INTRODUCTION

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In view of ever increasing population on this planet, the agriculturists in all parts of the world are continuously seeking for different ways and means to increase the productivity of crop plants. Chemical manipulation of crop growth and development with the help of Plant Growth Regulators has been found to be one of the fruitful strategies in achieving this goal. Plant Growth Regulators are either naturally occurring or synthetic organic compounds other than nutrients or vitamins and they are active at low concentration. An aromatic acid, salicylic acid has recently attracted the attention of botanist in this context, since it is found to be effective in influencing number of metabolic processes in plant. It is worthwhile to mention here that its derivative acetylsalicylic acid is one of the most popular and potent drugs in the world of medicine. Salicylic acid occurs naturally and it can also be synthesized in the laboratory.

After consideration of its various roles in plant metabolism, Raskin [1992a] has suggested that this compound should be given the status of phytohormone. However this suggestion is not accepted so far by the scientific world. This may be probably due to the fact that as compared to other phytohormones, relatively less research work has been done on this compound and the only aspect, which is studied in depth is about the involvement of salicylic acid in disease resistance in plants. Hence it was thought worthwhile to study the influence of salicylic acid on seed germination. For this purpose a monocot crop Wheat and dicot crop Moong were selected because it is well known that there are some major differnces in the germination physiology of these two types.

The investigation mainly encompasses studies of the influence of salicylic acid on the fate of some key enzymes in germination of Wheat and Moong. It is hoped that although this study is of preliminary nature, it would provide useful background for further detailed research at molecular level.

CHAPTER I

REVIEW OF LITERATURE

A] INTRODUCTION:

Salicylic acid (SA) is a basically an aromatic compound belonging to an extraordinary diverse group of secondary metabolites, plant phenolics. This compound attracted the attention of medical world from the prehistoric times as a constituent of medicinal plant willow (Plate-I). Latter on it was utilized on large scale as an active principle of popular drug aspirin. In recent years, plant physiologists have noticed that this compound exerts significant influence on various physiological processes in plants.

B] HISTORY:

There are reports in literature, which reveal that in 4^{th} century B.C. Hippocrates a Greek medical practitioner gave willow leaves to women during childbirth as pain reliever. From prehistoric times the natives of America, North American Indians were using marshed extracts of willow tree bark to relive pain, and fever. The mention of this practice in folklore aroused scientific interest in *Salix* species in early 20^{th} century in Britain, Germany and France.

Buchner (1828) successfully isolated a tiny amount of salicyl alcohol glucoside from willow bark. Raffaele Piria (1938) gave the name salicylic acid from Latin 'Salix' a willow tree (white willow *Salix alba*). The first commercial production of synthetic salicylic acid was commenced in Germany in 1874. The trade name Aspirin was given by 'Bayer' company in 1898 for a derivative of salicylic acid, acetylsalicylic acid.

Plate I

SALIX ALBA

A) Habit

B) Leaves

C) Flowers

D) Bark

C] GENERAL PROPERTIES:

Salicylic acid belongs to the group of secondary metabolites plant phenolics. It possesses an aromatic ring bearing a hydroxyl group.

Salicylic acid is a white crystalline solid with molecular formula C_6H_4 (OH) COOH. Molecular weight of salicylic acid is 138.1. Salicylic acid melts at 159^0C (319^0F). It is somewhat sweet in taste and sparingly soluble in water and increasingly soluble in alcohol, ether and chloroform. The pH of saturated aqueous solution of salicylic acid is 2.4. The structure of salicylic acid is given below.

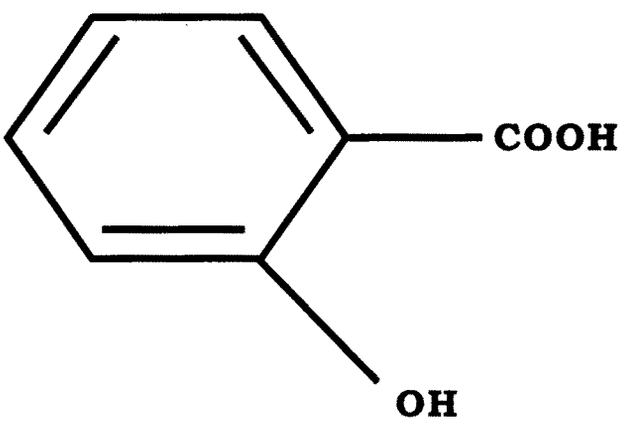

Figure.1. Structure of Salicylic acid.

