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Hormonal regulation of the growth and morphogenesis in potato

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Abstract

Modern techniques of plant propagation in solanaceae family provide with high capacity for in vitro culture owing to the associated tuber borne diseases during vegetative propagation. Phytohormones including brassinosteroids (BRs), regulate the growth and morphogenesis of callus by modulating gene expression, enzyme activities, including a significant level of overlapping signalling pathways and molecules amongst each other. There have been ample evidences generated that suggest a BRs cross talk significantly with auxins, ethylene, cytokinins and abscissic acid amongst others to bring qualitative changes in growth and patterning as well as in modulating cellular stress response. The effects of BRs seem to be concentration dependent and stage specific providing a lucrative tool for the benefit of agriculture having been reported to augment yield and quality of the crop under challenging conditions of high salinity, drought or nutrient stress. The BRs have also protective roles under biotic stresses such as viral infections and fungal pathogens supported by significant number of field trials. This review intend to address the molecular impact of BRs application and the observed affect on plant morphology.

Keywords: brassinosteroids, *in vitro* culture, hormonal cross talk, stress, potato

Introduction

Potato, like other Solanaceous crops, shows considerable regenerative ability in culture, producing adventitious shoots both directly from organ tissue and from callus under appropriate conditions (Wang and Huang, 1975) ^[1]. Potato, being vegetatively propagated crop, is prone to the built of tuber borne diseases that can be reduced by Propagation of stem cutting. The in vitro propagation of potato by nodal part has been extensively reported and has become an effective means of rapid and mass multiplication of new or existing cultivar under aseptic conditions (Nozeran *et al.*, 1977; Goodwin *et al.*, 1980; Hussey and Stacey, 1981) ^[2, 3, 4]. Alternative methods of potato propagation are now being achieved due to the recent advancement in tissue culture methods and the flexibility of organ development. Much work has been carried out on callus induction and growth in potatoes. A wide range of protocols and procedures are being established continuously as tissue culture has gained an considerable importance in plant propagation, conservation, and breeding (Ahloowalia 1982; Wareh *et al.*, 1989) ^[5, 6]. Callus is used for most of these transformation methods such as particle gun and *Agrobacterium tumefaciens* mediated transformation as well as initiation of cell culture. A callus is an unorganised mass of different cell types that develops from an explant tissue due to a set of dramatic changes in the appearance and metabolism of the cells (Aitchison *et al.*, 1978) ^[7]. Induction of callus, physical disorganization of cultured cells, is thought as a result of the breakdown of intercellular physical and chemical communication. Earlier reports suggest that the callus culture showed higher multiplication rate in comparison to other methods of in vitro culture. As in nodal culture, the major limiting factors are the rates of multiplication, short height of the plantlets and the low number of nodes on the plantlets. Considerable improvements have been made in callus culture by addition of growth regulators to the medium.

Emerging concepts of brassinosteroids in plant growth

Phytohormones are regulators of plant growth that in vanishingly small concentrations mediate important physiological responses including both biotic and abiotic stresses. BRs can induce plant tolerance to a variety of biotic and abiotic stresses, such as low and high temperatures, drought, salinity, heavy metal contamination and pathogen attack (Bajguz and Hayat, 2009) ^[8]. BRs can also regulate the rate of nitrogen fixation (Vardhini and Rao, 1999) ^[9] and enhance

the activities of nitrogenase and nitrate reductase (Ali *et al.*, 2007) ^[10] under normal condition or salt stress in groundnut and chickpea. BRs, constitute a group of steroidal phytohormones that regulate cell elongation, photomorphogenesis, xylem differentiation, and seed germination (Sasse, 2003) ^[11]. In addition to this, BRs have the ability to enhance biological yield and improve stress tolerance in plants so as to employ them in phytoremediation (Barbafieri and Tassi, 2011) ^[12].

Mitchell *et al.* 1970, ^[13] attributed hormonal status to the brassins “because they are specific translocatable organic compounds isolated from a plant and have induced measurable growth control when applied in minute amounts to another plant.”

Brassinolide, a plant steroid lactone, and its analogs enhance maturation and increased crop yield of several vegetables including pepper (Mandava, 1988) ^[14, 18, 39]. Since then, brassinolide has been regarded as a new plant growth regulator which is essential for normal plant growth and development. Brassinolide promotes cell elongation in organizing the cortical microtubules (Mayumi and Shibaoka, 1995) ^[15] and regulate gene expression (Clouse *et al.*, 1992) ^[16]. Brassinosteroids are involved in the process of cell enlargement through their effects on gene expression and on enzyme activity (Mussig and Altmann, 1999) ^[17]. A better understanding of the absolute requirement for BS in cell elongation has become possible from studies on mutants and from molecular genetics approaches. Many results suggest that Brassinosteroids are required for optimal productivity and resistance to unfavorable influences of the environment (stresses, diseases). A lack of brassinosteroids in mutants, caused by breaks in brassinosteroids biosynthesis, leads to dramatic deviations from normal development, which can often be rescued by application of exogenous brassinosteroids.

