bases (Figure 2a, 2b). Such similar observations were made by Cheng *et al*. [25] and Shan *et al*. [31]. Furthermore, Cheng *et al*. also showed that germination of soybean seeds in the presence of 10-50 µM BA did not only cause morphological differences among the developed seedlings but also stimulated multiple shoot bud formation. Shan *et al*. also reported significant differences in the growth and development of soybean seedlings established from seeds germinated on a medium containing BA. According to this study, seeds germinated on a culture medium supplemented with cytokinins germinated abnormally, while those germinated on hormone free MS medium were normal. The findings were consistent with Mangena *et al*.'s observation, whereby seedlings presented enlarged cotyledons, thick and short hypocotyls. In addition, with the thick and short roots which were swollen at the end with callus, and without lateral roots [31]. However, the observed morphological differences as illustrated in Figure 2a-2c may be beneficial for the production of stout seedlings that are suitably required for use as explant source [14]. The findings indicate that BA has a key role in seed germination, and as the most commonly used PGR either alone or in combination with low concentration of other cytokinin or auxins.

(2) Effect of BA on soybean shoot induction and regeneration

Other hormones, like TDZ were reported to efficiently induce multiple buds from cotyledonary node axillary meristems into shoots

in the presence of BA. The efficiency of shoot bud formation relied upon and was enhanced by supplementing the medium with BA. This follows reports by Sachs and Thimann [20], Ma and Wu [11], Paz *et al*. [10], Cheng *et al*. [25], and Islam *et al*. [30] who indicated that multiple shoot buds from cotyledonary nodes were successfully induced with the presence of BA. Moreover, findings were made that shoot induction and regeneration was improved when explants were transferred to MS medium with lesser amounts of BA. These studies have showed that BA remain widely used and has given better *in vitro* regeneration responses on soybean plantlets in comparison with any other kind of cytokinin or a hormone free medium, as well as culture medium supplemented with any other types of growth regulators. The stimulation of shoot buds remains a key role and function of BA. Early reports by Kimball *et al*. [32] showed that adventitious buds formation from soybean hypocotyl segments were successfully achieved with the use of BA in addition to other culture media additives. Although, in contrast, Yoshida [33] also reported a simple and efficient shoot induction system using 1 mm transversely cut hypocotyl sections on B_5 medium supplemented with 2-10 µM concentration of TDZ, also in soybean. It is still widely reported that vigorous shoot inductions were achieved when TDZ was combined with BA as described by Shan *et al*. [31], as well as Cheng *et al*. [25].

Most researchers who have sought to improve and optimize soybean tissue culture have done this with BA unequivocally being one of the hormones. In other plant species, Distabanjong and Geneve [34] reported the highest number of shoots (7.8 to 9.8 shoots per explant) when cotyledonary node explants were subcultured on Driver and Kuniyaki Walnut (DKW) medium containing 10 or 15 µM BA in combination with 0.5 or 1.0 µM TDZ for 20 days, than BA alone. Apart from the fact, this study used cotyledonary node explants from *in vitro* grown seedlings that showed high organogenic competencies or the shoots were formed from actively dividing cells located at the axillary bud region. This report also specifically highlighted the positive impact of the combination of TDZ and BA on wounded explants from seedlings of various ages which successfully induced multiple adventitious shoots. This was the case, although explant age (5 to 10 days old, compared to 2 days old seedlings) had negatively affected the number of shoots formed as previously observed by Kim *et al*. [35] in soybean.

A range of multiple shoot numbers achieved by culturing seedlings derived explants on DKW medium supplemented with BA in combination with TDZ was effectively high as reported by Kim *et al*. [35]. Kim *et al*. [36] also reported axillary shoot proliferation of the three *Fraxinus pennsylvanica* Marsh clones on MSB_5 medium containing 5 µM BA, 5 µM TDZ, and 1 µM IBA. BA alone or in combination with TDZ has efficiently improved multiple shoot induction and plantlets regeneration in soybean [6, 7, 10, 11, 13, 14]. Again, the efficacy of BA in regenerating multiple shoots was also demonstrated in other legumes by Polisetty *et al*. [37] in *Cicer arietinum* L., Malik and Saxena [38] in *Phaseolus acutifolius* A. *P. aureus* (L.) Wilczeck, *P. coccineus* L., and *P. wrightii* L., and Distabanjong and Geneve [34] in *Phaseolus vulgaris* L., *Pisum sativum*, *Vicia faba*, as well as *Cercis canadensis* L.

