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ORIGINAL ARTICLE

Role of Exogenous Application of Salicylic Acid on Medicinal Plants under Drought Stress: A Review

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Drastic and rapid climate changes causes water deficit condition (abiotic stress) in plants and alter growth and developmental processes. Salicylic acid (a plant growth regulator) a phenolic compound, is an essential signal molecule which is responsible for inducing tolerance for both biotic and abiotic stress. It accelerates restoration of growth and developmental process due to elicitor action of the synthesis of secondary metabolites. The aim of this review is to understand the efficacy of salicylic acid during drought stress at various morpho-physiological and biochemical processes on medicinal plants.

Key words: Medicinal plants, Drought stress, Salicylic acid, Plant growth regulator

Medicinal plants are well known as they include culinary herbs, spices and are greatly used in 'traditional medicine system' from ancient time. The ancient traditional medicine systems (e.g. Chinese, Egyptian and Ayurvedic) which mainly depend on plant based natural products and are widely used by around 3.4 billion peoples in the developing world (Sarkar and Nahar, 2007; Doughari *et al.,* 2009). They consists large number of chemical compounds which are mainly synthesized by them and enclose diverse functions which include defense against insects, fungi, diseases and herbivoroury (Tapsell *et al.*, 2006). They also have numerous phytochemicals with potential and established biological activity (Dillard *et al.,* 2000). The two different books *"Ebers papyrus"* from ancient Egypt documented 850 species while another book *"Dioscorides"* documented 600 species which describes the medicinal plants and results for the formation of pharmacopeias for some 1500 years (Alamgir, 2017). Ethnobotanical studies describe hundreds of pharmacologically useful compounds which have four major classes- alkaloids, glycosides, polyphenols and terpenes (Cowan, 1999; Sher, 2009; Das, 2010; Tariq *et al.,* 2015). After subsequent ethnobotanical uses of the plants, 74% of pharmacologically active plant derived components have been discovered which are used for medicinal purpose, whereas earlier it was estimated to be 14-28% for higher plant species only (Borah *et al.,* 2012). In the last couple of decades, interest of people for the herbal medicines has increased exponentially, which has resulted in a new progress in the research, formulation, production and promotion of plants based drugs (Bisset, 1994; Tyler, 1997; Singh, 2016). The non- industrialized society also widely uses herbs because they are readily available and cheaper than modern medicine (Olowokudejo *et al.,* 2008).

India comes under the category of world's top 12 mega biodiversity countries with 10 biogeographical regions. India itself includes two among the world's eight biodiversity hotspots. The variation in climate and altitude contribute to varied ecological habitats of this country which results in the development of immensely rich vegetation with a unique diversity in medicinal plants and provide the important source of medicinal raw materials for traditional medicine systems and also for pharmaceutical industries. World Health Organization (WHO) has listed over 21,000 plant species used around the world for medicinal purpose, while in India, about 2,500 plant species are being used in indigenous system of medicine. Majority of Indian medicinal plants comprise of about 50% of higher medicinal plant species which are widely used in preventive, primitive and curative applications (Pan *et al.,* 2014).

India has a great heritage of knowledge on plant based drugs since time immemorial. Medicinal and Aromatic plants have played key roles in the lives of tribal peoples living in the Uttarakhand (India) by providing products for both food and medicine. Their primary health care system depends on traditional knowledge of medical practices and medicinal herbs which serve as the remedy of many types of ailments. The geographical distribution of Uttarakhand shows 17.3% of India's total land area with 53,483 km^2 of which 92.57% is under hills and 7.43% under plains. It is located between 77˚34'27" to 81˚02'22"E longitude and 28˚53'24" to 31˚27'50"N latitude. It comes under the hill state, having international borders with China (Tibet) in the north and Nepal in the east. On its north-west lies Himachal Pradesh and while on the south is Uttar Pradesh. It has different types of geographical conditions and vast biodiversity ranging from the snowbound peaks of the Himalayas with the highest Nanda Devi (7817m) to the sub-tropical Terai region. The flora of Garhwal (Uttarakhand) has been already extensively explored and studied by several botanists (Naithani, 1984-85; Bawa, 1993; Gaur, 1999; Bhatt *et al.,* 2008). About 17% of flowering plants show medicinal values, out of which 15,000 species are found in India (Nadkarni, 1954; Pei, 2001), while several species are from the Indian Himalayan region and most of them are found in Uttarakhand only (Bentley, 1980; Kirtikar and Basu 1933). Himalayan region is commonly acknowledged for the high diversity of native, endemic, rare and endangered medicinal plants which are valued worldwide due to their unique active compounds of therapeutic use. Local people are partially or completely dependent on forest resources for medicine, food and fuel (Gaur, 1999) and therefore, medicinal species are progressively deteriorating due to anthropogenic activities (Chhetri *et al.,* 2005)**.**

