CHAPTER - I

REVIEW OF LITERATURE

A. INTRODUCTION

Plant growth and development is influenced by various endogenous and environmental factors. Among the endogenous factors, phytoharmones are of fundamental importance since these compounds regulate all the vital processes in the plants. The compounds such as salicylic acid are also found to be involved in these processes. The importance of salicylic acid as a plant growth regulator has been recognized relatively recently and the first review on this compound appeared in Annual Review of plant physiology in 1992 (Raskin, 1992b). Latteron in 2001 Moharikar has submitted M. Phil dissertation entitled “Physiological studies in seed germination of moong and wheat under the influence of salicylic acid” to Shivaji University, Kolhapur. In this dissertation he has presented a brief review of literature on the aspect of salicylic acid. Hence it is felt worthwhile to review the literature on salicylic acid published mainly after 2001. In order to maintain a continuation in the discussion of the subject matter, some relevant references before 2001 has been also cited in the discussion presented here.

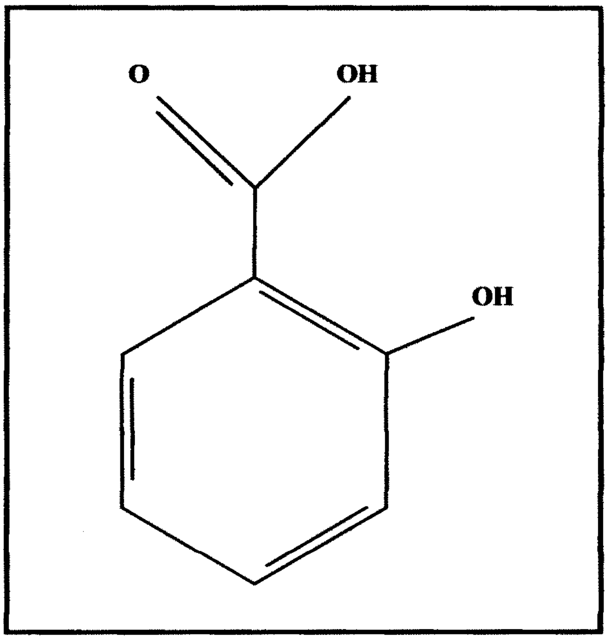
The existence of SA was first elucidated in Willow tree bark (*Salix alba*) which was having medicinal applications in the Red Indian tribes in USA. Raffaele Piria, an Italian Chemist and Henri Leroux, a French Pharmacist (1938) were given the name Salicylic acid to the active ingredient of willow bark from the Latin word *Salix* for willow tree. Salicylic acid was also isolated from the herb Meadowsweet (*Filipendula ulmaria* or it classified as *Spirarea ulmaria*) by German researchers in 1939. SA occurs naturally as well as it can be synthesized in the laboratory. It is a natural cellular component found in several plants.

Salicylic acid has drawn the great attention of researchers due to its involvement to induce the systemic acquired resistance (SAR) in plants leading to defense mechanism (Mauch- Mani and Slusarenko, 1996, Ryals *et al.*, 1996, Wobbe and Klessing, 1996, Mateo, 2006) enhancing resistance to biotic and abiotic stresses. Several attempts cleared that SA has been documented as the calorigen, responsible for the thermogenesity (heat production).There is evidence that SA can influence the antioxidant capacity in plants while it also reported to cause a morphological and physiological effects of plants (Pancheva *et al.,* 1996). The key roles of SA in signal transduction, disease resistance and thermogenesity suggested that SA should be qualifying criterion of phytohormone.

B. INACTIVATION OF SALICYLIC ACID:

Raskin was documented the inactivation of SA in two ways, by conjugation and metabolic inactivation. The plants are able to produce 0-glucosides or glucose esters of SA. Metabolic inactivation of SA occurs by additional hydroxylation of its aromatic ring.

SA has been recognized as an allelopathic chemical (Einhelling, 1986). In contrast to it Manthe *et al.* (1992) noticed the toxic properties of SA affecting the growth of *Vicia faba*, thus they should not be considered as an allelopathic compound.



IUPAC NAME : 2-Hydroxybenzoic Acid, $C_7H_6O_3$

Fig.No.1 : STRUCTURE OF SALICYLIC ACID

C. SALICYLIC ACID ANALOGUES

Salicylic acid and related compounds (Fig. No. 2) has been reported to indicate the significant effects on various biological aspects in plants in Table -1.

Table 1 : Compounds Related to Salicylic Acid.

Sr.No.	Related Compounds	References
1.	Salicylic Hydroxamic acid	Dekock and Grabowska, 1974
2.	Salicylic aldehyde	
3.	Salicylic alcohol	
4.	8- Hydroxyquinoline	
5.	o-Carboxyphenyl phosphate	
6.	4-hydroxyquinoline	
7.	O-hydroxy-phenyl- acetic acid	
8.	O-coumaric acid	
9.	Bismuth subsalicylate (pepto Bismol)	
10.	Coumarin	
11.	Melilotic acid.	
12.	3- amino salicylic acid	Strong, 1956, Dekock and Grabowska, 1974, Hartman, *et al.*, 1955
13.	4- amino salicylic acid	
14.	3,4 dihydroxycinnamoyl-salicylic acid	
15.	Benzoic acid	Leon *et al.*, 1995, Senaratna *et al.*, 2003.
16.	2- Acetyloxy benzoic acid (Acetyl Salicylic acid)	Day *et al.*, 2004, Caspersen, *et al.*, 2004, Tuna *et al.*, 2007
17.	2- Hydroxy 3- Methyl Benzoic Acid (3-Methyl Salicylic acid)	Senaratna *et al.*, 2003.
18.	2- Hydroxy Benzoic Acid (Salicylic acid)	Senaratna *et al.*, 2003, Khan *et al.*, 2003
19.	Gentisic acid (GTA)	Groenewald and Westhuizen, 1998. Khan *et al.*, 2003.
20.	2- Hydroxy -5 Sulphobenzoic Acid (Sulfosalicylic acid)	Guenzi and McCalla, 1966; Arora et al., 2007, Tuna *et al.*, 2007.
21.	Glucosyl salicylic Acid	Enyedi *et al.*, 1992

As the salicylic acid contains carboxyl (-COOH) and hydroxyl (-OH) groups the ester derivatives such as acetate esters (acetylsalicylic acid or aspirin), methyl ester (methyl salicylate or oil of wintergreen and menthyl salicylate) and phenyl esters (phenyl salicylate or salol) can be prepared from the salicylic acid. (The Columbia Electronic Encyclopedia, 6th ed. 2005, Columbia University Press.)

Salicylic acid, acetyl Salicylic acid (Aspirin) and gentisic acid induced photosynthetic rates, stomatal conductance, leaf area and plant dry mass in corn and soyabean (Khan *et al.*, 2003). Gentisic acid induces plant resistance to pathogen attack and inhibits prostaglandin biosynthesis (Groenewald and Westhuizen, 1998) whereas benzoic acid, aspirin and salicylic acid blocked the flow of electrons from the dehydrogenase to ubiquinone pool in isolated mitochondria. It confirms the role of these analogues in the inhibition of mitochondrial electron transport (Day *et al.*, 2004). Arora and Singh (1999) and Arora *et al.* (2007) have been demonstrated the antisenescence role of 5- sulphosalicylic acid in cut gladiolus flowers. Larque-Saavedra (1978) observed the antitranspirant role of acetyl salicylic acid in leaves of *Phaseolus vulgaris*. In addition to it acetyl salicylic acid also reported to inhibiting the opening of stomata in epidermal strips of *Commelina communis* (Larque-Saavedra, 1979).The aspirin (acetyl salicylic acid) is the most popular drug attracted the attention in the world of medicine.

Recently several workers has been documented the role of salicylic acid and related compounds in induction of multiple stress tolerance associated with protective mechanism (Senaratna et. al., 2000, 2003, Kumar and Klessing, 2003, Khalil and Mughrabi, 2008).

D. SALICYLIC ACID: BIOSYNTHETIC PATHWAYS IN PLANTS

Even though the presence of SA in plant reported long back, its biosynthesis in plants has been reported recently in various plants. It is synthesized from amino acid phenylalanine (Phe). The most common pathway for the SA biosynthesis is the Shikimate-Propanoid pathway (Raskin, 1992a).

i) Salicylic Acid Biosynthesis in Tobacco

The pathway of salicylic acid biosynthesis has been reported in tobacco (Yalpani *et al.*, 1993) in which SA is synthesized from Cinnamic acid via benzoic acid (BA) as a part of phenylpropanoid pathway. Benzaldehyde (BD) a potential intermediate of this pathway, was found in healthy and tobacco mosaic virus (TMV) - inoculated tobacco (*Nicotiana tabacum* L. Cv *Xanthi*-nc). The enzyme benzoic acid -2-hydroxylase (BA2H) catalyzes the conversion of BA to SA. Leon *et al.* (1993) reported approximately 10 fold induction in BA2H by pathogen infection and inhibition of blocking of activity by a protein synthesis inhibitor.

