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Review

# Brassinosteroids and their analogs: Feedback in plants under *in vitro* condition

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ABSTRACT

Brassinosteroids (BRs) are the sixth important plant hormones that induce their effect at micro-level concentration. The importance of its analogous under *in vitro* condition for many studies includes the study of response in plants aimed at improving various developmental, physiological, morphological, biochemical processes without the involvement of climatic factors, nutrients and diseases. BR improves and increases regeneration and multiplication rate by induction of callus growth, somatic embryos, tracheary elements, shoot elongation, formation of 2embryogenic tissue, stimulation of branch elongation, etc. These provide rapid disease-free clonal propagation of improved varieties within less time and space. BRs improve photosynthetic processes, antioxidant enzymes activities, synthetic seed production and elicitation of secondary metabolites production. Most importantly, it provides resistance from abiotic stresses, for example, oxidative and salinity stress. This review summarizes role of brassinosteroid and its various analogs in improving different morphological, physiological and biochemical attributes in various crop plants grown *in vitro*, both under normal as well as abiotic stress conditions.

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## 1. Introduction

Brassinosteroid (BR) is a plant steroid that shows its effect at the micro-level concentration (Kanwar et al., 2017). Its isolation was done from rape pollen (Mitchell et al., 1970). It can be perceived, synthesized and respond which is required for the functioning of plants i.e. growth and development (Peres et al., 2019). BRs play a very important biological role in a broad range of morphological processes, multiplication rate, metabolic role, photosynthetic processes, physiological processes (Ribeiro et al., 2020) and plant resistance to abiotic stresses (Ahammed et al., 2020). BRs can have high positive impact on the future of agriculture by regulating important horticultural and agronomic traits (Tong and Chu 2018).

All BRs can be distributed into three main categories based on carbon-number of each steroid molecule (i.e., $C_{27}$, $C_{28}$ and $C_{29}$) (Fujioka and Yokota 2003). Among all the BRs, Castasterone (CS), Brassinolide (BL), typhasterol (TY), 6-deoxoCS, teasterone (TE) and 28-norCS have shown to be broadly present in different plant species across different environments (Bajguz and Tretyn 2003). Considering the latest literature reports, 81 natural BRs were characterized which include five conjugates along with 137 BRs analogues and 8 metabolites (Liu et al., 2017). Brassinosteroids are derivatives of the 5-cholestane skeleton, with structural differences that result from the form and location of functional groups in the A and B rings and the side-chain (Fujioka and Yokota 2003). The synthetic BRs analogs are those which are synthesized by keeping the common patterns of organic functions in the A/B rings and cis-trans fusion between them, which occurs in some natural BRs, but with moderate or dramatic structural changes (shorter side chains, different oxygenated functions, spirostanic, aromatic and cyclic substituents, methyl esters, carboxylic acids (Wang et al., 2015; Bajguz, et al., 2020; Ferrer et al., 2021). The analogs are used as an alternative to manipulate plant height and offer the potential to improve production of crops (Duran et al., 2017). Its analogs have been synthesized for the physiological and biochemical studies and laboratory experiments (Ekinci et al., 2012). In this review, we discuss the effect of different BRs and their analogs, for example, Brassinolide(BL), 24-epibrassinolide(24-EBL), 28-Homobrassinolide (28-HomoBL), 3$\alpha$-monofluorohomotyphasterol (3F-HTY), 5$\alpha$-MonofluoroHomotyphasterol (5F-HTY), 5fluoro-28-homocastasterone(5F-HCTS), 28-homocastasterone (28-HCTS), Biobras-6 (BB-6) or MH-5 (spirostane analogs of BRs), under *in vitro* condition.

## 2. Importance of testing different responses of BRs and their analogs in plants under *in vitro* condition

The backbone of molecular science and genetic engineering is plant tissue culture (Teixeira et al., 2018). Tolerant species are selected based on changes in the environment as well as soil physico-chemical

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**Abbreviations**

| | |
|---|---|
| BB-6 or MH-5 | Biobras-6 |
| BL | Brassinolide |
| BRs | Brassinosteroids |
| BrZ | Brassinazole |
| 2,4-D | 2,4-Dichlorophenoxyacetic acid |
| 24-EBL | 24-epibrassinolide |
| 5F-HCTS5 | fluoro-28-homocastasterone |
| 28-HBL | 28-Homobrassinolide |
| 28-HCTS | 28-Homocastasterone |
| HTY | Homotyphasterol |
| $\mu$g | Micro-gram |
| $\mu$M | Micro-molar |
| $\mu$mol$^{-1}$ | Micro per mole |
| mg dm$^{-3}$ | milli gram per decimeter |
| ml L$^{-1}$ | Millilitre per litre |
| M | Molar |
| 3F-HTY | 3$\alpha$-monofluorohomotyphasterol |
| 5F-HTY | 5$\alpha$-monofluorohomotyphasterol |
| MS | Murashige and Skoog (1962) |
| ng | Nano gram |

properties. The response of particular hormones/elicitors and stress effects creates an actual challenge in the studies. Thus, the *in vitro* grown plants help to overcome such problems by managing required temperature, light, nutrients, elicitors, etc according to the need of the study. The preservation of plant's genetic materials could be done for a long time under *in vitro* and can be used whenever needed (Cruz et al., 2013). It is easy to produce disease-free plants under *in vitro* conditions within less time (Naik and Karihaloo 2007). The main aim of plant tissue culture is to increase the number of plant quantities on a large scale in labs (Xu and Huang 2014). *In vitro* approaches reduced many such limitations (Karan and Subudhi 2012). Many researches are being done on different analogs of BRs under *in vitro* condition which prove beneficial in many plants in several ways (Table 1; Fig 1). This review covers various aspects of the role of BR and its analogs in many families of the plants under *in vitro* conditions.

### 2.1. Family: Apocynaceae

#### 2.1.1. Periwinkle

BL increased the effective root number per plant and rooting rate during *in vitro* rooting of *Vinca minor* seedlings in the test tube (Da-Zhi et al., 2012).

### 2.2. Family: Arecaceae

#### 2.2.1. Coconut

Coconut is grown as cash crop in tropical areas but due to loss of senescence in some palms, its susceptibility to diseases and low yield from seed propagation, it requires regular planting. An alternative method for its massive propagation is *in vitro* cloning. For this plant, plumule is used as explant and was tested for callus initiation; the formation of the somatic embryo *in vitro* was possible by application of 22(S), 23(S)-HBL. By supplementation of BL @ 0.01 or 0.1 $\mu$M per explant for three days, the highest amount of somatic embryos was produced (Azpeitia et al., 2003).

