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Evolving role of synthetic cytokinin 6-benzyl adenine for drought stress tolerance in soybean (*Glycine max* L. Merr.)

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The enhanced growth and productivity of soybeans during the past decades were possible due to the application of agrichemicals such as bio-fertilizers, chemical fertilizers, and the use of high yielding, as well as disease resistant transgenic and non-transgenic varieties. Agrichemicals applied as seed primers, plant protectants, and growth regulators, however, had a diminutive significance on growth and productivity improvements across the globe. The utilization of plant growth regulators (PGRs) for vegetative growth, reproduction and yield quality improvements remains unexplored, particularly, the use of cytokinins such as 6-benzyl adenine (6-BAP) to improve soybean response to abiotic stresses. Therefore, an understanding of the role of 6-BAP in the mediation of an array of adaptive responses that provide plants with the ability to withstand abiotic stresses must be thoroughly investigated. Such mitigative effects will play a critical role in encouraging exogenous application of plant hormones like 6-BAP as a mechanism for overcoming drought stress related effects in soybean. This paper discusses the evolving role of synthetic cytokinin 6-benzyl adenine in horticulture, especially the implications of its exogenous applications in soybean to confer tolerance to drought stress.

KEYWORDS

6-benzyl adenine, drought stress, foliar application, reactive oxygen species, leaf senescence, soybean

Introduction

Soybean (*Glycine max* L. Merr.) is one of the most important leguminous crops belonging to the Fabaceae family, and cultivated globally as a primary source of food, feed, oil, and proteins. In the past, soybean mainly constituted the crucial components of the traditional popular diets in China and other East and South-eastern Asian countries. Currently, it serves as a major industrial crop accounting for roughly more than 50% of the total oil and 40% protein (Yao et al., 2020). Soybean oil contained in seeds is mainly composed primarily of triacylglycerides, thinly dispersed quantities of mono- and di-acylglycerols, as well as significant amounts of essential fatty acids as indicated in Figure 1B. The quality of foods and feeds derived from this crop also depend on the composition of globulin (2S, 7S, 11S, and 15S) proteins (Figure 1A),

oil fatty acids (Figure 1B), carbohydrates, lipids and lesser amounts of anti-nutritional factors. Overall, soybean seeds contain 25% of carbohydrates, 20% lipids (with 95–98% triglycerides, 1.5–2.5% phospholipids, and 0.5–1.6% unsaponifiable matter), 5% crude fiber, and at least 1.5% phytic acid (Qin et al., 2017; Li et al., 2020). According to Yao et al. (2020), the unsaturated fatty acids shown in Figure 1B play a critical role in human health by regulating blood clotting, cholesterol metabolism, neurotransmission problems, and significantly contribute to the structure of membranes and membrane-phospholipids found in the brain and the retina. However, the nutritional contents in soybean may be reduced by fatty acid oxidation, including anti-nutritional factors such as phytic acid, and susceptibility of cultivated soybean varieties to various abiotic stress factors, especially water shortage or water logging. Soybeans are undergoing breeding to confer tolerance to drought and the various types of abiotic stresses that this crop has endured for decades. Many studies in genetic engineering have been conducted by introducing *Bacillus thuringiensis* (*Bt*) genes such as *Cry4Aa*, *Cry4Ba*, *Cry10Aa*, and *Cry11Aa*, encoding *Cry* toxins. Trypsin inhibitors, serine (Kunitz-type) protease inhibitors and cystatins (*Oryzacystatins I* and *II*) genes have also been investigated for abiotic and biotic stress resistance (Parmar et al., 2017; Abbas, 2018; Nair et al., 2018). Although, not as widely reported as genetic engineering, the use of agrichemicals such as primers, osmoprotectants, and plant growth regulators (PGRs) like auxins and cytokinins also offer a myriad of applications in the improvement of horticultural crops for trait and yield improvements, as well as for stress resistance. In the demand for increased cereals and pulses, including reduced pesticides use, chemical fertilizers and carbon footprint, plant modifications through exogenous application of PGRs may also increase crop yields. In view of the fact that, auxins inhibit formation of axillary shoot buds while cytokinins such as 6-BAP efficiently regulate bud proliferation, shoot branching, flowering, fruiting and tissue/organ senescence. In cereal grains, exogenous application of cytokinins contributed to the development of spikelets to mature grains after fertilization in rice. The reported cytokinin effects included increased cell number and filling in seed endosperms (Panda et al., 2018). Apart from seed setting, grain yields in cereals and other crops is determined by the number of branches on the stem, which is influenced by exogenous levels of 6-BAP used. In soybean, based on two-year field trials conducted to assess the impact of different irrigation regimes on plant growth, net photosynthesis and yield, El-Metwally et al. (2021) reported increased plant height, net assimilation rates and enhanced pod number per

plant using 100–200 mgL⁻¹ 6-BAP under low water supply conditions. This study recommended the use of 150 mgL⁻¹ 6-BAP as part of the irrigation program to compensate reduced water supply of up to 80% of crop evapotranspiration in soybean. Other studies that have exploited 6-BAP due to its crucial role in soybean plant growth and development include Abdallah (2020), Mangena (2020), Ibrahim et al. (2021), and Amoanimaa-Dede et al. (2022).

During the past two decades, a large number of transgenic soybean lines have been developed, including those still being optimized through genome editing (CRISPR/Cas9), but only a limited number of varieties were enhanced using plant growth regulators as major role players during the crop improvement (Liu et al., 2017; Parmar et al., 2017). Although, a large body of information is widely available on the role of plant growth regulators such as cytokinins on improving root and shoot growth, biomass, antioxidant capacity, and other physiological processes. Additional work still need to be done to elucidate growth and developmental responses of plants treated with these PGRs like 6-BAP under environmentally constraining conditions. Therefore, the role of 6-BAP in the mediation of an array of adaptive responses that provide plants with the ability to withstand abiotic drought stress is discussed in this review. Such mitigative effects may also play a critical role in encouraging exogenous application of plant hormones as alternative mechanisms for overcoming drought stress related effects in soybean. In summary, this paper discusses the evolving role of 6-benzyl adenine (6-BAP) in horticulture, paying particular attention to the implications of exogenously optimizing 6-BAP levels in soybean treatments to confer tolerance to drought stress.

