Effects of salicylic acid on growth and biochemical characters of salt stressed tomato varieties

THESIS

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in

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by

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SELF ATTESTATION

This is to certify that I have personally worked on the research work entitled, **"Effects of salicylic acid on growth and biochemical characters of salt stressed tomato varieties".** The data mentioned in the Thesis have been generated during the work and are genuine. Data/information obtained from other agencies have been duly acknowledged. None of the findings/information pertaining to the work has been concealed. The results embodied in this thesis have not been submitted to any other University or Institution for the award of any degree or diploma.

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Date: 12·01·2018

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(Sarita Tripathi)

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LIST OF ABBREVIATIONS

%	per cent
/	per
@	at the rate of
µM	micromolar
ANOVA	analysis of variance
CD	critical difference
cm	centimeter
d.f.	degree of freedom
DAS	days after sowing
ESS	error sum of square
et al.	and others
etc.	and the rest
F (cal.)	calculated value of F
F (tab.)	tabulated value of F
g	gram
i.e.	that is
mg g-1	miligram per gram
ml.	mililitre
mM	milimolar
MSS	mean sum of square
spp.	species
TSS	total sum of square
U mg-1	unit per milligram
viz.	namely

ABSTRACT

The experiments for present research work were conducted in the botanical garden of Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad from a period during October, 2013 to February, 2014. The pot experiment was planned in complete randomized design with three replications for each treatment. Salinity stress hinders plant growth and cause significant yield losses and therefore, induction of salinity stress tolerance in crop plants is one of major goals of agriculture research. Tomato is a crop plant with high nutritive value and its cultivation is dependent on many environmental factors, viz. temperature, salinity, nutrients etc, which can affect the yield and reproductive potential of the plant. On the basis of some growth parameters and finally the yield, 20 tomato varieties were selected as salt efficient tomato varieties. Twenty tomato varieties were taken to record several growth and biochemical parameters under the treatments- 1) Control, 2) 25µM SA, 3) NaCl 50 mM, 4) NaCl 100 mM, 5) NaCl 150 mM, 6) NaCl 200mM, 7) NaCl 50 mM + 25µM SA, 8) NaCl 100 mM + 25µM SA, 9) NaCl 150 mM + 25µM SA and 10) NaCl 200 mM + 25µM SA. The interactive effects of salicylic acid (SA) as shoot spraying on NaCl stressed tomato plants grown in experimental pots under different salinity levels (0, 50, 100, 150 and 200 mM NaCl, respectively) were studied. An increase in the degree of salt tolerance induced by salicylic acid was indicated by finally the yield. The data provided evidence that salicylic acid treatment reduced the adverse effects of salt stress on tomato plants and might play a key role in providing stress tolerance by stimulation of the antioxidant system as a stress protection mechanism.

Key words: Tomato, salt stress, salicylic acid, antioxidants

Chapter One

Introduction

INTRODUCTION

Abiotic stresses are major constraints for global crop production. Among various abiotic stresses, salinity has become a severe threat to ensure food security by affecting about one-third of the irrigated land on earth **(Mengel *et al.*, 2001).** It is estimated that at least 20% of all irrigated lands are salt-affected **(Pitman and Läuchli, 2002).** Salinity is one of the most important abiotic stresses that cause reduction in plant growth, development and productivity worldwide in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching. The FAO estimates that 34 million hectares of irrigated land are salt-affected worldwide, and an additional 60–80 million hectares are affected by waterlogging and related salinity **(Payen *et al.*, 2014).** Soil and water salinity in the arid regions are continuously increasing **(Rus *et al.*, 2002).** The responses of plants to salinity vary according to the developmental stage **(Dumbroff and Cooper 1974; Munns 2002).** There are many natural and human factors contributing to increased salinity of soils, such as land clearing, the replacement of perennial vegetation with annual crops, low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices. Increased salinization may affect up to 50 % of arable land by the year 2050 **(Wang *et al.*, 2003).** Even more, the area affected by drought and salinity is still expanding **(Wang *et al.*, 2003).** Soil is generally considered saline when the electrical conductivity (EC) of the saturation extract in the root zone exceeds 4 dS m-1 at 25^0C with an exchangeable sodium percentage (ESP) of 15 **(Foolad, 2004).** Currently, more than 800 million ha of land are affected by salinity **(Munns, 2005)** and about 1/3 of the world's arable land has experienced yield reduction due to cyclical or unpredictable drought **(Chaves and Oliveira, 2004).** Among the soluble salts, NaCl is the major component contributing to salinity **(USSL, 2005).** The most important agent of soil salinity is sodium chloride therefore the study about it is necessary **(Dantas *et al.*, 2005).** It has a major impact on crop production in arid and semi-arid regions and causes agricultural problems under rain-fed conditions when annual precipitation is not enough to leach excessive salts and prevent salt accumulation in the root zone **(Parida and Das, 2005).**

Salinity is one of major abiotic stresses and affects almost every aspect of the physiology and biochemistry of plants. This significantly reduces yield. Great effort has been devoted to understanding physiological aspects of response to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes **(Cuartero *et al.*, 2006).** The high salinity of the soil affected the soil penetration, decreased the soil water potential and finally caused

physiological drought **(Yusuf *et al.*, 2007).** Salinity is a soil condition characterized by a high concentration of soluble salts. Soils are classified as saline when ion(s) concentration is such that osmotic pressure produced by ion(s) are equivalent to that generated by 40 mM NaCl that is 0.2 MPa or more **(USDA-ARS, 2008).** As NaCl is the most soluble and widespread salt, it is not surprising that all plants have evolved mechanisms to regulate its accumulation and to select against it in favour of other nutrients commonly present in low concentrations, such as K+ and NO3− **(Munns and Tester, 2008).** Plant responses to drought and salinity stress have much in common. Salinity reduces the ability of plants to absorb water, which creates a 'chemical drought', and quickly causes reductions in growth rate, along with a suite of metabolic changes identical to those caused by water stress, such as osmotic stress, oxidative damage, stomatal closure, inhibition of photosynthesis, and damage of cellular structures, and decreased gas exchange rates **(Wang *et al.*, 2008).** The increasing land area by salinity urges the need to develop strategies to enhance crop production under saline conditions. It is, therefore, necessary to understand physiological processes and molecular mechanisms that plants operate to develop salt resistance for sustainable crop production. High levels of sodium (Na^+) and chloride (Cl^-) contents are accumulated in plants growing under salt stress, which disturb the homeostasis of essential nutrients, and plants tend to maintain desirable K^+/Na^+ ratio in cytosol to adjust with salt stress **(Ghogdi *et al.*, 2012; Fageria *et al.*, 2008).** Salt stress limits plant growth and productivity, mainly by inducing osmotic effects, ion-specific effects and oxidative stress **(Okhovatian-Ardakani *et al.*, 2010).** Salinity affected cauliflower growth mainly when imposed in the first growth phase (**Giuffrida *et al.*, 2016).**

Tomato (*Lycopersicon esculentum* Mill.) is a major horticultural crop with an estimated global production of over 120 million metric tons **(F.A.O. 2007).** Tomato is one of the important plants, which plays a vital role in maintaining human health, vigor and helpful in healing wounds because of the antibiotic properties found in the ripe fruit. In addition, tomato is a rich source of Vitamin C, Vitamin B, fibers and a good source of β-carotene **(Raziuddin *et al.*, 2004).** Tomato fruit consists of approximately 20-50 mg of lycopene/100 g of fruit weight **(Kalloo, 1991).** This crop is cultivated in semi-arid regions in which soil and groundwater salinity are an increasing threat, affecting both tomato yield and quality **(Cuartero and Fernandez-Munoz, 1999).** Tomato (*Lycopersicon esculentum* Mill.) is the most prominent crop grown in greenhouses worldwide and in Mediterranean Region as well. The plants require high temperature and high photosynthetic active radiation conditions for

optimal production. These conditions are typical for arid and semi-arid regions where soil and groundwater salinity are insidious problems **(Cuertero & Fernandez, 1999).** The tomato plant is moderately tolerant to salinity stress **(Ayers & Westcot, 1989; Maas, 1986, 1990).** It has been determined that salinity causes several kinds of damage such as growth inhibition, metabolic disturbance and quality losses in addition to yield reduction on tomato plants **(Sanchez-Blanco *et al.*, 1991; Schwarz *et al.*, 1998; Navarro *et al.*, 2000; Li & Stanghellini, 2001; Romero-Aranda *et al.*, 2001; Tüzel *et al.*, 2003; Maggio *et al.*, 2007).** Salicylic acid (SA) is a common plant-produced phenolic compound and a potential endogenous plant hormone that plays an important role in plant growth and development **(Khan *et al.*, 2012; Alam *et al.*, 2013).** The role of SA is intensively studied in plant responses to biotic stress. In recent years, the involvement of SA in the response to abiotic stresses has come into light. Several studies support a major role of SA in plant adaptation to the changing environment, and induce plant tolerance to various abiotic stresses including elevated NaCl **(Stevens *et al.*, 2006; Arfan *et al.*, 2007; Gunes *et al.*, 2007).** The positive effects of SA have been well reported, including salinity tolerance in different plants **(Tari *et al.*, 2002; Gunes *et al.*, 2007; Azooz, 2009).** Application of salicylic acid affected tomato yield and quality characters of tomato fruits **(Javaheri *et al.*, 2012).**

To adapt to salt stress, new proteins in tomato seedlings are induced **(Amini *et al.*, 2007).** The cultivated tomato is classified as a moderately salt sensitive plant **(Katerji *et al.*, 2003).** Sucrose content was found to increase in tomato (*Solanum lycopersicum*) under salinity due to increased activity of sucrose phosphate synthase **(Gao *et al.*, 1998).** Transgenic tomato (*Lycopersicon esculentum* Mill) plants expressing a cation transport gene *HAL1* and a vacuolar Na+/H+ antiporter gene *AtNHX1* exhibited higher capacity for salt exclusion, and increased significantly their salt tolerance **(Gisbert *et al.*, 2000; Zhang and Blumwald, 2001).** Like many salt sensitive crop species, performance of tomato plant under salt stress is modulated by many physiological and agronomical characteristics through regulation of a complex genetic mechanism **(Foolad, 2004).** Genetic studies on salt tolerance in tomato dates back to the 1940's, when a different physiological mechanism was observed between the salt sensitive tomato (*L. esculentum* Mill.) and the tolerant wild relatives **(Lyon, 1941; Flowers, 2004).** Tomato responses to salinity have been documented **(Adams, 1991; Al Karaki, 2000; Romeroaranda *et al.*, 2001; Incrocci *et al.*, 2006).** Salt stress adversely affects chlorophyll fluorescence and the growth of stem, leaves, and roots, and subsequently, yield and fruit quality in tomato **(Cuartero and Fernandez-Munoz, 1999; Cuartero *et al.*,**

2006; Hajer *et al.*, 2006). The exogenous application of SA a significant increase in number of leaves either under different levels of salt stress or alone **(Tripathi *et al.*, 2016).**

Plants are classified as glycophytes or halophytes according to their capacity to grow on high salt medium. Most plants are glycophytes and cannot tolerate salt-stress. Salinity causes severe ion toxicity in glycophyte, since Na+ is not readily sequestered into vacuoles as in halophytes **(Khan *et al.*, 2000).** The tolerance of halophytes to salinity depends on maintaining low osmotic potentials through the controlled uptake and compartmentalization of ions (especially Na, K and Cl) and the synthesis of organic osmolytes that are compatible with metabolism; many are able to secrete excess salts through special salt glands **(Flowers and Colmer, 2008).** Only plants with modified adaptive mechanisms can avoid the adverse effect of salinity **(Blumwald, 2000).** Most plants can adapt to low or moderate salinities, but their growth is severely limited above 200 mM NaCl **(Hasegawa *et al.*, 2000).** Deleterious effects of salinity depend upon the stage of plant growth. There are several defense mechanisms against salt in salt–tolerant, or halophyte, species, such as osmoregulation (glycinebetaine and proline), antioxidants (enzymatic and nonenzymatic agents), ion homeostasis, and hormonal systems **(Cha-um & Kirdmanee, 2010; Hasegawa *et al.*, 2000; Singh *et al.*, 2008; Vaidynathan *et al.*, 2003).** Accumulations of carbohydrates such as sugars (e.g., glucose, fructose, fructans, and trehalose) and starch occur under salt stress **(Parida *et al.*, 2004).** To achieve salt-tolerance, the foremost task is either to prevent or alleviate the damage, or to re-establish homeostatic conditions in the new stressful environment **(Pardossi *et al.*, 2004).** During salt stress the accumulation of external sodium ions will hamper the intracellular potassium ion influx, thereby changing the sodium: potassium ratio. Besides it prevents the uptake of many fundamental nutrients due to competitive interactions and ion selection of membranes. This affects the photosynthetic electron transport system to a wide extent, thereby affecting photosynthesis **(Sudhir *et al.*, 2005).** Germination assessment of control and exposed to salt seeds are used as a sign of some species and varieties resistance to salinity. Root and shoot length are most important parameter for salinity stress. Salinity stress inhibits plants growth (The length and fresh weight of root and shoot) but root length is more influenced than shoot length **(Jamil *et al.*, 2006).**

Salt stress affects germination percentage, germination rate, and seedling growth in different ways depending on plant species **(Meloni *et al.*, 2008 and Ríos – Gómez *et al.*, 2010).** Typical agronomic selection parameters for salinity tolerance are yield, survival, plant height,

leaf area, leaf injury, relative growth rate, and relative growth reduction **(Ashraf and Harris, 2004; Okhovatian-Ardakani *et al.*, 2010).** Based on transcript profiling, it has been suggested that salinity may lead to a series of changes in basic photosynthesis, photorespiration, amino acid and carbohydrate synthesis **(Sengupta and Majumdar, 2009; Chaves *et al.*, 2009; Ahuja *et al.*, 2010).**

Changes in activities of various antioxidant enzymes under salinity stress have been reported **(Koskeroglu and Tuna, 2008; Venkatesan and Sridevi, 2009; Hernández *et al.*, 2000).** The capacity to scavenge ROS and to reduce their damaging effects on macromolecules such as, protein, DNA, lipids, chlorophyll and other important macromolecules appears to represent an important stress-tolerance **(Xiong *et al.*, 2002).** When plants are subjected to salinity stress, the balance between reactive oxygen production and destruction by antioxidant enzymes is perturbed, resulting in oxidative damage **(Roy *et al.*, 2005).** The levels and/or activities of antioxidative enzymes, such as catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, and superoxide dismutase, increase in salinity-stressed plants **(Chen *et al.*, 2012; Wang and Han, 2009).** GSH plays essential roles within plant metabolism and stress tolerance to ROS **(Szalai *et al.*, 2009).** Plants possess a complex antioxidative defense system comprising of nonenzymatic and enzymatic components to scavenge ROS. GSH, ascorbate (AsA), carotenoids, and tocopherols are non-enzymatic antioxidants. Enzymatic defense components include peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT), which together with the other enzymes of the ascorbate–glutathione cycle such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) promote the scavenging of ROS **(Gill and Tuteja, 2010).** The alleviating effect of glutathione might be through scavenging active oxygen species under salt stress. Therefore, proposed that glutathione may ameliorate salt alterations on the plasma membrane, which may enhance salt tolerance in plants **(Jui-Hung *et al.*, 2012).**

Proline is also considered to be the only osmolyte able to scavenge free radicals, thereby ensuring membrane stabilization and preventing protein denaturation during severe osmotic stress **(Szabados and Savouré, 2010).**

Under salinity stress, the accumulation of sugars and other compatible solutes (e.g., proline) allows plant to maintain the cellular turgor pressure necessary for cell expansion under stress conditions; such compounds also act as osmoprotectants **(Ruiz-Carrasco *et al.*, 2011).** It has been stated that genotypes with a high proline accumulation and chlorophyll content, high

K/NA ratio and low Na+ and Cl- accumulation are more tolerant to salt **(Mane *et al.*, 2011). Hatamnia *et al.*, (2013)** have concluded the decreased transpiration rate due to 200mM salinity stress in tobacco cultivars. NaCl stress induced remarkable reduction in transpiration in mustard has also been reported by **(Jamil and Rha, 2013).** Substantial information is available on the physiological, molecular, and metabolic changes in plants exposed to individual stresses. These studies have delineated the effect of each stress at cellular as well as plant level by exposing plants to different stress intensities at different growth stages under controlled laboratory conditions and the results have been translated to field situation **(Atkinson and Urwin, 2012; Mittler, 2006; Suzuki *et al.*, 2014).** Plants manifest themselves with various adaptive mechanisms (morphological, biochemical, enzymatic and physiological) to survive under stressed conditions. The extent of these mechanisms' adaptability is unique with every plant genotype **(Abbas *et al.*, 2015).** The salinity experiment in okra genotypes shows that NaCl-induced stress caused decreases in plant biomass, green pigments, photosynthetic activity, stomatal conduction, transpiration rate, number of stomata and stomatal size and resulted in alterations in enzymatic activities (SOD, POD and CAT) and osmolyte accumulation (proline, glycine betaine, total free amino acids and total soluble sugars). The increase in Na and Cl and lipid peroxidation under saline conditions is the indication of ion toxicity and oxidative damage. However, the oxidative damage is controlled by a defensive system comprising various antioxidants, such as SOD, POD and CAT. The results depicted that salt-tolerant and salt-sensitive genotypes exposed to NaCl stress showed the highest activities of SOD, POD and CAT, both in root and leaf tissues of okra genotypes **(Abbas *et al.*, 2015).** Salt stress markedly increased the proline content in salt treated plants as compared to non-salt treated plants **(Tripathi *et al.*, 2017).**

Genes involved in salinity adaptation can be divided into two groups: those that directly protect against stress and those that regulate gene expression during stress **(Ashraf, 1994; Winicov, 1998; Saki *et al.*, 2003).** It is important to understand how plants develop salt tolerance, so that vast areas of saline soils can be cultivated. One way of studying this is to compare the differential gene expression of salt tolerant and salt-sensitive plants under saline conditions **(Kong-Ngern *et al.*, 2005).** Salt tolerance effectors and regulatory components gain importance at this juncture. Despite recent advances in molecular marker technology, QTL mapping, marker assisted selection, genetic transformation, a successful salt tolerant tomato derivative from agronomic point of view hasn't come yet. Tomato plant is considered as a model crop to examine the potentiality of marker assisted selection and genetic

transformation as its genes are well studied than any other dicotyledons crop and it can be transformed through various methods **(Cuartero *et al.*, 2006).** Salt tolerance is a complex trait which involves numerous genes and various physiological and biochemical mechanisms **(Cuartero *et al.*, 2006).** mRNA profiling of NaCI-trcated tomato plants has shown that salt stress can affefect many different pathways **(Ouyang *et al.*, 2007; Zhou *et al.*, 2007).** As tolerance to stress is multifactorial syndrome rather than result of a single reaction or gene **(Mittler 2006; Shinozaki & Yamaguchi-Shinozaki; 2000),** tackling of the primary stress reactions by gene transfer can also alleviate the secondary stress and generate plant with higher stress tolerance **(Beck *et al.* 2007).**

Plants perceive and respond to stressful conditions by quickly altering their gene expression in parallel with physiological and biochemical modulation. To adapt to salt stress, new proteins in tomato seedlings are induced **(Amini *et al.*, 2007).** Molecular markers offer specific advantages in assessment of genetic diversity and in trait-specific crop improvement which control salt stress by bulk segregate analysis (BSA). Moreover, molecular markers are also associated with genes or quantitative trait loci (QTLs) to improve salt tolerance via marker assisted selection (MAS) in different crop species and acquiescent towards manipulation with current molecular genetic techniques **(Ashraf and Foodlad, 2013).**

The present research work comprised the following objectives as-

- To study the effects of salicylic acid on growth parameters of salt stressed tomato varieties.
- To study the effects of salicylic acid on biochemical and quality parameters of salt stressed tomato varieties.
- To study the effects of salicylic acid on the yield of salt stressed tomato varieties.

Chapter Two

Review of Literature

REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum* Mill) belongs to the family Solanaceae. It is a diploid plant with 2n = 24 chromosomes. It is grown in almost every country of the world. Nowadays, tomato is grown in an area of around 3.9 million–hectares worldwide **(Hassanein *et al., 2010).*** It is a perennial, often grown outdoors in temperate climates as an annual, typically reaching to 1-3m (3 to 10 ft) in height, with a weak, woody stem that often vines over other plants **(http://www.vegetableipmasia.org/Crops/Tomato.html).** Tomato is a rapidly growing crop with a growing period of 90 to 150 days. It is a day length neutral plant **(FAO, 2013).**

2.1. PRODUCTIVITY

Table.2.1. State wise area, production and productivity of tomato in India

STATE/UTs	Area (000' ha)	Production (000't)	Productivity (t/ha)
Andhra Pradesh	76.50	1453.50	19.00
Bihar	46.00	727.20	15.81
Chhattisgarh	29.20	365.80	12.53
Gujarat	29.30	650.00	22.18
Haryana	17.10	257.30	15.05
Karnataka	44.50	1188.10	26.70
Orissa	100.40	1332.20	13.27
Madhya Pradesh	20.40	306.70	15.03
Maharashtra	35.00	987.00	28.20
Tamil Nadu	22.00	277.70	12.62
West Bengal	50.00	857.20	17.14
Others	64.10	959.10	14.96
Total	534.50	9361.80	17.52

Source: NHB Data base (2005-06)

Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop next to potato. Present world production is about 100 million tons fresh fruit from 3.7 million ha**. (FAOSTAT, 2001; http://www.fao.org/nr/water/cropinfo_tomato.html).**

The tomato is now grown worldwide for its edible fruits, with thousands of cultivars having been selected with varying fruit types, and for optimum growth in differing growing conditions **(http://en.wikipedia.org/wiki/Tomato).**

Tomato is a major vegetable plant, and it is moderately sensitive to salinity. (**Basak *et al.*, 2011).** There are two types of tomato plants. Determinate tomatoes form a flower cluster at a terminal growing point and will then only grow laterally. Indeterminate tomato plants do not set terminal flower clusters and will continue growing in height indefinitely. The different varieties of tomato plant range from dwarf or patio shrubs to cherry, beefsteak or salad varieties, and tomato colors can vary immensely too **(http://www.ehow.com/facts_7715264_general-information-tomato-plant.html).**

The yield and quality of tomato appear to be regulated by the net assimilation rate of the crop, the rate of import into individual fruit and sink activity **(Shao-wei *et al.*, 2010).** Salinity affects some of physiological processes of plants such as increased respiration rate, changes in the plant growth, and changes in mineral distribution, membrane instability and failure in the maintenance of turgor pressure **(Joset *et al.*, 1996; Murphy and Durako 2003; Muranaka *et al.*, 2002; Hasegawa *et al.*, 2000).**

When a plant is exposed to salt stress, chemical potential, activity and salt concentration is higher than normal limits **(Porgali, 2001).** Changes in protein hydration are one of the results of high ion amounts in salt stress in plant cells. Salinity reduces both RNA amounts due to changes in cytoplasmic RNAaz activity and DNA levels as a result of disruption of synthesis mechanism **(Doganlar *et al.*, 2010)**.

Soil salinity imposes two types of stresses on plants. The first one is nutritional imbalance caused by saline ions and low soil water potential in both uptake and translocation process. The second one is a toxicity due to the high accumulation of Na+ and Cl- ion in the cytoplasm. Excess of Na+ and Cl− ions may lead to conformational changes in the protein structure, while osmotic stress leads to turgor loss and cell volume **(Errabii *et al., 2007).***

2.2. Nutrition

Tomatoes are now eaten freely throughout the world, and their consumption is believed to benefit the heart, among other organs. They contain the carotene lycopene, one of the most powerful natural antioxidants (**http://en.wikipedia.org/wiki/Tomato)**. Tomatoes are rich in vitamins A and C, low in calories, and an excellent source of lycopene, the pigment that makes tomatoes red and has been linked to the prevention of many forms of cancer (**http://www.fao.org/nr/water/cropinfo_tomato.html**). From the nutritional and health points

of view, tomato is characterised by high content in carotenoids (lycopene) and vitamin C **(Mittova *et al.*, 2000).** Lycopene is the carotenoid, which causes colours of fruits and vegetables. Lycopene is the most powerful antioxidant; consequently, it protects human cells from free radicals that degrade many parts of the body, and prevents cancer **(Block *et al.*, 1992; Gerster, 1997; Rao and Agarwal, 2000).** Yellow tomatoes have higher vitamin A content than red tomatoes, but red tomatoes contain lycopene, an anti-oxidant that may contribute to protection against carcinogenic substances **(Naika *et al.*, 2005).** The antioxidants play a fundamental role in the protection of plant cells from oxidative damage and are also of primary importance for animal cells. In fact, antioxidants like vitamin C, vitamin E and ß-carotene with their antagonist functions against free radicals are very useful in protection against various diseases (**De Pascale *et al.*, 2001).** Fruit texture, such as hardness, is also important to organoleptic characteristics of tomato fruits because tomato texture is one of the critical components of the consumer's perception of tomato fruit quality **(Chaïb *et al.*, 2007).** The intensity of taste of tomatoes is mainly determined by the amount of soluble solids, mostly reducing sugars and organic acids **(Krumbein and Auerswald, 1998; Malundo *et al.*, 1995).** A strong positive correlation has been observed between sweetness and reducing sugars or total soluble solids content **(Malundo *et al.*, 1995; Tandon *et al.*, 2003).** The sour taste of tomatoes has been attributed mainly to citric acid **(Petro-Turza, 1986).**

Table.2.2. Red tomatoes, raw

Tomato contents	**Nutritional value per 100 g (3.5 oz)**
Energy	74 kJ (18 kcal)
Carbohydrates	3.9 g
Sugars	2.6 g
Dietary fiber	1.2 g
Fat	0.2 g
Protein	0.9 g
Water	94.5 g
Vitamin A	42 µg (5%)
lutein and zeaxanthin	123 µg
Vitamin C	14 mg (17%)
Vitamin E	0.54 mg (4%)
Potassium	237 mg (5%)
Percentages are relative to. US recommendations for adults	

Source: USDA Nutrient Database (2013).

2.3. LYCOPENE

Lycopene belongs to the class of compounds known as carotenoids **(Rao and Agarwal, 2000)** and can be ingested by people as a component of certain foods, most notably tomatoes **(Rao and Agarwal, 2000)** as well as via supplements **(Hininger *et al.*, 2001; Paetau *et al.*, 1998)**. Lycopene, an aliphatic hydrocarbon, has received particular attention as a result of studies indicating that it has highly efficient antioxidant and free radical scavenging capacity **(Turk *et al.*, 2006).**

Lycopene, a carotenoid without provitamin-A activity, is present in many fruits and vegetables. It is a red, fat-soluble pigment found in certain plants and microorganisms, where it serves as an accessory light-gathering pigment and protects them from ultraviolet B radiation. Gac fruit (*Momordica cochinchinensis*); tomatoes (*Lycopersicon esculentum*); and tomato products, including ketchup, tomato juice, and pizza sauce, are the more bioavailable sources of lycopene **(Arab and Steck, 2000).** Lycopene (a natural organic compound that gives tomatoes their red colour) is an antioxidant which has gained increased attention for its healthgiving properties. Lycopene is one of the most powerful antioxidants found in foods. Antioxidants protect and repair cells and tissues against the damaging effects of free radicals which cause cell and tissue damage **(http://www.simplot.com.au/pdf/fact-sheets/FactSheet-Lycopene.pdf)**. The unique biochemical properties of lycopene may render it able to protect cellular components against specific types of damage from highly reactive oxygen species. The source of the reactive compounds differs by tissue type and includes smoking, sunlight, chronic inflammation, and normal metabolic processes **(Cerutti, 1985; Wiseman and Halliwell, 1996; Ames *et al.*, 1993).** Lycopene appears to be the most efficient quencher of singlet oxygen and free radicals among the common carotenoids *in vitro* **(Di Mascio *et al.*, 1989; Woodall *et al.*, 1997; Mortensen and Skibsted, 1997; Conn *et al.*, 1991).** Lycopene is a fat-soluble compound, absorption into tissues is improved when it is consumed with oil. Its concentration in body tissues is higher than all other carotenoids **(Gerster, 1997; Stahl *et al.*, 1998).** In one study, serum concentrations of lycopene increased after consumption of heated tomato juice mixed with oil, with a peak at 24–48 h after ingestion **(Stahl and Sies, 1992; Clinton, 1998).** In cell cultures, lycopene has been found to inhibit breast cancer tumors more efficiently when compared to alpha- and beta-carotene **(Zhang *et al.*, 1997; Levy *et al.*, 1995).** *In vitro* studies with lycopene have shown induction of apoptosis and inhibition of cell growth in androgen-sensitive (LNCaP) and androgen-independent (PC3 and VeCaP) prostate cancer cell lines

(Vaishampayan *et al.*, 2007). *In vitro,* animal, and clinical studies suggest that lycopene may attenuate liver injury and possibly prevent the development of hepatocellular carcinoma **(Seren *et al.*, 2008).**

2.4. TOMATINE

Leaves, stems, and green unripe fruit of the tomato plant contain small amounts of the toxic alkaloid tomatine **(Mcgee, 2009).** Tomatoes (unripe or plant part) - contain tomatine**,** an alkaloid related to solanine and atropine. As the fruit ripens, the tomatine is metabolized. Therefore, ripened, red tomatoes are not likely to be harmful to a dog when eaten **(http://www.dogheirs.com/dogheirs/posts/141-toxic-foods-for-dogs-fruits-vegetables-and-nuts).** Tomato plants (Lycopersicon esculentum) synthesize the glycoalkaloids dehydrotomatine and a-tomatine, possibly as a defense against bacteria, fungi and viruses, and insects **(Ito *et al.*, 2007; Simons *et al.*, 2006; Thorne *et al.*, 1985).** Tomatine is a saponin (steroidal glyco-alkaloid) produced by tomato and some other *Solanum* species **(Roddick, 1974).**

2.5. SALICYLIC ACID (SA)

Salicylic acid (SA) is a hormone-like substance that plays an important role in the regulation of plant growth and development **(Raskin, 1992; Kang *et al.*, 2006; Taguchi *et al.*, 2001).** Salicylic acid (SA) is a naturally occurring phenolic compound and acetyl salicylic acid is a derivative of SA with parallel properties. Role of acetyl salicylic acid has been documented for heat and drought tolerance in tomato **(Khan *et al.*, 2015, 2014; Senaratna *et al.*, 2000).** SA has attained more attention because of its involvement in plant defense mechanisms, such as establishment of systemic acquired resistance (SAR), (**Metraux *et al.*, 1990)** induction of pathogenesis related (PR) proteins **(Malamy *et al.*, 1990)** as well as hypersensitive response **(Horvath *et al.*, 2007).** The protective effect of SA against abiotic stress factors such as toxic metals **(Strobel and Kuc, 1995),** heat stress **(Dat *et al.*, 1998),** low temperature **(Janda *et al.*, 1999; Mora-Herrera *et al.*, 2005)** and oxidative damage **(Strobel and Kuc, 1995**; **Kusumi *et al.*, 2006)** has been demonstrated. SA has been reported to induce salinity tolerance in tomato (**Stevens *et al.*, 2006)** maize **(Gunes *et al.*, 2007)** carrot **(Eraslan *et al.*, 2007)** and wheat **(Arfan *et al.*, 2007).** It has been estimated that about 45 million hectares of irrigated land have been damaged by salinity stress worldwide and considerable area of land affected by salinity is increasing day by day worldwide **(Pitman and Lauchli, 2002; Munns and Tester, 2008).** In fact, the loss of plant productivity due to salinity stress is a consequence of imbalance in cellular ionic and osmotic balances **(Khan *et al.*, 2012b).** Major adverse effects of salinity

stress include increased ion toxicity, osmotic stress, and nutrient-acquisition and homeostasis/deficiency, impaired stomatal conductance, increased cell-turgor loss, decreased reduction in leaf water potential, altered physiological/biochemical processes, and elevated ROS-caused oxidative stress **(Munns and Tester, 2008; Nazar *et al.*, 2011; Khan *et al.*, 2014).** The role of SA in strengthening salinity stress-tolerance mechanisms has been extensively evidenced in many crops including *Vicia faba* **(Azooz, 2009),** *Brassica juncea* **(Nazar *et al.*, 2011, 2015),** *Medicago sativa* **(Palma *et al.*, 2013)**, and *V. radiata* **(Khan *et al.*, 2014).** Salicylic acid was reported to induce salinity tolerance and increased biomass of *Torreya grandis* as a result of enhanced chlorophyll content and the activity of antioxidant enzymes that eventually activated the photosynthetic process and alleviated oxidative stress **(Li *et al.*, 2014).** It has been suggested that SA has considerable agronomic potential to improve the stress tolerance of agriculturally important crops **(Ghazanfar *et al.*, 2016).**

2.6. STRESS

All the factors that inhibit plant growth are defined as stresses. Drought, saltiness, excess irrigation, high or low temperature, pH and heavy metals are common sources of stress. Those stresses create social and economic problems, especially in developing countries. Only 10% of the land that can be used for agriculture in the world isn't under the effect of any environmental stress element. For the rest 90%, the most common stress element is drought with 26%, followed by salt stress by 20% **(Blum, 1985; Ashraf, 1994).** Stress in plants could be defined as any change in growth conditions that disrupts metabolic homeostasis and requires an adjustment of metabolic pathways in a process that is usually referred to as acclimation **(Shulaev *et al.*, 2008).**

During their entire life cycle, plants are exposed to various environmental stresses. Two major categories can be distinguished: abiotic stress, which encompasses a variety of unfavorable environmental conditions, such as drought, submergence, salinity, heavy metal contamination and nutrient deficiency, and biotic stress caused by infectious living organisms, such as bacteria, viruses, fungi, or nematodes. Both types of stress negatively affect the productivity and survival of plants **(Shaik and Ramakrishna, 2014)**. Abiotic stress reduces agricultural yield, so that novel crop genotypes adapted to environmental stress need to be developed **(Wu *et al.*, 2014; Wang and Frei, 2011; Dolferus, 2014).**

Plants possess antioxidant defense systems, comprised of enzymatic and non-enzymatic components, which normally maintain ROS balance within the cell. For instance, they use a

diverse array of enzymes like superoxide dismutases (SOD), catalases (CAT) and peroxidises as well as low molecular mass antioxidants like ascorbate and reduced glutathione (GSH) to scavenge different types of ROS **(Foyer *et al.*, 1994).** In order to survive under stress conditions, plants are equipped with oxygen radical-detoxifying enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR). Oxidative stress is the result of ROS, such as superoxide, H_2O_2, and hydroxyl radicals, and causes rapid cell damage by triggering off a chain reaction. ROS scavenging is one among the common defence responses against abiotic stresses. Changes in antioxidants and protective molecules reflect the impact of environmental stresses on plant metabolism **(Jaleel *et al.*, 2007; Doğan *et al.*, 2010a, b).** Reactive oxygen species (ROS) homeostasis is changed in response to stress; they are regarded as molecules causing damage to cells at high concentration as well as ubiquitous signaling molecules at low concentration, thus participating in recognizing and responding to stress factors **(Wrzaczek *et al.*, 2013).**

2.7. STRESSES EXPERIENCED BY PLANTS

2.7.1. ABIOTIC STRESS

Plants are sessile organisms and as such must have mechanisms to deal with both abiotic and biotic stresses to ensure survival. The term “abiotic stress” includes many stresses caused by environmental conditions such as drought, salinity, UV and extreme temperatures. Due to global climate change it is predicted that a biotic stresses will increase in the near future and have substantial impacts on crop yields **(Intergovernmental Panel of Climate Change; http://www.ipcc.ch).**