D] SALICYLIC ACID BIOSYNTHESIS IN PLANTS:

Similar to most of the aromatic compounds, salicylic acid is synthesized from the precursor of phenylalanine. The scheme of biosynthesis of SA proposed by Raskin (1992a) is depicted in figure 2. Cinnamic acid and benzoic acid are two important intermediates in the pathway of salicylic acid biosynthesis. Chadha and Brown (1974) suggested separate pathways of salicylic acid biosynthesis for healthy and infected tomato plants. In healthy tomato plants synthesis via

benzoic acid takes place while in infected plants it occurs via o-coumaric acid after β-oxidation. However Yalpani *et al.*, (1993) reported only one pathway of salicylic acid biosynthesis from cinnamic acid via benzoic acid in healthy and tobacco mosaic virus inoculated tobacco plants. Silverman *et al.*, (1995) studied biosynthesis of salicylic acid in rice. They also noticed salicylic acid biosynthesis via benzoic acid. Radiolabeling studies of Philippe *et al.*, (1995) showed that salicylic acid is synthesized from phenylalanine via benzoic acid in cucumber plants inoculated with pathogens. According to Ribnicky *et al.*, (1998) salicylic acid biosynthesis was stimulated by benzaldehyde. Salicylic acid is synthesized from benzoic acid via phenylpropanoid pathway and the benzaldehyde is one of important intermediate of this pathway. But in healthy and infected tobacco plants, benzaldhyde is not the intermediate between benzoic acid and cinnamic acid. Recently Coquoz *et al.*, (1998) reported that salicylic acid biosynthesis starts from phenylalanine via cinnamic and benzoic acid.

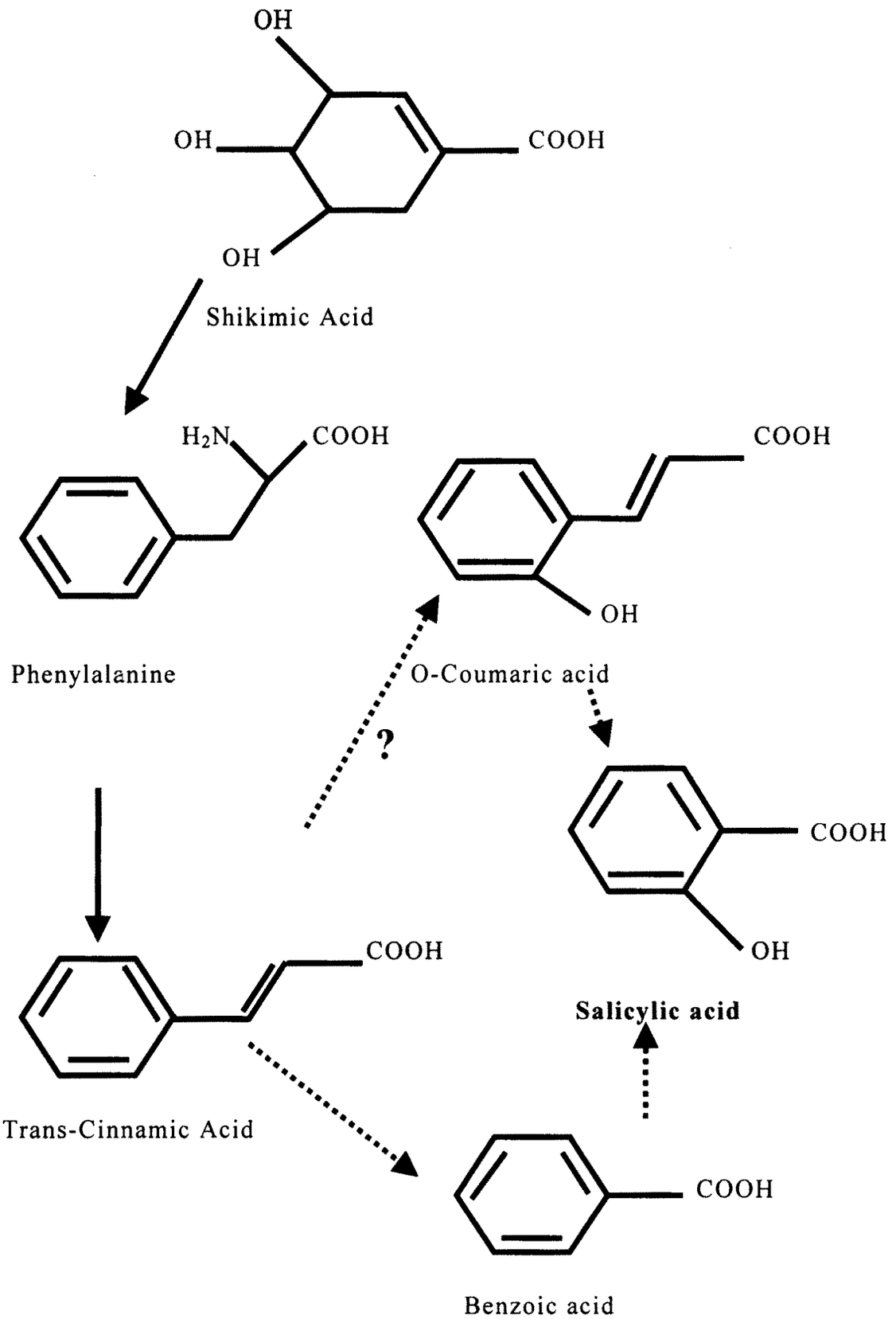

Figure: 2. Biosynthesis of SA in higher plants, after Raskin (1992).

