Critical Reviews in Plant Sciences, 29:162–190, 2010

ISSN: 0735-2689 print / 1549-7836 online
DOI: 10.1080/07352689.2010.483580

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The Physiological, Biochemical and Molecular Roles of Brassinosteroids and Salicylic Acid in Plant Processes and Salt Tolerance

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Plant hormones regulate plant growth and development by affecting an array of cellular, physiological, and developmental processes, including, but not limited to, cell division and elongation, stomatal regulation, photosynthesis, transpiration, ion uptake and transport, initiation of leaf, flower and fruit development, and senescence. Environmental factors such as salinity, drought, and extreme temperatures may cause a reduction in plant growth and productivity by altering the endogenous levels of plant hormones, sensitivity to plant hormones, and/or signaling pathways. Molecular and physiological studies have determined that plant hormones and abiotic stresses have interactive effects on a number of basic biochemical and physiological processes, leading to reduced plant growth and development. Various strategies have been considered or employed to maximize plant growth and productivity under environmental stresses such as salt-stress. A fundamental approach is to develop salt-tolerant plants through genetic means. Breeding for salt tolerance, however, is a long-term endeavor with its own complexities and inherent difficulties. The success of this approach depends, among others, on the availability of genetic sources of tolerance and reliable screening techniques, identification and successful transfer of genetic components of tolerance to desired genetic backgrounds, and development of elite breeding lines and cultivars with salt tolerance and other desirable agricultural characteristics. Such extensive processes have delayed development of successful salt-tolerant cultivars in most crop species. An alternative and technically simpler approach is to induce salt tolerance through exogenous application of certain plant growth–regulating compounds. This approach has gained significant interest during the past decade, when a wealth of new knowledge has become available on the beneficial roles of the six classes of plant hormones (auxins, gibberellins, cytokinins, abscisic acid, ethylene, and brassinosteroids) as well as several other plant growth–regulating substances (jasmonates, salicylates, polyamines, triacontanol, ascorbic acid, and tocopherols) on plant stress tolerance. Among these, brassinosteroids (BRs) and salicylic acid (SA) have been studied most extensively. Both BRs and SA are ubiquitous in the plant kingdom, affecting plant growth and development in many different ways, and are known to improve plant stress tolerance. In this article, we review and discuss the current knowledge and possible applications of BRs and SA that could be used to mitigate the harmful effects of salt-stress in plants. We also discuss the roles of exogenous applications of BRs and SA in the regulation of various biochemical and physiological processes leading to improved salt tolerance in plants.

Keywords abiotic stress tolerance, exogenous application, physiological processes, plant growth regulators, plant hormones, salt tolerance

I. INTRODUCTION

The use of plant growth regulators (PGRs) in agriculture to promote plant growth, production, and quality is becoming increasingly more common (Brosa, 1999; Wang *et al.*, 2005; Rajjou *et al.*, 2006; Tuna *et al.*, 2007; He and Zhu, 2008; Arora *et al.*, 2008; Ashraf *et al.*, 2008). Both beneficial and adverse effects of PGRs on growth and development as well as plant metabolism have been addressed extensively. Endogenous concentrations and ratios of different PGRs are impacted by numerous internal and external stimuli. For example, environmental stresses, which often cause a plethora of complex physiological, molecular, and biochemical changes in plants, may alter the levels and ratios of different endogenous PGRs (Wang *et al.*, 2005; Ashraf *et al.*, 2008), thereby modifying their signal transduction pathways. Such alterations often cause serious metabolic disorders leading to a general suppression in plant growth and development under stress conditions (Lerner and Amzallag, 1994). Salt-stress, for example, generally causes reduced synthesis, and in many cases also degradation of PGRs in plants (Kuiper *et al.*, 1988). Under environmental stress conditions, however, exogenous application of PGRs, either to the seed before planting or to the growing plant, may overcome much of the internal PGR deficiency and may lead to a reduction of the inhibitory effects caused by the stress (Ashraf and Foolad, 2007; Ashraf *et al.*, 2008). For example, exogenous application of many natural and synthetic PGRs appear to improve plant salt tolerance, or at least partially reduce the salt-induced harmful effects. However, the mechanisms underlying such effects remain largely unknown, and have not been directly related to the physiological roles of these compounds (Ashraf *et al.*, 2008). To date, it is not known whether exogenous application of PGRs overcomes the imbalance of regulatory substances caused by the stress (Khan *et al.*, 2000; Debez *et al.*, 2001), generates specific defense mechanisms against the stress, or just improves plant vitality (Ashraf *et al.*, 2008). Nevertheless, from a practical viewpoint, application of PGRs offers a potential approach to mitigating the inhibitory effects of salt-stress on plant growth and crop productivity.

Like many other known plant growth-regulating substances, brassinosteroids (BRs) and salicylates (salicylic acid, SA) play vital roles in promoting growth and development of plants exposed to saline conditions by modulating a number of metabolic phenomena affecting a plant's tolerance to salt-stress. For example, exogenously applied BRs can effectively ameliorate the adverse effects of several abiotic stresses, including salt-stress, in different plant species (Kulaeva *et al.*, 1991; Sasse *et al.*, 1995; Anuradha and Rao, 2001; Sakhabutdinova *et al.*, 2003; Khodary, 2004; El-Tayeb, 2005; Jason *et al.*, 2006; Yusuf *et al.*, 2008). Although the type or extent to which the physiological and biochemical processes are affected by exogenous BRs is unknown, it is clear that BRs promote a number of growth-related phenomena (described below) and are involved in the regulation of gene expression (Clouse, 1997; Nemhauser and Chory, 2004; Bajguz and Hayat, 2009; Chinchilla *et al.*, 2009; Vert, 2009). Recent signal transduction studies have revealed how the BRs' signals are perceived at the cell surface and transmitted to the

nucleus to regulate gene expression (discussed below), although it has not yet been determined how salt-stress affects these signal transduction processes. Similarly, SA is a key component of a signaling pathway that is triggered by a number of biotic and abiotic stresses in plants (Yordanova and Popova, 2007). SA is an endogenous regulatory signal, which is involved in plant defense mechanism against pathogens (Raskin, 1992). SA also is involved in the plant's general defense mechanism, though this role is not stress-specific and apparently is effective against multiple biotic and abiotic stresses (Janda *et al.*, 2007). Furthermore, numerous reports suggest that SA has an important role in the plant's response to salinity stress. However, there has not been any critical review of the potential use of BRs or SA as external agents to improve plant stress tolerance. Considering the major reduction to agricultural productivity worldwide caused by salt-stress, any approach to reduce such adverse effects may have significant positive economic impact globally. In the present article, we review the literature and discuss how exogenous application of BRs and SA could ameliorate the inhibitory effects of salt-stress. We also discuss the potential mechanisms underlying these ameliorative effects, which will help elucidate how plants respond to exogenous application of these plant growth-regulating substances.

II. BRASSINOSTEROIDS

In the early 1960s, John W. Mitchell and co-workers at the USDA Agricultural Research Center in Beltsville, Maryland, initiated screening pollen in search of new plant hormones. Pollen was known as a rich source of plant growth-regulating substances, thereby making it a logical choice for screening. Nearly 60 species of plants were screened and pollen crude extracts from half of them caused increased growth in the common bean (*Phaseolus vulgaris* L.) second internode bioassay. The largest increases were obtained from the alder tree (*Alnus glutinosa* L.) and rapeseed (*Brassica napus* L.) pollen. The researchers proposed that this was due to a new class of steroidal hormones, which they termed brassins (defined as a crude lipid extract from rapeseed pollen). In 1972, Mitchell and co-workers showed that brassins could in fact enhance seed vigor and crop yield (Mitchell and Gregory, 1972). However, Millborrow and Pryce (1973) suggested that since brassins was a crude extract it could possibly contain gibberellins or other compounds rather than just endogenous lipids such as brassins and therefore should not be considered a new class of plant hormones. Subsequently, in an effort to isolate the active component in brassins, 500 pounds of bee-collected rapeseed pollen, which was more readily available than alder pollen, was extracted and purified resulting in 10 mg of an active crystalline material, thereby identifying brassinolide as the active component in brassins (Grove *et al.*, 1979). All plant hormones discovered prior to 1979 were of nonsteroidal nature, and thus Grove and co-workers (1979) marked the discovery of the first plant steroidal hormone, brassinolide (BL). This was followed by the discovery of the second steroidal hormone, castesterone, in 1982 (Yokota *et al.*, 1982). Since then numerous analogs have been discovered and isolated from various plant species, of which approximately 60 are fully characterized (Haubrick and Assmann, 2006). All such naturally-occurring steroidal compounds now constitute an independent class of plant hormones, known as brassinosteroids (BRs), which are defined as a class of compounds having activity similar to BL. They are a group of polyhydroxy lactones with a common 5α-cholestane skeleton, which vary in their chemical structures by the kind and orientation of functional groups on the skeleton (Fujioka and Sakurai, 1997; Zullo and Adam, 2002). The classification of BRs as C_{27}, C_{28} or C_{29} usually depends on the alkyl-substitution pattern of the side chain (Yokota, 1997; Zullo and Adam, 2002) (Figure 1). For a BR to be active, the following structural requirements must be met: they must have a trans A/B ring system with a 5 alpha hydrogen; must have a 6-ketone or a 7-oxa-6-ketone system on the B ring; must have cis-oriented hydroxyl groups at the C_2 and C_3 positions; and must have cis hydroxy groups at C_{22} and C_{23} plus a methyl or an ethyl at C_{24}, In addition, the alpha orientation at C_{22}, C_{23}, and C_{24} are more active than the beta-oriented groups. These requirements have been shown in many experimental test systems (Thompson *et al.*, 1981, 1982; Arteca *et al.*, 1985; Takasuto *et al.*, 1983; Cutler, 1991). BRs can also occur in conjugated forms especially with sugars or fatty acids (Zullo and Adam, 2002; Zullo *et al.*, 2002). Brassinosteroids are considered a new class of growth hormones, which are ubiquitous in the plant kingdom (Clouse and Sasse, 1998; Khripach *et al.*, 1999; 2000; Sasse, 2003; Haubrick and Assmann, 2006; Vlasankova *et al.*, 2009) and affect a multitude of developmental and physiological processes. Thus far, more than 70 natural analogs of BRs have been identified and characterized in 37 angiosperms (9 monocots and 28 dicots) and 5 gymnosperms (Takatsatu *et al.*, 1990; Fujioka, 1999; 2002; Haubrick and Assmann, 2006; Bajguz, 2007; Vlasankova *et al.*, 2009). Due to their multiple effects, BRs are considered as plant hormones with pleiotropic effects (Sasse, 1997). They can affect general plant growth and many developmental processes such as seed germination, rhizogenesis, flowering, senescence, abscission and maturation, and physiological phenomena such as induction of cell expansion and elongation (Clouse and Sasse, 1998; Cortes *et al.*, 2003; Nemhauser and Chory, 2004). BRs also play a vital role in vascular differentiation and signal transduction (Cano-Delgado *et al.*, 2004) and are necessary for pollen tube formation (Hewitt *et al.*, 1985). In BR-deficient plants, senescence is delayed as compared to wild type plants, which indicates that this function may biologically provide some protection to plants against chilling and drought (Clouse, 1996; Li *et al.*, 1996; Clouse and Sasse, 1998). However, BRs can also confer resistance/tolerance to various other abiotic stresses.

A. Biosynthesis

The BR biosynthetic pathways were initially established based on metabolic conversion assay of radio-labeled compounds in BL-overproducing cell lines of Madagascar periwinkle (*Catharanthus sp.*) (Fujioka and Yokota, 2003). Subsequently, numerous mutants defective in BR biosynthetic

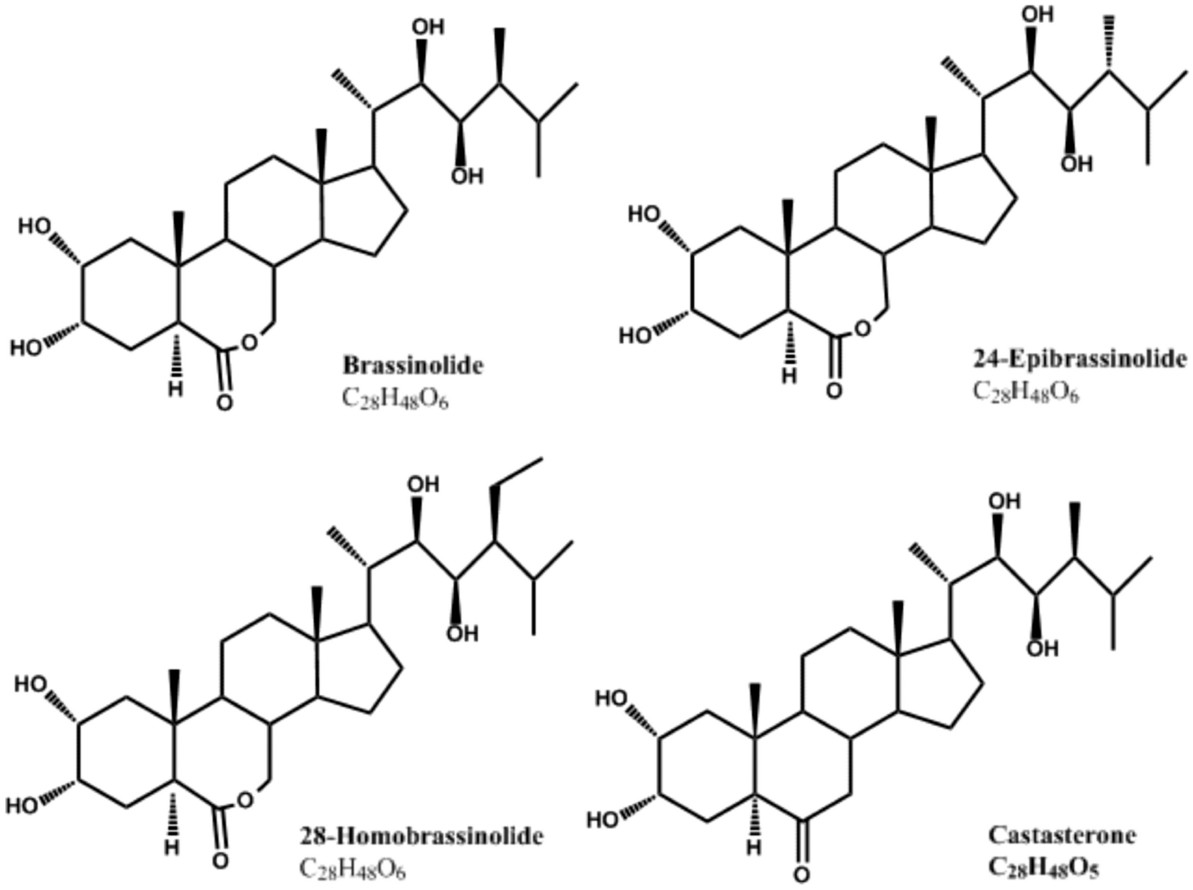


FIG. 1. Chemical structures of different types of BRs: brassinolide, 24-epibrassinolide, 28-homobrassinolide and castasterone (adopted from Zullo and Adam, 2002; Bajguz and Tretyn, 2003 with permission).

enzymes were discovered in other plant species (Bishop, 2003; Choe, 2004). Plants defective in BR biosynthesis or signal transduction pathways display specific phenotypes, including short stature, round and curled leaves, short petioles, short pedicels, and reduced fertility. When these mutants were grown in the dark, hypocotyls were short and cotyledons opened without an apical hook (Choe, 2004). Biochemical characterization of these mutants has facilitated further validation of previously existing pathways and also the discovery of previously unknown steps (Choe, 2006).