In the world only 9% of the area is conducive for crop production, while 91% is under stress. This includes 25% under drought, 22% has got shallow depth, 22% is under mineral stress and 14% is under freezing stress and 11% is water logged. Added pressure is that global population is likely to reach 7 billion by 2025 and 10 billion by 2050. The area under stress is likely to increase further due to land degradation and urbanization. Thus agricultural production has to be increased from these lands that are under stress **(http://www.niam.res.in/abiotic_stresses.aspx)**. Abiotic stresses such as salinity, drought, low temperature and ABA cause adverse effects on crop growth and productivity **(Suzuki *et al.*, 2005).** Both low and high temperatures, dehydration and salinity cause metabolic damage, generate reactive oxygen species (ROS) and cause inhibition of photosynthesis **(Hasegawa *et al.*, 2000).** Among abiotic stresses, drought and salinity cause a reduction in hydraulic

conductivity in plants (**Peyrano *et al.*, 1997; Steudle, 2000).** Generation of Reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals are inevitable under oxidative stress as does the level of ROS-induced oxidative damage to lipids, proteins, and nucleic acids **(Meloni *et al.*, 2003).**

Plant responses to abiotic stresses are dynamic and complex **(Skirycz and Inze, 2010; Cramer, 2010).** They are both elastic (reversible) and plastic (irreversible). The plant responses to stress are dependent on the tissue or organ affected by the stress. For example, transcriptional responses to stress are tissue or cell specific in roots and are quite different depending on the stress involved **(Dinneny *et al.*, 2008).**

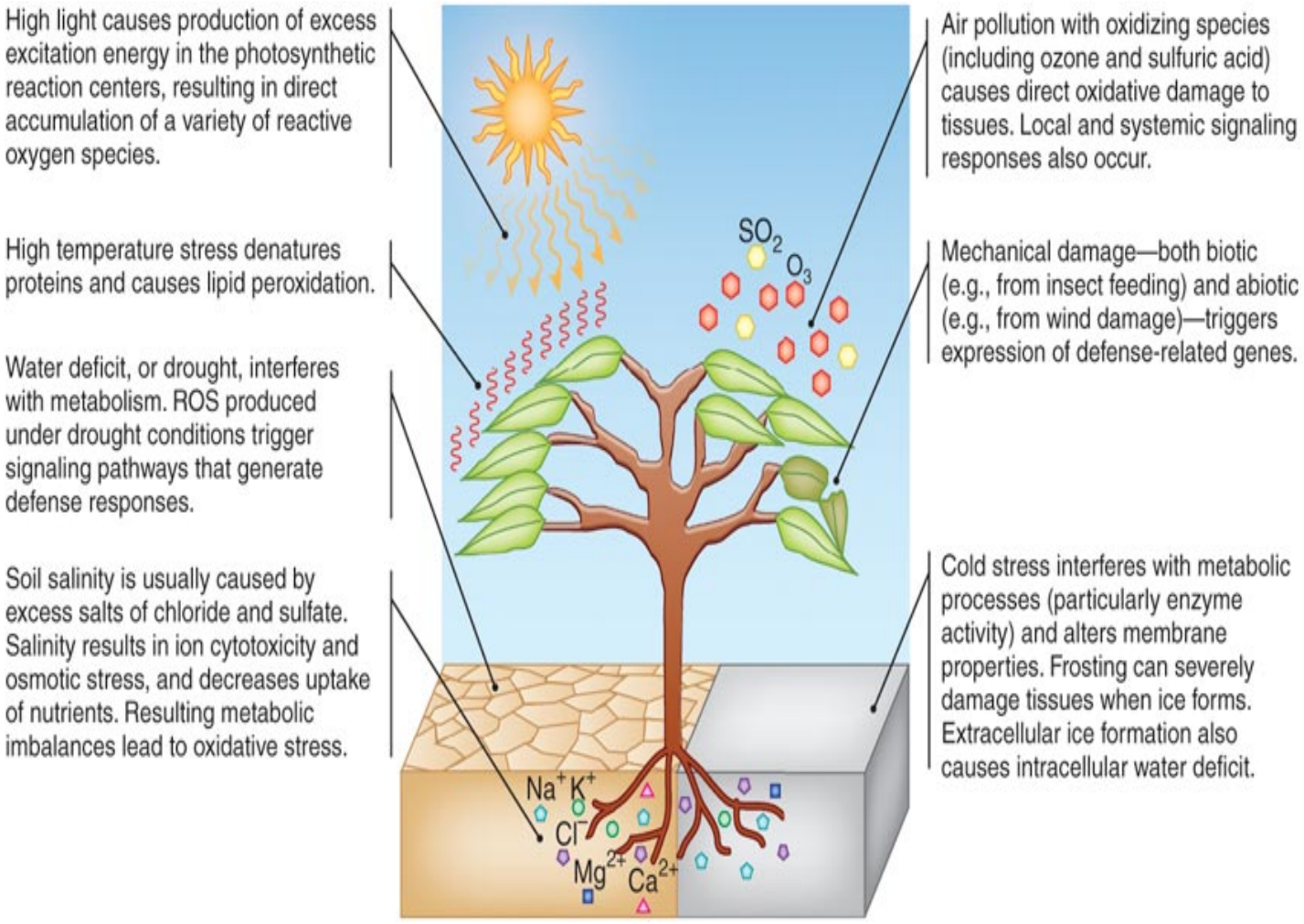

Figure 2.1 - Plants are exposed to a variety of abiotic stresses (http://www.nature.com/nchembio/journal/v5/n5/images/nchembio.158-F1.jpg).

Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by >50% **(Rodríguez *et al.*, 2005).** Drought, salinity, extreme temperatures

and oxidative stress are interconnected and affect the water relations of a plant on the cellular as well as whole plant level causing specific as well as unspecific reactions **(Beck *et al*., 2007).** This leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity **(Wang *et al.*, 2001).** Depending upon the extent of stress, the plants try to adapt to the changing environmental conditions. For example, under osmotic and ionic stresses, the plants must get adequate amount of water for their growth and development of reproductive structures. Therefore, under these conditions, the adaptive mechanisms should be directed to this objective. The closure of stomata limits water loss and the integrity of the photosynthetic and carbon fixation apparatus is maintained by the initiation of a series of physiological processes **(Bohnert and Zhu, 2001).** Exposure of plants to unfavourable environmental conditions such as alteration of temperature, high light intensity, water availability, air pollutants or salt-stress can increase the production of reactive oxygen species. Salt, drought, heat, cold stress and oxidative stress are accompanied by the formation of ROS causing extensive cellular damage and inhibition of photosynthesis. This phenomenon is called oxidative stress and is known as one of the major causes of plant damage as a result of environmental stresses **(Sunkar *et al*., 2003).** Organelles with a highly oxidizing metabolic activity or with an intense rate of electron flow, such as chloroplasts, mitochondria or microbodies, are a major source of ROS production in plant cells, and chloroplast and peroxisomes are thought to be the two major contributors to the oxidative load in plant cells during abiotic stress. Despite the negative effects of ROS, recent studies have shown that ROS play a key role in plants as signal transduction molecules involved in mediating responses to abiotic stress **(Mittler *et al.*, 2004).** In addition to the different proteins and enzymes that detoxify ROS, antioxidants such as ascorbate, glutathione and tocopherol play an important role in the regulation of the cellular ROS homeostasis. These antioxidants act as redox buffers and can influence gene expression associated with abiotic and biotic stresses **(Foyer and Noctor, 2005).** The identification of ROS-generating enzymes such as the plant homolog of respiratory burst NADPH oxidases (Rboh) demonstrated that plant cells can initiate and most likely amplify ROS production for the purpose of signaling **(Bailey-Serres and Mittler, 2006).** Normally, ROS are rapidly removed by antioxidative mechanisms, but this removal can be impaired by stresses themselves **(Allan & Fluhr, 2007),** causing a rise in their intracellular concentration and an increase of the damage. To prevent or repair these damages, plant cells use a complex defence system, involving a number of antioxidative stress-related defence

genes that, in turn, induce changes in the biochemical plant machinery **(Ciarmiello *et al.*, 2011).**

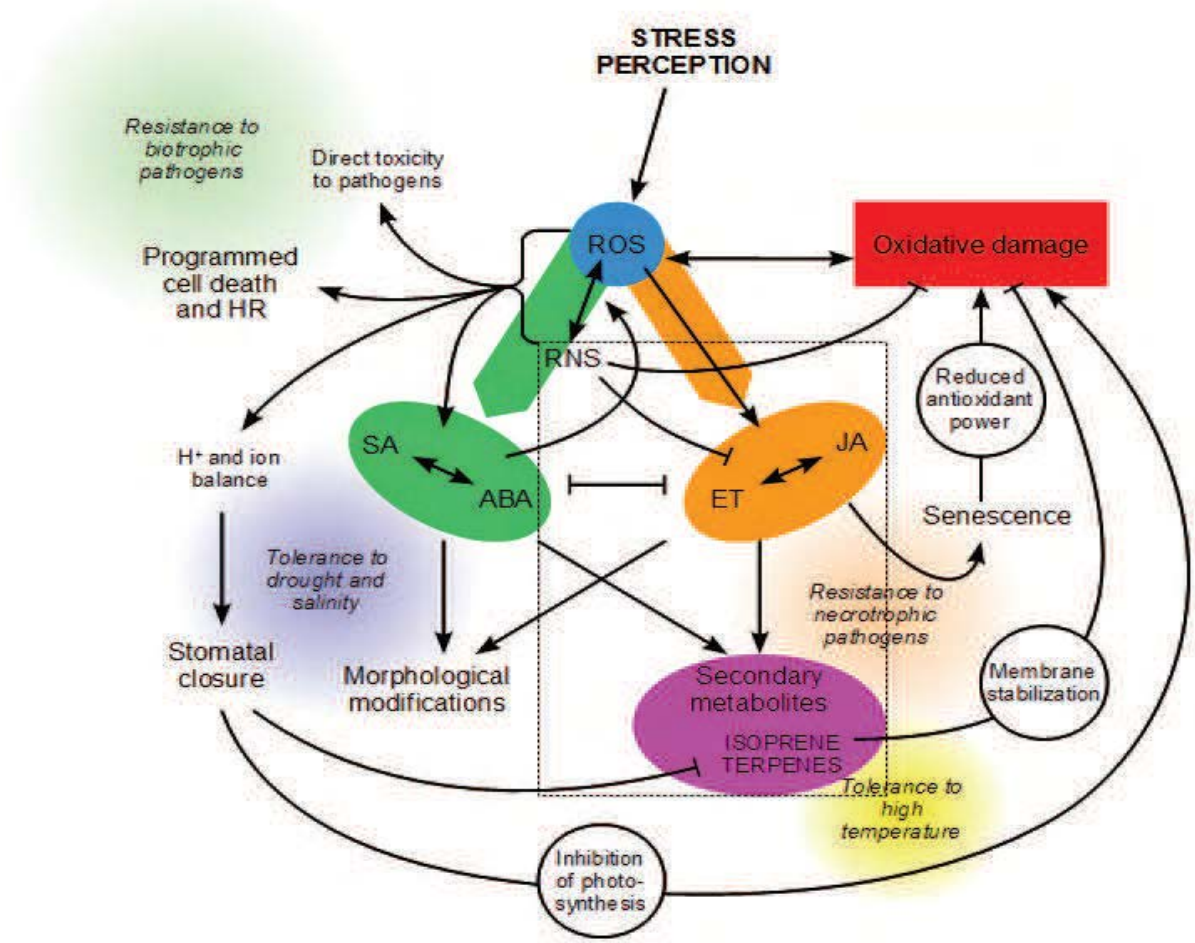

Fig. 2.2. Overview of the signalling events and reactions following the perception of stress. As a primary consequence, an oxidative burst occurs, due to the production of Reactive Oxygen Species (ROS). This early event causes oxidative damage and trigger the signal cascades leading to stress tolerance. The plant reacts to the ROS accumulation through two possible ways. On one side (green path), the interplay of ROS and Reactive Nitrogen Species (RNS) allows the induction of programmed cell death, the modulation of ion fluxes (including Ca2+), and the direct killing of noncompatible pathogens. Salicylic (SA) and abscisic acid (ABA) are long-ranged hormones mediating these responses in feedback with ROS and RNS. Several stressing agents, both abiotic and biotic, stimulate this pathway. A distinct and partially antagonistic signal cascade (orange path) involves the production of ethylene (ET) and jasmonates (JA). These hormones are required for the resistance to necrotrophic pathogens. Notably, the production of isoprene and terpenoids is stimulated by JA, and contributes to thermotolerance and stress mitigation. Apart

from their role as signal molecules, isoprenoids can also act as quenching molecules of ROS. The volatile messengers in the described processed, namely NO (the precursor of RNS), ET, JA, isoprene and terpenoids are evidenced in the dashed frame *(Spinelli et al., 2011)*.

Key enzymes involved in the detoxification of ROS are, namely, SOD, CAT, POD, APX and other enzymes implicated in the Halliwell and Asada cycle (ascorbate–glutathione pathway). Under stress conditions, these antioxidants enhance the activity of almost all of these enzymes **(Jaleel *et al.*, 2009).** It is generally observed that ROS production and ROS-induced damage increase during abiotic stress, but ROS are also important signaling molecules **(Moller *et al.*, 2007).** The production of ROS, such as superoxide (O_2●-), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO●), and singlet oxygen ($1O_2$), is an unavoidable consequence of aerobic metabolism **(Moller *et al.*, 2007),** and can be produced continuously as byproducts of various metabolic pathways that are localized in different cellular compartments such as chloroplasts, mitochondria and peroxisomes **(del Rio *et al.*, 2006; Navrot *et al.*, 2007).** During an increase in osmotic stress levels, plants utilise various antioxidant enzymes and increase their activity; thus these enzymes play vital roles in ROS removal. SOD, which is an important scavenger, is used for producing water and oxygen by catalysing O_2 afterwards the ROS hydrogen peroxide is catalysed by POD and CAT to produce water and oxygen as well **(Zhang *et al*., 2008).**

Under normal growth conditions, ROS are produced at a low level. However, during stress, their production rate is dramatically elevated **(Miller *et al.*, 2010).** Over-accumulation of ROS results in oxidative stress, which damages plant macromolecules and cell structures and leads to inhibition of plant growth and development **(Gill and Tuteja, 2010; Jaspers and Kangasjarvi, 2010; Suzuki *et al.*, 2011).**

Restriction of plant growth by retarding cell extension is often the earliest visible effect of stress. Plants have developed a range of morphological, physiological and biochemical mechanisms that enable them to avoid and/or tolerate stress factors and survive **(Potters *et al.*, 2006)**. Reactive oxygen species (ROS) homeostasis is changed in response to stress; they are regarded as molecules causing damage to cells at high concentration as well as ubiquitous signaling molecules at low concentration, thus participating in recognizing and responding to stress factors **(Wrzaczek *et al.*, 2013).** Under natural conditions, both the timing and the intensity of the stressors can vary; thus, appropriate fine-tuning of the defence responses is required to minimize detrimental effects on plant fitness **(Des Marais and Juenger, 2010; Brown and Rant, 2013).** Field crops are grown under the same variable conditions; however,

as they are bred and selected under relatively controlled conditions, several trade-offs might have been overlooked that can result in negative interactions under field conditions **(Brown and Rant, 2013; Huckelhoven *et al.*, 2013; Mc Grann *et al.*, 2014).** It is thus of great importance to examine plant responses to combinations of abiotic and biotic stress factors, important variables that are relevant to crop yields **(Soliman and Kostandi, 1998; Kissoudis *et al.*, 2014).** Plants in their natural environment are continuously exposed to a variety of stress factors, both abiotic and biotic, and thus have evolved a multitude of defence mechanisms in order to maintain their fitness **(Roux *et al.*, 2014; Mickelbart *et al.*, 2015).** When a plant is subjected to abiotic stress, a number of genes are turned on, resulting in increased levels of several metabolites and proteins, some of which may be responsible for conferring a certain degree of protection to these stresses **(Bhatnagar *et al.*, 2007).** Many transcription factors involved in stress responses have been identified. Often the expression of genes encoding these transcription factors responds rapidly to abiotic stress treatments **(Gadjev *et al.*, 2006; Kilian *et al.*, 2007).** Plant defences against different abiotic stresses have both common and unique elements. Common elements include increases in reactive oxygen species (ROS) and cytosolic Ca^2+ as well as activation of kinase casades. In addition, stresses can lead to increased concentrations of hormones such as salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA) and ethylene, all of which have been implicated in response to environmental conditions **(Hirayama & Shinozaki, 2010).** Many of the pathways will be affected in response to abiotic stresses like salt stress. Carotenoid biosynthetic pathway is one such pathway of paramount importance as it produces carotenoids, which plays a major role in photosynthesis. Photosynthesis is one of the mostly affected factors due to salt stress **(Sudhir and Murthy, 2004; Stoeva and Kaymakanova, 2008; Lu *et al.*, 2010).** They form essential components of photosynthetic antenna and reaction centre complexes. Thus they play a crucial role in harvesting light energy during photosynthesis as reported by **(Mimuro and Katoh, 1991).**

At the cellular level, calmodulins reduce the effective diffusion in the cytosol and restrict calcium to specific locations thereby regulating the activity **(Sanders *et al.*, 1999).** Consequently, calmodulin gene expression is induced by a variety of stresses like salinity, osmotic stress and wounding in different plants **(Phean-O-Pas *et al.*, 2005).** Calcium is another versatile signaling molecule that controls many aspects of plant growth, development, and stress adaptation **(White & Broadley, 2003; Dodd *et al.*, 2010).** The Ca^2+ signals take the form of transient increase in cytosolic free Ca^2+ ($[Ca^2+]cyt$) from the outside of cells and/or subcellular compartments, such as the vacuole, apoplast, and ER, in which the concentration of

Ca^{2}+ is much higher compared with the cytosol **(Dodd *et al.*, 2010).** Plants possess antioxidant defence systems, comprised of enzymatic and non-enzymatic components, which normally maintain ROS balance within the cell. For instance, they use a diverse array of enzymes like superoxide dismutases (SOD), catalases (CAT) and peroxidises as well as low molecular mass antioxidants like ascorbate and reduced glutathione (GSH) to scavenge different types of ROS **(Foyer *et al.*, 1994).**

2.7.1.1. DROUGHT STRESS

Insufficient availability of water, *i.e.*, drought, is presumably the most common stress experienced by terrestrial plants. On the cellular level drought stress will affect vital metabolic functions and maintenance of turgor pressure. Cell expansion and cell wall formation are therefore especially sensitive to water limitation. In order to minimise water loss, plants respond to lower water availability with the closure of stomata. However, this protective measure is not without drawbacks for the plant as this will also decrease the CO_2 supply within the plant leaves and finally affect photosynthesis. Within the chloroplasts, light is absorbed by the antenna pigments and funnelled to the reaction centres of photosynthesis. When CO_2 limitation causes the decrease of photosynthetic fixation, the absorbed light needs to be diverted to other processes, giving rise to additional plant stress. Even under non-stressed conditions, part of the absorbed energy is used for the reduction of oxygen, creating ROS. ROS including singlet oxygen ($1O^{2-}$), superoxide anions (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) are highly reactive and damage cells by ROS mediated oxidative processes such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage of nucleic acids **(Grene, 2002).** Drought-induced genes encode proteins involved in metabolic, osmotic or structural adjustment as well as proteins with damage control and repair functions **(Ingram and Bartels, 1996).** Biosynthesis of proline for instance increases the concentration of compatible osmo protectants in the cells, while aquaporins can facilitate water permeability of cellular membranes and maximise water uptake potential of the plant, and ROS scavenging proteins can limit damage by secondary oxidative stress **(Chaves *et al.*, 2003).** Drought tolerance in black pepper is attained through osmotic adjustment and better ROS scavenging machinery, functioning through different antioxidant enzymes. The activities of antioxidant enzymes such as SOD and POD become higher during stress in tolerant variety **(Vijayakumari and Puthur, 2014).**

2.7.1.2. SALT STRESS

In general, the term salinity includes all the problems due to salts present in the soil while in strict terms, these soils are categorized into two types: sodic (or alkali) and saline. (a third type can be referred to as saline-sodic soils).

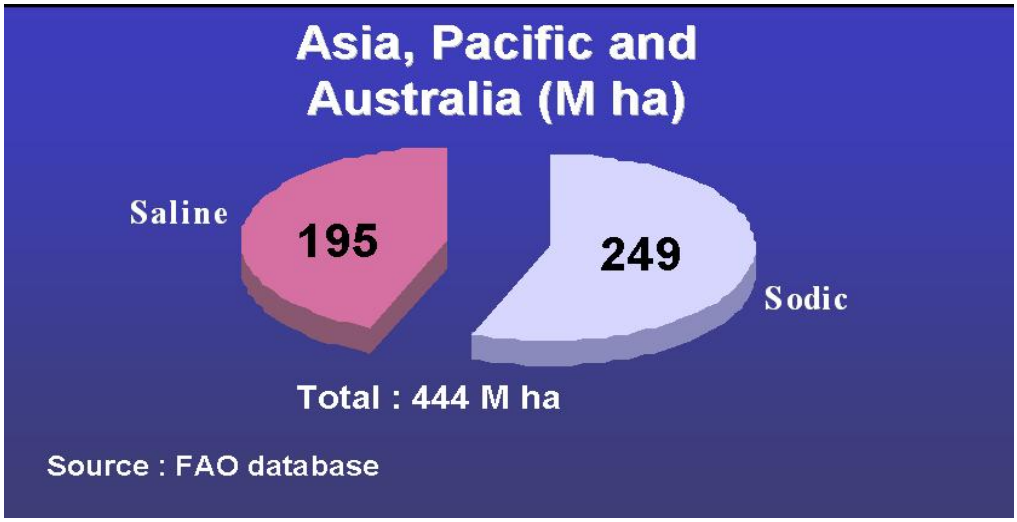

Fig.2.3. Partition of the total salt affected land in saline and sodic area

http://www.knowledgebank.irri.org/ricebreedingcourse/Breeding_for_salt_tolerance.htm

Soil salinity is widely reported to be a major agricultural problem, particularly for irrigated agriculture, but a few cultivars, resistant to saline soils can be developed. Approximately 850 million (6% of the total world area) hectares of land throughout the world are considered as salt affected **(FAO, 2011)**, so soil salinity is considered as one of the major problems that imbalances the sustainability of the system over a vast area **(Flowers, 2004).** Salinity is now taken as one of the major abiotic stresses for the agriculture **(Mahajan and Tuteja, 2005).** According to the USDA salinity laboratory, saline soil can be defined as soil having an electrical conductivity of solution extracted from the water-saturated soil paste ECe (Electrical Conductivity of the extract) of 4 dS m-1 (decisiemens per meter), where 4 dS m-1 ≈ 40 mM NaCl or more **(Chinnusamy *et al.*, 2005; Kotuby-Amacher *et al.*, 2000).** Arid and semi-arid regions compose 6.5 billion ha of this farm land and 1 billion ha of these regions are saline soils. With the increase of saline soils over the years, it is expected that by 2050, more than 50% of the available land for agriculture will be lost because of salinity. Salinity negatively

affects crop productivity and quality. So, it is an important limitation to food supply **(Gol, 2006).**

Salt stress induces various biochemical and physiological responses in plants and affects almost all plant processes **(Nemoto and Sasakuma, 2002; Megdiche *et al.*, 2007).** Salinity may cause hyperionic and hyperosmotic effects in plants leading to membrane disorganization, increase in reactive oxygen species (ROS) levels, and metabolic toxicity **(Jaleel *et al.*, 2007).** An important consequence of salt stress is the excessive generation of reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radicals (OH.) particularly in chloroplast and mitochondria **(Asada, 1994; Prochazkova and Wilhelmova, 2007).** The stress imposed by salts excess is an important restriction for the productive use of lands **(Sanderson *et al.*, 1997)** as it reduces plant growth and productivity at a soil conductivity over 4.5 dS/m (50 mM) **(Muscolo *et al.*, 2003).** Salt stress is one of the most important abiotic stresses that adversely affect natural productivity and causes significant crop loss worldwide. Almost every aspect of the plant's physiology and biochemistry is affected **(Darwish *et al.*, 2009).** Studies on the effects of salinity stress on plants have primarily focused on growth, proline accumulation, chlorophyll content, K/Na, Ca/Na ratio, Na+ and Cl accumulation It has been stated that genotypes with a high proline accumulation and chlorophyll content, high K/NA ratio and low Na+ and Cl- accumulation are more tolerant to salt **(Mane *et al.*, 2011).** Under salt stress, the plant height and leaf area of cultivated soybean decreased, the protein content and the quality of the seeds decreased, and the nitrogen fixation ability was inhibited, thus constraining growth and yield **(Ledesma *et al.*, 2016; Ren *et al.*, 2012).** The cyclic sugar alcohols, pinnitol and ononitol are stored in a variety of species, which are consistently exposed to saline conditions or accumulate in tolerant species when exposed to saline environments **(Paul and Cockburn, 1989).** Plants are classified as glycophytes or halophytes according to their capacity to grow on high salt medium. Most plants are glycophytes and cannot tolerate salt-stress. High salt concentrations decrease the osmotic potential of soil solution creating a water stress in plants. Secondly, they cause severe ion toxicity, since Na+ is not readily sequestered into vacuoles as in halophytes. Finally, the interactions of salts with mineral nutrition may result in nutrient imbalances and deficiencies. The consequence of all these can ultimately lead to plant death as a result of growth arrest and molecular damage **(McCue and Hanson, 1990)**. Morphology, anatomy, ultra-structure and metabolism of plant species are also deeply affected by salt stress **(Prat & Fathi-Ettai, 1990).** The most common anatomical response to salinity is related to cell wall modifications. In

cotton, an accelerated deposition of suberin in cells of the Casparian strip was observed **(Wilson and Peterson, 1983; Reinhardt and Rost, 1995).** Salinity reduces root length and diameter **(Neumann, 1995).** The deleterious effects of salinity on plant growth are associated with (i) low osmotic potential of soil solution (water stress), (ii) nutritional imbalance, (iii) specific ion effect (salt stress), or (iv) a combination of these factors **(Ashraf & Harris, 2004; Marschner, 1995).** Salinity stress causes an imbalance in the uptake of mineral nutrients and their distribution within the plants **(Grattan & Grieve, 1992; Glenn *et al.*, 1999).** Anatomically, it affects cell division and expansion processes **(Kurth *et al.*, 1986; Zidan *et al.*, 1990),** reduces the size of apical meristems, cortex and vascular cylinder. Additionally, stimulates exodermis and endodermis suberization **(Reinhardt and Rost, 1995; Sanderson *et al.*, 1997; Ramos *et al.*, 2004)** or the occurrence of a typical structures such as rhizodermis with phi-thickenings. **(Degenhardt and Gimmler, 2000).** Salinity can differentially affect the mineral nutrition of plants. Nutrient imbalances due to salinity diminish plant growth by affecting the availability, transport, and partitioning of nutrients. Nutrient deficiencies or imbalances result due to competition of Na and Cl with other nutrients such as K, Ca, Mg and NO_3 **(Hasegawa & Bressan, 2000; Hu & Schmidhalter, 1998; Hu & Schmidhalter, 2005; Munns, 2002; Netondo *et al.*, 2004).** Net photosynthesis, transpiration rate and stomatal conductance are significantly affected by salt stress due to changes in chlorophyll content and chlorophyll fluorescence, damage of photosynthetic apparatus and chloroplast structure **(Abd El Baki *et al.*, 2000; Fidalgo *et al.*, 2004; Kao *et al.*, 2003; Pinheiro *et al.*, 2008).** Crop yield response to soil salinity depends on soil water regime, which is modified by irrigation amounts, frequency and salinity of irrigation water **(Eynard *et al.*, 2005).** Salinity causes a range of deleterious effects such as inhibition of photosynthetic rate, chlorophyll content, damage to plasma membrane permeability and other metabolic disturbances **(Ashraf and Parveen, 2002; Karimi *et al.*, 2005).** Drought and salinity activate *de novo* ABA synthesis to prevent further water loss by evaporation through stomata **(Levitt, 1980),** mediated by changes in the guard cell turgor pressure. The mechanism involves fast changes in intracellular Ca2+concentration and stimulates further signalling in the cell. Under osmotic stress, ABA induces the accumulation of proteins involved in the biosynthesis of osmolytes (*e.g.*, proline, trehalose), which increases the stress tolerance of plants **(Nayyar *et al.*, 2005).**

Proline accumulation is one of the most frequently reported modifications induced by salinity and water deficit in plants **(Giridara Kumar *et al.*, 2000; Ramanjulu and Sudhakar, 2001),** and it is often considered to be involved in stress resistance mechanisms. Some workers did not

observe any appreciable increase in free proline content **(Koca *et al.*, 2007; Kumar *et al.*, 2003),** whilst others consider enhanced proline level merely a stress effect, rather than a cause of stress tolerance **(Moftah and Michel, 1987).** Proline accumulates in many plant species under a broad range of stress conditions such as water shortage, salinity, extreme temperatures, and high light intensity **(Aspinall and Paleg 1981; Delauney and Verna, 1993; Hare *et al.*, 1999; Mansour, 2000).** Proline is significantly accumulated under salt stress and performs the positive role in the adaptation of cells to salt and water stress **(Kaviani, 2008).** Proline is considered to be a compatible solute. It protects folded protein structures against denaturation, stabilises cell membranes by interacting with phospholipids, functions as a hydroxyl radical scavenger, or serves as an energy and nitrogen source **(Aspinall and Paleg, 1981; Samaras *et al.*, 1995).** Salinity also disturbs the levels of Ca2+, which increases in the cytosol. Increased cytosolic Ca2+ functions as a second messenger, resulting in changes in gene expression and metabolism in salt-affected cells. Elevated Ca2+ levels return to their original values through active efflux out of the cell mediated by Ca2+ pumps (Ca-ATPase), whose expression increases under salinity. Ca- ATPase is located in the endoplasmic reticulum, plasmalemma and tonoplast, and mediates Ca2+ sequestration in the cell **(Wimmers *et al.*, 1992).** Hormones are also important regulators of plant responses to abiotic stress . The two most important are abscisic acid (ABA) and ethylene **(Goda *et al.*, 2008).** Under saline conditions, the monovalent Na^+ cation competes mainly with K^+ due to their similar valance structure, and interferes in normal cellular processes **(Fonseca *et al.*, 2007).** In addition to being toxic, NaCl can cause osmotic stress and deficiency of essential nutrient elements **(Zhu, 2003).** The phytotoxicity of NaCl is likely due to its ability to generate reactive oxygen species (ROS) represented predominantly by superoxide anion (O2−), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) **(Huang *et al.*, 2005; Tanou *et al.*, 2009; Tuteja, 2007; Verma & Mishra, 2005).** The excess production of ROS during salinity stress results from impaired electron transport processes in chloroplast and mitochondria as well as from pathways such as photorespiration. The results of most studies have shown that the resistance to salt stress is usually correlated with a more efficient antioxidant system **(Shalata, 1998; Olmos *et al.*, 1994).** Moreover, the ability of certain species to increase production of antioxidant compounds and enzymes in response to salinity has been correlated with salt tolerance **(Lopez et *al.*, 1996; Shalata *et al.*, 2001).**

Salt tolerance can be defined as the ability of plants to survive and maintain growth under saline conditions. Plants have three mechanisms to tolerate high salt concentrations: cellular

homeostasis which includes ion homeostasis and osmotic adjustment; detoxification which includes neutralization of ROS; and growth regulation **(Zhu, 2001).** The role of transport proteins such as antiporters, ion channels, ABC-type transporters, Na and K transporters, plasma membrane and vacuolar ATPases is fundamental for salt tolerance in Na+ exclusion, ion homeostasis, and compartmentalization of solutes and amino acids under stress **(Apse *et al.*, 2003; Takahashi *et al.*, 2009).**

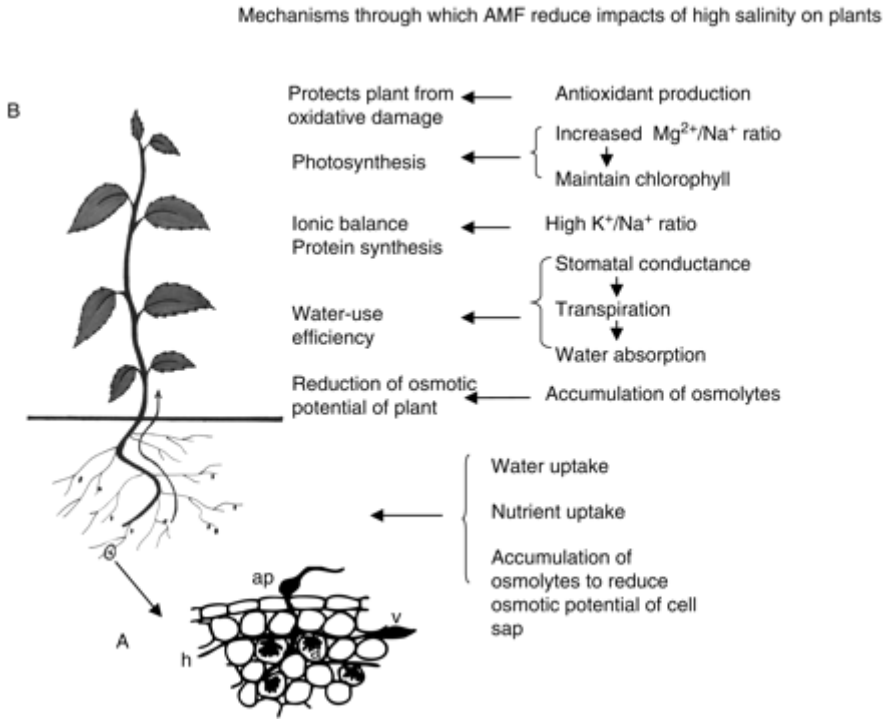

Fig.2.4. The intricate functioning of arbuscular mycorrhizal (AM) fungi in ameliorating salt stress in plants. In AM symbiosis, the fungus forms an appressorium (ap) on the root surface and enters the root cortex by extending its hyphae (h). The hyphae form arbuscules (a) and vesicles (v) in the cortex. Salinity deprives plants of the basic requirements of water and nutrients, causing physiological drought and a decrease in osmotic potential accompanied by nutrient deficiency, rendering plants weak and unproductive. Arbuscular mycorrhiza help plants in salt stress by improving water and nutrient uptake: a decrease in osmotic potential is countered by increasing accumulation of osmolytes, and water-use efficiency, photosynthesis and antioxidant production (to scavenge ROS) is more efficient in salt-stressed plants in the presence of AMF (Evelin *et al.*, 2009).

NO has a role in the plant response to drought and salinity stress.