Effect of drought stress in soybean

Drought, together with other non-living environmental constraints are adverse abiotic stress factors affecting soybean crop production worldwide in numerous ways (Table 1). Drought stress reduces soybean's survival rates, growth, and development by interfering with complex networks of coupled anabolic and catabolic pathways that direct the flow of energy and resources within, as well as between cells. Drought stress in plants is characterized by cell dehydration, reduced water potential, altered carbon partitioning, membrane and protein destabilization, production of reactive oxygen species (ROS), and tissue senescence (Abid et al., 2018; Zhang et al., 2018; Bashir et al., 2021). Plants exposed to moderate and severe water deficit stress experiences oxidative stress, which damages protein molecules, macromolecules such as nucleic acids and lipids, subsequently increasing the number of harmful reactive oxygen species (ROS) by-products, aggregated and misfolded proteins. In crops such as soybean, cowpea (*Vigna unguiculata*), chickpea (*Cicer arietinum*), common bean

Abbreviations: ABA, Abscisic acid; 6-BAP, 6-Benzyl adenine; CaM, Calmodulin; CAT, Catalase; ET, Ethylene; GA3, Gibberellic acid; IPT, Isopentenyl transferase; JA, Jasmonic acid; PGR, Plant growth regulators; POD, Peroxidase; ROS, Reactive oxygen species; SA, Salicylic acid; SOD, Superoxide dismutase, SUMO, Small ubiquitin-like modifier proteases.

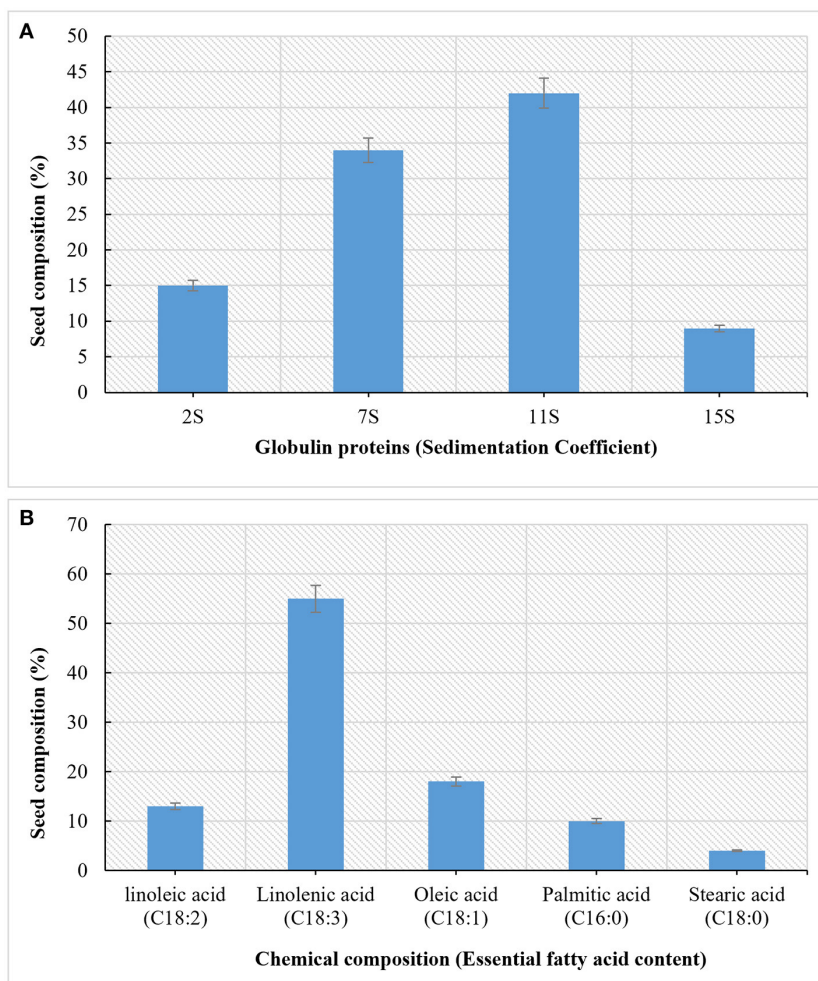


FIGURE 1
Composition of protein (A) and oil (fatty acids) (B) in soybean seeds. Data sourced from Yao et al. (2020) and Guan et al. (2021).

(*Phaseolus vulgaris*), and lentil (*Lens culinaris*), oxidative stress also rapidly induces protein damage by the activity of proteolytic enzymes which are mostly localized in the nodules, leaves, and roots.

Proteolytic enzymes, which form a diverse group of proteases capable of cleaving peptide bonds, are implicated in various essential processes including fine control of protein catabolism, selective degradation of damaged proteins during stress and bulk hydrolysis of dietary proteins (Mangena, 2020). Similarly, in monocot grains such as wheat (*Triticum aestivum* L.), small ubiquitin-like modifier proteases (SUMO) were reported to play a vital role in regulating pathway fluxes during plant growth and development under drought stress conditions. According to Le Roux et al. (2019), wheat plants transformed with a vector expression of *Arabidopsis thaliana* cysteine protease inhibitor (OVERLYTOLERANT TO SALT-1, OTS1) showed enhanced stress tolerance and delayed tissue

senescence compared to other lines of this species that only contained SUMO proteases. In soybean, drought negatively influences the various parameters shown in Table 1, in addition to causing significant changes in the shoot and root morphology, reduction of cortical tissue in stems, and causes a decrease in the number and size of flowers, fruit pods, and seeds (Du et al., 2020; Arya et al., 2021). Apart from these diminished vegetative and reproductive growth, soybeans also show serious inhibitions on the formation of cell protuberance containing nitrogen-fixing Gram-negative bacteria in the roots, discussed later in this paper. However, vegetative and reproductive soybean traits, including nodulation could be enhanced by treating seeds with cytokinins before sowing, followed by the exposure of plants to drought stress and higher temperatures as indicated by Kempster et al. (2020) and Zhang et al. (2022). These growth aspects still need to be thoroughly researched amid the criticism and challenges suffered by animals, humans and the

environment as post-impacts of using chemical pesticides and chemical fertilizers to improve crop productivity as highlighted by Isman (2019).