Regulation via cross talk; brassinosteroids and other phytohormones

The relationships between BRs and the other well-known plant hormones have been extensively explored. BR can induce ethylene production (Arteca *et al.*, 1998) ^[18] and enhance the level of abscisic acid in *Chlorella vulgaris* cultures exposed to heat stress (Bajguz, 2009) ^[19]. Physiological activity of BRs is largely consistent with physiological influences exerted by auxins (Cao and Chen, 1995) ^[20]. The signal transduction pathways of both phytohormones are connected and the physiological effects of these groups' compounds are synergistic. Both promote elongation, increase fresh weight and electrogenic proton extrusion in *Vigna angularis* epicotyls (Cerana *et al.*, 1983) ^[21] and ethylene production. BRs synergistically increase the seedling sensitivity to auxin and has been shown that combined treatment with both hormones can increase the magnitude and duration of gene expression (Vert *et al.*, 2008). Probably, BRs and auxin signalling pathways converge at the level of the transcriptional regulation of the common target genes. BRs have been shown to regulate the expression of PIN genes, which encode essential components in the polar transport of auxin. Similarly, auxins may have a role in the control of gene expression involved in BR signaling.

The crosstalk and coordinated regulation of site-specific cocktail of phytohormones co-regulate numerous genes. BRs and ABA both are considered as stress hormones. They have

been indicated to co-regulate hundreds of genes controlling many developmental processes. However, BRs appear to antagonize the effect of ABA. ABA induces seed dormancy during embryo maturation and inhibits seed germination while BRs reverts them. The ABA shows an antagonistic relationship with GA also during seed dormancy and germination. The increased level of ABA is presumed to be the reason for the delay in sprouting of potato tubers under dormancy, while a decline in its concentration with subsequent increase of GA was correlated with the breakdown of dormancy. GA biosynthesis in insensitive mutants was rescued by BR (24-EBL and BL) application. Sprouting is accompanied by the decline of ethylene as evident from the increase of endogenous ethylene synthesis prolong the ABA-mediated deep dormancy in potato tubers. BRs promote ethylene synthesis in intact potato tubers and ABA synthesis in the apical cells of tuber. In the study of thermotolerance of potato, carried out by 24-epibrassinolide protected *in vitro* cultured plants against heat stress.

Brassinosteroids in overall health and stress tolerance: A molecular insight

Potato cuttings cultured in a medium containing brassinolide showed a significant increase in all the parameters that characterize growth and development and this led to a higher yield of plants. One of the most important facts was a reduction of viral infection in the material, evident in all stages of plant development. Along with an essential lowering of viral infection, the plants obtained from BS-treated cuttings gave a higher crop yield when compared to plants from untreated cuttings. The seed or planting material, are vital need of farmer and if this requirement is fulfilled, farmers can definitely increase the yield potential manifold as the multiplication coefficient of *in vitro* propagation system is very high. The Application of brassinosteroids as the shot gun approach (pre-sowing seed soaking) improved the growth traits (fresh and dry mass of shoot and root) in stress-free plants as well as in Ni-stressed plants supposedly due to their involvement in cell elongation (Fariduddin *et al.*, 2013) ^[23]. The high biological activity of brassinosteroids (BRs) determines their importance in regulating different processes in a plant (Khripach *et al.*, 1996 a, b: 1999; 2000: Bajguz and Czerpak, 1998) ^[24, 25, 26, 27, 36, 37, 38, 28]. It is known that they change the membranous potentials and the enzyme activity, promotes the protein synthesis, nucleic and fatty acids, alter the balance of the other endogenic hormones, stimulating in this way, the cell elongation and division (Mussig *et al.*, 2002; Lisso *et al.*, 2005) ^[29, 30]. Alterations at a cell level are reflected in a whole plant by growth intensification, productivity rise and stress stability (Yu *et al.*, 2004; Hayat *et al.*, 2007) ^[31, 32].

