Chapter

Antioxidant Properties of Alpine Plant

Vijay Laxmi Trivedi and Mohan Chandra Nautiyal

Abstract

Alpines are the exceptional regions of the world's biomes. They have unique climatic and topographic conditions; they are the habitat of some of the highly specialized flora and fauna. The harsh environmental conditions and extreme fluctuations in them on a seasonal and diurnal basis created extremely stressful situations for the alpine plants. Such stress causes damage to biochemical structures and compounds of the plant cells leads to the production of free radicals, *i.e.* reactive oxygen species (ROS), which can further damage the plant cells or tissues. Alpine plants protect themselves from those ROS efficiently by their highly competent enzymatic and non-enzymatic antioxidant system. In general, this protection increases in alpine plants with altitudes; however, some exceptions are also reported. Antioxidant compounds *viz.* ascorbic acid, tocopherol, glutathione, carotenoids found in alpine flora in higher concentrations as compared to low land plants. Phenolic compounds protected the alpine plants from UV induced ROS by screening the UV radiations and blocking their entries in the cell's mesophyll. This higher antioxidant potential of the alpine plants is highly beneficial for the human being as most alpine plants are the sources of some life-saving drugs.

Keywords: enzymes, non-enzymatic compounds, UV radiation, medicinal plants, freeze–thaw cycles, flavonoids

1. Introduction

Alpine biomes of the world are characterized by their unique features and usually lie between an altitude of about 10,000 feet (3,000 meters) and where the snow line of a mountain begins. The Alpine and Arctic biomes cover 16% of the earth's surface area. Testolin *et al*. [1], based on regional tree line models, estimated their extent to 3.56 Mkm^2, corresponding to 2.64% of the total land area outside Antarctica. Asia hosts almost three-fourths of the global alpine area with 2.59 Mkm^2, followed by South America (15%; 0.55 Mkm^2), North America (9%; 0.32 Mkm^2), and Europe (2%; 0.08 Mkm^2), while Oceania and Africa together contribute to only 1% of the global alpine area. The climate of the alpine regions is dynamic and changes as you move above the lower to higher elevations. The most prominent environmental factor, *i.e.* the temperature normally drops by about 10 °C for every 1000 meters as we go up a mountain. The alpine regions experience a long and cold winter season that lasts about nine months in some alpine areas of the world from around October to May. Temperatures in summer normally ranges from 40 to 60 °F and may last from June to September. Temperature shows very high fluctuations and can normally drop from warm to freezing within a day. The alpine biome is usually dry, with an average precipitation of 12 inches (30 cm) each year. Topographical specialization such as physical gradients, rough terrain, and relative isolation, mountains created attitudinally segregated life zones, and harsh climatic factors of the regions make them very special and unique habitats of some wonderful floras around the world. However, to grow in such a harsh environment, they must have to cope up with the related constraints, including reduced O_2 and CO_2, strong winds, high solar irradiance, shallow rocky soils, low temperatures, and low water and nutrient contents, UV radiation, large temperature variations, *etc.* [2]. Combinations of high-altitude environmental stress lead to increased reactive oxygen species (ROS) production, which increases the risk of oxidative damages in the alpine floras. For performing the vital life functions, alpine flora must quench those ROS. Thus, the alpine flora showed specific adaptations such as accumulation of the secondary metabolites (SMs). Several enzymes such as ascorbate peroxidase, catalase, superoxide dismutase, and glutathione reductase, *etc*., and other specific molecules such as carotenoids, xanthophylls, *etc.*, play an important role in the protection of alpine flora from the ROS. In this chapter, we will adress the challenges related to ROS production and protection from them in alpine plants.

2. Challenges of alpine habitats that leads to ROS production

Reduced or lower partial pressures of physiological gases (Atmospheric pressure is reduced from sea level values of c. 1000 h Pa (1 bar) by c. 10% for every 1000 m in elevation.), and other associated factors, for example, lower temperatures, correspondingly more precipitation falling as snow, and less atmospheric attenuation of radiation affected the life processes of the alpine plants. Temperature directly affects the plant metabolism by affecting them at the molecular *viz.* DNA, proteins or supramolecular *viz.* membranes, chromosomal structures [3]. The chilling temperature causes decreased activities of several enzymes, including the kinases, carboxylases, *etc.* involved in respiration and photosynthetic processes. Further lowering of the temperature beyond the freezing point leads to the cessation of the vascular functions. Other cytosolic damages involve the complications like the formation of embolisms or cavitation in the xylem and injury to cell membranes.

Along with this alpine region experiences unusually higher diurnal temperature fluctuations, the temperature may range from 0–18 °C from dawn to dusk, on these conditions, leaf temperatures might increase up to 35 °C and lower up to −7 °C to 28 °C. The freeze–thaw cycles operated in plants due to fluctuating diurnal and seasonal temperature may inactivate the PS II and catalase [4] and can enhance the ROS formation in the alpine flora. Radiations affect the alpine environments positively and negatively; for example, positive longwave radiation balance warms leaves in the cool alpine, but the more common negative longwave balance cools minimum temperatures in an already cool environment. Visible shortwave (solar) radiation drives photosynthesis and warms leaves but can also cause photochemical problems [5]. The most destructive radiations in the alpine region are ultraviolet radiation that may cause photochemical damage and other somatic and molecular damages in the alpine flora. High irradiation coupled with low temperature and other stresses leads to the photosynthesis machinery's photoinhibition and halts in synthesis, transport, and storage of the resources. Desiccation is also a profound problem in the alpine plants that are experienced by the plants either in winters due to the freezing lead complications in the plants *viz.* root damage, loss of xylem conductivity, ice formation inside the plants, and in the summer when warmer temperatures can intensify the leaf-to-air vapor deficit and further stimulated the desiccation stress [6]. All the above factors lead to the formation of the ROS that may lead to damage to the general cellular structure and loss of function and block of basic metabolism and repair processes [7], and control of ROS formation and ROS detoxification might be of key importance for the survival of alpine plants.