Role of 6-BAP in soybean

The 6-benzyl adenine, also known as 6-benzylamino purine (Figure 2), is a synthetic N⁶-substituted adenine derived from the N⁶-aromatic ring compounds (Wang et al., 2016). The conversion or rearrangement of 3-bezyladenine to N⁶-benzyl adenine involves ring opening of both the imidazole and pyrimidine rings (Figure 2A) to produce the 6-benzyl adenine presented as 3D and 2D conformational chemical structures depicted in Figures 2B,C (SpectraBase, 2022). Generally, 6-BAP regulates various aspects of cell division, apical dominance, tissue senescence and translocation of photosynthates from the source to the sinks. 6-BAP in cooperation with other cytokinins or auxins can further control fundamental growth and developmental processes, including direct/indirect somatic embryogenesis, lateral shoot formation and callus initiation under controlled *in vitro* cell culture conditions (Solorzano-Cascante et al., 2018; Srilestari et al., 2020). However, the 6-BAP levels used in plant tissue culture are generally applied at concentration ranging from 0.01 to 10.0 mgL⁻¹ to stimulate axillary and adventitious shoot proliferation, regulate cell differentiation, activate RNA synthesis, and stimulate protein/enzyme activity (Naito et al., 2006). *In vitro* growth of seeds and explants is more sensitive to exogenous application of PGRs which induce significant stimulatory effects due to increased rate of absorption and endogenous hormone content in the cells. Moreover, differences exist among *in vivo* and *ex vitro* studies in the levels of 6-BAP used, at a range of 50–500 mgL⁻¹ and 0.01–10 mgL⁻¹, respectively. Physiological characteristics and growth regulation measures, however, indicated marked concentration differences because exogenously applied PGRs cannot enter plant tissues rapidly to directly promote or inhibit growth. Booker et al. (2003) and Cai et al. (2018) reported that indole-3-acetic acid (IAA) did not directly inhibit the growth of lateral buds because IAA applied at shoot apices could not enter inside the lateral buds. Although, information on the mechanism of hormonal absorption and translocation in plant tissues has not been widely reported, many studies nevertheless, found a positive correlation between growth and enhanced exogenous levels of 6-BAP which had tremendous effects on endogenous cytokinin content (Yuan et al., 2019; Li et al., 2021; Zhang et al., 2021).

Liu and Zhang (2017), reported foliar application of exogenous 6-BAP to improve plant drought stress resistance by increasing the rate of photosynthesis, stomatal conductance, and transpiration rates, reducing yield losses up to 46% in soybean. In another study, foliar spraying with 20 mg.L⁻¹ 6-BAP improved soybean growth and yield which were

negatively influenced by maize (*Zea mays* L.) shading during maize-soybean relay strip intercropping systems (Kai et al., 2020). According to Kai et al. (2020), maize shading inhibited soybean by influencing morphological characteristics, biomass, and physiological responses of this crop, including chlorophyll content, net photosynthetic rates, and leaf thickness. Furthermore, the results indicated that the application of suitable PGRs could alleviate the detrimental effects caused by maize shading, and other growth inhibitory factors serving as both biotic and abiotic stress.

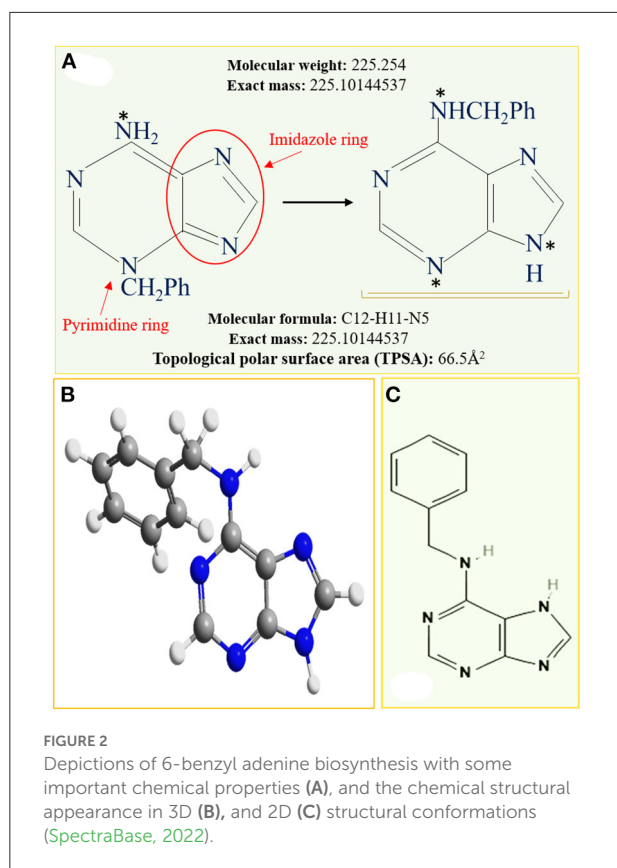
For instance, exogenous application of 6-BAP delayed the degradation of photosynthetic pigments by increasing the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) enzymes during seed filling and production in maize (Ahmad et al., 2019). Kempster et al. (2020) also reported seed priming and foliar application of cytokinins to improve yield and cold stress tolerance in soybean, cultivar DM50I17 and DM4OR16 in field trials with early (September and early November) and conventional (late November) sowing dates in Argentina. However, apart from lack of irrigation or rainfall, water deficit stress in plants can be caused by cold/chilling and salt/salinity stress. Therefore, cytokinins interactions, especially with other hormones, play a significant role in counteracting cellular dehydration and turgor pressure losses suffered by plants exposed to drought, salinity, and cold stress, especially frost (Beck et al., 2007). Although, several reports such as those highlighted above are widely interrogate these effects, only a handful of studies have emphasized the role of these growth regulators on vegetative growth, yield components and nodulation in numerous crop plants, such as leguminous pigeonpea, cowpea, and soybean.

Modulation of shoot and root architecture

Growth modulation of the root and shoot systems are among the most essential adaptations under abiotic drought stress conditions. Although, more literature is available that describe the immediate physiological and molecular pathways coordinating the changes in root and shoot growth, biomass (Figure 3) and nodulation (Figure 4), including branching because of ABA and auxin expressions in plants during their response to drought stress. Not much reported work is available regarding the role of cytokinins on growth adjustments in response to stress. Cytokinins, including 6-BAP, play a pivotal role in plant signaling and adaptation to the fluctuating availability of nutrients and water from different soil horizons. To cope with fluctuating temperatures and water availability, soybean plants integrate their systemic signals pertaining to their water status into developmental pathways that coordinate changes in the architecture of both roots and shoots. Typically, any change perceived by the plant concerning plant nutritional

TABLE 1 General biochemical and physiological growth parameters influenced by different abiotic stress factors in plants.

