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Influence of abiotic stress signals on secondary metabolites in plants

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Key words: abiotic stress, anthocyanin, climate change, cold stress, jasmonates, plant cell and tissue culture, polyamines, secondary metabolites

Abbreviations: ABA, abscisic acid; JA, jasmonic acid, MeJ, methyl jasmonate; Put, putrescine; Spd, spermidine; Spm, spermine; BRs, brassinosteroids

Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors, and industrially important biochemicals. Accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Secondary metabolites play a major role in the adaptation of plants to the environment and in overcoming stress conditions. Environmental factors viz. temperature, humidity, light intensity, the supply of water, minerals and CO_2 influence the growth of a plant and secondary metabolite production. Drought, high salinity and freezing temperatures are environmental conditions that cause adverse effects on the growth of plants and the productivity of crops. Plant cell culture technologies have been effective tools for both studying and producing plant secondary metabolites under in vitro conditions and for plant improvement. This brief review summarizes the influence of different abiotic factors include salt, drought, light, heavy metals, frost etc. on secondary metabolites in plants. The focus of the present review is the influence of abiotic factors on secondary metabolite production and some of important plant pharmaceuticals. Also, we describe the results of in vitro cultures and production of some important secondary metabolites obtained in our laboratory.

Introduction

Plant secondary metabolites are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense. However we are beginning to understand the crucial role played by them in plant growth and development. In higher plants a wide variety of secondary metabolites are synthesized from primary metabolites (e.g., carbohydrates, lipids and amino acids). They are needed in plant defense against herbivores and pathogens. Often they may confer protection against environmental stresses.[1] Secondary metabolites also contribute to the specific odors, tastes and colors in plants.[2] Plant secondary metabolites are unique sources for food additives, flavors, pharmaceuticals and industrially important pharmaceuticals.[3,4] Chemicals include calcium, abscisic acid (ABA), salicylic acid (SA), polyamines and Jasmonates (JA), nitric oxide are involved in stress responses in plants.[5] Accumulation of metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. Secondary metabolites have significant practical applications in medicinal, nutritive and cosmetic purposes, besides, importance in plant stress physiology for adaptation.[1] The production of these compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant.[6] Some of the plant derived natural products include drugs such as morphine, codeine, cocaine, quinine etc. Catharanthus alkaloids, belladonna alkaloids, colchicines, phytostigminine, pilocarpine, reserpine and steroids like diosgenin, digoxin and digitoxin, flavonoids, phenolics etc. In this communication we have reviewed the literature on the environmental influence on plant secondary metabolite production in in vitro and in vivo conditions, except where our studies are quoted, the information is largely based on others work from published literature.

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Abiotic Factors Influencing Secondary Metabolites

A wide range of environmental stresses (high and low temperature, drought, alkalinity, salinity, UV stress and pathogen infection) are potentially harmful to the plants.[1] Elicitation has been widely used to increase the production or to induce de novo synthesis of secondary metabolites in in vitro plant cell cultures.[7] A number of researchers have applied various elicitors for enhancement of secondary metabolite production in cultures of plant cell, tissue and organ.[8,9] Environmental stresses, such as pathogen attack, UV-irradiation, high light, wounding, nutrient deficiencies, temperature and herbicide treatment often increase the accumulation of phenylpropanoids.[10] Nutrient stress also has a marked effect on phenolic levels in plant tissues.[11] The concentrations of various secondary plant products are strongly dependent on the growing conditions and have impact on the metabolic pathways responsible for the accumulation of the related natural products. Exposure to drought or salt stress causes many common reactions