Plants enclosed in jars when applied with gaseous BD caused an increase in salicylic acid concentration about 13- fold. Ribnicky *et al* (1998) reported that BD was not the intermediate between cinnamic acid and BA. They showed that SA accumulation was promoted by BD and benzyl alcohol. It leads to expression of defense response in tobacco. These findings provide insight into the early reports of salicylic acid biosynthesis.

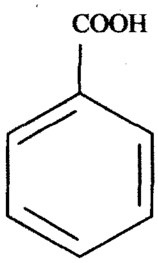
ii) Salicylic Acid Biosynthesis in Potato Plant

Coquoz *et al.* (1998) reported a rapid local synthesis of salicylic acid in potato leaves sprayed with Arachidonic acid (AA).

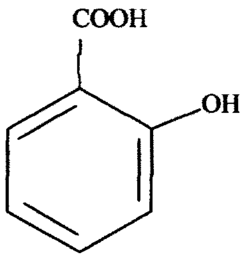
They also demonstarted with radiolabeling studies with untreated potato leaves that SA was synthesized from phenylalanine and cinnamic acid and benzoic acid were intermediates in biosynthesis of salicylic acid.

iii) Salicylic acid Biosynthesis in Cucumber

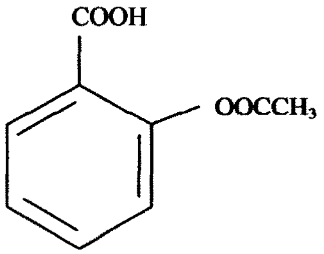
Radiolabeling studies of Meuwly *et al.* (1995) showed that salicylic acid is synthesized from phenylalanine (phe) via BA locally and systematically upon inoculation with totacco necrosis virus (*Pseudomonas lacrymnas*). Similar findings were reported by Philippe *et al.* (1995.) SA biosynthesis via benzoic acid in rice was also reported by Silverman *et al.* (1995). The direct correlation between the amount of endogenous salicylic acid and resistance to pathogens has been observed in potato (Coquoz *et al.*, 1998) and in rice (Silverman, 1995).



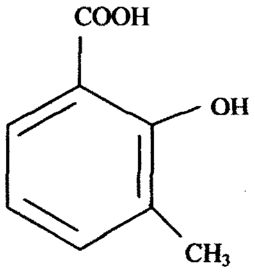
Benzoic Acid



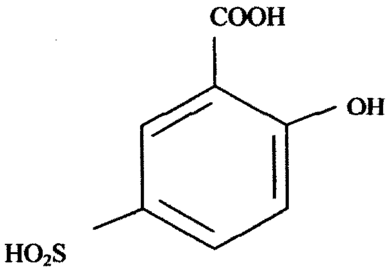
2- Hydroxy benzoic acid
Or
Salicylic acid



2- Acetyloxy benzoic acid
Or
Acetyl Salicylic acid
Or
Aspirin



2- hydroxyl 3- Methyl
benzoic acid
Or
3- Methyl Salicylic acid



2- Hydroxy 5-sulfobenzoic acid
Or
Sulfosalicylic acid

Fig. 2 : Molecular Structure of Salicylic acid and its related compounds (Senaratna *et al.*, 2003).

iv) Salicylic acid Biosynthesis in Tomato plants

In higher plants salicylic acid derives from the shikimate - phenylpropanoid pathway (Raskin, 1992a). The salicylic acid biosynthesis scheme - shikimate propanoid pathway is depicted in fig. 3a. Cinnamic acid and benzoic acid are two important intermediates in the shikimate acid pathway. Thus SA could be viewed as a derivative of cinnamic acid. Two routes from phenylalanine to salicylic acid have been found. These two routes differ in order of β-oxidation and ortho-hydroxylation reaction. Phenylalanin is converted into cinnamic acid (CA) and is either hydroxylated to Ortho-Coumaric acid or side chain of CA is oxidized to give Benzoic acid (BA).

Infection of young tomato plants with *Agrobacterium tumefaciens* increases conversion of cinnamic acid to O-coumaric acid through ortho-hydroxylation. In non infected plants, cinnamic acid converted to benzoic acid and finally to salicylic acid. According to Mauch-Mani and Slusarenko (1996), the inhibitors of phenyl ammonia – lyase (PAL) can affects the SA biosynthesis. The AIP (2-aminoindan-2-phosphonic acid), a highly specific PAL inhibitor can inhibit the synthesis of Cinnamic acid (SA precursor) and it leads to plants susceptible to pathogen attack.

v) Involvement of H_20_2 in SA biosynthesis

Leon *et al.* (1995) has been reported that hydrogen peroxide stimulated the accumulation of free benzoic acid (BA) and salicylic acid (SA) in *Nicotiana tabacum* L. Cv. *Xanthi*-nc leaves. They noticed two mechanisms for SA biosynthesis. First is biosynthesis of SA through the activation of benzoic acid - 2- hydroxylase (BA2H) directly by H_2O_2 or via formation of molecular oxygen (substrate for H_2O_2) in a catalase mediated reactions, whereas in the second mechanism maximum concentration of BA stimulates the accumulation of BAH2 protein in the cells and it provides maximum substrate for this enzyme.

According to Klessing *et al.* (1998) SA would synthesized from phenylalanine via transcinnamic acid and benzoic acid. Recently Wildermunt *et al.* (2001) has been proposed one pathway for SA biosynthesis (Fig No.3b). They reported that SA synthesized from chorismate via isochorismate synthase and pyruvate lyase. Ogawa *et al.* (2005) proposed the two different pathways of SA biosynthesis in ozone- exposed *Tobacco* and *Arabidopsis* plants. They reported accumulation of SA with the activation of PAL, mRNA levels and chorismate mutase transcripts in tobacco exposed to 0.2

ppm ozone i.e. in ozone exposed tobacco SA synthesizes from phenylalanine whereas in *Arabidopsis* it synthesizes from isochorismate under ozone stress.

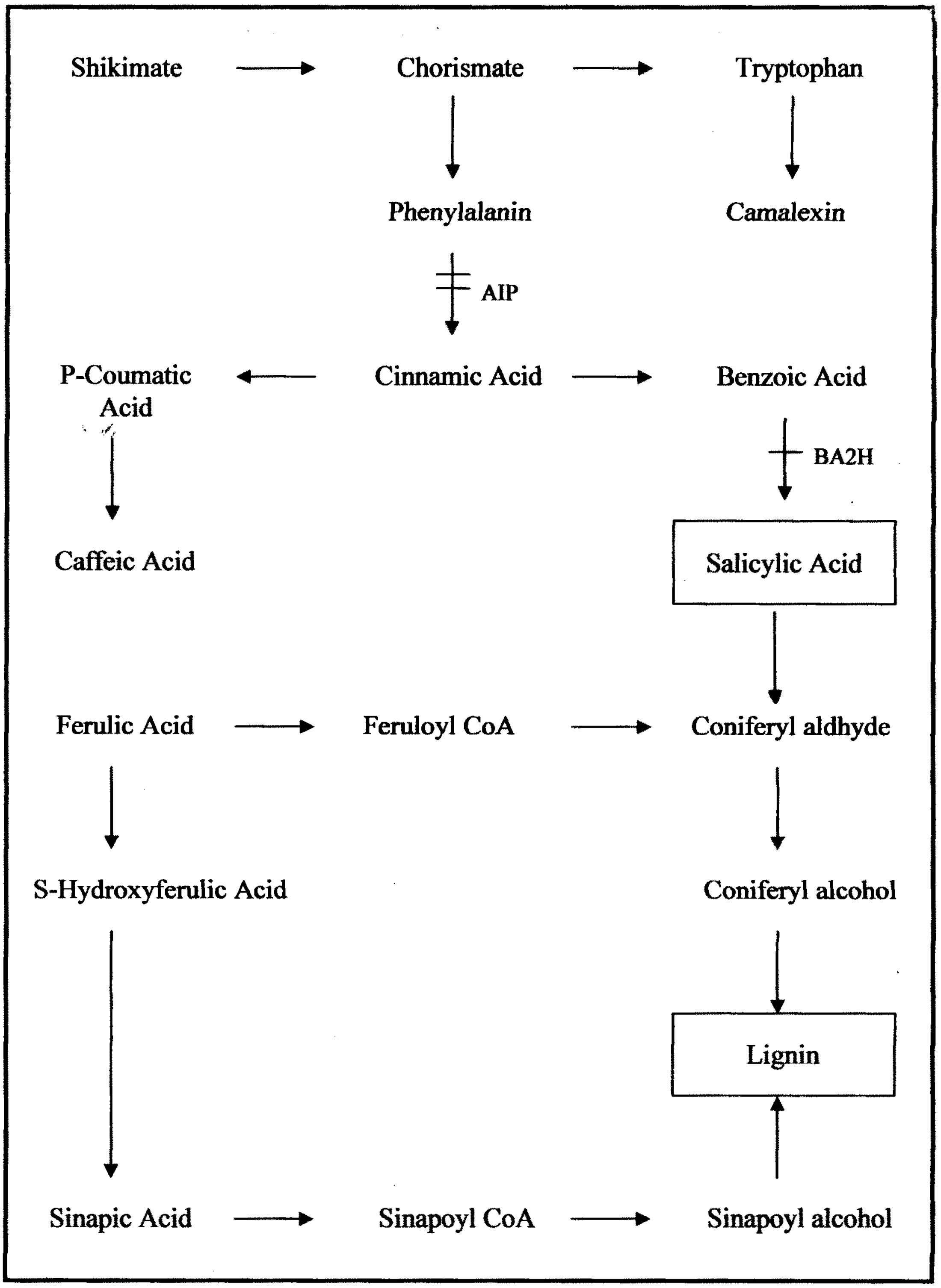
vi) Commercial production of salicylic acid:

SA is commercially prepared by treating the sodium salt of phenol (sodium phenolate) with a carbon dioxide under high pressure and at high temperature to form sodium salicylate. The reaction solution of sodium salicylate on acidification (treated with sulphuric acid) gives salicylic acid (The Columbia Electronic Encyclopedia, 6th ed., 2005, Columbia University Press). (Fig. 4).

E. EFFECT OF SALICYLIC ACID ON PLANTS

a) SALICYLIC ACID: A SIGNAL OF SYSTEMIC ACQUIRED RESISTANCE (SAR)

Systemic acquired resistance (SAR) is a signal which originates at the site of infection and moves throughout the plant (Ross, 1961 a,b). SAR is a mechanism of induced defense produced by an infected leaf (Dean and Kue, 1986 a, 1986 b). The detachment of infected leaf before the development of hypersensitive response (HR) blocks the induction of SAR and lead to cell death whereas HR is also common trigger for accumulation of salicylic acid and induction of SAR (Metraux *et al.*, 1990, Alvarez 2000.) Grafting and stem girdling experiments in Cucumber and Tobacco suggested that the SAR - signal is a phloem mobile (Guedes *et al*, 1980, Tuz~~a~~un and Ku~~e~~c, 1985). Salicylic acid (SA) induces production of some pathogenesis – related proteins (PR-Proteins), and plays an important role in the defense mechanism and in the acquisition of SAR (Fig. No.5) (Raskin, 1992a, Yalapani and Raskin, 1993, Klessing and Malamy, 1994). It has been found that highest systemic induction of SA observed in the youngest leaves located above the inoculated leaf. It confirms the phloem mobility of SA (Shulaev *et al.*, 1995). Klessing and Chen (1991) identified a soluble SA-binding protein in tobacco leaves and indicate its involvement in perceiving and transduction of SA signal to plant disease-resistance response. Whereas only small increase in SA observed in the leaves located approximately opposite to the inoculated leaf (Enyedi and Raskin, 1993, Varnooji *et al.*, 1994). As the SA is phloem mobile it can be easily transported from the infection site (production site) to the healthy part of the plant. According to Gaffney *et al.* (1993) the transgenic plants exhibit about a two fold



*** Inhibitor : AIP = 2-aminoindan-2phosphonic acid**

Fig.3a: Shikimate and Phenylpropanoid Pathways: Its branches leading to synthesis of Tryptophan, Phenyalanine, Lignin and Salicylic acid.

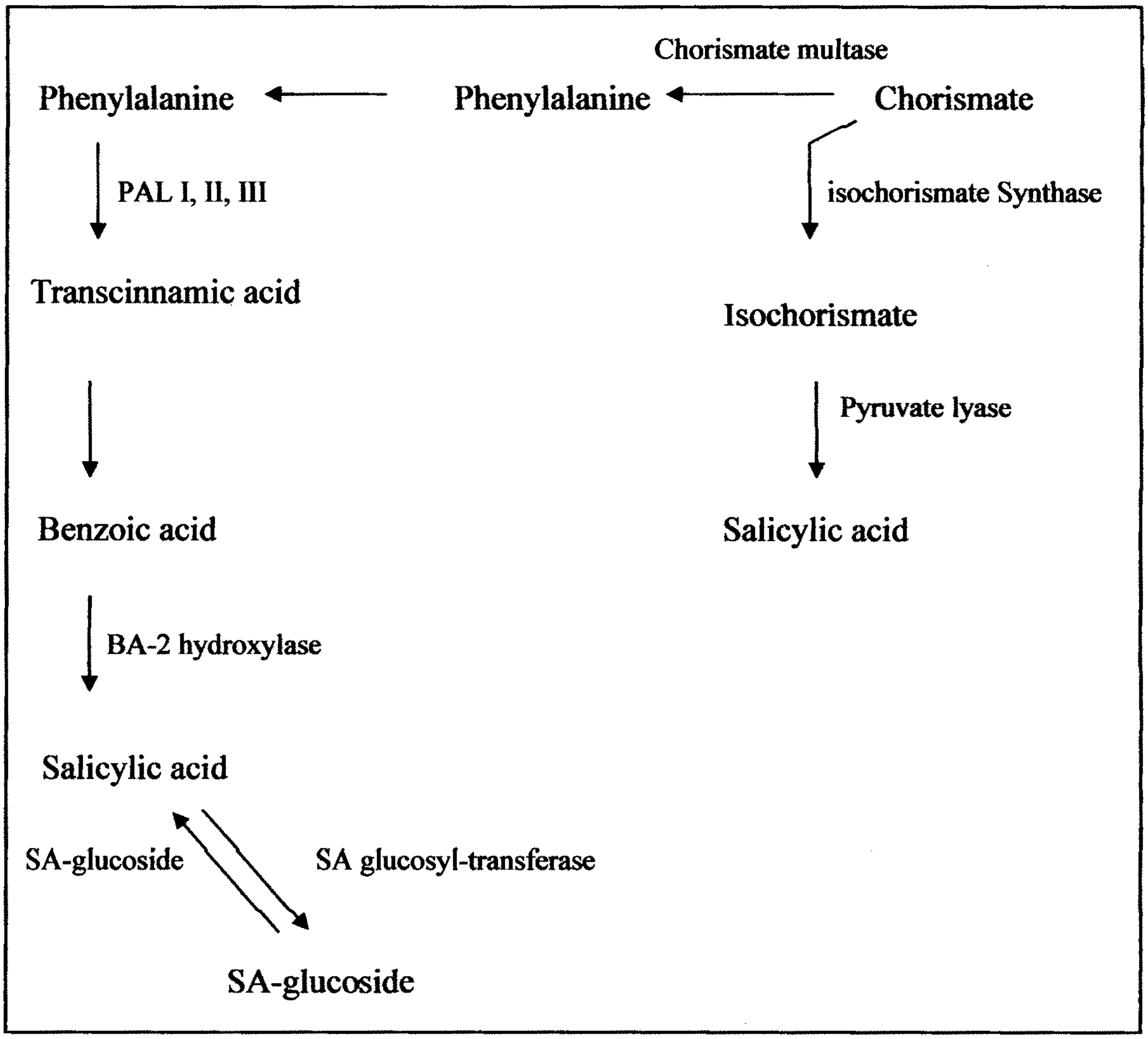


Fig. 3b: Salicylic Acid Biosynthesis

(Klessing *et al.*, 1998, Wildermunt *et al.*, 2001 and Ogawa *et al.*, 2005)