One of the first responses to these stresses is the ABA-mediated stomata closure. A simplified model for the process, not accounting for self-amplification and feedback effects, is the cascade H2O2 → NO → cGMP-cADPR → release of Ca2+. The K+- intaking channels are inactivated by Ca2+, whereas the outwards K+ channels open in response to H2O2. As a result, the rise in water potential causes the guard cell to collapse and close the stoma **(Bright *et al.*, 2006).** The processes regulated by NO include seed germination, growth and development, apoptosis, hypersensitive response and phytoalexin production **(Besson-Bard *et al.*, 2008).**

Salinity and osmotic stress share a common basis, so it is reasonable that plant adopt similar mechanisms to cope with them, consisting in the regulation of the water loss by transpiration, and of Na+ uptake, transport and redistribution. Proton concentration provides energy for Na+ sequestration in the apoplast or in the vacuole, carried out by a H+:Na+ antiport; NO activates the H+:ATPase and H+:pyrophosphatase activities, in concert with H2O2 and Ca2+ **(Tanou *et al.*, 2009; Zhang *et al.*, 2006; Zhang *et al.*, 2007; Zhao *et al.*, 2007).** One major antioxidant that plays a role in the detoxification of ROS and plant protection against oxidative damage is glutathione. There are two versions in which glutathione can exist in which are the oxidised disulphide version (GSSG) and the reduced version (GSH). The function of glutathione as an antioxidant is mainly assigned to its reduced (GSH) version as this form is oxidised to form the oxidised (GSSG) version during its function as an antioxidant. Therefore, keeping the concentration of reduced glutathione, from the ratio GSH/GSSG, high is important for plants. The production of GSH can occur both in cytosol and the chloroplast in the leaves of the plant. Furthermore, in the ascorbate–glutathione cycle, GR catalyses GSSG reduction into GSH via donation of electrons from NADPH molecules. ROS detoxification in the chloroplast is known to be mostly carried out by the ascorbate–glutathione cycle, which is thus accepted as the main pathway in this process. In this cycle, ascorbate is also considered to be a major antioxidant in addition to GSH **(Shu *et al.*, 2011).** The salinity experiment in tomato genotypes shows that NaCl-induced stress caused decreases in plant biomass, green pigments, photosynthetic activity, stomatal conduction, transpiration rate, number of stomata and stomatal size and resulted in alterations in enzymatic activities (SOD, POD and CAT) and osmolyte accumulation (proline, glycine betaine, total free amino acids and total soluble sugars). The increase in Na and Cl and lipid peroxidation under saline conditions is the indication of ion toxicity and oxidative damage. However, the oxidative damage is controlled by a defensive system comprising various antioxidants, such as SOD, POD and CAT. The results depicted that

salt-tolerant and salt-sensitive genotypes exposed to NaCl stress showed the highest activities of SOD, POD and CAT, both in root and leaf tissues of okra genotypes **(Abbas *et al.*, 2015).** Antioxidant enzymes such as SOD, POX and CAT are known to substantially reduce the levels of superoxide and hydrogen peroxide in plants. It is one of the most important enzymes used against oxidative stress in the plant defence system, and it occurs ubiquitously in every cell of all types of plants **(Ashraf, 2009).** It had observed the three isoforms for SOD and POX and one isoform for CAT that in salt stress, the main activities of SOD, POX and CAT isozymes are significantly higher than normal conditions in red bean (*Phaseolus vulgaris* L.) **(Moharramnejad and Valizadeh, 2014).** Several genes are expressed upon salt exposure and a number of proteins involved in salt-tolerance have been identified **(Bohnert and Jensen, 1996).** Molecular studies have since been conducted on QTL mapping of salt tolerance **(Foolad *et al.*, 1999),** and cloning salt responsive genes from salt tolerant and sensitive cultivars **(Ouyang *et al.*, 2007).** Several salt-induced proteins have been identified in plant species **(Ashraf and Harris, 2004).** It is suggested that stress proteins could be used as important molecular markers for the improvement of salt tolerance using genetic engineering techniques. However, proteins produced under salt stress are not always associated with salt tolerance; consequently, using proteins as a salt tolerance indicator depends on the nature of the plant species orcultivar **(Pareek *et al.*, 1997).** Agronomic characters represent the combined genetic and environmental effects on plant growth and include integration of the physiological mechanisms conferring salinity tolerance. Typical agronomic selection parameters for salinity tolerance are yield, survival, plant height, leaf area, leaf injury, relative growth rate, and relative growth reduction **(Ashraf and Harris, 2004; Okhovatian-Ardakani *et al.*, 2010).** In recent years, tissue culture has gained importance in the development of plants against various abiotic stresses as well as in elucidating mechanisms operating at the cellular level by which plants survive under various abiotic stresses including salinity **(Jain *et al.*, 2001; Daşgan *et al.*, 2009).**

2.7.1.3. TEMPERATURE STRESS

Extremely high or low temperatures affect vital cell functions such as enzyme activity, cell division and membrane integrity. Nevertheless, heat and cold acclimation is possible, as mild stress pre-treatment can significantly enhance the thermo-tolerance of plants **(Thomashow, 1999).**

We will consider temperature stresses as follows: heat stress and cold stress including freezing stress-

2.7.1.4. HEAT STRESS

Effects of high temperature stress can range from moderate effects such as oxidative stress and enhanced transpiration to fatal consequences for the plant, leading to tissue collapse and plant death. To cope with the high temperature stress, plants usually react with enhanced transpiration rates to achieve evaporative cooling. Since high temperatures are often accompanied with limited water availability, the water potential in the plant cell can decrease, leading to drought conditions and initiate responses similar to drought stress. Under these conditions, different events take place: small heat shock proteins (HSPs), acting as molecular chaperones, are synthesized at high abundance **(Vierling, 1997),** Ca2+ influx is enhanced and ROS accumulate rapidly in different parts of the cell **(Doke *et al.*, 1996, Foyer *et al.*, 1997).** ROS on the other hand, serve as fast messengers inside the cell, activating multiple downstream responses and increased ROS generation has been observed during heat shock in plants **(Dat *et al.*, 1998b)**. In *Arabidopsis*, even brief periods of heat stress can induce significant oxidative stress to the cells by membrane peroxidation (Larkindale and Knight 2002). The main source of ROS is probably the chloroplast, but the uncoupled electron transport chain or activation of an NAD(P)H-dependent oxidase can contribute to the oxidative burst **(Dat *et al.*, 2000).** ABA is also needed for protection against the oxidative damage of heat stress **(Larkindale and Knight, 2002).** Consequently, plant mutants, which are defective in the biosynthesis of ABA, have been shown to be less tolerant to environmental stresses such as low temperatures and heat **(Thomashow, 1999; Larkindale and Knight, 2002; Xiong *et al.*, 2002).** A clear sign for involvement of Ca2+ signals is the enhanced Ca2+influx into the cytoplasma and activation of calmodulins in heat stressed plants. Intact Ca2+ signalling is needed for heat survival, since Ca2+ inhibitors can reduce or even abolish heat tolerance in *Arabidopsis* **(Larkindale and Knight, 2002).**

2.7.1.5. COLD AND FREEZING STRESS

Chilling stress is a sub-optimal temperature, where the plant faces reduced enzyme activity and maybe water availability, yet the temperature is above the freezing point of water. Cold stress affects mainly metabolic processes, impairing enzyme reactions, substrate diffusion rates and membrane transport properties. Thereby some reactions are more affected by the cold then others. In particular, the dark reaction of photosynthesis and oxidative phosphorylation seem to be sensitive to chilling. The discrepancy between the speeds of biochemical reactions can cause ROS accumulation in the chloroplast and mitochondrion **(Scheller and Haldrup, 2005).** One

early response to cold and osmotic stress is the rapid Ca^{2+} influx into the cell. Physical alterations in the cellular structure may cause this Ca^{2+} influx by activation of Ca^{2+} channels and initiate downstream Ca^{2+} dependent signalling pathways **(Xiong *et al.*, 2002).** Comparing cold stress with other stresses, parallels in the responses are seen in the accumulation of compounds or activation of genes, which are multi-stress responsive: based on microarray studies, about 10% of the drought-inducible genes are also induced by cold stress **(Seki *et al.*, 2002).**

2.8. SALINITY & TOMATO

Salinity is the presence of salts such as sodium chloride, magnesium and calcium sulfates, and bicarbonates, in soil and water **(http://www.derm.qld.gov.au/salinity/index.html).**

FAO estimates that salt build up has severely damaged about 30 million of the world's 237 million hectares of irrigated farmland. As much as 80 million hectares more are affected to some degree, with about 1.5 million hectares of irrigated land lost each year to waterlogging and salinity **(http://www.fao.org/docrep/u8480e/U8480E0c.html).**

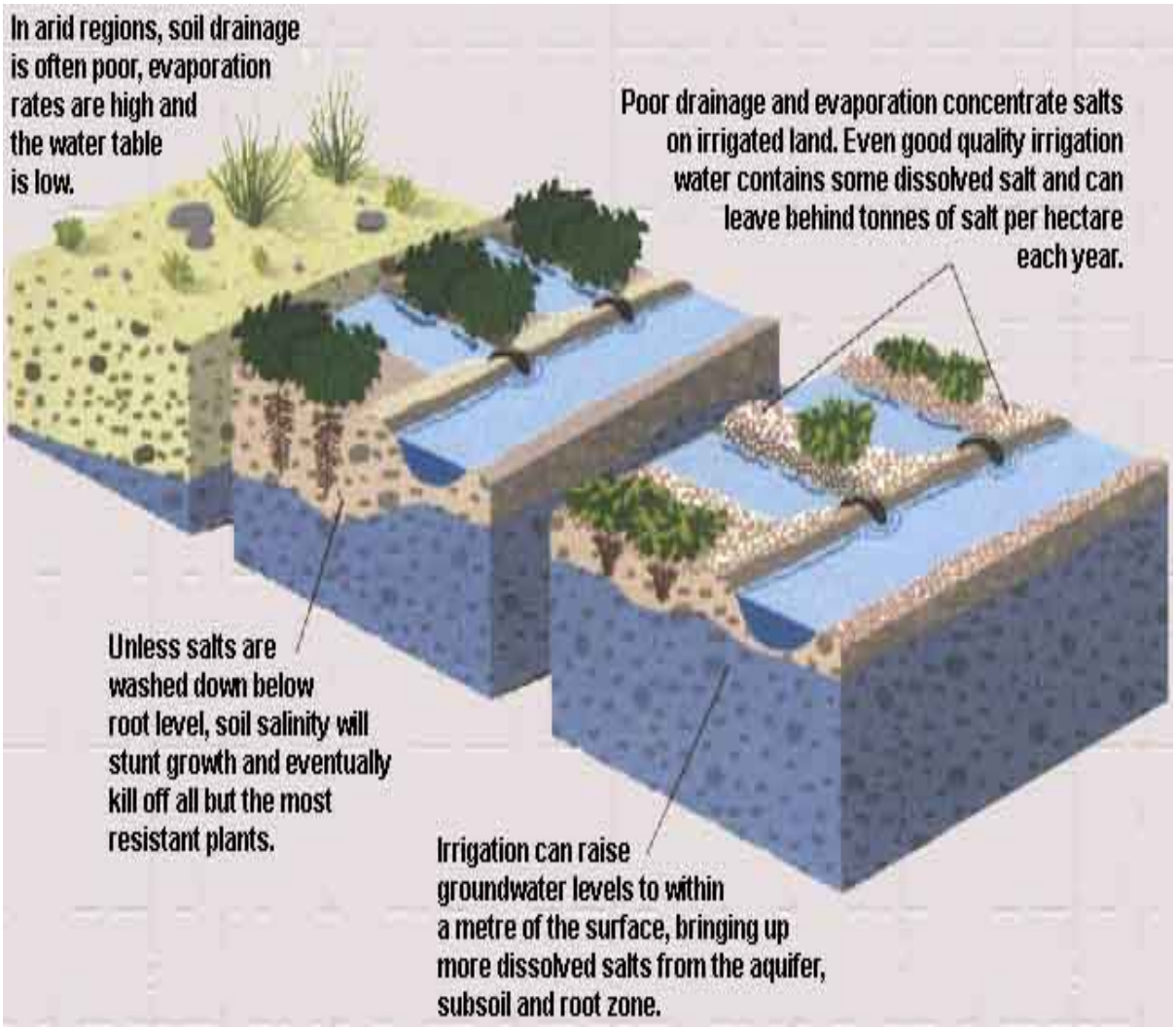

Fig.2.5. Salinization of soil. (http://www.fao.org/docrep/u8480e/U8480E0c.htm).

The salt affected soils were primarily located in the irrigated areas of the old alluvial plains and zones of low rainfall, shallow water table depth and hot and dry moisture regions **(Mandal and Sharma, 2005).**

Soil and water salinity in the arid regions are continuously increasing **(Rus *et al.*, 2002).** It induces osmotic and toxic effects leading physiological, morphological and biochemical modifications; it causes growth inhibition, crop yield reduction, lower photosynthesis and respiration, nutritional deficiencies and inhibition of protein synthesis **(Ashraf and Foolad, 2007).** These phenomena have been observed in agricultural and horticultural crops, including tomato **(Juan *et al.*, 2005).** The scarcity of water resources in most countries of the arid and semiarid regions has led many farmers to use poor quality water for irrigation. Considerable amounts of such marginal water are available and can be successfully used for irrigation under proper management **(Mitchell *et al.*, 1991).**

Salt – affected soils are widespread over the world especially in arid, semi arid and some sub-humid regions. Soil salinity and waterlogging are two of the main constraints present in irrigated agricultural lands. In India, the problem of salinity and alkalinity increases every year as a result of secondary salinisation.

Table.2.3. Extent and distribution of salt affected soils in India

Sr. No.	State	Saline soils (ha)	Alkali soils (ha)	Coastal saline soil (ha)	Total (ha)
1	Andhra Pradesh	0	196609	77598	274207
2	A & N islands	0	0	77000	77000
3	Bihar	47301	105852	0	153153
4	Gujarat	1218255	541430	462315	2222000
5	Haryana	49157	183399	0	232556
6	J & K*	0	17500	0	17500
7	Karnataka	1307	148136	586	150029
8	Kerala	0	0	20000	20000
9	Maharashtra	177093	422670	6996	606759
10	Madhya Pradesh	0	139720	0	139720
11	Orissa	0	0	147138	147138
12	Punjab	0	151717	0	151717
13	Rajasthan	195571	179371	0	374942
14	Tamil Nadu	0	354784	13231	368015
15	Uttar Pradesh	21989	1346971	0	1368960
16	West Bengal	0	0	441272	441272
	Total	**1710673**	**3788159**	**1246136**	**6744968**

Salinity increases the osmotic pressure in the root environment and significantly decreases fresh yield of tomato. It is known that salinity (high EC) reduces yield. Uptake of water into the fruits is reduced by a high osmotic pressure of the irrigation water, and as a result the fruit size is smaller. On the benefit side, mild saline irrigation water may improve the quality of horticultural products by increasing dry matter content and sugar concentration in the fruit **(Li, and Stanghellini, 2001; Mavrogianopoulos *et al.*, 2002).** Performance of tomato plants under salt stress is regulated by a complex genetic mechanism **(Foolad, 2004).** In photosynthetic tissues, in fact, Na+ accumulation affects photosynthetic components such as enzymes, chlorophylls, and carotenoids **(Davenport *et al.*, 2005).** Tomato is sensitive to moderate levels of salt stress and is produced in areas that are increasingly affected by salinity. Most of the wild relatives of tomato are easy to cross with cultivated tomato and provide a rich source of resistance and tolerance genes for biotic and abiotic stresses including salinity **(Hajjar and Hodgkin, 2007).**

Plants perceive and respond to stressful conditions by quickly altering their gene expression in parallel with physiological and biochemical modulation. To adapt to salt stress, new proteins in tomato seedlings are induced **(Amini *et al.*, 2007).** To maintain inner cellular osmotic status, tolerant genotypes can accumulate a higher content of inositol and sugars in their leaves **(Sacher and Staples, 1985).** mRNA profiling of NaCl-treated tomato plants has shown that salt stress can affect many different pathways **(Ouyang *et al.*, 2007; Zhou *et al.*, 2007).** The cyclic sugar alcohols, pinnitol and ononitol are stored in a variety of species, which are consistently exposed to saline conditions or accumulate in tolerant species when exposed to saline environments **(Paul and Cockburn, 1989).** The tomato plant is moderately tolerant to salinity stress **(Ayers & Westcot, 1989; Maas, 1986, 1990).** Thus, Maas (1986) reported that a 50% yield reduction at an electrical conductivity of the saturated soil extract of 7.6 dS m-1. It has been determined that salinity causes several kinds of damage such as growth inhibition, metabolic disturbance and quality losses in addition to yield reduction on tomato plants **(Sanchez-Blanco *et al.*, 1991; Schwarz *et al.*, 1998; Navarro *et al.*, 2000; Li & Stanghellini, 2001; Romero-Aranda *et al.*, 2001; Tüzel *et al.*, 2003; Maggio *et al.*, 2007).** The response to soil salinity in tomato is genetic and species dependant and most of researches have been focusing on the screening and breeding for higher salt tolerance germplasm **(Mohamed *et al.*, 2007).**

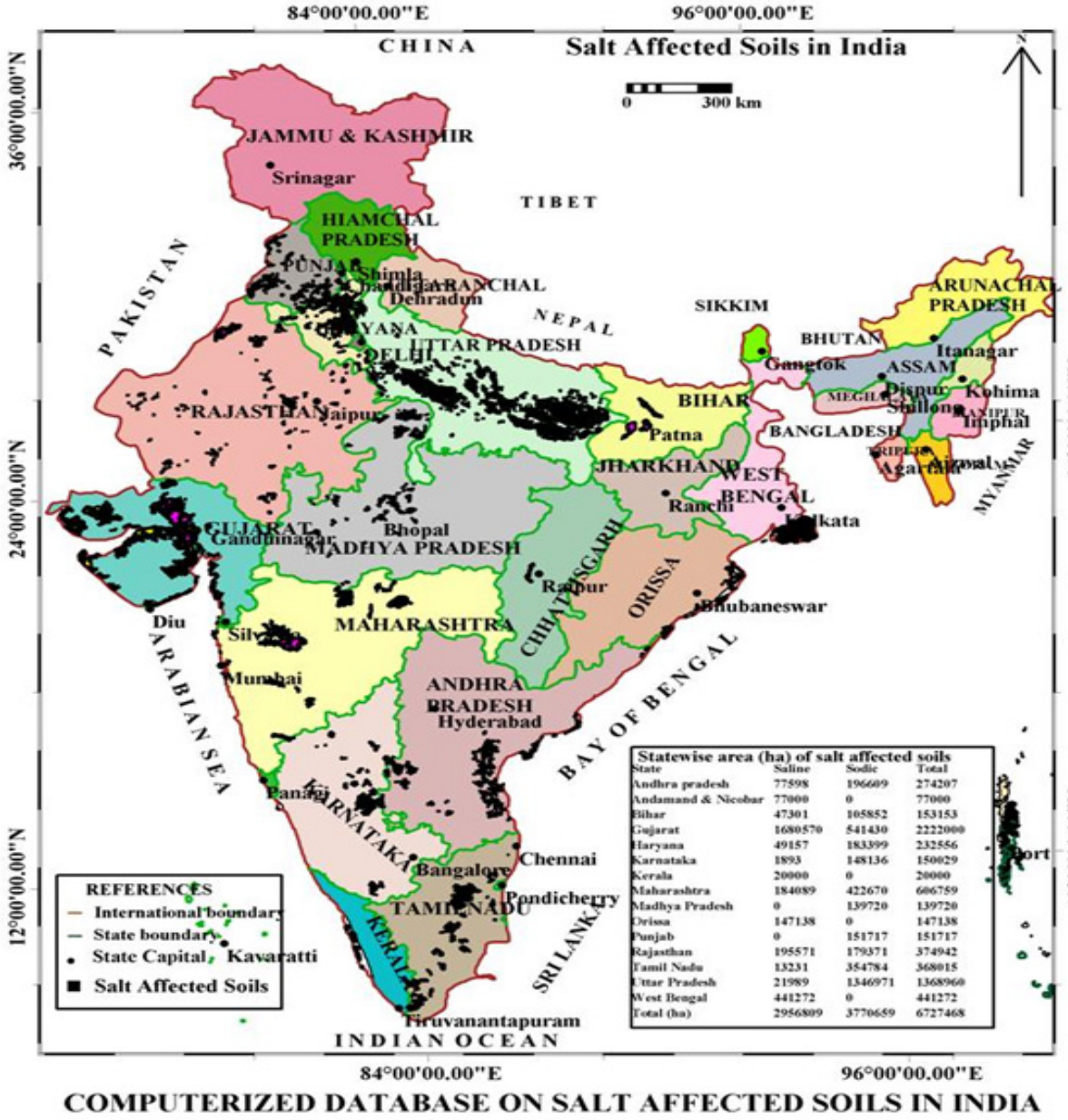

Fig.2.6.(http://www.cssri.org 2012). Central Soil Salinity Research Institute. Indian Council of Agricultural Research, Ministry of Agriculture, "Government of India"

The effects of the salinity on the tomato may be either harmful, reducing the yield and increasing the incidence of blossom-end rot, or beneficial (antioxidant), increasing fruits concentration of soluble solids **(Cuartero and Muñoz, 1999; De Pascale *et al.*, 2001)** and acidity **(De Pascale *et al.*, 2001),** resulting in larger profit at processing **(Boamah *et al.*, 2011).**

Salinity stress improves the fruit quality of tomato (*Solanum lycopersicum*) by increasing the level of total soluble solids, including sugars, organic acids, and amino acids in fruits **(Tal *et al.*, 1979; Ho *et al.*, 1987; Adams, 1991; Balibrea *et al.*, 1996, 1999; Gao *et al.*, 1998; Krauss *et al.*, 2006; Saito *et al.*, 2008)**. An increase in soluble solids enhances not only the market value of fresh fruit but also its processing efficiency, because it increases flavour and lowers water content **(Stark *et al.*, 1996).** Sugar content is most important in terms of fruit taste. Many studies have used model plants in an attempt to understand the mechanism of the effect of salinity stress in enhancing the accumulation of metabolites in plant tissues. However, in the tomato plant, most investigations into salinity stress have been agronomic and have tended to explain the phenomenon as a 'concentration effect' due to a reduction in the size of the fruit **(Ehret and Ho, 1986; Ho *et al.*, 1987; Sakamoto *et al.*, 1999).**

In tomato, salt tolerance is known to increase with plant age, plants being usually most tolerant at the fruit maturation stage **(Bolarin *et al.*, 1993).** As demonstrated by **(Cuartero *et al.*, 2002),** screening for genotype tolerance should involve the determination of several physiological parameters such as Na^+ accumulation and transport parameters, in addition to plant growth parameters **(Ashraf and Harris, 2004; Munns *et al.*, 2002; Sairam *et al.*, 2002).**

Measurements of ion contents in plants under salt stress revealed that halophytes accumulate salts whereas glycophytes tend to exclude the salts **(Zhu, 2007).**

Shoot growth is more sensitive than root growth to salt- induced osmotic stress probably because a reduction in the leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve soil moisture and prevent salt concentration in the soil **(Munns & Tester, 2008).**

An effective antioxidative system has also been found to be responsible for the salt tolerance of some wild tomato species. For example, the increase of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities, and the increased level of the reduced form of ascorbate and glutathione correlated with the decreased lipid peroxidation in a salt-tolerant wild genotype, *Solanum pennellii* , in comparison with cultivated tomato **(Shalata *et al.*, 2001 , Mittova *et al.*, 2004).**

Recently, based on the combination of suppression subtractive hybridization (SSH) and microarray, salt-induced changes in the transcriptome profile were compared in roots of two tomato cultivars with distinct salt tolerance **(Ouyang *et al.*, 2007).**

The highest concentrations of both Na^+ and Cl^- had previously been recorded in the shoots and the lowest in the roots (reviewed in **Munns and Tester, 2008).** One of the harmful effects of

salinity on plant growth is the excessive accumulation of Na^+ and Cl^- in the leaves **(Zhang and Blumwald, 2001; Munns *et al.*, 2002; Ashraf and Harris, 2004).** This accumulation under saline conditions depends on the plant's capacity to limit the uptake of these elements **(Koval and Koval, 1996). Alian *et al.*, (2000)** showed that, in tomato, salt stress induced the uptake of considerable amounts of sodium and chloride and these accumulations were cultivar-dependent and organ-specific. In the present work, K^+:(K^++Na^+) and Ca^{2+}:(Ca^{2+}+Na^+) ratios have been used as nutritional indicators for the salt tolerance of tomato plants according to previously published works **(Pérez-Alfocea *et al.*, 1996; Cuartero and Fernández-Muñoz, 1999; Maathuis and Amtmann, 1999; Asch *et al.*, 2000; Sairam *et al.*, 2002).**

Some of the salt-responsive genes are those that encode proteins involved in the regulation of other salt-responsive genes. Salt response regulatory genes are mostly transacting factors **(Urao *et al.*, 1993)** and protein kinase **(Mizoguchi *et al.*, 1996).**

Changes in activities of various antioxidant enzymes under salinity stress have been reported **(Koskeroglu and Tuna, 2008; Venkatesan and Sridevi, 2009; Hernández *et al.*, 2000).** It is well known that zinc is an important component of many vital enzymes, and a structural stabilizer for proteins, membrane and DNA-binding proteins **(Aravind and Prasad, 2004).** Zn deficiency is also recognized to cause higher levels of ROS in plants and relevant damages to plants **(Marschner, 1995; Cakmak, 2000).**

Most cultivated plants are glycophytes with limited compartmentation of NaCl. Glycophytes are not as effective as halophytes in ionic partitioning at the cellular level, but more effective at the plant and tissue level **(Lauchli & Epstein 1990).** The energy requirement for salt exclusion in glycophytes explains in part the stimulation of root respiration by soil salinity (salt respiration) and the loss of net synthesis of organic C **(Eynard *et al.*, 2005; Lambers *et al.*, 1998).** Sensitive cultivars accumulate ions more quickly than tolerant cultivars and this ion accumulation leads to leaf death and, progressively, death of the plant **(Flowers & Flowers 2005; Munns, 2002).** The reduced water potential at saline habitats creates in the plant a two–edged problem: a corresponding water and ion stress. The uptake and accumulation of Na+ and Cl− into the different plant organs is highly controlled **(Hasegawa *et al.*, 2000; Marschner, 1995).**

Only plants with modified adaptive mechanisms can avoid the adverse effect of salinity **(Blumwald, 2000)**. It is important to understand how plants develop salt tolerance, so that vast areas of saline soils can be cultivated. One way of studying this is to compare the differential gene expression of salt tolerant and salt-sensitive plants under saline conditions **(Kong-Ngern**

***et al.*, 2005).** Genes involved in salinity adaptation can be divided into two groups: those that directly protect against stress and those that regulate gene expression during stress **(Ashraf, 1994; Winicov, 1998; Saki *et al*., 2003).**

Salinity has three potential effects on plants: Lowering of water potential, specific ion toxicity (sodium and chloride) and interference with the uptake of essential nutrients. The latter may not be considered because it has no immediate effect due to mobile reserve nutrients present in plants **(Flowers and Flowers, 2005).** Two of the above reasons are important and have part in reduction of plant growth under salt stress. The first one is lowering of external water potential due to salt present outside the root. The second is the senescence of leaves due to the accumulation of ion in the older leaves; there is a true difference in salt tolerance appearance **(Akram *et al.*, 2007).** The mechanism of plant adaptation required to survive in saline conditions is the same in all the plants. How-ever, adaptations are at their extreme in halophytes, but can be found at different degress in glycophytes **(Flowers and Flowers, 2005).** Plants perceive and respond to stressful conditions by quickly altering their gene expression in parallel with physiological and biochemical modulation. To adapt to salt stress, new proteins in tomato seedlings are induced **(Amini *et al.*, 2007).** mRNA profiling of NaCl-treated tomato plants has shown that salt stress can affect many different pathways **(Ouyang *et al.*, 2007; Zhou *et al.*, 2007).** Previous studies revealed metabolic changes under different stresses based on metabolomics in tobacco **(Zhang *et al*., 2001)**, barley **(Wu *et al*., 2013)**, maize **(Sun *et al*., 2016)**, wheat **(Guo *et al.*, 2015)**, tomato **(Ampofo-Asiama, 2014)**, Arabidopsis **(Kim *et al*., 2007; Rizhsky *et al*., 2004)** and other test materials.

Transgenic tomato (*Lycopersicon esculentum* Mill) plants expressing a cation transport gene *HAL1* and a vacuolar Na+/H+ antiporter gene *AtNHX1* exhibited higher capacity for salt exclusion, and increased significantly their salt tolerance **(Gisbert *et al.*, 2000; Zhang and Blumwald, 2001).** Expression of genes involved in antioxidant and detoxification mechanism **(Avsian-Kretchmer *et al.*, 2004)**, intracellular vesicle trafficking system and ion transport **(Mazel *et al*., 2003; Vera-Estrella *et al.*, 2005),** accumulation of compatible solutes and polyamines **(Ferjani *et al.*, 2003; Pommerrenig *et al.*, 2007),** Genetic studies on salt tolerance in tomato dates back to the 1940's, when a different physiological mechanism was observed between the salt sensitive tomato (*L. esculentum* Mill.) and the tolerant wild relatives **(Lyon, 1941; Flowers, 2004).** The accumulation of plant metabolites and the secondary metabolism are not only closely related to plant growth, but are also regulated by other factors in the

environment. Thus, metabolomics reveals the connection between plant and environment, which is accomplished through a thorough understanding of the relationships among function, metabolic networks, metabolic regulation, phenotype and plant growth **(Fan *et al.*, 2013; Aghaei *et al.*, 2009).**

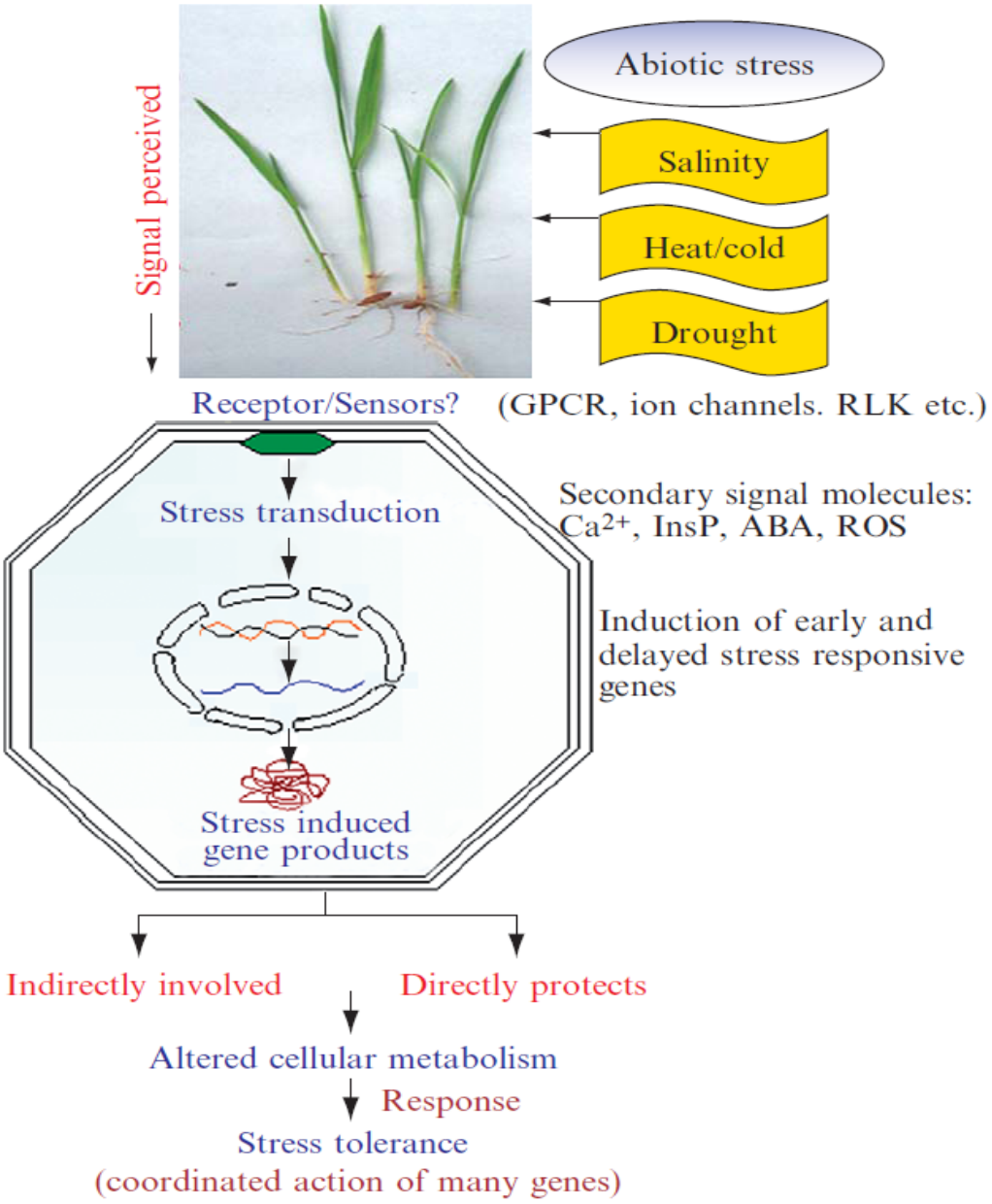

Fig.2.8. Generic pathway for plant response to stress.

Molecular studies have since been conducted on QTL mapping of salt tolerance **(Foolad *et al.*, 1999),** and cloning salt responsive genes from salt tolerant and sensitive cultivars **(Ouyang *et al.*, 2007).** The ability of plants to maintain low cytosolic sodium concentrations is controlled by their ability to selectively absorb Na and K by their roots, transport these ions to aboveground tissues, and exclude or compartmentalize Na intoliar vacuoles. These activities

are modulated through the functions of Na /K -ATPasc of several transmembrane proteins (transporters and antiporters) and H pumps **(Fonseca *et al.*, 2007: Zhu, 2003).** Genes involved in salinity adaptation can be divided into two groups: those that directly protect against stress and those that regulate gene expression during stress **(Ashraf, 1994; Winicov, 1998; Saki *et al.*, 2003).** In recent years, it became clear that ROS play a dual role in plants as both toxic compounds as well as key regulators of many biological processes such as growth, cell cycle, programmed cell death, hormone signaling, biotic and abiotic cell responses and development **(Foyer and Noctor, 2005, Fujita *et al.*, 2006; Mittler *et al.*, 2004).** Organelles with a highly oxidizing metabolic activity or with an intense rate of electron flow, such as chloroplasts, mitochondria or microbodies, are a major source of ROS production in plant cells, and chloroplast and peroxisomes are thought to be the two major contributors to the oxidative load in plant cells during abiotic stress (Mittler *et al.*, 2004). The extracellular stress signal is first perceived by the membrane receptors and then activates a large and complex signalling cascade intra cellularly, including the generation of secondary signal molecules. The signal cascade results in the expression of multiple stress responsive genes, the products of which can provide the stress tolerance directly or indirectly. Overall, the stress response could be a coordinated action of many genes, which may cross talk with each other. GPCR, G-protein-coupled receptor; RLK, receptor-like kinase; InsP, inositol phosphate;ABA, abscisic acid;ROS, reactive oxygen species **(http://www.unice.fr/EB/topic/ReviewSaltTolerance_.pdf).**

Under optimal growthconditions, intracellular ROS are mainly produced at a low level inorganelles. However, ROS are dramatically acclimated during stress. Under abiotic stress condition, limitation of CO_2 uptake, caused by stress-induced stomatal closure, favors photorespiratory production of H_2O_2 in the peroxisome and production of superoxide and H_2O_2 or singlet oxygen by the over reduced photosynthetic electron transport chain **(Apel and Hirt, 2004; Noctor *et al.*, 2014)**.

In addition, as a result of limited CO_2 fixation, reactive oxygen species, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-), can be overproduced in the chloroplasts and other organelles, thus leading to disruption of cellular metabolism through membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage to nucleic acids **(Prakash *et al.*, 2011; Sabra *et al.*, 2012)**. To detoxify active oxygen species, a highly efficient antioxidant defense system is induced in plant cells. Antioxidants can be divided into two classes: (1) non-enzymatic constituents, including lipid-soluble and membrane-associated tocopherols, water-soluble reductants, ascorbic acid and glutathione, and (2) enzymatic

constituents, including superoxide dismutase (SOD), catalase, peroxidase, ascorbate peroxidase (APX), and glutathione reductase **(Eyidogan and Öz, 2007)**. Numerous studies report increased activity of antioxidant enzymes in plants subjected to salt stress **(Meneguzzo and Navari-Izzo, 1999; Hernandez *et al*., 2000).**

Overproduction of ROS caused by stress conditions in plant cells is highly reactive and toxic to proteins, lipids, and nucleic acid which ultimately results in cellular damage and death **(Gill and Tuteja, 2010)**. On the other hand, the increased production of ROS during stresses also thought to act as signals for the activation of stress response pathways **(Baxter *et al*., 2014)**.