3. ROS protection in alpine plants

Plants operated several mechanisms to quench the ROS based on the enzymes and several other compounds, including several primary and secondary metabolites. Antioxidative enzymes enhanced their activities, and other lipid and water-soluble compounds, which serve as antioxidants, increase the tolerance in plants to several stresses responsible for ROS productions [8, 9]. Alpine plants also adopted numerous biochemical strategies to quench the ROS that may damage the lipid membrane generated during the physiological process. These strategies included oxidation of superoxide by cascades of enzymatic reactions and accumulation of the compounds like carotenoids, a-tocopherol, ascorbic acid, glutathione, flavonoids, *etc.* [10, 11, 12]. Mostly high altitudinal ecotypes consisted of higher antioxidants than their lower altitudinal counterparts mainly due to combined effect and higher light intensities and lower temperature as compared to lower elevations [13, 14].

3.1 Enzymatic antioxidant protection in alpine plants

The enzymatic antioxidative defense system is the cascade of the many enzymes; some of them catalyzes the ROS degradation [superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (POX, EC 1.11.1.7), glutathione peroxidase (GPX, EC 1.11.1.9), *etc.*] and other's regenerated the soluble antioxidants [ascorbate peroxidase (APX, EC 1.1.11.1), monodehydro ascorbate reductase (MDAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and glutathione reductase (GR, EC 1.8.1.7)] [15]. Alpine flora showed an increase in many of these enzymes, such as CAT, SOD, APX, DHAR, GR [16, 17] but some plants also showed a contradictory pattern. For example, the SOD, GR, and CAT activities were higher in the leaves of *Soldanella alpina* but lower in leaves of *Ranunculus glacialis* [18]. Other plants also showed the range of the variations in antioxidant enzyme activities; for example, extremely higher antioxidant enzyme activities were found in high altitudinal ecotypes of *Soldanella pusilla* moderate in *Poa laxa*, *Carex curvula* and lower in *Taraxacum alpinum, Dryas octopetala,* and some other species [6, 18, 19]. For example, *Polygonum viviparum* showed increased antioxidant enzyme's activities as the altitude increases in the Tianshsan Mountain [20]. During the winter acclimation and spring rewarming, the role of the antioxidant enzymes becomes more prominent, especially in the perennial plants where roots also efficiently takes part in ROS quenching as found in roots of perennial grasses *Poa sphyondylodes, Bromus inermis, Bromus sinensis, Elymus nutans* [21]. In alpine plants, antioxidant enzymes are also involved in the freezing resistance [22] and protected the plants from the ROS produced due to the freeze–thaw cycles of the alpine habitats.

3.2 Non-enzymatic antioxidant

Non-enzymatic antioxidant comprises the major cellular redox buffers which interact with various cellular constituents includes the compounds like ascorbic acid (AA), glutathione (γ-glutamyl-cysteinyl-glycine, GSH), tocopherol, carotenoids, and phenolic compounds. These commands also served as the cofactors of the various enzymes involved in ROS quenching [23]; along with this, these compounds also participate in UV protection.

3.2.1 Ascorbic acid

L-ascorbic acid (L-AA or vitamin C) is the most important antioxidant found in the plants that also takes part in various physiological and developmental processes in the plants protected critical macromolecules from the ROS. Alpine plants are very specific about the L-AA, and some contain extremely high L-AA content. For example in the young leaves of *Soldanella alpina* [12] and *Polygonum viviparum* [20] reported a very high L-AA range. In few alpine species, ascorbate/L-AA is the major C metabolite after sucrose; for example, in the leaves of *S. alpina* [12] in such plant species majority of L-AA found outside the chloroplasts [18]. Although in some alpine flora relatively lower level of L-AA was reported, such as in *Ranuculus glacialis* and *Homogyne alpina,* and most of their L-AA content was reported to present in the chloroplasts. The importance of ascorbic acid in antioxidant protection was determined by Wildi and Lutz [19]. They found an increasing level of antioxidant content in some alpine plant species as the altitude increases. The role of the ascorbic acid is most prominent in this increase where they correlated this increase to increase in light intensity as altitude increases. The concentration of L-AA within an alpine plant species also reflected the diurnal variations. The rhythm of L-AA concentration was reported to go along with light intensity; hence the lowest L-AA was observed at the time of lowest light intensities and lowest temperature in some alpine plants [19].

3.2.2 Glutathione

Glutathione is a tripeptide (γ-glutamyl-cysteinyl-glycine; γ-Glu-Cys-Gly) with long hydrophilic groups and a key low molecular weight non-protein thiol compound known to play a crucial role as an antioxidant [24]. Glutathione also helps in the regeneration of other antioxidants, ascorbic acid and tocopherol, it is the substrate of dehydroascorbate reductase that catalyzes the regeneration of reduced ascorbate, which is the substrate for ascorbate peroxidase regenerated α-tocopherol while detoxifying of H_2O_2 and arresting lipid peroxidation reactions [25, 26]. Potentially higher glutathione synthesis was observed in many plant species in stress conditions, especially in high light intensities, chilling, or freezing temperatures, as reviewed by Tausz [27] and Hasanuzzaman *et al*. [28]. Alpine plants did not show uniformity in the presence of glutathione concentrations; for example, it was higher in *Taraxacum alpinum* and *Soldanella alpina* and comparatively lower in the *Homogyne alpina* and *Ranunculus glacialis*. In general, the alpine plant showed an increase in glutathione concentrations with increasing altitudinal gradients [19], as found in *S. alpina* and *R. glacialis* [26]. Diurnal variations were also observed in studied alpine flora where with increased glutathione concentrations during the morning and maximum during the mid-day and the concentration decline continuously in the afternoon and reached a minimum in the night [19]. Wildi and Lutz [19] also reported the diurnal fluctuations in the glutathione concentration in the alpine plant-like *T. alpinum* and *H. alpina,* which depend on the light intensities and the temperature differences. Glutathione plays an important role in the chilling and freezing protection along with other antioxidants from low temperature in the non-acclimatized plants as well as the acclimatized [29, 30]. Glutathione also plays an important role in increasing the alternative antioxidative scavenging by ascorbate and enzymes like catalase [26], and the glutathione status also acts as a signal or modulator for stress response in the plants [25, 31].