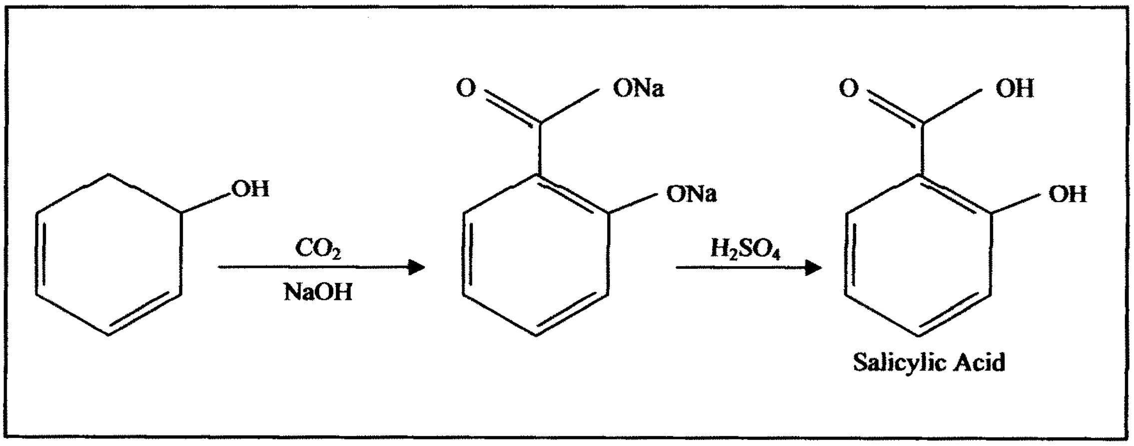


Fig. 4 : Commercial Production of Salicylic Acid.

increase in SA levels in inoculated leaves. The detachment of inoculated leaf before it begins to accumulate SA causes to blocks systemic SA increase in the upper leaves. Volatile derivative of SA-Methyl-Salicylate can be transported from the air to the neighboring plant. Klessing and Malamy (1994) indicated that SA is a natural signal molecule for the activation of plant defense responses. The defense function of SA was first observed in the tobacco (*Nicotiana tabacum* CV. *Xanthi-cv*). White (1979) was reported that the exogenously applied SA or acetyl salicylate induces resistance to totacco mosaic virus (TMV) in Tobacco. Evidances indicated that exogenous application of SA can induce resistance to pathogens in plants that locate a relevant resistance gene (Clarke *et al.*, 1998, Naylar *et al.*, 1998).

According to Ward *et al.* (1991) SAR in Tobacco and *Arabidopsis* is associated with expression of 'SAR' genes. These genes include genes which express PR-Proteins. (Cutt and Klessing, 1992, Stintzi *et al.*, 1993) and are identified as chitinase (PR-3) and β-1, 3-glucanase (PR-2). The molecular studies showed that SA induces nine classes of PR-protein mRNA in tobacco during the development of SAR. Similarly Metraux *et al.* (1990) found that TNV (Tobacco Necrosis Virus) inoculated in cucumber plant, developed SAR due to increased SA concentration in phloem on to increased SA concentration in phloem. On the basis of sequence of similarities, serologic or immunologic relationships and enzymatic properties the PR-proteins have been classified into 17 families (PR-1-PR-17) (Christensen *et al.*, 2002). These include proteins such as proteins - inhibitors (PTS), thionins, peroxidases and β-1, 3-glucanases (Van Loon, 1999).

Shulaev *et al.* (1995) reported that SA synthesized in the TMV-inoculated tobacco leaf was translocated to inoculated leaves where it performs as a translocated signal. In contrast to it, studies made by Ramsussen *et al.* (1991) and Vernooji *et al.*, (1994) suggest that SA may not be translocated SAR - inducing signal. Transgenic *Arabidopsis* plants carrying a bacterial salicylate hydroxylase gene showed susceptibility to different pathogens whereas transgenic tobacco plants carrying same gene do not develop SAR (Gaffney *et al.*, 1993).

Raskin (1992b) recorded highest concentration of SA on and around the hypersensitive lesions (Wounding portion). The findings made by various workers indicates that SA induces resistance to pathogens; induction of PR-Proteins; induction in SA concentration locally as well as systematically due to pathogen attack and phloem mobility of SA confirms that SA should be regarded as a signal molecule. Plants are exposed to many biotic stresses (infection by fungi, bacteria and viruses, damage by insects) or abiotic stresses (water, temperature or ionic stresses) imposed by their environment. Plants have devised sophisticated mechanism to cope many such environmental stresses (Sticher *et al.*, 1997). Endogenous elicitor, salicylic acid has crucial role in this mechanism (Gaffney *et al.*, 1993, Clarke *et al.*, 2002). Some workers have shown that exogenous SA can regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stresse (Li *et al.*, 1998, He *et al.*, 2002). They suggested that SA has a regulatory role in activating biochemical pathways related with tolerance mechanism. The application of SA has been reported to be involved in tolerance of plants to many abiotic and biotic stresses. (Table No. 2 and 3)

According to Senaratna *et al.* (2000) Salicylic acid, acetyl salicylic acid (aspirin), 3-methyl salicylic acid, sulphosalicylic acid and benzoic acid (precurssor of salicylic acid) are involved in multiple stress tolerance in plants.

Although SA has several roles in defense mechanism, excessive SA accumulation enhances a programmed cell death pathway (Rao and Davis, 1999). The reports made by Senaratna *et al.* (1998) were in agreement to previous reports. They observed adverse effects of SA (above 1mm) in tomato and bean plant.

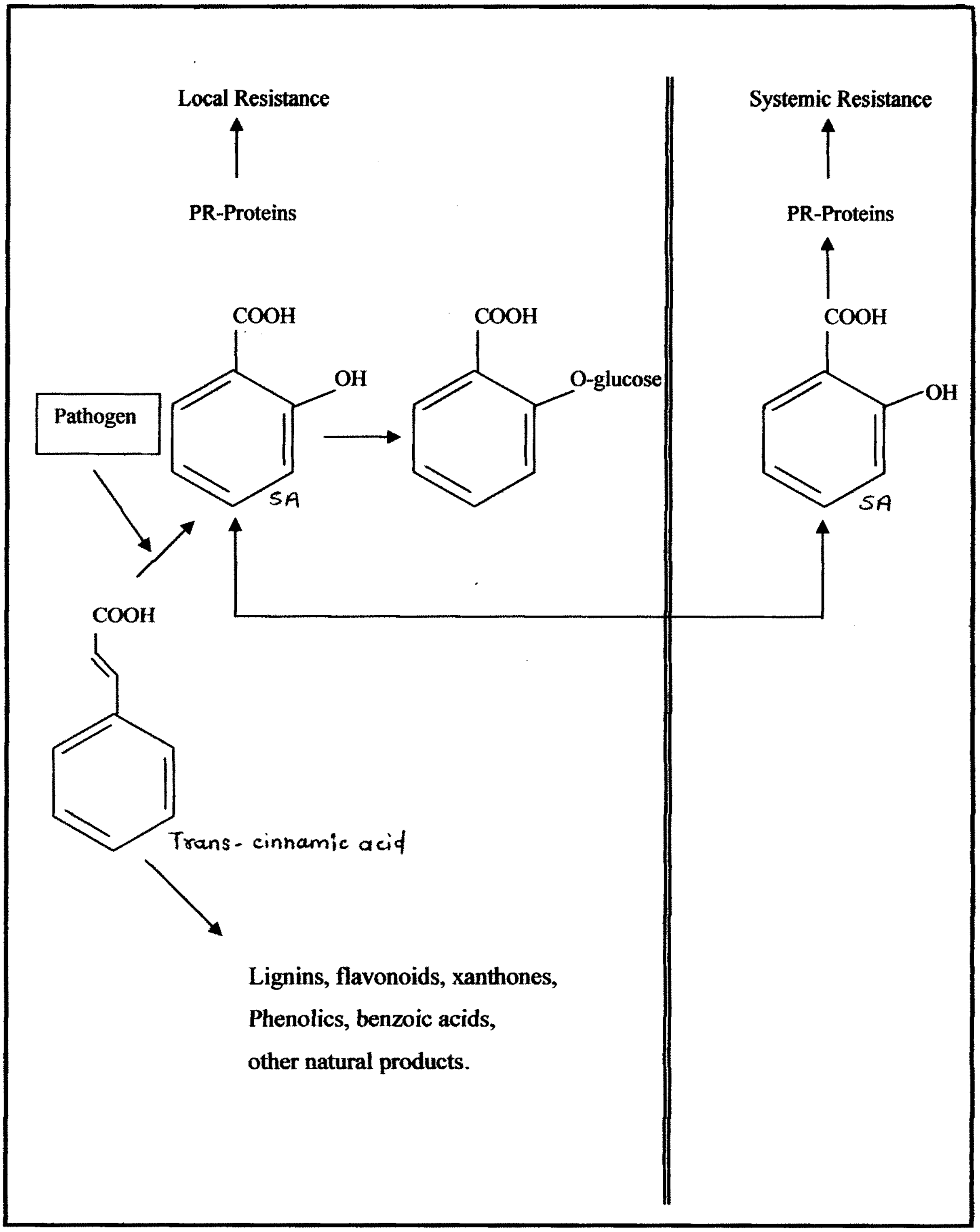


Fig.5 : Action of salicylic acid in disease resistance
(Raskin 1992a)

Table 2 : The Role of Salicylic Acid in Abiotic Stress Tolerance.