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Chapter Three

Materials and Methods

MATERIALS AND METHODS

The materials used and the methods adopted in the present study entitled **"Effects of salicylic acid on growth and biochemical characters of salt stressed tomato varieties"** are as follows:

3.1-Experimental site: The research work was carried out at Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad.

3.2. Germplasm: Tomato genotypes commercially used in various eco-zones of India were used during the present study and these genotypes were obtained from Indian Institute of Vegetable Research, (IIVR),Varanasi as follows:

Table – 3.1. Source of tomato genotypes.

S. No.	Varieties	Acquisition Source
1.	H-8878-1	IIVR, Varanasi
2.	WIR-13706	IIVR, Varanasi
3.	Anigoarlentha	IIVR, Varanasi
4.	Ec-521080	IIVR, Varanasi
5.	Arka marginal	IIVR, Varanasi
6.	Ec-520079	IIVR, Varanasi
7.	WIR-3957	IIVR, Varanasi
8.	P-6 chhu chara	IIVR, Varanasi
9.	VRT-2	IIVR, Varanasi
10.	H-24	IIVR, Varanasi
11.	Roma	IIVR, Varanasi
12.	Ec-520078	IIVR, Varanasi
13.	T-Loeal	IIVR, Varanasi
14.	Arka Saurabh	IIVR, Varanasi
15.	H-86	IIVR, Varanasi
16.	Ec-520061	IIVR, Varanasi
17.	DT-10	IIVR, Varanasi
18.	Agata-30	IIVR, Varanasi
19.	WIR-13708	IIVR, Varanasi
20.	WIR-3928	IIVR, Varanasi

3.2. Experimental and treatment details:

The experiments were conducted from a period during October, 2013-February, 2014 in two trials. The data obtained from two successive years were pooled and analysed.

Number of Treatments: 10

Number of varieties: 20

Number of Replication: 3

Season : Rabi

The experiments consisted of 20 varieties of tomato under each variety with ten treatments as follows:

T_0= Control,

T_1=SA (25µM),

T_2=50mM(salt),

T_3= 100mM(salt),

T_4=150mM(salt),

T_5=200mM(salt),

T_6=50mM(salt)+SA(25µM),

$T_{7=}$100mM(salt)+SA(25µM),

$T_{8=}$150mM(salt)+SA(25µM),

$T_{9=}$200mM(salt)+SA(25µM)

3.3. Growth conditions: Healthy and uniform sized seeds of all varieties were surface sterilized with 1% sodium hypochlorite solution for ten minutes, followed by repeated washings with double distilled water (DDW). The sterilized seeds were sown in earthen pots to create nursery. At 20 days after sowing (DAS), seedlings were subsequently transplanted to the maintained pots, filled with soil and farmyard manure (6:1). Irrigation was done by using tap water as and when required.

3.4. Treatments and harvest:

3.4.1. Salt treatments: 35-days-old seedlings were supplied with 0, 50, 100, 150 and 200 mM NaCl solution for six days in order to develop the required salinity level. Thereafter,

plants were irrigated with tap water only when necessary. Each treatment was replicated three times with three plants each time.

3.4.2. Salicylic acid (SA) Application: Salicylic acid (SA) was procured from sigma Aldrich Chemicals Pvt. Ltd., India. A solution of SA (25µM) was prepared by dissolving required quantity of SA in 5ml of ethanol in a 100 ml volumetric flasks, and the final volume was made up to the marked by using DDW. Tween-20 was added prior to the treatment.

The foliage of each plant was sprayed thrice at 7, 14 and 21 days after salt treatments. The nozzle of the sprayer was adjusted in such a way that it pumped out 1 ml (approx) in one sprinkle. Therefore, each foliage of plants received 3 ml SA solution. The plants in all the sets were harvested at 60-days stage of growth to assess various growth parameters and yield. These observations recorded in 3 replicates.

The plants were allowed to grow, to be assessed at 60 DAS for various growth, biochemical and quality parameters. The fully expanded third leaves of each plant were harvested, immediately frozen in liquid nitrogen, and stored at -80ºC until required for analysis. The experiment was conducted under completely randomized block design.

3.5. OBSERVATIONS UNDER CONSIDERATION

- **Analysis of growth and yield contributing parameters -**
  - Plant height (cm), 60 DAS
  - Number of leaves per plant, 60 DAS
  - Number of branches per plant, 60 DAS
  - Number of fruits per plants
  - Fruit-diameter (cm)
  - Yield per plant (g)
- **Analysis of Biochemical parameters**-
  - Chlorophyll a
  - Chlorophyll b
  - Total chlorophyll content
  - Carotenoids
  - Total protein content
  - Estimation of Proline
- **Analysis of enzymatic antioxidant**s -
  - Superoxide Dismutase (SOD)

- Peroxidase (POD)
- Catalase
- Glutathione reductase

- **Analysis of Stress parameters:**

- Relative water content
- Electrolytic leakage

- **Analysis of quality parameters-**

- Lycopene (mg/100g)
- ß-carotene (mg/100g)
- pH (mg/100g)
- total sugars (mg/100g)
- Hydrogen peroxide (mg/100g)
- Total phenol (mg/100g)

3.6. Methods for growth and yield contributing parameters -

3.6.1. Morphological traits:

Length of shoot was measured by using a meter scale and number of leaves counted per plants after 60 DAS for getting the morphological data. Number of branches/plant, number of fruits/plant also counted. The fruit diameter was measured weekly with the aid of a Vernier calliper. The yield per treatment was recorded by weighing and counting the total number of fruits per treatment at the time of harvest.

3.6.2. Biochemical parameters

3.6.2.1. Estimation of total chlorophyll content

Chlorophyll pigments viz., chlorophyll 'a', chlorophyll 'b' and total chlorophyll in leaves were determined by dimethyl sulfoxide method (DMSO) of **Hiscox and Israelstam (1979).** Top fully expanded leaf was brought from the field in polythene bags, kept in ice box and it was cut into small pieces. 100 mg of leaf was kept in test tubes containing 7 ml of dimethyl sulfoxide (DMSO). The test tubes were incubated at 650^0C for 30 minutes. Leaf residue was removed by decanting the solution and final volume was made to 10 ml with DMSO. The absorbance of the extract was measured at 645 and 663 nm in a UV-Vis spectrophotometer (Elico, CL-54) and a blank was run using DMSO.

The total chlorophyll content was calculated by using the following formula and expressed in mg g fresh weight-1;

$$\text{Chlorophyll 'a'} = 12.7 \times (A_{663}) - 2.69 \times (A_{645}) \times \frac{V}{1000 \times w \times a}$$

(mg g^{-1} fr.wt.)

$$\text{Chlorophyll 'b'} = 22.9 \times (A_{645}) - 4.68 \times (A_{663}) \times \frac{V}{1000 \times w \times a}$$

(mg g^{-1} fr.wt.)

$$\text{Total chlorophyll} = 20.2 \times (A_{645}) + 8.02 \times (A_{663}) \times \frac{V}{1000 \times w \times a}$$

(mg g^{-1} fr.wt.)

where,

A_{645} = Absorbance of the extract at 645 nm

A_{663} = Absorbance of the extract at 663 nm

a = Path length of cuvette (1 cm)

V = final volume of the chlorophyll extract (10 ml)

w = Fresh weight of the sample (0.10 g)

3.6.2.2. Estimation of carotenoid:

The method of **Hiscox and Israelstam (1979)**was used to estimate carotenoid content. Freshleaves weighing of 0.05 gm were taken and homogenized in 5 ml of DMSO and incubated at65 for 3 hours. The absorbance was recorded at 470 nm.

Related formula for calculation:

$$\textbf{Total carotenoids} = A_{470} \times (3.27 \times \text{chl a} + 104 \times \text{chl b}) \times \frac{V}{1000 \times w}$$

V = final volume of the chlorophyll extract (10 ml)

w = Fresh weight of the sample (0.05 g)

3.6.2.3. Leaf relative water content: Leaf relative water content (LRWC) was calculated based on the methods from **Yamasaki and Dillenburg (1999).** Leaves were always collected from mid-section of plant in order to minimise age effects. Individual leaves were first removed from stem and then weighed to obtain fresh mass (FM). In order to determine the turgid mass (TM), leaves were floated in distilled water inside a closed petri dish. During the

imbibition period, leaf samples were weighed periodically, after gently wiping the water from the leaf surface with tissue paper. At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80°C for 48 h, in order to obtain dry mass (DM). All mass measurements were made using an analytical scale, with precision of 0.0001g. Values of FM, TM and DM were used to calculate LRWC using the equation below:

LRWC (%) = [(FM–DM)/(TM–DM)] X 100

3.6.2.4. Electrolyte leakage: Electrolyte leakage was used to assess membrane permeability according to **Lutts *et al.*, (1996).** Electrolyte leakage was measured using an Electrical Conductivity Meter (EC). Leaf samples of two plant per replicate were taken and cut into 1cm segments. The samples were then placed in individual stoppered vials containing 10 ml of distilled water after three washes with distilled water to remove surface contamination and incubated at room temperature (25ºC) on a shaker (100 rpm) for 24 h. Electrical conductivity (EC) of bathing solution (EC1) was recorded after incubation. The same samples were then placed in an autoclave at 120ºC for 20 min and second reading (EC2) was taken after cooling the solution to room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed as percentage.

3.6.2.5. Total protein content: Protein was estimated from seeds using Lowry's method **(Lowry *et al.*, 1951)**.

Reagents Preparation: 1. Reagent A Prepared by dissolving 2% sodium carbonate in 0.1 N sodium hydroxide.

2. Reagent B Prepared by dissolving equal amount of 1% copper sulphate and 2.0% of potassium sodium tartarate.

3. Reagent C Prepared by mixing 50 ml reagent A and 1 ml reagent B and it was freshly prepared before use.

4. Reagent D Folin-Ciocalteu reagent available commercially was used after 1:1, v/v dilution with distilled water.

5.Protein standard Prepared by weighing 100 mg bovine serum albumin (BSA) and dissolving it in distilled water, thereafter, the volume was made upto 100 ml by distilled water. Final concentration was made 1 mg/ml.

Procedure: Estimation of protein was done by pipetting out 50 µl supernatant containing proteins into test tubes in replicates of three and the total volume was made up to 1 ml. A tube with 1 ml distilled water served as a blank. 3 ml reagent C was added to each tube including the blank and after proper mixing the solutions were allowed to stand for 30 min then 0.5 ml reagent D was added and after mixing, the tubes were left at room temperature in the dark for 60 min. Blue colour developed in the solution. The absorbance was taken at 660 nm in UV-visible spectrophotometer. With the help of the standard curve the amount of protein in tomato genotypes was estimated.

3.6.2.6. Determination of Proline: The proline content in fresh leaves was determined according to **Bates *et al.* (1973)** methods. The substance was extracted in sulfosalicylic acid for which an equal volume of glacial acetic acid and ninhydrin solutions were added. The sample was heated at 100ºC, to which 5 mL of toluene were added after cooling. The absorbance of the toluene layer was read at 528 nm on a spectrophotometer.

3.7.3. Antioxidant Enzyme Assays:

Preparation of Tomato Leaf Tissues

To determine antioxidative enzyme activity and total protein amount, 1 gram of fresh weight of tomato leaf tissues was homogenized for two minutes in 20 ml of cold 100 mM potassium phosphate buffer (pH 6.8) containing 0.1 g PVPP using a Waring blender. The homogenate was filtered through four layers of nylon cloth and samples were centrifuged at 35.000 X G for 20 minutes with all steps performed at +4°C. The supernatant was collected as clear phase into a tube and was kept in an ice bath.

3.7.3.1. Superoxide Dismutase (SOD) (EC 1.15.1.1):

SOD activity was determined as described by **(Bayer and Fridovich, 1987)** by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT). The assay mixture contained 1 ml of enzyme extract, 0.1 mM phosphate buffer (pH 7.5), 3 mM NBT and 60 mM riboflavin. The tubes were thoroughly shaken and placed under 15W fluorescent lamp for 10 min, then the lights were switched off and the tubes were covered with a black cloth. For the purpose of blank, the non-illuminated reaction mixture was used. Absorbance of the reaction mixture was read at 560 nm and 1U of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate. The results were expressed as U mg $^{-1}$ protien.

3.7.3.2. Catalase (CAT) (EC 1.11.1.6) Activity: CAT activity was measured following the method of **(Aebi, 1984).** The assay mixture contained 0.1 ml of enzyme extract, 0.1 mM

phosphate buffer (pH 7.5), 0.1 M EDTA and 0.3% H_2O_2 and the absorbance was measured at 240 nm. CAT activity was expressed as µmol H_2O_2 g F.W. For the calculation of CAT activity, the extinction coefficient of 0.036 mM cm was used.

3.7.3.3.Peroxidase (POD)(EC1.11.1.7) Peroxidase activity was determined by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm **(Ghanati *et al.*,2002)**. The reaction mixture contained 100 µL crude enzyme, 500 µL $H_2O_2$5 mm, 500 µL guaiacol 28 mm and 1900 µL phosphate buffer 60 mm (pH 7.0). Peroxidase activity of the extract was expressed as µmol tetraguacial $min^{-1}mg^{-1}$protein.

3.7.3.4. Glutathione Peroxidase (GPX) (EC 1.6.4.2) Activity: GPX was assayed as described by **(Foyer and Halliwell, 1976)**, with minor modifications.The reaction mixture (1.0ml) consisted of 100mM phosphate buffer (pH 7.8), 0.1µM EDTA, 0.05mM NADPH, 3.0mM GSSG and 50µL enzyme extract. The reaction was started by the addition of GSSG and the NADPH oxidation rate was monitored at 340nm for 1.0 min. Enzyme activity was expressed as µmol NADPH oxidation g F.W.

3.7.4.Estimation of quality parameters: For estimation of nutraceuticals qualities, twenty fruits in each replication were harvested.

3.7.4.1.Determination of lycopene: Lycopene was extracted from tomato samples after homogenization of whole fruit sample in a blender (Braun Multiquick, MR-400, Spain). One gram of the homogenized sample was weighted into screw top glass tube covered with aluminium foil to exclude light and the lycopene from the sample was extracted using the method **Sadler *et al.*, (1990).**For that, 25 mL of hexane-acetone—ethanol (2:1:1: V:V:V) was added to the samples, which were then placed on the rotary mixer for 30 min. Agitation was continued for another 2 min. after adding 10 ml of distilled water. The solution was then left to separate into distinct polar and nonpolar layers, and the absorbance of the hexane layer was measured at 503 nm on a spectrophotometer (Simadzu 1601 UV Vis spectrophotometer). The purity of extracted standard lycopene was checked using its extinction coefficient (E 1% 1 cm of 3450-**Davis, 1976**)**,** and a standard curve was prepared. The amount of lycopene in the tomato samples was determined from this standard curve, and the results were expressed as mg/100g FW.

$$\text{mg of lycopene per 100g} = \frac{3.1206 \times OD \times \text{Volume makeup} \times \text{Dilution} \times 10}{1 \times \text{Weight of sample} \times 1000}$$

3.7.4.2. Determination of beta carotene: Beta carotene from fruit sample were extracted using the method **(Sadler *et al.*, 1990)**. One gram homogenized samle was extracted with acetone then filter with whatmann No.2 filter paper. Filtrate was then placed on the rotary mixer for 30 min. Agitation was continued for another 2 min after adding 10 ml of 60-80% petroleum ether. The solution was then left to separate into distinct colour and colorless layers, and absorbance of the colored layer was measured at 452 nm on a spectrophotometer (Simadzu 1601 UV *Vis* spectrophotometer).

$$\text{Mg of carotene per 100 g} = \frac{3.857 \times OD \times \text{Volume makeup}}{\text{Weight of sample}} \times 100$$

3.7.4.3. Determination of total sugar: The total soluble sugars was the determined according to thephenol-sulphuric method **(Dubois *et al.*, 1956)**.

3.7.4.4. Determination of pH: The pH of sample was determined by using a microprocessor based pH tester (Eutech Instruments Pvt, Ltd. Singapore). The pH meter was dipped inside the pulp for few seconds and the stabilized pH reading was recorded. Before every observation, the bulb of the pH meter was washed with running tap water and then with distilled water to eliminate any residual effect.

3.7.4.5. Determination of total phenol: Total phenols were estimated according to procedure given by **Singleton and Rossi, (1965).** 5g sample was extracted with 50 ml 80% methanol. The extract (0.5 ml) were taken in test tubes and were added with 0.2 ml of Folin and Ciocalteau's Phenol Reagent. To that, 3.25 ml of distilled water was added and all the tubes were sheken well. Then , 1 ml of Sodium Carbonate (20%) solution was added to all the tubes and kept for incubation at room temperature for 30 min. The color so developed was read spectrophotometically at 700 nm. Standard curve was drawn using Gallic acid as standard. Different concentrations of Gallic acid were prepared and OD was read at 700 nm. The concentration of sample was calculated based on the standard curve.

$$\text{mg Gallic acid} = \frac{\text{Equivalence} \quad OD \times \text{Factor} \times \text{Volume makeup} \times 100}{\text{Weight of sample} \times 1000}$$

3.7.4.6. Determination of hydrogen peroxide:

Hydrogen peroxide (H_2O_2) concentration was determined according to **Loreto and Velikova (2001)**. Leaf samples of0.5 g were homogenized in 3 mL of 1% (w/v) tri-chloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm and 4°C for10 min. Subsequently,

0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphatebuffer (pH 7.0) and 1.5 mL of 1M KI. H_2O_2 concentration of the supernatant was evaluated by comparing its absorbance at 390 nm to a standard calibration curve. The concentration of H2O2 was calculated from a standard curve plotted in therange from 100 to 1000 µmol mL^{-1}. H2O2 concentration was expressed as µmol g^{-1} DW.

3.8. Statistical Analysis:

The experimental design was a Randomized Complete Block Design (RCBD) with three replications. The data obtained from three successive seasons were pooled and analysed usingWindostat Version 9.2 from indostat services, HYDERABADstatistical software. Two-wayANOVA was applied to evaluate the significantdifference in the parameters studied in thedifferent treatments. Least significant difference(Fisher's protected LSD) was calculated,following significant F-test (p=0.05).The analysis of variance technique was applied for drawing conclusions from the data **(Fisher and Yates, 1968).**

SKELETON OF ANOVA TABLE:

Table: 3.2.

Source of variation	**d. f.**	**S.S.**	**M. S. S.**	**F (cal)**	**F (tab)at 5%**	**Results**
Due to replications	(r-1)	R. S. S.	$\frac{R.S.S}{(r-1)}$ =M.S.S.R	$\frac{M.S.S.R}{M.E.S.S}$	F (r-1)	S/NS
Due to treatments	(t-1)	T. S. S.	$\frac{T.S.S}{(t-1)}$ =M.T.S.R	$\frac{M.T.S.S}{M.E.S.S}$	E(r-1) (t-1)	S/NS
Due to error	(r-1) (t-1)	E.S. S.	$\frac{E.S.S}{(r-1)\ (t-1)}$ =M.E.S.S	-	F (t-1), (r-1) (t-1)	-
Total	(r t-1)	T.S.S	-	-	-	-

To calculate the critical difference, standard error of difference between means (S. Ed.) has to be calculated, with the help of the following formula:

$$S.E\ (d)\ \ for\ treatment = \sqrt{\frac{2 \times MESS}{r}}$$

C.D. at 5% = S.E.d × $t_{(5\%)}$ edf.

Where,

d. f. = degree of freedom

S. S. = sum of square

M.S.S = Mean sum of square

F. (cal) = Calculated value of 'F'

F. (tab) = Table value of 'F'

R. S. S. = Sum of square due to replicate

E. S. S. = Error sum of squares

T. S. S. = Total sum of squares

M. R. S. S = Mean sum of squares (Replication)

S. S. T. = Sum of square due to treatment

MESS = Mean sum of square due to error

MSST = Mean sum of squares (Treatment)

Chapter Four

Results and Discussion

RESULTS AND DISCUSSION

The present investigation entitled "**Effects of salicylic acid on growth and biochemical characters of salt stressed tomato varieties**" was conducted to study the effects of salicylic acid on the growth, biochemical parameters and yield of salt stressed tomato varieties. The various outcomes during the course of investigation have been portrayed in the form of different tables and figures, are described and discussed in this chapter.

4.1. Morphological parameters in tomato genotypes:

This experiment was conducted to study differences in salt tolerance among different tomato genotypes and also the effect of salicylic acid alone and in combination with the different treatments of salt (NaCl).

4.1.1. Plant Height (cm):

It was evident from the table-4.1 that there were significant differences (at $P < 0.01$) for plant height (cm) among the treatments (NaCl salinity levels), tomato genotypes and interaction between salinity treatments and genotypes were also significant.

Interactions between salinity levels and genotypes presented in table–4.1 showed that all genotypes decreased plant height compared to control as salinity stress was increased from 50 mM to 200 mM. The genotype H-8878-1 showed the maximum plant height at control and it was followed by Arka marginal and WIR-13706 but the differences between both of them were non-significant whereas H-24 produced the minimum plant height at control. The maximum decrease was observed at 200 mM Salt stress in H-8878-1, whereas, the minimum decrease was shown by the variety Roma at the highest level of NaCl followed by H-24. Interaction of genotypes with salicylic acid showed great positive results as well as the interaction of genotype within the combination of salicylic acid and different levels of salt stress overcame the effect of salt on genotypes.

The tomato genotype H-24 showed the minimum height at control followed by the varieties Roma, VRT-2, Ec-520078, Ec-520061 and WIR-3957. These varieties also showed a gradual decrease at the highest level of salinity stress for height.

Tomato varieties WIR-3928, WIR-13706, Arka marginal, Agata-30 and WIR-3928 showed greater plant-height at control. These varieties showed higher reduction in their height at the maximum level of salinity stress. They also showed very good responce at the treatment of salicylic acid (25 µM) alone and also in the combination of different level of salt stress.

Table: 4.1. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on plant-height (cm) in different tomato varieties, after 60 days of salt treatment.

plant height (cm)											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	36.66	39.83	35.83	34.80	33.26	32.80^{ab}	38.00	37.43	36.60	35.20	36.04
WIR-13706	33.06	36.73	32.80	31.86	30.20	29.50^{abcd}	35.83	34.66	32.96	31.53	32.91
Anigoarlentha	28.60	31.46	25.96	25.00	26.70	44.10^{a}	30.76	29.33	27.80	26.00	29.57
Ec-521080	22.93	25.50	20.96	18.50	16.36	14.56^{d}	24.233	23.70	22.83	21.36	21.09
Arka marginal	26.93	29.96	25.96	23.56	21.43	18.46^{bcd}	28.700	27.50	26.43	25.50	25.44
Ec-520079	29.63	32.90	28.33	26.60	24.43	23.20^{bcd}	31.733	30.83	31.36	30.10	28.91
WIR-3957	29.23	36.73	28.63	27.76	26.36	23.90^{bcd}	35.36	34.33	33.43	25.36	30.10
P-6 chhu chara	25.43	28.20	24.40	23.56	21.63	19.60^{bcd}	27.80	26.73	25.96	24.40	24.77
VRT-2	25.23	34.73	23.73	21.50	19.13	15.66^{cd}	33.63	32.00	30.86	29.30	26.57
H-24	33.40	39.60	32.33	31.96	30.43	29.56^{abcd}	38.30	37.53	35.80	33.90	34.28
Roma	26.06	29.46	23.00	22.93	21.26	19.33^{bcd}	28.43	27.53	26.20	24.90	24.91
Ec-520078	30.23	42.46	29.36	27.10	26.83	24.00^{bcd}	41.96	40.66	38.56	36.86	33.80
T-Loeal	26.00	30.10	33.20	36.00	38.73	43.40^{a}	29.20	32.20	34.56	41.00	34.43
Arka Saurabh	34.83	39.70	33.16	32.03	27.03	30.63^{abc}	33.93	32.80	31.56	29.53	32.52
H-86	29.20	32.66	29.03	28.36	26.46	24.30^{bcd}	31.90	31.03	30.03	28.33	29.13
Ec-520061	24.73	29.96	24.66	21.83	19.83	17.93^{bcd}	28.63	27.06	26.23	25.06	24.59
DT-10	26.73	33.16	25.00	24.50	23.73	20.80^{bcd}	32.70	31.40	31.13	30.70	27.98
Agata-30	29.43	35.20	29.13	28.70	27.83	26.06^{bcd}	33.20	32.93	31.36	30.76	30.46
WIR-13708	33.83	42.76	33.56	33.20	31.93	30.60^{abc}	39.06	38.33	37.46	36.80	35.75
WIR-3928	19.80	28.46	31.90	34.73	37.80	42.43^{a}	29.00	31.30	34.60	40.26	33.02
Gen. Mean	**28.60**	**33.98**	**28.55**	**27.72**	**26.57**	**26.54 ****	**32.62**	**31.96**	**31.29**	**30.34**	
C.V.	28.43	26.06	30.94	28.81	34.60	35.54	27.53	23.05	26.90	32.54	
F Prob.	0.66	0.52	0.81	0.30	0.26	0.00	0.74	0.43	0.69	0.54	
S.E.M.	4.69	5.11	5.10	4.61	5.30	5.44	5.18	4.25	4.86	5.70	
C.D. 5%	_	_	_	_	_	15.59	_	_	_	_	
C.D. 1%	_	_	_	_	_	20.88	_	_	_	_	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

4.1.2. Number of leaves per plant:

In relation to leaves numbers, data in table-4.2 clearly indicated that salt stress at 200 mM level led to marked reduction in the leaves numbers of plants. On the other hand applications with salicylic acid (SA) at 25 µM concentration enhanced leaves numbers, and it was the maximum in Ec-521080 followed by Anigoarlentha and WIR-3957. The minimum value for leaves number was recorded in VRT-2 followed by H-24, Roma, Agata-30 and WIR-13708 at the highest salinity level as compared to control plants. WIR-3928, DT-10, T-Loeal and Ec-520078 showed the maximum number of leaves at the concentration of salicylic acid (25 µM) alone and also at the concentration of 200 mM (salt) + SA (25 µM).

Results showed that there was a dramatic decrease in number of leaves with the increasing concentration of NaCl. A negative relationship was detected between salt concentration in irrigation water and number of leaves (Table-4.2).

Application of salicylic acid imposed a positive result for number of leaves per plant which is directly related to the yield. Results showed the maximum recovery in number of leaves at the treatment of salicylic acid in combination with different levels of salt stress.

4.1.3. Number of branches per plant:

The results of analysis of variance showed that (Table-4.3) salicylic acid affected the number of branches significantly and also the different levels of salt stress affected the number of branches significantly but the interaction effect of both salt and salicylic acid on this one of the most important morphological parameters which directly affect the final yield was also significant. Number of branches was reduced by increase of salt stress levels as in stress level of salt 150mM and 200mM in the comparison with control plants but there were no considerable changes during the stress level of salt 50 mM. Exogenous application of salicylic acid (25 µM) increased considerable positive results comparison with control level (Table-4.3).

Genotypes VRT-2 and H-24 showed the minimum value of number of branches per plant at 200 mM concentration and the genotype H-8878-1 showed the maximum value of number of branches per plant at 200 mM. Genotype VRT-2 showed the minimum value at 200 mM (salt) + SA (25 µM) concentration.

Table: 4.2. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on number of leaves/plant in different tomato varieties, after 60 days of salt treatment

Number of Leaves/Plant											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	7.0	9.0^{bcd}	6.0	5.6	4.6	8.6	7.0	5.3	5.0	7.0	6.5
WIR-13706	8.0	10.6^{ab}	7.0	5.6	4.3	9.0	7.0	6.0	4.6	8.0	7.0
Anigoarlentha	8.3	10.6^{ab}	7.3	7.0	5.6	9.3	8.0	6.3	5.6	8.3	7.6
Ec-521080	8.6	10.6^{ab}	7.6	7.3	6.0	9.6	8.3	7.3	6.0	8.6	8.0
Arka marginal	6.0	9.3^{abcd}	6.3	5.0	4.6	8.0	6.3	6.0	5.0	6.0	6.2
Ec-520079	7.0	9.3^{abcd}	6.3	5.6	5.0	8.6	6.6	6.0	5.0	7.0	6.6
WIR-3957	8.3	10.3^{abc}	7.33	6.6	6.0	8.3	7.6	7.0	7.0	8.3	7.6
P-6 chhu chara	7.6	9.3^{abcd}	7.00	6.3	5.3	9.0	7.6	6.6	5.6	7.6	7.2
VRT-2	6.0	8.3^{cd}	5.3	4.6	4.0	7.3	6.0	5.6	6.0	6.0	5.9
H-24	6.3	8.0^{d}	6.0	5.3	4.3	7.6	7.0	5.0	5.0	6.3	6.0
Roma	6.6	9.0^{bcd}	5.6	5.0	4.0	7.6	6.3	5.6	5.0	6.6	6.1
Ec-520078	7.0	11.0^{ab}	6.3	5.6	5.0	8.3	7.6	7.0	5.3	7.0	7.0
T-Loeal	7.3	11.3^{a}	6.6	6.3	6.0	8.0	8.0	6.6	5.6	7.3	7.3
Arka Saurabh	6.6	10.6^{ab}	6.3	5.6	5.0	8.0	7.0	5.6	6.0	6.6	6.7
H-86	7.6	9.6^{abcd}	7.0	6.0	5.6	9.0	7.0	6.0	5.6	7.6	7.1
Ec-520061	7.0	9.0^{bcd}	6.0	5.6	5.3	8.0	7.0	7.0	4.6	7.0	6.6
DT-10	7.0	11.0^{ab}	7.0	6.0	4.6	8.6	7.3	6.6	4.6	7.0	7.0
Agata-30	6.3	9.0^{bcd}	5.6	5.0	4.0	7.6	6.3	5.6	5.0	6.3	6.1
WIR-13708	7.0	9.6^{abcd}	6.3	6.0	5.0	7.6	7.6	6.0	4.6	7.0	6.7
WIR-3928	8.0	11.3^{a}	7.0	6.0	5.0	9.6	8.0	5.6	5.6	8.0	7.4
Gen. Mean	**7.2**	**9.8 ***	**6.5**	**5.8**	**4.9**	**8.4**	**7.2**	**6.1**	**5.3**	**7.2**	
C.V.	18.9	12.8	20.2	16.5	17.8	19.4	14.8	17.5	17.8	18.9	
F Prob.	0.4	0.04	0.8	0.1	0.08	0.0	0.5	0.6	0.4	0.4	
S.E.M.	0.7	0.7	0.7	0.5	0.5	0.4	0.7	0.7	0.6	0.7	
C.D. 5%	_	2.0	_	_	_	_	_	_	_	_	
C.D. 1%	_	2.8	_	_	_	_	_	_	_	_	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant

Table: 4.3. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on number of branches/plant in different tomato varieties.

Number of branches/plant											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+ SA(25µM)**	**100mM(salt) +SA(25µM)**	**150mM(salt) +SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	19.3^{a}	23.3^{a}	18.6^{a}	18.0^{a}	17.0^{a}	15.0^{a}	20.3^{a}	19.3^{a}	18.3^{a}	16.0^{a}	18.5
WIR-13706	18.6^{ab}	22.0^{ab}	18.3^{a}	17.6^{a}	16.6^{a}	14.3^{a}	20.0^{ab}	19.6^{a}	18.3^{a}	15.6^{a}	18.1
Anigoarlentha	13.6^{cd}	17.6^{bcd}	13.0^{bcd}	12.3^{bc}	11.3^{bc}	10.3^{bc}	15.0^{bcd}	14.0^{bc}	13.0^{bc}	11.3^{b}	13.1
Ec-521080	13.0^{cd}	15.6^{cdef}	12.3^{bcd}	11.6^{bcd}	10.6^{bc}	8.6^{cde}	14.3^{cde}	13.3^{bcde}	12.3^{bcd}	10.0^{bc}	12.2
Arka marginal	9.3^{defgh}	12.3^{efghi}	9.0^{cdefg}	8.33^{defg}	7.33^{def}	6.0^{fghi}	10.3^{defgh}	10.3^{cdefghi}	9.6^{defg}	7.0^{efg}	8.9
Ec-520079	6.3^{gh}	9.3^{i}	6.0^{fg}	5.33^{g}	4.33^{f}	3.6^{jk}	7.6^{h}	7.3^{i}	6.0^{h}	5.0^{fg}	6.0
WIR-3957	7.3^{fgh}	10.6^{ghi}	6.6^{fg}	6.00^{g}	5.00^{f}	4.0^{ijk}	8.3^{gh}	8.0^{hi}	7.0^{fgh}	5.3^{fg}	6.8
P-6 chhu chara	9.6^{defgh}	13.0^{defgh}	8.6^{defg}	8.00^{efg}	7.00^{def}	5.6^{ghij}	10.6^{defgh}	9.6^{efghi}	8.3^{efgh}	6.6^{efg}	8.7
VRT-2	6.6^{gh}	10.3^{hi}	6.0^{fg}	5.33^{g}	4.33^{f}	3.3^{k}	7.6^{h}	7.0^{i}	6.6^{gh}	4.66^{g}	6.2
H-24	6.0^{h}	9.3^{i}	5.6^{g}	5.00^{g}	4.33^{f}	3.3^{k}	7.3^{h}	7.0^{i}	6.3^{h}	5.0^{fg}	5.9
Roma	7.6^{efgh}	11.0^{fghi}	7.3^{efg}	6.66^{fg}	5.66^{ef}	4.6^{hijk}	9.0^{fgh}	8.3^{ghi}	7.0^{fgh}	5.3^{fg}	5.9
Ec-520078	8.3^{efgh}	12.0^{fghi}	7.6^{efg}	7.00^{fg}	5.66^{ef}	4.3^{ijk}	9.3^{efgh}	8.6^{fghi}	8.0^{efgh}	5.3^{fg}	7.6
T-Loeal	9.3^{defgh}	12.0^{fghi}	8.6^{defg}	8.00^{efg}	7.00^{def}	5.6^{ghij}	10.6^{defgh}	10.3^{cdefghi}	8.3^{efgh}	7.33^{def}	8.7
Arka Saurabh	10.6^{cdefg}	15.0^{cdefgh}	10.3^{bcdef}	9.66^{cdef}	8.33^{cde}	7.0^{efg}	12.0^{cdefgh}	11.3^{bcdefgh}	10.0^{cdef}	8.00^{cde}	10.2
H-86	11.6^{cdef}	15.3^{cdefg}	11.3^{bcde}	10.66^{bcde}	9.33^{cd}	8.0^{def}	13.0^{cdefg}	12.0^{bcdefg}	10.6^{bcde}	9.6^{bcd}	11.1
Ec-520061	9.3^{defgh}	12.0^{fghi}	9.0^{cdefg}	8.33^{defg}	7.33^{def}	6.6^{efgh}	10.6^{defgh}	10.0^{defghi}	8.6^{efgh}	7.3^{def}	8.9
DT-10	12.0^{cde}	15.0^{cdefgh}	11.3^{bcde}	10.66^{bcde}	9.33^{cd}	7.6^{defg}	13.3^{cdefg}	12.3^{bcdef}	10.6^{bcde}	7.3^{def}	10.9
Agata-30	13.6^{cd}	17.0^{cde}	13.3^{bc}	12.6^{bc}	11.3^{bc}	9.6^{bcd}	14.6^{cd}	13.6^{bcd}	12.6^{bcd}	10.6^{b}	12.9
WIR-13708	14.6^{bc}	18.0^{bc}	14.3^{ab}	13.6^{b}	12.6^{b}	11.0^{b}	16.0^{abc}	15.0^{b}	13.66^{b}	12.0^{b}	14.1
WIR-3928	13.0^{cd}	17.3^{bcd}	12.3^{bcd}	11.6^{bcd}	10.6^{bc}	9.6^{bcd}	13.6^{cdef}	13.3^{bcde}	12.3^{bcd}	11.0^{b}	12.5
Gen. Mean	**11.0 ****	**14.4 ****	**10.5 ****	**9.8 *****	**8.7 *****	**7.4 *****	**12.2 *****	**11.5 ****	**10.4 *****	**8.5 ****	
C.V.	24.8	19.8	25.1	22.2	22.5	18.9	25.0	20.5	18.6	17.5	
F Prob.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
S.E.M.	1.5	1.6	1.5	1.2	1.1	0.8	1.7	1.3	1.1	0.8	
C.D. 5%	4.5	4.7	4.3	3.6	3.2	2.3	5.0	3.9	3.2	2.4	
C.D. 1%	6.0	6.3	5.8	4.8	4.3	3.1	6.7	5.2	4.3	3.3	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

4.2. Physiological parameters:

4.2.1. Electrolyte Leakage:

Electrolyte leakage was used to assess membrane permeability. Addition of 150 mM NaCl into nutrient solution induced significant increases in electrolyte leakage (table-4.4). Electrolyte leakage of tomato leaves increased following the NaCl treatment, and it was higher in the plants treated with 200 mM NaCl concentration. Moreover, compared to NaCl treatment, salicylic acid treatment resulted in a decrease in electrolyte leakage by significant value in all the genotypes (table-4.4). The genotype Arka Saurabh showed the maximum value of electrolyte leakage at the highest salinity level. The variety WIR-13708 showed the minimum value of electrolyte leakage at the treatment of salicylic acid of concentration of 25 µM while the variety Arka marginal showed the maximum value of electrolyte leakage at the treatment of salicylic acid of concentration of 25 µM. The tomato genotypes VRT-2, WIR-13706, Anigoarlentha, Ec-521080, Roma and H-24 showed a moderate increase in electrolyte leakage at the highest salinity level while the tomato varieties H-8878-1, WIR-3957, H-86 showed greater increase in electrolytic leakage at 200 mM NaCl concentration as compared to control.