Though, India has a rich biodiversity, the growing demand is putting a heavy strain due to climate change, poor seed germination percentage, habitat destruction and over-collection to meet the market demand on the existing medicinal plant resources which is decreasing the population size of medicinal plants at an alarming rate and hence threatening their existence in their natural habitat as they are restricted to their native place. Therefore it requires the priority conservation from the Himalayan region due to over exploitation, habitat degradation and other biotic and abiotic interferences in its distribution ranges (Sudharani *et al.,* 2013; Polunin *et al.,* 1987)**.**

Water stress and its effects on the plant growth and yield

Stress distorts the physiological state of a plant. It is caused by biotic and abiotic factors that tend to disrupt the equilibrium state in plants. (Gaspar *et al.,* 2002). Abiotic stresses become dominant subject of study for the improvement of agriculture and sustainable food supply due to its ailing effect which decreases the yield of crop plants and some active constituents of medicinal and aromatic herbs. They disturb the normal equilibrium state and lead to a series of morphological, physiological, biochemical and molecular changes in the plants, which adversely affect their growth and productivity. Drought, salinity, extreme temperatures, chemical toxicity, oxidative stress etc., are different types of stress which cause imbalance in the natural status of the environment. Water stress is an abiotic stress either caused by the water deficit (drought or high soil salinity) or through flooding or low soil temperature and causes the 'physiological drought' where water exists in soil solution but plants are unable to uptake it. Among all, the physical stress, drought stress is the global issue that results in a drop of water potential and leads to a decline in growth and productivity of plants (Chaves *et al.,* 2003).. Drought is a meteorological term and is commonly defined as a period without significant rainfall and it generally occurs when the available water in the soil is reduced due to transpiration or evaporation. Plants show their tendency for tolerance but its extent varies from species to species and even within species.

Mild to severe drought has been one of the major production limiting factors in the advancement in agriculture. Thus, the ability of plants to survive under stressful condition is of immense economic importance. On the one hand, stress is very destructive for crop plants but for medicinal plants it can be proved beneficial due to the presence of active constituents i.e, secondary metabolites which are actually protective for plants, (Wink, 1999) which defend plants against a variety of herbivores and pathogenic microorganisms as well as various natural abiotic stresses (Mazid *et al.,* 2011). Plants have great ability to escape quickly and mitigate the stress condition by early maturity and flower production (Taiz and Zeiger 1998). During drought, moderate loss of water results in stomatal closure and limitation of gas exchange while during desiccation (i.e, more loss of water), bumpy disruption of metabolism and cell structure takes place which eventually leads to the termination of enzyme catalyzed reactions (Smirnoff 1993; Jaleel *et al.,* 2007).

Water is one of the very essential components for the survival of living beings as the entire metabolism reactions take place through water, therefore; water stress has a great impact on the physiological and biochemical processes of plants as it is a very important limiting factor at the initial phase of plant's growth and establishment (Lisar *et al.,* 2012). It is characterized by a decline of water content, diminished leaf water potential and turgor loss, stomata closure and inhibition of cell enlargement more than cell division and growth. It affects the plant growth by reduction in photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel *et al.,* 2008; Farooq *et al.,* 2009). Severe water stress may result in detention of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel *et al.,* 2008). Furthermore, decrease in photosynthesis due to drought stress is associated with disrupted activities of enzymes, loss of membrane integrity and stomatal closure. Productivity of important cereals is also negatively influenced by drought- induced osmotic stress (Valentovic *et al.,* 2006). A number of secondary metabolites produced in plants are also helpful in the induction of drought tolerance **(**Verma and Shukla

2015). The rise in endogenous levels of plant secondary metabolites in response to drought stress was recorded in various medicinal plants such as *C. roseus, H. perforatum, and Artemisia annua.* For instance, drought stress caused increase in phenolics and photosynthetic pigments and decrease in plant fresh and dry biomass in *T. ammi* (Azhar *et al.,* 2011). In another study, imposition of drought stress resulted in improved quality of key secondary metabolites such as rutin, quercetin and betulinic acid in *Hypericum brasiliense,* and that of artemisinin in *Artemisia* (Verma and Shukla 2015). Likewise, exposure of *St. John's wort* plants to water-limited conditions resulted in a significant decrease in photosynthesis along with concomitant increase in the concentrations of secondary metabolites including pseudohypericin, hypericin, and hyperforin (Zobayed *et al.,* 2007). Likewise, increase in total flavonoids was observed in *Glechoma longituba* grown under drought conditions (Zhang *et al.,* 2012). Water-limited conditions in *Ocimum americanum* and *Ocimum basilicum* significantly affected concentrations of different macronutrients, proline, carbohydrates and essential oils (Khalid, 2006).