Stress	Parameter	Biological effects	References
Drought	Antioxidant; catalases, glutathione/ascorbate-glutathione, superoxide dismutase	Inhibit enzyme activity, and reduce antioxidant capacity levels, causing oxidative stress	Sarker and Oba (2018), Khaleghi et al. (2019), and Kapoor et al. (2020)
	Malondialdehyde (MDA)	MDA content is increased due to high ROS accumulation	Baccari et al. (2020)
	Abscisic acid (ABA), alkaloids or phenols, proline content	Increased overexpression of ROS causes increases in ABA, alkaloids/phenols and proline content serving as oxidative stress markers	Honório et al. (2021)
	Amino acids	Accumulates under drought, e.g., tryptophan, which plays a role in osmotic adjustments and ROS scavenging activity	Mansoor et al. (2022)
	Carotenoids content, Chlorophyll (a and b) content	Cause changes in pigment content by affecting chlorophyll components and damaging chlorophyll apparatus	Wang et al. (2018) and Iqbal et al. (2019)
	Sugars, inorganic ions (Na ⁺ , K ⁺ , N, Ca, Mg, Fe, Zn, Mn, and B), reactive oxygen species (ROS)	Reduces sugar content and causes alterations in metabolism, ionic balance, osmolarity, and membrane stability	Baccari et al. (2020) and Kapoor et al. (2020)
High temperatures	Amino acids	Increase protein degradation, and lowers protein synthesis	Hu et al. (2020)
	Heat shock proteins (HSP)	Apparent increase in the levels of HSPs	Kapoor et al. (2020)
	Sugars/ phenols	Decrease sugar content and increased amounts of minor phenolic acids	Kim et al. (2020)
	Chlorophyll content	Damages photosynthetic apparatus	Honório et al. (2021)
	ROS	Induces oxidative stress due to accumulation of ROS	Mansoor et al. (2022)
Salinity	Flavonoids	Increased average total flavonoid content	Vosnjak et al. (2021)
	Osmotic content, protein content	Increases osmotic stress and affect synthesis of ribosomes	You et al. (2019)
	Relative water content (RWC)	Reduce net photosynthetic rate and increased solutes and ROS accumulation	Kapoor et al. (2020)
Chilling	Cryoproteins	Activate defense proteins to protect membrane structure and delicate balance of ROS and scavenging	Beck et al. (2007)
	Sugars	Causes nutrients imbalance, restricting photosynthesis	Khaleghi et al. (2019)
	ABA, jasmonic acid (JA), Ca ²⁺	Inhibit carbon reduction cycle and induces increased levels of metabolic markers	You et al. (2019)
	ROS	Increases JA, reduces electron transport, and causes changes in protein structure and enzyme activity	Mansoor et al. (2022)



and water status, in relation to the external supply firstly modulate the root system. As reported by Giehl et al. (2013), such changes occur over time and they determine the degree of root plasticity based on the individual root system components, including root phenotype, extension, placement, and the direction of root growth.

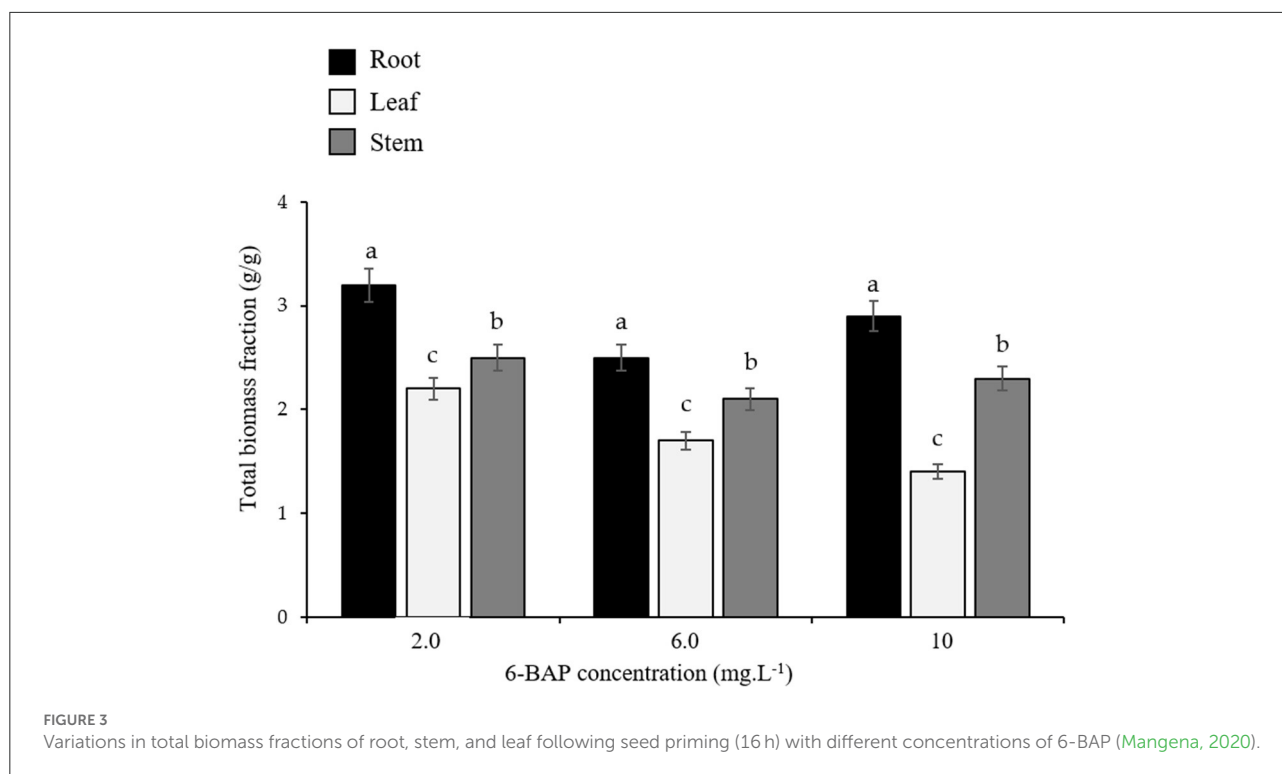
Furthermore, some organ modifications may be stress specific and acted upon by local specific signals. The role of root system modulation in soybean, like many other crop plants can be demonstrated by cell proliferation and differentiation that take place when root growth is observed in response to drought stress. In general, increased root growth biomass (like as exemplified in Figure 3 in relation to 6-BAP treatments) and depth are predominantly observed in water-stressed soybean plants. Müller et al. (2021) reported greater proportions of root growth in the first centimeters of soil-depth in response to soil water-related properties. Many studies also concurred with findings by Dong et al. (2019), Hanum and (2020), and Amoanimaa-Dede et al. (2022) who reported that drought tolerance mechanism in soybean is closely related to cytokinin activities in combination with other hormones, which closely regulate the rooting and shooting patterns. Furthermore, the lack of high-level cytokinins in plant tissues also immediately inhibit root extension because of halted cell

cycle and other cellular activities. In terms of shoots, drought affect nutrient uptake and availability to soybean plants, which completely diminishes their physiological, and biochemical growth responses as indicated previously in Table 1.