The effect of SA alone and in combination with different concentration of NaCl was studied on electrolytic leakage in different tomato varieties. The result showed that application of salicylic acid alone showed a positive response on electrolyte leakage in different tomato varieties. Result also showed that there was a dramatic increase in electrolytic leakage with the increasing concentration of NaCl. Application of 25µM salicylic acid exhibited positive response on electrolytic leakage in different levels of salt stress in tomato varieties. Therefore, in general salicylic acid was notified to overcome the adverse effect imposed by salt stress.

4.2.2. Leave Relative Water Content (LRWC):

LRWC was significantly decreased with increasing salinity levels (table-4.5). On the other hand, salicylic acid application either alone or with salinity increased LRWC as compared to saline conditions only. It was recorded that the genotype Arka Saurabh showed the maximum relative water content while the genotype DT-10 showed the minimum value of that in control. Dramatic reduction in leave relative water content was recorded at the NaCl concentration of 200 mM. The tomato genotype H-8878-1, H-24, WIR-3957, WIR-13706

and Anigoarlentha showed a very less effect of salinity at lower concentration but at highest level of salinity, it showed greater reduction in relative water content.

The effect of SA alone and in combination with different concentration of NaCl was studied on leave relative water content in different tomato varieties. Varietal mean for the leave relative water content at different concentration of NaCl, SA alone and in combination with different concentration of NaCl showed the maximum value in variety T-Loeal and that of the minimum value in the variety DT-10.

All such means, which share a common english letter, are not significantly different at $P<0.01$.

4.2.3. Protein content:

In the present study the minimum protein content was observed in genotype H-24 at the highest concentration of 200 mM NaCl and the highest values was recorded at 200 mM NaCl in the genotype WIR-13706 as compared to control. VRT-2, H-24 and Agata-30 showed lower protein content as compared to control at the highest salinity level. H-8878-1, WIR-13706 and showed greater increase in protein content at highest salinity level as compared to control. The high salt concentration showed a significant decrease in total protein content among all the genotypes of tomato plant. Data presented in (Table-4.6) indicated that salinity significantly increased leaf total soluble proteins content with increasing salinity concentration up to 200 mM NaCl compared to control treatment. The proteins at salt stress condition accumulate and act as osmotic regulator. Salinity stress increases amino acid content in tomato plant. Also, salicylic acid either alone or in combined with salinity levels significantly increased total soluble proteins and also.

Table: 4.4. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on electrolyte leakage in different tomato varieties.

Electrolyte Leakage (%)											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	28.10^{ghi}	24.83^{ghi}	30.26^{ghij}	33.50^{ef}	37.90^{fgh}	42.86^{efg}	26.33^{ef}	30.93^{fg}	34.90^{efgh}	40.76	33.04
WIR-13706	46.26^{abcd}	41.70^{abcd}	48.50^{abc}	50.76^{ab}	53.70^{ab}	57.53^{abc}	45.46^{ab}	49.06^{abc}	51.36^{ab}	56.16	50.05
Anigoarlentha	47.43^{abc}	43.93^{abc}	48.83^{abc}	51.20^{ab}	54.23^{ab}	58.56^{abc}	44.80^{ab}	48.03^{abc}	52.16^{ab}	55.43	50.46
Ec-521080	42.76^{abcde}	38.60^{abcdef}	44.53^{abcde}	47.13^{abcd}	50.16^{abcd}	53.30^{abcde}	40.46^{abcd}	45.33^{abcd}	47.26^{abcd}	51.03	46.06
Arka marginal	51.63^{ab}	46.83^{ab}	53.03^{ab}	55.40^{a}	58.20^{a}	61.73^{ab}	49.73^{a}	52.26^{ab}	55.86^{a}	60.16	54.48
Ec-520079	40.66^{bcdef}	36.56^{bcdefg}	41.86^{bcdef}	43.73^{bcde}	46.43^{bcdef}	50.76^{bcdef}	40.56^{abcd}	41.06^{bcdef}	44.30^{bcde}	48.76	43.47
WIR-3957	27.40^{ghi}	24.46^{ghi}	29.00^{hij}	35.53^{ef}	38.56^{efgh}	42.66^{efg}	31.20^{cdef}	32.533^{fg}	35.36^{efgh}	41.60	33.83
P-6 chhu chara	48.20^{abc}	43.70^{abc}	49.16^{abc}	52.10^{ab}	54.53^{ab}	57.63^{abc}	45.60^{ab}	48.93^{abc}	52.50^{ab}	56.167	50.85
VRT-2	25.90^{hi}	23.46^{hi}	27.70^{ij}	29.033^{f}	31.43^{gh}	34.63^{g}	24.06^{ef}	26.16^{g}	29.33^{gh}	32.63	28.43
H-24	29.80^{fghi}	26.83^{efghi}	31.23^{fghij}	33.80^{ef}	35.60^{fgh}	39.96^{fg}	28.33^{def}	30.43^{fg}	32.96^{gh}	37.66	32.66
Roma	45.53^{abcd}	42.23^{abcd}	47.16^{abcd}	48.93^{abc}	50.83^{abc}	54.96^{abcd}	43.20^{abc}	46.20^{abc}	48.60^{abc}	53.33	48.09
Ec-520078	30.26^{fghi}	26.66^{fghi}	32.53^{fghij}	35.60^{ef}	38.26^{fgh}	41.93^{efg}	28.66^{def}	32.10^{fg}	35.26^{efgh}	39.70	34.10
T-Loeal	38.56^{cdefg}	35.53^{bcdefgh}	41.03^{cdefg}	43.00^{bcde}	45.60^{bcdef}	50.63^{bcdef}	36.83^{bcde}	38.96^{def}	43.30^{bcdef}	48.13	42.15
Arka Saurabh	53.53^{a}	49.90^{a}	54.66^{a}	57.467^{a}	59.20^{a}	62.33^{a}	50.50^{a}	54.63^{a}	57.66^{a}	60.40	56.03
H-86	37.13^{cdefgh}	33.96^{cdefgh}	39.93^{cdefgh}	43.10^{bcde}	45.83^{bcdef}	50.13^{cdef}	35.93^{bcde}	39.83^{cdef}	44.13^{bcdef}	47.73	41.77
Ec-520061	44.76^{abcd}	40.16^{abcd}	46.90^{abcd}	48.40^{abcd}	50.93^{abc}	54.63^{abcd}	42.93^{abc}	44.50^{abcd}	49.43^{abc}	52.16	47.48
DT-10	32.83^{efghi}	30.33^{defghi}	35.26^{efghij}	37.53^{def}	39.56^{defgh}	42.36^{efg}	30.86^{cdef}	34.36^{defg}	37.33^{defg}	40.66	36.11
Agata-30	35.10^{defgh}	32.33^{cdefgh}	36.93^{defghi}	37.93^{cdef}	40.63^{cdefg}	43.83^{defg}	33.00^{bcdef}	34.33^{defg}	38.333^{cdefg}	41.03	37.34
WIR-13708	22.46^{i}	19.83^{i}	24.70^{j}	26.93^{f}	28.60^{h}	32.33^{g}	20.30^{f}	23.50^{g}	26.06^{h}	30.53	25.52
WIR-3928	42.03^{abcde}	38.83^{abcde}	44.90^{abcde}	47.20^{abcd}	49.46^{bcde}	52.23^{abcde}	40.20^{abcd}	43.93^{abcde}	46.56^{abcd}	49.90	45.52
Gen. Mean	**38.52*** **	**35.03*** **	**40.40*** **	**42.91*** **	**45.48*** **	**49.25*** **	**36.95*** **	**39.85*** **	**43.13*** **	**47.20**	
C.V.	18.43	21.00	17.31	16.03	14.86	14.18	20.94	17.94	15.69	14.68	
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
S.E.M.	4.10	4.24	4.04	3.97	3.90	4.03	4.46	4.12	3.90	4.00	
C.D. 5%	11.73	12.16	11.56	11.37	11.17	11.54	12.78	11.82	11.19	11.45	
C.D. 1%	15.72	16.29	15.49	15.23	14.96	15.46	17.13	15.83	14.98	15.34	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.5. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200mM) and different levels of NaCl & SA on leave relative water content in different tomato varieties.

Relative water content (%)											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	74.53^{ab}	81.63^{ab}	71.53^{ab}	68.40^{abc}	65.20^{abc}	58.03^{abc}	73.03^{abcd}	71.00^{abc}	68.96^{ab}	62.96^{abc}	69.52
WIR-13706	68.43^{abcd}	76.54^{abcd}	65.56^{abcd}	62.86^{abcde}	59.36^{abcde}	51.933^{abcdef}	69.26^{abcde}	65.80^{abcd}	71.90^{a}	53.36^{abcdef}	64.50
Anigoarlentha	60.80^{bcde}	73.93^{abcd}	57.26^{bcde}	54.16^{cdef}	51.26^{cdef}	43.90^{cdefg}	63.16^{cdef}	57.20^{cde}	54.03^{bcde}	45.73^{defg}	56.14
Ec-521080	60.30^{bcde}	68.05^{bcde}	58.40^{bcde}	55.06^{cdef}	50.33^{cdef}	45.367^{defg}	63.03^{cdef}	59.80^{cde}	53.30^{bcde}	47.93^{cdefg}	56.15
Arka marginal	66.76^{abcd}	75.36^{abcd}	63.93^{abcd}	60.70^{abcde}	57.20^{abcde}	50.56^{abcdef}	67.96^{abcde}	64.20^{abcd}	59.40^{abcd}	52.16^{bcdef}	61.82
Ec-520079	72.43^{abc}	79.76^{abc}	70.46^{ab}	67.20^{abcd}	62.36^{abcd}	55.96^{abcd}	75.50^{abc}	70.40^{abc}	64.56^{abc}	57.96^{abcd}	67.66
WIR-3957	70.70^{abc}	75.93^{abcd}	68.26^{abc}	65.56^{abcd}	61.86^{abcd}	54.20^{abcde}	73.06^{abcd}	68.76^{abcd}	64.16^{abc}	56.10^{abcde}	65.86
P-6 chhu chara	60.13^{bcde}	67.46^{bcde}	57.93^{bcde}	53.76^{cdef}	50.80^{cdef}	45.40^{cdefg}	62.83^{cdef}	59.50^{cde}	53.43^{bcde}	47.30^{cdefg}	55.85
VRT-2	67.10^{abcd}	74.43^{abcd}	64.76^{abcd}	62.53^{abcde}	58.66^{abcde}	52.03^{abcdef}	69.10^{abcde}	67.56^{abcd}	60.56^{abcd}	54.46^{abcdef}	63.12
H-24	53.96^{cde}	63.00^{cde}	50.93^{cde}	46.93^{ef}	43.86^{ef}	39.20^{efg}	55.56^{def}	52.06^{de}	46.30^{de}	41.66^{efg}	49.35
Roma	80.62^{a}	86.73^{a}	77.80^{a}	74.23^{ab}	68.70^{ab}	62.26^{ab}	81.60^{ab}	78.20^{ab}	71.83^{a}	64.73^{ab}	74.67
Ec-520078	53.93^{cde}	60.80^{de}	52.33^{cde}	50.13^{def}	46.43^{def}	40.03^{defg}	56.80^{def}	54.50^{cde}	49.00^{cde}	42.00^{defg}	50.59
T-Loeal	82.17^{a}	88.46^{a}	78.83^{a}	75.86^{a}	71.26^{a}	65.93^{ab}	85.03^{a}	80.26^{a}	73.33^{a}	68.56^{a}	76.97
Arka Saurabh	82.93^{a}	88.03^{a}	79.13^{a}	75.26^{a}	70.73^{a}	66.83^{a}	83.16^{a}	78.56^{a}	73.46^{a}	68.83^{a}	76.69
H-86	61.46^{bcde}	66.26^{bcde}	58.46^{bcde}	56.50^{bcdef}	52.40^{bcdef}	45.06^{cdefg}	64.06^{bcdef}	60.60^{bcde}	54.23^{bcde}	47.53^{cdefg}	56.66
Ec-520061	67.70^{abcd}	74.96^{abcd}	65.46^{abcd}	63.13^{abcde}	58.23^{abcde}	50.00^{bcdef}	70.26^{abcde}	67.00^{abcd}	60.66^{abcd}	52.03^{bcdef}	62.94
DT-10	47.92^{e}	55.16^{e}	43.93^{e}	40.63^{f}	37.16^{f}	32.40^{g}	48.00^{f}	45.26^{e}	41.00^{e}	34.06^{g}	42.55
Agata-30	51.47^{de}	60.46^{de}	49.13^{de}	49.26^{def}	43.56^{ef}	37.56^{fg}	54.26^{ef}	53.96^{cde}	46.00^{de}	39.60^{fg}	48.53
WIR-13708	67.40^{abcd}	72.83^{abcde}	64.86^{abcd}	60.93^{abcde}	55.90^{abcde}	50.70^{abcdef}	68.50^{abcde}	65.23^{abcd}	57.53^{abcde}	52.86^{abcdef}	61.67
WIR-3928	68.03^{abcd}	74.00^{abcd}	65.80^{abcd}	62.63^{abcde}	57.36^{abcde}	52.96^{abcdef}	68.93^{abcde}	67.33^{abcd}	60.46^{abcd}	55.40^{abcdef}	63.29
Gen. Mean	**65.94 ***	**73.19 ***	**63.24 ***	**60.29 ***	**56.13 ***	**50.01 ****	**67.65 ***	**64.36 ***	**59.20 ****	**52.26 ****	
C.V.	17.02	14.66	17.31	18.13	18.54	19.70	16.16	16.58	17.56	18.50	
F Prob.	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.00	
S.E.M.	6.48	6.19	6.32	6.31	6.00	5.69	6.31	6.16	6.00	5.58	
C.D. 5%	18.55	17.73	18.09	18.06	17.20	16.29	18.07	17.64	17.18	15.98	
C.D. 1%	24.84	23.75	24.24	24.20	23.04	21.82	24.21	23.63	23.02	21.41	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.6. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on Protein (mg/g fresh leaves) in different tomato varieties.

Protein (mg/g fresh leaves)											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	26.66	29.83	33.16	36.80	39.60	44.46	28.66	34.10	36.60	41.86	35.17
WIR-13706	33.06	36.73	39.80	42.86	46.53	49.16	34.83	40.00	42.96	47.20	41.31
Anigoarlentha	28.60	31.46	34.96	38.00	40.03	44.10	30.76	34.66	37.13	41.66	36.14
Ec-521080	22.93	25.50	29.30	33.50	37.36	41.23	26.23	30.36	33.83	38.36	31.86
Arka marginal	26.93	29.96	33.30	36.23	40.76	44.46	28.70	32.50	38.43	42.16	35.34
Ec-520079	29.63	32.90	37.33	39.93	42.10	45.53	31.73	36.50	39.36	42.43	37.74
WIR-3957	27.90	30.06	33.30	36.76	38.36	41.90	30.03	31.66	36.43	39.36	34.58
P-6 chhu chara	25.43	28.20	31.06	35.56	37.96	41.26	27.80	32.06	34.63	38.73	33.27
VRT-2	25.23	28.06	29.73	31.50	34.13	38.00	25.63	28.33	30.86	34.96	30.64
H-24	26.73	29.60	27.33	32.30	35.10	37.90	24.63	28.20	31.46	33.90	30.71
Roma	26.06	29.46	34.00	36.60	38.93	42.33	29.10	32.20	35.20	38.90	34.28
Ec-520078	30.23	32.46	36.03	38.76	40.16	42.66	31.96	34.00	36.23	40.20	36.27
T-Loeal	26.00	30.10	33.20	36.00	38.73	43.40	29.20	32.20	34.56	41.00	34.44
Arka Saurabh	31.50	34.03	38.16	41.70	43.70	47.30	33.93	37.46	40.23	44.53	39.25
H-86	29.20	32.66	34.70	38.36	41.13	43.63	30.90	35.70	38.36	42.66	36.73
Ec-520061	26.73	29.96	33.33	37.16	39.83	43.93	30.30	32.40	36.23	42.06	35.19
DT-10	29.73	32.16	36.66	39.83	42.06	46.13	31.36	35.40	38.13	43.36	37.48
Agata-30	23.43	27.20	29.80	33.36	35.50	38.06	26.53	29.60	31.36	39.10	31.39
WIR-13708	33.83	36.10	38.56	38.86	40.93	45.26	33.06	35.00	38.46	42.46	38.25
WIR-3928	25.13	28.46	31.90	34.73	37.80	42.43	29.00	31.30	34.60	40.26	33.56
Gen. Mean	**27.75**	**30.74**	**33.78**	**36.94**	**39.53**	**43.16**	**29.72**	**33.18**	**36.25**	**40.76**	
C.V.	30.25	26.09	22.17	19.34	18.08	15.66	23.25	20.97	18.81	17.12	
F Prob.	0.98	0.98	0.88	0.93	0.94	0.89	0.96	0.90	0.87	0.90	
S.E.M.	4.84	4.63	4.32	4.12	4.12	3.90	3.99	4.01	3.93	4.02	
C.D. 5%	_	_	_	_	_	_	_	_	_	_	
C.D. 1%	_	_	_	_	_	_	_	_	_	_	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

4.2.4. Chlorophyll contents:

Salinity stress gradually reduced, chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of tomato leaves (Table-4.7, 4.8 and 4.9). The effect of SA alone and in combination with different concentration of NaCl was studied on chlorophyll *a*, chlorophyll *b* and total chlorophyll contents in different tomato varieties. The result showed that application of SA alone showed a positive response on chlorophyll *a*, chlorophyll *b* and total chlorophyll contents in different tomato varieties. Result also showed that there was a dramatic decrease in all the pigment parameters *i.e.* chlorophyll *a*, chlorophyll *b* and total chlorophyll contents with the increasing concentration of NaCl. Application of 25 µM SA exhibited positive response on all the pigment parameters in different levels of salt stress in tomato varieties. Therefore, in general SA was notified to overcome the adverse effect imposed by salt stress. Positive results with the application of salicylic acid alone as well as in combination with different levels of salt stress imposed positive response on final yield which is directly related to the pigment system of plant. The tomato genotypes H-8878-1, Arka marginal, Ec-520079, P-6 chhu chara, VRT-2, Roma, Arka Saurabh, H-86 and WIR-3928 showed greater value of chl a at highest salinity level as copared to control. The maximum value of chl b was recorded in the tomato genotype P-6 chhu chara at the highest NaCl concentration while the minimum value was recorded in the tomato genotype WIR-3957 as compared to control.

4.2.5. Carotenoid contents:

The tomato genotypes Agata-30, H-24, T-Loeal, DT-10 and WIR-13708 showed highly increased value of carotenoid at the highest salinity level as compared to control while the minimum carotenoid content was recorded in the tomato genotypes WIR-3928, Ec-520061, Roma and Ec-520079 as compared to control. The maximum value of total chlorophyll was recorded in the tomato genotypes H-8878-1, Arka Saurabh, Arka marginal, P-6 chhu chara, Roma and VRT-2 at the highest level of salinity (table-4.10). All the genotypes showed positive response at the treatment of salicylic acid alone very well as well as at the treatment of salicylic acid in combination with different levels of salt stress.

Table: 4.7. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on chlorophyll a in different tomato varieties.

Chlorophyll- a (mg g-^{1}) Fresh weight											
varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt) +SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt) +SA(25µM)**	**V. mean**
H-8878-1	1.75^{abc}	1.81^{abcde}	1.71^{abc}	1.68^{abcd}	1.62^{bcde}	1.52^{bcd}	1.84^{ab}	1.72^{bcd}	1.65^{abcde}	1.56	1.68
WIR-13706	1.38^{fgh}	1.48^{ghi}	1.36^{fgh}	1.33^{fgh}	1.28^{ghi}	1.22^{fg}	1.46^{ef}	1.38^{fg}	1.33^{gh}	1.25	1.35
Anigoarlentha	1.54^{defg}	1.60^{fghi}	1.52^{defg}	1.58^{cde}	1.53^{cdef}	1.44^{cde}	1.59^{de}	1.63^{cde}	1.58^{cdef}	1.48	1.55
Ec-521080	1.36^{gh}	1.41^{hi}	1.33^{gh}	1.31^{gh}	1.26^{hi}	1.19^{fg}	1.41^{ef}	1.37^{fg}	1.31^{gh}	1.22	1.32
Arka marginal	1.73^{abcd}	1.82^{abcde}	1.72^{abc}	1.72^{abc}	1.65^{abcd}	1.54^{bcd}	1.77^{abcd}	1.76^{abc}	1.70^{abcd}	1.56	1.70
Ec-520079	1.73^{abcd}	1.82^{abcd}	1.71^{abcd}	1.67^{bcd}	1.61^{bcde}	1.54^{bcd}	1.76^{abcd}	1.71^{bcd}	1.66^{abcde}	1.58	1.67
WIR-3957	1.51^{efgh}	1.61^{efgh}	1.49^{efg}	1.46^{efg}	1.41^{fgh}	1.31^{efg}	1.57^{de}	1.51^{efg}	1.45^{fgh}	1.53	1.48
P-6 chhu chara	1.84^{ab}	1.92^{a}	1.82^{ab}	1.79^{ab}	1.75^{ab}	1.60^{abc}	1.88^{ab}	1.83^{ab}	1.80^{ab}	1.64	1.78
VRT-2	1.83^{ab}	1.88^{ab}	1.79^{ab}	1.76^{abc}	1.72^{abc}	1.70^{ab}	1.86^{ab}	1.79^{abc}	1.78^{abc}	1.73	1.78
H-24	1.38^{fgh}	1.49^{ghi}	1.36^{fgh}	1.33^{fgh}	1.28^{ghi}	1.21^{fg}	1.42^{ef}	1.38^{fg}	1.33^{gh}	1.25	1.34
Roma	1.81^{ab}	1.90^{ab}	1.79^{ab}	1.76^{abc}	1.73^{ab}	1.65^{ab}	1.83^{ab}	1.82^{abc}	1.78^{abc}	1.67	1.77
Ec-520078	1.32^{h}	1.40^{i}	1.29^{h}	1.26^{h}	1.22^{i}	1.16^{g}	1.35^{f}	1.33^{g}	1.28^{h}	1.21	1.28
T-Loeal	1.53^{efg}	1.63^{defg}	1.51^{efg}	1.48^{efg}	1.44^{efgh}	1.36^{def}	1.48^{ef}	1.51^{efg}	1.50^{efg}	1.41	1.48
Arka Saurabh	1.92^{a}	1.97^{a}	1.89^{a}	1.87^{a}	1.82^{a}	1.76^{a}	1.94^{a}	1.94^{a}	1.85^{a}	1.80	1.87
H-86	1.82^{ab}	1.91^{ab}	1.80^{ab}	1.78^{ab}	1.71^{abc}	1.60^{abc}	1.85^{ab}	1.81^{abc}	1.76^{abcd}	1.64	1.77
Ec-520061	1.66^{bcde}	1.72^{bcdef}	1.65^{bcde}	1.62^{bcde}	1.52^{def}	1.45^{cde}	1.69^{bcd}	1.65^{bcde}	1.57^{def}	1.49	1.60
DT-10	0.92^{i}	1.04^{j}	0.88^{i}	0.86^{i}	0.84^{j}	0.78^{h}	0.95^{g}	0.90^{h}	0.90^{i}	0.82	.89
Agata-30	1.57^{cdef}	1.67^{cdefg}	1.53^{cdef}	1.51^{def}	1.45^{efg}	1.36^{def}	1.61^{cde}	1.55^{def}	1.49^{efg}	1.40	1.51
WIR-13708	1.44^{fgh}	1.55^{fghi}	1.36^{fgh}	1.33^{fgh}	1.27^{hi}	1.20^{fg}	1.41^{ef}	1.38^{fg}	1.32^{gh}	1.24	1.35
WIR-3928	1.79^{ab}	1.84^{abc}	1.72^{abc}	1.69^{abcd}	1.60^{bcde}	1.54^{bcd}	1.81^{abc}	1.73^{bcd}	1.64^{bcdef}	1.57	1.69
Gen. Mean	**1.59 *****	**1.67 *****	**1.56 *****	**1.54 *****	**1.48 *****	**1.40 *****	**1.62 *****	**1.58 *****	**1.53 *****	**1.45**	
C.V.	7.65	7.39	7.26	7.49	7.51	8.37	7.70	7.42	7.65	8.50	
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
S.E.M.	0.07	0.07	0.06	0.06	0.06	0.06	0.07	0.06	0.06	0.07	
C.D. 5%	0.20	0.20	0.18	0.19	0.1	0.19	0.20	0.19	0.19	0.20	
C.D. 1%	0.27	0.27	0.25	0.25	0.24	0.26	0.27	0.26	0.26	0.27	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.8. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on chlorophyll b in different tomato varieties.

Chlorophyll- b (mg g-^{1}) Fresh weight											
varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	0.65	0.72	0.62	0.58	0.56^{abcde}	0.49^{bcde}	0.70	0.62	0.61^{abcde}	0.51	0.60
WIR-13706	0.64	0.72	0.61	0.57	0.52^{bcdef}	0.41^{def}	0.74	0.62	0.50^{def}	0.45	0.58
Anigoarlentha	0.60	0.67	0.57	0.53	0.50^{bcdef}	0.43^{cdef}	0.65	0.58	0.54^{bcdef}	0.47	0.55
Ec-521080	0.62	0.73	0.60	0.55	0.51^{bcdef}	0.46^{cdef}	0.68	0.62	0.57^{bcdef}	0.48	0.58
Arka marginal	0.70	0.81	0.68	0.62	0.59^{abc}	0.49^{bcde}	0.76	0.71	0.63^{abcd}	0.52	0.65
Ec-520079	0.64	0.70	0.62	0.59	0.53^{abcdef}	0.41^{def}	0.66	0.66	0.57^{bcdef}	0.44	0.58
WIR-3957	0.51	0.61	0.49	0.46	0.40^{f}	0.34^{f}	0.54	0.54	0.45^{ef}	0.37	0.47
P-6 chhu chara	0.83	0.88	0.80	0.74	0.69^{a}	0.63^{a}	0.85	0.78	0.74^{a}	0.67	o.76
VRT-2	0.63	0.74	0.59	0.55	0.50^{bcdef}	0.42^{def}	0.66	0.60	0.54^{bcdef}	0.44	0..56
H-24	0.74	0.79	0.72	0.67	0.63^{abc}	0.55^{abc}	0.80	0.71	0.68^{abc}	0.58	0.63
Roma	0.59	0.68	0.55	0.52	0.48^{cdef}	0.38^{ef}	0.63	0.56	0.52^{cdef}	0.40	0.53
Ec-520078	0.50	0.59	0.46	0.44	0.40^{ef}	0.35^{f}	0.57	0.47	0.44^{f}	0.38	0.46
T-Loeal	0.66	0.74	0.62	0.59	0.54^{abcdef}	0.44^{cdef}	0.69	0.64	0.59^{abcdef}	0.47	0.60
Arka Saurabh	0.71	0.78	0.68	0.65	0.58^{abcd}	0.49^{bcde}	0.73	0.69	0.63^{abcd}	0.51	0.64
H-86	0.67	0.77	0.65	0.61	0.55^{abcdef}	0.48^{bcde}	0.73	0.64	0.60^{abcdef}	0.52	0.62
Ec-520061	0.74	0.80	0.70	0.68	0.58^{abcd}	0.46^{bcdef}	0.77	0.75	0.61^{abcde}	0.50	0.66
DT-10	0.59	0.65	0.72	0.68	0.62^{abc}	0.520^{bcd}	0.77	0.66	0.67^{abc}	0.54	0.64
Agata-30	0.55	0.65	0.52	0.50	0.42^{def}	0.35^{f}	0.57	0.54	0.47^{ef}	0.38	0.49
WIR-13708	0.79	0.84	0.73	0.71	0.64^{ab}	0.58^{ab}	0.79	0.74	0.69^{ab}	0.62	0.71
WIR-3928	0.64	0.71	0.60	0.55	0.48^{bcdef}	0.40^{def}	0.66	0.61	0.53^{cdef}	0.43	0.56
Gen. Mean	**0.65**	**0.73**	**0.63**	**0.59**	**0.54 ***	**0.45 *** **	**0.70**	**0.64**	**0.58 ***	**0.48**	
C.V.	17.35	15.48	18.55	19.37	18.24	16.22	15.20	16.46	16.54	15.04	
F Prob.	0.07	0.22	0.09	0.13	0.04	0.00	0.06	0.08	0.02	0.00	
S.E.M.	0.06	0.06	0.06	0.06	0.05	0.04	0.06	0.06	0.05	0.04	
C.D. 5%	_	_	_	_	0.16	0.12	_	_	0.15	0.12	
C.D. 1%	_	_	_	_	0.21	0.16	_	_	0.21	0.162	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.9. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on total chlorophyll in different tomato varieties.

Total Chlorophyll (mg g^{-1}) Fresh weight											
varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt) +SA(25µM)**	**100mM(salt) +SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	2.40^{abcdef}	2.53^{abcde}	2.33^{abcd}	2.26^{abcde}	2.19^{abcd}	2.02^{abcd}	2.54^{abc}	2.34^{abcde}	2.26^{abcde}	2.07	2.29
WIR-13706	2.03^{defg}	2.21^{def}	1.97^{cdef}	1.91^{defg}	1.80^{efgh}	1.64^{ef}	2.20^{cde}	2.00^{def}	1.83^{gh}	1.70	1.93
Anigoarlentha	2.14^{cdefg}	2.27^{cdef}	2.09^{bcde}	2.12^{bcde}	2.04^{bcdef}	1.87^{bcde}	2.24^{bcde}	2.21^{bcde}	2.12^{bcdefg}	1.96	2.10
Ec-521080	1.99^{fg}	2.14^{ef}	1.94^{def}	1.86^{efg}	1.77^{fgh}	1.65^{ef}	2.10^{de}	1.99^{ef}	1.88^{fgh}	1.71	1.90
Arka marginal	2.44^{abcd}	2.63^{abcd}	2.40^{ab}	2.35^{abc}	2.24^{abcd}	2.03^{abcd}	2.53^{abc}	2.47^{ab}	2.33^{abcd}	2.08	2.35
Ec-520079	2.38^{abcdef}	2.53^{abcde}	2.43^{ab}	2.27^{abcde}	2.14^{abcde}	1.95^{abcde}	2.42^{abcd}	2.37^{abcde}	2.23^{abcdef}	2.03	2.27
WIR-3957	2.02^{efg}	2.22^{def}	1.98^{cdef}	1.92^{defg}	1.81^{efgh}	1.66^{ef}	2.12^{de}	2.06^{cdef}	1.91^{efgh}	1.90	1.96
P-6 chhu chara	2.68^{a}	2.80^{a}	2.62^{a}	2.53^{a}	2.44^{a}	2.24^{a}	2.73^{a}	2.61^{a}	2.54^{a}	2.31	2.55
VRT-2	2.46^{abc}	2.62^{abcd}	2.38^{ab}	2.31^{abcd}	2.22^{abcd}	2.12^{ab}	2.52^{abc}	2.39^{abcd}	2.32^{abcd}	2.18	2.35
H-24	2.13^{cdefg}	2.28^{cdef}	2.09^{bcde}	2.00^{cdef}	1.917^{defg}	1.76^{cdef}	2.22^{bcde}	2.10^{bcdef}	2.01^{cdefg}	1.83	2.03
Roma	2.40^{abcdef}	2.59^{abcd}	2.35^{abc}	2.28^{abcd}	2.21^{abcd}	2.07^{abc}	2.47^{abcd}	2.38^{abcde}	2.30^{bcd}	2.08	2.31
Ec-520078	1.82^{gh}	2.00^{fg}	1.75^{ef}	1.70^{fg}	1.63^{gh}	1.51^{fg}	1.92^{ef}	1.80^{fg}	1.89^{fgh}	1.59	1.76
T-Loeal	2.19^{cdefg}	2.37^{bcdef}	2.14^{bcde}	2.07^{cdef}	1.99^{cdefg}	1.81^{bcdef}	2.18^{cde}	2.15^{bcdef}	2.09^{cdefg}	1.88	2.29
Arka Saurabh	2.63^{ab}	2.76^{ab}	2.58^{a}	2.52^{ab}	2.40^{ab}	2.25^{a}	2.67^{a}	2.63^{a}	2.48^{ab}	2.31	2.52
H-86	2.50^{abc}	2.69^{abc}	2.45^{ab}	2.39^{abc}	2.26^{abc}	2.08^{abc}	2.59^{ab}	2.46^{ab}	2.36^{abc}	2.16	2.39
Ec-520061	2.40^{abcdef}	2.52^{abcde}	2.35^{abc}	2.30^{abcd}	2.11^{abcdef}	1.91^{bcde}	2.46^{abcd}	2.41^{abc}	2.19^{abcdefg}	1.99	2.26
DT-10	1.51^{h}	1.70^{g}	1.60^{f}	1.55^{g}	1.47^{h}	1.30^{g}	1.72^{f}	1.56^{g}	1.57^{h}	1.37	1.53
Agata-30	2.13^{cdefg}	2.32^{cdef}	2.06^{bcde}	2.01^{cdef}	1.88^{defg}	1.71^{def}	2.19^{cde}	2.09^{bcdef}	1.96^{efg}	1.78	2.01
WIR-13708	2.23^{bcdefg}	2.39^{abcdef}	2.09^{bcde}	2.04^{cdef}	1.91^{cdefg}	1.78^{cdef}	2.20^{cde}	2.12^{bcdef}	2.02^{cdefg}	1.86	2.06
WIR-3928	2.43^{abcde}	2.56^{abcde}	2.32^{abcd}	2.24^{abcde}	2.09^{abcdef}	1.94^{abcde}	2.47^{abcd}	2.34^{abcde}	2.17^{abcdefg}	2.00	2.25
Gen. Mean	**2.24 *****	**2.41 *****	**2.19 *****	**2.13 *****	**2.02 *****	**1.86 *****	**2.32 *****	**2.22 *****	**2.12 *****	**1.94**	
C.V.	11.24	10.62	11.05	11.46	10.85	10.63	9.75	10.62	10.79	10.09	
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
S.E.M.	0.14	0.14	0.14	0.14	0.12	0.11	0.13	0.13	0.13	0.11	
C.D. 5%	0.41	0.42	0.40	0.40	0.36	0.32	0.37	0.39	0.38	0.32	
C.D. 1%	0.56	0.56	0.53	0.54	0.48	0.44	0.50	0.52	0.50	0.43	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.10. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100mM, 150 mM & 200 mM) and different levels of NaCl & SA on carotenoid in different tomato varieties.