It is evident from the foregoing account that although general outline of SA biosynthesis is known the detailed studies about enzymes and regulation of the pathways are not yet performed.

E] FACTORS INFLUENCING SALICYLIC ACID CONTENT IN PLANTS:

Procter and Griffiths (1958) indicated for the first time the presence of salicylic acid in plants. Mendez (1971), Cleland (1974) and Bardseth and Russwurm (1978), confirmed presence of salicylic acid in plants by using different analytical techniques. Raskin *et al.*, (1990) analyzed the level of salicylic acid in leaves and reproductive organs of 34 plant species and concluded universal distribution of salicylic acid in plants. Paul (1995) reported that rice seedling had highest level of salicylic acid (between 0.01 and 37.19 µg/g fresh weight).

Margot *et al.*, (1993) observed triphasic uptake of salicylic acid by roots of *Vicia faba* and *Fagopyrum esculantum.* They further noticed that *V. faba* detoxify salicylic acid by glycosylation to form O-beta-D-glucosyl hydroxy benzoic acid and *F. esculantum* detoxify SA by oxidizing it to 2,5-dihydroxy benzoic acid and glucosylated this product as 5 OH group and here detoxification occurs during the last phase. Recently Dudreva *et al.*, (1998) noticed that fragrance of *Clarkia breweri* is due to three benzoid esters: benzyl acetate, methylsalicylate which is emitted by petal tissue and benzyl benzoate from pistil. They found that adenosyl I methionine: salicylic acid carboxyl methyltransferase catalyses the methyl esterification of salicylic acid and the activity of this enzyme is higher in petals and

absent in leaves. Thus it is clear that both biosynthetic potential and conversion of this compound in to other derivatives influence the level of SA in plants. The level of salicylic acid in plant tissue is elevated by number of factors. These have been summarized in Table no. 1.

Table No. 1: Induction of salicylic acid accumulation in plants.

Name of Plant	**Causal agent**	**Reference**
Tobacco cv. Xanthi nc Genotype 'NN'	Tobacco mosaic virus	Enyedi *et al.* 1992.
Tobacco cv. Xanthi nc	Tobacco mosaic virus	Enyedi and Raskin 1993.
Tobacco (virus resistant)	Ultraviolet light and Ozone	Yalpani *et al.* 1994.
Carrot suspension culture	Ca^{++} from fungal elicitor	Schneide *et al.* 1994.
Potato	Arachidonic acid	Coquoz *et al.* 1995.
Tobacco	Fungal glycoprotein	Bailievl *et al.* 1995.
Tomato (Disease resistant)	*Cladosporium Fulvum*	Hammond *ea al.* 1996.
Phaseolus vulgaris	*Colletotrichum lindemuthianum* and 2-6,Dichloro isonocotinic acid	Dann *et al.* 1997.
Bean	*Pseudomonas aeruginossa* 7NSK2	De-Meyer and Hofte 1997.
Cucumber and tobacco	Bacterial strain marcescens 90-166	Press *et al.* 1997.
Rice	*Pseudomonas* species	Krishnamurthy and Gnanamanickam 1998.
Trasgenic tobacco	Cauliflower mosaic virus	Tanga *et al.* 1999.
Tobacco	*Pseudomonas aeruginasa* 7NSK2	Geert *et al.* 1999.
Tomato	Fusicoccin	Schaller *et al.* 1999.
Tobacco Genotype Nagh9	Cryptogein and salicylic acid treatment	Dong *et al.* 2000.
Tobacco	β-aminobutyric acid	Seigrist *et al.* 2000.

F] ROLE OF SALICYLIC ACID IN PHYSIOLOGICAL PROCESSES OF PLANTS:

a) EFFECT OF SALICYLIC ACID ON SEED GERMINATION, GROWTH AND DEVELOPMENT:

In 1971 De Bell and Dean found reduced seed germination and seedling growth of the sweet gum in soil beneath cherry bark. Their further analysis revealed that salicylic acid in the leaf extract of cherry bark was the primary inhibitory substance. Chopra and Kumar (1986) found inhibition of vegetative growth of *Riccia gangetica* by salicylic acid. Asthana and Srivastava (1978) observed inhibition of germination in maize by presowing soaking treatment with salicylic acid alone or in combination with ascorbic acid.

Gaur and Pareer (1976) found decrease in development of radicle and plumule of paddy seed by salicylic acid, vanillic acid, p-hydrobenzoic acid, p-coumaric acid and fumaric acid at low concentration. Cleland and Ben-Tal (1982) noticed decrease in growth rate of *Lemna gibba* G_3 strain by salicylic acid treatment. Marinos and Hemberg (1960) isolated natural growth inhibitor, which comprised of salicylic acid and cinnamic acid from dormant potato tuber. Anandhi and Ramanujam (1997) recently found inhibition of seed germination by salicylic acid in Black gram. They noticed that presowing soaking treatment was more inhibitory.