3.2.3 Tocopherols and tocotrienols

Tocopherols and tocotrienols, collectively termed tocochromanols are lipid-soluble molecules with a polar chromanol ring and a hydrophobic polyprenyl side chain [32]. They are synthesized in the shikimate and 1-deoxy-D-xylulose 5-phosphate (DOXP) pathways. The tocopherols are the tocochromanols having a fully saturated side chain. In contrast, tocotrienols are with an unsaturated side chain, and the naturally occurring form of tocopherols *viz.* α, β, γ, and δ is determined by the number of methyl groups in the chromanol ring. Tocochromanols participate in the quenching of peroxyl radicals and other ROS [33] and an important thylakoid-bound radical scavenger. The levels of tocopherols are generally high in some alpine and arctic plants; however, some exceptions are also present, for example, leaves of *Ranunculus glacialis* in which it is extremely low [19]. However, range of the variations was also observed in the tocopherols in the alpine plants [12, 18, 19] for example, Sickel *et al*. [34] showed the range of concentrations of the tocopherols in the dominants fodder species of the alpine pastures of the Norwegian alpine region from 2 to 664 $\mu g\ g^{-1}$ DW as the lowest level in *Avenella flexuosa* and tocopherol pool in all studied species was dominated by α-tocopherol. However, *Vaccinium myrtillus* showed relatively higher γ-tocopherol [34]. Tocopherol concentration also showed the diurnal rhythm in alpine plants. That was light and temperature-dependent in the study of the Wildi and Leutz [19], in which midday was represented by higher tocopherol concentrations and lower during the night. The fluctuations in the a-tocopherol content may be due to the rapid turnover of this compound. The plants react with rapid changes in the level of tocopherols to the slight differences in the environment (e.g., shading and chilling) [19, 35]. Light conditions affected the levels of tocopherols in the plants, and plants are grown in higher light exposer generally have higher tocopherol levels than plants from shady habitats. Concentrations of tocopherols increase typically with increasing altitudes in alpine plants as found in *Soldanella alpina* leaves in which a-tocopherol content showed a 2-fold increase at 2000 m as compared to 1000 m [19]. Tocopherol plays an important role in cold hardening, chilling, and freezing resistance in the various plants, including the alpine flora, as reported in Scots pine, where cold acclimatized older needles contain higher a-tocopherol levels than younger ones [36]. γ-tocopherol may also play an important role as an antioxidant in tissue that exhibits desiccation stress and an indicator of senescence and senescence-related changes.

3.2.4 Carotenoids

Carotenoids are lipophilic pigment and antioxidants which can quench the ROS like toxic oxygen and hydroxyl radicals [37] and broadly classified as hydrocarbon carotenes (included α-carotene, β-carotene, γ-carotene, lycopene, phytoene, and phytofluene) and oxygenated xanthophyll (lutein, zeaxanthin, β-cryptoxanthin, astaxanthin, and fucoxanthin) [38, 39]. Carotenoids are the parts of the light-harvesting complexes (LHC) and play a prominent role as photo-protectant by harvesting light efficiently along with chlorophyll [40] and quench the ROS before these species initiate the oxidative damage. The carotenoids quench the excess light energy and subsequently the ROS production via active non-photochemical quenching (NPQ) or by the dissipation of heat [40, 41]. Singlet oxygen formation at the PSII may damage the D1 protein of the PSII during photo-inhibition and initiate the lipid peroxidation in the chloroplast and carotenoids along with tocopherol protect chloroplast from this peroxidation [41, 42] and among all the carotenoids zeaxanthin reported to have the higher antioxidant capacity [43]. Singlet oxygen produced due to the low temperature-induced photoinhibition is reported either efficiently scavenged or its formation is avoided, which have a high implication on the alpine plants due to their low-temperature habitat. Under high light intensities, ROS production increases at PSI, including hydrogen peroxide (H_2O_2) and singlet oxygen at the PSII in the chloroplast [41]. Alpine plants are exposed to very high light intensities in their habitats. In that scenario role of carotenoids became most prominent from ROS protection in such high light intensities and, along with other antioxidants, carotenoids such as zeaxanthin, neoxanthin, and lutein [43] efficiently quenching ROS in alpine plants. That's why sun-exposed plants of higher altitude are characterized by the higher ratio of Chl a/b and b-carotene/xanthophyll accompanied and a lower ratio of Chl/Car, which results mainly from higher contents of xanthophylls and is interpreted as a higher capacity for non-radiative dissipation of excitation energy and antioxidative protection by carotenoids [6]. ROS are highly reactive and therefore accelerate photoinhibition through direct oxidative damage to PS II. However, the highly variable carotenoid content, especially the zeaxanthin contents reported in alpine plants, for example, a study by Streb *et al*. [18], reported a very high level of carotenoids and xanthophyll cycle pigments in leaves of *Soldanella alpine* and *Homogyne alpina* as compared to *Ranunculus glacialis* leaves. Oncel *et al* [44], in their study also reported the presence of the high b-carotene and xanthophyll in the alpine plants, and among the various alpine plant forms the higher b-carotene content was reported in tree and brush as compared to the herbaceous plants. The diurnal variations observed in the pigment content and carotenoids in alpine plants represented a light-dependent control study by Wildi and Leutz [19], showed all carotenoids except lutein and neoxanthin were showed diurnal rhythm in selected high alpine plant species. An increase in carotenoid content, especially the xanthophyll cycle pigments, also reported in the alpine plants as reported by Wildi and Leutz [19]. However, no effect of altitude in *R. glacialis*, and even decrease in some species such as in *Dryas octopelata* was reported. Seasonal variations also observed in the carotenoid content as reported by Gonzalez *et al.* [45] in *Polylepis tarapacana* where carotenoids content increase with altitude and exhibited seasonal variations, and the highest value recorded in winter. Hence increased carotenoids content in alpine and high altitudinal plants as a safety valve venting the excessive PAR energy before it can damage the photosynthetic system [46] against the large amounts of solar energy coupled with a low temperature in the alpine habitats [43, 47] along with their role in photosynthesis.