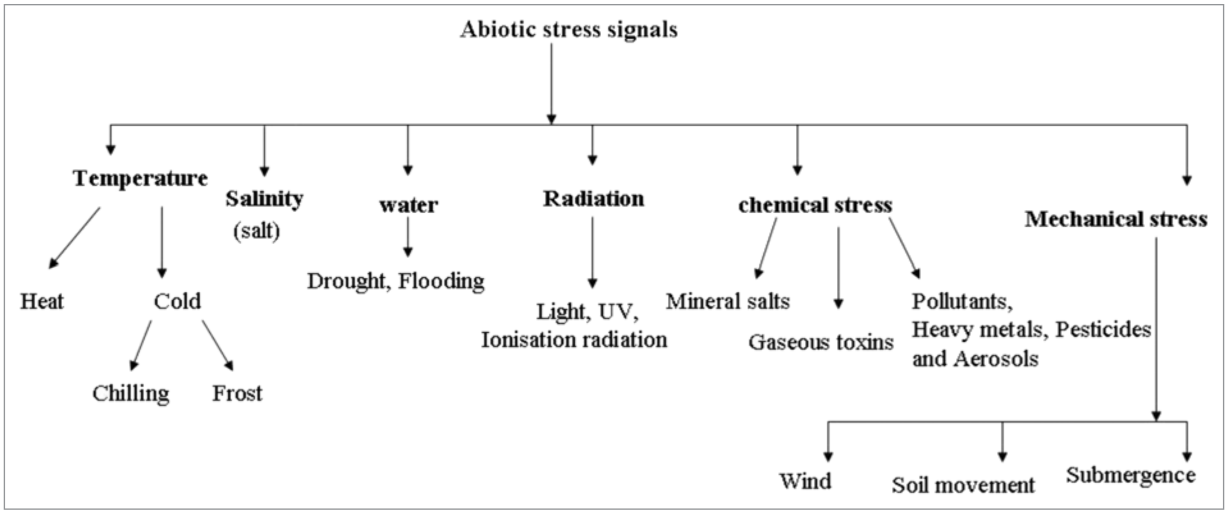

Figure 1. Various abiotic stress signals creating stress in plants (adapted from Mahajan and Tuteja 2005).[13]

Table 1. Influence of various abiotic signals on secondary metabolites in plants

Abiotic signals	Reference
Methyl jasmonate	97
Jasmonic acid	94
Salicylic acid	45
Calcium	110
Polyamines	90
Nitric oxide	5
Melatonin	130
Serotonin	134
Brassino steroids	135
Abscisic acid	5
Metal ions	41
Plant growth regulators	9
Light	71
Nutrient stress	11
Climate change	116
Temperature	61
Cold	50
Drought	25
Salt	12
Chemical stress	13

in plants. Both stresses lead to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm to vacules. Different abiotic stress factors creating stress is depicted in **Figure 1 and Table 1**.

Deficiencies in nitrogen and phosphate directly influence the accumulation of phenylpropanoids.[10] Potassium, sulfur and magnesium deficiency are also reported to increase phenolic concentrations. Low iron level can cause increased release of phenolic acids from roots.[11] Calcium levels have been implicated in plant response to many abiotic stresses including cold, drought and salinity. Expression levels of certain genes have been shown to increase in response to reactive oxygen species, cold temperature, high temperature and osmotic stress.[12] Salt stress in soil or water is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity.[13] Bryant et al.[14] have hypothesized that when plants are stressed, an exchange occurs between carbon to biomass production or formation of defensive secondary compounds. A stress response is induced when plants recognizes stress at the cellular level. Secondary metabolites are involved in protective functions in response to both biotic and abiotic stress conditions. Formation of phenyl amides and dramatic accumulation of polyamines in bean and tobacco under the influence of abiotic stresses were reported, suggesting antioxidant role of these secondary metabolites.[15] Similarly anthocyanin accumulation is stimulated by various environmental stresses, such as UV, blue light, high intensity light, wounding, pathogen attack, drought, sugar and nutrient deficiency.[16]

Salt Stress

Salt environment lead to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm resulting in a reduction of the cytosolic and vacuolar volumes. Salt stress often creates both ionic as well as osmotic stress in plants, resulting in accumulation or decrease of specific secondary metabolites in plants.[13] Anthocyanins are reported to increase in response to salt stress.[17] In contrast to this, salt stress decreased anthocyanin level in the salt-sensitive species.[18] Petrusa and Winicov[19] demonstrated that salt tolerant alfalfa plants rapidly doubled their proline content in roots, whereas in salt sensitive plants the increase was slow. However, Aziz et al.[20] reported a correlation between proline accumulation and salt tolerance in *Lycopersicon esculentum* and *Aegiceras corniculatum* respectively. In tomato cultivars under salt stress endogenous JA was found to accumulate.[21] Polyphenol synthesis and accumulation is generally stimulated in response to biotic or abiotic stresses.[10,22] Increase in polyphenol content in different tissues under increasing salinity has also been reported in a number of plants.[17] Navarro et al.[23] showed increased total phenolics content with moderately saline level in red peppers. Plant polyamines have been shown to be involved in plant response to salinity. Salinity-induced changes of free and bound polyamine levels in sunflower (*Helianthus annuus* L.) roots was reported.[24] The influence of salt stress on secondary metabolites in plants are shown in **Table 2**.