### 2.3. Family: Asteraceae

#### 2.3.1. Indian head

It is a famous medicinal plant that is used all over the world in the form of capsulated root extracts or teas for the treatment of various diseases, especially upper respiratory diseases. The valuable metabolites production (total flavonoids, total phenolics, cichoric acid, p-coumaric acid caftaric acid and echinacoside) are found in this plant which are synthesized mainly in root parts. Seeds of this plant cultured on MS medium supplemented with 24-EBL @ 1 mg L$^{-1}$ increased the secondary metabolites by *Agrobacterium rhizogenes* mediated Indian head (*Echinacea purpurea* L. Moench) hairy roots as compared to l-phenylalanine @ 500 $\mu$M. Moreover, 24-EBL @ 0.5 mg L$^{-1}$ increased the fresh root weight, dry growth index and root growth was obtained from the roots, while no influence on root growth with l-phy was noticed (Demirci et al., 2020).

#### 2.3.2. Lettuce

In lettuce, using cotyledons as explants, the BB-6 and MH-5 @ 0.1 mg L$^{-1}$ along with 6-benzylamino purine in the culture medium increased both the formation of callus and regeneration of shoot. They were only active in combination with cytokinin (Nunez et al., 2003).

### 2.4. Family: Cruciferae

#### 2.4.1. Mustard

Mustard is an important oilseed crop that is used widely all over India for its consumption as cooking oil mainly. BR is applied in mustard crop to know whether any positive effect on this crop occurred concerning its improvement in regeneration capacity and multiplication processes, the composition of fatty acids, etc. The novel way for the regeneration of *Brassica juncea* was achieved by thidiazuron (TDZ) which is an analog of cytokinin @ 6.8 $\times$ 10$^{-3}$ $\mu$mol$^{-1}$ that promoted callus for the formation of adventitious buds. The regeneration was promoted when their interaction (Hormones; TDZ and BL) affected the content of BL to mediate CYCD3-1 (Lu et al., 2020).

In winter oilseed rape, 24-EBL affected fatty acids and sugar content at 20°C and 5°C when callus was cultured. The action of 24-EBL depends on the high temperature. The sugar content was decreased by 24-EBL @ 100 nM only in calli cultured at 20C°. At 5°C, 24-EBL increased sugar content. 24-EBL at 20°C had the same effect on the composition of fatty acids of phospholipids as the cold in the process of ice-hardening of oilseed rape calli. 24-EBL decreased palmitic acid (18:1) and linoleic acid (18:2) and increased 18:3 fatty acid content. At 5°C, 24-EBL (100 nM) did not influence fatty acid composition. In the case of digalactosyldiacylglycerols and monogalactosyldiacylglycerols, the effect of 24-EBL on the fatty acid composition is still temperature-dependent (Janeczko et al., 2009).

In *Brassica napus*, the endogenous BL level regulates the development of microspore-derived embryos (MDEs). When treated with BL or brassinazole (BrZ), nucleotides synthesis and degradation in this crop was calculated based on the development of MDEs. The substrates of salvage pathway adenine and adenosine metabolic fate were identified by purine metabolism. The de novo (orotic acid), degradation (uracil) and salvage (uridine and uracil) were used as markers for pyrimidine pathways. Their utilization increased with BL treatment on the 15$^{th}$ day and remained high during the culture period. The salvage enzymes activities: adenine phosphoribosyltransferase, adenosine kinase and uridine kinase occurred by the treatment of BL application. BL induced the salvage synthesis by the degradation pathways reduction. The embryos treated with BrZ decreased the salvage activity of both purine and pyrimidine nucleotides. BrZ performed poor embryonic performance and structural abnormalities (Belmonte et al., 2010).

In Pakchoi (*Brassica rapa* var. multiceps), in the three Pakchoi genotypes - $XM_1$, $XM_2$, $XM_3$ the four different concentrations of BL in NLN liquid medium were applied. The major constraints like the frequency of embryogenesis in micropore and regeneration rate of plant was induced with the treatment of BL @ 0.01 mg L$^{-1}$ in $XM_2$. The rate of callus formation decreased in '$XM_1$' and '$XM_3$' when treated with BL @ 0.02 mg L$^{-1}$. The doubling rate from the three genotypes of