3.2.5 Phenolic compounds

Phenolics or polyphenols are the compounds found in plants that belong to secondary metabolites of the plants. Plant phenolics mainly belong to aromatic metabolites with one or more acidic phenolic hydroxyl groups. Their structure ranges from simple phenols such as salicylic acid to complex polymers such as suberin and lignin. Phenolics in plants included hydroxycinnamic acids (HCAs), flavonoids, anthocyanins, and tannins that are widely distributed in the plant kingdom to the classes that are limited taxonomic distribution such as isoflavones, stilbenes, coumarins, furanocoumarins, and styrylpyrones [48]. Phenolics have several functions in plants; they are an integral part of some structural components of the plants, component of plant–animal interactions, plant–plant interactions, act as signaling molecules, screening of highly visible and UV light, pathogens defenses, and general protection against oxidative stress [49, 50]. The role of phenolic compounds in the alpine became most important, especially for protecting against the harmful UV- radiations that are abundant in alpine regions and increase with elevation [51]. Mostly higher content of phenolic compounds was reported in alpine plants than the plants of the lower altitudes, and the reason for this was sighted there need to adapt to the harsh changing environment [52]. The phenolic compounds may also compensate for the lowering of the radical scavenging activity when temperatures become very low in alpine habitats [53]. Phenolic compounds showed great diversity in the interspecies and intra-species level; such variations were reported by Lefebvre *et al*. [54] on three alpine species *Dryas octopetala, Rhododendron ferrugineum,* and *Vaccinium myrtillus*, where flavonoid content and its diversity is very high in *Rhododendron ferrugineum*. *In vitro* evaluation of the phenolic content in extracts of several alpine plants represented their high antioxidant protection, such as in *Potentilla fulgens* [55]. A study in *Gaultheria trichophylla* an alpine Himalayan plant showed a positive correlation between the altitude with total phenolics, tannins, flavonoids, and flavonols, and a direct relationship with the antioxidant potential of the extract prepared from the species [56]. Seasonal variation in total phenolics in *Acorus calamus* and antioxidant activity was reported by Bahukhandi *et al.* [57]. This high phenolic content and diversity may be responsible for medicinal properties of some of the high valued plants of alpine areas such as *Nardostachys jatamansi, Aconetum, Picroriza Kurroa, Rheum* sp., *Hippophae* sp., etc. [58]

4. Freeze and thaw cycle, ROS production and antioxidant protection in alpine plants

Alpine plants have to cope with the phase shifts that are frequent events in the alpine regions, mainly the freeze–thaw cycles that can happen daily during the late winter to early spring. The freezing–thawing cycle (FTC) is a phenomenon in which the soil undergoes repeated freezing and melting due to seasonal or diurnal temperature change [59]. Alpine habitats are represented by the various growth forms such as small prostrate woody shrubs, grasses, sedges, tussocks, herbaceous perennials and annuals, cushion plants, etc. Some of them experienced the diurnal freeze–thaw cycles, and some escaped the seasonal cycle, mainly the herbaceous annuals. Still, almost all of them faced the diurnal freeze–thaw as well as freeze–thawing cycles in spring or autumn [52]. In the alpine regions the most important environmental phenomenon that enflunces the vegetation growth and survival is the freeze–thaw cycle. This cycling causes major complications in plants, such as injuries due to the leakage of cellular solutes [physico-molecular perturbations in cell membranes, and oxidative injury to macromolecules due to cellular accumulation of reactive oxygen species (ROS; e.g., superoxide, singlet oxygen, etc.)] [60]. In the alpine areas, plants mostly experienced two kinds of freeze–thaw moments. One is due to extreme day-night temperature variation, mainly during September–October. The other occurs from November to April, where the temperature is freezing in November and increasing in April [21]. Zhou *et al.* [21] analyzed four grass species from the alpine region for seasonal fluctuation in ROS and antioxidant protection. They found that this freeze–thaw cycle in the autumn destroyed the aerial part of those grasses due to the membrane damage, loss of membrane integrity, and higher electrolyte and lipid peroxidation [21]. This lipid peroxidation resulted due to the decrease in the antioxidant enzyme activities assayed in their work and leads to senescing process. Although roots of those perennial grasses resistant to such freeze–thaw cycle induced ROS with the help of their highly efficient antioxidant enzyme system. Most of the alpine herbaceous perennial plants survived winter with rhizomes, stolons, or other underground storage organs. Those organs may have high antioxidant enzyme activities; besides this, they are rich in secondary metabolites, which also ensures antioxidant protection to them. Complications of the freeze–thaw cycles are more challenging for the alpine woody shrubs, where roots and evergreen leaves have to experience and overcome these freeze–thaw cycles. The diurnal freeze–thaw cycle can also affect the reproductive shoots of several woody and herbaceous plants [61]. Somehow, natural antioxidant protection, either enzymatic or non-enzymatic, is genetically or environmentally induced in the alpine plants to protect them from diurnal and seasonal freeze–thaw cycles. However, the climate changing scenario disturbing this protection, such as unusual late spring frosts, early fall frosts events, a warmer- fall than normal, leaves the alpine plants in the vulnerable stages and subjected them to recurrent freeze–thaw conditions leads to more damage to plants due to more ROS production and little antioxidant protection.