Among all BRs thus far identified, BL is the most biologically active compound and has been found in a large number of plant species (Kim *et al.*, 2008). BL is a C_{28} complex molecule, possessing an S-methyl group at C_{24} of the side chain of its 5α-ergostane structure, which has been the focus of much research on BRs. BL biosynthesis normally takes place in the endoplasmic reticulum. In plants, BRs are synthesized from three sterols; campesterol, sitosterol and cholesterol (Schaller *et al.*, 1998; Diener *et al.*, 2000; Shimada *et al.*, 2003; Kim *et al.*, 2004; Taiz and Zeiger, 2006). Campesterol and sitosterol are found abundantly in plant membranes. However, the most common biosynthetic pathway revealed thus far is that originating from campesterol, which is converted to campestanol involving the DET2 (De-etiolated-2) enzyme, 5-α-reductase (Figure 2). Campestanol is then converted to castasterone, the immediate precursor of BL, via two analogous pathways, the early and the late C-6 oxidation pathways. The difference between these two pathways is that, in the early C-6 oxidation pathway oxidation at C-6 of the B ring takes place before the hydroxylation at C-22 and C-23 of the side chain (Fujioka *et al.*, 2002), whereas in the late C-6 oxidation pathway C-6 is oxidized after the addition of hydroxyls at the side chain and C-2 of the A ring (Choi *et al.*, 1997; Taiz and Zeiger, 2006). These two oxidation pathways have been found to occur not only in higher plants but also in the green alga *Chlorella vulgaris* (Bajguz, 2009). However, both the early and the late pathways are linked to each other at multiple steps, forming the very intricate BR biosynthetic network as has been observed in *Arabidopsis* (*Arabidopsis thaliana* L.), pea (*Pisum sativum* L.), and rice (*Oryza sativa* L.) (Fujioka and Yokota, 2003). This complexity, however, could be advantageous particularly under stressful conditions. Also, besides the BR intermediates noted in Figure 2, some other BR intermediates in the biosynthesis of castasterone have been identified, including secasterone in rye (*Secale cereale* L.) (Schmidt

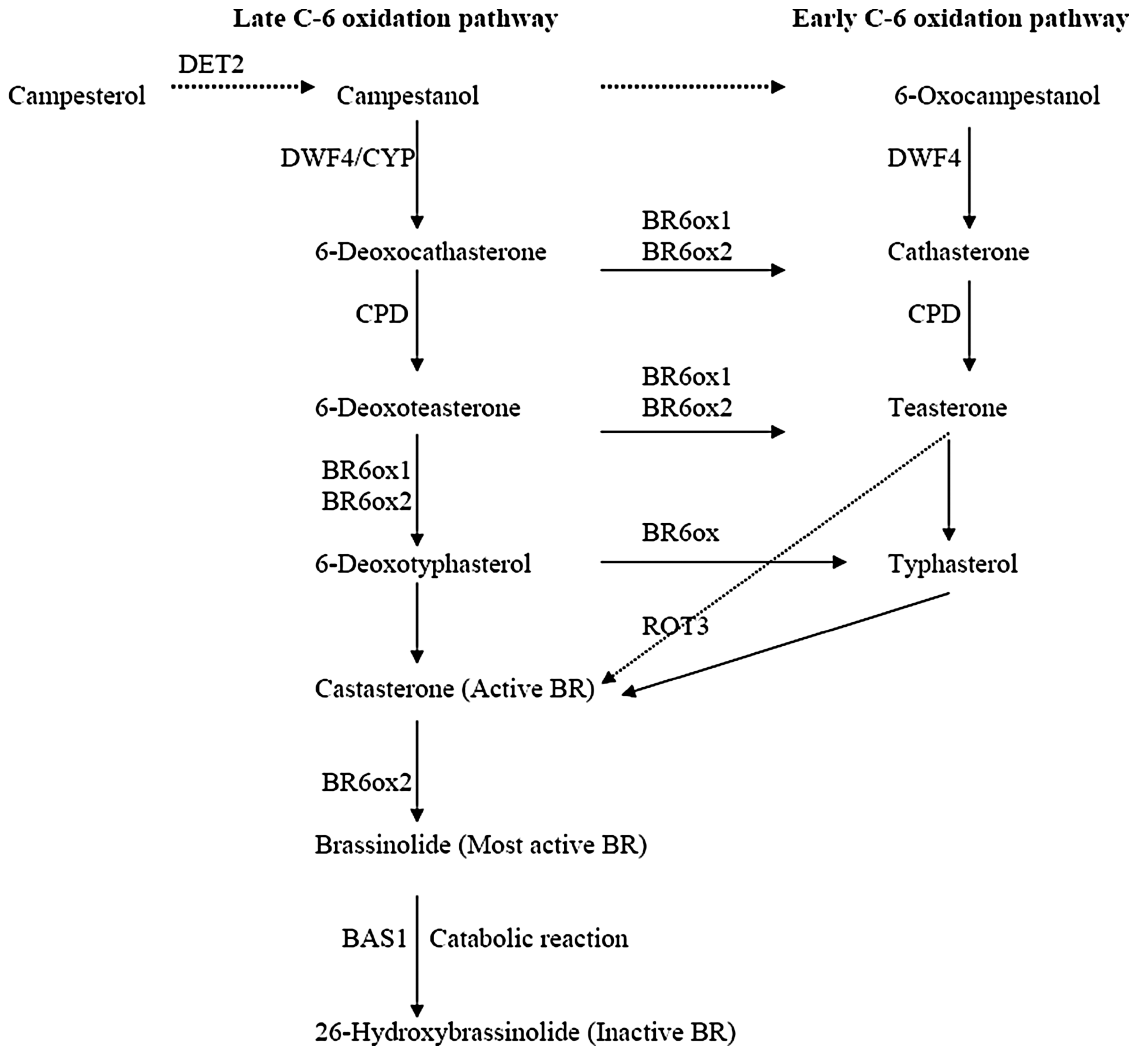


FIG. 2. Biosynthesis of biassinosteroids. **Enzymes used:** DET2 (De-etiolated-2); DWF4 (DWARF-4); CYP (Cytochrome monooxygenase P450); ROT3 (Rotundifolia3); CPD (Constitutive Photomorphogenesis and Dwarfism); BAS1 (phyB activation tagged suppressor1); BR6oxl (BR-6-oxidasel); BR6ox2 (BR-6-oxidase2). (Adopted from Taiz and Zeiger, 2006 and modified according to Divi and Krishna, 2009 with permission).

et al., 1995; Antonchick *et al.*, 2005) and 24-episecasterone in sticky catchfly (*Lychnis viscaria* L.) (Friebe *et al.*, 1999). In addition to the two oxidation pathways described above, two other branching pathways, i.e., the early C-22 oxidation pathway (Fujioka *et al.*, 2002) and a shortcut pathway from campesterol to 6-deoxytyphasterol involving C-23 oxidation (Ohnishi *et al.*, 2006), have been reported (Divi and Krishna, 2009). The reactions involving C-22 and C-23 hydroxylation and the C-6 oxidation play a central role in the regulation of BR biosynthesis. Thus, the enzymes catalyzing these reactions have gained considerable interest for biotechnological studies relating to BR metabolism (Choe, 2006; Divi and Krishna, 2009).

B. Signaling

Detailed genetic and molecular studies in different plant species, particularly in *Arabidopsis*, have led to the identification of BR-receptors and a variety of intermediates involved in BRs signal transduction. The kinase brassinosteroid-insensitive 1 (BRI1), which is a leucine-rich repeat (LRR) serine/threonine kinase receptor and located on the plasma membrane, is the specific receptor for BL (Figure 3). Recently, *in vitro* and *in vivo* studies in *Arabidopsis* showed that recombinant cytoplasmic domains of BRI1 and BAK1 (BRI1-associated receptor kinase 1) could autophosphorylate tyrosine, in addition to serine and threonine, suggesting that these kinases were dual-specific in action (Oh *et al.*, 2009). These findings negated the earlier perception that while plant receptor kinases were predominantly serine/threonine protein kinases animal receptor kinases were tyrosine kinases (Dievart and Clark, 2004). However, both LRR (located externally) and kinase (located internally) domains of the BRI1 receptors are necessary for the effective transmission of signals (Taiz and Zeiger, 2006). The receptor BRI1 becomes

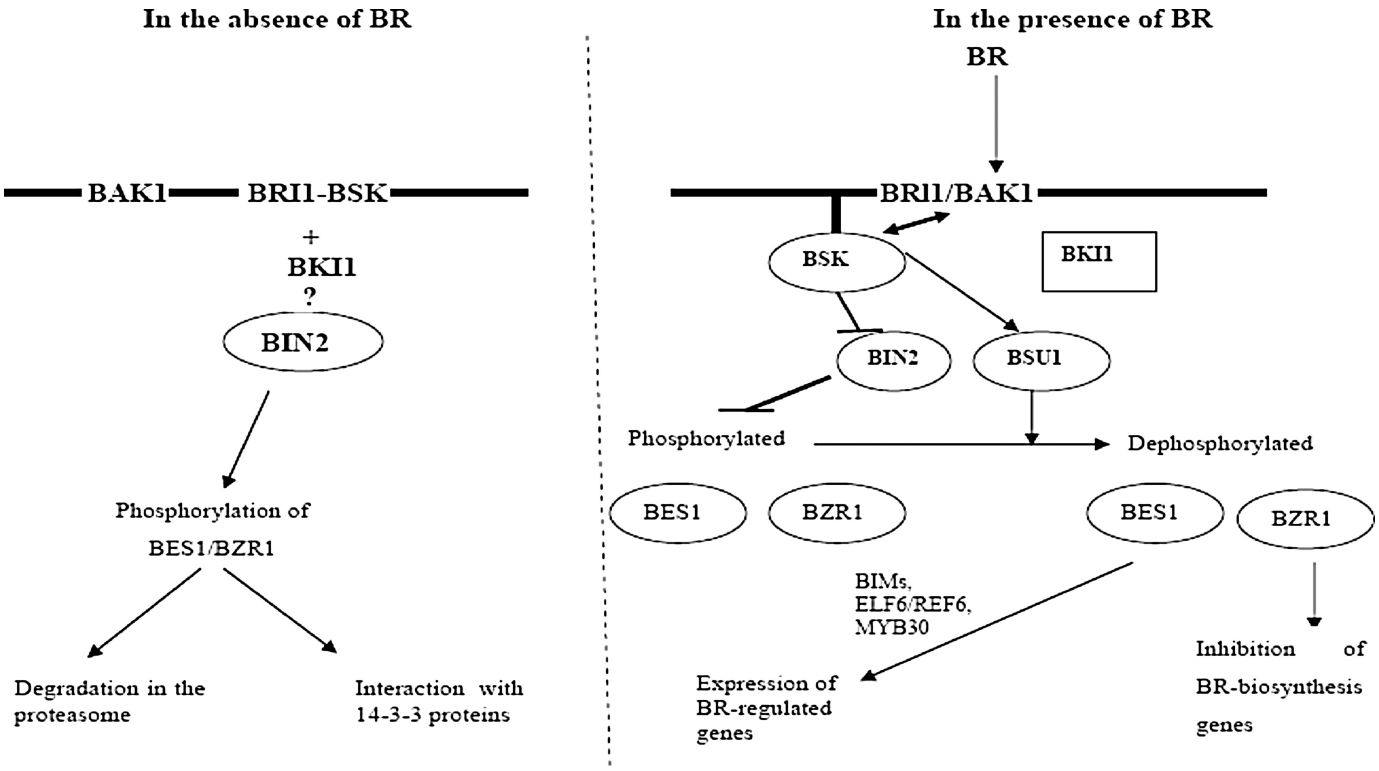


FIG. 3. Brassinosteroids signaling. **Abbreviations used:** BRI1 (Brassinosteroid-insensitive 1): BKI1 (BRI1 kinase inhibitor 1); BIN2 (Brassinosteroid-insensitive 2); BZR1 (Brassinazole-resistant 1) and BES1 (*bril* EMS-suppressor 1); BSU1 (*bril* suppressor 1); BIMs (BES1-interacting Myc-like proteins); MYB30 (MYB domain protein 30); ELF6 (Early flowering 6); REF6 (Relative of early flowering 6); BSK (Brassinosteroid signaling kinases). (Reproduced with some modification from Tang et al., 2008 and Divi and Krishna, 2009 with permission).

active after binding to BL, due to its improved autophosphorylation and association with a second membrane localized receptor BAK1. BAK1 probably acts as a co-receptor with BRI1 (Cano-Delgado *et al.*, 2004) and it can positively regulate BRI1 function due to physical interactions and transphosphorylation (Chinchilla *et al.*, 2009). However, it has not yet been clearly determined whether the main role of BAK1 is either to activate the BR receptor, link the receptor with downstream signaling cascades, or promote receptor endocytosis (Vert, 2009). Wang and co-workers (2008) revealed that BR-dependent activation of BRI1 preceded its association with BAK1 in plants, and that BRI1 positively regulated BAK1 phosphorylation levels. In addition, BAK1 transphosphorylates BRI1, thereby quantitatively increasing BRI1 kinase activity towards a specific substrate.