(3) Effect of BA on *in vitro* elongation and rooting of induced shoots

For shoot elongation and rooting, individual shoots are normally separated and transferred to either a basal culture medium without PGRs or a culture containing gibberellins or auxins. The auxin and gibberellin hormones are preferably used exogenously because they have been found to promote shoot elongation and rooting. Auxins are required during plant tissue culture to promote cell extension, embryogenesis, swelling of tissues and cell division for callus formation, formation of adventitious roots, as well as inhibition of the formation of axillary shoots [8, 17]. However, the absolute and relative amounts of the different growth hormones (cytokinins /auxins/gibberellin) needed for use in a culture must be thoroughly determined to avoid the failure to attain intended cultures. But these

compounds are usually required in smaller quantities to influence growth and development in *in vitro* tissue culture [39].

In vitro elongation and rooting of shoots induced on a culture media containing growth regulators may be affected by habituation. This is a phenomenon in which an *in vitro* culture that initially required a particular regulator for growth or organ formation no longer needs it, but instead becomes self-perpetuating. Christou [40] reported that habituation relative to auxins has been found to occur in many plants including *Nicotiana tabacum*, *Vitis vinifera*, *Helianthus annuus*, *Lilium longiflorum*, and *Zea mays* than encountered in cytokinins supplemented cultures. In soybean, it was reported that habituation can be induced rapidly, especially in callus tissues cultured for a longer period [40]. Another study by Kevers *et al*. [41] showed a loss of requirement of cultured plant cells for cytokinins. This report highlighted the cause of such autonomous growth to be attributed to either an increased biosynthesis of the growth substance, altered sensitivity of the cells, or the decrease in the degradation rate of the compound. Furthermore, many reports earlier speculated that habituated tissues accumulated higher amounts of auxins than cytokinins or gibberellins.

Gibberellins, however, remain well known for determining relative growth of *in vitro* cultured tissues by regulating the orientation of cortical microtubules, promoting their transverse alignment which enabled cell elongation [42]. Both gibberellins and cytokinins are still not confirmed to play a critical role in vigorously proliferating habituated plant tissues or having altered phenotype attributed to hormone imbalances than as reported for auxins. However, cytokinin-habituation was also gradually reported [39, 40], demonstrating that plant cells may become habituated based on organic compounds added on the culture media to support cell proliferation and regeneration. Furthermore, the induced effects could be reversible depending on the different level or degree of habituation [41]. In soybean cells, habituation is commonly reported to have been caused by zeatin, thiamine and its precursors than BA [39, 43].

(4) Flexibility and acclimation of plantlets regenerated from BA supplemented medium

It is widespread practice to vary the amounts of growth regulators to which plant tissues are exposed to *in vitro* in order to induce a desired morphogenesis. In addition, this may include decreasing the amounts of hormones used during *in vitro* elongation and rooting stages to avoid the formation of callus cells instead of cell expansion or inhibition of adventitious root formation. As indicated above, the inclusion of BA in this regard is rare, reduced to zero or used at very minimal quantities, and usually do not results in any significant morphological changes on the regenerated plantlets. Although it was indicated on section (3) above, both the duration and nature of the first and subsequent treatments may play a critical role in the regulation of further physiological processes and growth. So far, there are no apparent findings that attribute any efficient or inefficient flexible physiological adjustments and acclimatization to the pre-treatment of soybean explants or shoots with BA during shoot induction and regeneration stages.

George *et al*. [39] showed that morphogenic development of *in vitro* regenerated plants changed rapidly and differed significantly with *ex vitro* plant morphology, especially in their stems and roots. This study indicated that *in vitro* plantlets were generally smaller in size than their counterparts, further showing little stem collenchyma in the cortex, thicker layer of sclerenchyma that were associated with primary phloem of the stele and contained an even number of thin and thick-walled pith cells. However, endogenous cytokinins and auxins may additionally be responsible for further growth and development of all *in vitro* regenerated plants including soybean. According to Soukup *et al*. [44], the increase in various cellular growth components such as pigmented dermal and

cortical tissues, formation of numerous intercellular spaces, developed vascular system, enhanced photosynthetic rates, and even cell wall lignification occur in *in vitro* plants until they reached levels similar to plantlets grown in soil. Earlier report by Sprent [45] has indicated that BA may only has an effect by altering the distribution of nutrients required for shoot and root growths during acclimatization, which can cause branching if the amount is increased.

