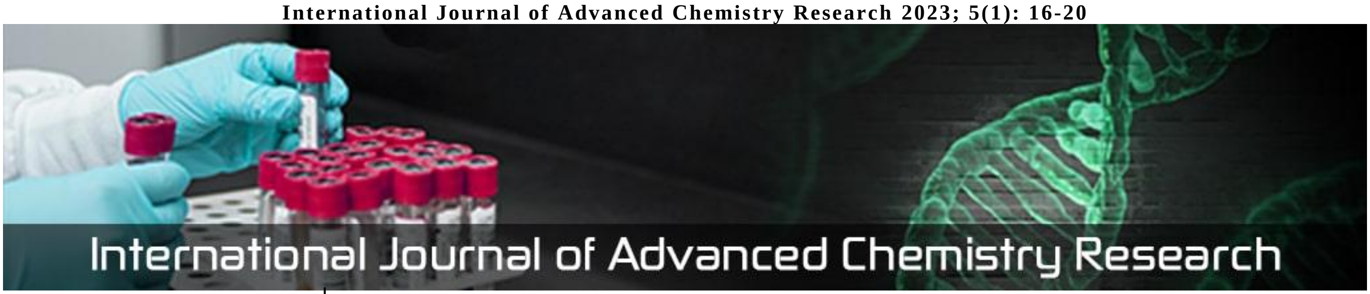

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Miracle molecule: Salicylic acid

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Abstract
Salicylic acid, a naturally occurring plant hormone acting as an important signaling molecule adds to tolerance against abiotic stresses. It plays a vital role in plant growth, ion uptake and transport. Salicylic acid is also involved in endogenous signaling to trigger plant defense against pathogens. This positive effect of SA could be attributed to an increased CO_2 assimilation and photosynthetic rate and increased mineral uptake by the stressed plant under SA treatment. The application of salicylic acid, acetylsalicylic acid or other analogues of SA, to leaves of plants accelerated their leaf area and dry mass production, but plant height and root length remained unaffected. SA reduced the Na uptake of plants and/or increased the uptake of N, P, K, Ca, Mg and the other minerals as compared to control treatment under salt stress. Salicylic acid (SA) plays many roles in plant physiology. Besides pathogenesis-related resistance, SA is involved in the response to abiotic stress. However, the effects of SA on plant resistance to abiotic stress were found contra dictionary, and the actual role of SA in abiotic stress remains unresolved. Generally, deficiency of SA or a very high level of SA increase the plant susceptibility to abiotic stress. The optimal levels for the highest stress tolerance range from 0.1 mm to 0.5 mm for most plants. But the role of SA at a certain level in moderate and severe abiotic stress may be different. This can be attributed to redox regulations in plant cells. In this paper, we discuss the relationship between reactive oxygen species (ROS) and SA, and propose a subsequent intracellular signal transduction network of SA and ROS under abiotic stress. Anti-stress substances besides antioxidant enzymes induced by SA are also summarized.

Keywords: Salicylic acid (SA), Miracle molecule, Salicylic acid, reactive oxygen species (ROS)

Introduction
Salicylic acid (SA) is a common plant-produced phenolic compound. Compounds in this group can function as plant growth regulators (Arberg 1981) [5]. Exogenous application of SA may influence a range of diverse processes in plants, including stomatal closure (Larque-Saaverda 1979) [40], ion uptake and transport (Harper and Balke 1981) [28], ethylene synthesis (Leslie and Romani 1986) [42], seed germination, fruit yield, glycolysis (Cutt and Klessig 1992) [12] and the growth-inhibitory effect of abscisic acid (ABA) (Rai *et al.* 1986) [47]. SA is also involved in induction of an alternative respiratory pathway (Ethlon *et al.* 1989) [18] and expression of a nuclear gene encoding the alter.

Miracle effects of salicylic acid
SA is involved in induction of an alternative respiratory pathway (Ethlon *et al.* 1989) [18] and expression of a nuclear gene encoding the alternative oxidase protein in Suaromantum gut Tatum (Rhoads and McIntosh 1991) [52]. Salicylic acid has been shown to play a role in plant thermogenicity (Raskin *et al.* 1987) [50]. It has also been shown that SA may play a part in the flowering of cocklebur plants, since its concentration increased in the phloem when they were induced to flower by manipulating day length (Cleland and Ajami 1974) [11]. Acetylsalicylic acid (ASA, 2-acetyloxy-benzoic acid; an artificial analogue of SA) and resorcylic acid (2,6dihyroxybenzoic acid) have shown thermo genic activity comparable to SA (Raskin *et al.* 1989) [51]. Flowering in these plants can also be stimulated by benzoic acid (Raskin 1992) [49]. The involvement of SA in the induction of systemic acquired resistance (SAR) is a topic of intense research (Sticher *et al.* 1997) [63]. ASA has been used in preference to SA in in vitro germplasm programs, as a component of culture media, in order to retard growth and induce tuberization or shoot organogenesis in potato micro plants (Lopez-Delgado and Carrillo Castaneda 1996) [43].