Carotenoid (mg g^{-1}) Fresh weight											
varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt) +SA(25µM)**	**100mM(salt) +SA(25µM)**	**150mM(salt) +SA(25µM)**	**200mM(salt)+SA(25µM)**	**V.mean**
H-8878-1	3.45^{abc}	3.52^{abc}	3.47^{abc}	3.41^{abc}	2.98^{bcde}	2.84^{bcde}	3.56^{abc}	3.51^{abc}	3.08^{bcde}	2.91	3.27
WIR-13706	3.27^{bcd}	3.37^{bcd}	3.27^{bcd}	3.21^{bcde}	3.06^{bcd}	2.98^{abcd}	3.42^{abc}	3.35^{abcd}	3.16^{abcde}	3.04	3.21
Anigoarlentha	2.73^{ef}	2.82^{ef}	2.68^{ef}	2.60^{fgh}	2.51^{efgh}	2.71^{cdef}	2.79^{ef}	2.69^{ef}	2.61^{efgh}	2.77	2.69
Ec-521080	3.38^{abc}	3.49^{abc}	3.32^{abc}	2.92^{defg}	2.82^{cdef}	2.70^{cdef}	3.45^{abc}	3.01^{cde}	2.90^{defg}	2.74	3.07
Arka marginal	3.14^{cde}	3.24^{cde}	3.07^{cde}	3.01^{cdef}	2.89^{cdef}	2.72^{cdef}	3.18^{cde}	3.08^{bcde}	2.97^{cdefg}	2.75	3.00
Ec-520079	2.63^{f}	2.75^{f}	2.59^{f}	2.51^{gh}	2.40^{fgh}	2.25^{f}	2.69^{f}	2.62^{ef}	2.49^{fgh}	2.33	2.52
WIR-3957	2.62^{f}	2.68^{f}	2.56^{f}	2.50^{gh}	2.41^{fgh}	2.28^{f}	2.69^{f}	2.61^{ef}	2.49^{gh}	2.31	2.51
P-6 chhu chara	3.36^{bc}	3.47^{bc}	3.32^{abc}	3.28^{abcd}	3.18^{abcd}	3.03^{abc}	3.42^{abc}	3.37^{abcd}	3.26^{abcd}	3.09	3.27
VRT-2	3.38^{abc}	3.50^{abc}	3.39^{abc}	3.32^{abcd}	3.24^{abc}	3.05^{abc}	3.51^{abc}	3.39^{abcd}	3.32^{abcd}	3.15	3.32
H-24	3.63^{ab}	3.70^{ab}	3.59^{ab}	3.53^{ab}	3.44^{ab}	3.28^{ab}	3.73^{ab}	3.66^{a}	3.54^{abc}	3.36	3.54
Roma	2.47^{f}	2.59^{f}	2.41^{f}	2.34^{h}	2.21^{h}	2.40^{ef}	2.52^{f}	2.45^{f}	2.30^{h}	2.37	2.40
Ec-520078	2.88^{def}	3.02^{def}	2.84^{def}	2.77^{efgh}	2.72^{defg}	2.60^{cdef}	2.93^{def}	2.90^{def}	3.06^{bcdef}	2.69	2.84
T-Loeal	3.64^{ab}	3.69^{ab}	3.60^{ab}	3.51^{ab}	3.43^{ab}	3.27^{ab}	3.73^{ab}	3.60^{ab}	3.57^{ab}	3.33	3.54
Arka Saurabh	3.28^{bcd}	3.41^{bcd}	3.23^{bcd}	3.16^{bcde}	3.07^{bcd}	2.94^{abcd}	3.36^{bcd}	3.25^{abcd}	3.11^{bcde}	3.03	3.18
H-86	3.45^{abc}	3.60^{abc}	3.40^{abc}	3.32^{abcd}	3.19^{abcd}	3.07^{abc}	3.54^{abc}	3.39^{bcd}	3.30^{abcd}	3.12	3.34
Ec-520061	2.52^{f}	2.70^{f}	2.48^{f}	2.40^{h}	2.33^{gh}	2.20^{f}	2.60^{f}	2.87^{def}	2.45^{gh}	2.26	2.48
DT-10	3.52^{abc}	3.72^{ab}	3.47^{abc}	3.38^{abc}	3.30^{abc}	3.13^{abc}	3.62^{abc}	3.51^{abc}	3.40^{abcd}	3.20	3.42
Agata-30	3.80^{a}	3.92^{a}	3.75^{a}	3.67^{a}	3.60^{a}	3.40^{a}	3.86^{a}	3.78^{a}	3.68^{a}	3.44	3.69
WIR-13708	3.49^{abc}	3.78^{ab}	3.45^{abc}	3.38^{abc}	3.27^{abc}	3.11^{abc}	3.57^{abc}	3.48^{abc}	3.38^{abcd}	3.19	3.41
WIR-3928	2.76^{ef}	2.87^{ef}	2.71^{ef}	2.64^{fgh}	2.54^{efgh}	2.47^{def}	2.84^{ef}	2.87^{def}	2.64^{efgh}	2.56	2.69
Gen. Mean	**3.174 *****	**3.29 *****	**3.13 *****	**3.04 *****	**2.93 *****	**2.82 *****	**3.25 *****	**3.173*****	**3.03 *****	**2.88**	
C.V.	8.40	8.00	8.66	9.04	9.97	11.45	8.47	10.29	11.37	10.92	
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
S.E.M.	0.15	0.15	0.15	0.15	0.16	0.18	0.15	0.18	0.20	0.18	
C.D. 5%	0.44	0.43	0.44	0.45	0.48	0.53	0.45	0.54	0.57	0.52	
C.D. 1%	0.59	0.58	0.60	0.61	0.64	0.71	0.61	0.72	0.76	0.69	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

4.3. Fruit-quality parameters:

The results of analysis of variance showed that (Table-4.11, 4.12, 4.13, 4.14, 4.15, 4.16) salicylic acid, different levels of salt stress and the interaction of salicylic acid with different levels of salt stress affected the lycopene, ß-carotene, hydrogen peroxide, total sugars and total phenol content and also the pH of the fruit significantly which directly affect the fruit quality. These quality parameters increased with increased salinity levels and varies according to the genotype. The maximum increament was recorded in salinity levels of 150 mM and 200 mM in the comparison with control plants but there were no significant changes during the stress level of salt 50 mM. Exogenous application of salicylic acid (25 µM) increased considerable positive results comparison with control level.

Lycopene content was estimated the maximum in the tomato cultivar H-8878-1 in control as well as under salicylic treatment. It was recorded the minimum in the genotype Ec-520079.

Total sugar and total phenol content also varies according to the tomato genotypes as total sugar content was found the maximum in tomato cultivar WIR-13708 in control (table-4.15). Increased value was recorded for the mentioned quality parameters in different tomato genotypes.

Table: 4.11. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on lycopene content (mg/100g) in the fruits of different tomato varieties.

Lycopene (mg/100g) Fresh weight											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	11.22^{a}	13.06^{a}	10.85^{a}	10.14^{a}	9.22^{a}	8.74^{a}	12.70^{a}	12.13^{a}	11.41^{a}	10.79^{a}	11.02
WIR-13706	5.78^{cde}	7.76^{bcde}	5.47^{cde}	4.83^{cde}	3.81^{def}	1.97^{defg}	6.17^{bc}	5.26^{bc}	4.71^{bc}	4.18^{bc}	4.99
Anigoarlentha	5.60^{def}	7.41^{cde}	5.14^{cdefg}	4.55^{cdefg}	3.74^{defg}	2.72^{defg}	6.16^{bc}	5.60^{bc}	5.03^{bc}	4.52^{bc}	5.04
Ec-521080	4.80^{defg}	6.09^{def}	4.38^{defghi}	3.74^{defgh}	3.11^{defgh}	2.28^{defg}	4.79^{c}	4.17^{c}	3.46^{c}	2.94^{c}	3.97
Arka marginal	6.68^{bcd}	8.57^{bcd}	6.34^{bcd}	5.68^{bcd}	4.93^{bcd}	4.09^{bcd}	6.84^{bc}	6.54^{bc}	5.76^{bc}	5.12^{bc}	6.05
Ec-520079	3.28^{g}	4.76^{f}	2.67^{hi}	2.01^{h}	1.70^{gh}	1.24^{fg}	4.02^{c}	3.70^{c}	3.15^{c}	2.78^{c}	2.93
WIR-3957	4.14^{efg}	5.97^{ef}	3.66^{efghi}	3.01^{efgh}	2.63^{efgh}	1.96^{defg}	5.15^{bc}	4.68^{bc}	4.20^{bc}	3.47^{c}	3.88
P-6 chhu chara	4.75^{defg}	5.70^{ef}	4.00^{efghi}	3.33^{efgh}	2.81^{efgh}	2.34^{defg}	4.80^{c}	3.93^{c}	3.31^{c}	2.64^{c}	3.76
VRT-2	5.63^{def}	7.15^{cdef}	5.25^{cdef}	4.64^{cdef}	4.06^{cdef}	3.28^{def}	6.40^{bc}	5.92^{bc}	5.25^{bc}	4.65^{bc}	5.22
H-24	7.61^{bc}	8.96^{bc}	6.77^{bc}	6.46^{bc}	6.13^{bc}	5.72^{bc}	7.07^{bc}	6.49^{bc}	5.84^{bc}	5.280^{bc}	6.63
Roma	3.13^{g}	5.27^{ef}	2.32^{i}	1.93^{h}	1.50^{h}	0.90^{g}	5.18^{bc}	4.74^{bc}	4.14^{bc}	3.57^{c}	3.26
Ec-520078	4.907^{defg}	6.91^{cdef}	5.00^{cdefg}	4.64^{cdef}	4.15^{cde}	3.15^{def}	5.91^{bc}	5.38^{bc}	4.77^{bc}	4.32^{bc}	4.91
T-Loeal	3.52^{g}	5.34^{ef}	2.87^{hi}	2.56^{gh}	2.15^{efgh}	1.29^{fg}	5.31^{bc}	4.75^{bc}	4.21^{bc}	3.49^{c}	3.54
Arka Saurabh	7.83^{b}	10.19^{b}	7.69^{b}	7.39^{b}	6.89^{b}	6.31^{b}	8.33^{b}	7.79^{b}	7.27^{b}	6.79^{b}	7.64
H-86	4.40^{efg}	6.69^{cdef}	3.93^{efghi}	3.40^{efgh}	2.82^{efgh}	2.10^{defg}	5.60^{bc}	4.94^{bc}	4.52^{bc}	3.76^{bc}	4.21
Ec-520061	3.89^{efg}	6.25^{def}	3.38^{fghi}	3.02^{efgh}	2.47^{efgh}	1.97^{defg}	6.03^{bc}	5.48^{bc}	4.99^{bc}	4.17^{bc}	4.16
DT-10	3.69^{fg}	5.63^{ef}	3.13^{ghi}	2.76^{fgh}	2.04^{fgh}	1.61^{efg}	4.95^{bc}	4.62^{bc}	4.15^{bc}	3.37^{c}	3.59
Agata-30	5.72^{cde}	7.28^{cdef}	5.12^{cdefg}	4.89^{cde}	4.19^{cde}	3.84^{cd}	6.80^{bc}	5.80^{bc}	5.37^{bc}	4.83^{bc}	5.38
WIR-13708	4.74^{defg}	7.240^{cdef}	4.71^{cdefgh}	4.43^{cdefg}	3.87^{def}	3.64^{cde}	6.61^{bc}	5.71^{bc}	5.22^{bc}	4.87^{bc}	5.10
WIR-3928	4.64^{efg}	6.25^{def}	4.15^{efghi}	3.68^{defgh}	2.87^{defgh}	3.10^{defg}	6.12^{bc}	5.64^{bc}	5.01^{bc}	4.19^{bc}	4.56
Gen. Mean	**5.30 *****	**7.12 *****	**4.84 *****	**4.35 *****	**3.75 *****	**3.11 *****	**6.25 ****	**5.66 ****	**5.09 ****	**4.48 ****	
C.V.	22.48	21.48	25.83	28.76	33.72	43.27	32.25	35.02	37.87	41.07	
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	
S.E.M.	0.68	0.88	0.72	0.72	0.73	0.77	1.16	1.14	1.11	1.06	
C.D. 5%	1.97	2.53	2.06	2.07	2.09	2.22	3.33	3.28	3.18	3.048	
C.D. 1%	2.63	3.38	2.77	2.77	2.80	2.98	4.46	4.39	4.27	4.08	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.12. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on lycopene content (mg/100g) in the fruits of different tomato varieties.

ß-carotene (mg/100g) Fresh weight											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	13.34^{a}	14.90^{a}	12.84^{a}	12.26^{a}	11.53^{a}	10.59^{a}	13.95^{a}	13.54^{a}	13.26^{a}	12.62	12.91
WIR-13706	7.62^{b}	9.43^{b}	7.32^{b}	6.82^{b}	6.16^{bc}	5.36^{b}	13.55^{a}	13.25^{a}	13.07^{a}	12.36	9.81
Anigoarlentha	5.63^{b}	6.76^{bc}	5.19^{bc}	4.60^{bcd}	3.68^{bcde}	3.27^{bcd}	5.92^{b}	5.16^{b}	4.70^{b}	4.10	4.88
Ec-521080	4.91^{b}	6.43^{bc}	4.56^{bc}	3.98^{bcd}	3.31^{bcde}	3.46^{bcd}	5.12^{b}	4.88^{b}	4.60^{b}	3.98	4.53
Arka marginal	5.01^{b}	6.46^{bc}	4.67^{bc}	4.19^{bcd}	3.62^{bcde}	2.78^{bcd}	5.68^{b}	5.28^{b}	4.92^{b}	4.35	4.71
Ec-520079	5.15^{b}	6.77^{bc}	4.79^{bc}	4.25^{bcd}	3.54^{bcde}	2.92^{bcd}	5.13^{b}	4.73^{b}	4.45^{b}	3.893	4.55
WIR-3957	4.83^{b}	6.41^{bc}	4.28^{bc}	3.89^{bcd}	3.31^{bcde}	2.83^{bcd}	5.13^{b}	4.68^{b}	4.29^{b}	3.79	4.33
P-6 chhu chara	6.09^{b}	7.53^{bc}	5.64^{bc}	5.12^{bcd}	4.48^{bcde}	3.75^{bcd}	6.89^{b}	6.44^{b}	5.95^{b}	5.34	5.74
VRT-2	4.75^{b}	6.41^{bc}	4.23^{bc}	3.68^{bcd}	2.78^{bcde}	1.82^{cd}	6.27^{b}	5.92^{b}	5.36^{b}	4.80	4.67
H-24	7.52^{b}	9.25^{b}	7.12^{bc}	6.70^{bc}	6.31^{b}	5.68^{b}	7.46^{b}	5.32^{b}	5.09^{b}	4.70	6.38
Roma	3.74^{b}	4.84^{c}	3.17^{c}	2.70^{d}	2.20^{e}	1.27^{d}	4.37^{b}	4.00^{b}	3.67^{b}	3.19	3.34
Ec-520078	5.73^{b}	6.99^{bc}	5.26^{bc}	4.82^{bcd}	4.13^{bcde}	3.31^{bcd}	6.92^{b}	6.52^{b}	5.92^{b}	5.42	5.54
T-Loeal	5.55^{b}	6.97^{bc}	4.95^{bc}	4.60^{bcd}	4.19^{bcde}	3.19^{bcd}	5.44^{b}	4.75^{b}	4.27^{b}	3.95	4.73
Arka Saurabh	7.16^{b}	8.91^{b}	6.89^{bc}	6.37^{bcd}	5.86^{bcd}	5.37^{b}	7.98^{b}	7.68^{b}	7.45^{b}	6.91	7.09
H-86	6.45^{b}	7.88^{bc}	5.82^{bc}	5.16^{bcd}	4.45^{bcde}	3.71^{bcd}	7.18^{b}	6.71^{b}	5.95^{b}	5.67	5.90
Ec-520061	4.00^{b}	5.48^{bc}	3.61^{bc}	2.97^{cd}	2.41^{de}	1.64^{d}	5.16^{b}	4.64^{b}	4.09^{b}	3.77	3.80
DT-10	6.21^{b}	7.31^{bc}	6.49^{bc}	5.94^{bcd}	5.15^{bcde}	4.28^{bcd}	6.23^{b}	5.60^{b}	5.30^{b}	4.90	5.70
Agata-30	7.16^{b}	8.76^{bc}	6.81^{bc}	6.25^{bcd}	5.81^{bcde}	5.30^{b}	7.15^{b}	5.11^{b}	4.83^{b}	4.38	6.03
WIR-13708	5.68^{b}	6.96^{bc}	6.48^{bc}	6.05^{bcd}	5.63^{bcde}	5.09^{bc}	6.38^{b}	4.85^{b}	4.57^{b}	4.05	5.48
WIR-3928	4.22^{b}	5.91^{bc}	3.89^{bc}	3.42^{bcd}	2.69^{cde}	1.74^{cd}	5.03^{b}	4.67^{b}	4.10^{b}	3.18	3.90
Gen. Mean	**6.04 ***	**7.52 ***	**5.70 ***	**5.19 ****	**4.56 ****	**3.87 ****	**6.85 ****	**6.18 ****	**5.79 ****	**15.37**	
C.V.	40.52	32.15	42.64	43.89	47.98	52.77	38.87	44.77	47.78	511.58	
F Prob.	0.02	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.49	
S.E.M.	1.41	1.39	1.40	1.31	1.26	1.17	1.53	1.60	1.59	45.40	
C.D. 5%	4.04	3.99	4.02	3.766	3.62	3.37	4.40	4.58	4.57	_	
C.D. 1%	5.42	5.35	5.38	5.04	4.85	4.52	5.89	6.13	6.13	_	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.13. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on pH level in the fruits of different tomato varieties.

pH level in the fruits											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	8.56^{a}	10.73	8.10^{a}	7.23^{a}	6.06^{a}	5.83^{a}	10.46^{a}	7.40^{a}	5.00^{abc}	3.50^{abc}	7.28
WIR-13706	6.03^{abcdef}	7.06	5.46^{bcd}	4.70^{bcde}	4.03^{bcde}	3.63^{bc}	6.20^{bcde}	5.13^{bcde}	4.46^{abcd}	3.00^{abcd}	4.97
Anigoarlentha	4.73^{bcdef}	5.10	3.93^{cde}	3.53^{bcdefg}	2.86^{defgh}	1.86^{def}	4.83^{defg}	4.26^{cdefg}	3.36^{cdefg}	2.46^{bcd}	3.69
Ec-521080	5.50^{abcdef}	6.53	4.86^{bcde}	4.33^{bcdefg}	3.76^{bcdef}	2.83^{bcde}	5.83^{bcde}	4.96^{bcde}	4.36^{abcd}	3.63^{abc}	4.65
Arka marginal	6.30^{abcde}	7.06	5.86^{abc}	4.53^{bcdef}	3.80^{bcdef}	2.30^{bcdef}	6.43^{bcd}	5.26^{bcd}	4.43^{abcd}	2.80^{bcd}	4.87
Ec-520079	4.80^{bcdef}	5.30	3.46^{de}	3.33^{cdefg}	2.80^{efgh}	2.26^{bcdef}	4.93^{def}	4.20^{defg}	3.53^{bcdef}	2.30^{bcd}	3.69
WIR-3957	3.80^{cdef}	4.43	3.30^{de}	2.46^{fg}	2.36^{efgh}	2.00^{cdef}	3.00^{hij}	2.66^{fgh}	2.16^{efgh}	1.90^{d}	2.80
P-6 chhu chara	4.63^{bcdef}	5.43	4.50^{bcde}	3.50^{bcdefg}	3.10^{defg}	2.53^{bcdef}	5.00^{def}	4.20^{defg}	3.80^{bcde}	3.03^{abcd}	3.97
VRT-2	3.13^{f}	3.63	3.06^{e}	2.80^{efg}	2.16^{fgh}	1.63^{ef}	2.20^{ij}	2.30^{h}	1.86^{gh}	1.76^{d}	2.45
H-24	3.63^{def}	3.96	3.40^{de}	2.90^{efg}	1.20^{h}	1.10^{f}	1.76^{j}	1.86^{h}	1.86^{gh}	1.70^{d}	2.33
Roma	3.16^{f}	3.53	3.00^{e}	2.63^{efg}	2.13^{fgh}	1.63^{ef}	2.20^{ij}	1.86^{h}	1.93^{fgh}	1.53^{d}	2.36
Ec-520078	4.00^{cdef}	4.93	3.43^{de}	3.20^{defg}	2.76^{efgh}	1.76^{ef}	2.80^{hij}	2.63^{fgh}	2.40^{efgh}	1.63^{d}	2.95
T-Loeal	3.56^{ef}	4.73	3.00^{e}	2.63^{efg}	2.13^{fgh}	1.70^{ef}	3.73^{fghi}	3.33^{efgh}	3.10^{defgh}	2.30^{bcd}	3.02
Arka Saurabh	3.90^{cdef}	4.50	3.30^{de}	3.03^{efg}	2.83^{efgh}	2.03^{cdef}	3.86^{fghi}	3.30^{efgh}	2.83^{defgh}	1.90^{d}	3.14
H-86	4.63^{bcdef}	5.06	4.13^{cde}	3.86^{bcdefg}	3.30^{cdefg}	2.46^{bcdef}	4.60^{efgh}	4.26^{cdefg}	3.50^{bcdefg}	2.23^{cd}	3.80
Ec-520061	3.13^{f}	3.76	2.63^{e}	2.20^{g}	1.63^{gh}	1.13^{f}	3.03^{ghij}	2.46^{gh}	1.70^{h}	1.63^{d}	2.33
DT-10	5.06^{bcdef}	5.80	4.53^{bcde}	4.00^{bcdefg}	3.03^{defg}	2.00^{cdef}	5.13^{cdef}	4.36^{cdef}	3.53^{bcdef}	2.40^{bcd}	3.98
Agata-30	6.70^{abcd}	7.40	5.93^{abc}	5.23^{abcd}	4.63^{abcd}	3.23^{bcde}	6.60^{bcd}	5.80^{abcd}	5.13^{ab}	3.6^{abc}	5.42
WIR-13708	7.40^{ab}	8.033	6.56^{ab}	5.63^{ab}	5.10^{ab}	3.80^{b}	7.50^{b}	6.53^{ab}	5.70^{a}	4.53^{a}	6.07
WIR-3928	6.83^{abc}	7.800	6.16^{abc}	5.46^{abc}	4.93^{abc}	3.53^{bcd}	6.86^{bc}	6.13^{abc}	5.66^{a}	3.83^{ab}	5.71
Gen. Mean	**4.97 ***	**5.742**	**4.43 *****	**3.86 ******	**3.23 *****	**2.465 *****	**4.85 *****	**4.14 *****	**3.51 *****	**2.58 ******	
C.V.	37.30	41.437	30.77	34.04	33.48	41.03	22.53	27.25	28.20	36.49	
F Prob.	0.02	0.065	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
S.E.M.	1.07	1.374	0.78	0.75	0.62	0.58	0.63	0.65	0.57	0.54	
C.D. 5%	3.06	_	2.25	2.17	1.79	1.67	1.80	1.86	1.64	1.56	
C.D. 1%	4.11	_	3.02	2.91	2.39	2.24	2.41	2.50	2.19	2.09	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.14. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on hydrogen peroxide content in the fruits of different tomato varieties.

Hydrogen peroxide (µmol g-^{1}) fresh weight											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	32.10^{defg}	37.83^{bcde}	32.26^{defg}	31.83^{def}	30.90^{de}	29.53^{f}	36.66^{abcdef}	35.93^{bcdefg}	34.23^{bcdef}	32.76^{cde}	33.40
WIR-13706	39.60^{bcdef}	44.36^{abc}	39.16^{bcdef}	38.63^{bcdef}	37.03^{cde}	36.86^{cdef}	45.46^{ab}	44.06^{abcde}	43.03^{abcd}	40.16^{abcd}	40.83
Anigoarlentha	37.43^{bcdef}	42.26^{abc}	36.16^{cdefg}	33.86^{cdef}	30.90^{de}	29.23^{f}	41.46^{abcd}	38.53^{abcdefg}	37.76^{abcde}	34.76^{bcde}	36.23
Ec-521080	39.43^{bcdef}	41.93^{abc}	44.53^{bcde}	47.13^{abcd}	50.16^{abc}	53.30^{abc}	40.46^{abcde}	45.33^{abcd}	47.26^{abc}	51.03^{abc}	46.05
Arka marginal	51.63^{ab}	46.83^{ab}	53.03^{ab}	55.40^{a}	58.20^{a}	61.73^{a}	43.06^{abc}	52.26^{a}	55.86^{a}	60.16^{a}	53.81
Ec-520079	40.66^{bcde}	36.56^{bcdef}	41.86^{bcdef}	43.73^{abcde}	46.43^{abcd}	50.76^{abcd}	34.23^{abcdef}	41.06^{abcdef}	44.30^{abcd}	48.76^{abc}	42.83
WIR-3957	27.40^{efg}	24.46^{ef}	29.00^{fg}	35.53^{cdef}	38.56^{cde}	42.66^{bcdef}	24.53^{ef}	32.53^{cdefgh}	35.36^{bcde}	41.60^{abcd}	33.16
P-6 chhu chara	48.20^{abc}	43.70^{abc}	49.16^{abc}	52.10^{ab}	54.53^{ab}	57.63^{ab}	43.93^{abc}	48.93^{ab}	52.50^{ab}	56.16^{ab}	50.68
VRT-2	25.90^{fg}	23.46^{f}	27.70^{fg}	29.03^{ef}	31.43^{de}	34.63^{def}	22.73^{f}	26.16^{fgh}	29.33^{cdef}	32.633^{cde}	28.30
H-24	33.13^{defg}	40.16^{abcd}	32.90^{defg}	30.46^{ef}	29.60^{e}	28.30^{f}	38.33^{abcdef}	30.43^{defgh}	32.96^{cdef}	37.66^{bcd}	33.39
Roma	45.53^{abcd}	42.23^{abc}	47.16^{abcd}	48.93^{abc}	50.83^{abc}	54.96^{ab}	41.20^{abcd}	40.86^{abcdef}	38.60^{abcde}	34.00^{cde}	44.43
Ec-520078	30.26^{efg}	26.66^{def}	32.53^{defg}	35.60^{cdef}	38.26^{cde}	41.93^{bcdef}	25.33^{def}	23.43^{gh}	22.60^{ef}	20.36^{de}	29.69
T-Loeal	38.56^{bcdef}	35.53^{bcdef}	41.03^{bcdef}	43.00^{abcde}	45.60^{abcd}	50.63^{abcd}	33.50^{abcdef}	31.63^{defgh}	29.96^{cdef}	26.13^{de}	37.55
Arka Saurabh	53.53^{a}	49.90^{a}	54.66^{a}	57.46^{a}	59.20^{a}	62.33^{a}	48.83^{a}	47.96^{abc}	44.00^{abcd}	60.40^{a}	53.82
H-86	37.13^{cdef}	33.96^{bcdef}	39.93^{bcdef}	43.10^{abcde}	45.83^{abcd}	50.13^{abcde}	31.60^{bcdef}	30.56^{defgh}	28.46^{cdef}	26.06^{de}	36.67
Ec-520061	44.76^{abcd}	47.83^{ab}	46.90^{abcd}	48.40^{abc}	40.93^{bcde}	54.63^{abc}	45.60^{ab}	44.50^{abcde}	42.76^{abcd}	38.83^{abcd}	45.51
DT-10	32.83^{defg}	30.33^{cdef}	35.26^{cdefg}	37.53^{bcdef}	39.56^{bcde}	42.36^{bcdef}	29.20^{bcdef}	28.36^{efgh}	27.86^{def}	25.33^{de}	32.86
Agata-30	35.10^{cdefg}	32.33^{cdef}	36.93^{cdef}	37.93^{bcdef}	40.63^{bcde}	30.50^{f}	28.00^{cdef}	27.66^{fgh}	26.00^{def}	41.03^{abcd}	33.61
WIR-13708	22.46^{g}	23.16^{f}	21.36^{g}	26.93^{f}	28.60^{e}	32.33^{ef}	21.96^{f}	19.50^{h}	16.06^{f}	15.53^{e}	22.78
WIR-3928	34.700^{cdefg}	38.83^{abcd}	31.56^{efg}	47.20^{abcd}	49.46^{abc}	52.23^{abcd}	35.53^{abcdef}	33.93^{bcdefgh}	32.233^{cdef}	29.90^{cde}	38.55
Gen. Mean	**37.52 ****	**37.12 ****	**38.65 ****	**41.19 ****	**42.33 ****	**44.83 *****	**35.58 ***	**36.18 ****	**36.06 ***	**37.66 ****	
C.V.	23.37	22.87	23.40	23.18	22.77	24.34	28.22	27.13	32.35	34.84	
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.01	0.00	
S.E.M.	5.06	4.90	5.22	5.51	5.56	6.30	5.79	5.66	6.73	7.57	
C.D. 5%	14.49	14.03	14.95	15.78	15.93	18.04	16.60	16.23	19.28	21.69	
C.D. 1%	19.41	18.80	20.03	21.14	21.34	24.16	22.23	21.73	25.83	29.05	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.15. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on total sugars content (mg/100g) in the fruits of different tomato varieties.

Total sugars (mg/100g)											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	38.66	45.16	36.50	34.13	33.60	31.80	42.00	41.10	40.60	39.86	38.34
WIR-13706	33.06	38.23	32.13	30.20	29.53	26.50	37.50	36.00	42.96	40.86	34.69
Anigoarlentha	28.60	31.46	27.96	25.66	24.70	22.10	30.76	30.00	37.13	34.66	29.30
Ec-521080	22.93	25.50	21.30	20.50	19.70	16.90	24.23	23.70	33.83	29.36	23.79
Arka marginal	26.93	29.96	26.63	26.23	24.10	19.80	28.70	27.16	38.43	35.50	28.34
Ec-520079	29.63	32.90	28.33	26.60	21.80	17.10	31.73	30.83	39.36	34.10	29.23
WIR-3957	26.87	31.40	25.30	24.76	21.89	17.46	30.03	29.66	36.43	32.03	27.58
P-6 chhu chara	22.43	28.20	21.73	20.56	17.63	12.60	27.80	27.40	34.63	28.73	24.17
VRT-2	29.46	34.73	29.06	28.16	25.13	21.30	33.63	28.33	30.86	31.63	29.22
H-24	32.96	39.60	28.00	27.96	25.10	18.56	37.96	35.20	31.46	29.56	30.63
Roma	34.56	39.46	34.00	33.26	28.93	22.66	37.43	36.20	35.20	32.23	33.39
Ec-520078	39.93	42.46	36.03	35.76	32.16	27.00	41.96	37.33	36.23	33.53	36.23
T-Loeal	26.86	30.10	25.86	24.33	20.73	15.73	29.20	28.86	27.90	26.00	25.55
Arka Saurabh	37.73	44.03	36.50	35.03	33.36	27.30	43.93	37.46	34.90	25.86	35.61
H-86	33.26	32.66	32.70	31.70	31.13	25.63	30.90	29.03	27.36	25.66	30.00
Ec-520061	38.23	41.96	33.33	30.50	26.50	23.26	40.30	32.40	30.56	28.73	32.57
DT-10	37.74	38.83	36.66	36.50	35.40	30.33	36.03	35.40	34.80	31.03	35.27
Agata-30	28.26	33.86	27.80	26.70	25.50	22.73	33.20	29.60	28.70	27.10	28.34
WIR-13708	40.10	42.76	38.90	35.53	34.26	29.60	41.40	39.00	38.46	37.13	37.71
WIR-3928	30.73	35.13	30.23	29.06	27.80	23.76	29.00	28.63	34.60	40.26	30.92
Gen. Mean	**31.95**	**35.92**	**30.45**	**29.16**	**26.95**	**22.60**	**34.38**	**32.16**	**34.72**	**32.19**	
C.V.	29.55	23.65	25.99	28.30	30.83	33.76	22.08	22.96	22.08	32.95	
F Prob.	0.45	0.20	0.28	0.43	0.31	0.17	0.08	0.29	0.56	0.87	
S.E.M.	5.45	4.90	4.56	4.766	4.79	4.40	4.38	4.26	4.42	6.12	
C.D. 5%	_	_	_	_	_	_	_	_	_	_	
C.D. 1%	_	_	_	_	_	_	_	_	_	_	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.16. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on total phenol content (mg/100g) in the fruits of different tomato varieties.