In addition to BAK1, another protein, BKI1 (BRI1 kinase inhibitor 1), has recently been found in plants, which may act as a negative regulator in BR signaling (Wang and Chory, 2006). BKI1 may prevent the association of BRI1 with BAK1 and other BRI1 substrates, thus blocking the downstream BR signaling (Li and Jin, 2007). Besides BAK1 and BKI1, two other regulatory proteins have been discovered in *Arabidopsis*, a transthyretin-like protein (TTL) (Nam and Li, 2004) and a homolog of the TGFb-receptor-interacting protein 1 (TRIP1) (Ehsan *et al.*, 2005), however, the exact contribution of these two proteins to BR signaling remains unknown. Furthermore, Tang and co-workers (2008) identified three homologous plasma membrane-bound BR-signaling kinases, BSK1, BSK2, and BSK3, which are a small family of kinases acting as the substrates for BRI1 kinase. The BSKs are actively involved in triggering BR signaling downstream of BRI1.

In the absence of BRs, BIN2, a GSK3 kinase repressor protein, present in the cytosol, nucleus and plasma membrane, phosphorylates two nuclear transcription factors, BZR1 (brassinozole-resistant 1) and BZR2/BES1 (*bri1*-EMS-suppressor 1), thereby inhibiting their activities (Figure 3). Therefore, the association of BZR1 and BZR2/BES1 with other proteins or transcription factors is prevented making them unable to function as transcription regulators (Vert and Chory, 2006). BIN2-catalyzed phosphorylation of BZR1/BES1 not only prevents DNA binding but also enhances binding to the 14-3-3 proteins (phosphopeptide-binding proteins highly conserved in all eukaryotes) (Gampala *et al.*, 2007). However, upon BR perception, both BRI1 and BAK1 are phosphorylated and they induce the BR response by inactivating BIN2, resulting in the accumulation of biologically active forms (dephosphorylated) of BES1 and BZR1. Dephosphorylation occurs due to the activity of plant-specific serine/threonine phosphatase, BSU1 (*bri1* suppressor 1). However, the dephosphorylated BES1 and BZR1

can activate or inhibit BR-regulated genes. The dephosphorylated BES1 along with three BIM (BES1-interacting Myc-like 1) transcription factors binds to the E-box motif (CANNTG) in the SAUR-AC1 promoter to trigger gene expression, whereas BZR1 recognizes the BR-response element [CGTG(T/C)G] and acts as a go-between in the feedback inhibition of a number of genes involved in BR biosynthesis (Li and Jin, 2007; Divi and Krishna, 2009).

The above-mentioned studies are related to the occurrence of BR signal trasnduction in plants grown under normal (nonstress) conditions. However, information on BR signaling in plants subjected to stress conditions is scarce. Thus, it is pertinent to determine the role of stress-related signaling pathways in the modulation of BR biosynthesis and its role in mitigating the adverse effects of stress on plant growth, in order to fully understand the mechanism of stress tolerance in plants.

C. Physiological Roles

After its discovery, BL was evaluated for its biological activity in different bioassay systems designated for auxins, gibberellins, and cytokinins (Arteca, 1995). One of the main effects of BL appeared to be its close relationship with indole-3-acetic acid (IAA). Typically these two hormones acted synergistically. Although in many cases BL acts in a similar manner to auxins, gibberellins, and cytokinins, in auxin bioassays, based on root formation in mung bean (*Vigna radiata* L.), pea shoot lateral decapitated bud growth, and cress (*Lepidium sativum* L.) seedling root elongation, BR and IAA act differently. In the dock (*Rumex obtusifolius* L.) leaf disc senescence bioassay for gibberellins, BR promotes senescence whereas gibberellins delay senescence. In cytokinin bioassays using the dwarf pea apical hook and tip expansion, pigweed (*Amaranthus sp.*) betacyanin formation and cockelbur (*Xanthium strumarium* L.) leaf disc senescence bioassays, BR and cytokinin acted differently (Arteca, 1995). However, more recently it has become evident that BRs have an independent mode of action, possibly through cross-talk that may occur between BRs and other endogenous plant hormones, including auxins (Mandava, 1988; Arteca and Arteca, 2001; Swarup *et al.*, 2002). In general, BRs play important roles in regulating plant growth and development at very low concentrations, ranging from nanomolar to micromolar (Clouse and Sasse, 1998). They affect a multitude of physiological and metabolic processes, including coordination of morphogenic events throughout plant ontogeny, from seed germination and seedling elongation to maturity and seed development. Specific physiological processes affected by BRs include cell elongation, division and differentiation, enhancement of crop yield, reproductive biology (flowering), senescence, induction of ethylene biosynthesis, root growth and development, pollen tube growth, activation of proton pump, activation of photosynthesis and antioxidant system (Cao *et al.*, 2005; Houimli *et al.*, 2008; Shahbaz *et al.*, 2008).

Brassinosteroids are known to promote elongation of shoot tissues in a number of plants at very low concentrations. Wang and co-workers (1993) demonstrated that BRs could stimulate hypocotyl elongation by increasing wall relaxation without a concomitant change in wall mechanical properties in pakchoi (*Brassica chinensis* L.). In a subsequent study, Zurek and coworkers (1994) showed that BRs stimulated wall loosening in soybean (*Glycine max* L.) epicotyl segments. However, they found that the loosening in soybean appeared to alter mechanical properties of the wall, since they observed an increase in plastic extensibility, as measured by Instron analysis. The promotive effect of BRs on elongation has clearly been shown under white, green or soft red light. However, little or no effects have been found in complete darkness suggesting that BRs action may result by overcoming the inhibitory effects of light (Mandava, 1988; Cutler, 1991; Kamuro and Inada, 1991).

The primary cell walls in dicots and monocots are comprised of cellulose microfibrils entwined in a network via non-covalent attachment to hemicelluloses (primarily xyloglucans), which are in turn embedded in a pectic gel matrix (Carpita and Gibeaut, 1993). Plant hormones are thought to regulate the biosynthesis and activity of cell wall modifying enzymes and other proteins such as xyloglucan endotransglucosylase/hydrolase (XTHs), cellulose synthase, expansins, sucrose synthase and glucanases, thereby regulating cell elongation. It has been reported that BRs are involved in the regulation of genes encoding XTHs and expansins in *Arabidopsis*, tomato (*Solanum lycopersicum* L.), soybean and rice. In addition, physiological measurements revealed that BRs could stimulate wall loosening in epicotyls of soybean and hypocotyls of *Brassica chinensis* and *Cucurbita maxima* (Bishop and Koncz, 2002; Clouse and Sasse, 1998; Sakurai, 1999). The dwarf nature of BR-deficient mutants, and the ability to return to normal phenotype with the application of BRs shows the key role of BRs in plant growth and development. Evaluation of cell orientation in the wild-type *Arabidopsis* as well as BR mutants *cbb*, *dwf4*, *cpd* and *dim* using light- or electron-microscopy indicated that longitudinal cell expansion was markedly impaired in the BR mutants (Altmann 1999; Sakurai, 1999). The overexpression of *dwf4* gene, which encodes an enzyme responsible for regulating a putative rate-limiting step in BR biosynthesis, has been shown to promote hypocotyl length in *Arabidopsis* (Choe *et al.*, 2001). This suggests that enhancing the endogenous biosynthesis of BR leads to enhanced cell expansion. The arrangement of cortical microtubules is one of the key factors involved in the regulation of cell elongation. Physiological and genetic studies have revealed that BRs play an important role in the reconfiguration of microtubules to the transverse orientation, which permits longitudinal cell growth (Clouse and Sasse, 1998). Some studies have demonstrated that BRs also may promote cell elongation by regulating the transport of water via aquaporins as well as regulating the activity of a vacuolar H^+-ATPase subunit (Friedrichsen and Chory, 2001; Morillon *et al.*, 2001).

Brassinosteroids also have been reported to promote cell division in the second internode of bean plants (Arteca, 1995). In addition, BRs have been found to promote cell proliferation in

combination with auxins and cytokinins in cultured parenchyma cells of Jerusalem artichoke (*Helianthus tuberosum* L.) and in protoplasts of Chinese cabbage (*Brassica rapa* L., a.k.a. *B. chinensis* L.) and petunia (*Petunia sp*. L.) (Clouse and Sasse, 1998; Sakurai, 1999). It has been suggested that BRs play a key role in *Arabidopsis* cell division in mutant *det2* (*de-etiolated2*) suspension cultures, where it was shown that epibrassinolide (24-EBR) caused an increase in transcript levels of the gene encoding cyclin-D3, a regulatory protein of the cell cycle. Cyclin-D3 is also regulated by cytokinins, and it may be significant that 24EBR can efficiently substitute for zeatin (a naturally occurring cytokinin) in the growth of *Arabidopsis* callus and suspension cultures (Hu *et al.*, 2000). Furthermore, BRs have been determined to be involved in many major physiological processes, including the following:

1. Cell Differentiation

Research conducted in different plant species has provided evidence that BRs play active roles in vascular differentiation. In Jerusalem artichoke explants and isolated mesophyll cells of zinnia (*Zinnia elegans* L.), low concentrations of BRs effectively promote tracheid formation (Fukuda, 1997). In addition, BRs are involved in the regulation of expression of several genes involved in xylem development in zinnia mesophyll cells. BRs also play a key role in xylem formation in soybean epicotyls (Zurek *et al.*, 1994). Microscopic examination of BR mutants has shown an active role of endogenous BRs in vascular differentiation. For example, the *Arabidopsis* BR-deficient mutant *cpd* showed unequal division of the cambium, because it produced additional layers of phloem cells at the cost of xylem cells (Szekeres *et al.*, 1996). Similarly, the sterol and BR-deficient *Arabidopsis* mutant *dwf7* also exhibited an increase in phloem as opposed to xylem cells, and the number of vascular bundles was down from eight in the wild type to six in the mutant, with irregular spacing between vascular bundles (Choe *et al.*, 1999).

2. Reactive Oxygen Species

The involvement of BRs in the regulation of reactive oxygen species (ROS) metabolism is evident as they can induce and regulate the expression of certain antioxidant genes and increase the activities of key antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Mazorra *et al.*, 2002; Nunez *et al.*, 2003; Cao *et al.*, 2005; Ogweno *et al.*, 2008). However, whether BRs directly or indirectly alter responses of plants to oxidative stress remains unknown (Cao *et al.*, 2005). Although both BRs and ROS act as vital secondary messengers for the induction and regulation of antioxidant systems in plants under stress (Mazorra *et al.*, 2002), the association between BRs and ROS in stress-signal transduction is unknown. Currently, however, detailed molecular investigations are underway in various laboratories to reveal the mechanisms by which endogenous or exogenous BRs induce and/or control the stress response in plants by regulating ROS.

3. Enhancement of Crop Yield

Sakamoto and co-workers (2006) showed that the manipulation of BR biosynthesis might provide a new and very effective approach to enhancing rice yield under a high-density planting. They showed that a rice phenotype with a more erect leaf type, which is related to an increase in the rate-limiting step in BL biosynthesis, increased productivity without any discernable side effects. However, thus far, there has not been any successful effort to biologically engineer the BR pathway to enhance crop yield without having an adverse effect on plant's phenotype.

In the 1970s, the significance of BRs in cell elongation revealed insight into manipulating their endogenous levels in plants. A major proportion of the early work was based on exogenous application of BR to promote crop yield, but the results were inconsistent under field conditions thereby leading to a loss of interest in such research (Cutler, 1991). However, recent advances in the identification of *Arabidopsis* and rice genes involved in BR biosynthesis and signal transduction have resulted in renewed interest in engineering the BL pathway in plants (Fujioka and Yokota, 2003). This is more feasible since it is now known that most plant genes involved in BL biosynthesis or function are present as single copies. Since BRs are involved in the regulation of cell elongation, most BR-deficient and signaling mutants are dwarf to varying degrees. This is obvious in that the loss of function of the *Arabidopsis DWARF4* gene causes severe dwarfism.

Sakamoto and co-workers (2006) identified two rice C-22 hydroxylases, one that was involved in shoot elongation and reproductive development (OsDWARF4L1) and the second which regulated leaf inclination (OsDWARF4). Loss of function of OsDWARF4L1 resulted in a semi-dwarf plant with small seeds, whereas the loss of function of OsDWARF4 resulted in a plant that was only slightly shorter with erect leaves. The more erect orientation of leaves probably promotes photosynthesis and improves yields under high plant density (Sakamoto *et al.*, 2006). From these findings it is evident that an agronomically important trait may be improved by manipulating endogenous BL levels without any adverse side effects. Sakamoto and co-workers (2006) carried out small-scale field trials involving wild type and OsDWARF4 plants at two planting densities and three N levels. In the densely planted plots with the highest N level, biomass in OsDWARF4 was improved by about 40% as compared to the wild type control. With all levels of N evaluated, the densely planted OsDWARF4 plots yielded 17%– 20% more grain than did the corresponding wild type plots. However, under normal planting densities the differences in yields were not significant. Manipulation of BL biosynthesis or other signal transduction pathways could prove to be a viable approach for increasing crop yield.