Name of Plant	**Abiotic Stress**	**References**
Maize	Chilling Stress	Janda *et al.*, 1999.
Rice	Heavy metal stress	Mishra and Choudhary, 1999
Arabidopsis thaliana	Oxidative Stress	Rao and Davis, 1999.
Tomato and Bean	Low and High temperature stress	Senaratna *et al.*, 2000
Wheat	Water deficit	Bezrukova *et al.*, 2001
Tomato	Salt and Osmotic stress	Borsani *et al.*, 2001
Kentucky Bluegrass	Heat stress	He *et al.*, 2001
Cucumber, Rice, Maize	Chilling Stress	Kang and Saltveit, 2002
Wheat	Salt stress	Sakhabutdinova *et al.*, 2003 Shakirova *et al.*, 2003
Wheat and Maize	Osmotic stress	Bhupinder and Usha, 2003
Arabidopsis thaliana	Paraquat tolerance	Kim *et al.*, 2003
Banana	Chilling Stress	Zhang and Chou, 2003
Maize	Salt Stress	Khodary, 2004
Agrostis solonifera	Heat Stress	Larkindale and Huang *et al.*, 2004
Barley	Salt stress	El-Tayeb, 2005
Sunflower	Copper stress	El-Tayeb et al., 2006
Cucumis sativa	Heat Stress	Shi *et al.*, 2006
Tomato	Salinity stress	Stevens and Senaratna, 2006
Cucumber	Salt Stress	Zhang *et al.*, 2006
Banana	Chilling Stress	Kang *et al.*, 2007
Maize	Salt stress	Gunes *et al.*, 2007

Table 3. The Role of Salicylic Acid in Biotic stress Tolerance.

Name of Plant	**Biotic-Stress**	**References**
Phaseolus vulgaris	Clover mosaic virus (WCIMV)	Clarke *et al.*, 1998
Tomato	Cauliflower Mosaic Virus	Tanga *et al.,* 1999
Arabidopsis thaliana	Turnip Crinkle Virus	Doonera *et al.,* 2000
Cowpea	Meloidogyne incognita (Root-Khot infection)	Nandi *et al.*, 2000
Phaseolus vulgaris	Clover mosaic virus	Galis *et al.*, 2004
Anacardium occidentale (Cashew)	Anthracnose disease	Lopez and Lucas, 2002.
Tomato	Cotton Bollworm (*Helicoverpa armigera*)	Peng *et al.*, 2004
Arabidopsis thaliana	Pathogen defense signalling	Mateo *et al.,* 2006
Cucurbita pepo	ZYMC	Radwan *et al.*, 2007
Vitis vinifera	Downey mildew	Kedge, 2008
Potato	*Rhizoctonia solani*	Khalil Al- Mughrabi, 2008

b) SALICYLIC ACID: AN INDUCER OF ACIDIC PR- PROTEINS

The plants have two distinct signal transduction pathways for pathogen and wound induced responses (Malamy and Klessing, 1992). Biosynthesis of Pathogenesis related-proteins (PR) in plants is one of the self-defense system developed by plants against the pathogen attack and wounding. The synthesis of PR-Proteins considered as a molecular indicator for the activation of defense mechanism. PR-proteins were initially classified into 5 groups : the PR-1 to PR-5 proteins (Van Loon *et al.,* 1987). In addition to previous 5 groups, recently 6 more groups have been identified. First five groups consist of both acidic and basic isoforms (Brederode *et.al.*, 1991, Ohashi and Ohashima, 1992).

The TMV infection as well as salicylic acid induces PR proteins in plants, auxin and cytokinin also induce it but in minor quantity (Ohashi and Matsuoki, 1987). The wounding or Jasmonic acid or ethylene causes induction of basic PR-proteins (Pena-cortes *et al.,* 1995, Suzuki *et al.*, 1995) whereas, cytokinin and auxin found to inhibit the accumulation of basic PR-proteins in wounded plant (Shinshi *et al.,* 1987). Wound signals are transmitted to jasmonic acid which causes induction of basic PR-proteins, whereas pathogenic signals transmitted to jasmonic acid and it leads to formation of acidic PR proteins via accumulation of salicylic acid. Bergmann *et al.* (1994) found that SA can induce polygalactourinase inhibiting proteins in Phaseolus vulgaris L. against wounding or fungal infection.

Salicylic acid is considered as a natural inducer for acidic PR proteins and Jasmonic acid (JA) as a natural inducer for basic PR-proteins (Varnooji *et al.*, 1994, Pena-cortes *et al.,* 1995). Sano *et al.* (1996) reported that cytokinin acts as a signal switch which causes the transmission of wound signal to either jasmonic acid biosynthesis or salicylic acid biosynthesis pathway.

c) ROLE IN THERMOGENECITY

Salicylic acid plays an important role in thermogenecity (Fig No.6). Lamark (1778) was to described the thermogenecity i.e. heat production which was found in the inflorescence of some angiospermic families including Annonaceae, Araceae, Aristolochiaceae, Cyclanthaceae, Nymphaeceae, Palmae and male reproductive structures of cycades (Meeuse and Raskin, 1988, Raskin, 1992). Van Herk (1937) reported the prescence of water soluble substance, 'Calorigen' in staminate flower of Vodoo lily which was found to triggers the burst of metabolic activities in the appendix of Vodoo lily. Later on he has been identified and purified, 'Calorigen' the natural trigger of heat production in thermogenic plants and named as salicylic acid. Further they reported 14^0c increase in temperature above the ambient temperature on the blooming day in Vodoo lily spadix. According to James and Beevers (1950) the thermogenecity is associated with induction in the cyanide - insensitive non - phosphorylating electron transport pathway (alternate respiratory pathway). Kapulnic *et al.* (1992) have been reported the role of SA in the induction of cyanide resistant pathway of mitochondrial electron transport in Tobacco cell suspention cultures. They found that SA induces the activity of cyanide resistant respiration without altering the capacity of the cytochrome c respiration. SA induced heat evolution was significantly affected by salicylhydroxamic acid (SHAM), which is an inhibitor of cyanide resistant respiration, but was not affected by cyanide. Thermogenecity causes activation of enzymes of glycolysis and Krebs cycle and also the activation of alternate oxidase. Such activated respiratory enzymes believed to provide subsrates for alternate respiratory pathway. The heat is a product of cyanide insensitive respiration, the enzymes of Krebs cycle and glycolysis induced by salicylic acid (Raskin 1992a).

The prime role of thermogenecity was thought to be the volatilization of amines and indols that of foul smelling attractive to insect pollinators.

Chen and Klessing (1991) regarded SA as an endogenous regulator of heat and odour production in the inflorescence of some thermogenic plants. The chemical identity of calorigen with SA was shown by Rhods and McIntosh (1991), as the both causes induction of alternate oxidase gene. Raskin *et al.* (1990) estimated differential levels of SA in the inflorescence of five aroid species and male cones of four thermogenic cycads during heat production. Wagner *et al.* (1998) has measured the *in vivo* ubiquinone reduction levels at the time of the development of the inflorescence of *Arum maculate* and *Amorphophallus krausei.* They reported 100-fold increase in respiration via alternative pathway which caused more than $12\text{-}20^0c$ increase in temperature of appendix. Among the studied 33 analogs of SA only 2, 6-dihydroxy benzoic acid and acetyl salicylic acid (aspirin) were reported as thermogenic (Raskin *et al.* 1989).

Recently Gibernau *et al.* (2005) investigated a logarithmic relationship between the volume of the thermogenic inflorescence and highest temperature difference between spadix and ambient air.

The earlier reports clears that the thermogenecity is due to the operation of cyanide resistant alternate respiration pathway involving prime role of alternate oxidase (Meeuse and Raskin, 1988, Siedow and Umbach, 1995).