Shetty *et al.*, (1992) reported stimulation of shoot organogenesis by salicylic acid and aspirin in *Cucumis melo* on MS medium but higher concentration of salicylic acid proved inhibitory.

Salicylic acid was found to have no effect on stem elongation of *Hysocyamus niger* in the experiments of Warm (1980).

At the same time, there are some reports, which reveal positive effect of salicylic acid on plant growth and development Datta and Nanda (1985) found increase in plant height of Cheena millet by 100 ppm SA. Ray *et al.*, (1985) reported that inhibition of seedling growth by ABA was reversed by salicylic acid in *Raphanus sativus*. Datta *et al.*, (1986) reported increase in leaf area of pearl millet by salicylic acid treatment. The experiments of Mishra and Cahudhary (1997) revealed that inhibitory effect of heavy metals on seed germination and seedling growth of rice was reduced by salicylic acid. Gutierrez *et al.*, (1998) observed increase in shoot and root growth of soybean after 7 days of treatment with salicylic acid. Rao *et al.*, (1998) observed that foliar spray of salicylic acid hastened growth and development in green gram and black gram. Pathmanabhan and Thangaraj (1999) reported that foliar spray of salicylic acid caused increase in plant height, leaf area, leaf weight and dry matter of soybean.

Briand and Kapoor (1989) found that sodium salicylate induced nuclear and a chromosomal aberrations in *Allium sativum* and this effect was concentration dependent.

b) LEAF SENESCENCE AND ABSCISSION:

Plant extracts have been shown to contain senescence promoting substances. The senescence promoting substance is mainly abscisic acid but other substances such as salicylic acid are also found to be effective in this respect. (Housley and Taylor, 1958, Hemberg, 1961, Ryugo 1969 and Holst, 1971).

Pawar and Laloraya (1982) reported that salicylic acid and other phenolics like caffeic acid, resorcinol and others had no effect on abscission in *Phaseolus vulgaris.* On the other hand, Apate and Laloraya (1982) observed reversal of ABA induced leaf abscission in kidney bean by salicylic acid. Enzyme cellulase plays a key role in the process of leaf abscission and level of this enzyme protein increases in the abscission zone. The work of Ferrarese *et al.,* (1996) has revealed that salicylic acid treatment prevented increase in cellulase activity and reduced abscission in peach and pepper plants.

c) ROOTING:

The experiments of Bolarczuk and Jankiewicz (1975) revealed that the number of roots per cutting and total length of roots per cutting were increased by salicylic acid or pyrogallol when used alone or in combination with auxin in *Populus abla* L. and *Populus canescens* Sm. He further found that phenolic compounds are effective in late summer when natural ability to form root was very low. Morsink and Smith (1975) observed rooting in *Tilia americana* L. under mist by IBA (indole butyric acid) in combination with salicylic acid. Adventitious root initiation was accelerated by salicylic acid, catechol, pyrogallol and tannic acid in mung bean (Kling and Meyer 1983). They further noticed that salicylic acid in

combination with IBA caused similar effect. Dhavan and Nanda (1985) noticed that salicylic acid increased rooting in hypocotyls cuttings of *Impatiens balsamina* L. but this effect was dependent of endogenous level of auxin and nutrition in cutting.

In contrast to the above observations, Kakkar and Rai (1986) found inhibition of rooting of hypocotyls in *Phaseolus vulgaris* L. by 10^{-5} M salicylic acid.

d) FLOWERING:

Flowering is one of the most complex developmental processes in higher plants, which is influenced by several environmental and endogenous factors. Lee and Skoog (1965) recorded for the first time flower-inducing effect of salicylic acid in tobacco tissue culture supplemented with kinetin and indole acetic acid (IAA). The experiments of Cleland (1974 b) employing phloem-feeding aphids clearly demonstrated involvement of salicylic acid in flowering. By using *Lemna gibba* bioassay system of honeydews he analyzed flower inducing as well as flower inhibiting compounds from the honeydew of aphids feeding on *Xanthium straumarum*. When aphids fed on synthetic food, flowering effect was not seen. Cleland and Ajami (1974) identified salicylic acid as flower inducing substance from *Xanthium straumarum*. Salicylic acid was found to accelerate actual flowering as well as rate of flower development in *Lemna* (Cleland 1974, Cleland and Ajami, 1974 and Cleland and Tanaka *et al.*, 1979).

After these initial studies several experiments demonstrated promotion of flowering by salicylic acid. These are summarized in Table no. 2.

Table No.: 2. Reports about involvement of salicylic acid in flowering.