5. Ultraviolet radiations ROS production and antioxidants in alpine plants

High ultraviolet radiations are the characteristic feature of the alpine regions [62]. That can be quite stressful to the alpine lives because of shade-devoid environments and high albedo due to the snow. Increasing UV doses affected the vital processes in plants such as productivity, carbon assimilation, stomatal function, etc. [63] that becomes more relevant when the ozone layer is depleting continuously. Exposure to higher UV radiations leads to the production of free radicals or ROS and other chemically active harmful molecules in the cells that can bring damage to the membrane lipids, nucleic acids proteins including enzymes, etc. [47, 64]. However, alpine plants are protected naturally from the UV radiation's harmful impact with its enzymatic and non-enzymatic equipment. Accumulation of the phenolic compounds such as flavonoids and derivatives of the phenylpropanoid, induction of photo-repair system either prevent the UV from penetrating the cells or help to cells to overcome all the UV driven difficulties. An increase in UV-B absorbing compounds such as pigments like carotenoids, chlorophylls, flavonoids contents was observed in alpine plants with increasing altitude as well as in different seasons [65]. Chanishvili [65] screened various alpine plants, for example, *Tripholium pratense, Plantago major Taraxacum officinale, Achillea millefolium, Polygonum aviculare* for antioxidant compounds content for different altitudes with different level of UV radiations and concluded that the numbers of stress factors in the alpine interacted with UV radiation stress with these plants and the mechanisms of these plant to adapt in such conditions are species-specific [65]. An antioxidant like glutathione, ascorbic acid, phenolic compounds efficiently protect these plants from UV induced ROS, but the protection mechanism or level was species-specific. In the comparison of the herbaceous species, other alpine or subalpine life forms generally have a higher screening capacity of UV radiations [66] leads to more ROS formation in herbaceous plants due to increased penetration of the UV radiations in the underlying mesophyll [67]. In an artificial UV simulation experiment, it was observed that in some alpine plants such as *Carex firma, Dryas octopetala, Ranunculus alpestris, Salix retusa* several striking changes were observed, such as the reduction in glutathione content in most of the studied species. In contrast, other antioxidants such as ascorbic acids, tocopherols, flavonoids seemed to remain unaffected, which means alpine plants are genetically fixed to cope up with such conditions [68].

6. Alpine plants antioxidant and medicinal importance

The alpine plants are packed with antioxidants, and enzymatic as well as non-enzymatic antioxidant makes them quench the ROS produced due to the stressful conditions. Human utilizes this potential of these unique floras for their purposes like food and medicines. The popularity of antioxidants as food and nutraceuticals is increasing globally, and there is a continuous spike in the research on plant-based antioxidants and their medicinal potentials. Several alpine plants were used in various traditional and modern health care systems for their antioxidant activities, which comprises their other properties such as anti-cancerous, immune-modulatory, anti-stress, etc. A range of studies are available on the alpine plants' antioxidant potential [56, 57, 58, 69] and revived by Bhatt *et al*. [70]. Alpine plants from the Himalayan region are most remarkable for their medicinal properties plants like *Picrorhiza kurrooa* [71, 72] *Aconitum* sp. [72, 73], *Polygonum bistorta* [73, 74] *Nardostachys jatamansi* [75], etc.

7. Conclusion

Unique features of the alpine habitats make them home to some of the extraordinary flora and fauna in the world. In their habitats, alpine plants experience extremely stressful conditions, which causes the production of the free radicals or ROS species in them. However, these plant species genetically or phenotypically adapted to scavenge those ROS. Enzymatic as well non-enzymatic antioxidant system efficiently protecting the alpine floras, although this protection is species-specific, as a generalized feature the antioxidant potential in alpine plants increase with altitudes. Alpine vegetation showed a range of variation in the antioxidant protection plants like *Ranunculus glacialis* has very low antioxidant protection despite this, the plant species can survive in some of the highest altitudes of the world. On the other hand, species like *Soldanella alpina* showed very high antioxidant potential with high ascorbic acid, glutathione, phenolic, and other antioxidants. Alpine plants adapted to diurnal as well as seasonal fluctuations to ROS production due to the fluctuating environmental conditions of their habitats, especially during the daily freeze–thaw cycle of the early spring and late winter as well as the seasonal cycle. However, the changing climate is interfering with this seasonal acclimation. For example, a heatwave during the winter can deacclimatized the dormant buds of woody alpine trees, and a cold wave during summer may destroy the reproductive shoots. All these disturbances interrupt the ROS-antioxidant protection dynamics in those alpine plants. Most of the alpine floras are currently facing extinction due to climate change scenarios and anthropogenic activities, and we need to protect those unique habitats and related flora and fauna.

Conflict of interest

The authors declare no conflict of interest.

Author details

Vijay Laxmi Trivedi* and Mohan Chandra Nautiyal
Hemvati Nandan Bahuguna Garhwal University,
Srinagar Garhwal, Uttarakhand, India

*Address all correspondence to: vijaylaxmitrivedi@gmail.com

References

[1] Testolin R, Attorre F, Jiménez-Alfaro B. Global distribution and bioclimatic characterization of alpine biomes. Ecography. 2020; 43: 779-788. DOI: 10.1111/ecog.0501

[2] Roupioz L, Jia L, Nerry F, Menenti M. Estimation of Daily Solar Radiation Budget at Kilometer Resolution over the Tibetan Plateau by Integrating MODIS Data Products and a DEM. Remote Sensing. 2016; 8(6): 504. DOI: 10.3390/rs8060504

[3] Nievola CC, Carvalho CP, Carvalho V, Rodrigues E. Rapid responses of plants to temperature changes. Temperature (Austin). 2017; 4(4):371-405. DOI:10.1080/23328940.2017.1377812

[4] Feierabend J, Schaan C, Hertwig B. Photoinactivation of Catalase Occurs under Both High- and Low-Temperature Stress Conditions and Accompanies Photoinhibition of Photosystem II. Plant.1992; 100: 1554-1561. DOI: 0032-0889/92/100/1 554/08/$01 .00/

[5] Ball MC. The role of photoinhibition during tree seedling establishment at low temperatures. In: Baker NR, Bowyer JR, editors. Photoinhibition of photosynthesis from molecular mechanisms to the field. Oxford BIOS Scientific; 1994. p. 365-76.