**Table 1**
Application of Brassinosteroids and their analogues under *in vitro* condition in various plants

| S.No | Crop/Plants | BRs concentration | Feedback | Reference (s) |
|---|---|---|---|---|
| 1. | Marubakaido apple rootstock (*Malus prunifolia* (Willd.) Borkh] | 5F-HCTS @ 500 ng per shoot | Multiplication rate enhanced | Schaefer et al. (2002) |
| 2. | Coconut (*Cocosnucifera* (L.) | 22(S), 23(S)-HBL (0.01 or 0.1.$\mu$ M) | Induce somatic embryogenesis | Azpeitia et al. (2003) |
| 3. | Rice (*Oryza sativa*) | BB-16 (0.01 mg dm-3)+ NaCl (75 mM) | Increase activities of antioxidant enzymes | Nunez et al. (2003) |
| 4. | Lettuce (*Latuca sativa*) | BB-6 and MH-5 @ 0.1 mg $L^{-1}$ + 6-Benzylaminiourine- | Formation of callus and regeneration of shoot and only active in combination with cytokinin | Nunez et al. (2003) |
| 5. | Cord grass (*Spartina patens*) | BL @ 0.03 mg $L^{-1}$<br>BL @ 0.005 mg $L^{-1}$ | Growth of callus and regeneration of shoot enhanced, enhance the growth of regenerated shoot growth | Lu et al. (2003) |
| 6. | Marubakaido apple rootstock (*Malus prunifolia*) | 28-homocastasterone (5F-HCTS) @ 0.5 $\mu$g per shoot) | Elongation of branch stimulated in shoots grown under *in vitro* condition | Pereira-Netto et al., 2006a |
| 7. | Eucalyptus Hybrid (*Eucalyptus grandis* × *E. urophylla*) | 28-HCTS<br>28-HCTS (5F-HCTS) | BRs change shooting patterns in Eucalyptus. Elongation and new main shoots formation enhanced<br>Elongation or new main shoots formation are not stimulated | Pereira-Netto et al., 2006b |
| 8. | Himalayan pine (*Pinus wallichiana* A. B. Jacks) | 24-EBL @ 2.0 $\mu$M + 9.0 $\mu$M 2,4-D | Embryogenic tissue formation from mature zygotic embryos enhanced | Malabadi and Nataraja (2007) |
| 9. | Sugarcane (*Saccharum* spp.) | BB-6 and MH-5 @ 0.001 and 0.01 mg $L^{-1}$ | No role in somatic embryo production but proline levels (changes indicates role in stress tolerance), storage proteins and total soluble proteins were influenced by both of them | Nieves et al., (2008) |
| 10. | Winter oilseed rape (*Brassica napus* L.) | 24-EBL @ 100 nM | Composition of fatty acids and sugar content increased at high temperature (20°C) and decreased at lower temperature (5°C) | Janeczko et al. (2009) |
| 11. | *Brassica napus* | BL @ $10^{-7}$ M,<br>BrZ @ 4 × $10^{-6}$ | Salvage synthesis promoted by BL<br>Salvage activity of both purine and pyrimidine nucleotides is reduced in BrZ-treated embryos | Belmonte et al. (2011) |
| 12. | *Brassica napus* (Bactool variety) | BL Exogenous applications<br>BrZ, a BL biosynthetic inhibitor @ 4 × $10^{-6}$ M | The activity of APX increased in microspore-derived embryo APX activity decreased | Khamiss et al. (2011) |
| 13. | Groundnut *(Arachis hypogaea* L.) Genotypes (M-13 and PBS24030) | BL in combination:<br>BL @ 1 mg $L^{-1}$ + 3 mg $L^{-1}$ BA<br>BL @ 2 mL $L^{-1}$ + 3mg $L^{-1}$BA.<br>BL only<br>Highest @ 2 mL $L^{-1}$ with BA in M-13<br>BA @ 1.0 mL $L^{-1}$ | Increase shoot multiplication in both cultivars<br>Flowering in PBS24030 genotypes<br>Rhizogenesis<br>Total chlorophyll content and HRA in M-13 in PBS24030 | Verma et al. (2011) |
| 14. | Periwinkle (*Vinca minor*) | BL | Increase the effective root number per plant and rooting rate. | Da-zhi (2012) |
| 15. | Yam (*Dioscorea* Spp.) *Dioscorea alata, D. rotundata*, one *D. cay-enensis*and *D. trifida* | 24-EBL @ 0.1 $\mu$M + 0.23 $\mu$MGibberellic acid | Reduce the oxidation level in four of the five accessions during *in vitro* culture. | Engelmann-Sylvestre and Engelmann (2013) |
| 16. | Hybrid poplar (*Populus sieboldii, P. grandidentata*) | BL @ 1.0 mM | Tracheary elements formation in calli derived from petioles is induced | Yamagishi et al. (2013) |
| 17. | Orchid (Hybrid *Cymbidium*) | 24-EBL and HBL | Inhibit protocorm-like body development | |
| 18 | Dwarf Pear (*Pyrus comuunis*) | BL @ 1 mg $L^{-1}$ | Increases height and internode length of the plantlet, acceleration of differentiation of vascular bundles in stems and primary veins of leaves, increased the density of mesophyll cells and the thickness of cuticle | Chen et al. (2014) |
| 19. | Common bean *Phaseolus vulgaris* L. cv. Brunca | 24-EBL @ 0.1 mg $L^{-1}$ + TDZ @ 2 mg $L^{-1}$ | Improves shootlets percentage, leaf number, photosynthetic pigments, number and length of shootlets (cm) | Taha et al. (2014) |
| 20. | Banana cv. Rasthali | BL @ 1 $\mu$M | Tracheary elements differentiation from embryogenic cells in the form of elongated cells with secondary wall deposition as characteristic mark<br>Important genes of lignin pathway genes like CCoAOMT, CCR, and COMT were increased at 5$^{th}$ day of BL treatment | Negi et al. (2015) |

(*continued*)