Drought Stress

Drought stress is one of the most significant abiotic stress that affect plant growth and development.[25] Drought stress occurs when the available water in the soil is reduced to such critical levels and atmospheric conditions adds to continuous loss of water. Drought stress tolerance is seen in all plants but its extent varies from species to species. The drought stress arises due to the water deficit, usually accompanied by high temperatures and solar radiation.[25] Water deficit and salt stress are global issues to ensure survival of agricultural crops and sustainable food production.[26] Drought often causes oxidative stress and was reported to show increase in the amounts of flavonoids and phenolic acids in willow leaves.[27] Drought stress influenced changes in the ratio of chlorophyll "a" and "b" and carotenoids.[28] A reduction in chlorophyll content was reported in cotton under drought stress[29] and *Catharanthus roseus*.[30] Drought conditions decreased the content of saponins in *Chenopodium quinoa* from 0.46% dry weight (dw) in plants growing under low water deficit conditions to 0.38% in high water deficit plants.[30] Anthocyanins are reported to accumulate under drought stress and at cold temperatures. Plant tissues containing anthocyanins are usually rather resistant to drought.[31] For example, a purple cultivar of chilli resists water stress better than a green cultivar.[32] Flavonoids have protective functions during drought stress. Flavonoids are implicated to provide protection to plants growing in soils that are rich in toxic metals such as aluminum.[16] The influence of drought stress on various secondary metabolites are given in **Table 3**.

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Table 2. Salt stress increases various secondary metabolites in plants

Secondary metabolite	Plant species	Reference
Sorbitol	*Lycopersicon esculentum*	140
GABA	*Sesamum indicum L.*	141
Flavonoids	*Hordeum vulgare*	142
Jasmonic acid	*Lycopersicon esculentum*	21
Polyphenol	*Cakile maritima*	143
Tropane alkaloids	*Datura innoxia*	144
Anthocyanins	Grevillea spec.	17
Trigonelline	*Glycine max*	145
Glycinebetaine	*Trifolium repens*	146
Polyamines	*Oryza sativa*	147
Glycine betaine	*Triticum aestivum*	148
Sucrose and Starch	*Cenchrus pennisetiformis*	149

Adapted from Parvaiz and Satyavati 2008.[150]

Table 3. Influence of drought stress on various plant secondary metabolites

Secondary metabolite	Plant species	Reference
Glycosides	*Scrophularia ningpoensis*	151
Morphine alkaloids	*Papaver somniferum*	152
Trigonelline	*Glycine max*	153
Glucosinolates	*Brassica napus*	154
Chinolizidin alkaloids	*Lupinus angustifolius*	155
Epicatechins	*Camellia sinensis*	156
Betulinic acid	*Hypericum brasiliense*	157
Rutine	*Hypericum brasiliense*	157
Flavonoids	*Prisms sativum*	27
Anthocyanins	*Pisum sativum*	158
Chlorogenic acid	*Helianthus annuum*	159
Rosmarinic acid	*Salvia miltiorrhiza*	160

Adapted from Bartels and Sunkar 2005.[161]

Influence of Heavy Metal Stress on Secondary Metabolites

Metal ions (lanthanum, europium, silver and cadmium), and oxalate are also influenced secondary metabolite production.[33] The trace metal nickel (Ni) is essential component of urease enzyme, is needed for plant development.[33] However, elevated Ni concentrations reduce plant growth.[34] The significant decrease in anthocyanin levels due to Ni stress has been reported by Hawrylak et al.[35] Moreover, Ni has been shown to inhibit accumulation of anthocyanins.[36] Trace metals obviously limit anthocyanin biosynthesis by inhibiting activity of l-phenylalanine ammonia-lyase (PAL).[36] Effective accumulation of metals (Cr, Fe, Zn and Mn) also produced an increase of oil content up to 35% in *Brassica juncea*.[37] Cu^{2+} and Cd^{2+} have been shown to induce higher yields of secondary metabolites such as shikonin[38] and also on the production of digitalin.[39] Cu^{2+} also stimulated the production of betalains in *Beta vulgaris*.[40] Co^{2+} and Cu^{2+} exhibit stimulatory effect on the production of secondary metabolites.[40] In an attempt to enhance betalaines production, the hairy roots were exposed to metal ions.[41] Obrenovic[42] has demonstrated stimulatory effects of Cu^{2+} on the accumulation of betacyanins in callus cultures of *Amaranthus caudatus*. Addition of Zn^{2+} (900 μM) enhanced the yield of lepidine in cultures of *Lepidium sativum*.[42] However, Cu proved more effective than Zn in enhancing the yield.[43] $AgNO_3$ or $CdCl_2$ elicited overproduction of two tropane alkaloids, scopolamine and hyoscyamine, in hairy root cultures of *Brugmansia candida*.[44] Rare-earth metal (lanthanum) had influence on production of taxol in cell culture of Taxus sp.[45] Oat and bean plants treated with cadmium and copper significantly increased putrescine (Put) content.[46] However, a decrease in Put level in Cd^{2+} or Cu^{2+} treated sunflower leaf disks has been reported. Sunflower leaf disks showed a significant decreased in spermidine (Spd) content and no variation in spermine (Spm) level when they were treated with Cd^{2+} or Cu^{2+} respectively.[47] However, Jacobsen et al.[48] reported no changes in Spd or Spm content in chromium-exposed leaves of barley and rape plants, but Put accumulated with increasing chromium concentrations or exposure time. Lin and Kao[49] reported that copper treatment increased Put, but a decrease in Spm concentration in rice leaves.