Postembryonic growth involves consecutive stages of cell proliferation, elongation, and subsequent differentiation. Although it is now established that brassinosteroid activity affects each of these processes, much of the brassinosteroid related research has focused on cell elongation, as primarily inferred from changes in hypocotyl length, in whole-plant-based genomic studies. The various datasets revealed changes in the expression of hundreds of genes belonging to different functional groups, including prominent gene groups encoding for cell wall biosynthesis and remodeling enzymes as well as for cytoskeleton-related functions, consistent with the central role of brassinosteroids in cell elongation [30–34]. An

additional group of genes linked brassinosteroids to hormonal and light signaling pathways, as exemplified by the negative regulation of many genes involved in chloroplast development and photo-morphogenesis upon exposure to brassinosteroids (Yu, *et al.*, 2011; Sun, *et al.*, 2010) [33, 34]. The physiological action of brassinosteroids depends on their concentration in a plant and the medium.

Application of Brassinosteroids in Agriculture

The potential applications of BS in agriculture and horticulture are based not only on their ability to increase crop yield but also to stimulate other physiological processes. As a result, it may become feasible to grow crops under unfavorable (stressful) conditions, such as high salinity, drought or insufficient nutrients (Prusakova *et al.*, 1999) [35]. The treatment of potato plants with BS decreased the level of Phytophthora infection: it was shown, in field experiments, that spraying plants with BS (brassinolide, 24-epibrassinolide, and 28-homobrassinolide) solutions at the beginning of the bud formation period was most effective (Khripach *et al.*, 1996, 2000) [24, 27, 36, 37, 38]. In some cases, the protective effect of BS against fungi was even higher than for plants treated with standard fungicides. Crop yield was higher for the plants treated with 28 homobrassinolide than for those treated with Arcerid, and about 20% greater than the untreated control. Others, (Saveleva *et al.*, 1999) [38] have confirmed the efficiency of BS application in the reduction of Phytophthora infection (up to maxima 140%). Usually, the higher the level of pest development, the higher the expression of the protective properties of BS is observed. Treatment with 24-epibrassinolide prolongs dormancy of potato tubers and increases their resistance to sprouting and diseases, changes that are associated with enhancement of ABA and ethylene levels, and also the presence of phenolic and terpenoid protective substances. A newly discovered aspect of the protective action of BS on plants is related to their ability to stimulate resistance to viral infection.. This indicates that exogenous BS can act efficiently in plants as immunomodulators when applied at the appropriate dose and at the correct stage of plant development. As in other cases of the BS-regulated stress response, the pathogen protective action of BS is the result of a complex sequence of biochemical shifts such as activation or suppression of key enzymatic reactions, induction of protein synthesis, and the production of various chemical defense compounds. Protective and deprotective both types of roles have been attributed to BRs, depending upon the time and method of application at the point of interaction of host with its pathogen (Korableva *et al.*, 2002). In case of late blight of potato, BRs have been reported to suppress the immune status of plant tissues, inhibiting wound reparations. BRs stimulated the mycelial growth and sporulation intensity of *P. infestans* thereby induced susceptibility of potato tuber tissues. Post-harvest treatment of potato plants or tubers with BRs was shown to prolong the dormancy and subsequent enhancement of resistance to *P. infestans*. These relatively little-investigated properties of BS are promising from a practical point of view. They open up new approaches for plant protection, based on the employment of very small amounts of environmentally friendly natural substances instead of traditional pesticides, which are often environmentally-unfriendly. Brassinosteroids can be applied for the treatment of plants or seeds using existing equipment and technologies. The growth-promoting

activity of BS usually takes place only after treatment of plants in the appropriate phase of development and within a certain concentration range, which is different for each plant species and type of BS. For large-scale field application, two modes of BS application are possible: seed soaking and foliar spray. Both methods have been investigated extensively, but results with the later method were found to be highly dependent on the phase of plant development. Generally, better results can be obtained when young rather than old plants are treated. The formulation of the spraying solution is very important, and additives are necessary to facilitate the spreading of active substance, to prevent early drying and to ensure penetration of BS via cell walls. The working solution should be prepared by dilution with water shortly before application, which can be done with normal agricultural sprayers. The ability to combine BR treatment of plants with other pesticides, for example with fungicides for potato treatment, allows BRs to be used with existing technologies for plant protection, with no significant additional expense. A new variant of BR application has been developed recently, based on the use of combinations of BRs with mineral fertilizers (Pirogovskaya *et al.*, 1996). Exogenous application of BRs stimulated inner potentials of plants and is helpful not only for better survival in stressful conditions and quality improvement but also in diminishing disease damage. The potential of BRs to enhance plant resistance against fungal pathogen infection was documented in several studies (Khripach *et al.*, 2000).