**Table 1** (*Continued*)

| S.No | Crop/Plants | BRs concentration | Feedback | Reference (s) |
|---|---|---|---|---|
| 21. | Tomato (*Lycopersicon esculentum*) | Natural and synthetic BRs | Nitric oxide production in tomato cells triggered by it | Tossi et al. (2015) |
| 22. | Potato (*Solanum tuberosum* L.) cultivars Cardinal and Desiree | Pretreatment with 24-EBL @ 1 $\mu$M Combination of both 2 $\mu$M 24-EBL addition directly into the MS medium + NaCl @ 0, 40, 60, or 80 mM | For total soluble protein: Suitable for Cardinal cultivar Suitable for Desiree cultivar Plant growth subjected to NaCl stress is reduced | Khalid and Aftab (2016) |
| 23. | Potato (*Solanum tuberosum*) | BL @ 0.1 and 0.01 $\mu gL^{-1}$ BL @ 1–100 $\mu gL^{-1}$ BL @ 50 $\mu gL^{-1}$ | Elongation of root and lateral root development is promoted Inhibits root elongation Tolerance to salt stress | Hu et al. (2016) |
| 24. | Potato (*Solanum tuberosum*) Sante and Agria cultivars | 24-EBL @ $10^{-6}$ M | Increase the shoot length, regrowth rates and speed in both cultivars | Ghanbarali et al. (2016) |
| 25. | *Dendrobium* cultivars (Rungnappa Red, Miss Snow White and Earsakul) | HBL @ 0.5, 1.0, 2.0 and 4.0 mg $L^{-1}$ | Induces regeneration of three different cultivars at different concentrations | Kumari et al. (2016) |
| 26. | Sugarcane Co.86032 (*Saccharum* spp.). | BL @ 5 $mgL^{-1}$ | Increases shoot length, leaf number and fresh weight | Chavan et al. (2018) |
| 27. | Potato (*Solanum tuberosum*) | Gibberellic acid (GA) + Napthalene acetic acid (NAA) + 24-EBL at + 0.1 $\mu$M +0.1 $\mu$M concentration mixture | Number of nodes, the number of branches, shoot length, number of leaves, culture survival increased | Harshlata Tomar and Sai, (2018) |
| 28. | Coffee (*Coffea Arabica* L.) | 24-EBL @ 1.0 $\mu$M + 2-Ip | Cytokinin efficiency increases in leaf explants for somatic embryogenesis induction directly | Chone et al. (2018) |
| 29. | Potato (*Solanum tuberosum*) cv Désirée | 24-EBL @ 0.3 $\mu$M 24-EBL @ 0.04 $mgL^{-1}$ | Genes were highly expressed in leaves, downstream transcription factor gene *StBZR1-2* was highly expressed in stolons and tubers, The number and the total weight of potato tuber due to the activated light-period signal for tuberization, An important role in potato tuberization | Zhu et al. (2019) |
| 30. | *Coffea arabica*L. (Rubiaceae) | 24-EBL @ 1.0 $\mu$M + 2-Ip | Cytokinin efficiency increases in leaf explants for somatic embryogenesis induction directly | Chone et al. (2018) |
| 31. | Potato (*Solanum tuberosum*) cv Lugovskoy (identifier, 8301891) | 24-EBL and HBL concentration range from $10^{-11}$ to $10^{-8}$ M. | Delay salinity stress by compounds accumulation having antioxidant activity and by increasing salinity tolerance | Efimova et al. (2019) |
| 32. | Marubakaido apple rootstock (*Malus prunifolia*) | 2.5 $\mu$g per shoot of 5F-HTY, HTY & HTY and 3F-HTY | Main shoots and primary lateral shoots length increased, Stimulates elongation of the main shoot | Pereira Netto et al. (2019) |
| 33. | Pakchoi (*Brassica rapa* var. multiceps) Three Pakchoi genotypes: $XM_1$, $XM_2$,$XM_3$ | BL $XM_1$ and $XM_3$=0.02 $mgL^{-1}$, $XM_2$=0.01 $mgL^{-1}$ | Induced frequency of embryogenesis of microspore and regeneration rate of the plant, rate of callus formation decreased, Traits were identified from $XM_2$ Double Haploid lines, increased number of leaf and bright leaf color | Jia et al. (2019) |
| 34. | Potato (*Solanum tuberosum*) | 24-EBL @ $10^{-10}$ M+ 100 mMNaCl | The activity of POD and $K^+/Na^+$ ratio in potato leaves is improved | Kolomeichuk et al. (2020) |
| 35. | Indian head (*Echinacea purpurea* L. Moench) | 24-EBL @ 1.0 mg $L^{-1}$ 24-EBL @0.5 mg $L^{-1}$ | By *Agrobacterium rhizogenes* mediated hairy roots Increased secondary metabolites production Increase the growth index, fresh root weight, dry root weight | Demirci et al. (2020) |
| 36. | Mustard (*Brassica juncea*) | TDZ @ 6.8 × 10−3 $L^{-1}\mu$mol) | BRs content influenced by TDZ in the callus and their interactions mediate *CYCD3-1* for promotion ofregeneration | Lu et al. (2020) |

Pakchoi was maximum in $XM_1$ and $XM_3$ and minimum in $XM_2$. The self-compatibility, high stability, consistency and agricultural traits like the number of leaf and bright leaf colors were identified from double haploid lines which proved helpful in microspore technology in hybrid breeding (Jia et al., 2019).

Different levels of BL were examined for ascorbate peroxidase (APX) activity in *Brassica napus* (Bactool variety) during embryo development; APX activity increased in the microspore-derived embryo by exogenous application of BL. BrZ @ 4 × $10^{-6}$ M decreased the APX activity and the content of ascorbate increased in the reduced form by increasing the amount of toxic compound i.e. hydrogen peroxide. The physiological studies related to androgenesis system in this crop involved a large number of matched embryos released in less time from immature pollens *in vitro* (Khamiss et al., 2011).

### 2.5. Family: Discoreaceae

#### 2.5.1. Yam

The shoot tips of Yam species were grown in culture medium to analyze the effect of growth regulators of two different combination media. The medium 24-EBLhad 0.23 $\mu$M gibberellic acid and 0.1 $\mu$M 24-EBL while medium S contained 0.6 $\mu$M benzyl adenine, 1.07 $\mu$M naphthalene acetic acid, 0.23 $\mu$M gibberellic acid. The medium 24-EBL did not have any positive role regarding growth but it reduced the oxidation level in a two months-old plant (Engelman et al., 2013).

### 2.6. Family: Poaceae

#### 2.6.1. Rice

BB-16 modulates the antioxidant enzyme activity and provides tolerance from salt stress. When three days old *in vitro* rice seedlings were grown under salt stress for 16 days in the culture medium containing NaCl @ 75 mM and BB-16 @ 0.001 to 0.01mg $dm^{-3}$, afterwards for four days the seedlings were exposed to the same concentration of BB-16 and then further grown in the medium containing NaCl, but not BB-16. The activities of catalase, superoxide dismutase, glutathione reductase, ascorbate reductase were enhanced in seedlings exposed to BB-16 for 16 days, whereas those of superoxide dismutase and catalase enhanced by exposing seedlings to BB-16@ 0.001 mg $dm^{-3}$ for 4 days (Nunez et al., 2003).

#### 2.6.2. Cordgrass

The cultured calli of this grass having hormones indole-3-acetic acid (0.2 mg $L^{-1}$) and 6-benzyl amino purine (3.0 mg $L^{-1}$) in MS medium when treated with BL @ 0.03 mg $L^{-1}$ enhanced both the growth of callus and shoot regeneration capacity. BL @ 0.05 mg $L^{-1}$ after 40 days of culture increased the shoot height ratio whereas BL @ 0.005 mg $L^{-1}$ enhanced the growth of regenerated shoot growth (Lu et al., 2003).

#### 2.6.3. Sugarcane

Treatment with BB-6 and MH-5 @ 0.001 and 0.01 mg $L^{-1}$, respectively showed no role in somatic embryo production in sugarcane. However, the proline levels, storage proteins and total soluble proteins were influenced by both of them. The changes or increase in proline levels indicated that the application of these BRs had a role in stress tolerance rather than vegetative propagation in sugarcane (Nieves et al. 2007). Sugarcane is an important cash crop and its improvement regarding growth is a necessary step for increasing its yield. The growth of Co.86032 variety under *in vitro* condition was affected by BL either directly or through auxin and cytokinin stimulation. The length of shoot, the number of leaves and fresh weight increased when grown on a medium having BL @ 5 mg $L^{-1}$ (Chavan et al., 2018).

