ABSTRACT

Metal toxicity and tolerance in plants is a subject, which is being reviewed broadly for last 30 years. The toxification of plants with metal pollutants results in oxidative stress because they are involved in several reactive oxygen species generating mechanisms. Plant hormones, such as ethylene, abscisic acid, salicylic acid, jasmonic acid, auxins and brassinosteroids, have been found to be involved in modulating the plant responses to oxidative stress. Brassinosteroids (BRs) are the upcoming group of phytohormones, which are regarded as sixth group of plant growth regulators. The structure of BRs is similar to animal steroid hormones and these are widely distributed among the plant kingdom. BRs have been explored for their promising role in reducing the effects of various stresses like thermal, drought, salt, pesticides, infection and heavy metals. Since BRs have been reported to play an important role in stress amelioration, an attempt was made in the present study to isolate and characterize various BRs expressed in Brassica juncea L. plants at various growth stages (30, 45 and 60 days) grown under different concentrations of heavy metals viz., nickel (0.2, 0.4, 0.6 mM), chromium (0.1, 0.3, 0.5 mM) and arsenic (0.1, 0.2, 0.3 mM). The effect of heavy metal stress on B. juncea plants have been studied by assessing the modulation in activities of antioxidative enzymes. Further, the isolated BRs were evaluated for their potential as anticancer as well as free radical scavenging compounds. The preparation of extracts of B. juncea plants involved partitioning with chloroform, water, hexane and methanol. The extracts were subjected to purification with silica gel and sephadex LH-20 and the purified fractions were subjected to radish hypocotyls bioassay to obtain the bioactive fractions. The bioactive fractions were identified, pooled together and further processed for the detection of their masses using ESI-QTOF-MS and GC-MS. The BRs isolated and characterized from metal stressed B. juncea plants include 24-epibrassinolide, castasterone, dolicholide, teasterone, tyhpasterol and 28-homocastasterone. The effect of heavy metal stress on B. juncea plants was studied by observing the morphological parameters (shoot length and number of leaves), studying the metal uptake and biochemical stress markers, viz. protein content, assessment of antioxidative enzyme activities, lipid peroxidation and total osmolytes. The treatment of
different doses of heavy metals (Ni, Cr, As) to B. juncea plants resulted in a decrease in the shoot length and leaves count as compared to untreated control plants except 0.2mM dose in Nickel where there was increase in shoot length. The B. juncea plants showed considerable uptake of all heavy metals and increasing trend in metal uptake was seen in shoots and leaves. The protein content in metal stressed plants was found to increase in 30 and 45 days old metal treated plants, and decrease in protein content was visible in all the 60 days old plants. Modulation in the activities of different enzymes of stressed plants in comparison to the enzyme activities of control plants was also noticed. The metal stress resulted in altered activity of antioxidative enzymes as compared to control plants and an enhancement in the MDA content and osmolalities was also noticed. The isolated BRs were analysed for antiproliferative activity and it was found that the compounds effectively suppressed the growth of cancerous cells of Lung (A-549), Ovary (IGR-OV-1), Breast (MCF-7), Prostrate (PC-3), CNS (SF-295) and Leukemia (THP-1), C-6 (glioma) cell lines. Maximum growth inhibition was seen in case of Lung cell line and minimum inhibition (2%) was observed in Leukemia cell line. The potential of isolated BRs to scavenge free radicals was assessed by employing in vitro assays viz., DPPH radical scavenging assay, ferric ion reducing power assay and molybdate ion reduction assay. Among the DPPH assay, 24-EBL was found to have more potential to scavenge the DPPH radicals with its IC50 which was 357µgml⁻¹ followed by Typhasterol (470.13µgml⁻¹) and Teasterone (853.32µgml⁻¹). The reducing power of all these test compounds increased in a concentration-dependent manner though it was low as compared to L-Ascorbic acid. Maximum reduction was observed for 24-EBL with its IC50 and it was 224µgml⁻¹. In case of molybdate ion reduction assay, maximum activity in reducing molybdate ion was shown by DL with its IC50 which was value 29.13µgml⁻¹ followed by CS (29.13µgml⁻¹) with very less difference in its reducing capacity. The study revealed the presence of BRs in metal stressed B. juncea plants during different growth stages and strengthened the stress ameliorating properties of BRs as evident from the responses of antioxidative defence system enzymes to metal stress. The isolated BRs hindered the proliferation of cancerous cells significantly as well as scavenged the free radicals, thus emerging as ecofriendly compounds having promising medicinal properties.