4. Reproductive Biology and Senescence

It has been well documented that there is reduced fertility and male sterility in BR-deficient and insensitive mutants (Hewitt *et al.*, 1985; Clouse and Sasse, 1998; Clouse, 2002;

Sasse, 2003; Sakamoto *et al.*, 2006). The highest levels of BRs have been found in pollen, where they were first discovered, and in immature seeds. The BR-deficient mutant *cpd* was reported to be male sterile, which was thought to be due to the inability to develop pollen tube following pollen germination (Szekeres *et al.*, 1996). However, in the *Arabidopsis* BR biosynthesis mutant *dwf4*, which is affected at the step just prior to *cpd*, the pollen is viable and sterility is caused by reduced length of stamen filaments, thereby resulting in inserted stamens and deposition of pollen on the ovary wall instead of the stigmatic surface. The sterol- and BR-deficient mutant *dwf5-1* has similar fertility as the wild type, and is the only BR mutant that has stamens significantly longer than the gynoecium (Choe *et al.*, 2000). However, the *dwf5-1* mutant produces seeds that do not undergo normal development, and require exogenous BR for normal germination and seedling development.

5. Induction of Ethylene Biosynthesis and Epinasty

In etiolated mung bean hypocotyl segments, BR increases ethylene biosynthesis at the step between s-adenosyl methionine (AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC) by stimulating ACC synthase activity (Arteca, 1995; Joo *et al.*, 2006). BR-induced ethylene can be inhibited by aminooxyacetic acid (AOA), fusicoccin (a fungal toxin) and the transport inhibitors 2,3,4-tri-iodobenzoic acid and 2-(p-chlorophenoxy)-2-methylpropionic acid. BR acts synergistically with active auxins (Arteca *et al.*, 1983; Yi *et al.*, 1999; Swarup *et al.*, 2002) and calcium, whereas it has an additive effect when used in combination with cytokinins in the stimulation of ethylene production (Arteca, 1995). Light has been reported to inhibit BR-induced ethylene synthesis while having little effect on ethylene production in response to IAA (Arteca, 1990). BRs application to the roots of hydroponically-grown tomato plants also has been shown to promote a marked increase in the step between AdoMet and ACC, resulting in an increase in ACC, ethylene and petiole bending (Schlagnhaufer and Arteca, 1985). BRs show similar effects in the promotion of ethylene production in plant parts as well as in a whole plant system. This is unlike auxin, which is typically more effective in eliciting a response in detached plant parts.

Although auxins and BRs have been reported to enhance ethylene production alone or when applied in combination (Arteca *et al.*, 1983; Swarup *et al.*, 2002), most of the work has been conducted in mung bean, which has several disadvantages such as difficulties in genetic analysis in this plant. Recently, an experimental system utilizing *Arabidopsis* inflorescences has been developed that shows interactions among BRs, auxins and cytokinins (Arteca and Arteca, 2008), thereby paving the way for further research on the involvement of BRs in the regulation of ethylene production in plants.

6. Effects on Shoot Gravitropic Bending

Brassinosteroids are involved in gravitropic bending responses in plant roots and shoots. Meudt (1987) was the first to report that exogenous application of BRs enhanced gravitropic curvature in common beans (*Phaseolus vulgris* L.). Later, additional studies with tomato hypocotyls (Park, 1998) and the lamina joint of rice (Yamamuro *et al.*, 2000) demonstrated that BRs played an active role in shoot gravitropic bending. To better elucidate the role of BRs in shoot gravitropic and reorientation bending, recently a novel detached inflorescence system using *Arabidopsis* BR-biosynthetic mutants has been developed (Arteca and Arteca, submitted). In this study, it was shown that BR had a dramatic effect on gravitropic and reorientation bending, while auxins and gibberellins had no such effect. This work has laid the foundation for future studies at the molecular level to better understand the role of BRs in gravitropic bending.

7. Effects on Root Growth, Development, and Gravitropic Bending

Following their first identification in corn (*Zea mays* L.) roots (Kim *et al.*, 2000), BRs have been detected in the root tissues of *Arabidopsis*, pea and tomato (Yokota *et al.*, 2001; Bancos *et al.*, 2002; Shimada *et al.*, 2003). Also, genes involved in BR biosynthesis and signaling are shown to express in plant roots (Bancos *et al.*, 2002; Nam and Li, 2002), thereby providing evidence on their vital physiological roles in the root. Furthermore, exogenous applications of BRs and auxins to BR-deficient mutants of *Arabidopsis* promote elongation of the root (Mussig *et al.*, 2003). In maize (*Zea mays* L.) roots, BRs increase the gravitropic response by improving their sensitivity to IAA (Kim *et al.*, 2000). Li and co-workers (2005) showed that BRs actively take part in the control of the gravitropic response of *Arabidopsis* roots. They showed that externally applied BRs increased the activity of ROP2, a GTPase, and that this protein mediated BR-modulated polar auxin transport resulting in an improved gravitropic response. More recently, Kim and coworkers (2007) presented additional evidence that BRs were involved in root gravitropic bending in *Arabidopsis*. They showed that BRs interacted with auxin differently in the root elongation as in gravitropic responses. In addition, they demonstrated that BRs promoted an increased gravitropic response in *Arabidopsis* roots when the IAA concentration was low and had reduced activity when IAA levels were high. However, additional work is necessary to build upon the existing studies to better elucidate the role of BRs in root gravitropic bending.

8. Enhancement of Plant Stress Tolerance

Brassinosteroids generally play a multitude of protective and stimulatory roles in improving plant quality and quantity (Khripach *et al.*, 2000). The potential application of BRs in agriculture to improve crop growth and seed yield under various stress conditions, including drought, salinity, extreme temperatures, nutrient deficiency and toxicity, is well documented (Khripach *et al.*, 2000). For example, the beneficial effects of BRs application have been reported in plants exposed to chilling stress, mild water deficit, and salt-stress (Clouse and Sasse, 1998; Krishna, 2003; Kagale *et al.*, 2007). The 24-EBR has

been reported to enhance tolerance to both cold and heat stress in bromegrass (*Bromus inermis*) and tomato (Wilen *et al.*, 1995; Singh and Shono, 2005; Dhaubhadel *et al.*, 1999), and it also alleviates the adverse effects of salt-stress on growth, pigmentation, and nitrate reductase activity in rice (Anuradha and Rao, 2003). Furthermore, treatment with 24-EBR has been reported to protect barley (*Hordeum vulgare* L.) and cucumber (*Cucumis sativus*) plants against pathogenic fungi (Pshenichnaya *et al.*, 1997; Khripach *et al.*, 2000) and viruses (Bobrick *et al.*, 1998).

Brassinosteroids also have been shown to protect plants against pesticides and herbicides (Cutler, 1991; Krishna, 2003). For example, BRs reduce herbicidal injury to rice caused by symetrin, simazine, pretilachlor, and butachlor (Hamada, 1986), possibly by overcoming inhibition in photosynthesis caused by the herbicides (Mandava, 1988). In a more recent report by Pinol and Simon (2009), the effects of 24-EBR on some key physiological attributes in broad beans (*Vicia faba* L.) plants treated with Terbutryn, a photosynthesis-inhibiting herbicide, were investigated. The authors demonstrated that pre-sowing treatment of *V. faba* seeds with 24-EBR effectively mitigated the adverse effect of Terbutryn on chlorophyll fluorescence and net photosynthetic efficiency. The higher dose of 24-EBR also overcame the Terbutryn-induced reduction in plant growth (Pinol and Simon, 2009). In the present article, however, we focus mainly on the beneficial effects of BRs on plants grown under salt-stress.

9. Other Biological Effects

Brassinosteroids also have a number of other biological effects, including causing antiecdysteroid effects (insecticidal effects), promoting seed germination, reducing fruit abortion and drop, enhancing growth in tissue culture, and affecting general plant growth and development (Iwahori *et al.*, 1990; Cutler, 1991; Sasse, 2003).

D. Exogenous Application

Exogenous application of BRs by seed soaking (seed priming), root treatment, or foliar spray has been extensively studied in various plant species, which indicates that BRs substantially improve plant growth and development under various nonstress and stress conditions (Clouse and Sasse, 1998; Yu *et al.*, 2004; Cao *et al.*, 2005; Houimli *et al.*, 2008). For example, seed germination was accelerated by exogenous application of BRs in several plant species. Leubner–Metzger (2001) reported that BR application was highly effective in enhancing the germination of tobacco (*Nicotiana tabacum* L.) seed. Steber and McCourt (2001) determined that exogenous application of BL or 24-EBR promoted the germination of *Arabidopsis* BR-biosynthetic mutant *det2-1* and BR-insensitive mutant *bri1-1*. Sasse and co-workers (1995) reported enhanced seed germination in the river redgum (*Eucalyptus camaldulensis* Dehnh.) under saline regimes due to application of 24-EBR. Similarly, Anuradha and Rao (2001) demonstrated that rice seeds treated with 24-EBR or 28-homobrassinolide (28-HBL) exhibited enhancement in germination, shoot size, fresh and dry weights and the quantity of soluble protein in the resulting seedlings.

Brassinosteroid application at very low concentrations (nanomolar to μmolar) promoted elongation of different growing parts of germinating seeds, including hypocotyls, epicotyls and peduncles in dicot seeds, and coleoptiles and mesocotyls in monocot seeds (Clouse, 1996). Uozu and co-workers (2000) reported that exogenous application of BL enhanced expression of xyloglucan genes *OsXTR1* and *OsXTR3*, leading to enhanced internode elongation in rice seedlings. Nakaya and co-workers (2002) discovered that exogenous application of 24-EBR upregulated a particular cyclin gene, *CycD3*, which was involved in the cell cycle of *Arabidopsis*, leading to enhanced seed germination by stimulating cell expansion and cell proliferation. Similarly, the mitotic processes were accelerated in bread wheat (*Triticum aestivum* L.) roots with exogenous application of 24-EBR (Fatkhutdinova *et al.*, 2002), leading to increased nucleolar organizing region (NOR) activity. Also in wheat, application of 24-EBR prevented degradation of nuclei and chloroplasts under salt-stress by protecting cell ultra-structure (Kulaeva *et al.*, 1991). Collectively, these reports support the active role of BRs in cell division and cell elongation and thus enhancement of growth and development in *Arabidopsis* and wheat plants. In contrast to their promoting effects on shoot growth, BRs generally inhibit root growth in many plant species (Davies, 1995), although occasional promotion of root elongation and adventitious root formation also has been reported with very low (picomolar) concentrations (Clouse *et al.*, 1993; Özdemir *et al.*, 2004; Kagale *et al.*, 2007; Arora *et al.*, 2008). In summary, exogenous application of BRs appears to accelerate seed germination and plant growth and development, however, the extent of their effects may vary with plant species and the concentration applied.

E. Exogenous Application to Enhance Plant Salt Tolerance

Brassinosteroids have recently gained considerable interest due to their important roles in affecting plant tolerance to a variety of abiotic stresses, including salt (Dhaubhadel *et al.*, 1999; Nunez *et al.*, 2003; Ozdemir *et al.*, 2004), drought (Li and Van Staden, 1998), chilling (Wilen *et al.*, 1995; Dhaubhadel *et al.*, 1999; Yu *et al.*, 2002), and oxidative stresses (Cao *et al.*, 2005). The exogenous application of BRs can effectively reduce the adverse effects of abiotic stresses or induce plant stress tolerance (Cutler, 1991; Hayat *et al.*, 2000; Rao *et al.*, 2002). BRs can be exogenously applied via at least three different ways—seed treatment, root treatment, and foliar spray; however, seed treatment and foliar spray are most common. Each mode of application has its advantages and disadvantages, as discussed below.

1. Pre-sowing Seed Treatment

It is well established that priming or pretreating seeds with PGRs generally improves germination rate under both stress

and nonstress conditions (for a review see Ashraf and Foolad, 2005). Seed priming not only improves the rate and uniformity of seed germination, but it also improves seedling establishment and crop performance under field conditions. BRs have been employed as a seed-priming agent in numerous plant species grown under different conditions (Table 1). For example, a marked increase in germination rate was observed in *Eucalyptus camaldulensis* (riverred gum tree) seeds treated with 24-EBR (Sasse *et al.*, 1995). Similarly, priming seeds of barley and common bean with BRs resulted in enhanced seed germination under saline conditions (Abd El-Fattah, 2007). In this study, a further examination indicated high accumulations of betaine (a potential osmoprotectant) and glutathione (a nonenzymatic antioxidant) in the BR-treated germinating seeds of both species. Pre-sowing treatment of *B. napus* and *A. thaliana* seeds with 24-EBR helped promote germination under salt-stress (Kagale *et al.*, 2007). In rice, pre-soaking seeds with NaCl and BRs (24-EBR or 28-HBL) mitigated the salt-induced inhibitory effects on seed germination and seedling growth (Anuradha and Rao, 2001). Such improvement was determined to be associated with enhanced levels of nucleic acids and soluble proteins in the rice kernels. It is noteworthy that pre-sowing seed treatment with BRs not only does improve germination, but also it may lead to enhanced seedling establishment and plant growth at later stages of development. For example, rice seeds pretreated with 28-HBL and sown under saline conditions exhibited a marked improvement in germination and led to increases in the length and number of primary roots in the seedlings (Takematsu and Takeuchi, 1989). In another study, root weight and rooting ability were markedly improved in pakchoi plants (*Brassica chinensis* L.) raised from seeds treated with 28-HBL (Wang *et al.*, 1993). Similarly, pre-sowing treatment of chickpea (*Cicer arietinum* L.) seeds for 8 h with 28-HBL resulted in enhanced salt tolerance, as such plants exhibited considerably higher root and shoot biomass as well as higher seed yield under salt-stress, when compared with plants grown from untreated seeds (Ali *et al.*, 2007). Furthermore, roots of the treated plants exhibited significantly higher activities of nitrate reductase, carbonic anhydrase, and nodule nitrogenase, when compared with the untreated plants.