### 2.7. Family: Fabaceae

#### 2.7.1. Groundnut

The combination of BL alone or with Benzylamino-purine (BA) had some positive effect on groundnut cultivars (M-13 and PBS24030) concerning the multiplication of shoot, anti-oxidant activities (catalase, peroxidase, ascorbate peroxidase, polyphenol oxidase), chlorophyll content and Hill reaction activity. The cotyledonary node was used as explants and put on culture medium containing BL @ 1 ml $L^{-1}$ with BA @ 3 mg $L^{-1}$ which enhanced the shoot multiplication in both the cultivars. BL @ 2 ml $L^{-1}$ with BA @ 3 mg $L^{-1}$ induced flowering in PBS24030, BA @ 2 ml $L^{-1}$ in M-13 and BA @ 1.0 ml $L^{-1}$ in PBS24030 enhanced the total chlorophyll content, Hill reaction activity, BL alone induced rhizogenesis, BL alone or with BA enhanced the antioxidant activities (Verma et al., 2011).

#### 2.7.2. Common bean

It is an important legume crop used worldwide for its low-cost nutritional properties, so its production needs to be increased. It's *in vitro* shootlets regenerated via direct organogenesis in culture media containing 2 mg $L^{-1}$ thidiazuron (TDZ) and 0.1 mg $L^{-1}$24-EBL proved best for shootlets percentage, photosynthetic pigments, number of leaves, shootlets number and length of shootlets (cm). The stem explants gave the best results of the percentage of the shoot proliferation compared with root or shoot explants, respectively (Taha et al., 2014).

### 2.8. Family: Orchidaceae

#### 2.8.1. Orchid

28-HBL induced shoot regeneration when treated with BL @ 0.5, 1.0, 2.0 and 4.0 mg $L^{-1}$ in half-strength basal medium. From different cultivars 'Rungnappa Red' and 'Earsakul' BL @ 4.0 mg $L^{-1}$, 'Miss Snow White' BL @ 0.5 mg $L^{-1}$ shoot multiplication was gained in minimum days of 28-HBL concentration. The maximum shoots number gained in 'Rungnappa Red', Miss Snow White' and 'Earsakul' from 28-HBL concentrations at 0.5, 2.0 and 4.0 mg $L^{-1}$ respectively (Kumari et al., 2016).

### 2.9. Family: Musaceae

#### 2.9.1. Musa species

For induction in the differentiation of tracheary elements in banana, BL was applied and tracheary elements were formed when BL @ 1 $\mu$M was supplied in embryogenic cell suspension culture of banana for about ten days which helped in understanding the genes responsible for secondary wall thickening. Some important genes (COMT, CCR, CCo, and AOMT) were expressed early by the BL treatments as indicated by lignin biosynthesis pathway expression analysis (Negi et al., 2015).

### 2.10. Family: Myrtaceae

#### 2.10.1. Eucalyptus hybrid

The comparative effects of 28-HCTS and 5F-HCTS revealed that the exogenously supplied BRs were able to change the shooting pattern in Eucalyptus which gives the idea about the improvement in the micropropagation process. The *in vitro* grown shoots of this plant treated with 28-HCTS at very low doses enhanced the formation and elongation of new main shoots (the shoots originating directly from the initial explant) and reduced the formation and elongation of the primary lateral shoot (shoots originating from the main shoot). However, 5F-HCTS neither stimulated nor formed the new main shoots but stimulated elongation of the primary shoot (Pereira-Netto et al., 2006a).

### 2.11. Family: Pinaceae

#### 2.11.1. Himalayan pine

For the conservation of indigenous and commercial forest species of *Pinus* where its frequency is very low *in vitro*, the response of 24-EBL was studies for inducing somatic embryogenesis; it was observed that 24-EBL @ 2 $\mu$M with 9.0 $\mu$M 2,4-D enhanced the formation of embryogenic tissue from mature zygotic embryos on half-strength MSG basal medium, inHimalayan pine (Malabadi and Nataraja 2007).

### 2.12. Family: Rosaceae

#### 2.12.1. Pyrus communis

Dwarfing pear tube seedling as a material was taken and different concentrations of exogenous BL were added to the media, and the effect on phenotypes of the plantlets *in vitro* was studied. The higher