Influence of Cold Stress on Secondary Metabolites

Low temperature is one of the most harmful abiotic stresses affecting temperate plants. These species have adapted to variations in temperature by adjusting their metabolism during autumn, increasing their content of a range of cryo-protective compounds to maximize their cold tolerance.[50] In the cryopreservation process, environmental changes including osmotic injury, desiccation and low temperature can impose a series of stresses on plants.[50] During over wintering, temperate plant metabolism is redirected toward synthesis of cryoprotectant molecules such as sugar alcohols (sorbitol, ribitol, inositol) soluble sugars (saccharose, raffinose, stachyose, trehalose), and low-molecular weight nitrogenous compounds (proline, glycine betaine).[50] Cold stress increases phenolic production and their subsequent incorporation into the cell wall either as suberin or lignin.[51] In addition, apple tree adaptation to cold climate was found to be associated with a high level of chlorogenic acid.[52] Lignification and suberin deposition are also shown to increase resistance to cold temperatures. A mechanism by which suberin and lignin may protect plants from freeze damage.[51] Christie et al.[53] reported the accumulation of anthocyanins during cold stress. Pedranzani et al.[54] reported that cold and water stresses produce changes in endogenous jasmonates in *Pinus pinaster*. Lei et al.[55] reported that melatonin protect against cold-induced apoptosis in carrot suspension cells by upregulation of polyamines (putrescine and spermine). Moreover, Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress.[56] Recently, Zhao et al.[57] reported that melatonin improves the survival of cryopreserved callus of *Rhodiola crenulata.* The survival rate of the cryopreserved callus increased when the callus was pretreated with 0.1 μM melatonin.

Recently, the effect of cold stress on polyamime accumulation was reported.[58] When leaves of wheat (*Triticum aestivum* L.) are exposed to a cold temperature, accumulation of putrescine (6–9 times), spermidine accumulates to a lesser extent and, spermine decreases slightly. Moreover, alfalfa (*Medicago sativa* L.) also accumulates putrescine under low temperature stress.[59] Hummel et al. (2004)[60] reported that cold tolerance was associated with increased levels of polyamines (agmatine and putrescine) and their levels could be a significant marker of chilling tolerance in seedlings of *P. antiscorbutica*.

Temperature Variations Influence Plant Growth and Secondary Metabolite Production

Temperature strongly influences metabolic activity and plant ontology, and high temperatures can induce premature leaf senescence.[61] Carotenoids in Brassicaceae, including β-carotene, were found to be slightly decreased after thermal treatments.[61] Elevated temperatures increase leaf senescence and root secondary metabolite concentrations in the herb *Panax quinquefolius*.[62] Elevated temperatures by 5°C would reduce photosynthesis and biomass production of *P. quinquefolius*, on the contrary storage ginsenoside is reported to be enhanced.[63]

Several studies have examined the effects of increased temperatures on secondary metabolite production of plants.[61] Lower soil temperatures caused an increase in levels of steroidal furostanol and spirostanol saponins.[64] Temperature variations has multiple effects on the metabolic regulation, permeability, rate of intracellular reactions in plant cell cultures.[61] Changing the culture temperature may change the physiology and metabolism of cultured cells and subsequently affect growth and secondary metabolite production.[61] Temperature range of 17–25°C is normally used for the induction of callus tissues and growth of cultured cells.[6] Yu et al.[65] reported the influence of temperature and light quality on production of ginsenoside in hairy root culture of *panax ginseng.* Chan et al.[66] reported that *Melastoma malabathricum* cell cultures incubated at a lower temperature range (20 ± 2°C) grew better and had higher anthocyanin production than those grown at 26 ± 2°C and 29 ± 2°C. Optimum temperature (25°C) maximizes the anthocyanin yield as demonstrated in cell cultures of *Perilla frutescens*[67] and strawberry.[68] Lower temperature favors anthocyanin accumulation, but reduces cell growth. For strawberry cell culture, maximum anthocyanin content was obtained at 15°C and it was about 13-fold higher than that obtained at 35°C.[68] For suspension cultures of *Perilla frutescens*, anthocyanin production was remarkably reduced at the relatively high temperature of 28°C, whereas 25°C was optimal for the productivity of the pigment.[67] Similar observations on optimal productivity of anthocyanin in cell suspension cultures of *Daucus carota* was reported.[69] Pigment release from hairy root cultures of *Beta vulgaris* under the influence of different temperatures was reported.[70]