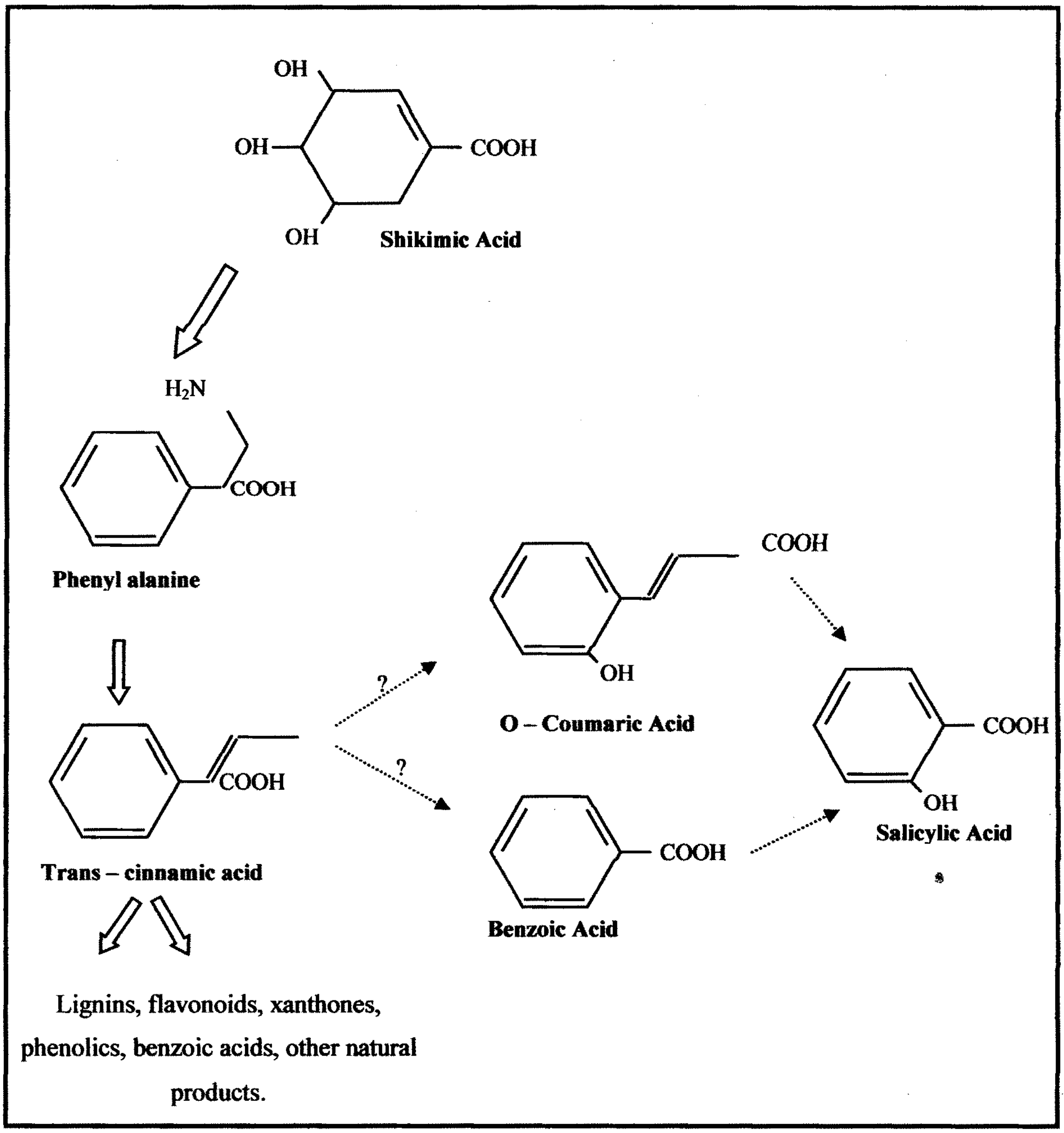


Fig. 6 : Action of salicylic acid in Thermogenesis

d) SALICYLIC ACID AND THERMOTOLERANCE

Plants are often exposed to changes in temperature at differtent growth stages. Temperature level beyond an optimum level i.e. heat stress is a major growth limiting factor. High temperature causes excessive excitation of the respiratory and photosynthetic electron transport systems, it leads to the production of Reactive Oxygen Species (ROS) such as superoxide and hydrogen peroxide (Dat *et al.*, 1998), which are toxic to plants. The concentrations of ROS are controlled by antioxidant defense systems. Recent studies revealed that SA can regulate the activities of antioxidant enzymes inducing tolerance of plants to heat stress (Dat *et al.*, 1998 and Clarke *et al.*, 2004, He *et al.*, 2002, Senaratna, 2003).

Ma *et al.* (1998) documented that heat stress increases salicylic acid concentration in leaves of cucumber seedlings. According to Dat *et al.* (1998), there was a transient increase in endogenous SA levels in heat shocked plants thus SA was involved in heat acclimation in mustard seedlings. Induction in SA causes synthesis of heat-shock proteins at osmotic stress (Mikolajlcyk *et al.*, 2000). Shi *et al.* (2004) studied the influence of exogenous salicylic acid on heat tolerance in cucumber seedlings. Heat stress also causes stimulation of scavenging enzymes like, catalase, peroxidase, dismutase and several antioxidants (Chaitanya *et al.* 2002). Clarke *et al.* (2004) documented that rather than acquired thermotolerance, SA signaling plays an important role in the acquisition of basal thermotolerance. Supportive conclusion was also made by Larkindale and Knight (2002). Heat acclimation and exogenous SA may causes the enhancement of thermotolerance in Pea leaves (Liu *et al.*, 2006 b). They studied the inter-relationship between salicylic acid, abscisic acid and P_1P_2-phospholipase C in induction of thermolotolerance.

Some plant species including potato, rice and tomato contains abundant SA for the establishment of protective mechanism (SAR) against biotic and abiotic stress, but their levels are poor in SA perception and transduction (Raskin *et al.*, 1990). On the other hand Tobacco and *Arabidopsis* contains very low basal SA levels are still effective in SA perception and transduction (Enyedi *et al.* 1992, Vernooji *et al.*, 1994). Senaratna *et al.* (2003) reported induced chilling tolerance and drought tolerance whereas Dat, *et al.* (1998) and Senaratna *et al.* (2003) has been found induction in heat tolerance in several plants by the application of Salicylic acid. It has been reported that salicylic acid confers the protection in mungbean plants to heat stress injury by inducing antioxidant enzymes such as catalase, peroxides and ascorbate peroxidase and superoxide dismutase and also by enhancing carotenoid and GSH content (Sahel *et al.*, 2007).

e) INFLUENCE ON GROWTH AND DEVELOPMENT

Exogenous application of salicylic acid has great influence on various growth processes and yield performance (Raskin, 1992 b).

SA improved the growth of barley seedlings (Pancheva *et al.*, 1996). Increased growth by SA application was observed by Gutierrez-coronado *et al.* (1998) in soyabean and Rajaskekaran and Balke (1999) in jack pine. Dhaliwal *et al.* (1997) and Zhou *et al.* (1999) reported that SA treatment causes increase in leaf area in sugarcane plant. It has been reported that foliar application of salicylic acid (SA) enhanced growth and development in green gram and black gram (Rao *et al.*, 1998). Hiremath *et al.* (1998) also observed that SA increase seed yield and leaf area in greengram. Gutierrez *et al.* (1998) reported increased shoot-root growth of soyabean by SA treatment. Exogenous application of SA caused early induction of floral bud and pod formation along with enhanced number of flowers and pods per plant as well as harvest index in soyabean (Kumar *et al.*, 1999). Foliar spray of SA elevetes plant height, leaf area, leaf weight and dry weight of soyabean (Pathmanabhan and Thangaraj, 1999). Ghai *et al.* (2002) found improved seed yield and other yield parameters with the foliar application of SA in *Brassica napus*. Exogenously applied SA was found to increase number of flowers, fruitset and fruit weight in tomato (Kalarani *et al.*, 2002 a). They further reported the positive effect of SA in improving grain yield in Pearl millet (2002 b).

Khan *et al.* (2003) have been investigated the effects of SA, acetyl salicylic acid (ASA) and Gentisic acid (GTA) on growth of soyabean and corn. They found increase in leaf area and plant dry mass. Fariduddin *et al.* (2003) observed positive impact of SA on growth parameters in *Brassica juncea*. Rajjou *et al.* (2006) noticed increased seed germination in *Arabidopsis* under salt stress, due to SA. Whereas, Scott *et al.* (2004) reported inhibition of growth in *Arabidopsis* due to accumulation of salicylate under chilling stress. SA improved the salt tolerance in maize by improving the measured plant growth criteria (Khodary, 2004). SA induced increase in bioproductivity of horticultural and ornamental plants, was attributed to positive effect of SA on root length and its density (Alfonso and Rodolfo, 2007). SA increased root cap size and appearance of lateral roots in the *Catharanthus roseus* transformed roots (Echevarria-Machado *et al.*, 2007). Barde *et al.* (2007) reported that foliar spray of SA caused the enhancement of growth components in soyabean plant. The seed treatment and foliar application of SA increased all yield components in pea (Ghulam *et al.*, 2007).

In contrast to majority of growth promoting effects Asthana and Srivastava (1978) have reported an inhibitory effect of SA on maize seed germination. Similarly, Anandhi and Ramanujam (1997) reported inhibitory action of SA which caused decrese in germination, root growth, seedling growth and biomass in black gram.

f) EFFECT ON PHOTOSYNTHESIS

Photosynthesis is the process of great importance contributing to the survival of the life. On this planet there have been number of efforts to increase photosynthesis in crop plants to bring about increase in plant productivity. There are several reports indicats the role of salicylic acid in photosynthesis.