Role of BA on *in vitro* genetic improvement of soybean

Attempts to produce new breeding materials in soybean using a variety of plant tissue culture-based transformation protocols have been pursued. But soybean has constantly proved to be highly recalcitrant or resistant to genetic transformation. A high throughput transformation system of fertile transgenic regenerants that prove positive to selectable markers still relies on the chemical composition of the culture media used. An insufficiently optimized tissue culture media generates various challenges that include genotype-dependent protocols, formation of chimeras, low stable transgene expression, prolonged culture periods, and the unresponsiveness of explants due to bacterial overgrowth that kill proliferating tissues [10, 46, 47]. In addition to these factors, the low tissue plasticity and reduced totipotency caused by unsuitable culture conditions like the pH, light, and temperature also affect the responsiveness of cultures. However, *in vitro* cultures are still tested for soybean transformation using basal culture medium and PGRs despite the challenges mentioned above. Yan *et al*. [48], Olhoft *et al*. [49], Paz *et al*. [10], Meurer *et al*. [50], and Board and Kahlon [51] reported culture optimization in order to increase transformation efficiencies in soybean. Furthermore, BA and axillary meristematic cells found on the cotyledonary junctions are the two leading role players targeted for this purpose. The method of soybean transformation using cotyledonary explants in the presence of BA was first reported by Hinchee *et al*. [52] and is now successfully used for the transformation of other legumes. This type of cytokinin has long been used to determine the type and extent of organogenesis in Agrobacterium-mediated genetic transformation of soybean through plant cell culture. This remains the case because the recovery of transgenic soybean plants using cotyledonary node explants was attained in the presence of BA [10, 49, 52]. These studies achieved an average of 6% of the recovered shoots that were transgenic on media also supplemented with L-cysteine, dithiothreitol, and acetosyringone to reduce genotype specificity and improved bacterium-explant surface contact in most recalcitrant soybean cultivars.

Future prospects of BA in soybean tissue culture establishment

The advancement of soybean plant cell culture, particularly as a prerequisite for genetic improvement, appears to be very gradual. The regeneration and transformation frequencies so far achieved in soybean are still at very low rates compared to some of its legume counterparts, and the recent improvement observed in corn, rice, and other cereal crops via plant tissue culture. There are clear indications that there is a need for improved and efficient tissue culture protocol for soybean regeneration. Such protocol could specifically cater for the needed improvement of transformation culture conditions to promote *in vitro* recovery of transgenic soybean plants. Paz *et al*. [10] intensively examined the conditions required for soybean plant tissue culture establishment. The report recommended a culture medium optimization and standardization through the use of plant hormones like BA and other organic additives. If all the regeneration problems already highlighted in this paper are not controlled, low transformation rates will persist. This in turn will severely limit the utilization of *in vitro* tissue culture protocols for crop growth and yield improvement against environmental stress,

and prohibit measure used to facilitate genetic studies.

Cytokinin hormones, such as BA, already play and continue to serve for a very critical role in the successful micropropagation of many crop, medicinal and ornamental plant species [39]. These observations warrant more advancements to make further expansions on the use of *in vitro* plant tissue culture on micropropagation of soybean and other species. This is particularly required to produce disease-free soybean plants, and to improve genetic improvement strategies for abiotic/biotic stress tolerance as discussed previously. So far, many reported studies widely use and involve the use of BA in combination with other hormones to enhance efficiency in culture responses across all genotypes using different explant types and culture media [1, 3, 4, 6-15]. Such studies should be further expanded to establish routine protocols that may integrate several propagation or breeding approaches that may include (a) *in vivo* applications, (b) hydroponic and aeroponic systems, and (c) foliar applications, which may all continue to explore the effects of BA on growth and reproductive responses under different conditions.

Conclusions

This review showed that BA can cause and intrinsically signal morphogenesis at varied degree from plant tissues cultured on media supplemented with this hormone at different concentrations. Such outcomes are also reliant on the high plasticity and totipotency of cells, while usually influenced the genotype, age of explants, and other culture conditions. Consequently, the factors discussed above should be carefully considered and also the excessive amounts of BA may lead to abnormal plant responses (particularly, during germination and seedling development), habituation, and other developmental aberrations fostered by prolonged exposure of plant materials to such *in vitro* conditions.

Acknowledgments

The Author would like to thank the Department of Research Administration of the University of Limpopo for sponsoring the article processing charge.

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