Influence of Light on Secondary Metabolite Production

It is well known that light is a physical factor which can affect the metabolite production. Light can stimulate such secondary metabolites include gingerol and zingiberene production in *Z. officinale* callus culture.[71] A positive correlation between increasing light intensity and levels of phenolics has been reported.[11] Larsson et al.[72] reported decreases in foliar tannin and phenolic glycosides in shaded willow foliage. Arakawa studied the effect of UV light on anthocyanin accumulation in light colored sweet cherry. In apples, UV light from 280–320 nm synergistically stimulate anthocyanin synthesis when it was combined with red light.[74] Effect of light irradiation on anthocyanin production in cell suspension cultures of *Perilla frutescens* was reported.[75] Chan et al.[66] investigated the effects of different environmental factors, such as light intensity, irradiance (continuous irradiance or continuous darkness), on cell biomass yield and anthocyanin production in cultures of *Melastoma malabathricum*. Moderate light intensity (301–600 lx) induced higher accumulation of anthocyanins, the cultures exposed to 10-d continuous darkness showed the lowest pigment content, while the cultures exposed to 10-d continuous irradiance showed the highest pigment content. Light

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irradiation exhibited significant influence on the accumulation of anthocyanins by cell cultures of strawberry,[76] *Daucus carota*[69] and *Centaurea cyanus*.[77]

UV-B have been seen to increase in flavonoids in barley,[78] and in polyamines in cucumber.[79] Hagimori et al.[80] reported the effect of light and plant growth regulators on digitoxin formation in *Digitalis purpurea* L. Moreover, effect of light irradiation influenced artemisinin biosynthesis in hairy roots of *Artemisia annua*.[81] Fett-Neto et al.[82] reported the effect of white light on taxol and baccatin III accumulation in cell cultures of *Taxus cuspidate*. UV-B irradiation enhanced the concentration of flavonols in Norway spruce (*Picea abies*).[83] *Catharanthus roseus* plants, exposed to UV-B light show significant increases in the production of vinblastine and vincristine, which have proven effective in the treatment of leukemia and lymphoma.[84] UV-B radiation could increase flavonoid content and phenylalanine ammonia-lyase (PAL) activity, associated with a decrease in chlorophyll content.[85] UV (300–400 nm) increased flavonoids in the roots of pea plants.[86] UV-B was also shown to induce the production of flavonols in silver birch and grape leaves.[87] Moreover, under six different daily doses of UV-radiation (UV-A and UV-B), photosynthetic pigments, condensed tannins were accumulated whereas its precursor, (+)-catechin, decreased significantly.[88] Our recent report suggests that photoperiod regimes influence endogenous indoleamines (serotonin and melatonin) in cultured green algae *Dunaliella bardawil*.[89]

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Influence of Polyamines on Secondary Metabolites

Polyamines, putrescine, spermine and spermidine are found in a wide range of organisms-bacteria, plants and animals. In plants, polyamines are involved in various physiological events such as development, senescence and stress responses.[90] High cellular levels of polyamines correlate with plant tolerance to a wide array of environmental stresses. Moreover, as compared with susceptible plants, stress-tolerant ones generally have a large capacity to enhance polyamine biosynthesis in response to abiotic stress.[90] Conversely, treatments with polyamine biosynthesis inhibitors reduce stress tolerance, but this effect is reversed by concomitant application of exogenous polyamines.[91] The influence of polyamines on in vitro morphogenetic response and caffeine biosynthesis were reported in *Coffea canephora*.[91] Apart from primary metabolic functions, external feeding of certain polyamines are known to act as elicitors.[91] Spermidine and putrescine, each at 0.75 mM significantly enhanced betalaine production in hairy root cultures of red beet.[92] Moreover, putrescine at 0.6 mM treatment stimulated polysaccharide synthesis in suspension cultures of *Dendrobium huoshanense*.[93]

Influence of Methyl Jasmonate and Jasmonic Acid on Secondary Metabolites

It is well known that jasmonic acid (JA) and methyl jasmonate (MeJ) are signal molecules in biotic and abiotic stresses.[94] Their broad effectiveness can be explained by the fact that these molecule acts as elicitors in a wide spectrum of signaling pathways. MeJ and JA have been proved to be able to elicit the production of several compounds (alkaloids, terpenoid and phenolic phytoalexins, coumarins and taxanes) in many plant species.[94] Exogenous jasmonates applied to plants have been shown to exhibit morphological and physiological effects.[94] Jasmonates have been associated with the accumulation of secondary metabolites, which are also part of the defense response.[94] MeJA increased the content of shikonin and its derivatives (red naphthoquinonone) in *Onosma paniculatum* cultured cells. MeJA can also promote the biosynthesis of endogenous IAA in plants.[95] Exogenous application of jasmonates greatly stimulated the biosynthesis of a wide range of secondary metabolites in cell suspension cultures, and in intact plants.[94] MeJA induced anthocynin accumulation was reported in *Arabidopsis thaliana*,[96] strawberry fruits,[97] *Vaccinium pahalae*,[98] *Vitis vinifera*[99] and tulip leaves.[100] Moreover, MeJA and salicylic acid induce anthocyanin production in in vitro callus cultures of *D. carota*.[101]

MeJA inhibited the cell growth and promoted the secondary metabolite production in root cultures of *Bupleurum falcatum* L.,[102] Taxus spp.[103] and rice.[104] The effects of exogenously applied MeJ on the content of biogenic amines include putrescine, spermidine, tyramine, cadaverine and 2-phenylethylamine in seedlings of common buckwheat (*Fagopyrum esculentum*) were investigated.[105] Influence of different abiotic factors on secondary metabolites in various plant species were shown in **Table 4**.