[6] Germino MJ. Plants in Alpine Environments. In: Monson R, editors. Ecology and the Environment. Springer, New York, NY. DOI: 10.1007/978-1-4614-7612-2_12-4

[7] Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI. Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta. 2007. 1767: 414-42. 10.1016/j.bbabio.2006.11.019.

[8] Noctor G, Foyer C H. Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Biology. 1998; 49: 249-279. DOI: 10.1146/annurev.arplant.49.1.249.

[9] Sharma P, Jha AB , Dubey RS, Pessarak M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany. 2012; DOI: 10.1155/2012/2170.

[10] Munné-Bosch. The role of α-tocopherol in plant stress tolerance. Journal of Plant Physiology. 2005; 162: 743-748. DOI: 10.1016/j.jplph.2005.04.022.

[11] Ort D, Baker N. A photoprotective role of O2 as an alternative electron sink in photosynthesis? Current Opinion in Plant Biology. 2002; 5:193-198. DOI: 10.1016/s1369-5266(02)00259-5

[12] Streb P, Aubert S, Gout E, Bligny R. Reversibility of cold- and light-stress tolerance and accompanying changes of metabolite and antioxidant levels in the two high mountain plant species Soldanella alpina and *Ranunculus glacialis*. Journal of Experimental Botany. 2003; 54:405-418. DOI: 10.1093/jxb/erg048

[13] Orabi S, Shaban A. Antioxidant defense mechanisms enhance oxidative stress tolerance in plants. A review. Current Science International. 2019; 8: 565-576.

[14] Zhou X, Chen S, Wu H, Yang Y, Xu H. Biochemical and proteomics analyses of antioxidant enzymes reveal the potential stress tolerance in Rhododendron chrysanthum Pall. Biology Direct. 2017;12(1):10. DOI:10.1186/s13062-017-0181-6

[15] Caverzan A, Casassola A, Brammer SP. Antioxidant responses of wheat plants under stress. Genetics and Molecular Biology. 2016;

39(1):1-6. DOI:10.1590/1678-4685-GMB-2015-0109.

[16] Bowler C, Van Montagu M, Inze D. Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology. 1992; 43: 83-116. DOI: 10.1146/annurev.pp.43.060192.000503.

[17] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012; 5(1):9-19. DOI:10.1097/WOX.0b013e3182439613

[18] Streb P, Feierabend J, Bligny R. Resistance to photoinhibition of photosystem II and catalase and antioxidative protection in high mountain plants. Plant Cell Environment. 1997; 20: 1030-1040. DOI: 10.1111/j.1365-3040.1997.tb00679.x

[19] Wildi B, Lütz, C, Antioxidant composition of selected high alpine plant species from different altitudes. Plant Cell Environment. 1996; 19: 138-146. DOI: 10.1111/j.1365-3040.1996.tb00235.x

[20] Wang Y, He W, Huang H et al. Antioxidative responses to different altitudes in leaves of alpine plant *Polygonum viviparum* in summer. Acta Physiology Plantarum. 2009; 31: 839-848 DOI: 10.1007/s11738-009-0300-9.

[21] Zhou R, Zhao H. Seasonal pattern of antioxidant enzyme system in the roots of perennial forage grasses grown in alpine habitat, related to freezing tolerance. Physiologia Plantarum. 2004; 121(3): 399-408. DOI: 10.1111/j.0031-9317.2004.00313.x

[22] Guo J, Liu X, Li X, Chen S, Jin Z, Liu G. Overexpression of VTE1 from Arabidopsis resulting in high vitamin E accumulation and salt stress tolerance increase in tobacco plant Journal of Applied Environmental and Biological Sciences. 2006; 12:468-471. DOI: 10.1007/s10529-008-9672-y

[23] De Pinto MC, De Gara L. Changes in the ascorbate metabolism of apoplastic and symplastic spaces are associated with cell differentiation. Journal of Experimental Botany. 2004; 55 (408): 2559-2569. DOI: 10.1093/jxb/erh253

[24] Foyer CH, Noctor G. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum. 2003; 119 (3): 355-364. DOI: 10.1034/j.1399-3054.2003.00223.x

[25] Foyer CH, Noctor G.] Ascorbate and Glutathione: The Heart of the Redox Hub. Plant Physiology. 2011; 155 (1) 2-18. DOI: 10.1104/pp.110.167569

[26] Laureau C, Meyer S, Baudin X, Huignard C, Streb P. In vivo epidermal UV-A absorbance is induced by sunlight and protects Soldanella alpina leaves from photoinhibition. Functional Plant Biology. 2015; 42:599-608. DOI: 10.1071/FP14240.

[27] Tausz M, Wonisch A, Peters J, Jiménez MS, Morales D, Grill D. Short-term changes in free-radical scavengers and chloroplast pigments in *Pinus canariensis* needles as affected by mild drought stress. Journal of Plant Physiology. 2001b; 158: 213-219. DOI: 10.1078/0176-1617-00178

[28] Hasanuzzaman M, Nahar K, Anee TI, Fujita M. Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. Physiology and Molecular Biology of Plants. 2017; 23(2): 249-268. DOI:10.1007/s12298-017-0422-2.

[29] Kocsy G, von Ballmoos P, Rüegsegger A, Szalai G, Galiba G, Brunold C. Increasing the glutathione content in a chilling-sensitive maize genotype using safeners increased protection against chilling-induced

injury. Plant Physiology. 2001; 127:1147-1156. DOI: 10.1104/pp.010107.