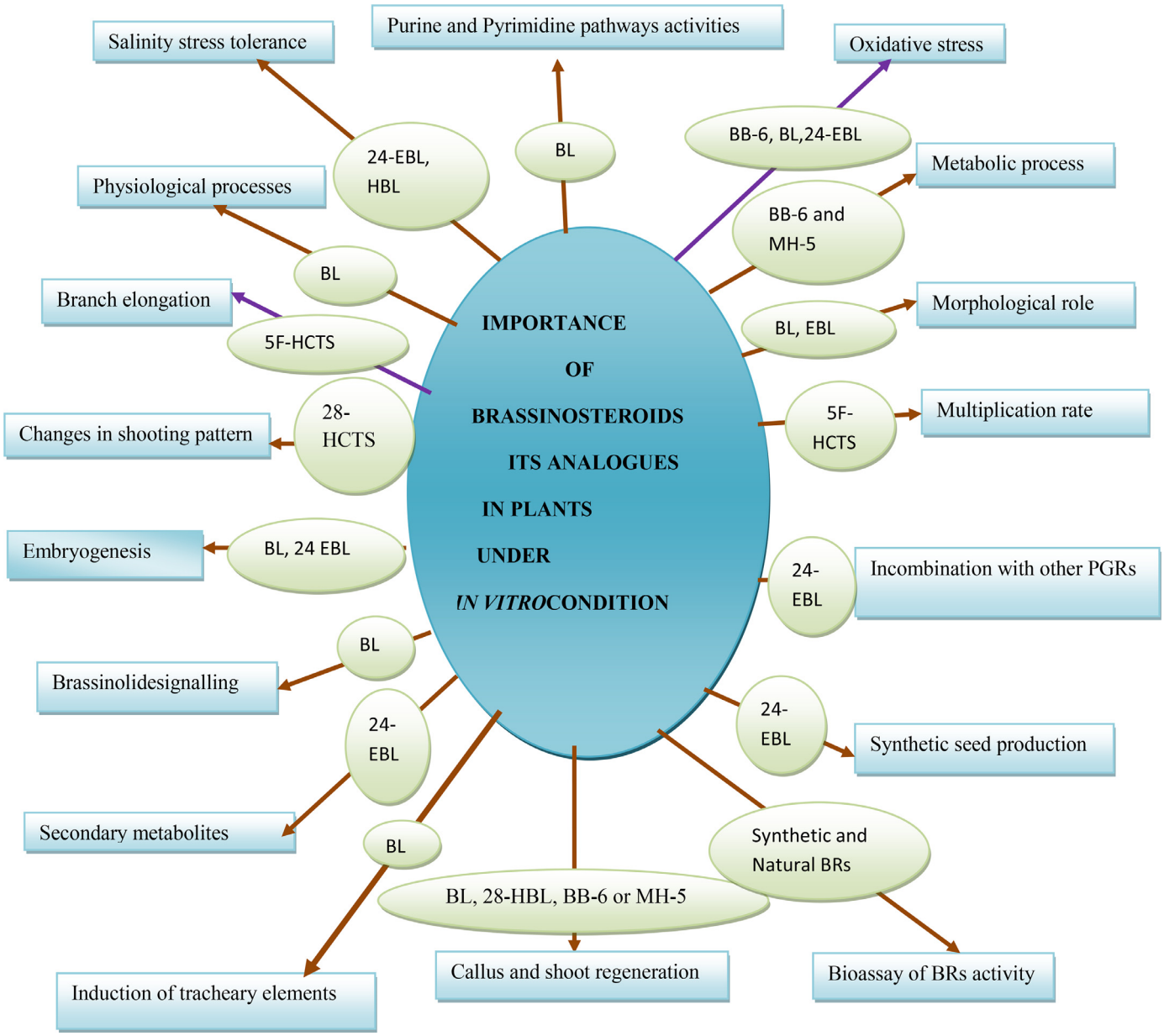



**Fig. 1.** Importance of BRs and their analogs in nut shell

concentration of BL increased the height and internode length of the plantlet. By the technique of paraffin section, it was observed that the treatment of BL @ 1 mg $L^{-1}$ distinctively accelerated the differentiation of vascular bundles in stems and primary veins of leaves and increased the density of mesophyll cells and the thickness of cuticle (Chen et al., 2014).

### 2.12.2. *Marubakaido apple rootstock*

The *in vitro* multiplication rate in this plant was increased by the application of 5F-HCTS in the range of 100–10,000 ng per shoot. The optimum concentration for multiplication enhancement of 5F-HCTS @ 500 ng per shoot was noticed when culture medium was enriched with N6-benzyl adenine @ 2.2 $\mu$M (Schaefer et al., 2002). It is a woody plant species and branch elongation is the main constraint. Improvement of micropropagation in Marubakaido apple rootstock is done through the stimulation of branch elongation by the treatment of *in vitro* grown shoots using (5F-HCTS) 0.5 $\mu$g per shoot (Pereira-Netto et al., 2006a and 2006b).

The rate of rootstock multiplication increased by the stimulatory effect of growth by the application of 5F-HTY and HTY @ 2.5 $\mu$g per *in vitro* shoot which increased the formation of the primary lateral shoot, increased length of primary lateral shoots and main shoots. The main shoot elongation was stimulated by both HTY and 3F-HTY. Hence, the compound 5F-HTY has the capacity for the improvement of micropropagation to increase the rate of multiplication (Pereira Netto et al., 2019).

## 2.13. *Family name: Rubiaceae*

### 2.13.1. *Coffee*

Coffee is an important economic and beverage crop. For the improvement in the regeneration capacity in this crop, the application of 24-EBL alone or together with N6-(2-isopentnyl) adenine (2-iP; cytokinin) was tested. The leaf was used as an explant cultured in MS medium containing 2-iP @ 0 or 10 $\mu$M and 24-EBL @ 0.01, 0.10 or 1.0 $\mu$M; EBL @ 1.0 $\mu$M together with 2-iP produced 6.8 times more somatic embryos than the explants cultured with only 2-iP (Chone et al., 2018).

### 2.14. Family: Salicaceae

#### 2.14.1. Hybrid poplar

Tracheary elements play an important role in water conduction in plants; their anatomical feature is determinant of wood quality. Poplar is grown for wood mainly and here it acts as a model plant for the analysis of differentiation in secondary xylem cells. The tracheary elements are formed in calli which are derived from petioles of hybrid poplar after ten days in a culture medium that lacks auxin but contained brassinolide @ 1 $\mu$M. The development of tracheary elements was formed within clusters of cells (Yamagishi et al., 2013).

### 2.15. Family: Solanaceae

#### 2.15.1. Potato

Salt stress has a bad impact on the roots of potato plants as roots have an important role regarding water and nutrient uptake that ultimately affect the growth and development of potato. Application of BL at low concentration (0.1 and 0.01 $\mu$g $L^{-1}$) enhanced root elongation and lateral root development whereas decreased at high concentration (1−100 $\mu$g$L^{-1}$). Treatment with BL @ 50 $\mu$g $L^{-1}$ enhanced the salinity tolerance by increasing antioxidant activities, protein content, and decreased malon-dialdehyde content in the shoots of potato, and also by maintaining and balancing the ratio of potassium and sodium in roots and shoots (Hu et al., 2016). The potato cultivars 'cardinal and Desiree' nodal explants pretreated with 24-EBL @ 0, 1, 2 $\mu$M for 2 h and then cultured into the medium containing 0, 40, 60, 80 mMNaCl [EBR treatment (PT)] and same concentration of 24-EBL and NaCl added directly into the MS medium before placing the nodal explants into the medium [EBR and NaCl concentrations were added directly in the MS medium (IM)]. Their effect was analyzed on various morphological (root length and number, shoot length and number, number of nodes and leaves number and fresh weight) and biochemical parameters (protein content) and antioxidant enzymes activity (superoxide dismutase, peroxidase) on 30 days old plant. The growth of a plant is affected by NaCl stress and pretreatment with 24-EBLalleviates the harmful effect of NaCl stress on a plant by increasing antioxidant activities, total soluble protein and growth of a plant. For total soluble protein, growth and antioxidant activity, the 24-EBL@ 1 $\mu$M was suitable for cardinal cultivar in case of PT treatment, while 24-EBL@ 2 $\mu$M was found suitable for 'Desiree cultivar' in case of IM treatment. The combination of both 24-EBLand NaCl together was not found much suitable as compared to the exogenous treatment of 24-EBL(Khalid and Aftab 2016).