Influence of Plant Growth Regulators on Secondary Metabolites

The production of useful secondary metabolites via plant tissue and organ culture has been reported by many researchers. Many efforts have been made to improve the productivity of the plant tissue cultures, such as studies on hormone-dependency, media composition and light exposure.[5,9] Many researchers have tried to enhance anthocyanin accumulation through the manipulation of phytohormones in cell suspensions of strawberry *(Fragaria ananassa)*,[106] *Daucus carota,*[69] *Ipomoea batatas*[107] and *Oxalis reclinata*.[108] Plant cell cultures are an excellent source for anthocyanin production in view of the higher productivity ranging from 10 to 20% on dry weight basis.[109] The influence of different growth regulators on biomass accumulation and anthocyanin content in solid-state and liquid state batch cultures of *Daucus carota* was studied.[69] While growth regulators such as 2,4-D, IAA and NAA supplemented at different levels, supported growth as well as anthocyanin synthesis. Among the cytokinins, kinetin (0.1 and 0.2 mg l^{-1}) supported highest productivity. The combinations of IAA at 2.5 mg l^{-1} and kinetin at 0.2 mg l^{-1} was superior to other combinations.[69] Lower 2,4-D concentration in the medium limited cell growth and enhanced both anthocyanin production and anthocyanin methylation.[69,107] The most significant enhancement in anthocyanin synthesis was obtained when treated with MeJ.[98]

Calcium is an ubiquitous molecule involved in various signal transduction pathways in plants. Calcium have been found to increase in response to stress such as light, salinity, cold and drought.[110] The influence of calcium on anthocyanin

Table 4. Effect of different abiotic elicitors on the production of various secondary metabolites in plants

Plant species	Abiotic factor	Secondary metabolite	Reference
Ocimum basilicum	Methyl Jasmonate	Rosmarinic acid, Caffeic acid	162
Beta vulgaris	Calcium, magnesium, manganese, zinc, copper, iron and cobalt	Betalain	163
Dioscorea bulbifera	$CuSO_4$	Diosgenin	164
Beta vulgaris	Metal ions	Betalaines	41
Beta vulgaris	Polyamines and Mej	Betalaine	165
Taxus chinensis	Lanthanum	Taxol	166
Beta vulgaris and Tagetes patula	Micro algal extracts	Betalaine	167
Vitis vinifera suspension cultures	Jasmonic acid and light irradiation	Anthocyanin	99
Beta vulgaris	Spermidine, Putrescine and Cu^{2+}	Betalaine	91
Beta vulgaris	polyamines	Betalaine	91
Brugsmansia candida	Salicylic acid	Scopolamine Hyposcyamine	45
Lepidium sativum	Zn^{2+}	Lepidine	43
Cichorium intybus	Polyamines	Coumarins	168
Capsicum	Nitrate and phosphate	Capsaicinoids	169
Capsicum	Cinnamic acid, coumaric acid, caffeic acid and ferulic acid	Capsaicin production	170
Vanilla planifolia	Blue light	Vanillin	171
Amaranthus caudatus	Cu^{2+}	Betacyanins	42
Vitis vinifera	Sucrose Osmotic stress	Anthocyanin	110

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accumulation was studied by Sudha and Ravishankar.[8] The treatment of *Daucus carota* cell cultures with low levels of calcium resulted in the enhancement of both growth and anthocyanin production. The accumulation of anthocyanin in cell cultures of *Daucus carota* and the enzymes involved in their biosynthesis were investigated. Our recent report suggest that exogenously administered calcium enhance somatic embryogenesis in in vitro cultures of *C. canephora*.[111] Exogenously applied melatonin stimulates root growth and raises endogenous indole-3-acetic acid (IAA) in roots of etiolated seedlings of *Brassica juncea*.[112] The influence of different growth regulators on secondary metabolites were given in **Table 5**.