[30] Zhao S, Blumwald E. Changes in oxidation-reduction state and antioxidant enzymes in the roots of jack pine seedlings during cold acclimation. Physiologia Plantarum. 2002; 104: 134-142. DOI: 10.1034/j.1399-3054.1998.1040117.x.

[31] Foyer CH, Shigeoka S. Understanding Oxidative Stress and Antioxidant Functions to Enhance Photosynthesis. Plant Physiology. 2011; 115: 93-100. DOI: 10.1104/pp.110.166181

[32] Falk J, Munné-Bosch S. Tocochromanol functions in plants: antioxidation and beyond, Journal of Experimental Botany. 2010; 61(6) : 1549-1566. DOI: 10.1093/jxb/erq030.

[33] Fritsche S, Wang X, Jung C. Recent Advances in our Understanding of Tocopherol Biosynthesis in Plants: An Overview of Key Genes, Functions, and Breeding of Vitamin E Improved Crops. Antioxidants (Basel). 2017; 6(4):99. DOI:10.3390/antiox6040099.

[34] Sickel H, Bilger W, Ohlson M. High Levels of α-Tocopherol in Norwegian Alpine Grazing Plants. Journal of Agricultural and Food Chemistry. 2012; 60 (31) 7573-7580. DOI: 10.1021/jf301756j.

[35] Franzen J, Bausch J, Glatzle D, Wagner E. Distribution of vitamin E in spruce seedlings and mature tree organs, and within the genus. Phytochemistry. 1991; 30: 147-151.

[36] Wingsle G, Hällgren JE. Influence of SO2 and NO2 exposure on glutathione superoxide dismutase and glutathione reductase activities in Scots pine needles. Journal of Experimental Botany. 1993; 44: 463-470.

[37] Young A J. The photoprotective role of carotenoids in higher plants. Physiologia Plantarum. 1991; 83(4): 702-708. DOI:10.1111/j.1399-3054.1991.tb02490.x

[38] Merhan O. The Biochemistry and Antioxidant Properties of Carotenoids, Carotenoids, Dragan J. Cvetkovic and Goran S. Nikolic, IntechOpen, DOI: 10.5772/67592. [BOOK]

[39] Zaripheh S, Erdman Jr. JW. Factors that influence the bioavailability of xanthophylls. Journal of Nutrition. 2002; 132: 531S–534S. DOI: 10.1093/jn/132.3.531S

[40] Croce R, Weiss S, Bassi R. Carotenoid-binding sites of the major light-harvesting complex II of higher plants. Journal of Biological Chemistry. 1999b; 274: 29613-29623. DOI: 10.1074/jbc.274.42.29613

[41] Niyogi KK. Photoprotection revisited: Genetics and molecular approaches. Annual Review of Plant Physiology and Plant Molecular Biology. 1999; 50: 333-359. DOI: 10.1146/annurev.arplant.50.1.333

[42] Aro EM, Virgin I, Andersson B. Photoinhibition of PS II. Inactivation, protein damage and turnover. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1993; 1143 (2): 113-134. DOI: 10.1016/0005-2728(93)90134-2.

[43] Havaux M, Dall'Osto L, Bassi R. Zeaxanthin Has Enhanced Antioxidant Capacity with Respect to All Other Xanthophylls in Arabidopsis Leaves and Functions Independent of Binding to PSII Antennae. Plant Physiology. 2007; 145 (4) 1506-1520. DOI: 10.1104/pp.107.108480.

[44] Öncel I, Yurdakulol E, Keleş Y, Kurt L, Yıldız A. Role of antioxidant defense system and biochemical adaptation on stress tolerance of high mountain and steppe plants. Acta Oecologica. 2004; 26: 211-218. DOI: 10.1016/j.actao.2004.04.004

[45] González JA, Gallardo MG, Boero C, Cruz ML, Prado FE. Altitudinal and seasonal variation of protective and photosynthetic pigments in leaves of the world's highest elevation trees Polylepis tarapacana (Rosaceae). Acta Oecologica. 2007; 32:36-41. DOI: 10.1016/j.actao.2007.03.002

[46] Gilmore AM. Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplast and leaves. Physiologia Plantarum. 1997; 99:197-209.DOI: 10.1111/j.1399-3054.1997.tb03449.x.

[47] Asada K. The water-water cycle as alternative photon and electron sinks. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 2000; 355(1402): 1419-1431. DOI: 10.1098/rstb.2000.0703

[48] Grace SC (2005) Phenolics as antioxidants. In: Smirnoff N (ed) Antioxidants and reactive oxygen species in plants. Blackwell Scientific Publishers, Oxford, pp 141-168

[49] Winkel-Shirley B. Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology. 2002; 5:218-23. DOI: 10.1016/s1369-5266(02)00256-x.

[50] Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM. How plants communicate using the underground information superhighway. Trends in Plant Science. 2004; 9(1):26-32. DOI: 10.1016/j.tplants.2003.11.008.

[51] Robberecht R, Caldwell M, Billings W. Leaf Ultraviolet Optical Properties Along a Latitudinal Gradient in the Arctic-Alpine Life Zone. Ecology. 1980: 61(3), 612-619. DOI :10.2307/1937427.

[52] Körner, C. (2003). Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. Berlin: Springer.

[53] Albert A, Sareedenchai V, Heller W, Seidlitz HK, Zidorn C. Temperature is the key to altitudinal variation of phenolics in *Arnica montana* L. cv. ARBO. Oecologia. 2009 ; 160(1):1-8. DOI: 10.1007/s00442-009-1277-1.