With short pretreatment of potato plants with two BRs i.e. 24-EBL and 28-HBL having concentration range from $10^{-11}$ to $10^{-8}$ M delayed salinity stress by increasing salt tolerance and with the accumulation of the compounds through antioxidant activity (Efimova et al., 2019).

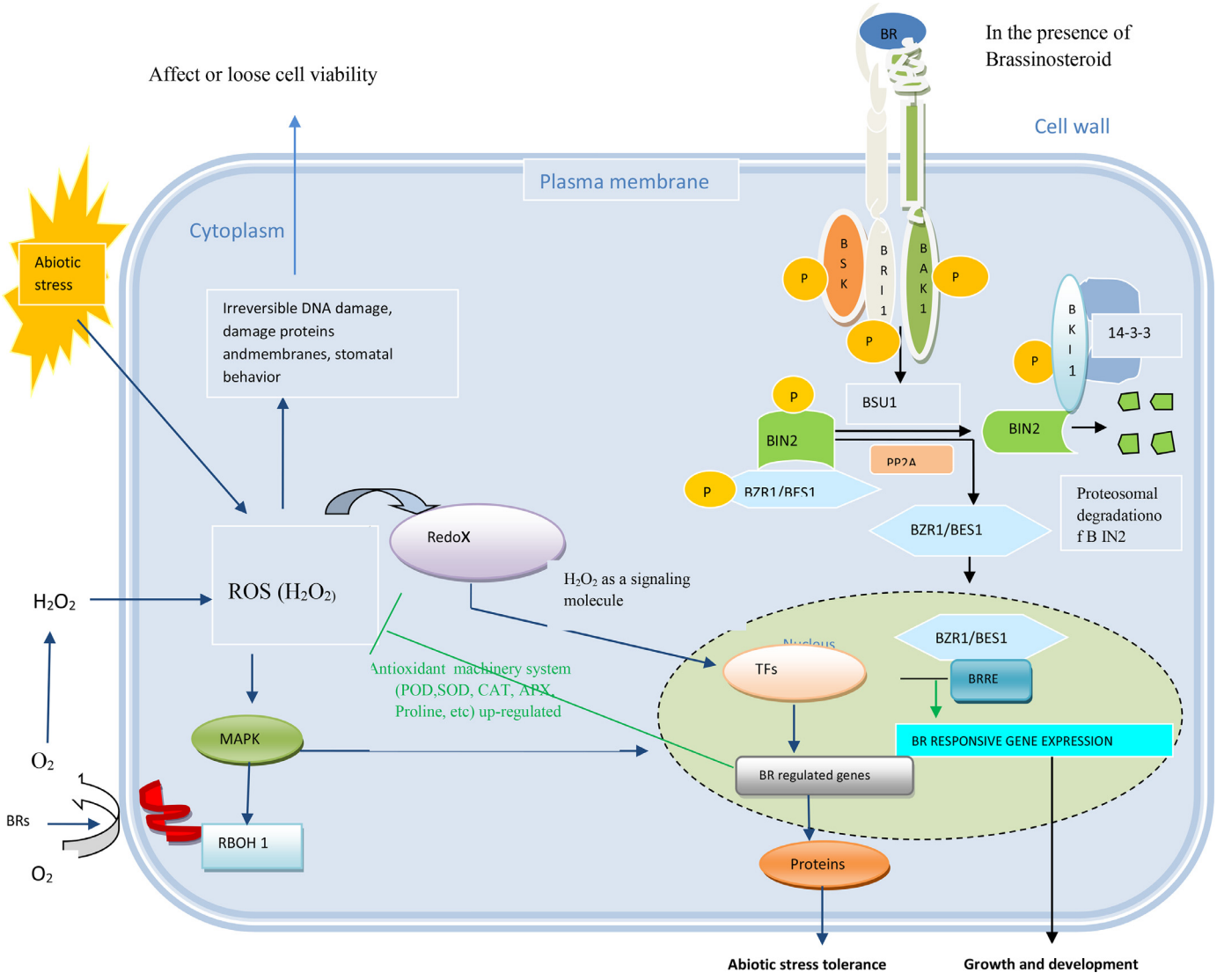



**Fig. 2.** A simple outline of BRs mechanism of action and its signaling pathway in response to some abiotic stresses (Source: Pandey et al. 2020)

For the cultivation of potato, the seeds proved the costliest input as it is sometimes marred by virus-infections or poor quality. To circumvent from tuber borne diseases the *in vitro* propagation of potato is found to be an alternative solution for this problem. The rate of shoot length, nodes number, leaves number, culture survival were enhanced when gibberellic acid (GA), naphthalene acetic acid (NAA) were supplied together with 24-EBL. For growth and multiplication of potato the preferable concentration found suitable was GA @ 0.5 $\mu$M, NAA @ 0.1 $\mu$M and 24-EBL @ 0.1 $\mu$M (Harashlata et al., 2018).

It has a low multiplication rate and quickly attacked by viral diseases during clonal propagation. To overcome these both limitations, synthetic seed production was applied in potato to produce large quantities of disease-free plant propagules from *in vitro* grown plantlets. For the synthetic seed production, axillary buds derived from potato mini tuber sprouts (PMSs) were used as plant material. Besides the material required for encapsulation (sodium alginate, calcium chloride, MS medium as the matrix), 24-EBL was applied to 'Sante' cultivar of potato to study its role in the synthetic seed production. For this, axillary buds of 2-3 mm in size from both cultivars were treated with different concentrations of 24-EBL (0, $10^{-6}$, $10^{-7}$ and $10^{-8}$ M) at three different stages: at the time of pre-culture of axillary buds for 2 days, in the matrix culture medium, during encapsulated buds in regrowth medium for 2 days; it noticed that the concentration of 24-EBL @ $10^{-6}$ M increased the shoot length and improved the regrowth properties in potato synthetic seeds (Ghanbarali et al., 2016).