Plants develop different approaches in order to cope with osmotic stress by synthesizing and accumulating large number of small, neutral and non- toxic compound called osmolytes like proline, glutamate, glycine-betaine, carnitine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose, oligosaccharides along with certain inorganic ions like K^+, Na^+ and Cl^-. To maintain the ionic balance in the vacuoles, different osmolytes like proline, glycine betaine, sugars and Polyols etc are accumulated (Parida and Das, 2005). High level of accumulation of compatible solutes in the cytosol can help plants in resistance against increased drought stress by maintaining the cell hydrated state (Hoekstra *et al.,* 2001). The structural integrity of membrane can be maintained by osmolytes through substitution of hydroxyl group of sugar alcohols with the hydroxyl group of water and by maintaining the hydrophilic interaction with membrane lipids and proteins (Hoekstra *et al.,* 2001; Reddy *et al.,* 2004). But they don't collapse the normal metabolic process of the cell. The low concentration of osmolytes in response to drought can protect the macromolecules by either stabilizing the tertiary structure of protein or by scavenging ROS (Zhu, 2001). However, in the absence of stress, the higher accumulation of osmolytes can cause impaired growth in the transgenic plants (Maggio *et al.,* 2002). These compounds may play role in osmotic adjustment but most importantly act as osmoprotectant such as, mannitol which is widely distributed sugar alcohol in nature and is used to stabilize the macromolecule and functions to scavenge the ROS, hydroxyl radicals (Mahajan and Tuteja, 2005). Under drought condition osmolytes make hydrogen bonds with macromolecules and prevent intra-molecular hydrogen bonds formation which irreversibly damages the 3-dimensional structure of protein. Among osmolyte solutes, proline plays important role because it acts as a free radical scavenger, a cell redox balancer, a cytosolic pH buffer and a stabilizer for subcellular structures, during osmotic stress (Szabados and Savoure, 2010; Trovato *et al.,* 2008). The accumulation of proline is known to be a good indicator of drought in *Corniculatus* (Diaz *et al.,* 2010). The accumulated proline under drought stress, either scavenge or reduce atomic oxygen production and thereby reduce the chance of photodamage in the thylakoid membranes (Lawlor and Cornic, 2002). With reduced leaf water potential, proline concentrations increase many fold and photosynthesis is known to be quite reduced (Munns, 2002).

Role of Salicylic acid as a plant growth regulator in medicinal plant (under water stress)

The derivative of the group of phenolic acid compounds originated from the secondary metabolism that are distributed in many species of monocotyledonous and dicotyledonous plants is known as salicylic acid (SA). Therefore, it is considered as a potential enzymatic antioxidant agent, related to the defense of the plant under stress conditions (Noreen *et al.,* 2008). For example: application of salicylic acid into tobacco leaves enhanced resistance to subsequent infection by Tobacco Mosaic Virus (TMV). In different physiological processes like photosynthesis, transpiration, nutrient uptake, chlorophyll synthesis and plant development salicylic acid shows its regulatory role. It is also an important component in signaling molecule which potentially influences the plant tolerance to water stress because of its influence on the regulation of metabolic and physiological activities (affecting its growth parameters and bio-productivity) during the entire lifetime of the plant. These responses are directly activated by changing the water status of plant tissues while others are triggered by signalling molecule transduction and hence are responsible for the physiological adaptation of the plant to stress conditions. Besides growth promotion, the exogenous application of salicylic acid to plants (like wheat) can play a role to enhance the abiotic stresses i.e., salinity and drought stress (Shakirova *et al.,* 2003). It has been demonstrated that exogenous treatment of salicylic acid with different levels in drought-stressed plants caused a decline of the adverse effects in yellow Maize (*Zeamays L.*) and rice plants, but also stimulated physiological traits, productivity and plant resistance to drought stress (Farooq *et al.,* 2009). Different environmental stresses such as high and low temperature, drought, alkalinity, salinity, UV stress and pathogen infection are potentially harmful to the plants. Therefore, along with many physiological processes, salicylic acid also confers various environmental stress tolerances such as drought, chilling and heat stress (Hussain *et al.,* 2008). It is reported that in tomato, drought tolerance is promoted through salicylic acid treatment as low concentration of salicylic acid enhanced photosynthetic parameters, membrane stability index (MSI), leaf water potential, activity of nitrate reductase (NR), carbonic anhydrase (CA), chlorophyll and relative water content (RWC) (Hayat *et al.,* 2007).