Total phenol (mg/100g)											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	60.50	71.10^{a}	74.06^{a}	76.46^{a}	81.60^{a}	85.93^{a}	63.40^{a}	70.53^{a}	72.80^{a}	78.13^{a}	73.45
WIR-13706	54.00	62.40^{abc}	65.16^{ab}	67.26^{abc}	69.46^{abc}	74.23^{abc}	56.76^{abc}	59.70^{abc}	62.73^{abc}	68.56^{abc}	64.02
Anigoarlentha	36.96	42.06^{de}	44.53^{e}	46.96^{e}	49.40^{e}	54.60^{ef}	36.93^{ef}	40.46^{e}	43.10^{e}	49.30^{g}	44.43
Ec-521080	58.20	63.93^{abc}	66.10^{ab}	67.63^{abc}	70.03^{abc}	73.63^{abc}	58.86^{abc}	61.90^{ab}	68.66^{ab}	71.56^{ab}	66.05
Arka marginal	41.83	48.10^{cde}	51.66^{bcde}	53.5^{cde}	56.16^{cde}	59.70^{cdef}	43.70^{cdef}	46.70^{cde}	50.03^{cde}	55.60^{defg}	50.69
Ec-520079	44.36	49.53^{bcde}	52.66^{bcde}	54.93^{cde}	57.46^{cde}	60.73^{cdef}	45.53^{bcdef}	49.26^{bcde}	53.66^{bcde}	58.73^{cdef}	52.68
WIR-3957	49.13	56.43^{abcde}	59.06^{abcde}	60.86^{bcde}	63.93^{bcde}	68.10^{bcdef}	51.03^{abcde}	52.53^{bcde}	56.26^{bcde}	61.56^{bcde}	57.88
P-6 chhu chara	38.93	43.03^{de}	46.90^{de}	49.26^{de}	50.30^{e}	53.66^{f}	38.96^{def}	41.43^{de}	45.900^{de}	51.63^{defg}	46.00
VRT-2	53.20	57.50^{abcd}	60.96^{abcd}	63.56^{abcd}	65.93^{bcd}	70.90^{bcd}	53.30^{abcd}	56.50^{abcd}	59.46^{abcd}	54.43^{defg}	59.57
H-24	48.96	55.66^{abcde}	63.36^{abc}	66.40^{abc}	68.03^{abcd}	72.20^{abcd}	52.13^{abcde}	54.76^{bcde}	57.23^{bcde}	62.36^{bcd}	60.10
Roma	48.53	55.36^{bcde}	58.80^{abcde}	61.90^{abcde}	63.96^{bcde}	68.97^{bcde}	50.36^{abcdef}	53.46^{bcde}	56.66^{bcde}	54.63^{defg}	57.26
Ec-520078	48.40	52.26^{bcde}	54.63^{bcde}	57.26^{bcde}	60.36^{bcde}	62.73^{cdef}	47.23^{bcdef}	49.60^{bcde}	53.76^{bcde}	50.06^{efg}	53.62
T-Loeal	57.60	65.40^{ab}	67.23^{ab}	70.83^{ab}	74.63^{ab}	80.00^{ab}	60.63^{ab}	64.36^{ab}	68.00^{ab}	70.13^{abc}	67.88
Arka Saurabh	36.70	42.03^{de}	44.60^{e}	47.73^{e}	49.53^{e}	53.93^{f}	37.13^{ef}	40.60^{e}	43.40^{e}	43.93^{g}	43.95
H-86	55.30	62.30^{abc}	65.23^{ab}	66.86^{abc}	68.46^{abcd}	71.93^{abcd}	48.03^{abcdef}	50.90^{bcde}	55.16^{bcde}	60.83^{cdef}	60.5
Ec-520061	46.33	52.37^{bcde}	55.90^{bcde}	58.36^{bcde}	60.13^{bcde}	62.70^{cdef}	47.93^{bcdef}	51.50^{bcde}	54.16^{bcde}	53.76^{defg}	54.31
DT-10	42.66	51.93^{bcde}	56.20^{bcde}	59.10^{bcde}	60.96^{bcde}	66.23^{bcdef}	47.43^{bcdef}	50.80^{bcde}	54.53^{bcde}	56.33^{def}	54.61
Agata-30	47.70	53.26^{bcde}	56.53^{bcde}	59.13^{bcde}	62.06^{bcde}	65.26^{bcdef}	47.90^{bcdef}	50.70^{bcde}	54.56^{bcde}	62.83^{bcd}	55.99
WIR-13708	41.20	45.13^{de}	48.20^{cde}	50.60^{de}	53.13^{de}	58.66^{def}	41.30^{def}	43.43^{de}	46.70^{de}	54.30^{defg}	48.26
WIR-3928	36.76	40.50^{e}	43.70^{e}	47.00^{e}	49.23^{e}	53.60^{f}	35.30^{f}	41.10^{e}	46.26^{de}	51.46^{defg}	44.49
Gen. Mean	**47.36**	**53.51 ***	**56.77 ***	**59.28 ****	**61.74 ****	**65.88 ****	**48.19 ***	**51.51 ***	**55.15 ****	**58.51****	
C.V.	20.58	18.29	17.22	15.78	15.11	13.56	19.32	17.83	16.53	12.40	
F Prob.	0.07	0.01	0.01	0.00	0.004	0.00	0.01	0.01	0.00	0.00	
S.E.M.	5.62	5.65	5.64	5.40	5.38	5.16	5.37	5.30	5.26	4.19	
C.D. 5%	_	16.18	16.16	15.46	15.42	14.77	15.39	15.18	15.07	12.00	
C.D. 1%	_	21.68	21.65	20.71	20.65	19.78	20.61	20.34	20.19	16.07	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

4.4. Antioxidant (non-enzymatic):

4.4.1. Proline content (mg g^{-1} fresh weight): It is evident from analysis of variance that proline content was significantly affected by salinity levels, tomato genotypes and their interaction at P < 0.01%. Treatment means (table-4.17) showed the significant increase in proline content with increase in salinity and maximum value for proline content was observed at the highest salinity level in Agata-30 and Arka saurabh whereas the lowest was in H-8878-1 at control treatment. The proline content in different tomato genotypes was increased as compared to control when salinity level was increased. At 200 mM salinity level increase in proline content ranged greatly among various genotypes. The maximum proline content was recorded in the genotype Agata-30 at the highest level of salinity (200 mM) and that was recorded the minimum in the genotype H-8878-1. Significant decrease in proline level was observed in all the genotypes at the treatment of salicylic acid (25 µM) alone. Significant decrease in the value of proline was recorded in all the genotypes even at the salinity level 200 mM along with the treatment of salicylic acid of concentration 25 µM. The tomato genotypes Agata-30, Arka Saurabh, H-86, Ec-520079, Ec-521080, Anigoarlentha showed a little increase in proline content but at higher level of NaCl showed greater proline content as compared to control. The tomato genotypes WIR-3957, H-24, T-Loeal and WIR-13708 showed moderate increase in proline content as compared to control.

4.5. Antioxidant enzyme activities: The results presented in Table-4.18, 4.19, 4.20 and 4.21, showed the effect of different concentrations of NaCl in tomato plants and also treated with salicylic acid alone and in combination with salicylic acid (25 µM) and different concentrations of salt on the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (PX) and glutathione peroxidase (GPX). The activities of SOD, CAT, POD and GPX showed progressively increased with increasing salinity level, Salicylic acid treatment induced significant decreases in the activities of SOD, CAT, POD, and GPX compared with those of the reference controls and among the antioxidant defence system, SOD showed the high value, followed by POD, GPX and CAT respectively.

4.5.1. SOD activity: The salinity induced a marked increase in SOD activity in tomato plants, especially at high salinity level (150 and 200 mM NaCl). Data in the table-4.18 revealed that, phytohormones, salinity, and their interactions significantly affected the SOD antioxidant enzyme. The application of salicylic acid in most salinity levels resulted in a stimulation of SOD activity. The maximum SOD activity was recorded in the variety H-8878-1 at salt level 200 mM (salt). Genotype WIR-3928 showed the minimum SOD activity at control. WIR-13706, Ec-521080, VRT-2, H-24, T-Loeal and H-86 showed greater increase

in SOD activity at the highest salinity level as compared to control. Anigoarlentha, Arka marginal, P-6 chhu chara, Arka Saurabh, WIR-3928 and WIR-13708 showed a moderate increase in SOD activity at the highest salinity level. Application of salicylic acid minimizes the effect of different levels of salt stress in all the genotypes.

4.5.2. Peroxidase (POD) activity: Salinity stress resulted a marked increase in POD activity of tomato plants, as compared with control plants (Table-4.19). Application of salicylic acid alone resulted a positively marked increase in POD activity in all the genotypes. The tomato variety Arka marginal showed the maximum value of POD activity at 200 mM (salt) + SA (25 µM) and the minimum value of that was showed by the tomato variety H-86 at the highest salinity level as compared to control. Arka marginal, H-24, Ec-521080, Ec-520079 and Arka Saurabh didn't showed greater effect on peroxidase activity at lower level but at the concentration of 200 mM NaCl they showed the maximum value of peroxidase activity. The tomato varieties Agata-30, H-86, and T-Loeal showed a moderate increase in POD content at higher salinity level as compared to control.

4.5.3. Catalase (CAT) activity: The interaction of phytohormone with salinity as revealed by table-4.20 showed the salinity significantly affected the antioxidant enzyme activity. The salinity inhanced a marked increase in CAT activity in tomato plants, especially at high salinity level (150 and 200 mM NaCl). Treatments with salicylic acid in most salinity levels resulted in a stimulation of CAT activity. The maximum CAT activity was showed by the variety H-8878-1 at stress level 200 mM (salt) + SA (25 µM). Genotype Arka marginal showed the minimum CAT activity at control. The tomato genotype Arka Saurabh showed the minimum catalase activity at the highest salinity level.

4.5.4. Glutathione peroxidase (GPX) activity: Data in the table-4.21 showed that, phytohormones, salinity, and their interactions significantly affected the studied antioxidant enzyme. The salinity induced a marked increase in GPX activity in tomato plants, especially at high salinity level (150 and 200 mM NaCl). Treatments with salicylic acid in most salinity levels resulted in a stimulation of GPX activity. The maximum GPX activity was recorded in the variety T-Loeal at stress level 200 mM (salt) and this salt concentration, genotype WIR-13708 showed the minimum GPX activity. The tomato genotypes Arka marginal, WIR-3957, P-6 chhu chara, H-24, DT-10 and WIR-13708 didn't showed very higher increase in glutathione peroxidase activity at the highest salinity level as compared to control. The tomato genotypes H-8878-1, WIR-13706, Anigoarlentha, VRT-2, Roma and T-Loeal showed marked increase in glutathione peroxidase activity at higher salinity level which was significant.

Table: 4.17. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on proline content in different tomato varieties.

Proline (µM g^{-1})											
varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	3.70^{k}	2.46^{f}	4.867^{l}	6.400^{i}	8.467^{j}	11.50^{j}	3.56^{j}	4.66^{i}	6.70^{j}	9.90	6.22
WIR-13706	12.93^{efgh}	10.16^{cde}	14.20^{efghi}	15.83^{defg}	17.60^{defgh}	19.43^{efgh}	12.63^{efgh}	14.90^{defg}	15.633^{efgh}	17.43	15.07
Anigoarlentha	17.56^{cdef}	15.10^{bc}	19.30^{cdef}	21.30^{bcde}	23.46^{bcd}	25.73^{bcde}	17.10^{bcde}	19.63^{bcde}	20.86^{cdef}	24.26	20.42
Ec-521080	18.66^{bcde}	15.23^{bc}	20.26^{bcde}	22.46^{bcd}	23.86^{bcd}	26.40^{bcd}	17.76^{bcde}	21.23^{abcd}	22.30^{bcd}	24.46	21.26
Arka marginal	17.30^{cdef}	14.23^{bc}	17.66^{cdefg}	19.70^{cdef}	21.86^{cde}	24.86^{bcdef}	16.20^{cdef}	18.40^{cde}	19.86^{cdefg}	22.66	19.27
Ec-520079	21.70^{abc}	18.16^{ab}	23.46^{abc}	26.20^{abc}	27.86^{abc}	29.46^{abc}	21.46^{abc}	24.33^{abc}	25.96^{abc}	27.56	24.62
WIR-3957	5.26^{jk}	3.60^{f}	6.66^{kl}	8.30^{hi}	10.00^{ij}	12.40^{ij}	5.30^{ij}	7.33^{hi}	8.33^{ij}	10.03	7.72
P-6 chhu chara	18.23^{bcdef}	15.20^{bc}	19.70^{bcdef}	21.60^{bcde}	22.96^{cd}	25.73^{bcde}	17.66^{bcde}	19.56^{bcde}	21.50^{bcde}	23.56	20.57
VRT-2	12.60^{efgh}	10.30^{cde}	14.00^{efghi}	16.06^{defg}	18.16^{defgh}	20.73^{defgh}	11.80^{efghi}	14.43^{defgh}	16.10^{defgh}	15.43	14.96
H-24	7.00^{hijk}	5.33^{ef}	8.36^{ijkl}	10.10^{ghi}	11.86^{hij}	14.90^{hij}	6.40^{hij}	8.63^{ghi}	10.16^{hij}	12.76	8.53
Roma	14.73^{defg}	12.16^{bcd}	16.83^{defgh}	19.26^{cdef}	20.76^{def}	22.90^{cdefg}	14.56^{defg}	17.33^{cdef}	18.50^{defg}	20.40	13.97
Ec-520078	10.267^{hij}	8.00^{def}	11.90^{ghijk}	14.03^{fgh}	15.80^{efghi}	18.633^{fghi}	10.06^{fghij}	12.76^{efgh}	14.13^{efghi}	15.50	11.69
T-Loeal	5.96^{ijk}	4.86^{ef}	7.40^{jkl}	9.76^{ghi}	12.16^{ghij}	15.53^{hij}	5.96^{hij}	8.23^{ghi}	10.33^{hij}	13.20	9.34
Arka Saurabh	24.43^{ab}	23.16^{a}	26.00^{ab}	27.83^{ab}	30.03^{ab}	33.93^{a}	23.73^{ab}	25.70^{ab}	28.43^{ab}	31.13	27.43
H-86	20.13^{abcd}	17.73^{ab}	21.90^{abcd}	25.56^{abc}	28.00^{abc}	30.73^{ab}	20.23^{abcd}	23.10^{abc}	26.36^{abc}	28.10	24.18
Ec-520061	9.40^{ghijk}	7.83^{def}	10.93^{hijkl}	12.56^{fghi}	15.90^{efghi}	19.56^{efgh}	9.16^{ghij}	10.86^{fghi}	14.10^{efghij}	17.43	12.77
DT-10	11.83^{fghi}	9.83^{cde}	13.56^{fghij}	15.90^{defg}	18.93^{defg}	22.96^{cdefg}	11.16^{efghi}	14.13^{defgh}	17.00^{defgh}	21.06	15.64
Agata-30	25.56^{a}	22.50^{a}	27.733^{a}	30.03^{a}	32.43^{a}	35.90^{a}	25.06^{a}	27.63^{a}	30.90^{a}	32.90	29.06
WIR-13708	7.50^{hijk}	6.30^{def}	9.16^{ijkl}	11.93^{ghi}	14.20^{fghij}	16.96^{ghij}	7.56^{hij}	10.00^{ghi}	12.66^{ghij}	15.20	11.15
WIR-3928	9.26^{ghijk}	7.23^{def}	11.56^{ghijk}	14.83^{efgh}	17.20^{defgh}	20.46^{defgh}	10.20^{fghij}	13.26^{efgh}	13.83^{fghij}	18.20	13.60
Gen. Mean	**13.70*** **	**11.47*** **	**15.27*** **	**17.48*** **	**19.57*** **	**22.43*** **	**13.38*** **	**15.80*** **	**17.68*** **	**20.06**	
C.V.	28.33	31.6	25.91	24.78	21.15	18.20	30.56	27.39	25.36	18.84	
F Prob.	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
S.E.M.	2.242	2.09	2.28	2.50	2.39	2.35	2.36	2.50	2.58	2.18	
C.D. 5%	6.41	6.00	6.54	7.16	6.84	6.75	6.76	7.15	7.41	6.24	
C.D. 1%	8.59	8.04	8.76	9.59	9.16	9.04	9.05	9.58	9.93	8.37	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.18. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100mM, 150 mM & 200 mM) and different levels of NaCl & SA on SOD in different tomato varieties.

SOD (U mg-^{1} Protein)											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	65.50^{a}	71.10^{a}	74.06^{a}	76.46^{a}	81.60^{a}	85.933^{a}	63.40^{a}	70.53^{a}	72.80^{a}	78.13	73.95
WIR-13706	56.66^{abc}	62.40^{abc}	65.16^{ab}	67.26^{abc}	69.46^{abc}	74.233^{abc}	56.76^{abc}	59.70^{abc}	62.73^{abc}	68.56	64.29
Anigoarlentha	36.96^{e}	42.06^{de}	44.53^{e}	46.96^{e}	49.40^{e}	54.60^{ef}	36.93^{ef}	40.46^{e}	43.10^{e}	49.30	43.43
Ec-521080	58.20^{ab}	63.93^{abc}	66.10^{ab}	67.63^{abc}	70.03^{abc}	73.63^{abc}	58.86^{abc}	61.90^{ab}	68.66^{ab}	71.56	66.05
Arka marginal	41.83^{cde}	48.10^{cde}	51.66^{bcde}	53.53^{cde}	56.16^{cde}	59.70^{cdef}	43.70^{cdef}	46.70^{cde}	50.03^{cde}	55.60	50.70
Ec-520079	44.36^{bcde}	49.53^{bcde}	52.66^{bcde}	54.93^{cde}	57.46^{cde}	60.73^{cdef}	45.53^{bcdef}	49.26^{bcde}	53.66^{bcde}	58.73	52.69
WIR-3957	49.13^{bcde}	56.43^{abcde}	59.06^{abcd}	60.86^{bcde}	63.93^{bcde}	68.10^{bcdef}	51.03^{abcde}	52.53^{bcde}	56.26^{bcde}	61.56	57.89
P-6 chhu chara	38.93^{de}	43.03^{de}	46.90^{de}	49.26^{de}	50.30^{e}	53.66^{f}	38.96^{def}	41.43^{de}	45.90^{de}	51.63	46.00
VRT-2	53.20^{abcd}	57.50^{abcd}	60.96^{abcd}	63.56^{abcd}	65.93^{bcd}	70.90^{bcd}	53.30^{abcd}	56.50^{abcd}	59.46^{abcd}	54.43	59.57
H-24	48.96^{bcde}	55.66^{abcde}	63.36^{abc}	66.40^{abc}	68.03^{abcd}	72.20^{abcd}	52.13^{abcde}	54.76^{bcde}	57.23^{bcde}	62.36	60.11
Roma	48.53^{bcde}	55.36^{abcde}	58.80^{abcde}	61.90^{abcde}	63.96^{bcde}	68.96^{bcde}	50.36^{abcdef}	53.46^{bcde}	56.66^{bcde}	54.63	57.31
Ec-520078	48.40^{bcde}	52.26^{bcde}	54.63^{bcde}	57.26^{bcde}	60.36^{bcde}	62.73^{cdef}	47.23^{bcdef}	49.60^{bcde}	53.76^{bcde}	50.06	53.63
T-Loeal	57.60^{ab}	65.40^{ab}	67.23^{ab}	70.83^{ab}	74.63^{ab}	80.00^{ab}	60.63^{ab}	64.36^{ab}	68.00^{ab}	70.13	67.88
Arka Saurabh	36.70^{e}	42.03^{de}	44.60^{e}	47.73^{e}	49.53^{e}	53.93^{f}	37.13^{ef}	40.60^{e}	43.40^{e}	43.93	44.95
H-86	55.30^{abc}	62.30^{abc}	65.23^{ab}	66.86^{abc}	68.46^{abcd}	71.93^{abcd}	48.03^{abcdef}	50.90^{bcde}	55.16^{bcde}	60.83	60.50
Ec-520061	46.33^{bcde}	52.36^{bcde}	55.90^{bcde}	58.36^{bcde}	60.13^{bcde}	62.70^{cdef}	47.93^{bcdef}	51.50^{bcde}	54.16^{bcde}	53.76	54.31
DT-10	42.66^{bcde}	51.93^{bcde}	56.20^{bcde}	59.10^{bcde}	60.96^{bcde}	66.23^{bcdef}	47.43^{bcdef}	50.80^{bcde}	54.53^{bcde}	56.33	47.99
Agata-30	47.70^{bcde}	53.26^{bcde}	56.53^{bcde}	59.13^{bcde}	62.06^{bcde}	65.26^{bcdef}	47.90^{bcdef}	50.70^{bcde}	54.56^{bcde}	62.83	55.99
WIR-13708	41.20^{cde}	45.13^{de}	48.20^{cde}	50.60^{de}	53.13^{de}	58.66^{def}	41.30^{def}	43.43^{de}	46.70^{de}	54.30	44.14
WIR-3928	36.76^{e}	40.50^{e}	43.70^{e}	47.00^{e}	49.23^{e}	53.60^{f}	35.30^{f}	41.10^{e}	46.26^{de}	51.46	44.49
Gen. Mean	**47.74 ***	**53.51 ***	**56.77 ***	**59.28 ****	**61.74 ****	**65.88 ****	**48.19 ***	**51.513 ***	**55.15 ****	**58.51**	
C.V.	19.71	18.29	17.22	15.78	15.11	13.56	19.32	17.83	16.53	12.40	
F Prob.	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	
S.E.M.	5.43	5.65	5.64	5.40	5.38	5.16	5.37	5.30	5.26	4.19	
C.D. 5%	15.55	16.18	16.16	15.46	15.42	14.77	15.39	15.18	15.07	12.00	
C.D. 1%	20.83	21.68	21.65	20.71	20.65	19.78	20.61	20.34	20.19	16.07	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.19. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on peroxidase in different tomato varieties.

Peroxidase (µmol tetraguacial min^{-1} mg^{-1} Protein)											
varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	5.56^{abcd}	5.93^{bcde}	6.46^{bcdef}	7.03^{bcdef}	7.53^{bcdef}	8.36^{bcdefg}	4.80^{bcde}	5.30^{bcdef}	5.86^{bcde}	6.33	6.32
WIR-13706	4.30^{defg}	5.13^{bcdef}	5.86^{cdef}	6.53^{cdefg}	7.33^{bcdef}	8.10^{cdefg}	4.53^{cde}	5.00^{cdef}	5.66^{cde}	6.40	5.88
Anigoarlentha	5.50^{abcde}	6.13^{abcd}	6.80^{abcd}	7.33^{bcde}	8.13^{abcde}	8.96^{abcde}	5.60^{abcd}	6.03^{bcde}	6.40^{bcde}	7.03	6.79
Ec-521080	4.96^{bcdef}	5.80^{bcde}	6.60^{abcde}	7.26^{bcde}	8.53^{abc}	9.43^{abcd}	5.36^{bcde}	5.96^{bcde}	6.60^{bcd}	7.36	6.79
Arka marginal	7.23^{a}	8.00^{a}	8.56^{a}	9.33^{a}	9.73^{a}	10.20^{a}	7.53^{a}	8.03^{a}	8.63^{a}	9.63	8.68
Ec-520079	5.66^{abcd}	6.60^{abc}	7.43^{abc}	8.00^{abc}	8.46^{abc}	9.00^{abcde}	6.06^{abc}	6.40^{abcd}	6.90^{abcd}	7.60	7.21
WIR-3957	4.33^{defg}	5.36^{bcdef}	5.93^{cdef}	6.73^{bcdefg}	7.20^{cdef}	7.96^{defg}	4.86^{bcde}	5.33^{bcdef}	6.10^{bcde}	6.66	6.05
P-6 chhu chara	4.33^{defg}	4.86^{cdef}	5.60^{cdef}	6.16^{cdefg}	6.70^{defg}	7.40^{efgh}	5.03^{bcde}	5.36^{bcdef}	5.86^{bcde}	6.46	5.78
VRT-2	4.90^{bcdef}	5.66^{bcdef}	6.26^{bcdef}	6.90^{bcdefg}	7.36^{bcdef}	8.36^{bcdefg}	5.20^{bcde}	5.76^{bcdef}	6.23^{bcde}	6.83	6.35
H-24	6.63^{ab}	7.06^{ab}	7.93^{ab}	8.53^{ab}	9.33^{a}	10.03^{ab}	6.56^{ab}	6.73^{abc}	7.43^{abc}	8.10	7.83
Roma	3.63^{efg}	4.50^{def}	5.23^{def}	5.86^{defg}	6.50^{efg}	7.63^{defg}	4.10^{de}	4.40^{ef}	5.10^{de}	5.86	5.28
Ec-520078	4.90^{bcdef}	5.73^{bcdef}	6.03^{bcdef}	6.80^{bcdefg}	7.30^{bcdef}	8.06^{cdefg}	5.30^{bcde}	5.70^{bcdef}	6.23^{bcde}	6.83	6.28
T-Loeal	3.43^{fg}	4.13^{ef}	4.66^{ef}	5.56^{efg}	6.20^{fg}	7.10^{fgh}	3.76^{de}	3.96^{f}	4.53^{e}	5.50	4.88
Arka Saurabh	6.13^{abcd}	6.73^{abc}	7.40^{abc}	7.86^{abc}	8.96^{ab}	9.86^{abc}	5.00^{bcde}	4.90^{def}	5.26^{de}	5.90	6.80
H-86	2.96^{g}	3.80^{f}	4.60^{f}	5.00^{g}	5.06^{g}	5.76^{h}	3.46^{e}	3.96	4.63^{e}	5.23	4.45
Ec-520061	6.16^{abcd}	6.80^{abc}	7.23^{abc}	7.60^{abcd}	8.13^{abcde}	8.56^{abcdef}	6.26^{abc}	6.70^{abcd}	7.30^{abc}	8.00	7.27
DT-10	3.63^{efg}	4.00^{ef}	4.60^{f}	5.13^{fg}	6.00^{fg}	6.60^{gh}	3.50^{e}	3.96^{f}	4.53^{e}	5.03	4.69
Agata-30	4.66^{cdefg}	5.33^{bcdef}	5.93^{cdef}	6.43^{cdefg}	6.83^{cdef}	7.46^{efgh}	4.96^{bcde}	5.30^{bcdef}	5.86^{bcde}	6.80	5.96
WIR-13708	6.40^{abc}	6.96^{ab}	7.46^{abc}	8.03^{abc}	8.40^{abcd}	8.86^{abcdef}	6.53^{ab}	6.96^{ab}	7.70^{ab}	8.30	7.56
WIR-3928	5.20^{bcdef}	5.86^{bcde}	6.73^{abcd}	7.13^{bcde}	7.06^{cdef}	7.70^{defg}	5.40^{bcde}	5.86^{bcde}	6.63^{bcd}	7.26	6.48
Gen. Mean	**5.02 ****	**5.72 ****	**6.36 ****	**6.96 ****	**7.54 *****	**8.27 *****	**5.19 ***	**5.58 ****	**6.17 ****	**6.85**	
C.V.	23.14	20.68	18.80	16.84	13.76	13.23	22.78	19.76	18.64	17.36	
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	
S.E.M.	0.67	0.68	0.69	0.67	0.59	0.63	0.68	0.63	0.66	0.68	
C.D. 5%	1.92	1.95	1.97	1.93	1.71	1.81	1.95	1.82	1.90	1.96	
C.D. 1%	2.57	2.62	2.65	2.59	2.29	2.42	2.62	2.44	2.54	2.63	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.20. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on catalase in different tomato varieties.

Catalase (U mg-^{1} Protein)											
varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V.mean**
H-8878-1	0.41	0.57^{a}	0.66^{a}	0.70^{a}	0.72^{a}	0.75^{a}	0.51^{a}	0.55^{a}	0.61^{a}	0.70	0.62
WIR-13706	0.31	0.39^{b}	0.42^{b}	0.4zz^{b}	0.51^{b}	0.53^{b}	0.35^{b}	0.38^{b}	0.44^{b}	0.50	0.43
Anigoarlentha	0.23	0.29^{bcd}	0.35^{bcd}	0.39^{bcd}	0.43^{bcd}	0.49^{bcd}	0.25^{bcd}	0.28^{bc}	0.34^{bc}	0.42	0.35
Ec-521080	0.34	0.38^{bc}	0.42^{bc}	0.46^{bc}	0.46^{bc}	0.52^{bc}	0.35^{bc}	0.38^{b}	0.44^{b}	0.51	0.42
Arka marginal	0.14	0.19^{d}	0.23^{d}	0.29^{d}	0.33^{cd}	0.38^{cd}	0.16^{d}	0.19^{c}	0.24^{c}	0.30	0.24
Ec-520079	0.15	0.19^{d}	0.24^{d}	0.29^{d}	0.31^{cd}	0.36^{d}	0.16^{d}	0.19^{c}	0.23^{c}	0.30	0.24
WIR-3957	0.22	0.29^{bcd}	0.33^{bcd}	0.37^{bcd}	0.41^{bcd}	0.44^{bcd}	0.25^{bcd}	0.28^{bc}	0.33^{bc}	0.41	0.33
P-6 chhu chara	0.20	0.27^{bcd}	0.31^{bcd}	0.34^{bcd}	0.39^{bcd}	0.43^{bcd}	0.24^{bcd}	0.27^{bc}	0.34^{bc}	0.40	0.32
VRT-2	0.24	0.29^{bcd}	0.32^{bcd}	0.38^{bcd}	0.41^{bcd}	0.45^{bcd}	0.26^{bcd}	0.31^{bc}	0.36^{bc}	0.43	0.34
H-24	0.19	0.24^{bcd}	0.28^{bcd}	0.33^{bcd}	0.38^{bcd}	0.42^{bcd}	0.22^{bcd}	0.26^{bc}	0.33^{bc}	0.43	0.38
Roma	0.15	0.20^{d}	0.23^{d}	0.29^{d}	0.34^{cd}	0.40^{bcd}	0.17^{d}	0.20^{c}	0.26^{c}	0.36	0.26
Ec-520078	0.21	0.27^{bcd}	0.30^{bcd}	0.33^{bcd}	0.35^{bcd}	0.39^{bcd}	0.23^{bcd}	0.27^{bc}	0.32^{bc}	0.41	0.31
T-Loeal	0.20	0.25^{bcd}	0.29^{bcd}	0.32^{bcd}	0.36^{bcd}	0.38^{cd}	0.21^{bcd}	0.25^{bc}	0.30^{bc}	0.38	0.29
Arka Saurabh	0.19	0.23^{cd}	0.26^{cd}	0.31^{bcd}	0.34^{cd}	0.34^{d}	0.21^{bcd}	0.24^{bc}	0.30^{bc}	0.39	0.28
H-86	0.19	0.23^{cd}	0.26^{cd}	0.31^{cd}	0.33^{cd}	0.38^{bcd}	0.21^{bcd}	0.27^{bc}	0.34^{bc}	0.42	0.27
Ec-520061	0.21	0.26^{bcd}	0.30^{bcd}	0.35^{bcd}	0.38^{bcd}	0.43^{bcd}	0.23^{bcd}	0.25^{bc}	0.29^{bc}	0.39	0.31
DT-10	0.17	0.22^{d}	0.26^{cd}	0.28^{d}	0.31^{d}	0.36^{d}	0.20^{cd}	0.24^{bc}	0.31^{bc}	0.40	0.27
Agata-30	0.23	0.27^{bcd}	0.32^{bcd}	0.35^{bcd}	0.37^{bcd}	0.42^{bcd}	0.24^{bcd}	0.28^{bc}	0.35^{bc}	0.43	0.33
WIR-13708	0.60	0.21^{d}	0.23^{z}	0.26^{d}	0.28^{d}	0.34^{d}	0.19^{d}	0.21^{c}	0.27^{c}	0.37	0.30
WIR-3928	0.16	0.19^{d}	0.22^{d}	0.26^{d}	0.29^{d}	0.35^{d}	0.15^{d}	0.19^{c}	0.26^{c}	0.35	0.24
Gen. Mean	**0.24**	**0.27 ****	**0.31 *****	**0.35 ****	**0.38 ****	**0.43 ****	**0.24 ****	**0.278 ****	**0.33 ****	**0.41**	
C.V.	73.74	34.68	30.35	27.84	24.23	21.64	36.47	33.11	28.02	26.92	
F Prob.	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.06	
S.E.M.	0.10	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	
C.D. 5%	_	0.15	0.15	0.16	0.15	0.15	0.14	0.15	0.15	_	
C.D. 1%	_	0.21	0.21	0.22	0.20	0.20	0.19	0.20	0.20	_	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.21. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on glutathione peroxidase in different tomato varieties.

Glutathione peroxidase (U mg-^{1})											
varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	2.30	3.83	4.80	5.76	6.63	7.40	3.16	3.50	4.20	4.96	4.65
WIR-13706	2.83	4.13	4.80	5.66	6.16	7.20	3.66	4.23	4.76	5.60	4.90
Anigoarlentha	2.86	3.86	4.93	6.06	6.76	7.63	3.36	3.90	4.70	4.23	4.83
Ec-521080	2.89	4.00	4.56	5.06	5.60	6.33	3.63	4.03	4.80	5.36	4.62
Arka marginal	2.26	3.26	3.90	4.46	5.00	5.66	2.83	3.33	4.16	5.26	4.01
Ec-520079	3.10	4.00	4.80	5.40	5.83	6.76	3.63	4.03	4.80	5.86	4.82
WIR-3957	1.89	2.70	3.43	4.33	5.06	5.96	2.46	3.00	3.70	4.23	3.67
P-6 chhu chara	2.93	3.66	4.06	4.66	5.13	5.96	3.90	4.40	4.96	5.73	4.54
VRT-2	3.20	4.83	5.73	6.26	6.66	7.16	4.00	4.46	5.13	6.20	5.36
H-24	1.77	2.93	3.80	4.33	4.90	5.70	2.16	2.60	3.36	4.40	3.59
Roma	3.00	3.96	4.46	5.26	5.96	7.10	2.93	3.60	4.23	5.50	4.60
Ec-520078	2.76	3.76	4.36	5.13	5.56	6.63	3.20	3.70	4.40	5.73	4.52
T-Loeal	2.80	3.66	4.26	5.13	7.40	7.90	3.33	3.80	4.36	5.36	4.50
Arka Saurabh	2.47	3.86	4.26	4.53	5.06	6.03	2.83	3.26	4.06	5.06	4.14
H-86	2.70	3.40	3.83	4.53	5.26	6.16	3.36	3.80	4.50	5.36	4.29
Ec-520061	3.02	3.83	4.66	5.46	5.93	6.63	3.30	3.73	4.33	5.30	4.62
DT-10	2.31	3.06	3.70	4.26	4.73	5.93	2.60	3.10	3.66	4.43	3.78
Agata-30	3.16	4.23	4.66	5.40	5.90	6.86	3.73	4.23	5.00	5.76	4.89
WIR-13708	1.24	2.03	2.53	3.16	3.76	5.00	1.63	1.96	2.83	4.03	2.82
WIR-3928	2.86	3.76	4.46	5.06	5.63	6.56	3.30	3.70	4.30	5.23	4.49
Gen. Mean	**2.62**	**3.64**	**4.30**	**5.00**	**5.65**	**6.53**	**3.15**	**3.62**	**4.31**	**5.18**	
C.V.	41.83	33.70	29.64	25.63	24.16	21.64	35.24	31.80	26.28	22.11	
F Prob.	0.82	0.75	0.66	0.51	0.36	0.67	0.61	0.62	0.70	0.62	
S.E.M.	0.63	0.70	0.73	0.74	0.78	0.81	0.64	0.66	0.65	0.66	
C.D. 5%	_	_	_	_	_	_	_	_	_	_	
C.D. 1%	_	_	_	_	_	_	_	_	_	_	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

4.6. Yield parameters:

4.6.1. Number of fruits per plant:

Results presented in table-4.22 revealed that salinity levels, genotypes, salicylic acid and interaction of salinity and genotypes, interaction of salicylic acid and genotypes, as well as the combined effect of salicylic acid and different salt concentrations affected the number of fruits per plant and finally the yield significantly at $P < 0.01\%$. The genotypes means (table-4.22) showed that the maximum number of fruits were produced by tomato genotype H-8878-1, WIR-13708 and WIR-3928 followed by WIR-13706 and DT-10 even at the highest salinity level, whereas, the minimum value was observed in VRT-2 and H-24. Treatment means showed significant decrease in number of fruits with increase in salinity and the minimum value was observed at the highest salinity level. The results on interaction between genotype and salinity along with salicylic acid (table-4.4) showed positive results for the morphological character i. e. number of fruits per plant which is directly related to the yield of the tomato varieties. WIR-13706, Anigoarlentha, Ec-521080, Arka marginal, H-86 showed a insignificant effect on number of fruits per plat at low salinity level but at the highest NaCl concentration (200 mM), greater decrease in number of fruits was recorded. Application of salicylic acid minimized the efeect of salt stress in all the 20 tomato genotypes also at the highest salinity level.