Treatment of 24-EBL @ 0.3 $\mu$M for two hours to a 30 days old leaf of the potato plant and the heat treatment by exposing potato seedlings at the temperature of 35°C for 2, 6, 24, 48 h was given to detect the synthesis and signaling genes. 24-EBL @ 0.04 mg/L was sprayed on a plant at 50th and 70th day and harvesting was done at 90th day to record the number and weight of tuber per plant. Through bioinformatics and molecular technologies, namely StCPD, StDWF4, StDWARF, StDET2, StBRI1, StBIN2, StBZR1-2 and StBZR1-4, a series of putative homologs of BRs synthesis and signaling genes were identified in potato. Exogenous BR treatment decreased the expression of all synthesis and signaling genes except the negative signal gene StBIN2 which was increased. In leaves, these genes were highly expressed by tissue expression analysis, and only the downstream transcription factor gene StBZR1-2 was highly expressed in stolons and tubers. The expression of these genes after heat treatment showed the opposite results from BRs treatment, suggesting that BRs signaling can be activated by heat stress resulting in increase in both the number and the total weight of potato tuber due to activation of light-period signal for tuberization (Zhu et al., 2019).

Salinity stress produced negative effects in the potato which ultimately leads to reduced yield. Pretreatment with 24-EBL @ $10^{-10}$ M for 4 h was given to a six-week-old potato plant and then transferred to a medium containing 100 mM NaCl. The photosynthetic and biochemical analysis was done from leaves after 1-4 days of NaCl treatment while inorganic ion estimation was done at the 4th day of treatment only. The salinity stress decreased the photosynthetic pigments, photosynthetic electron transport, photosystem-II. These all ill effects of salinity stress due to the ionic effect become toxic for the oxidation level in plants. Pretreatment with 24-EBL enhanced the oxidation level in plants like peroxidase activity and by balancing the ratio of sodium and potassium levels in plants, but there was no effect on photosynthetic pigments (Kolomeichuk et al., 2020).

*2.15.2. Tomato*

Role of the synthetic and natural BRs by fluorimetric methods that triggered nitric oxide (NO) production in cells of tomato under *in vitro* condition was demonstrated. In comparison to methodologies like Rice lamina inclination test (RLIT) assay, it is new, quick and sensitive bioassay having so many advantages (Tossi et al., 2015).

***In vitro* and *in vivo* BRs application:** The *in vivo* and *in vitro* application of BRs and their analogues in plants depends on the time/developmental stage when BR is applied, concentrations of BRs used, and mode of treatment. The mode of treatment of BRs for *in vivo* can be done by foliar application, seed soaking, soaking of various plant parts (root, shoot, leaf segments) in BRs solution, while for *in vitro* growth of plants, in a liquid medium containing nutrients and BRs. *In vivo* BRs application enhances seed germination (quality of the seedlings), regulate flowering, frutification and process of senescence, photosynthetic rate, provide resistance to various diseases, help to overcome from various stresses like water, heat cold and also alleviate stress caused by pesticides, reduce accumulation of heavy metal and radioactive elements in plants (Gomes 2011). These also improved the productivity of various crop plants such as vegetables, fruits and oil seed plants (Khripach et al., 2000; Vardhini et al., 2006; Divi et al., 2009), whereas *in vitro* BRs application affect plant elongation, cell division and vascular development influencing morphogenesis, secondary metabolite production, synthetic seed production, etc. The use of this hormone under *in vitro* as well as *in vivo* condition is a promising supplementation in all above aspects.

## 3. Mechanism of action and signaling of the Brassinosteroids

When the plants respond to any abiotic stress they acclimatize themselves by the production of Reactive oxygen species (ROS) such as Hydrogen Peroxide ($H_2O_2$). ROS are by-products of plant aerobic metabolism and generated in the different cell organelle compartments such as chloroplasts, mitochondria, peroxisomes (Huang et al., 2016) and affects different physiological and chemical reactions in plants. Photosynthetic electron transport system leads to the production of $O_2^-$ by the membrane-dependent NADPH oxidase (RESPIRATORY BURST OXIDASE HOMOLOG proteins) systems, which reacts with hydrogen ions to form oxygen molecules to form $H_2O_2$, an important redox molecule which also participate in cell signaling (Miller et al., 2010). The BR-mediated ROS signal maintains the homeostasis which turns the activation of transcriptions factors (TFs) that regulate stress responsive genes which enhance the tolerance from different abiotic stresses by the accumulation of protective proteins and up-regulation of the antioxidant machinery system (Fig 2).

The perception of BRs takes place by the receptor and co-receptors located on the plasma membrane

↓

The ligand of BRs binds with its receptors Brassionosteroid Insensitive 1 (BRI1) and its co-receptor BRI 1 Associated Receptor Kinase 1 (BAK1)

↓

This binding activates BRs dependent signaling events

↓

These signaling events allow the dissociation of BRI-1 Kinase Inhibitor (BKI-1), i.e. BKI-1 from the BRI-1 receptor

↓

This dissociation leads to the formation of BRI 1-BAK 1 complex

↓

This complex initiates a series of phosphorylation/ dephosphorylation dependent downstream signaling events

↓

Brassinosteroid Signaling Kinases (BSKs) phosphorylation activates BRI-1 Supressor 1 (BSU-1) and BSU-1 phosphatase inhibits BR INSENSITIVE 2 (BIN 2) activity and stability

↓

Dephosphorylation of BIN 2 inhibits its activity and promotes proteosome-mediated degradation, in a sense activating the BR signaling transcription factors BZR 1/BES 1 which is dephosphorylated by protein phosphatase 2A (PP2A)

↓

BZR1/BES 1 regulates BR dependent gene expression which helps in growth and development of the plant

## 4. Conclusion

The report provides information on the BRs response under *in vitro* condition. Their importance arises when they are found to be environmentally friendly i.e. non-toxic and can induce their effect at a micro concentration which brings a new direction for the *in vitro* application of BR analogous in agriculture, forestry and horticulture crops. The combination of BRs with or without distinct plant hormones shows valuable effects for the induction of *in vitro* morphogenetic pathways. It produces a large number of disease-free *in vitro* plantlets by synthetic seed technology. Their essential role has been found in the context of physiological, morphological, metabolic, photosynthetic and developmental processes. It is used to provide resistance in plants against various abiotic stresses mainly salinity and oxidative stress. It also helps in the elicitation of secondary metabolites in medicare plants. It is potentially useful to improve regeneration rates, multiplication rates and micropropagation techniques for the proliferation of plant clones of so many different species, especially woody species, in which it is impossible to effectively stimulate shoot production, which otherwise is often a limitation for commercial *in vitro* propagation. Their application under *in vitro* condition is tremendous as an aid for the improvement of various genetic, molecular, morphological and physiological processes to increase the production, directly or indirectly related to agronomical, horticultural, forestry and medicinal plants.

## Declaration of Competing Interest

All authors declare that there are no conflicts of interest.

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