Lesile and Romani (1988) observed that SA enhanced the photosynthesis and chlorophyll contents. Sinha *et al.* (1993) reported increased chlorophyll and carotenoid content by SA treatment in maize leaves. Zhao *et al.* (1995) and Liu *et al.* (1999) documented that SA stimulated the pigment contents as well as photosynthetic rates. It has been reported by Rajasekaran and Balke (1999) that SA enhances the rate of photosynthesis under stress conditions. The treatment of auxin and salicylic acid significantly enhanced the chl. a, b and carotenoids- beta carotene, and lutein + zeaxanthin content in *Wolffia arrhiza* (Czerpak, 2002). Ghai *et al.* (2002) observed that foliar application of paclobutrazol and salicylic acid (10 mug. ml.) stimulated chlorophyll content and Hill activity in leaves of *Brassica napus.* Foliar application of SA, brassinosteroids, triacontanol, NAA and mepiquat chloride found to increase the chlorophyll contents (Sivakumar *et al.,* 2002). According to Khan *et al.* (2003) foliar application of SA, acetyl salicylic acid (ASA) and gentisic acid (GTA) enhances photosynthetic rates in corn and soyabean. They also reported increased photosynthetic rates by chronic injection of SA in corn. Similary, Smith *et al.* (2001) found that chronic injection of SA into the pith of the corn stems increased rates of photosynthesis. It has bean reported that SA enhanced the activity of RuBISCO (RuBISCO catalyzes the biological fixation of atmospheric CO_2), chlorophyll a, b, carotenoid contents, rate of CO_2 fixation and carbohydrate metabolism in maize plants under salt stress. These results confirmed that SA conflict the salt stress in maize plant by improving photosynthetic machinery. Singh and Usha (2003) reported induction of Rubisco activity and total chlorophyll content by SA especially at 1-3 mM concentration. Agarwal (2005) noticed increased carotenoid and chlorophyll contents in wheat plants under the influence of SA treatment.

The exogenous application of 1mM SA increased maximum yield of PSII photochemical reactions (Fv/Fm) and quantum yield of the PSII electron transport of *Cucumis* seedlings under heat stress. In contrast to it, SA in combination with nutrient solution and both folior application as well as addition in nutrition solution showed negative effects on PSII (Shi *et al.*, 2006).

Stevens and Senaratna (2006) noticed that 0.1 mM SA increased photysynthetic rates in salt stressed tomato plants and it leads to improv its survival efficiency. Tuna *et al.* (2007) indicated that 1 and 2 mM 5-sulphosalicylic acid, 1 mM acetyl-salicylic acid increased total chlorophyll content in maize plants.

In contrast to the above observations, some inhibitory findings are available. Anandhi and Ramanujam (1997) found inhibition of Chl. a, b, total chlorophyll and carotenoid contents in blackgram by SA treatment. SA pretreatments caused reduction in net photosynthesis in maize (Janda *et al.*, 2000). Heat stress causing transient enhancement in photosynthesis was suppressed in SA-treated plants (Larkhindale and Huang, 2004). Mateo (2006) investigated that SA inhibits photosynthetic electron transport under low light and short day conditions in *Arabidopsis* leaves. According to El-Tayeb (2006) SA reduced chl. a/b ratio in Cu-stressed sunflower plants by SA.

g) EFFECT ON FLORAL DEVELOPMENT

There are number of findings providing evidences about the involvement of salicylic acid in flowering. The effects of SA on flowering have been reviewed by Raskin (1992 b). Participation of SA in the regulation of flowering was also suggested by the experiments made by Cleland and Ajami (1974).

According to Oota (1975), SA acts as a chelating agent and induces flowering. Nanda *et al.* (1976) and Sood and Nanda (1979) reported that SA, GA3 and β- naphthol induces floral bud formation in *Impatien balsamina* (short-day plant) under non-inductive photoperiods. SA at 1 and 100 mg l^{-1} concentrations induced floral bad formation. Watanable and Takimoto (1979) found that benzoic acid and other non-chelating phenolics induces flowering. The relationship between the structure and flower-inducing activity of benzoic acid derivatives in *Lemna paucicostata* has been studied by Watanable *et al.* (1981). The size of benzyl-ring substitution of benzoic acid is related with the flower inducing effect. Khurana and Maheshwari (1980) found that SA, aspirin and related phenolic compounds stimulates the flowering under non-inductive photoperiods in *Spirodela phlyrrhiz, Spirodela punctata* (Scharfetter *et al.*, 1978). Kharana and Cleland (1992) found that SA or benzoic acid at 10 μM concentration induced flowering of *L. paucicostata.*

It is well known phenomenon that the tablet of aspirin dissolved in water increases the vase life of cut flowers. SA was found to be induces the flower longevity, these evidences are in agreement with the reports made by Lesile and Romani, (1986), they also reported that SA blocks the ethylene biosynthesis in pearl cell suspension culture.

Recently, Kumar *et al.* (1999) in soyabean, Rao *et al.* (1999) in green gram and black gram; Kalarani *et al.* (2002 a) in tomato observed that SA treatment causes early induction of flowers and also increases number of flowers and fruits. It has been noticed that SA inhibits gravitropic bending of cut Snapdragod, lupins and anemone flowering shoots (Friedman *et al.*, 2003). Heitholt *et al.* (2001) observed that single spray of sodium salicylate caused two to three weeks prior flowering in cotton. Martin Mex *et al.* (2005) found that 0.1 mM salicylate induced number of flower primordial and flowering at 74-days of plant age earlier than control plants. Chandra and Das (2007) found hastened floral induction by SA treatment in *Toria.*

h) EFFECT ON ENZYME ACTIVATION

Since almost all biochemical reactions in plants catalysed by enzymes, effect of salicylic acid on activities of different enzyme systems have been invetigated by some workers.

Rajjou *et al.* (2006) observed abundant isocitrate lyase and malate synthase enzymes in seeds germinated in presence of SA. These enzymes are key enzymes of glyoxylate cycle involved in the synthesis of carbohydrates from storage lipids during seed germination (Eastmond and Graham, 2001). During the seed germination, SA influences the enzymes of citric acid cycle, pentose phosphate pathway, glycoysis and gluconeogenesis.

Abiotic stresses can induce the accumulation of reactive oxygen species such as O_2, H_2O_2 and hydroxyl ions (Hasegawa *et al.*, 2000). The two enzymes i.e. catalase and peroxidase have capability of decomposition of H_2O_2 in the higher plants. Several evidences indicate the involvement of salicylic acid in the establishment of abiotic stress tolerance through its participation in influenzing oxidative enzymes. Superoxide dismutase and catalase are the most effective antioxidant enzymes in scavenging AOS (Bowler *et al*., 1992).

Shi *et al.* (2006) observed that foliar application of SA stimulated catalase activity whereas addition of SA to the nutrient solution and it in combination with foliar application reduced activity of catalase in cucumber seedlings under heat stress. It indicated the involvement of SA in heat tolerance by the removal of H_2O_2. The report made by several workers indicates that SA can regulate the activities of antioxidant enzymes and plant tolerance to abiotic stresses (Ma *et al*., 1998, He *et al*, 2002, Rajjou *et al*., 2006). SA enhanced the peroxidase activity in *Vicia faba* (Mori *et al.*, 2001). Fernandes *et al.* (2006) reported the induction of anionic peroxidase in *Vigna unguiculata* by exogenous SA. Repka (1996) made reports in agreement with it, who reported induction of anionic peroxidases in TNV (Tobacco Necrosis Virus) infected cucumber. Kim *et al.* (2003) have reported that SA-pretreatment caused slight increase in superoxide dismutase, inactivation of catalase and increase in activity of guaicol peroxidase in *Arabidopsis* plants. The root injection and foliar spray of SA improved the activities of superoxide dismutase, peroxidase and catalase and reduced the Malondialdhyde (MDA) content in cucumber seedlings (~~Liu~~ Zhang *et al*., 2006). Sudhakar *et al.* (2007) indicated that tomato plants infected with cucumber mosaic virus when treated with various concentrations of ozone increased the activities of SA, phenylalanine ammonia lyase (PAL) and peroxidase. It shows induction of systemic resistance in tomato.