SA is well known as a key signaling molecule in response to both biotic and abiotic stress factors and which is synthesized via phenylalanine lyase (PAL) and isochorismate synthase (ICS) pathways and therefore controls resistance in the plants by regulating the accumulation of some secondary metabolites. Plants frequently undergo various kinds of stresses and therefore, SA can regulate the physiological processes of plants under a variety of stresses by altering their resistance towards these changes due to the expression of many genes which results in alterations in the accumulation of some secondary metabolites and consequently, improve the tolerance of plants towards stress (An and Mou, 2011).

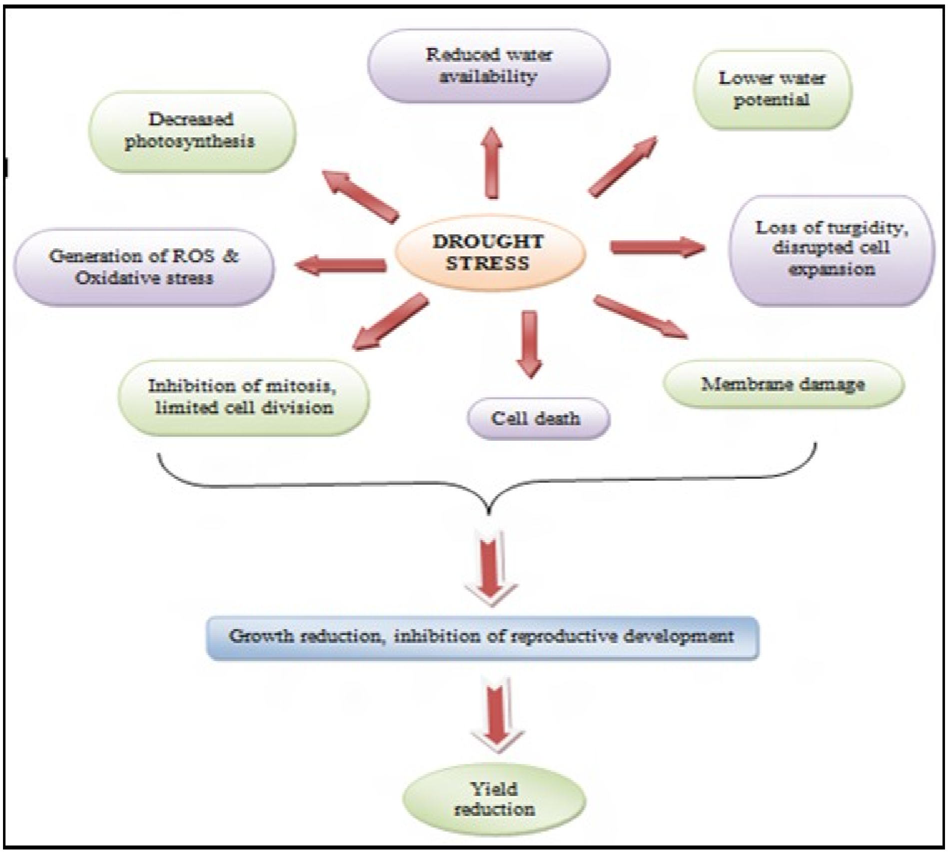

Figure 1. Effect of drought stress in plants and on yield production

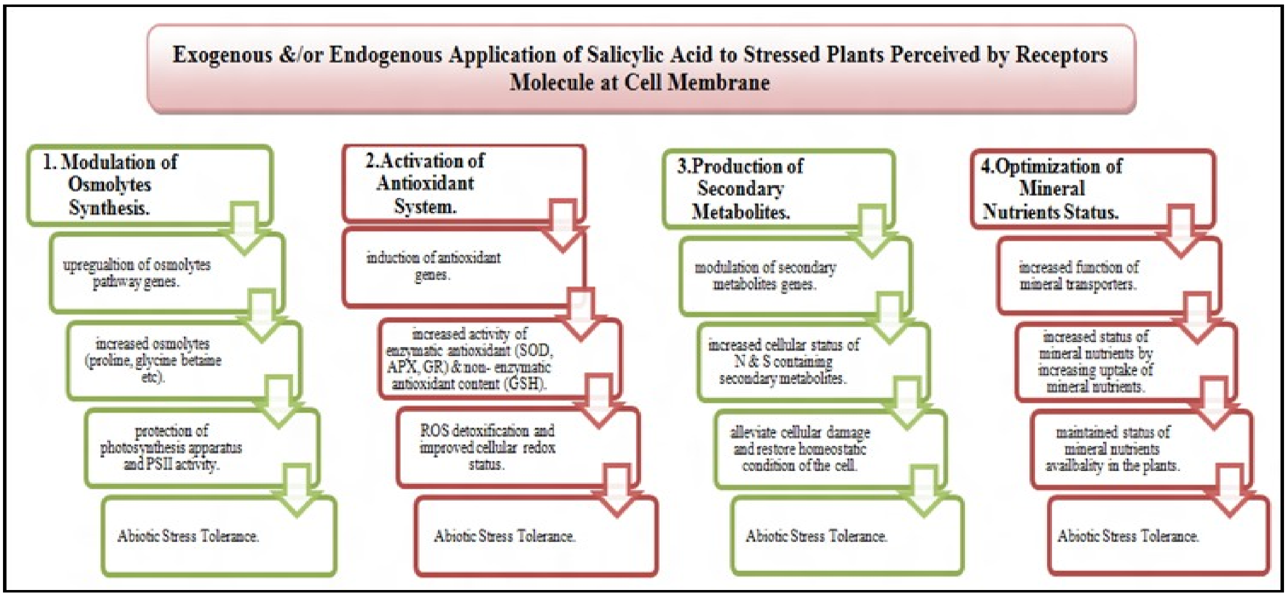

Figure 2. Exogenous and endogenous application of salicylic acid on plants under drought stress

Foliar feeding of SA has some agronomic advantages over the application of solutes in soil. The leaves can absorb the required nutritional substances through the foliar application. Foliar nutrition can reduce the lag time between application and uptake by the plant, which could be important during a phase of rapid growth. It can also circumvent the problem of restricted uptake of a nutrient from the soil (Taiz and Zeiger, 1998) and can reduce the wastage of the nutrients. Nutrient movement into the plant seems to involve diffusion through the cuticle and uptake by leaf cells and whereas, uptake through the stomatal pore could provide a pathway into the leaf (Oosterhuis, 2009). Carbohydrate solutions, mainly sucrose are absorbed by plants which could be an extra source of metabolites and can enhance the crop better. The secondary metabolites of plants are by products of main biochemical processes therefore if somehow we can increase the amount of primary metabolites then there will be probability of more synthesis of secondary metabolites in plant body. Extra nourishment may also increase dry matter and can promote better vegetative growth (Hardy and Norton, 1968).

CONCLUSION AND FUTURE PERSPECTIVES