Low water content in the cells causes leaves to wilt and drop (Wang et al., 2022), have a negative impact on plant's architecture by affecting stem elongation, branching and subsequently blossoming as well as fruit production (Du et al., 2020; Arya et al., 2021). All of these growth effects appears to be modulated or regulated by cytokinins as illustrated later in Figure 5. Root and shoot growth was inhibited by the enhanced concentrations of 6-BAP (2–10 mgL⁻¹) applied through seed priming. The decrease in leaf, stem, and root biomass was inversely proportional to the increase in 6-BAP levels during a 16 h priming period of soybean seeds (Figure 3). These results confirms the inhibitory role of 6-BAP during seed germination, seedling development, and plant establishment as reported by Gu et al. (2022) in which 6-BAP concentration of 5 mgL⁻¹ and less promoted germination, while higher levels caused seedling retardation. In contrast, foliar application of 6-BAP solutions at concentration ranging between 20 and 200 mgL⁻¹ presented maximum plant establishment and net assimilation rates, together with higher yields in soybean plants subjected to induced drought stress. 6-BAP was reported to improve water use efficiency progressively with decreased irrigation and gradually increasing the amounts of the hormone (Bozso and Barna, 2021; El-Metwally et al., 2021; Amoanimaa-Dede et al., 2022; Gu et al., 2022). Therefore, more scientific evidence is required to strengthen exogenous application of 6-BAP and other synthetic and naturally occurring hormones in playing a pivotal role in the modulation of shoot and root development, especially in response to biotic and abiotic stress.

Effect of 6-BAP on lateral bud break

The control of changes in bud dormancy along stems may as well be attributed to the variations in endogenous hormonal concentrations. Generally, cessation of branching or lateral shoot growth, and frequent occurrence of abscission of both leaves and apices may result from the apical dominance, but the precise hormone regulated mechanisms still need to be elucidated. Although, the regulation of lateral bud formation is not extensively studied, numerous reports have demonstrated the role of drought on the inhibition of apical/lateral buds during shoot formation. In soybean, Dong et al. (2019) showed that drought stress inhibited increases in plant height and leaf area by halting bud activity. Other shoot related characteristics that develop from lateral buds that are affected by drought include stem diameter, leaf formation, and node density (Gao et al., 2020; Carrera et al., 2021). Stem elongation in plants is usually controlled by the intercalary meristems. However, cytokinins such as 6-BAP and kinetin play a significant role in regulating



the activities of apical meristem, lateral meristem (vascular and cork cambium) and intercalary meristem that are responsible for shoot growth (Wu et al., 2021; Yang et al., 2021).

Cytokinins influence lateral bud growth by promoting nuclear shuttling of transcriptional factors that activate mitotic gene expression (Yang et al., 2021). Under drought conditions, high cytokinin activity was reported in the veins of poplars (*Populus x canescens*), with areas of leave scars exhibiting low cytokinin activity. Other high cytokinin activities were observed in the differentiation zones of stem pith, mature phloem, and primary meristematic tissues in drought-stressed plants (Paul et al., 2017). As expected, cytokinin concentrations will be high in the bud meristematic tissues as these hormones support primary and secondary growth through bud initiation, a process predetermined to take place regardless of drought stress (Prerostova et al., 2018). Furthermore, failure of lateral buds on the shoots of soybean plants to grow into lateral branches may reduce various yield components and yield quality (Yoshihira and Shiraiwa, 2016; Xu et al., 2020).

Influence of cytokinins on soybean sex expression

Male sterility constitutes the most common type of reproductive mutations observed in soybean. Previous studies suggested that the high frequencies of male sterility reported in soybean result from the numerous genes that are involved in

mega- and micro- gametogenesis (Thu et al., 2019; Zhao et al., 2019). However, in agricultural breeding, such sex mutations are very valuable for the production of hybrid seeds. These seeds tend to perform much better compared to their normal counterparts, particularly in terms of yield quantity, quality, disease, and pest resistance, as well as tolerance to abiotic stress factors (Liu et al., 2021). The use of hybrid seeds has greater commercial value since male sterility increases the effectiveness of first filial generation (F1) hybrid seed production. This takes place without pollination and reduces production costs for many farmers (Zhao et al., 2019). Huang et al. (2003) reported the involvement of cytokinins in male reproductive development of all flowering plants. Although, elucidating the role of specific hormones on particular tissue types or developmental stages remains a daunting task due to the fact that these hormones operate in interaction/ cooperation with each other. Furthermore, no reports have been found on the role of 6-BAP on sex expressions in soybean. Seesangboon et al. (2018) reported that the application of 6-BAP on *Jatropha curcas* L. flower buds increased the number of female flowers and seed yield. This crop had initially exhibited dwindling number of female flowers which led to insufficient seed yields.

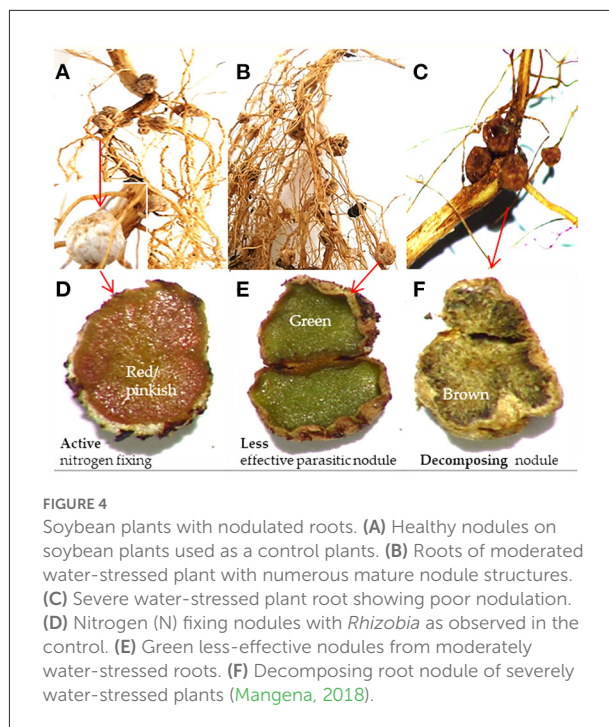
All of the abovementioned studies clearly indicated that exogenous applications of cytokinins and increased accumulations in reproductive tissues of transgenic maize and *Jatropha curcas* plants resulted in altered sex expressions. In maize plants, this further enhanced male-sterility whose restoration was also achieved by further application of this