Brassinosteroids are also known to modulate enzyme activities in certain key metabolic pathways. For example, when mung bean plants were treated with 28-HBL, they exhibited higher carbonic anhydrase activity and higher carboxylation efficiency (Fariduddin *et al.*, 2003). Similarly, lentil (*Lens culinaris* Medik.) seeds treated with 28-HBL produced plants with increased activity of nitrate reductase in their leaves at the adult stage (Hayat and Ahmad, 2003). Enhanced nitrate reductase activity was also observed in germinating seeds of chickpea treated with 28-HBL (Ali *et al.*, 2005). In bread wheat, treatment with 28-HBL substantially enhanced activities of α-amylase, catalase, and peroxidase, as well as the contents of soluble sugars and proteins in germinating kernels (Hayat and Ahmad, 2003). In maize, pre-sowing seed treatment with 28-HBL resulted in enhanced activities of antioxidative enzymes, superoxide dismutase, guaiacol peroxidase, glutathione reductase, and ascorbate peroxidase in plants growing under salt-stress (Arora *et al.*, 2008).

In conclusion, it is apparent that BR application as a pre-sowing seed treatment plays an important role in enhancing seed germination and ameliorating salt-induced oxidative stress in plants grown under salt-stress. For practical purposes, therefore, pre-sowing treatment with BRs may be employed as an effective approach to improving plant growth and crop production under saline conditions.

2. *Application Through the Root Growing Media*

Application of BRs through the root growing media has been determined to be an effective approach to ameliorating the harmful effects of salt-stress on seed germination, vegetative growth and development, and final yield in many plant species (Table 1). For example, Kagale and co-workers (2007) discovered that the injurious effects of salt-stress on seed germination in *Brassica napus* could be markedly reduced by the addition of 24-EBR to the germination medium. Similarly, application of 24-EBR or 28-HBL through the root growing media was highly effective in accelerating seed germination and seedling growth of sorghum (*Sorghum bicolor*) under osmotic stress (Vardhini and Rao, 2003). In this study, the seedling growth enhancement was attributable to elevated levels of soluble proteins and free proline content in the seedlings. In barley plants grown under salt-stress, it was determined that salt-induced damage to nuclei and chloroplasts could be significantly reduced by the application of BRs through the rooting medium (Kulaeva *et al.*, 1991). It is important to note that the beneficial effects of exogenous application of BRs may differ with differing concentrations of BRs. For example, when rice seedlings grown in NaCl-enriched culture media were exposed to 0.01 mg dm^{-3} of a BR analog (polyhydroxylated spirostanic brassinosteroid, BB-16) for 16 days, they exhibited a marked enhancement in the activities of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and a small increase in ascorbate peroxidase (APX) and glutathione reductase (GR). However, when rice seedlings were exposed to 0.001 mg dm^{-3} BB-16 for only 4 days, they showed enhanced activities of SOD and CAT, but not of GR (Nunez *et al.*, 2003).

The above-mentioned studies suggest that BRs applied exogenously through the growing media in plants grown under salt-stress could significantly reduce the adverse effects of salinity by modulating important physiological and biochemical processes. However, it should be noted that this conclusion is based on a limited number of studies, and manifestation of such effects may require optimizing BR concentrations and period of applications. This assessment is not unusual and is similar to that for the effects of traditional PGRs on plant growth and development, which exhibit a dose/response curve that is often bell shaped (Arteca, 1995), indicating that they are potentially harmful at higher concentrations (Oh and Clouse, 1998). Furthermore, there are other issues concerning the use of BRs as

TABLE 1
Modulation of growth and various physiological and biochemical processes in different plant species under salt-stress by exogenous application of brassinosteroids (BRs) through different modes, including seed treatment, root treatment, and foliar spray

Mode of BR application	BR level	Salt level imposed	Plant species	Effects	Reference
Pre-sowing seed treatment	3 μM brassinolide, 24-epibrassinolide and 28-homobrassinolide	150 mM NaCl	Rice	Improved growth, restored pigment levels and increased nitrate reductase activity	Anuradha and Rao, 2003
	0, 10^{-8}, 10^{-6} and 10^{-4} mM 28-homobrassinolide	0, 25, 50 and 75 mM NaCl	Maize	Enhanced activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) as well as concentrations of proteins, while decreased lipid peroxidation	Arora *et al.*, 2008
	0.5, 1 and 3 μM 24-epibrassinolide and 28-homobrassinolide	150 mM NaCl	Rice	Improved germination and seedling growth and enhanced levels of nucleic acids and soluble proteins	Anuradha and Rao, 2001
	5 μM brassinosteroids	150 mM NaCl	Beans and barley	Enhanced growth, betaine level, and chlorophyll content	Abd El-Fattah, 2007
	10^{-10} and 10^{-8} M 28-homobrassinolide	1 and 10 mM NaCl	Chickpea	Improved activities of leaf nitrate reductase and carbonic anhydrase, and increased dry biomass, leaf nodule number, and nodule fresh and dry weight	Ali *et al.*, 2007
	3 μM 24-epibrassinolide	120 mM NaCl	Rice	Improved seedling growth, soluble protein content, and activity of ascorbate peroxidase, while reduced lipid peroxidation and oxidative damage	Özdemir *et al.*, 2004
	3 μM 24-epibrassinolide	0, 0.30 and 0.35 M NaCl	Barley	Improved germination percentage, radicle elongation, and seedling fresh weight	Kilic *et al.*, 2007
	5 μM L^{-1}brassinolide	13.6 dS/m NaCl	Lucerne	Improved germination percentage, germination index, vigor index, shoot fresh weight, shoot dry weight, root dry weight, root length, root vigor and activities of antioxidant enzymes (POD, SOD, CAT), and reduced malondialdehyde (MDA)	Zhang *et al.*, 2007
	3 μM 24-epibrassinolide	0.30, 0.35, 0.40, and 0.45 M NaCl	Barley	Improved germination percentage, radicle elongation, and seedling fresh weight	Cavusoglu and Kabar, 2008

(Continued on next page)

TABLE 1
Modulation of growth and various physiological and biochemical processes in different plant species under salt-stress by exogenous application of brassinosteroids (BRs) through different modes, including seed treatment, root treatment, and foliar spray *(Continued)*

Mode of BR application	BR level	Salt level imposed	Plant species	Effects	Reference
Rooting medium	1 and 2 μM 24-epibrassinolide	50, 100, 150, 200, 250 and 300 mM NaCl	Rapeseed	Increased germination and seedling growth	Kagale *et al.*, 2007
	0.1, 1, and 10 nM 24-epibrassinolide	150 mM NaCl	Surghum	Modified initiation, duration and intensity of critical periods of reorganization of leaf number and leaf sheath, and developmentally perturbed leaf index	Amzallag, 2004
	0.001 and 0.01 mg dm-3 brassinosteroid analog (BB-16)	75 mM NaCl	Rice	Increased activities of CAT, SOD, GR, and APX	Nunez *et al.*, 2003
	0.5, 1.0, and 3.0 μM 24-epibrassinolide	50, 100, 150, and 200 mM NaCl	Cyanophyta	Improved growth	Saygideger and Deniz, 2008
Foliar spray	0, 0.01, 0.05, 0.1, and 0.5 mg L^{-1} 24-epibrassinolide	0.4 g L^{-1}NaCl	Pepper	Improved shoot and root length, leaf area, and shoot and root fresh and dry weight	Houimli *et al.*, 2008
	0.0125, 0.025, and 0.0375 mg L^{-1} 24-epibrassinolide	150 mM NaCl	Wheat	Increased plant biomass, leaf area, photosynthetic rate, photosystem-II efficiency (F_v/F_m), and activities of POD and CAT	Shahbaz *et al.*, 2008
	0.0125, 0.025, and 0.0375 mg L^{-1} 24-epibrassinolide	150 mM NaCl	Wheat	Improved shoot fresh and dry weight	Shahbaz and Ashraf, 2007
	0, 0.0125 and 0.025 mg L^{-1} 24-epibrassinolide	150 mM NaCl	Wheat	Enhanced chlorophyll *a* and *b* contents, while decreased transpiration rate and stomatal conductance	Qayyum *et al.*, 2007

root supplements, which deserve careful attention. For example, addition of BRs to the soil in the field may not be efficient as they may be partially or completely degraded by soil microorganisms (Ashraf *et al.*, 2008). Additionally, soil supplementation of BRs at optimal concentrations may not be economically feasible, due to their high cost. Currently, 24-EBR may cost up to US$22 per mg. Supplementation of a hectare of land with a concentration of 0.0375 mg L^{-1} of 24-EBR may cost more than US$3,000 (calculated from Shahbaz *et al.*, 2008). For most agronomic crops this cost is too great when considering the yield benefits of such applications. Therefore, further research is needed to fine-tune the efficiency of the use of BRs as a soil supplement to reduce the adverse effects of any abiotic stress, including salinity.

3. Foliar Application

Similar to many organic and inorganic compounds, PGRs could be applied as a foliar spray for a variety of agricultural purposes. Foliar applications of PGRs have produced encouraging results in terms of improving plant stress tolerance, including tolerance to salt-stress (Table 1). For example, foliar application of 24-EBR increased plant biomass of bread wheat plants under both non-saline and saline conditions, though it did not alter leaf Na^+, K^+, Ca^{2+} or CI^- content or the K^+/Na^+ ratio (Shahbaz and Ashraf, 2007). More recently, Shahbaz and co-workers (2008) observed a significant effect of foliar application of 24-EBR on growth and photosynthetic capacity of a salt-tolerant and a salt-sensitive cultivar of wheat. Under non-saline (normal) conditions leaf area and plant growth were improved in both tolerant and sensitive cultivars; however, under saline conditions improvement was observed only in the salt-tolerant cultivar. Furthermore, under saline conditions the photosynthetic capacity and photosystem-II efficiency, measured as Fv/Fm ratio, were markedly improved in both cultivars (Table 1). In a similar study, Houimli and co-workers (2008) determined that foliar application of 24-EBR to pepper (*Capsicum annuum* L.) plants grown under salt-stress significantly reduced the inhibitory effects of the salt on shoot growth and leaf relative-water-content and had a nonsignificant positive effect on root growth and chlorophyll fluorescence.

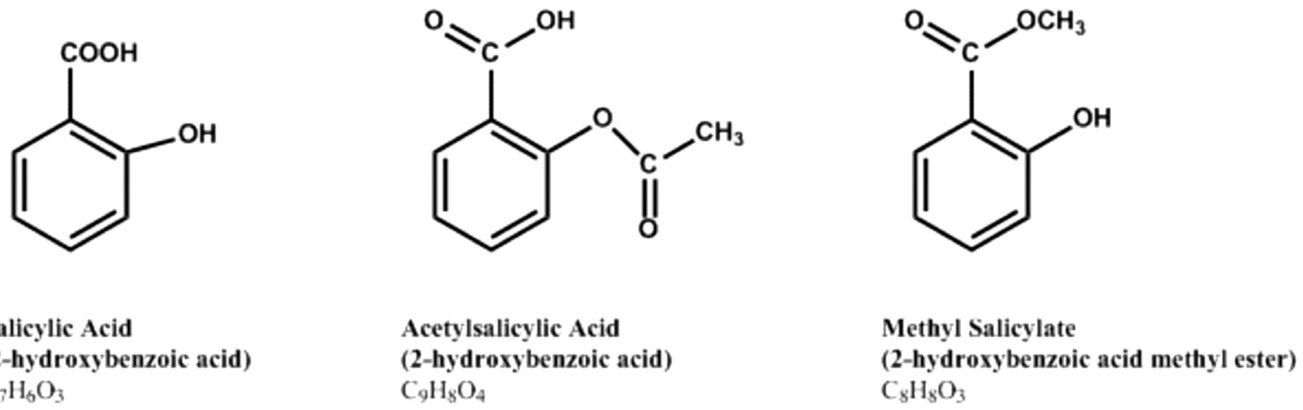
Exogenous application of BRs can also alter the activities of key antioxidant enzymes, particularly under salt-stress (Zhang *et al.*, 2007; Shahbaz *et al.*, 2008). For example, foliar application of 28-HBL or 24-EBR increased catalase activity in groundnut (*Arachis hypogaea* L.) (Vardhini and Rao, 2000) and Indian mustard (*Brassica juncea* L.) (Hayat *et al.*, 2000). In a recent study, Shahbaz and co-workers (2008) examined the effect of foliar applications of 24-EBR on the antioxidant system of two wheat cultivars differing in salt tolerance. Under salt-stress, activities of SOD, CAT and peroxidase (POD) were increased in both cultivars. Also, exogenous applications of 24-EBR reduced the effects of salt-stress in both cultivars by increasing POD and CAT activities, while SOD activity was unchanged. The overall results from these studies suggest the potential benefits of using foliar applications of BRs in reducing the adverse effects of salt-stress in different plant species.

4. Comparison of Different Methods of Application

For practical reasons, application of BRs via seed treatment or foliar spray is more convenient and more economical than root application. However, the effectiveness of foliar application is highly dependent upon the stage of plant development and the concentration of BRs used (Table 1). For example, most studies suggest that spraying during early vegetative growth is more effective than spraying at later stages (Zhang *et al.*, 2006; Ashraf and Foolad, 2007; Ali *et al.*, 2008). Furthermore, before application on a large scale, information on the proper formulation for spraying the solution is essential (Khripach *et al.,* 2000). For example, the spraying solution must contain additives, which could effectively facilitate spreading of the active substance(s) on the leaf surface (Shahbaz *et al.*, 2008; Akram *et al.*, 2009). Additives are included to delay dryness of the leaf surface and to ensure rapid penetration of BR through the cell walls. Similarly, the efficacy of pre-sowing treatment depends not only on the concentration of BR but also the length of the time seeds are soaked in the BR solution. BRs also could be added to the fertilizers or applied via fertigation under field conditions (Pirogovskaya *et al.*, 1996; Khripach *et al.*, 2000). This method of application not only may prolong the treatment period but also may minimize the required labor (Khripach *et al.*, 2000). However, it is important to note that the effectiveness of BR application may also vary depending on plant cultivars, climatic conditions, types of soil, and levels of applied fertilizers. For example, in a number of field trials, the effectiveness of BR on rice differed substantially at different temperatures and light conditions (Kamuro and Takatsuto, 1999). Therefore, it is imperative that, prior to field application on a large scale, the proper application protocols and BR concentrations for target growing conditions and plant species are determined.