Plant species	**Year**	**Reference**
Impatiens balsamina	1976	Nanda *et al.*, 1976.
Lemna gibba	1977	Pietersr 1977.
Setaria italica	1977	Nanda *et al.*, 1977.
Panicum miliaceum	1978	Datta *et al.*, 1978.
Spirodela punctata 05	1978	Scharfetter *et al.*, 1978.
Lemna paucicostata	1978	Khurana and Maheshwari 1978.
Pistia stratiotes	1978	Pietersr 1978.
Lemna paucicosata 151	1979	Watanabe *et al.*, 1979
Spirodela polyrhiza	1980	Khurana and Maheshwari (1980)
Lemna paucicostata 6746	1981	Tanaka *et al.*, 1981
Lemna minor M601	1979	Watanabe and Takimoto, 1979
Wolfia microscopica	1987	Khurana and Maheshwari 1987
Pennisetum typhoides	1986	Datta *et al.*, 1986
Wolfia hyaling 738	1983	Tamot *et al.*, 1987
Oncidium sps.	1987	Hew 1987.
Glycine max L.	1998	Kumar *et al.*, 1998
Phaseolus mungo	1999	Rao *et al.*, 1999

At the same time some contradictory reports are also available. Groenewald and Visser (1978) observed inhibition of flowering by salicylic acid (0.1 mM) in *Pharbitis nil*. Also salicylic acid had no effect on flowering of *Xanthium stramarum* and there was no change

in level of salicylic acid in honeydew of vegetative or flowering plant.

The mechanism of salicylic acid induced flowering is still unsolved question. Watanbe *et al.*, (1981) noticed that undissociated form of salicylic acid was more active from the florigenic point of view. They further proposed that flower inducing effect of benzoic acid is related with increase in size of benzyl ring substitution. They found that in *Lemna paucicostata* 151 salicylic acid shows higher activity than expected from the model. According to Ootta (1975) salicylic acid acts as a chelating agent and induces flowering. This suggestion is supported by experiments of many workers (Set *et al.*,, 1970; Ootta 1972; Pieters, 1978 and Khurana and Maheshwari, 1986). But benzoic acid and other non chelating phenolics also promoted flowering (Watanbe and Takimoto, 1979). Fujiok *et al.*,, (1983) proposed that there may be other possible mechanism involved in flower inducing effect of salicylic acid.

Kumar and Nanda (1981 e, f) observed that the action of salicylic acid in induction of flowering of *Impatiens balsamina* involved increase in RNA content and increase in acid phosphatase activity. They also noticed synthesis of some novel proteins in shoot apex in response to salicylic acid treatment. Thus it is apparent from these studies that salicylic acid might be acting at a gene level.

e) ETHYLENE BIOSYNTHESIS:

Methionine is a precursor of biosynthesis of gaseous phytohormone ethylene and enzyme ACC oxidase (ethylene forming enzyme), plays a key role in the biosynthetic pathway. The enzyme

ACC oxidase brings about conversion of aminocyclopropane 1 carboxylic acid to ethylene. There are several reports, which indicate inhibition of ACC oxidase by salicylic acid. Betty and Lesile (1983) in apple discs and moong hypocotyls, Lesile and Romani (1986) Lesile and Roger (1988) in pear cell suspension culture, Lesile and Romani (1989), Li *et al.*, (1992) in tomato and Huang (1993) in detached rice leaves has made such observations. Hyeok *et al.*, (1999) suggested that salicylic acid and polyamine may specifically regulate ethylene biosynthesis at level of ACC synthase transcript. The above findings form the basis of a common observation about delaying of petal senescence by aspirin or salicylic acid. Romani *et al.*, (1989) noticed that inhibition of ethylene biosynthesis was more at pH 3.5 and 4.5.

In contrast to above reports, Pennazio and Roggero (1991) noticed that non-phytotoxic level of salicylic acid did not affect ethylene biosynthesis in soybean cuttings. Sheng *et al.*, (1997) reported that salicylic acid at pH 6.4 enhanced endogenous ethylene formation in aged potato tuber slices.

f) MEMBRANE FUNCTION AND NUTRIENT UPTAKE:

Singh *et al.*, (1985) observed enhanced pigment efflux (Betacynin) from vacuoles and increase in conductivity of the medium due to the treatments of kinetin, ethephon and salicylic acid. Mishra and Chaudhari (2001) noticed that the damaging effects (increase in leakage of electrolytes, injury index and extent of lipid peroxidation) of lead and mercury on membrane in rice cells were ameliorated by salicylic acid treatment. Chen and Kuo (1999) while

monitoring metabolism of exogenously applied salicylic acid noticed a Ca^{++} dependent excretion of salicylic acid from tobacco cell suspension culture.

Tillberg *et al.*, (1970) found small decrease in total inorganic phosphate uptake in *Scenedesmus* a unicellular green alga due to salicylic acid treatment. They further reported that ATP level in the cells was increased due to the treatment. Glass (1973) found decrease in phosphate uptake in barley roots due to salicylic acid. Tanaka *et al.*, (1986) found that decrease in phosphorus concentration in *Lemna gibba* G3 grown on E medium was stopped by salicylic acid, but salicylic acid did not increase phosphorous concentration. Harper and Balke (1981) observed that SA inhibited potassium absorption in beet roots and this effect was concentration and pH dependent.

g) STOMATAL BEHAVIOUR:

Larque (1978) noticed decrease in transpiration of *Phaseolus vulgaris* leaves by 1 and 10 mM salicylic acid treatment. In another species *Commelia communis* Larque (1979) observed decrease in transpiration by salicylic acid treatment. Pennazio and Roggero (1985) recorded increase in stomatal resistance due to salicylic acid treatment in detached tobacco leaves kept in light. Bhatia *et al.*, (1986) noticed reduction in stomatal aperture and complete stomatal closure in isolated epidermal peeling of *Euphorbia hirta*. Barbara *et al.*, (1992) found that long-term treatment of salicylic acid at concentration more than 3.5 mM affected transpiration rate, which was related to stomatal pore width. They further noticed that guard cells and epidermal peeling are more sensitive to the salicylic acid

even at concentration 0.001 mM which resulted in stomatal closure. Lee (1998) has made detailed investigation of the mechanism of stomatal closure by salicylic acid treatment in *Commelina communis* leaf epidermis. He found that in the guard cell cytoplasm, H_2O_2 concentration increased due to inhibition of catalase activity by salicylic acid. The membrane permeability for K^+ increased due to oxidizing capacity of H_2O_2 to the plasma membrane and thus mass efflux of potassium ions took place, which induced the loss of turgor pressure and it resulted in stomatal closure.

In contrast to above observations Ray *et al.*, (1986) reported that ABA induced stomatal closure was reversed by salicylic acid.

h) RESPIRATION:

Heat production or thermogenicity in the inflorescence of some angiosperm species is now well known and well-understood fact. In 1778 Lamarck observed heat production in the inflorescence of *Arum* genus for the first time. Flower or inflorescence of some angiosperm species belonging to families Anonaceae, Araceae, Aristolochiaceae, Cyclanthaceae, Nympheacae, Palmae and male reproductive organ of some Cycads display heat production. Now it is very well realized from the experiments of various workers like Van Herk (1937), James and Beevers (1950) Meeuse and Raskin (1988) that the heating is due to the operation of cyanide resistant pathway of mitochondrial electron transport, which is due to a key role of a alternate oxidase (Siedow and Umbach, 1995).

Van Herk (1937) indicated that a water-soluble substance 'Calorigen' is responsible for the metabolic heat burst in Voodo lily.

After fifty years Raskin *et al.*, (1987) concluded the story of Calorigen identification. By mass spectroscopic analysis of highly purified Calorigen extracted from the male flowers of Voodo lily. Raskin *et al.*, identified Calorigen as salicylic acid. They further noticed that application of salicylic acid to immature appendix led to increase in temperature of appendix in Voodo lily.

Goyal and Tolbert (1989) for the first time observed induction of the alternative oxidase path in *Chlamydomonas* (non thermogenic plant) by salicylic acid. Kapulnik *et al.*, (1992) noticed that salicylic acid induced capacity and activity of cyanide resistant respiration without affecting the capacity of the cytochrome respiration pathway in tobacco cell suspension culture. Wen and Liang (1994) observed increase in cyanide resistant respiration in dormant potato slices and slices undergoing breaking of dormancy. They further noticed involvement of alternative pathway in potato slices undergoing breaking of dormancy and in isolated mitochondria. Guo *et al.*, (1995) observed salicylic acid induced increase in activity of alternate pathway during aging of potato tuber slices. They observed this effect in percoll-purified mitochondria also.

Salicyl Hydroxamic acid (SHAM) is inhibitor of alternate oxidase activity. Chivasa *et al.*, (1997) noticed that salicylic acid interferes with tobacco mosaic virus replication via a novel salicyl hydroxamic acid sensitive mechanism. According to Xie and Chen (1999) salicylic acid induced inhibition of respiration may play role in salicylic acid mediated biological process including plant defense response.

i) ENZYME ACTIVITIES:

Salicylic acid is found to have marked influence on variety of enzymes involved in different metabolic pathways. The recent work has revealed that this influence is mediated through the impact of SA on gene expression. Kumar and Nanda (1981a) observed that SA treatment caused decrease in peroxidase activity and increase in activity of polyphenol oxidase in stem and leaves of *Impatiens balsamina*. The isozyme studies revealed that new peroxidase isozyme with 0.38 and 0.8 rf and polyphenol isozyme with 0.81 rf. appeared due to GA_3 and SA treatments. Kumar and Nanda (1981 b) observed an increase in amylase activity in leaves of *I. balsamina* by GA_3 and SA treatment under inductive as well as no inductive photoperiodic conditions. They further reported synthesis of a new isozyme of amylase in GA_3 and GA_3 + SA treated plants which was also observed in water treated plants under inductive photoperiod. Kumar and Nanda (1981 c) also recorded increase in activities of alkaline and acid phosphatase by GA_3, SA and GA_3 + SA. An increase in activity of enzyme IAA oxidase in stem and leaves of *I. balsamina* treated with GA_3, SA and GA_3 + SA was also evident in the experiments of these workers. (Kumar and Nanda 1981 d). These workers tried to correlate the alterations in activities of above enzymes with induction of flowering in *I. balsamina* by SA.