Bi *et al.* (1995) recorded that tobacco plants infected with *Pseudomonas syringae* or leaf discs treated with SA both did not show reductions in catalase activity. It has been reported that, heat stress caused a significant increase in endogenous H_2O_2 with reduction in catalase. SA and heat acclimation caused reduction in both H_2O_2 and catalase conferring thermotolerance in mustard seedlings (Dat *et al*., 1998). Kumar *et al.* (1999) found increased activities of enzymes catalase and peroxidase in soyabean plants in response to foliar application of phenolic compounds indluding SA, gallic acid, resorcinol (Res) and tannic acid (TA). Foliar sprays of SA were found to inhibit the activites of catalase, ascorbate peroxidase and increased the activity of peroxidase whereas the activity of superoxide dismutase remained unaffected. On the other hand pretreatments of SA increased the activities of catalase, alternate peroxidase (APX) and reduced the H_2O_2 production (Zhang *et al*., 2003). Larkindale and Huang (2004) also studied the effect of foliar application of SA on antioxidant enzyme activities in *Agrostis stolonifera.* SA also increased the peroxidases in bean plants (Faheed and Mahamoud 2006). Recently Tuna *et al.* (2007) found that different SA derivatives decreased activities of antioxidant enzymes.

Several reports indicated the impact of SA on photosynthetic reactions. Khodary (2004) investigated the significant increase in RuBISCO activity in response to foliar spray of SA ameliorating the deterious effects caused by salt stress in maize. SA controls the breakdown of Rubisco (Pancheva and Popova, 1998). Galis *et al.* (2004) noticed that SA significantly increased the expression of PAL (Phenyl alanine ammonia lyase) in *Phaseolus valgaris*. SA pretreatment prior to paraquate application enhanced the activities of detoxifying enzymes particularly in chlorplasts, cytosol and peroxisomes (Ananieva *et al.*, 2004).

Singh and Usha (2003) reported that SA induces the activities of Rubisco (Ribulose 1,5, biphosphate carboxylase) and superoxide dismutase (SOD) and it also maintains and protects the activity of nitrate reductase enzyme in seedlings of wheat under water stress.

Nitrate reductase is the rate limiting enzyme in nitrogen assimilation. Several reports are available on the influence of SA on the activity of nitrate reductase. Presowing soaking treatments with ascorbic acid and ascorbic acid in combination with SA enhanced NRA in maize seedlings (Asthana and Srivastava, 1978). Kaur (1987) found stimulation of nitrate reductase activity in groundnut cultivars in response to phenolic compounds. Jain and Srivastava (1981) found increase in NRA in roots of maize seedlings due to by SA treatments. Ramanujam *et al.* (1998) recorded enhancement of NRA and reduction in nitrogenase in black gram by SA presowing soaking treatment. Lower concentration of SA caused induction in NRA in *Vigna mungo* (Jaleel *et al.*, 1998). In addition to NRA, Jaleel *et al.* (1998) reported reduction in nitrogenase activity and nodulation in *Vigna mungo* plants. Moharikar (2001) reported stimulation of NRA by higher doses of SA in germinating mung seeds. Kalarani *et al.* (2002 a) noticed increased NRA by foliar application of SA in tomato seedlings. Awasti and Garg (2007) studied the effect of SA on enzymes of ammonium assimilation in maize seedlings.

The application of SA increased the dehydrogenase activity in soyabean. Awasti and Garag (2007) noticed that glutamate dehydrogenase (GDH) activity increased at lower concentration of SA. Glutamine synthtase activity was significant at

5o and 100 μM concentration whereas glutamate synthase (GOGAT) activity was decreased due to SA treatment. From these results they suggested concentration dependant effect of SA.

i) EFFECT ON TRANSPIRATION

There are number of reports available in literature showing impact of SA on stomatal functioning influencing transpiration rates in plants.

Barbara *et al.* (1972) reported that SA at concentration 0.001 mM causes stomatal closure in *Vicia faba.* Larque Saavedra (1979) observed stomatal closure and reduction in rate of transpiration in *Commelina communis* by SA treatment within 13 min after application. They further found increased H_2O_2 concentration in cytoplasm of guard cells leading to increased membrane permeability and efflux of K^+ inducing stomatal closure. Barkosky and Einhelling (1993) observed that SA at lower concentration decreased stomatal aperture Aldesuguy *et.al.* (1998) and Aktas (2001) reported that SA and its derivatives decreased the transpiration rate. Similarly, Mori *et al.* (2001) found induced stomatal closure with exogenous application of SA in *Vicia faba.* Singh and Usha (2003) indicated that SA treatment at 3mM concentration maintains higher moisture content in Wheat plants grown in water stress. Agarwal *et al.* (2005) and Tuna *et al.* (2007) found elevation in relative water content by SA and its related compounds.

In contrast to above findings Rai *et al.* (1986) found that SA reversed the ABA induced closure of stomata in *Commelina communis*. Manthe *et al.* (1992) investigated the effects of SA on stomatal behaviour in *Vicia faba*. They noticed closure of stomata at 0.001 mM concentration whereas SA concentration more than 3.5 mM affects the transpiration rates. Khan *et al.* (2003) found increased stomatal conductance and transpiration by foliar application of SA, acetyl salicylic acid (ASA) and gentisic acid (GTA) in corn and soyabean. Stevens and Senaratna (2006) observed that optimum dosage of SA did not alters the transpiration rates in unstressed tomato plants whereas SA application to salinity stressed plants showed significantly higher transpiration rates.

Larque-Saavedra (1978, 1979) demonstrated that different concentrations of acetyl-salicylic acid functions as antitranspirant in leaves of *Phaselus vulgaris*.

j) OTHER KEY FUNCTIONS OF SALICYLIC ACID

There are several reports showing the interaction of salicylic acid in some basic physiological functions including membrane functioning, nutrient uptake, ethylene biosynthesis, protein content and seed germination.

Kang and Saltveit (2002) and Stevens and Senaratna (2006) reported that SA reduced electrolyte leakage in corn and rice leaf and in cucumber *hypocotyls under chilling stress and also in salt stressed tomato leaves*. Fariduddin *et al.* (2003) reported that higher doses of SA caused permanent change in the cells particularly at the levels of membrane organization and they concluded from these findings that such change may be injurious for plant metabolism and growth. Similar findings were made by Uzunova and Popova (2000). El-Tayeb (2006) found that SA caused reduction in membrane damage in copper-stressed sunflower plants. Tuna *et al.* (2007) observed that salicylic acid, sulphosalicylic acid and acetyl salicylic acid reduced the ion leakage in maize plant under salt stress condition. These reports indicate the role of SA in maintaining the membrane function under salt stress condition. Similarly the reduction in ion leakage in salt stressed tomato plants by SA facilitating the maintenance of membrane functions.

Exogenous application of salicylic acid controlled ion uptake and their transport (Haper and Balke, 1981). Aly and Soliman (1998) found that SA reduced the iron chlorosis in soyabean. Al-Hakim *et al.* (2001) reported positive impact of SA on the uptake of Na, Ca, K and Mg in wheat plants under salinity stress. Alpaslan *et al.* (2005) studied the stimulatory action of SA on mineral nutrient concentrations. SA increased the accumulation of nitrogen and also stimulated the uptake of P, K. Mg and Mn in maize under multiple stress condition. Application of different concentrations of SA reported to increase nitrogen content in maize seedlings (Awasthi and Garg, 2007). SA improved the accumulation of macro and micro-elements in leaves and roots of maize-seedlings (Tuna *et al.*, 2007).

Similarly Enyedi *et al.* (1992) and Yalapani *et al.* (1994) reported that SA induced the number of genes coding for pathogenesis related proteins under the influence of biotic and abiotic stresses. Kumar *et al.* (1999) noticed that SA increased the total soluble proteins in soyabean. Purohit and Gehlot (2001) observed increase in protein content in *Sesamum indicum* Cv. RT-54 by 5 and 200 ppm SA treatment. Foliar application of growth regulators namely salicylic acid, triacontanol, brassinosteroid, Naphthalene acetic acid (NAA) and mepiquat chloride enhanced soluble protein, grain protein and total sugar content in pearl-millet (Kalarani *et al.* 2002b). Similarly Sivakumar *et al.* (2002) found that foliar application of SA increased soluble proteins and grain proteins of Pearl-Millet. Garg and Awasthi (2007) indicated that SA caused enhancement in protein content up to 50 µM concentration in the shoot and roots of maize seedlings. SA was found to induce the synthesis of heat shock proteins in tobacco plants in cell suspension culture under osmotic stress (Mikolajcyk *et al.*, 2000). Singh and Usha (2003) observed that SA maintained the protein content in Wheat in response to water stress.

Apte and Laloraya (1982) have found that 10^{-4} to 10^{-10} M SA reduced abscisic acid- induced leaf abscission in ***Phaseolus vulgaris*** L. Lesile and Romani (1988) reported inhibition of ethylene biosynthesis by SA. Srivastava and Dwivedi (2000) noticed SA induced delaying of ripening of banana fruits by the inhibition of biosynthesis of ripening harmone, ethylene.