[54] Lefebvre T, Millery-Vigues A, Gallet C. Does leaf optical absorbance reflect the polyphenol content of alpine plants along an elevational gradient? Alpine Botany. 2016; 126(2), 177-185. DOI: 10.1007/s00035-016-0167-5

[55] Jaitak V, Sharma K, Kalia K, Kumar N, et al. Antioxidant activity of Potentilla fulgens: An alpine plant of western Himalaya. Journal of Food Composition and Analysis. . 2010. 23(2): 142-147. DOI:10.1016/j.jfca.2009.02.013

[56] Bahukhandi A, PandeyA, Sekar KC, Bhatt I. Polyphenolics, Nutrients and Antioxidant Activity of Gaultheria trichophylla Royle: A High Value Wild Edible Plant of Trans Himalaya. Horticulture International Journal. 2017; 1: 1-6. DOI: 10.15406/hij.2017.01.00007.

[57] Bahukhandi A, Rawat S, Jugran AK, et al. Seasonal Variation in Phenolics and Antioxidant Activity of *Acorus calamus* Linn.: An Important Medicinal Plant of Himalaya. National Academy Science Letters. 2021; 44, 13-15. DOI: 10.1007/s40009-020-00959-3

[58] Nautiyal MC and Nautiyal BP. Agrotechniques for High Altitude Medicinal & Aromatic Plants. M/s Bishen Singh Mahendra Pal Singh; First Edition. 2004.

[59] Mayr S, Hacke U, Schmid P, Schweinbacher F, Gruber A. Frost Drought in Conifers at the Alpine Timberline: Xylem Dysfunction and Adaptations. Ecology. 2006; 87(12): 3175-3185. DOI: 10.1890/0012-9658(2006)87[3175:FDICAT]2.0.CO;2

[60] Arora R. Mechanism of freeze-thaw injury and recovery: a cool retrospective

and warming up to new ideas. Plant Science . 2018; 270: 301-313. DOI: 10.1016/j.plantsci.2018.03.002

[61] Neuner G. Frost resistance in alpine woody plants. Frontiers in plant science. 2014; 5: 654. DOI: 10.3389/fpls.2014.00654.

[62] Caldwell MM. Solar Ultraviolet Radiation as an Ecological Factor for Alpine Plants. Ecological Monographs. 1968(38)1968: 243-268. DOI: 10.2307/1942430.

[63] Sedej T T, Erznožnik T, Rovtar J. Effect of UV radiation and altitude characteristics on functional traits and leaf optical properties in Saxifraga hostii at the alpine and montane site in the Slovenian Alps. Photochemical & Photobiological Sciences. 2020; 19(2):180-192. DOI: 10.1039/c9pp00032a.

[64] Frohnmeyer H, Staiger D. Ultraviolet-B Radiation-Mediated Responses in Plants. Balancing Damage and Protection. Plant Physiology.2003; 133 (4) 1420-1428; DOI: 10.1104/pp.103.030049.

[65] Chanishvili S, Badridze G, Rapava L, Janukashvili N. Effect of altitude on the contents of antioxidants in leaves of some herbaceous plants. Russian Journal of Ecology. 2007; 38(5): 367-373. DOI:10.1134/s1067413607050128.

[66] Day T A, Vogelmann T C, Delucia E H. Are some plant life forms more effective than others in screening out ultraviolet-B radiation. Oecologia. 1992; 92(4): 513-519. DOI: 10.1007/BF00317843.

[67] Barnes PW, Ryel RJ, Flint SD. UV Screening in Native and Non-native Plant Species in the Tropical Alpine: Implications for Climate Change-Driven Migration of Species to Higher Elevations. Frontiers in Plant Science. 2017; 8:1451. DOI: 10.3389/fpls.2017.01451

[68] L€utz C, Seidlitz HK. Physiological and Ultrastructural Changes in Alpine Plants Exposed to High Levels of UV and Ozone. IN: Plants in Alpine Regions Cell Physiology of Adaption and Survival Strategies . 2012 : 29-42 . Springer-Verlag/Wien, Newyork

[69] VL Trivedi, J Sati, D Chand , Nautiyal MC. Antioxidant Potential of Herbal Tea from Rhododendron anthopogon D. Don and Hippophae salcifolia D. Don. Journal of Non-Timber Forest Products. 2017; 3: 131-135.

[70] Bhatt ID, Rawat S, Rawal RS. (2013) Antioxidants in Medicinal Plants. In: Chandra S., LATA H., Varma A. (eds) Biotechnology for Medicinal Plants. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-29974-2_13

[71] Rajkumar V, Gunjan R, Ashok R. Antioxidant and antineoplastic activities of Picrorhiza kurroa extracts. Food Chemical Toxicology. 2011; 49:363 369. DOI: 10.1016/j.fct.2010.11.009

[72] Konda VG, Eerike M, Raghuraman LP, Rajamanickam MK. Antioxidant and Nephroprotective Activities of Aconitum heterophyllum Root in Glycerol Induced Acute Renal Failure in Rats. Journal of Clinical and Diagnostic Research for doctors. 2016; 10(3):FF01-2. doi: 10.7860/JCDR/2016/10798.7388.

[73] Munir N, Ijaz W, Altaf I, Naz S. Evaluation of antifungal and antioxidant potential of two medicinal plants: Aconitum heterophyllum and *Polygonum bistorta*, Asian Pacific Journal of Tropical Biomedicine. 2014; 4 (2) : 639-S643. DOI: 10.12980/APJTB.4.201414B182.

[74] Wang ST, Gao WF , Ya-Xi , Xinguang & LKD, et al. Phenol profiles and antioxidant capacities of Bistort

Rhizoma (*Polygonum bistorta* L.) extracts. RSC Advances . 2016; 6(33)10.1039/C6RA00687F.

[75] Sharma SK, Singh AP. In vitro antioxidant and free radical scavenging activity of Nardostachys jatamansi DC. J Acupunct Meridian Stud. 2012 ;5(3):112-8. DOI: 10.1016/j.jams.2012.03.002.