Gentisic acid (GTA, 2, 5-dihydroxybenzoic acid) occurs in a wide range of plant species (Griffith 1959) and has been demonstrated to have antifungal activity in vitro. Benzoic acid and SA are normally converted into gentisic acid, where SA is the immediate precursor of GTA (Belles, *et al.*1999) [8].

Under pathogen attack conditions the metabolism of phenolic compounds and the functioning of the photosynthetic apparatus play key roles (Prokharchik and Marshakova 1985) [46]. Previous experiments conducted in our laboratory have shown that the chronic injection (Zhou and Smith 1996) [70] of SA (10–2mol/L from shortly after tasseling until physiological maturity) increases the photosynthetic rate of corn plants (Zhou *et al.* 1999) [69].

Plants are the major source of reduced carbon compounds directly or indirectly used by all animals and thus any possible increase in the photosynthetic activity will be beneficial and requires particular attention, especially if it can be enhanced by the simple application of exogenous chemicals. The known effects of SA on stomatal function, chlorophyll content, transpiration rate and respiratory pathways indicate that SA and related phenolic compounds may be involved in regulation of some photosynthetic reactions. The objective of this work was to test the possibility that the exogenous foliar application of SA and related phenolic compounds affect metabolic processes related to gas exchange and growth by plants. We investigated the effects of exogenous application of SA and related compounds on net photosynthetic rate, stomatal conductance and transpiration rate in two diverse crop plants, corn (C_4) and soybean (C_3), following exogenous application of salicylic acid and related compounds. Plant growth regulators can improve the physiological efficiency including photosynthetic ability and thereby helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops (Solamani *et al.* 2001) [61].

Foliar feeding of plants can effectively supplement soil fertilization. It has been found that element foliar application is more influential compared to soil application (Kazemi 2013) [35].

Salicylic acid (SA) is an endogenous plant growth of phenolic nature that possesses an aromatic ring with a hydroxyl group or its hormone plays a vital role in plant growth, ion uptake and transport (Hayat *et al.* 2010) [22]. Enhanced germination and seedling growth were recorded in wheat, when the grains were subjected to pre-sowing seed-soaking treatment in salicylic acid (Shakirova 2007) [58]. In cucumber and tomato, the fruit yield enhanced significantly when the plants were sprayed with lower concentrations of salicylic acid (Larque-Saavedra and Martin-Mex 2007) [38].

Salicylic acid was also found to enhance the activities of antioxidant enzymes such as peroxidase (POD), superoxidase dismutase (SOD) and catalase (CAT), when sprayed exogenously to the drought stressed plants of tomato (Hayat *et al.* 2008) [29] or to the salinity stressed plants (Szepesi *et al.* 2008; Yusuf *et al.* 2008) [64, 68]. The exogenous SA application also enhanced the growth and photosynthetic rate in wheat (Hussein *et al.* 2007) [34] under water stress. However, numerous studies have demonstrated that the effect of exogenous SA depends on various factors, including the species and developmental stage, the mode of application and the concentration of SA used (Vanacker *et al.* 2001; Horvth *et al.* 2007) [66, 32].

Fariduddin *et al.* (2003) [19] also reported that the dry matter accumulation was significantly increased in Brassica juncea, when lower concentrations of salicylic acid were sprayed. However, higher concentrations of salicylic acid had an inhibitory effect. Khodary (2004) [37] observed a significant increase in growth characteristic, pigment contents and photosynthetic rate in maize, sprayed with salicylic acid. Eraslan *et al.*, (2007) [17] also reported that exogenous application of salicylic acid, enhanced growth, physiological process and antioxidant activity of carrot plants grown under salinity stress.

Soybean (*Glycine max* (L.) merril) is an important grain legume due to its high protein (35%), oil content (21%) and nitrogen fixing ability (17-24 kg/ha/yr.). Increasing plant productivity is one the main target in Indian Agricultural policy, which could be achieved through fertilization and plant growth regulators. However, very little is known about the effect of SA applied to foliage on plant growth and development and especially about the effect of SA on influencing the activities of antioxidative enzymes at different growth stages. Keeping in view the diverse physiological roles played by SA, the present research was undertaken to improve our understanding of the effect of the various concentrations of SA applied as foliar spray on the pigment and protein content, lipid peroxidation and activities of antioxidative enzymes in soybean at different growth stages.