III. SALICYLIC ACID

Salicylic acid (SA), an ortho-hydroxybenzoic acid [C_6H_4(OH)COOH] (Figure 4), is a phenolic compound, first identified in the bark of willow tree (*Salix sp.*) in 1828. It is a β-hydroxy acid, a colorless organic and crystalline material derived from the metabolism of salicin. Salicin ($C_{13}H_{18}O_7$) is an alcoholic β-glycoside that contains D-glucose with anti-inflammatory activities that is produced from all willow bark (Uchytil, 1991). SA is chemically analogous to the biologically active component of aspirin (acetylsalicylic acid). The salts and esters of SA are known as salicylates. SA naturally occurs in plants and plays important roles in plant growth and development, photosynthesis-related processes (Arfan *et al.*, 2007; Gemes *et al.*, 2008; Noreen and Ashraf, 2008; Noreen *et al.*, 2009) and ion uptake and transport (Kaydan *et al.*, 2007). SA also causes changes in leaf anatomy and chloroplast ultrastructure. It is involved in endogenous signaling and in the plant



Salicylic Acid
(2-hydroxybenzoic acid)
$C_7H_6O_3$

Acetylsalicylic Acid
(2-hydroxybenzoic acid)
$C_9H_8O_4$

Methyl Salicylate
(2-hydroxybenzoic acid methyl ester)
$C_8H_8O_3$

FIG. 4. Chemical structures of different types of salicylic acid (adopted from Taiz and Zeiger, 2006 with permission).

defense response against pathogens (Hayat and Ahmad, 2007). It is actively involved in plant resistance to pathogens by inducing the synthesis of pathogenesis-related (PR) proteins (Hooft Van Huijsduijnen *et al.*, 1986). It also plays a role in systemic acquired resistance (SAR) (Taiz and Zeiger, 2006). SA is moderately soluble in water, but highly soluble in polar organic solvents (Shalmashi and Eliassi, 2008). It is widely present in most agronomic plant species (Petersen *et al.*, 2000) and has also been found in many fruit, vegetable, herb, and spice crops (Robertson and Kermode, 1981; Petersen *et al.*, 2000). When applied exogenously, SA can actively move from the site of application to other parts of the plant where it is then metabolized or conjugated (Popova *et al.*, 1997). A variety of SA conjugates, which are formed by glucosylation or esterification have been found in many plant species. For example, a large quantity of SA glucosides was found in sunflower (*Helianthus annuus L.*), common bean, and common oat (*Avena sativa* L.) roots (Yalpani *et al.*, 1992). SA has been shown to act as a signaling molecule, modulating plant responses to various external biotic and abiotic stimuli (Ganesan and Thomas, 2001; Singh and Usha, 2003).

A. Biosynthesis

Salicylic acid may be synthesized via the phenylalanine or isochorismate pathways (Figure 5) (Kawano *et al.*, 2004; Mustafa *et al.*, 2009). The phenylalanine pathway is the most common pathway in plants. After a series of reactions, SA is produced by the enzyme benzoic acid 2-hydroxylase, which catalyzes the hydroxylation of benzoic acid at the ortho position (at C-2 position). Benzoic acid is synthesized through a series of reactions starting from cinnamic acid (trans-cinnamic acid) either via a β-oxidation of fatty acids or a non-oxidative pathway (Verberne *et al.*, 1999; Hayat *et al.*, 2007; Mustafa *et al.*, 2009). Trans-cinnamic acid is produced from phenylalanine by the action of the enzyme phenylanaline ammonia lyase (PAL; EC 4.3.1.5). This enzyme is known to be induced by different types of abiotic and biotic stresses and is a key regulator of the phenylpropanoid pathway, which gives rise to various types of phenolics with multiple functions (Yalpani *et al.*, 1993).

In the isochorismate pathway, chorismate is converted to isochorismate by the activity of isochorismate synthase (ICS; EC 5.4.99.6), which is subsequently converted to SA by isochorismate pyruvate lyase (EC 4.1.3.-) (Mustafa *et al.*, 2009). For example, the biosynthesis of the SA-analog 2,3-dihydroxybenzoic in Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don) takes place via isochorismate, whereas in some plants belonging to Rubiaceae family ICS gives rise to anthraquinones (Moreno *et al.*, 1994; Budi Muljono *et al.*, 2002). In *Arabidopsis*, ICS has been found to be involved in the biosynthesis of SA during the plant defense process (Wildermuth *et al.*, 2001).

B. Signaling

Since SA plays an essential role in plant defense against pathogens, a primary focus of research has been to uncover the mechanism of SA signal transduction with respect to pathogen infection. As a part of host-pathogen interactions, in the presence of a relevant resistance gene in the host, the binding of a pathogen to the plasma membrane of a host cell triggers a signal transduction pathway (STP), which leads to a hypersensitive response (HR) and destruction of the infected plant cells. During this process and before cell death, the cells release antimicrobial molecules to kill the invading pathogen (Verberne *et al.*, 2000). Simultaneouly, the dying cells are believed to release SA, which is frequently transported throughout the whole plant system and triggers the synthesis of antimicrobial molecules leading to an increased resistance against the pathogen (Figure 6). This mechanism, often referred to as systemic acquired resistance (SAR), prevents further infection of the plant by the pathogen (Mauch-Mani and Metraux, 1998). Although initially SA was believed to be a mobile signal involved in SAR, later studies have shown that SA is not a mobile signal and molecules other than SA are playing this role (Park *et al.*, 2007).

It has been proposed that SA binds to and inhibits catalases, which leads to the accumulation of H_2O_2 that may act as an antibiotic agent against pathogens. Alternatively, some derivatives of SA may act as potential intermediates in signal transduction

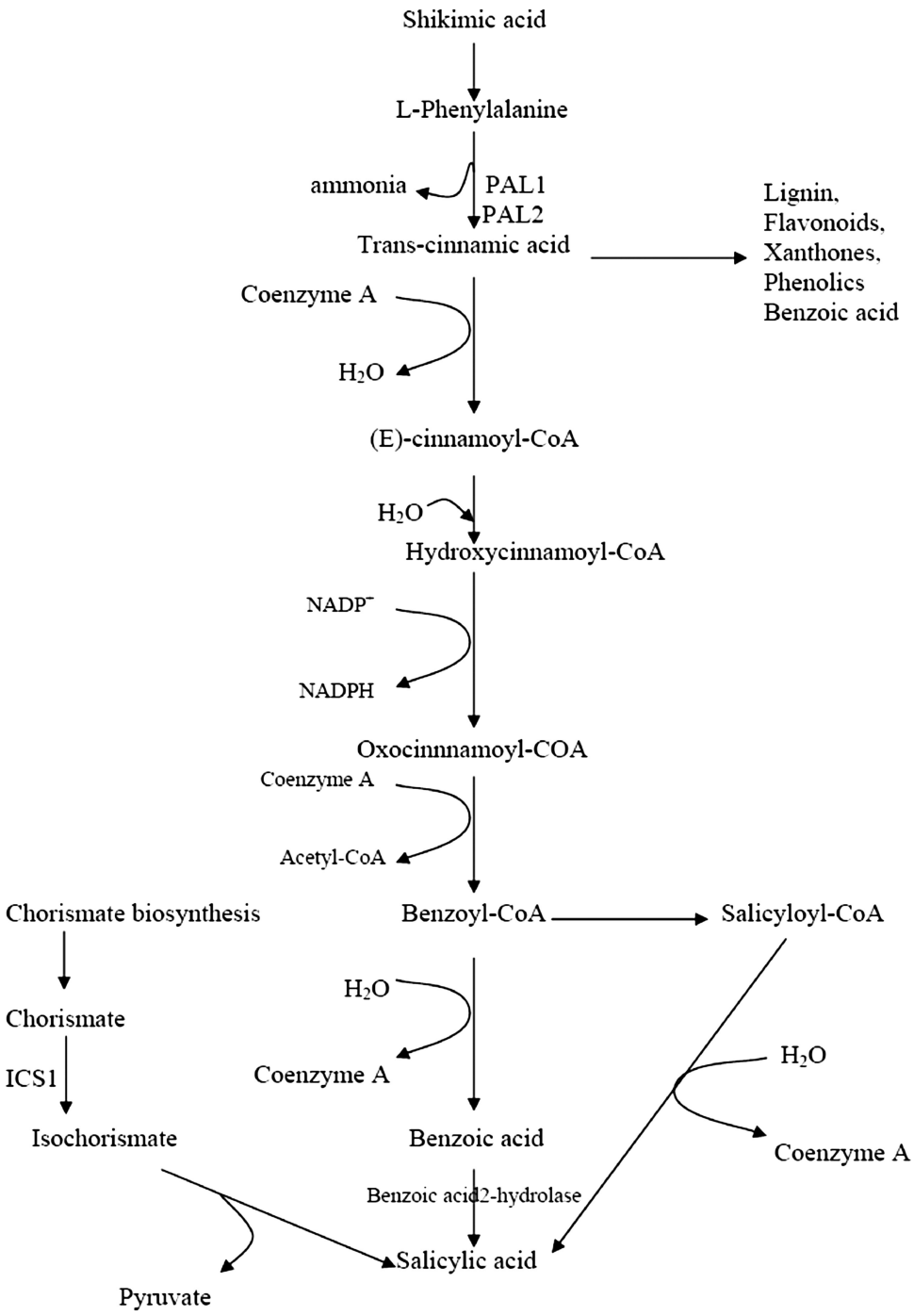


FIG. 5. Biosynthesis of salicylic acid. **Abbreviations used:** PAL1 and PAL2 (Phenyl ammonia-lyase 1 and 2): ICS1 (Isochorismate synthase). (Adopted from http://www.arabidopsis.org:1555/ARA/NEW-IMAGE?type=PATHWAY&object=PWY-981 accessed on 30/09/09 and modified according to Hayat et al., 2007 with permission).

pathways involved in defense-related gene expression (Chen *et al.*, 1993; Durner *et al.*, 1997). However, several reports are in disagreement with the SA-induced catalase inhibition hypothesis (Bi *et al.*, 1995; Leon *et al.*, 1995; Neuenschwander *et al.*, 1995; Tenhaken and Rubel, 1997), and some research suggests that SA gives rise to SA-free radicals that cause inhibition of heme-containing antioxidative enzymes such as catalases or peroxidases (Durner *et al.*, 1997). Such SA-free radicals may lead to lipid peroxidation, which could generate products that may trigger defense reactions (Goodman and Novacky,

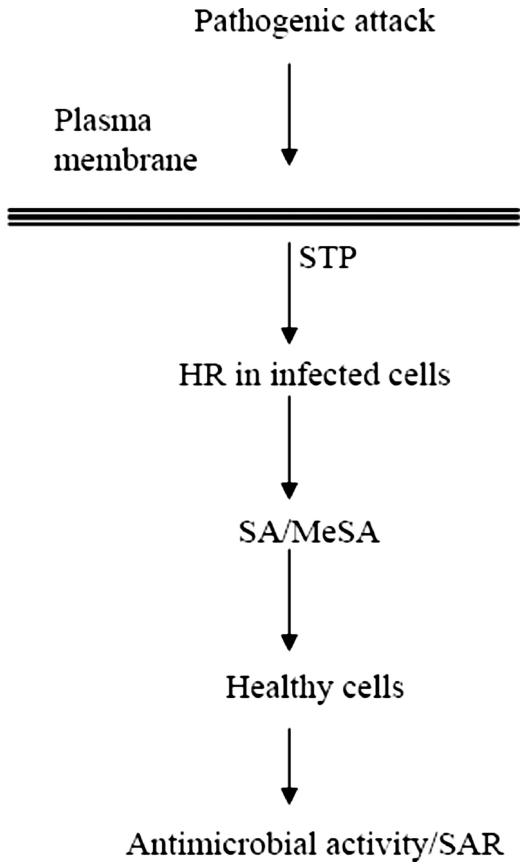


FIG. 6. Salicylic acid signaling. **Abbreviations used:** STP (Signal transduction pathway), HR (Hypersensitive response), SA (Salicylic acid), MeSA (Methyl salicylate), SAR (Systemic Acquired Resistance). (Reproduced with some modifications from Mauch-Mani and Metraux, 1998 and Park et al., 2007 with permission).

1994). However, it is unknown whether the products of lipid peroxidation would accumulate to sufficient levels to trigger defense responses effectively (Mauch-Mani and Metraux, 1998).

Du and Klessig (1997) found a 25-kDa soluble SA-binding protein in tobacco leaves that showed a reversible affinity for SA that was 150-fold higher than that for catalase. However, a variety of SA-binding proteins have been identified in different plant species, including SA binding protein 2 (SABP2) that has been shown to play a vital role in SAR (Chen *et al.*, 1993; Slaymaker *et al.*, 2002). SABP2 is an esterase with a strong preference for methyl salicylate (MeSA). Park and co-workers (2007) proposed the putative role of MeSA as a mobile signal in SAR. In addition, they proposed that there might be two mobile signals that induce SAR, i.e., a lipid-derived molecule (most likely jasmonic acid) and MeSA.

Although the role of SA in plant tolerance against a variety of biotic and abiotic stresses has been widely studied, very limited information is currently available regarding SA signaling in plants with respect to tolerance to abiotic stresses.