Influence of Nutrient Stress on Secondary Metabolites

When plants are stressed, secondary metabolite production may increase because growth is often inhibited more than photosynthesis, and the carbon fixed is predominantly allocated to secondary metabolites.[1] The *Daucus carota* callus subjected to phosphate stress produced 7.2% dry wt anthocyanin against 5.4% dry weight (DW) in the control.[113] Nutrient stress also has a marked effect on phenolic levels in plant tissues.[11] Deficiencies in nitrogen and phosphate lead to the accumulation of phenyl propanoids and lignification.[10] In tomato, the 3-fold increase in anthocyanidins level and the simultaneous doubling of quercetin-3-O-glucoside occurs under nutrient stress stress.[114] Zeid (2009)[115] reported that the increased urea concentration in the nutrient solution markedly increased putrescine contents in *Phaseolus vulgaris* cell suspensions. Osmotic stress created by sucrose and other osmatic agents was found to regulate anthocyanin production in *Vitis vinifera* cultures.[110]

Influence of Climate Change on Secondary Metabolites

Climate change is the major threat to biodiversity and one of the main factors affecting human health and well-being over the coming decades.[116] Cold weather crops like rye, oats, wheat and apples are expected to decline their productivity by about 15% in the next 50 y and strawberries will drop as much as 32% simply because of projected climate changes.[116] Plants are extremely sensitive to such changes, and do not generally adapt quickly. Ozone exposure has been shown to increase conifer phenolic concentrations,[117] but low ozone exposure had no effect on monoterpene and resin acid concentrations.[118] Changes in crop quality due to ozone exposure have been studied in a limited number of crops. For example, in wheat, ozone reduced yield but increased grain protein concentration.[119] Moreover, ozone was found to have positive effects on the quality of potato tubers by reducing sugars and increasing the vitamin C content.[120] In contrast, O_3 has been found to reduce the oil, protein and carbohydrate contents in rape seeds.[121] Moreover, in leaves of *Ginkgo biloba* ozone fumigation increased the concentrations of terpenes, decreased the concentrations of phenolics.[122]

Plants grown at high CO_2 levels exhibit significant changes of their chemical composition.[123] A prominent example of a CO_2 effect is the decrease of the nitrogen (N) concentration in vegetative plant parts as well as in seeds and grains resulting in the decrease of the protein levels.[123] Previous studies have shown that elevated CO_2 increases phenolics and condensed tannins in the leaves. In conifers, elevated CO_2 influenced a decrease/increase in concentrations of some individual monoterpenes[124] and an increase in total phenolics have been reported.[123] Increased concentrations of the monoterpene a-pinene was noticed in elevated

Table 5. Increased secondary metabolite production from in vitro plant tissue and organ culture

Plant species	Plant growth regulator	Secondary metabolite	Reference
Psoralea cordifolia	MS + TDZ + BA	Isoflavones	172
Vitis vinifera	MS + IAA + GA_3 + UV	Resveratrol	173
Azadirachta indica	MS + 2,4-D	Azadirachtin	174
Catharanthus roseus	MS + 2,4-D + UV-B	Catharathine	175
Rauvolfia serpentina	MS + BAP + IAA	Serpentine	176
Rauvolfia serpentina	MS + IAA + Cu^{2+}	Reserpine	177
Stevia rebaudiana	MS + BA + NAA	Stevioside	178
Capsicum annum	MS + 2,4-D + Kin	Capsiacin	179
Zataria multiflora	MS + IAA + Kinetin	Rosmarininc acid	180
Vitis vinifera	MS + BAP + NAA	Anthocyanin	181
Gymnema sylvestre	MS + 2,4-D + IAA	Gymnemic acid	182
Gymnema sylvestre	MS + IAA + BA	Gymnemic acid	183
Catharanthus roseus	MS + 2, 4-D + GA_3	Vincristine	184
Hydrocotyle bonariensis	2,4-D + Kinetin	Flavonoids	185
Daucus carota	IAA +Kn	Anthocyanin	69
Fabiana imbricate	MS + NAA + 2,4-D	Rutin	186
Cichorium intybus	NAA + Kn	Esculin, Esculetin	187
Capsicum annum	MS + 2,4-D + GA3	Capsaicin	188
Cassia acutifolia	MS + 2,4-D + Kinetin	Anthraquinones	189
Phytolacca americana	MS + 2,4-D	Betacyanin	190
Taxus *spp*	B5 + 2,4-D + BA	Taxol	191
Catharanthus roseus	MS + IAA	Indole alkaloids	192
Gynostemma pentaphyllum	MS + 2,4-D + BA	Saponin	193
Coscinium fenustratum	LS + NAA + 2,4-D + BA	Berberin	194
Beeta vulgaris	MS + IAA	Betalain	195
Anisodus luridus	MS + 2,4-D + BA	Tropane alkaloids	196
Capsicum annuum	MS + 2,4-D + Kn	Capsaicin	197
Catharanthus trichophyllus	MS + IAA + GA_3	Indole alkaloids	198
Catharanthus roseus	MS + 2,4-D + GA_3 + Vanadium	Indole alkaloid	199
Mucuna pruriens	MS + IAA	L-Dopa	200

Adapted from Karuppusamy, 2009[8]; Vijaya Sree et al. 2010.[201]

CO_2 composition.[123] In contrast to this, Williams et al.[124] found decreased concentrations of b-pinene in needles under elevated CO_2. Several studies have been reported the effect of temperature on secondary metabolite production in plants. Secondary metabolites increase in response to elevated temperatures.[60,62,63] In contrast to this Snow et al.[125] reported that high temperature decreases monoterpene levels in Douglas fir (*Pseudotsuga menziesii*).