Exogenous application of plant growth regulators is considered effective technique for improving the plant productivity. Salicylic acid has been found to induce significant effects on various biological aspects in plants. Our results state that SA have stimulatory effects on vegetative growth parameters of Soybean plant. Application of SA leads to increase in number of branching and leaves per plant along with increase in the dry weight also. Regarding foliar application of SA, obtained results are similar to those describe by Salarizdah *et al.* (2012) [55] on canola,

Dawood *et al.* (2012) [13] on sunflower and Ali and Mahmoud (2012) [3] on mungbean. In the present study SA showed elevated effects and leads to manifold increase in the level of biochemical and antioxidant enzymes. The chlorophyll content of Soybean leaves was increased due to foliar application of SA (Khan *et al.* 2003) [36]. Lower SA concentration was generally more effective in enhancing photosynthetic rate and biochemical parameters. Application of lower concentration of SA might leads to synthesis of more carbohydrate in treated plants. Chlorophyll pigments play a key role in light capturing for photosynthesis whose content forced a direct impact on the intensity of photosynthesis. The stimulatory effects of SA are in agreement with those of Barakat (2011) [7] on wheat and Saeidnejad *et al.* (2012) [54] on maize. However, declined in chlorophyll content under influence of SA in certain crops like *Vigna mungo* has been reported by Anandhi and Ramanujam, (1997) [4].

The reduction of total chlorophyll content occurs due to increased in activity of the enzyme chlrophyllase. The Role of SA deficiency is associated with reduced damage to the photosynthetic apparatus as well as chlorophyll level. MDA content was estimated to determinate the extent of lipid peroxidation. The data showed that increased level of MDA content was achieved from 45 to 60 DAS over that of control. It has been postulated that low level of the induced leakiness of membrane is caused by lipid peroxidation resulting from uncontrolled ROS increase (Rodriques-Rosales *et al.* 1999) [53].

Delvari *et al.* (2010) [14] showed that pretreatment will decrease the level of lipid peroxidation induced by oxidative stress in basil plants. Agarwal *et al.* (2005) [2] also showed that SA treatment of wheat leaves under water stress conditions resulted in less production of MDA. So, the lipid peroxidation induced by drought stress was ameliorated by SA treatments. Antioxidant enzymes of Soybean were increased in response to different concentration of SA. Oxidative stress generated in the plants can be removed with the help of antioxidative enzymes. It was found that application of low concentration of SA increased the activity of antioxidant enzymes like CAT, APOX, GPOX, SOD. This increase in the activity of antioxidant enzymes might be due to the regulatory role of SA at the level of transcription/Translation. Foliar spray of SA to soybean plant leads to significant increase in SOD and CAT activity. Among the enzymes measured here, CAT and SOD most effective in preventing cellular damage by converting superoxide anion to H_2O_2 and H_2O_2 to H_2O (Scan-Dalios 1993) [57]. It was found that increased SOD activity was accompanied by increase in CAT and POD because of high demands of H_2O_2 quenching. It was cleared that increment in SOD and POD simultaneously affect each other. First line of defense was provided by SOD against the cellular due to environmental stress along with its major superoxide scavenger. Catalase seems to be a key enzyme in SA induced stress tolerance.

Tenhakan and Rubel (1997) and Rao *et al.* (1997) have reported that SA caused hypersensitive reaction or enhanced H_2O_2 produced leading to cell death was not associated with the inhibition of these H_2O_2 scavenging enzymes, similarly there was no inhibition of GPOX activity by SA thus conforming the results reported by Durner and Klessing (1995). So these antioxidant enzymes protect plant cell from oxidative damage by being scavenging of ROS. So from above discussion, it was observed that foliarapplication of SA increased antioxidant enzymes activity (SOD, GPOX, CAT) which was further related to decrease in oxidative stress (H2O2). From above results, it was observed that foliar feeding of soybean (*Glycine max* L. merril) varieties Pusa-9612 with SA at lower concentration can stimulate the growth through increasing in the activities of antioxidant enzymes, preventing protein loss and enhancing photosynthetic pigment thereby increasing overall growth parameter of the plant. Thus SA at 10-6 M concentration can positively affect the growth of the Soybean plant as compared to 10-5 or 10-4 M application.

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