C. Physiological Roles

Salicylic acid plays prominent roles in different aspects of plant growth and development, photosynthesis, stomatal regulation, and ion uptake. Such roles have been verified in many plant species, including rice, tobacco, sunflower, beans and duckweed (*Spirodella polyrrhiza* (L.) Schleiden) (Yalpani *et al.*, 1993; Silverman *et al.*, 1995; Popova *et al.*, 1997). In duckweed, SA induces flowering and also modulates the production rate and content of anthocyanin and chlorophyll (Khurana and Maheshwari, 1980). In common oat, Harper and Balke (1981) observed that SA inhibited K^+absorption in excised roots, and the extent of inhibition was found to be both pH- and concentration-dependent. At a low pH, the inhibitory effect of SA was more pronounced. Similarly, in barley and oat roots, SA decreased K^+ absorption; however, this was dependent on concentration and pH of the SA solution. Furthermore, SA can greatly perturb the trans-membrane electrochemical potential of mitochondria and the ATP-dependent proton gradient of tonoplast-enriched vesicles (Macri *et al.*, 1986). In addition, a physiological function of SA in flower induction and bud formation in tobacco cell cultures has been observed (Eberhard *et al.*, 1989).

Recent research on the molecular aspects of SA-mediated growth and development in plants has helped provide a better understanding of the physiological roles of SA as well as its mechanisms of action. Generally, at the site of plant infection by a pathogen, a rapid change in ion flux and reactive oxygen species takes place, which subsequently initiates a signaling cascade leading to the activation of transcription factors that are involved in the activation of defense response genes. Many of these defense-related genes have been shown to be directly or indirectly involved in the synthesis of SA (Dangl and Jones, 2001; Metraux, 2002). SA plays an important role in the control of gene expression during leaf senescence (Morris *et al.*, 2000) and in advancing flowering time in plants such as *Arabidopsis* (Martınez *et al.*, 2004). The recent use of transgenic plants and mutants to study the modulation of developmental processes by applying SA has helped in the elucidation of the mechanism of action of SA in plant metabolism. For example, Abreu and Munne-Bosch (2009) used *NahG* (a bacterial *NahG* gene encoding SA hydroxylase) and SA-induction-deficient (*sid2*) mutants of *Arabidopsis* to assess the role of SA in plant development. The researchers suggested that the *sid2* gene, which encodes for isochorismate synthase, played a key role in SA biosynthesis during plant development and that SA modulated growth, senescence, and seed production. They also reported cross-talk between SA and plant hormones during plant development.

D. Exogenous Application

Exogenous application of SA has been reported to influence many processes in plants, including seed germination (Cutt and Klessing, 1992), glycolysis (Raskin, 1992), transpiration (Klessig and Malamy, 1994), stomatal closure (Larque-Saavedra, 1979), uptake and transport of nutrients (Harper and

Balke, 1981), membrane permeability (Barkosky and Einhellig, 1993), flowering and thermogenesis (Dempsey *et al.*, 1999; Raskin, 1992), and photosynthetic and growth rate (Khan *et al.*, 2003). In addition, it has been shown that SA is involved in leaf senescence (Morris *et al.*, 2000), fruit ripening (Srivastava and Dwivedi, 2000), gravitropism (Medvedev and Markova, 1991) and local systemic acquired resistance (SAR) by either evoking PR genes (Metraux, 2001; Yang *et al.*, 2004) or scavenging reactive oxygen species such as $^1O_2$, $O_2^{\cdot-}$, $\cdot OH$ and H_2O_2 (Chen *et al.*, 1993).

Stomatal regulation is a key process involved in the maintenance of photosynthetic capacity in plants (Athar and Ashraf, 2005; Arfan *et al.*, 2007; Noreen and Ashraf, 2008). Regulation of stomatal closure and opening affects the plant's transpiration and photosynthetic capacity, and thus its adaptation to different environmental conditions. Larque-Saavedra (1978) determined that exogenous application of acetylsalicylic acid (ASA) to common bean plants dramatically reduced the transpiration rate. The author speculated that ASA might have decreased the CO_2 concentration within the leaf tissues, thereby causing stomatal closure. Such reduced transpiration may be beneficial in reducing water loss under drought stress conditions. Rai and co-workers (1986) reported that SA antagonized the ABA-induced stomatal closure in the epidermis of Asiatic dayflower (*Commelina communis* L.). However, the prominent effects of SA on photosynthesis rate, stomatal regulation, chlorophyll content and respiratory pathways suggest that SA is possibly involved in the regulation of key photosynthetic reactions such as activity of RuBP carboxylase (rubisco) and CO_2 compensation point (Popova *et al.*, 1997). It has been determined that photosynthetic capacity in plants is regulated by stomatal and non-stomatal factors such as stomatal conductance, intercellular CO_2 concentration, transpiration rate, chlorophylls, carotenoids, efficiency of photosystem II, rubisco enzyme activity and concentration, supply of ATP and NADPH to carbon reductive pathway and exploitation of assimilation products (Dubey, 1997; 2005; Athar and Ashraf, 2005). Pancheva and co-workers (1996) showed that SA applied to barley seedlings for a 7-day period caused a marked reduction in photosynthetic rate and rubisco activity and enhanced CO_2-compensation point and stomatal resistance. In contrast, no significant effect was observed on these processes when SA was applied only for 2 h or less. The authors suggested that the 7-day treatment with SA might have caused stomatal closure, leading to a reduced supply of CO_2 to the photosynthetic machinery. However, because the concentration of substomatal CO_2 (C_i) remained unchanged in the SA-treated plants, the SA-induced regulation of photosynthesis was likely not due to stomatal factors. In another study with barley, SA-treated plants showed decreased levels of rubisco, confirming the involvement of SA in the regulation of photosynthesis through non-stomatal factors (Pancheva and Popova, 1997). Similarly, the carboxylation efficiency of Indian mustard was found to be a key aspect of the non-stomatal (metabolic) mechanism of photosynthesis, stimulated when 30-day-old plants were treated with aqueous solutions of SA (Fariduddin *et al.,* 2003). In conclusion, the above-mentioned studies clearly demonstrate that SA is involved in a multitude of physio-biochemical processes during plant growth and development; however, the extent of the regulation of specific metabolic processes depends on the plant species and environmental conditions.

E. Exogenous Application to Enhance Plant Salt Tolerance

There are numerous reports in the literature showing the beneficial effects of exogenous applications of SA in reducing the adverse effects of abiotic stresses in different plant species (Raskin, 1992; Munne-Bosch, 2007; Ahmed *et al.*, 2009). For example, it has been determined that exogenous application of SA improves tolerance/resistance to: ozone pollution in tobacco (Koch *et al.*, 2000), UV radiation (Surplus *et al.*, 1998) or high temperatures in *Arabidopsis* (Clarke *et al.*, 2004), water deficit in wheat (Bezrukova *et al.*, 2001; Singh and Usha, 2003), oxidative stress in rice, wheat, and cucumber (Shim *et al.*, 2003), salt or osmotic stress in wheat, *Arabidopsis,* barley, and tomato (Shakirova and Bezrukova, 1997; Borsani *et al.*, 2001; El-Tayeb, 2005; Ahmed *et al.*, 2009), and heavy metal stress in rice (Mishra and Choudhuri, 1999). However, in this review we focus only on the exogenous application of SA to improve plant salt tolerance. For such intended improvement, SA could be applied at the pre-sowing stage, through the rooting medium or as foliar spray during plant growth, as discussed below.

1. Pre-sowing Seed Treatment

Pre-sowing seed treatment with SA has been shown to be an effective means in counteracting harmful effects of salt-stress in many plant species (Table 2). Aldesuquy and co-workers (1998) reported that priming wheat kernels with SA reduced the inhibitory effects of salt-stress on seedlings by reducing the size and number of stomata, decreasing transpiration and maintaining turgidity. Afzal and co-workers (2005) demonstrated that SA-treated wheat kernels exhibited enhanced germination rate and produced vigorous seedlings under saline conditions. Similarly, Kaydan and co-workers (2007) determined that the application of SA to wheat kernels prior to sowing enhanced seedling emergence and increased leaf solute potential, shoot and root dry mass, K^+/Na^+ratio, and chlorophyll a, b, and carotenoid contents in salt-stressed seedlings. Deef (2007) showed that pre-treatment of wheat or barley kernels with SA resulted in elevated levels of glutathione, a potential non-enzymatic antioxidant, as well as salt tolerance of the resulting seedlings. In this study, accumulation of betaine was also high in the salt-stressed seedlings of both species raised from pre-treated kernels. Dolatabadian and co-workers (2008) observed that treating wheat kernels with SA prior to sowing significantly improved germination under both salt-stress and nonstress conditions. In this study, SA treatment of kernels also accelerated cell division in the growing roots and shoots, which resulted in enhanced growth of the plants. Furthermore, the authors observed that

TABLE 2
Modulation of growth and various physiological and biochemical processes of different plant species under salt-stress by exogenous application of salicylic acid (SA) through different modes, including seed treatment, root treatment and foliar spray

Mode of SA application	SA level	Salt-stress imposed	Plant species	Effects	Reference
Pre-sowing treatment	0.05 mM	150 mM NaCl	Wheat and barley	Increased growth rate and chlorophyll content, and improved photosynthetic rate and activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), and ascorbate peroxidase (APX)	Deef, 2007
	0, 0.5, and 1 mM	0, 50, 100, and 200 mM NaCl	Wheat	Increased seed germination, plant growth and activities of SOD, CAT and POX, and decreased polyphenol oxidase and lipid peroxidation	Dolatabadian *et al.*, 2008
	0.05 mM	2% NaCl	Wheat	Restored growth processes, increased cell division, accumulation of abscisic acid (ABA), indole acetic acid (IAA) and proline, and reduced damaging effects of salinity	Sakhabutdinova *et al.*, 2003
	0, 10^{-2}, 10^{-4} and 10^{-6} M	8 dS/m = 80 mM NaCl	Wheat	Improved seedling emergence, shoot and root dry weight, K^+/Na^+ratio, osmotic potential, photosynthetic pigments chlorophyll *a*, *b*, and carotenoids under saline conditions	Kaydan *et al.*, 2007
	50 mg L^{-1}	15 dS/m NaCl	Wheat	Enhanced seedling emergence, root and shoot length, fresh and dry weight, and decreased electrolyte leakage	Afzal *et al.*, 2005
	10^{-7}–10^{-4} M	100 mM NaCl	Tomato	Improved activities of SOD and CAT, and decreased accumulation H_2O_2	Szepesi *et al.*, 2008
	10^{-7}–10^{-4} M	100 mM NaCl	Tomato	Increased leaf water potential, chlorophyll *a* and carotenoid contents, photosynthetic electron transport and quenching, and decreased thiobarbiturate (TBA)	Tari *et al.*, 2002
	10^{-7}–10^{-4} M	100 mM NaCl	Tomato	Restored CO_2 fixation rate, and improved photosynthetic pigments and accumulation of soluble sugars	Gemes *et al.*, 2008
	10^{-7}–10^{-4} M	100 mM NaCl	Tomato	Higher accumulation of compatible osmolytes, improved photosynthetic efficiency, enhanced activities of ascorbate and guaiacol peroxidases in roots and carotenoids and polyamines in shoots.	Szepesi, 2006
	1 mM	0, 50, 100, 150 and 200 mM NaCl	Barley	Improved relative water content, fresh and dry weight, photosynthetic pigments, insoluble saccharides, phosphorus content and peroxidase activity, and decreased Na^+, soluble proteins, lipid peroxidation and electrolyte leakage	El-Tayab, 2005

	0.5 mM	0 and 100 mM	*Arabidopsis*	Improved seed germination	Rajjou *et al.*, 2006
Rooting medium	0, 0.25, 0.50, 0.75 and 1.00 mM	0 and 150 mM NaCl	Wheat	Improved growth and yield and photosynthetic capacity at 0.25 and 0.75 mM SA	Arfan *et al.*, 2007
	0.05 mM	100 mM NaCl	Wheat	Improved growth, cell division, accumulation of ABA and IAA, reduced salt-induced changes in phytohormones and enhanced proline accumulation	Shakirova *et al.*, 2003
	0.10 g L^{-1}	1.20% = 205.1 mM NaCl	Wheat	Enhanced ATP content	Shi-Gong *et al.*, 1999
	0.1 mM	150 and 200 mM NaCl	Tomato	Improved survival, relative shoot growth rate, photosynthetic rate, transpiration rate and stomatal conductance, and reduced electrolyte leakage	Stevens *et al.*, 2006
Foliar spray	10^{-2} M	0, 50, 100 and 150 mM NaCl	Maize	Increased emergence percentage, shoot and root dry weight, K^+/Na^+ ratio, chlorophyll *a*, *b*, and carotenoid content and leaf osmotic potential	Khodary, 2004
	1 and 2 mM (5-sulfo salicylic acid and acetylsalicylic acid)	125 mM NaCl	Maize	Improved total chlorophyll content, shoot dry matter, relative water content, ear weight and accumulation of macro- and micronutrients, and decreased antioxidative capacity	Tuna *et al.*, 2007
	0, 100, 200, and 300 mg L^{-1}	120 mM NaCl	Sunflower	Improved shoot and root fresh and dry weight, photosynthetic rate, stomatal conductance, and water use efficiency	Noreen and Ashraf, 2008
	0, 100, 200, and 300 mg L^{-1}	120 mM NaCl	Sunflower	Improved growth and photosynthetic capacity and activities of leaf SOD and POD	Noreen *et al.*, 2009
	0, 100, and 200 mg L^{-1}	150 mM NaCl	Wheat	Improved shoot and root fresh and dry weight, chlorophyll content, photosynthetic rate, and transpiration rate	Jabeen *et al.*, 2007
	1 mM (138 mg L^{-1})	0, 100 mM NaCl	Tomato	Decreased lipid peroxidation and Na^+ content, increased K^+ and Mg^{2+} contents, activities of SOD, CAT, GPX, DHAR and contents of ascorbate and glutathione	He and Zhu, 2008
	10 μM	0, 50, 100 and 150 mM NaCl	Indian mustard	Increased root and shoot length, leaf area, fresh and dry weight, and activities of catalase, peroxidase, superoxide dismutase, and accumulation of proline	Yusuf *et al.*, 2008
	0, 0.1, 0.5 and 1.0 mM	40 mM NaCl	Maize	Decreased lipid peroxidation, membrane permeability, and Na^+ and Cl^- accumulation, and increased UV-absorbing substances, H_2O_2, N, Mg, Fe, Mn and Cu levels	Gunes *et al.*, 2005

while salt-stress significantly increased the activities of key antioxidant enzymes such as superoxide dismutase, catalase and peroxidase, it decreased the activity of polyphenol oxidase in the resulting seedlings. This observation suggests that SA might have acted as a potential antioxidant and scavenged the reactive oxygen species (ROS) in the wheat seedlings exposed to saline conditions. The overall conclusion from these studies is that pre-sowing seed treatments with SA can be an effective means of enhancing seedling emergence, plant vigor and activities of enzymatic and non-enzymatic antioxidants.