Drought stress is one of the global issues to ensure survival of agricultural crops and medicinal plants for sustainable food production and yield. Under drought stress, there is an increase in flavonoids and phenolic acids is reported in willow leaves often causes oxidative stress. Water deficit condition influenced changes in the ratio of chlorophyll “a” and “b” and carotenoids. It is reported the reduction in chlorophyll content in cotton and *Catharanthus roseus* under drought stress. In *Chenopodium quinoa,* reported the decreased in the content of saponins from 0.46% dry weight (dw) in plants growing under low water deficit conditions to 0.38% in high water deficit plants. Under both at drought stress and cold temperatures the accumulation of anthocyanins are reported. Plant tissues containing anthocyanins are usually quite resistant to drought. For example, a purple cultivar of chilli resists water stress better than a green cultivar. Flavonoids have protective functions during drought stress. Maintenance of low levels of MDA (product of the lipid peroxidation) is associated with better resistance to drought (Lima *et al.,* 2002) as peroxidation of lipids disrupts the membrane integrity of a plant cell. Proline is thought to play a cardinal role as an osmoregulatory solute in plants subjected to drought stress. It not only acts as an osmolyte for osmotic adjustment but it also contributes to stabilize sub-cellular structures (e.g., membranes and proteins) under stress conditions. Thus relates the tolerance power of plants against stress (Hayat *et al.,* 2012). In vitro techniques are also used to resist drought condition. Simultaneously, MDA content and electrolyte leakage were measured as the indicator of membrane damage and proline estimation was done from in vitro generated calluses to check the tolerance capacity with increasing levels of stress as well as time interval. Knowledge of the biochemical and molecular responses to drought is essential for a holistic perception of plant resistance mechanisms to water limited conditions. New approaches including effective use of water, selection of drought resistant species, cultivars and root stocks have been considered to reduce the effects of water stress. Similarly, exogenous application of Salicylic acid (elicitor compound) can be useful to overcome the ailing effect of drought stress as it has been found to generate metabolic and physiological responses in growth and development and can be exploit as a helpful strategy in order to increase antioxidant enzymes, sugars, proline, protein and essential oil content. And it may help to preserve the structure of membrane and proteins against ROS under drought stress.

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