class of PGRs. According to Huang et al. (2003), exogenous application of kinetin at 0, 20, and 100 mg restored male sterility when used with or without 3 mg thidiazuron (TDZ). The results obtained in this study, together with other uncited reports provided the evidence of direct involvement of cytokinin in sex expressions, and further showed that combined effects of multiple hormones control this phenomenon. In *Jatropha curcas* L. again, similar observations of a strong dependence on cytokinin 6-BAP on flowering, which increased the number of male and female flowers per inflorescence, were also reported by Fröschle et al. (2017). Other specific sex expressions related to traits reported in species such as *Cucumis sativus* (cucumber) and *Momordica charantia* L. (bitter melon) included improved female to male flower ratio, number of seeds per fruit, seed size, seed weight, and seed oil content (Amiriam et al., 2019; Abbas et al., 2020).

Role of 6-BAP on flowering, fruit development, and yield

The increase in crop production during the past years, until currently, were made possible by the application of agrichemicals like fertilizers and cultivation of genetically improved transgenic varieties. However, exogenous chemicals like plant growth regulators such as ethylene, jasmonic acid, ABA, GA₃, and 6-BAP had little to no use in the promotion of crop growths, especially for the enhancement of flowering, fruiting, and yield. Increments in the utilization of PGRs for reproductive growth, yield increases and stress tolerance could offer an optimistic view of the future beneficial effects of these agrichemicals to farmers. Jasmonic acid (JA) for instance, was attributed to several positive effects in response to biotic and abiotic stress. To accurately demonstrate the beneficial role of high JA levels in plant tissues, Wang et al. (2020) reported the synergistic and antagonistic effects of exogenously applied JA with ABA, ethylene (ET), salicylic acid (SA), and other synthetic hormones in the process of resisting environmental stress. Although, soybean produces a large number of flowers per plant, one of its major challenges is that the plant also experiences a great deal of flower abortions (Cho et al., 2019). Fruit and flower abortions under unfavorable water deficit conditions has been a serious problem in commercial production of soybean and other leguminous and non-legume crops. Nagel et al. (2001) indicated that exogenous application of cytokinin (6-BAP) to raceme tissues stimulated flower production and prevented abortions in soybean, SD-87001 line that has been proved to be highly sensitive to external treatment of cytokinins.

Exogenous application of 6-BAP (300 mgL⁻¹) at end of flowering stage significantly reduced flower abortion, thus increasing productivity by increasing the number of seeds per



plant, seed weight, and seed diameter (Larrisa et al., 2014). In another study, El-Metwally et al. (2021) reported enhanced pod number per plant, and increased overall yield when 100 and 150 mgL⁻¹ 6-BAP interacted with 80 and 100% of crop evapotranspiration water regimes, respectively. The findings indicated that lowering water supply up to 80% of crop evapotranspiration (saving 20% of irrigation water) could be compensated by folia spraying of plants with 6-BAP. This study provides significant insights showing that exogenous 6-BAP application could serve as a potential practice for reducing flower abortion, improving fruit and productivity on fields cultivated with soybean.

Role of 6-BAP on nodulation

All leguminous crop species, including soybean play a critical role toward sustainable agriculture and maintenance of soil fertility through a biological nitrogen fixation via a highly specialized symbiotic relationship with *Rhizobia* bacteria (Figure 4). A sophisticated signaling exchange mediated by glycopeptides and phytohormones regulate soybean root infection by *Rhizobia* and induce the formation of novel organs termed nodules by cytokinin mediated cell proliferation (Figures 4A,D). A high nitrogen supply is required to achieve high yields in soybean. Kempster et al. (2020) increased early biological nitrogen fixation and total nodule area by priming soybean seeds with 2 mL of 10⁻⁷ (high) and 10⁻⁹ (low) molL⁻¹ kinetin solution for 4 h in cultivar DM50I17 and DM40R16.

Root drenching and petiole feeding technique were also used to increase nodule number in soybean using 6-BAP and trans-zeatin (Mens et al., 2018). The *isopentenyl transferase (IPT)* genes and their homologous duplicate gene partners (*GmIPT5* and *GmIPT6*) involved in cytokinin biosynthesis for controlling nodule number were also identified in soybean genome (Ye et al., 2006). The proteins encoded by *GmIPT1* gene are responsible for the production of active cytokinins during nodulation. *IPT* genes, especially the constitutive expression of the *GmIPT* gene in roots, act as key regulator of cytokinin homeostasis and phytohormones crosstalk in soybean under both biotic and abiotic stress together with delaying senescing of nodules as indicated in Figures 4B,C,E,F (Nguyen et al., 2021). Recent research has revealed other regulatory functions of cytokinins during nodulation such as nodule cell proliferation and tissue differentiation, root system architecture and regulation of auxins expression during this process (Dolgikh et al., 2020). Therefore, both cytokinins and auxin are essential for regulating rhizobial infection and nodule organogenesis, while synthetic hormones such as 6-BAP plays a fundamental role in ensuring that specific recognition of symbiotic partners occur, initiate root cells infection by symbiont, and inception of nodules in the root cortex of all legumes, including soybeans (Gamas et al., 2017). However, more research is still required to elucidate the role of exogenous 6-BAP treatments on nodulation in soybean and other legume genotypes as reported by Mens et al. (2018), Kempster et al. (2020), and Amoanimaa-Dede et al. (2022).