Enzyme nitrate reductase plays a key role in nitrogen assimilation in plants. Asthana and Srivastava (1978) reported slight increase in nitrate reductase activity in maize endosperm by SA presowing soaking treatment, but ascorbic acid alone and SA in combination with ascorbic acid caused increased activity of nitrate

reductase. Jain and Srivastava (1981b) noticed that SA induced nitrate reductase activity in roots of maize seedling. Sharma *et al.*, (1984) also noticed that SA and β-naphthol were able to cause increase in activity of enzyme nitrate reductase. Jaleel *et al.*, (1998) recorded increase in nitrate reductase activity by lower concentration of SA in *Vigna mungo*. Ramanujam *et al.*, (1998) noticed increase in nitrate reductase activity in black gram due to SA presowing soaking treatment. Kumar *et al.*, (1998) found that foliar spray of SA after 12, 24 and 36 days enhanced nitrate reductase activity in soybean. Similar observation was recently made by Pathmanabhan and Thangaraj (1999).

The process of ammonia assimilation in higher plants is catalyzed by glutamate synthase. Singh *et al.*, (1987) found that SA treatment caused increase in activity of NADH glutamate synthase activity in root and leaf tissue of maize seedling.

In legumes enzyme nitrogenase in root nodules plays a major role in nitrogen fixation. Jaleel *et al.*, (1998) found reduced nitrogenase activity and reduced nodulation in 15 and 30 days old *Vigna mungo* plants raised from seed pretreated with SA. Ramanujam *et al.*, (1998) also reported reduced nitrogenase activity in response to SA treatment in black gram.

Recent years have witnessed a great surge of interest in the influence of SA on oxidative enzymes and in view of involvement of SA in plant defense mechanism. This is mainly related to role of free radical source hydrogen peroxide in hypersensitive reactions. The level of H_2O_2 is the cell is controlled by the activities of two enzymes catalase and ascorbate peroxidase. The inhibition of

catalase by SA was evident for the first time in the experiments of Chen *et al.*, (1993) in tobacco. They noticed that SA binding protein is SA inhibitable catalase.

The observation of Sanchez and Klessing (1994) in *Arabidopsis*, tomato and cucumber also supported this finding. Rao *et al.*, (1997) also indicated SA induced inactivation of catalase. Liang (1998) observed a slight decrease in catalase activity in SA treated cucumber leaves. Durner and Klessing (1995) reported that SA inhibited ascorbate peroxidase. They further noticed that inhibition of ascorbate peroxidase was reversible but guaicol peroxidase was not inhibited. According to Kraratskhelia *et al.*, (1999), SA is not effective inhibitor of ascorbate peroxidase. In cucumber, Liang (1997) found increase in superoxide dismutase and peroxidase in SA treated and untreated second leaf. Rao *et al.*, (1997) noticed that SA enhanced H_2O_2 production, lipid peroxidation and oxidative damage to proteins and caused increase in Cu, Zn, superoxide dismutase in *A. thaliana*.

j) PROTEIN LEVEL:

Asthana and Srivastava (1978) found increase in protein nitrogen by ascorbic acid and ascorbic acid plus SA presowing soaking treatment but SA pretreatment alone reduced protein nitrogen content. Jain and Srivastava (1981a) observed increase in total nitrogen content by calcium nitrate supplemented with SA in maize embryonic axis, but higher concentration of SA inhibited accumulation of organic nitrogen. SA in combination with Cholrogenic acid improved nodulation and also induced nitrogen

fixation (Garg *et al.*, 1989). Ramanujam *et al.*, (1998) noticed that presowing soaking treatment with SA caused decrease in total nitrogen content in black gram. Anandhi and Ramaujam (1997) recorded that salicylic acid caused decrease in protein content of black gram. Ramanujam (1998) noticed that presowing soaking with SA caused decrease in protein content in black gram. Pathmanabhan and Thangaraj (1998) reported increase in soluble protein content of soybean in response to SA treatment. Pancheva and Popova (1998) recently noticed inhibition of synthesis of total soluble protein and more pronounced synthesis of RUBISCO when SA was supplied to the *Hordeum vulgare* L. seedlings.

k) DISEASE RESISTANCE:

The disease resistance is ability of host plant to create an environment, which is unsuitable and hostile for pathogen. As we have already seen the level of salicylic acid is increased due to infection of several pathogens in general and viruses in particular (Table I). It is now very well established that SA plays a key role in acquired disease resistance through its participation in hypersensitive reaction.

Hypersensitive reaction is restricted to the infected cells, which are immediately killed in response to infection. Oxidative burst and generation of free radicals mainly cause the death of cell. H_2O_2 is a main source of free radicals (OH^* ions) and its level is controlled by enzyme catalase and ascorbate peroxidase. As we have seen earlier, SA is reported to cause inhibition of enzyme catalase and ascorbate peroxidase.