In a recent study, young tomato seedlings were pretreated with SA for three weeks before they were exposed to salt-stress (Szepesi *et al.*, 2008). The pretreatment enhanced the activities of certain antioxidant enzymes, including superoxide dismutase, catalase, and peroxidase, in tomato plants when exposed to salt-stress. In a similar study, Szepesi (2006) noted that SA pretreatment of tomato seedlings resulted in a marked accumulation of Na^+ and compatible osmolytes (e.g., glucose, fructose, sorbitol, and proline) in the leaves of plants subjected to a saline regime, which enhanced osmoregulation and promoted better plant growth. The author also determined that the salt-induced reduction in leaf water potential led to the enhanced accumulation of ABA in the roots, which in turn enabled the plants to trigger the ABA signal transduction pathways and gene expression under saline conditions. Additionally, under saline conditions, the SA-pretreated plants accumulated higher amounts of compatible osmolytes such as glucose, fructose, sorbitol, and proline resulting in better photosynthetic efficiency and plant growth as compared with the untreated plants. Following a similar method of pretreating plants with SA and subsequently exposing them to salt-stress, Gemes and co-workers (2008) determined that the SA-treated tomato plants had improved photosynthetic capacity, enhanced photosynthetic pigments, and increased accumulation of soluble sugars under saline conditions, compared to the untreated plants.

2. *Application Through the Root Growing Media*

Salicylic acid application to growing media has been determined as an effective way to reduce the harmful effects of salt-stress in different plant species (Table 2). It has been shown that such applications may not only promote seed germination and seedling emergence, but also may accelerate subsequent plant growth, leading to enhanced crop yield. In *Arabidopsis*, for example, seed germination was improved with the addition of SA to the saline growth medium (Rajjou *et al.*, 2006). In tomato, a 4-fold increase in growth rate as well as significantly higher photosynthetic and transpiration rates were observed in SA-treated plants compared with the nontreated plants under saline conditions (Stevens *et al.*, 2006). In wheat, application of SA to the growing medium in combination with 2% sucrose mitigated the damaging effects of salt-stress on the seedling growth (Shakirova *et al.*, 2003). This treatment also enhanced the accumulation of ABA, IAA, and proline in plant tissues during the seedling stage. Shi-Gong and co-workers (1999) related the SA-induced growth promotion with the enhanced synthesis of ATP in some wheat genotypes under saline conditions, suggesting that SA maintains energy balance in plants under salt-stress. Recently, Ahmed and co-workers (2009) demonstrated that the application of SA to the growing medium of tomato plants grown under salt-stress considerably reduced the harmful effects of the stress by maintaining membrane integrity (as measured by ion leakage) and photosynthetic pigments. In addition, there was an increase in the uptake of K^+, Ca^{2+} and Mg^{2+} and a decrease in the uptake of Na^+ and Cl^-. Similarly, Misra and Saxena (2009) examined the effect of root-applied SA on plant growth and activities of key enzymes in proline metabolism in lentils. In this study, the SA application led to accelerated plant growth under both non-saline and saline conditions, and approximately a 5-fold increase in shoot proline content, which was ascribed to SA-induced increase in the activities of pyrroline-5-carboxylate reductase (EC: 1.5.1.2) and γ-glutamyl kinase (EC: 2.7.2.11). In conclusion, SA application to the growing medium appears to play a significant role in enhancing seed germination and early and late vegetative growth in different plant species grown under saline conditions. Unfortunately, however, there have been only a limited number of studies evaluating the beneficial effects of SA on crop production under field conditions. In particular, information is needed regarding the economical use of SA under field conditions. The high cost of SA may be a prohibitive factor for its field application. For example, an application of 200 mg L^{-1} SA may cost up to US$350 per ha (calculated based on US$ 4.75/g of SA, from Arfan *et al.*, 2007). Thus, further research is needed to determine ways in which exogenous application of SA could be done more efficiently. If successful, SA may be considered as a promising and environmentally friendly compound for crop protection and yield promotion in agriculture.

3. *Foliar Application*

Foliar applications of SA have been shown to be more effective than the other modes of application in promoting plant growth, or modulating different physiological processes for better adaptation under salt-stress. For example, in various studies on wheat plants grown under salt-stress, foliar applications of SA were determined to reduce the adverse effects of salt in different ways, including promotion of seedling growth, restoring plant growth and promoting accumulation of proline, ABA, IAA, and cytokinin (Hamada and Al-Hakimi, 2001; Sakhabutdinova *et al.*, 2003; Arfan *et al.*, 2007). In maize, foliar application of SA counteracted the damaging effects of salt on the plants by improving most of the growth attributes in addition to improving photosynthetic capacity and rubisco activity (Khodary, 2004) (Table 2). In sunflower, photosynthetic efficiency was improved under salt-stress with foliar application of SA (Noreen and Ashraf, 2008). In this study, application of SA did not alter sub-stomatal CO_2 concentration, thus it was concluded that stomatal opening and closing were probably not the determining factors in improving the photosynthetic efficiency.

Foliar application of SA has also been shown to alter the uptake and transport of inorganic ions in different plants. For example, in SA-treated maize plants grown under salt-stress, accumulation of N, Mg^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} increased significantly, whereas that of $Na^{+}$and Cl^{-} decreased (Gunes *et al.*, 2005). In tomato plants grown under saline (NaCl) conditions, foliar application of SA significantly reduced NaCl toxicity effects by decreasing Na^{+} and increasing K^{+} and Mg^{2+} in the roots and shoots (He and Zhu, 2008).

Salicylic acid is also an effective antioxidant, similar to many other non-enzymatic antioxidants known in plants (Rao and Davis, 1999). In addition to its direct effects as an antioxidant, SA may induce plant defense mechanisms by stimulating different antioxidant enzymes. By altering the activity of these enzymes, SA plays an important role in plant protection against osmotic, ionic and many other abiotic as well as biotic stresses (You-Sheng *et al.*, 2004; Horvath *et al.*, 2007; Catinot *et al.*, 2008; Yusuf *et al.*, 2008). However, the mode of action of SA may vary depending on its concentration. Generally, at lower concentrations, SA stimulates the activities of antioxidant enzymes, whereas at higher concentrations it acts as an antioxidant itself and reduces antioxidant enzymatic activities. For example, in the cultivated vanilla (*Vanilla planifolia*), lower concentrations of SA enhanced activities of SOD and POD, whereas at higher concentrations it reduced the activity of these enzymes (Chuan-Jai *et al.*, 2003). In tomato plants grown under salt-stress, foliar application with moderate concentrations of SA decreased lipid peroxidation and increased activities of the antioxidant enzymes SOD, CAT, GPX, and DHAR as well as the contents of ascorbate and glutathione (He and Zhu, 2008). In Indian mustard, foliar sprays at very low concentrations of SA to seedlings grown under NaCl-stress resulted in enhanced photosynthetic capacity and increased activities of two key enzymes of plant metabolism, carbonic anhydrase and nitrate reductase (Yusuf *et al.*, 2008). In addition, the activities of the antioxidant enzymes SOD, CAT and POX were also enhanced.

In addition to upregulating growth and various key metabolic phenomena under stress conditions, endogenous SA in low concentrations is effective in promoting growth under normal (nonstress) conditions. For example, by comparing SA-deficient *NahG* transgenic line and a SA-induction-deficient (*sid2*) mutant of *Arabidopsis* with wild type plants (which produce very high endogenous levels of SA), it was determined that low endogenous SA levels in *NahG* and *sid2* (both partially SA deficient) led to increased growth and decreased ABA levels, compared to the wild type plants (Abreu and Munne-Bosch, 2009). Low levels of endogenous SA in *NahG* and *sid2* also resulted in an average of 4-fold increase in seed yield, compared to the wild type plants. Furthermore, the *NahG* plants accumulated higher levels of α- and γ-tocopherol (vitamin E) and β-carotene (pro-vitamin A) in their seeds, compared to the wild type plants.

In addition to SA, a variety of SA analogs have been isolated from different plant species (Weissmann, 1991; Choh *et al.*, 2006; Park *et al.*, 2009), which may have beneficial effects on plant growth and development under salt-stress. For example, foliar application of SA analogs 5-sulfo SA and acetyl-SA resulted in increased total chlorophyll, shoot dry mass, relative water content and ear weight in salt-stressed maize plants (Tuna *et al.*, 2007). In this study, the levels of macro- and micro-elements in the roots and shoots of the treated plants were generally increased, whereas the activities of antioxidant enzymes SOD, POX and CAT decreased. The suppression in the activities of antioxidant enzymes may be due to the fact that SA analogs directly act as antioxidants. Therefore, it is possible that SA analogs have similar beneficial effects by modulating growth and physiological processes in plants.

4. Comparison of Different Methods of Application

Exogenous application of SA via seed treatment, root growing media or foliar spray exerts diverse physiological effects on plants with respect to their growth and development under normal (nonstress) and stress conditions, as discussed above. Specifically, it appears that exogenous application of SA is effective in triggering a variety of metabolic processes involved in plant salt tolerance, or improving plant growth and development under salt-stress. Although the exact mechanisms by which exogenous SA exerts its effects are unknown, its contribution to enhanced plant growth and productivity is clear. However, no study has been conducted to compare the effectiveness or efficiency of the different methods of SA application, though foliar application appears to be generally more effective in accelerating different physiological processes and improving plant growth and development. A major advantage of foliar application of SA, as compared to root application, is that it may be a less expensive approach to achieve improved crop growth under stressful environments. However, the effectiveness and efficacy of SA application may vary with its concentration, pH of the solution, solvent used for solution preparation, plant species, environmental conditions, and the plant developmental stage at which SA is applied. Therefore, before SA can be adopted as a routine commercial procedure to improve crop growth and productivity under different conditions, the factors affecting its efficiency must be optimized for each plant species.

IV. CONCLUSION AND FUTURE PROSPECTS

Exogenous application of BRs or SA is an effective approach to promoting plant growth and development under different growing conditions. However, different plant species may vary in their responses to exogenous application of BRs or SA. For any given species, there is no consensus as to the optimal concentration or treatment duration for BRs or SA to maximize crop productivity under stressful environments. It is likely that optimal concentrations of BRs and SA are species- or even cultivar-dependent. Furthermore, the effectiveness of exogenous applications of these growth substances may vary with plant developmental stages. Therefore, it is imperative that before

any commercial recommendation is made as to the exogenous application of BRs or SA to improve plant salt tolerance or to reduce the adverse effects of salt-stress, optimum rates and duration of application, as well as the most suitable plant developmental stage for application, is determined for each plant species of interest.

Although there have been several reports in the literature on exogenous application of BRs or SA as a useful approach improving plant growth and productivity under salt-stress, the mechanism(s) underlying this improvement remains unknown (Ashraf *et al.*, 2008). For example, it is unknown whether exogenous application of BRs or SA could compensate for the imbalance in other plant growth substances normally caused by salt-stress (Khan *et al.*, 2000; Debez *et al.*, 2001; Ashraf *et al.*, 2008), upregulate specific defense mechanisms against salt-stress, or merely enhance plant vigor. Such determinations will require extensive molecular and physiological examination of BR or SA treated and untreated plants grown under saline conditions.

To benefit from their effects, BRs or SA can be exogenously applied as a pre-sowing treatment, in the growing media or as a foliar spray. Each method has advantages and disadvantages and may be useful under specific growing conditions. For example, addition of BRs or SA to the soil at the field level may not be beneficial because of their possible degradation by soil microorganisms or their prohibitive cost. In comparison, foliar application of these plant growth substances may be more cost-effective as it requires relatively lower concentrations compared to their use as a soil amendment. Yet, the most cost- and labor-effective approach is probably the pre-sowing seed treatment, although its effects on improving crop salt tolerance may not be as pronounced as those with foliar application or incorporation through the root growing media. To improve the efficiency of the pre-sowing treatment in different plant species, it is necessary to optimize the contributory factors, including seed preparation (e.g., temperature and humidity during storage) and the concentration and duration of seed treatment with the BRs or SA. Furthermore, considering the high cost of pure BRs or their derivatives, there is a need to explore less expensive sources of BRs or effective synthetic analogs.

Although BRs have been shown to be essential for normal plant growth and development, there are still large gaps in knowledge between the perception of this hormone, the resulting signal transduction pathways, and the physiological responses in plants growing under stress conditions. Therefore, unraveling the salt-induced BR or SA signal transduction pathways appears to be a promising area of future research, and to this end, detailed biochemical and genetic studies need to be conducted to uncover various steps of such signal transduction pathways in plants exposed to salt-stress. Moreover, an improved knowledge of the underlying mechanisms of action of exogenously applied BRs or SA will certainly promote their efficient use in crop production under stressful conditions.

ACKNOWLEDGMENTS

The authors would like to thank Professors Dennis Decoteau, Mark Guiltinan, Dawn Luthe and James Sellmer for critical review of the manuscript before submission and helpful suggestions. This review article contains information gathered from numerous published and unpublished resources, and thus we would like to extend our appreciations to all authors of the references used in this manuscript.

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