Role of 6-BAP on delayed cell, tissue and organ senescence

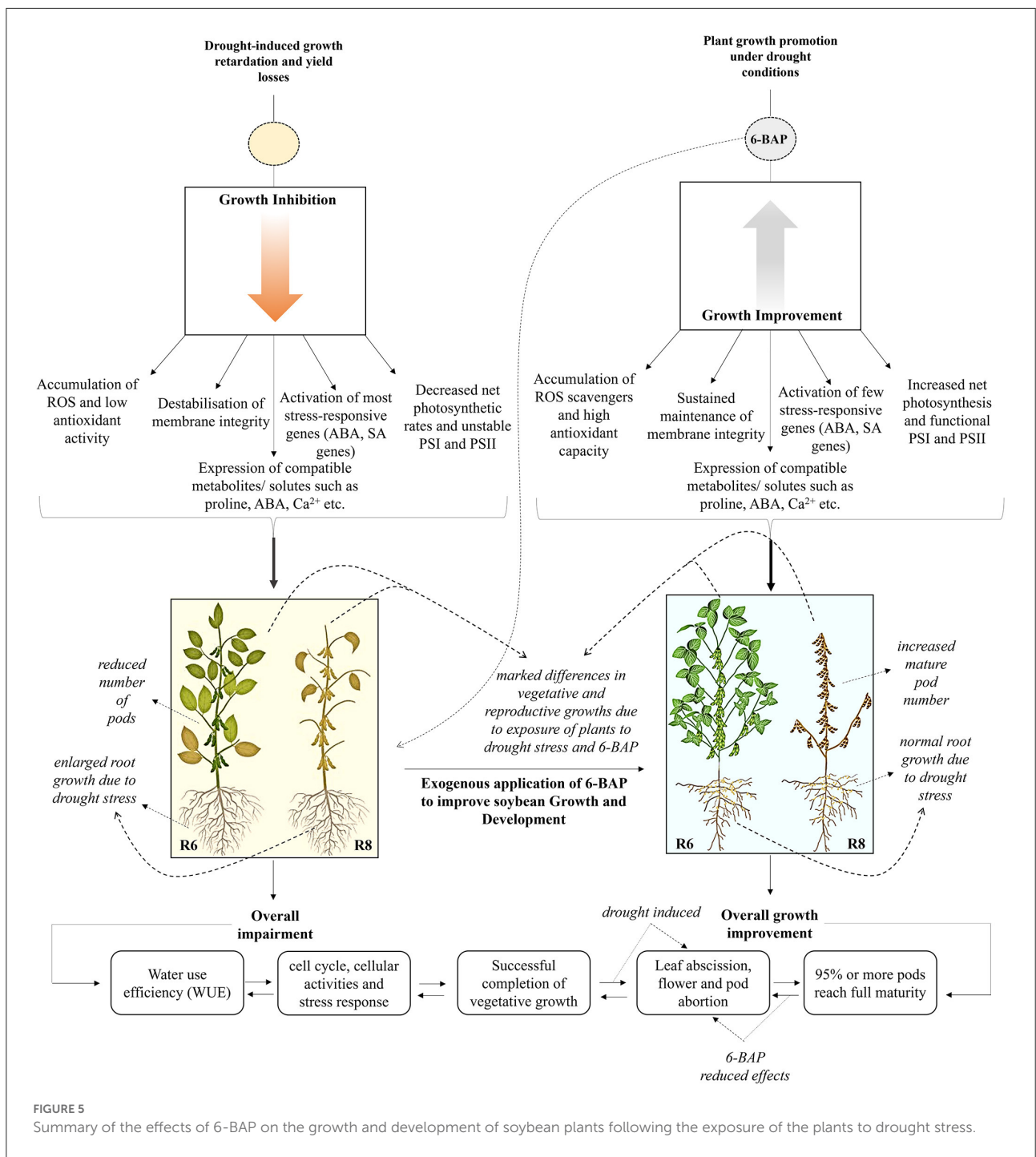
Cytokinins have become important chemicals not only as yield or growth enhancers as shown in Figure 5 but also as quality improvers. These plant hormones play a crucial role in delaying the development of cell, tissue and organ senescence, and eventually plant death. Senescence of plant cells, tissues or organs is often understood as an evolutionary acquired process that is critical for plant fitness (Woo et al., 2018). Under natural circumstances, plant regulate senescence by timing initiation, rate of progression and nature of senescence. This is more evident on hormonal regulation of leaf and petal abscission by plants for the reason of enhancing yield, biomass and maintaining high nutritional value (Ma et al., 2018; Patharkar and Walker, 2018). In this case, the process proceeds naturally to shed some leaves, flowers, floral organs, and fruits that are no longer required. In soybean, flower abscission of about 50% or more is considered a natural process since the crop profusely produces flowers, but only set a limited number of pods and seeds. Furthermore, the agronomic significance of this phenomenon is still yet to be fully understood. Different soil water level regimes and elevated temperatures influence

senescing of nodules (Figures 4E,F) and abscission of flowers and pods in soybean. Hoque et al. (2016) and Kim et al. (2020) reported some of these effects in flower and pod abscission caused by both hot temperatures and drought. Ethylene-induced leaf and petiole abscission revealed the expression of several families of cell wall modifying enzymes. The enzymes were produced by about 188 abscission-specific transcription factors (TFs) encoded by pathogenesis-related (*PR*) genes in soybean. These TFs are involved in determining cell separation, balancing organ polarity within the abscission zone and regulating plant hormones, which plays a key role in cell differentiation (Kim et al., 2016). The TF regulated hormones act as chemical messengers that coordinate signaling pathways and many cellular processes, including facilitating the adaptation of plants under environmental stress conditions.

According to Amoanimaa-Dede et al. (2022), exogenous application of cytokinins before or during the exposure of plants to stress modulates endogenous hormone levels activating systemic response to stress. When 6-BAP was investigated for the formation of senescence on intact leaves, like in many herbaceous plants, this hormone was found to retard senescence in the detached leaves (Kai et al., 2020; El-Metwally et al., 2021; Wu et al., 2021). However, modulation of senescence by cytokinins in intact soybean cells or tissues could be easily demonstrated using plant materials or explants grown under *in vitro* plant tissue culture conditions (Aremu et al., 2012; Jablonska-Trypuc et al., 2016; Damanik et al., 2018; Solorzano-Cascante et al., 2018; Teixeira da Silva et al., 2020; Desta and Amare, 2021). The application of 10–4 M 6-BAP to *Phaseolus vulgaris* germinated seeds under etiolating conditions markedly delayed initiation of cotyledon senescence (Gilbert et al., 2011). Furthermore, Jablonska-Trypuc et al. (2016) also reported stimulatory effects of 6-BAP and kinetin on antioxidant enzyme activity, reduced glutathione and thiol group content that influence tissue and organ senescence, not in plant cell culture, but in *in vitro* cell culture of fibroblast cells. This evidence warrant further studies on 6-BAP's signaling role in mediating cell senescence and response to environmental stresses which may lead to improved nitrogen accumulation through nodulation and increased yield, especially in soybean genotypes that are more sensitive to hot temperatures and drought stress conditions.