Another aspect of role of SA in disease resistance is the induction of synthesis of low molecular weight proteins commonly known as Pathogenesis

Related Proteins or PR proteins (Group-1, Group -2, Group-3, Group-4 and Group-5). The nature of PR proteins has been described by Gehalot *et al.,* (1999) in following words.

Group-1

Group -1 consists of three well characterised acidic proteins and two basic proteins. The three acidic PR-1 proteins, 1a, 1b and 1c have similar immunological properties and a molecular weight of 16 KD (Antoniw *et al.,* 1980, Matsuoka and Ohashi 1984). They exhibit 94% homology or more in terms of their amino acid sequences (Cornelissen *et al.,* 1987, Pfitzener and Goodman 1984).

Group-2

The evidences indicate that the members of PR-2 group have beta-1, 3-glucanase activity (Kauffman *et al.,* 1987). Five acidic proteins (2, 0, 0' and Q') and a basic one (glue b) have been reported, with molecular weights of 40, 40, 41, 25, 36, and 33 KD, respectively. Protein sequence homologies among the five different members of the acidic group are greater than 90% but between acidic group and the basic proteins it is only about 50% (Linthorst *et al.,*1990).

Group-3

The members of group 3 have chitinase activity (Legrand *et al.,* 1987). Two acidic members, P\Q, have molecular weights of 28 and 29 KD, respectively, and are 93% homologous in terms of their amino acid sequence (Payne *et al.,* 1990). However , the homology is only 57% between the acidic and the basic enzymes (Ch 32 and 34).

Group-4

Little is known about the members of group 4, which have with the lowest molecular weights (13-14.5 kD) of tobacco PR proteins. All four members, s1, s2, r1 and r2 have been reported as being acidic and secreted into the intercellular spaces. The sequences of proteins in group 4, as deduced from nucleotide sequences of the cDNAs, exhibit 75% homology to *win* proteins of potato (Linthorst *et al.,* 1991). These *win* proteins are basic and contain extra domains that resemble wheat germ agglutin and/or basic chitinase like domains at their N-termini (Stanford *et al.,* 1989).

Group-5

PR-R and PR-S are acidic and are secreted into the intercellular spaces. They exhibit 98% homology in terms of the amino acid sequences encoded by their open reading frames, and they each contain putative signal peptide of 25 amino acid residues in the N- terminal region.

Thus enzyme like chitinase and β–1–3 glucanase released in intercellular places can inhibit the pathogen growth through their attack on pathogen cell wall. Salicylic acid is phloem mobile and hence it can be easily transported from the site of production (infection site) to the healthy part. Further a volatile derivative of SA, in methyl salicylate can be transported through the air to the neighbouring plants. It is observed that SA as wel as its derivatives induce the synthesis of PR proteins in healthy leaves and the neighbouring plants and thus confer systemic acquired resistance. Such reports have been summarized in the following Table no. 3

Table No.- 3: Reports about involvement of salicylic acid in systemic acquired resistance.

Name of Plant	**Causal Organism**	**Reference**
Cucumber	*Colletotrichum lagenarium*	Mills and Wood 1985
Tomato	*Meloidogyna incognita* and *M. javanica*	Sitramaiah and Pathak 1981.
Tobacco (detached leaves)	Tobacco mosaic virus	Pennaizio *et al.*, 1987.
Tobacco	Alfaalfa mosaic virus	Huijsdujnen *et al.*, 1986
Nicotiana tabacum cv. Samson WN	Tobacco mosaic virus	Matsuka and Ohashi 1986.
Tobacco suspension Culture	Tobacco mosaic virus	Ohashi *et al.*, 1987.
Vigna sesquipedalis	Tobacco mosaic virus	Pennaizio *et al* 1987.
Tobacco	Tobacco mosaic virus	Yalpani *et al.*,1993, Chivasa *et al.*, 1997.
Tobacco	*Erwinia carotovora* subspecies carotovora	Palva *et al.*, 1994, Vidal *et al.*,1997.
Cucumis sativus	*Spaerotheca fuliginea*	Conti *et al.*, 1996.
Nicotiana langsdorfi	Virus	Du and Klessig1997.
Potato	*Phytophthora infestans*	Diqiu *et al.*, 1997.
Rice	*Pyricularia oryzae*	Manandhar *et al.*, 1998
Cucumber	Cucumber mosaic virus	Naylor *et al.*, 1998
Tomato	*Fusarium oxysporum*	Attitalla *et al.*, 1998,
Mangifera indica L.cv.Zihva	*Colletotrichum gloesporioides*	Jianliang *et al.*, 1998.
Groundnut	*Puccinia arachidis* speg.	Sathiyabama and Balsubramanion *et al.*, 1999.
Tomato	Cauliflower mosaic virus	Tanga *et al.*,1999.
Arabidopsis thaliana	Turnip crinkle virus	Doonera *et al.*, 2000

It is evident from the foregoing account that the phenolic acid, salicylic acid influences multitude of physiological processes in plants. Raskin (1992) suggested that salicylic acid should be included in the list of phytohormones. The review of literature on involvement of SA in various physiological processes, presented here clearly justifies above suggestion.