Influence of Environmental Factors on Secondary Metabolites

The local geoclimate, seasonal changes, external conditions such as light, temperature, humidity affect composition of secondary metabolites.[61] The synthesis of secondary metabolites, including saponins, response to environmental factors and part of an adaptative strategy leading to tolerance of abiotic stresses. Saponins occur in roots, leaves, stems, bulbs, flowers and fruit of *Panax ginseng,* and their content influence by environmental abiotic factors.[64] The accumulation of saponins in plant reproductive organs, play a role in chemical protection and the plant response to environmental factors.[126] American ginseng plants exposed to longer sunlight were found to have higher root ginsenoside contents than those exposed to shorter periods of direct sunlight.[127] He et al.[128] reported the effect of CO_2 or ozone on endogenous hormones in the leaves of *Ginkgo biloba.* Huang et al.[129] reported that elevated O_3 reduce the concentrations of the isorhamnetin aglycon (7%), but increase the concentration of quercetin aglycon (6%). Elevated CO_2 reduce the concentrations of keampferol aglycon (10%), isorhamnetin aglycon (15%).

Melatonin, a neurohormone produced by the pineal gland, has recently been reported in the plant kingdom.[130,131] Melatonin is an environmentally friendly-molecule with broad spectrum antioxidant capacity. High levels of melatonin exist in an aquatic plant, the water hyacinth, which is highly tolerant of environmental pollutants.[132] Elevated levels of melatonin probably help plants to protect against environmental stress caused by water and soil pollutants. Recently, the potential relationships between melatonin supplementation and environmental tolerance in plants was reported.[132] In pea plants treated with high levels of copper in the soil. Copper contamination kills pea plant, however, melatonin added to the soil significantly enhanced their tolerance therefore, increased their survival.[132] Serotonin is an indoleamine neuroharmone in vertebrates. Recently, serotonin has also been reported in wide range of plant species.[133] Serotonin involved in various physiological functions in plants viz. protect from environmental stress, protective against pathogenic infection. Serotonin is believed to play a protective role against reactive oxygen species (ROS) leading to a delay in the process of senescence.[133] In *D. metel* serotonin acts as an antioxidant in protecting the young reproductive tissues from environmental stress. The exposure of Datura flower to a cold stress significantly increased the concentrations of serotonin.[134]

Influence of Brassino Steroids on Secondary Metabolites

Brassinosteroids (BRs) are a group of naturally occurring plant steroidal compounds with wideranging biological activity.[135] Several reports suggest that treatment with BRs enhances plant resistance to a variety of environmental stresses.[136] And also confer resistance to plants against various abiotic and biotic stresses.[137] The chlorophyll content was maintained in BR-treated seedlings during the cold treatment, increasing even further during recovery from cold.[138] The possible role of BRs to enhance plant resistance against fungal pathogen infection has been investigated.[139] The increase in resistance in BR-treated potato tubers was associated with enhancement of ABA, ethylene levels, phenolics and terpenoids. BR-induced disease resistance was also noted in barley and cucumber plants. In cucumber plants increased activities of peroxidase and polyphenoloxidase enzymes, which are involved in the metabolism of polyphenols, was suggested as a factor contributing to BR-induced disease resistance.[137]

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Conclusion

Thus it is evident that abiotic stress factors influence growth and secondary metabolite production in higher plants. The influences are well marked. In fact, productivities depend on the changed ecosystem also. For example, influence of climate change on bees, butterflies, soil microflora, etc. also effect plant antogeny, adaptation and productivities. Such holistic studies are lacking. Most importantly, climate change drastically influence water availability, salinity and several adverse soil conditions which will have direct bearing on original yields. The major advantage of the cell cultures include synthesis of bioactive secondary metabolites, independently of environmental and soil conditions. The use of in vitro plant cell culture for the production of chemicals and pharmaceuticals has made great strides. The use of genetic tools and regulation of pathways for secondary metabolism will provide the basis for the commercial production of seconadary metabolites. The increased level of natural products for medicinal purposes coupled with the low product yields and supply concerns of plant harvest has renewed interest in large-scale plant cell culture technology. Biotic and abiotic factors which influence secondary metabolite production have a bearing on enhancing the potential to over produce useful phytochemicals for varied applications. Moreover, molecular understanding of stress response will be useful in plant improvement with enhanced adaptation and efficacy.

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