Overall signaling and regulation of drought stress by 6-BAP

As already discussed, cytokinins are plant-specific chemical messengers that play a critical role in the regulation of numerous plant developmental processes. Although, many studies focused on the role that these hormones play on processes involving physiological and metabolic activities, a limited number of



reports attempted to unravel the understanding of cytokinin signal transduction. Recent studies proposed that calmodulin and the G-protein-linked receptors were involved in cytokinin signaling. However, calmodulin (CaM, Ca²⁺ sensing protein) has been implicated on the regulation of metal ions, ROS and modulation of transcription factors such as CAMTA3, GTL1, and WRKY39 as reported by [Virdi et al. \(2015\)](#) and [Kolling et al.](#)

(2019). Furthermore, the interactions between cytokinins and CaM cannot be denied, particularly, given the effects of these hormones on regulating cytosolic Ca²⁺ levels. [Hooley \(1998\)](#) earlier showed that G-proteins signaling in plants implicated their heterotrimeric forms on influencing gibberellins and possibly auxin signaling. Moreover, the antisense suppression of the *Arabidopsis* putative G-protein-coupled receptor (GCR1)

revealed the influence of these proteins on cytokinin signaling (Hooley, 1998; Pandey and Assman, 2004). It should, however, be noted that scientific evidence describing the involvement of these factors on cytokinins signaling is very scant. This is similar to the comparison of cytokinin signaling with auxin, ABA and ethylene signaling frequently reported in literature due to their versatility on germination, plant growth and stress responses.

In fact, according to Feng et al. (2017), major obstacles still exist in determining responses that mainly or specifically involve cytokinin signal transduction pathways, and this is because exogenous cytokinin applications evoke ethylene biosynthesis and most components of cytokinin signaling pathways are deemed highly redundant. Indeed, these issues have made it difficult to use biochemical, physiological or genetic approaches to investigate and elucidate cytokinin signaling, even under different environmental conditions, let alone signaling of individual hormones such as 6-BAP. Even though, the biochemical and physiological phytohormonal crosstalk occurring during the exposure of plants to drought stress are still not fully understood, exogenous treatment of plants with 6-BAP reveals widespread effects on morphological, physiological, and biochemical indices that ameliorated drought stress symptoms in plants (Ghaleh et al., 2020). Regulation was also observed in waterlogged maize, cultivar DengHai, following the application of 100 mg dm^{-3} 6-BAP that resulted in changes on leaf protein abundance levels at the tasselling stage (Hu et al., 2020). Plant tissues take up 6-BAP actively as free-hormone in the cells with the exception of only small portions of it being transformed into small nucleotide forms (Centeno et al., 1998). The free 6-BAP remains active in the cells to trigger the induction and initiation of plant cell cycle, cell maturation and differentiation states of the basal and apical meristematic cells. Specifically, the presence of 6-BAP in water-stressed soybean plant tissues is necessary to induce antioxidant activity (El-Metwally et al., 2021) depending on the amount of exogenously applied 6-BAP. Furthermore, Hurny and Benková (2017), Amoanimaa-Dede et al. (2022), and Wang et al. (2022) reported that the application of appropriate amounts of 6-BAP at suitable growth periods relieves abiotic stress such as drought by modulating senescence associated with chlorophyll breakdowns, oxidative cell damage and inhibition of important enzyme activities.

Conclusions and future perspectives

A great deal of work involving other cytokinins and 6-BAP have been reported, particularly, on cell division and differentiation, bud formation, shoot proliferation and direct/indirect somatic embryogenesis under controlled *in vitro* cell culture conditions in soybean (Phat et al., 2015; Raza et al., 2017; Mishra et al., 2020). In this review, an attempt

was made to present current knowledge and improve the state of understanding of the role of synthetic cytokinin 6-BAP in modulating different drought-stress related cellular processes and its applications in horticulture for abiotic stress tolerance. 6-BAP regulate numerous cellular processes that influence the morphology, physiology, and yield of many plants, including recalcitrant crops such as soybean, with or without drought stress. Similar to other methods of crop improvement, seed priming or plant treatments with 6-BAP have to be encouraged to exploit its crucial influence on stress response during plant growth and development. The current trend clearly indicates that cytokinins remain less used as primers or in foliar applications despite the demand for low cost, environmentally friendly methods of improvement, and their natural influence on almost all of the developmental stages of plant life.

Some of those stages already discussed above include vasculature formation, embryonic cell differentiation, shoot formation, and delays in leaf senescence (Saleiman et al., 2021). Although, the discovery of this distinguished class of growth regulators by Gottlieb Haberlandt precisely 109 year ago, now well-known as cytokinins have been widely used in plant tissue culture, plant biotechnology (including *in vitro* cell genetic engineering), pharmacology, and cosmetic industry, more still need to be done particularly, on commercially targeted traits in horticultural crops such as soybean. Nevertheless, as the various biotechnological strategies remain targeted toward the manipulation of soybean tolerance to drought stress, the application of plant growth regulators as an evolving strategy for potential engineering of soybean plants showing tolerance to abiotic stress must also be developed (Figure 5). Presently, this cytokinin-based compound (6-BAP) has also been widely tested for conferring resistance against temperature and salinity stress in grain crops, such as maize, rice, and wheat (Liu et al., 2020; Saleiman et al., 2021). The use of 6-benzyl adenine in plant growth and development plays an important role as an alternative approach for inducing abiotic stress resistance (Figure 5). This cytokinin enhances stress resistance by effectively functioning as a regulatory hormone, directing cell division, elongation, controlling dormancy, and influencing other biological responses necessary for plants to cope with adverse growth conditions. All above-mentioned biological functions of cytokinins, particularly 6-BAP, were revealed through research progress made thus far, and the fact that auxin and cytokinin crosstalk remains a critical regulatory function for these hormones in differentiation of plant organs and response to stress (Hurny and Benková, 2017). Furthermore, it is primarily on the best interest of farmers and consumers that plant biotechnologists and crop breeders continue their work on deciphering the roles played by cytokinins in general. These must take place at a physiological and molecular level to provide insights into mechanisms of action, regulation, and to develop cost-effective approaches needed to best regulate

plant growth and development in soybean under drought stress conditions.

Author contributions

The author wrote the manuscript and verified the submitted version.

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