Experiments to investigate the potential of BS for use in agriculture began in the 1970s in the USA and showed beneficial effects (Mandava, 1991). In the early eighties, studies on BS in Japan and the USSR confirmed their usefulness as agricultural chemicals (Khripach *et al.*, 1996). Since then, numerous reports from all over the world have appeared and many potential practical uses have been patented. Along with growth alteration, BS can influence plant development, in particular, reproduction, maturation, senescence, and seed yield. Brassinolide, 24-epibrassinolide, and 28-homobrassinolide and some other BS have been tested in field trials to determine their influence on plant growth and development and on crop yield in natural conditions. The results obtained in field trials do not always coincide with those predicted from bioassays. Under field conditions, better results can be obtained with 24-epibrassinolide and 28-homobrassinolide, even though the activity of brassinolide is usually higher in bioassays. One reason for this effect could be the lower stability of brassinolide compared to 24-epibrassinolide and 28-homobrassinolide under field conditions. This, together with the fact that 24-epibrassinolide and 28-homobrassinolide can be synthesized more cheaply than brassinolide, has promoted the use of these two natural hormones in agricultural applications. Nowadays, 24-epibrassinolide is used as the active ingredient in preparations that are socially registered and have large scale application. Nevertheless, rapid development of the application of 28-homobrassinolide in agriculture might be expected because of its specific action in plants (Khripach *et al.*, 2000) [27, 37].

An important feature of BS is their ability to increase not only the yield but also the quality of crops (Prusakova *et al.*, 1999) [35]. Thus, the application of brassinolide, 28-homobrassinolide, and 24-epibrassinolide to potato gives, along with a 20% rise in productivity, a better quality of crop with regard to a diminished nitrate content and enhanced

starch and vitamin C content (Khripach *et al.*, 1996b) ^[25]. Treated plants are damaged less by Phytophthora and have better consumer properties. A similar influence of BR has been reported for tomato, cucumber, sugar beet and some other plants. New promising findings in food and non-food applications are growth stimulation in mushrooms, production of ornamental plants, enhanced resistance of lawns in contaminated environments (Kleinkopf, 1990), improvement of the introduction and adaptation of new crops in new areas and production of grapes. In Russia and Belarus, it was reported that EBR is the active ingredient of the plant growth promoter Epin2, which has been officially registered since 1992 and is recommended for treatment of agricultural plants such as tomato, potato, cucumber, pepper, and barley. A spirostane analogs of brassinosteroids applied in the culture medium as an alternative to cytokinin promotes embryogenic callus induction and growth of coffee and potato (Garcia, 2000; Moree *et al.*, 2001) ^[40, 41]. However, there is little information about the role of these new compounds on shoot formation.

Several BRs, mainly brassinolides and homobrasinolides, have been evaluated in the field and have produced significant yield increases in diverse crops (Khripach *et al.*, 1999) ^[38]. However BRs occur naturally in very low concentrations in plants, and analog compounds have been synthesized for possible commercial use. Two of these BRs analogs are BB-6 and BB-16. Application of these BRs has increased yields, in tomato (Nuñez *et al.*, 1995) ^[43], garlic (Nuñez *et al.*, 1994) ^[42], potato (Torres and Nuñez, 1997) ^[44] and corn (Almenares *et al.*, 1999) ^[45].

BRs have also been reported to increase plant resistance to biotic and abiotic stresses. However, the mechanisms underlying such protective properties of BRs remain largely unexplored. It has been observed that 24-epibrassinolide (EBR) could protect in vitro grown potato plants from heat stress. It is also apparent that EBR effectiveness in protection is affected by the concentration of ethanol—the solvent for EBR—in the growth medium. In suspension cultured cells of *Chorisporabungeana*, Liu *et al.*, (2009) reported that BRs treatment promoted the activities of antioxidant enzymes and increased the contents of ascorbic acid (AsA) and reduced glutathione (GSH) under chilling stress, thus scavenging excess reactive oxygen species (ROS) accumulation and decreasing the content of malondialdehyde (MDA). From these results, it is clear that BRs could result in alleviating oxidative injury and improving the resistance of cells to chilling stress.

The effect of BR on the functionality of the photosynthetic apparatus is studied in more details on BR-treated higher plants. When exogenously applied to leaves and roots BRs improve stress tolerance and thus stimulate crops growth and yield (Vert, *et al.*, 2005) ^[46]. The decreased photosynthetic rate and photosystem II (PSII) activity under heat, cadmium and oxidative stresses are compensated by pre-treatment with 24-epibrassinolide that is the most active BR. The positive effects of BRs are attributed mainly to increased Rubisco activity and carboxylation efficiency and enhanced antioxidant system.

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