4.6.2. Average fruit diameter (cm):

The results of analysis of variance showed that (table-4.23) salicylic acid affected the fruit diameter significantly and different levels of salt stress affected the plant-height as well as the number of leaves significantly but the interaction effect of both salt and salicylic acid on fruit diameter, the most important morphological parameters which directly affect the final yield was also significant. Fruit diameter reduced with the increase in salt stress levels as (150 mM and 200 mM) in comparison with control but there were no considerable changes in stress level of salt 50 mM. Exogenous application of salicylic acid increased considerable positive results comparison with control level (table-4.23). The genotype P-6 chhu chara showed the highest value of fruit diameter at control and even also at the highest level of salinity 200 mM (salt) and the minimum value of that was showed by the genotype Ec-520078 at the highest salinity level (200 mM NaCl concentration). Arka marginal, P-6 chhu chara, VRT-2, H-24 and Arka Saurabh showed greater value of average fruit- diameter at the highest salinity level. H-8878-1, WIR-13706, Anigoarlentha and Ec-520079 showed an insignificant effect of salt stress even at the highest salinity level in combination with salicylic acid treatment.

Application of salt delivered reduced value of fruit diameter in all the genotypes while the application of salicylic acid ameliorated the negative effect of different levels of salt stress.

4.6.3. Fruit yield per plant:

Data presented in table-4.24 showed average fruit yield as affected by different soil salinity conditions and treated by salicylic acid of concentration 25 µM concentrations. It is clear that increasing salinity levels resulted in outstanding reductions in average fruit yield per plant. So it could be concluded that average fruit yield per plant decreased considerably under saline conditions compared with non-saline one. Moreover, it was noticed that as the soil salinity increased the more outstanding decreases were found in growth attributes. The maximum yield was reported in the genotypes Arka Saurabh and H-8878-1 followed by WIR-13706 and DT-10 at the highest salinity level 200 mM (salt). Application of salicylic acid imposed great positive effect on fruit yield per plant. The effect of salicylic acid alone and in combination with different concentration of NaCl was studied on fruit yield per plant in different tomato varieties. The result showed that application of SA alone showed a positive response on fruit yield per plant in different tomato varieties significantly. Results also showed that there was a dramatic decrease in fruit yield per plant with the increasing concentration of NaCl. Therefore, in general salicylic acid was notified to overcome the adverse effect imposed by salt stress. Ec-520079, Anigoarlentha and WIR-3928 showed the maximum effect of salt on fruit yield per plant. H-8878-1, Anigoarlentha, Ec-521080, Arka marginal, P-6 chhu chara, Arka Saurabh, WIR-3928 showed greater effect of salicylic acid application at the concentration of (25 µM) on the most important morphological parameter i. e. fruit yield per plant.

Table: 4.22. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on number of fruits/plant in different tomato varieties.

Number of fruits/plant											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	16.0^{a}	19.0^{a}	15.3^{a}	13.6^{ab}	12.3^{a}	9.6^{ab}	17.0^{a}	15.0^{ab}	13.6^{ab}	10.6^{ab}	15.3
WIR-13706	14.3^{abcd}	16.6^{abc}	13.3^{abcd}	12.3^{abc}	10.6^{abc}	8.6^{abcd}	16.0^{abc}	13.6^{abcd}	12.0^{abcd}	9.6^{abc}	12.7
Anigoarlentha	13.0^{abcd}	16.0^{abcde}	12.3^{abcde}	11.0^{abcd}	9.6^{abcd}	8.0^{bcdef}	14.0^{abcde}	12.6^{abcde}	11.0^{abcd}	9.0^{bcde}	11.6
Ec-521080	133^{abcd}	16.6^{abc}	12.0^{abcde}	10.6^{abcd}	9.6^{abcd}	8.0^{bcdef}	14.0^{abcde}	12.3^{abcde}	11.3^{abcd}	9.3^{abcd}	11.7
Arka marginal	13.0^{abcd}	15.6^{abcde}	12.3^{abcde}	11.3^{abcd}	10.3^{abc}	8.3^{abcde}	14.0^{abcde}	13.0^{abcde}	11.6^{abcd}	9.3^{abcd}	11.8
Ec-520079	11.0^{bcdef}	15.0^{abcde}	10.3^{cdef}	9.3^{cde}	8.0^{bcde}	6.3^{defg}	12.3^{bcdef}	11.0^{cdef}	9.6^{cde}	7.6^{cdef}	10.0
WIR-3957	10.0^{def}	12.0^{def}	9.0^{ef}	8.0^{de}	6.6^{de}	5.0^{g}	11.0^{def}	10.0^{def}	8.6^{de}	6.0^{f}	8.6
P-6 chhu chara	11.0^{bcdef}	14.0^{cdef}	10.0^{cdef}	9.0^{cde}	8.0^{bcde}	6.3^{defg}	11.6^{cdef}	11.0^{cdef}	9.3^{cde}	7.6^{cdef}	9.8
VRT-2	7.6^{f}	10.0^{f}	7.3^{f}	6.3^{e}	5.6^{e}	4.3^{g}	9.0^{f}	8.0^{f}	7.0^{e}	5.6^{f}	7.1
H-24	9.0^{ef}	11.6^{ef}	7.6^{f}	7.6^{de}	7.6^{cde}	6.0^{efg}	9.0^{f}	9.3^{ef}	8.6^{de}	7.0^{def}	8.3
Roma	10.3^{cdef}	14.0^{cdef}	9.3^{def}	8.6^{cde}	7.6^{cde}	5.6^{fg}	11.0^{def}	10.0^{def}	9.0^{cde}	6.6^{ef}	9.2
Ec-520078	11.3^{bcdef}	14.3^{bcdef}	10.3^{cdef}	9.6^{cde}	8.3^{bcde}	6.3^{defg}	9.6^{ef}	11.3^{bcdef}	10.3^{bcde}	7.0^{def}	9.8
T-Loeal	10.6^{cdef}	12.0^{def}	10.0^{cdef}	9.0^{cde}	8.0^{bcde}	6.0^{efg}	11.6^{cdef}	10.3^{cdef}	9.3^{cde}	6.6^{ef}	9.3
Arka Saurabh	13.3^{abcd}	15.6^{abcde}	12.0^{abcde}	11.0^{abcd}	9.6^{abcd}	7.6^{bcdef}	13.6^{abcde}	12.0^{bcde}	11.3^{abcd}	8.6^{bcde}	11.5
H-86	13.6^{bcd}	16.0^{abcde}	12.6^{abcde}	11.3^{abcd}	10.0^{abc}	8.3^{abcde}	14.3^{abcd}	13.3^{abcd}	11.0^{abcd}	9.3^{abcd}	11.9
Ec-520061	11.6^{abcdf}	15.3^{abcde}	10.6^{bcdef}	10.0^{bcde}	8.6^{bcde}	6.6^{cdefg}	12.6^{abcdef}	11.6^{bcdef}	10.3^{bcde}	7.3^{cdef}	10.5
DT-10	14.0^{abcd}	15.3^{abcde}	13.0^{abcde}	12.0^{abc}	10.6^{abc}	8.0^{bcdef}	14.6^{abcd}	13.3^{abcd}	12.0^{abcd}	9.0^{bcde}	12.2
Agata-30	14.6^{abc}	16.3^{abcd}	13.6^{abc}	12.3^{abc}	11.0^{ab}	9.0^{abc}	15.6^{abc}	14.0^{abc}	12.3^{abc}	9.6^{abc}	12.8
WIR-13708	16.0^{a}	18.6^{ab}	15.0^{a}	14.0^{a}	12.6^{a}	10.6^{a}	17.0^{a}	16.0^{a}	14.3^{a}	11.6^{a}	14.6
WIR-3928	15.3^{ab}	18.0^{abc}	14.6^{ab}	13.6^{ab}	12.3^{a}	10.0^{ab}	16.3^{ab}	16.0^{a}	14.0^{a}	11.0^{ab}	14.1
Gen. Mean	**12.4***	**15.1 ***	**11.5 ****	**10.5 ****	**9.3 ****	**7.45 *****	**13.2 ****	**12.20 ****	**10.8 ****	**8.4 *****	
C.V.	21.2	18.2	21.1	21.5	20.1	20.9	20.0	18.8	19.4	18.5	
F Prob.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
S.E.M.	1.5	1.5	1.4	1.3	1.0	0.9	1.5	1.3	1.2	0.9	
C.D. 5%	4.3	4.5	4.0	3.7	3.1	2.5	4.3	3.8	3.4	2.5	
C.D. 1%	5.8	6.1	5.4	5.0	4.1	3.4	5.8	5.0	4.6	3.4	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.23. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on average fruit diameter (cm) in different tomato varieties.

Average Fruit Diameter (cm)											
Varieties	**Control**	**SA(25µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	3.5^{defgh}	4.7^{cd}	3.4^{defg}	3.2^{efgh}	3.1^{efgh}	2.6^{defg}	4.0^{efgh}	3.7^{efg}	3.7^{def}	3.1^{fgh}	3.5
WIR-13706	3.5^{defgh}	4.4^{cde}	3.3^{defg}	3.2^{efgh}	3.0^{efgh}	2.5^{efgh}	4.2^{defg}	3.8^{efg}	3.6^{ef}	3.0^{fghi}	3.4
Anigoarlentha	3.4^{efgh}	3.8^{ef}	3.3^{defg}	3.2^{fgh}	3.1^{defg}	2.8^{def}	4.2^{defg}	3.8^{defg}	3.6^{ef}	3.3^{efg}	3.4
Ec-521080	2.8^{hij}	3.3^{fg}	2.7^{ghi}	2.7^{hij}	2.4^{hij}	2.2^{fghi}	3.3^{hi}	3.2^{ghi}	2.8^{hi}	2.6^{ghi}	3.9
Arka marginal	3.9^{cdef}	4.2^{de}	3.8^{de}	3.7^{de}	3.5^{de}	3.0^{de}	4.7^{de}	$4.3s^{de}$	4.4^{cd}	3.5^{def}	3.9
Ec-520079	2.5^{j}	3.0^{g}	2.4^{hi}	2.3^{ij}	2.1^{j}	1.9^{hi}	3.2^{hi}	3.1^{ghi}	2.7^{hi}	2.5^{hi}	2.6
WIR-3957	3.1^{ghij}	3.8^{ef}	3.0^{fgh}	2.9^{ghij}	2.7^{fghi}	2.4^{efghi}	3.8^{fghi}	3.5^{fgh}	3.3^{fgh}	2.7^{ghi}	3.1
P-6 chhu chara	6.5^{a}	6.8^{a}	6.2^{a}	5.9^{a}	5.7^{a}	5.3^{a}	6.8^{a}	6.4^{a}	6.2^{a}	5.9^{a}	6.2
VRT-2	5.5^{b}	6.0^{b}	5.2^{b}	4.9^{b}	4.7^{b}	4.3^{b}	5.9^{b}	5.5^{b}	5.4^{b}	4.7^{b}	5.2
H-24	5.5^{b}	5.9^{b}	5.1^{b}	4.8^{b}	4.6^{b}	4.0^{b}	5.8^{b}	5.4^{b}	5.2^{b}	4.4^{bc}	5.1
Roma	4.0^{cde}	4.5^{cde}	3.9^{d}	3.7^{def}	3.5^{de}	2.8^{def}	4.5^{def}	4.4^{de}	4.0^{de}	3.4^{efg}	3.9
Ec-520078	2.5^{j}	3.2^{fg}	2.3^{i}	2.2^{j}	2.0^{j}	1.7^{i}	3.0^{i}	2.7^{i}	2.5^{i}	2.3^{i}	2.4
T-Loeal	3.2^{fghi}	3.8^{ef}	3.1^{efgh}	3.0^{fghi}	2.4^{hij}	2.0^{ghi}	3.9^{efgh}	3.4^{fghi}	2.9^{ghi}	2.5^{hi}	3.0
Arka Saurabh	4.2^{cd}	4.8^{cd}	4.0^{cd}	3.9^{cd}	3.7^{cd}	3.3^{cd}	4.8^{cd}	4.5^{cd}	4.2^{de}	3.9^{cde}	4.1
H-86	4.5^{c}	5.0^{c}	4.7^{bc}	4.5^{bc}	4.3^{bc}	3.7^{bc}	5.5^{bc}	5.2^{bc}	5.0^{bc}	4.2^{bcd}	4.7
Ec-520061	2.5^{ij}	2.6^{g}	2.5^{hi}	2.4^{ij}	2.1^{ij}	1.8^{hi}	3.3^{hi}	3.3^{ghi}	2.6^{hi}	2.4^{hi}	2.5
DT-10	3.8^{defg}	4.3^{cde}	3.7^{def}	3.4^{defg}	3.2^{defg}	2.8^{def}	4.3^{defg}	4.1^{def}	3.7^{def}	3.5^{def}	3.7
Agata-30	3.8^{defg}	4.5^{cde}	3.6^{def}	3.5^{defg}	3.3^{def}	2.8^{def}	4.3^{defg}	4.1^{def}	3.6^{efg}	3.4^{efg}	3.7
WIR-13708	2.5^{j}	2.9^{g}	2.4^{hi}	2.3^{ij}	2.0^{j}	1.8^{i}	3.1^{i}	2.9^{hi}	2.5^{i}	2.4^{hi}	2.5
WIR-3928	2.9^{hij}	3.3^{fg}	2.8^{ghi}	2.7^{hij}	2.6^{ghij}	2.2^{fghi}	3.6^{ghi}	3.3^{ghi}	3.0^{fghi}	2.8^{fghi}	2.9
Gen. Mean	**3.7*****	**4.2*****	**3.6*****	**3.4*****	**3.2*****	**2.8*****	**4.3*****	**4.0*****	**3.7*****	**3.3*****	
C.V.	11.6	10.8	11.7	12.0	12.0	14.6	11.0	11.1	11.0	12.8	
F Prob.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
S.E.M.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	
C.D. 5%	0.7	0.7	0.7	0.6	0.6	0.6	0.7	0.7	0.6	0.7	
C.D. 1%	0.9	1.0	0.9	0.9	0.8	0.9	1.0	1.0	0.9	0.9	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

Table: 4.24. Effect of salicylic acid (25 µM), different levels of NaCl (50 mM, 100 mM, 150 mM & 200 mM) and different levels of NaCl & SA on fruit yield per plant (g) in different tomato varieties.

Fruit yield/plant (g)											
varieties	**Control**	**SA(25/µM)**	**50mM(salt)**	**100mM(salt)**	**150mM(salt)**	**200mM(salt)**	**50mM(salt)+SA(25µM)**	**100mM(salt)+SA(25µM)**	**150mM(salt)+SA(25µM)**	**200mM(salt)+SA(25µM)**	**V. mean**
H-8878-1	1840.33^{a}	2084.00^{a}	1737.66^{a}	1608.66^{a}	1471.00^{a}	1227.33^{a}	1865.00^{a}	1721.33^{a}	1524.66^{a}	1286.66^{a}	1636.66
Ec-520079	1608.33^{b}	1880.66^{a}	1464.66^{b}	1297.33^{b}	1161.00^{b}	950.00^{b}	1611.33^{bc}	1415.00^{b}	1232.33^{b}	1016.33^{b}	1363.69
Anigoarlentha	1402.00^{c}	1576.66^{b}	1365.33^{c}	1207.33^{b}	1045.66^{bc}	844.66^{bcd}	1470.33^{cde}	1342.33^{bcd}	1122.33^{bc}	908.66^{bcd}	1228.53
Ec-521080	1341.33^{cde}	1563.00^{b}	1265.00^{cdef}	1134.00^{bcd}	955.00^{bc}	782.66^{cde}	1423.00^{cdef}	1280.66^{bcd}	1078.66^{bc}	858.33^{bcde}	1168.1
Arka marginal	1395.00^{c}	1639.66^{b}	1301.66^{cde}	1192.66^{bc}	1062.33^{bc}	788.00^{cde}	1470.33^{cde}	1355.66^{bcd}	1122.00^{bc}	861.00^{bcde}	1218.83
Ec-520079	1193.66^{def}	1425.33^{bcd}	1079.66^{fg}	939.66^{def}	740.66^{def}	632.33^{efg}	1269.00^{efg}	1033.66^{ef}	833.33^{def}	699.66^{efg}	984.70
WIR-3957	1259.333^{cdef}	1476.33^{bcd}	1140.66^{efg}	1104.33^{bcd}	929.00^{cd}	761.00^{cde}	1289.66^{defg}	1226.00^{cd}	1009.00^{cd}	818.66^{cde}	1101.4
P-6 chhu chara	1289.00^{cdef}	1511.33^{bcd}	1188.66^{defg}	1057.33^{cd}	904.66^{cde}	724.66^{cde}	1352.66^{def}	1192.00^{de}	980.33^{cd}	812.33^{cde}	1101.3
VRT-2	1137.33^{f}	1368.00^{cd}	997.33^{gh}	844.33^{efg}	713.33^{ef}	563.00^{fgh}	1124.33^{ghi}	970.66^{fg}	765.66^{efg}	635.33^{fgh}	911.93
H-24	847.66^{g}	1079.66^{ef}	770.00^{i}	655.33^{gh}	554.00^{f}	445.66^{h}	986.66^{i}	789.66^{g}	620.33^{g}	505.66^{h}	725.46
Roma	788.00^{g}	940.66^{f}	750.66^{i}	670.33^{gh}	558.33^{f}	439.66^{h}	982.66^{i}	818.00^{g}	632.00^{fg}	504.333^{h}	708.46
Ec-520078	837.33^{g}	1017.33^{f}	742.33^{i}	634.00^{h}	590.66^{f}	455.66^{h}	942.00^{i}	801.00^{g}	682.33^{fg}	510.66^{h}	721.33
T-Loeal	844.66^{g}	971.66^{f}	773.667^{i}	697.00^{gh}	588.00^{f}	494.33^{gh}	1020.00^{i}	831.66^{g}	661.66^{fg}	559.33^{gh}	744.20
Arka Saurabh	1693.00^{ab}	1965.00^{a}	1596.00^{ab}	1542.66^{a}	1422.66^{a}	1231.00^{a}	1778.33^{ab}	1639.33^{a}	1493.66^{a}	1294.33^{a}	1565.6
H-86	1233.33^{cdef}	1419.00^{bcd}	1159.66^{efg}	1087.33^{cd}	956.33^{bc}	765.00^{cde}	1301.33^{defg}	1253.66^{bcd}	971.66^{cde}	826.66^{cde}	1097.4
Ec-520061	892.66^{g}	1060.33^{ef}	823.66^{hi}	749.66^{fgh}	636.00^{f}	514.33^{gh}	1032.33^{hi}	866.33^{fg}	707.00^{fg}	564.00^{gh}	784.63
DT-10	1254.00^{cdef}	1403.66^{bcd}	1176.66^{defg}	1099.00^{bcd}	967.33^{bc}	810.67^{bcd}	1399.66^{cdef}	1202.00^{cde}	1049.33^{bc}	893.00^{bcd}	1125.53
Agata-30	1272.33^{cdef}	1468.33^{bcd}	1190.66^{defg}	1073.33^{cd}	959.33^{bc}	763.00^{cde}	1392.33^{def}	1212.66^{cde}	1034.66^{bcd}	862.00^{bcde}	1122.86
WIR-13708	1156.00^{ef}	1291.66^{de}	1093.00^{fg}	1024.33^{cde}	896.00^{cde}	710.33^{def}	1245.00^{fgh}	1207.00^{cde}	962.00^{cde}	787.33^{def}	1037.26
WIR-3928	1384.66^{cd}	1527.00^{bcd}	1310.33^{cde}	1220.66^{bc}	1020.66^{bc}	872.33^{bc}	1499.66^{cd}	1378.66^{bc}	1080.00^{bc}	960.33^{bc}	1225.43
Gen. Mean	**1233.50*** **	**1433.46*** **	**1146.36*** **	**1041.96*** **	**906.60*** **	**738.78*** **	**1322.783*** **	**1176.86*** **	**978.15*** **	**808.23*** **	
C.V.	9.46	10.16	10.59	12.14	14.06	13.23	9.84	9.49	13.02	12.26	
F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
S.E.M.	67.41	84.14	70.10	73.06	73.60	56.43	75.20	64.52	73.55	57.22	
C.D. 5%	193.01	240.90	200.70	209.17	210.72	161.57	215.31	184.73	210.58	163.83	
C.D. 1%	258.52	322.68	268.83	280.17	282.25	216.41	288.39	247.44	282.06	219.44	

Numerical values with similar alphabets are statistically non-significant and dissimilar alphabets show statistically significant.

4.5. Discussion:

Plants in their natural environment are continuously exposed to a variety of stress factors, both abiotic and biotic, and thus have evolved a multitude of defence mechanisms in order to maintain their fitness **(Roux *et al.*, 2014; Mickelbart *et al.*, 2015).** Under natural conditions, both the timing and the intensity of the stressors can vary; thus, appropriate finetuning of the defence responses is required to minimize detrimental effects on plant fitness **(Des Marais and Juenger, 2010; Brown and Rant, 2013).** The results reported here may be transferable and translated to other crops, as the core stress tolerance/defence response genetic regulation appears to be universal, despite the existence of species-specific responses. However, stress (abiotic) has some unique properties (*e.g.* toxic effects of Na^+ and Cl^- on plants are unique to salt stress) that need to be taken into account. Moreover, studies should be extended to cover the entire life cycle of plants, as plant age might significantly influence the phenotypic response and senescence in particular.

4.5.1. Morphological parameters in tomato genotypes:

This experiment was conducted to monitor the salt efficient tomato varieties which show the survival of fittest among different tomato genotypes and also the effect of salicylic acid alone and in combination with the different treatments of salt.

Analysis of variance showed that application of salicylic acid significantly affected tomato plant morphological traits such as plant-height, number of leaves, number of branches, number of fruits per plant and fruit diameter and fruit weight also (P<0.01).

The growth performance of the plants was estimated by measuring the plant height on the 60th DAS. Under 100 mM NaCl stress, the growth of the plants was highly affected and reduced in tomato variety H-8878-1 when compared to control plants (Table-4.1). Excess foliar accumulation of Na+ inhibits plant growth and development **(Ashraf and Harris, 2004; Munns, 2002)**. In the present study, NaCl significantly decreased plant-height, number of leaves per plant and number of branches per plant (Table-4.1, 4.2 and 4.3). Similar results were also reported for tomato **(İnal, 2002; Rahman *et al.*, 2006; Saeed and Ahmad, 2009)** and strawberry **(Khayyat *et al.*, 2007).**

Application of salicylic acid enhanced all growth parameters and yield in all the genotypes compared with salinity treatment only. This positive effect of salicylic acid may be attributed to the increase of CO_2 assimilation and photosynthetic rate and increased mineral uptake in stressed plant under SA application **(Szepesi *et al.*, 2005).** Salicylic acid significantly increased plant growth either under stress or without stress conditions. This effect probably related to salicylic acid inhibition of Cl- and Na+ absorption and its help for Mg, Fe, Mn, N and Cu absorption or results from its effect on lipid peroxidation and membrane permeability. In cucumber and tomato, the fruit yield was enhanced

significantly when the plants were sprayed with lower concentrations of salicylic acid **(Larque-Saavedra and Martin-Mex, 2007).** Salinity may directly or indirectly inhibit cell division, cell enlargement, which reflects in reduction of plant height **(Shahid *et al.*, 2011)**. It is reported that the reduction of shoot height may be due to dehydration of protoplasm, less relative turgidity associated with turgor loss and decreased cell expansion and cell division **(Raza *et al.*, 2014)**. In Moringa plants found that there are negative relationship between salt stress degree and plant growth characters i.e. plant height, leaves area and dry weight of root, stem and leaves, which decreased as the salt concentration increased in the diluted seawater **(Hussein and Abou-Baker, 2014)**.

The results obtained from these experiments herein show that salt stress caused a significant reduction in plant growth parameters and fruit yield (Table 4.1, 4.2 and 4.3). However, supplementary salicylic acid enhanced these parameters compared to stressed plants. Salicylic acid has been reported to protect plants from various environmental stresses, including drought, salinity, and heavy metals **(Fletcher *et al.*, 2000; Zhang *et al.*, 2007)**. Present study demonstrate that exogenous application of salicylic acid improved the shoot length, number of leaves, number of branches per plant and finally the yield of tomato plants under salt stress.

4.5.2. Physiological parameters:

4.5.2.1. Electrolyte Leakage:

The presence of NaCl in the rooting medium causes a disturbance in membrane permeability, as expressed by an increase in solute leakage or membrane permeability. A major effect of environmental stress (i.e., salt, drought) on plant is membrane modification, which results in cell membrane disturbed function or total dysfunction. Changes in membrane leakage and injury can be measured by the extent of EC (Electrolyte Leakage) in tissues. In the present study, membrane permeability (EC%) of tomato was measured (table-4.4).

High concentrations of Na caused membrane disorganization **(Greenway & Munns, 1980).** It is pointed out that molar percentages of strerols and phospholipd decreased with increasing salinity **(Wu *et al.*, 1998)**. Electrolyte leakage enables cell membrane injury to be assessed when plants are subjected to salinity stress. Maintaining integrity of the cellular membranes under salt stress is considered as an integral part of the salinity tolerance mechanism **(Stevens *et al.*, 2006)**. Similar reports were presented by (**Parida & Das, 2005)** and (**Yildirim *et al.*, 2008)** for several crops.

In the present study the application of 25µM SA showed positive response on electrolytic leakage in different levels of salt stress in tomato varieties. From the tables and data of present study, it is concluded that application of salicylic acid protect the tomato genotypes from membrane injury caused by different levels of salt stress.

4.5.2.2. Leave Relative Water Content (LRWC):

Leaf water content (RWC) decreased with increased NaCl concentration. However, the genotypes did not differ significantly in this water relation attribute, and comparison among two salt levels (100 and 200 mM NaCl) indicates dramatic reduction in leaf relative water content as compared to control plant, respectively (Table-4.5).

External NaCl salinity lowered LRWC of tomato plants ($p < 0.05$). It was reported that the cause of higher LRWC in tolerant cultivars is ability to absorb more water from the soil and compensate transpiration was done from plant leaves **(Siddique *et al.*, 2000)**. Water stress often results when plants were subject to high salt concentrations **(Gonzalez & Gonzalez-Vilar, 2001)**. It was reported that the relative water content, water potential and osmotic potential of plants become more negative with an increase in salinity **(Parida & Das, 2005)**. This study showed that salicylic acid treatment induced an increase in LRWC of the salt stressed plants as compared to the control plants. Increases in LRWC of tomato plants treated with SA were also reported for other crops grown under salt stress including barley **(El-Tayeb, 2005)**, tomato **(Tari *et al.*, 2002; Szepsi *et al.*, 2005)** and cucumber **(Yildirim *et al.*, 2008).** Exposure of plants to NaCl reduced the availability of water for the plants, thus causes osmotic stress. The dominance of Na+ and Cl- ions inhibits the uptake of other minerals vital for plant's growth **(Ghanem *et al.*, 2008; Albacete *et al.*, 2008; Hamdia and Shaddad, 2010)**.

Application of salicylic acid imposed a positive effect on leaves water content of all the genotypes even after the treatment of salt of different concentrations (table-4.5).

4.5.2.3. Protein content:

The results of present study indicate a positive effect for sodium chloride using various concentrations on total protein of tomato plant leaves after 60 days. It appears from the data that there was a general increase in protein content that corresponded with the increase in salt concentrations as well as in salicylic acid application and also in combination with different concentrations of salt and salicylic acid (table-4.6).

Proteins accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress is over and may play a role in osmotic adjustment **(Pareek *et al.*, 1997)**. The results obtained, in general agree with what **Chao *et al.*, (1999)** had presented. They noticed an increase of protein content of the tomato plant in response to salt treatment.

There are many reports about protein changes along with compatible stages that adapt the plants with changed environment **(Kong-Ngern *et al.*, 2005)**. Compatible solutes in plant stress responses is not limited to conventional osmotic adjustment, but also includes some other regulatory or osmoprotective functions. One such function is in maintaining cytosolic K+ homeostasis by

preventing NaCl induced K+ leakage from the cell **(Cuin and Shabala, 2005)**. NaCl at 50-150 mM increased proline contents in *Salventia natans* L **(Jampeetong and Brix, 2009 and Patel *et al.*, 2010 on *Ceriops tagal*)**. Salt stress 18000 and 20000 ppm increased amino acids contents **(Darwesh *et al.*, 2011** on *Phoenix canariensis*)**.** Again, **Sibole *et al.*, (2003)**, reported that the treatment of clover plant (*Medicago citrna* L.) for 30 days with concentrations of zero, 1, 50, 100, 200 mM of NaCl increased soluble protein content in the seedlings, compared with control plants. Also, **Tort and Turkyilmaz, (2004)** recorded a big increase in protein content when treating barley plant (Hordeum vulgare L.) with 120 mM of sodium chloride. Results of a recent study by **Kapoor and Srivastava, (2010)** on *Vigna mungo* (L.) support the previous results. They observed an increase in protein content when increasing salt concentration. The accumulation of compatible solutes is often regarded as a basic strategy for the protection and survival of plants under abiotic stress conditions, including both salinity and oxidative stress **(Zhonghua *et al.*, 2014)**. Salicylic acid of 50-75 mM with NaCl at 60 mM increased proteins of *Solanum tuberosum* L. **(Sajid and Aftab, 2012)**, total soluble proteins of sunflower increased under NaCl 150 mM and L-argenine **(Havva *et al.*, 2014).**

4.5.2.4. Chlorophyll and carotenoid contents:

In the present study, NaCl treatment caused a major decline in the chlorophyll content while salicylic acid treatment of the NaCl-stressed plants significantly increased not only chlorophyll content, but also the carotenoids compared to control (table-4.7, 4.8, 4.9 and 4.10).

The reduction in leaf chlorophyll under salinity is attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex **(Levitt, 1980)**. The severe reduction in the photosynthetic pigments (*Chl* a, b and carotenoids) in tomato plants in the present investigation of the control treatments may be attributed to the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and/or maintaining damage of the chloroplast thylakoid. These results are in harmony with those observed by **(Quartacci and Navari-Izzo, 1992; Rao and Rao, 1981)**. One of the most important reasons that reduce chlorophyll content in salt stressed plants is destructive effect of reactive oxygen species (ROS). Salicylic acid decreases the damaging influences of reactive oxygen species on chlorophyll by activating antioxidant systems **(Kaya *et al.*, 2001).** It was observed that salicylic acid provides a pool of compatible osmolyts in the presence of salinity. The increase in production of photosynthetic pigments in SA treated plants was concomitant with the accumulation of saccharides and growth yield of wheat plant under salinity levels as compared with control plants **(Tari *et al.*, 2002)**. It has been shown that the use of salicylic acid increased the content of chlorophyll a in bean plants in salinity stress **(Türkyilmaz *et al.*, 2005).** Salicylic acid is supposed to increase the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or chlorophyll biosynthesis **(Shi *et al.*, 2006)**.

Excessive absorption of toxic ion (Na^+) may result in Na^+ toxicity resulting in enhanced senescence and decreased photosynthesis **(Munns, 2002; Chookhampaeng *et al*., 2007). Azooz (2009)** in bean and **Arfan (2007)** in spring wheat showed that treatment with salicylic acid significantly increased the total chlorophyll content in salinity condition. The chlorophyll and total carotenoid contents of leaves generally decrease under salt stress **(Karimi *et al*., 2005; Yokas *et al*., 2008).**

Many authers, **Shakirova *et al*. (2003)** and **Abdel-Wahed *et al*., 2006; El-Mergawi and Abdel-Wahed, 2007)** on maize plants found that salicylic acid caused significant increased in chlorophyll content. This accumulation of photosynthetic pigments as a result of exogenous application of SA may be due to increase in photosynthetic efficiency as reflected by increasing in both chl a, chl. b and carotenoids content in the leaves of stressed wheat plants.

In present results regarding a decrease in chlorophyll 'a', 'b', and total chl., agree with what **Tort and Tuwrkyilmaz (2004)** reported, that the exposure of barley (*Hordeum vulgare L.*) to zero, 120, and 240 mM of sodium chloride led to the decrease in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content. Also **Lee *et al*., (2004)** in their study on *Paspalum vaginatum* (L.) and **Siler *et al*., (2007)** in their study on *Centaurium erythraea* (L.) reported that chlorophyll 'a', 'b' and total chlorophyll decreased with the increase of salt concentrations. Supporting results also include what **Turan *et al*. (2007),** on bean plant *Phaseolus vulgaris* (L.), **Cheruth *et al*., (2008)**, on *Catharanthus roseus* (L.), **Taffouo *et al*., (2009)**, on cowpea (*Vigna unguiculata* L.) and **Taffouo *et al*., (2010)**, on *Vigna ubterranean* (L.), demonstrated that salt stress caused a decrease in total chlorophyll content.

Application of salicylic acid did not only alleviate the inhibitory effect of salinity stress on the biosynthesis of photosynthetic pigments, but also induced a significant stimulatory effect greater than observed in the corresponding controls, a response which may contribute directly to the effectiveness on photosynthetic apparatus and in some way can alter plant productivity. It had been observed that the high concentration of NaCl respond as increased senescence **(Yao and Greenberg, 2006; Stael *et al*., 2015)**. Na^+ and Cl^- concentrations in the leaves were slightly more increased under stress combination than under only salt stress, which may additionally contribute to the augmented growth penalty under these conditions.

4.5.3. Quality parameters:

There is correlation between fruit colour and lycopene content **(Brandt *et al*., 2006).** Red-fruiting cultivers also have higher lycopene content than yellow **(Cox *et al*., 2003).** It was reported that the lycopene content and antioxidant activity of tomatoes varies between cultivers and is highest in cherry **(Kaur *et al*., 2004; Molyneux, 2004).** Under stress conditions total soluble sugars and total phenols content increased with increased salinity level **(Toor *et al*., 2006).** Both the hydrogen peroxide and pH level of fruit influence the storability of processed tomatoes and tomato products by

inhibiting the spore germination of thermophilic organisams. The rise in salinity levels caused the rise in total phenolic content (table-4.11, 4.12, 4.13, 4.14, 4.15 and 4.16).

4.5.4. Antioxidant (non-enzymatic):

4.5.4.1. Proline content:

Salt stress markedly increased the proline content in salt treated plants compared with non-salt-stressed plants (Table-4.17). The magnitude of the increase was directly proportional to NaCl concentration. Salicylic acid treatment of plants grown under different concentrations of salt resulted in a significant reduction in proline content compared with those of the reference controls.

It is possible that, enhanced proline accumulation may regulate multiple processes required for survival in salt stress Conditions. The salt tolerance of tomato cultivars is dependent on the ability to synthesize proline under salinity stress conditions **(Maggio *et al.*, 2002).** The results of this study are in agreement with other investigations **(Behnamnia *et al.*, 2009; Zhang *et al.*, 2006).** Proline is generally accumulated under salt stress condition and plays positive role in adaptation of cells to salt and water stress **(Kaviani, 2008).** Proline acts as an osmolite beside enzymes and other macromolecules, and therefore, protects the plant against low water potential and causes osmotic regulation in plant organs. Also proline can act as an electron receptor preventing photosystems injuries in dealing with ROS function. Proline accumulation in foliage was seen in olive cultivars (*Olea europea* L.) in low water condition **(Bacelar *et al.*, 2009).** Proline accumulation could be a protective response, not only because of the osmoprotectant role of proline that prevents water-deficit stress under high salinity, but also as a result of the radical scavenger and protein stabilization properties of proline **(Kuznetsov and Shevyakova, 1997; Ben Ahmed *et al.*, 2010).**

In present study, proline content increased with salinity intensity on growth medium (table-4.17). Significant decrease in the value of proline was recorded in all genotypes even at the salinity level 200 mM in combination with the treatment of salicylic acid of concentration 25µM.

Many authors were reported the increase of proline accumulation under salt stress in different plants such as tomato **(Amini and Ehsanpour, 2005; Mohamed *et al.*, 2007),** potato **(Potluri and Devi Prasad, 1994),** green gram **(Misra and Gupta, 2005)** and Jojoba plant **(Fayek *et al.*, 2010).**

4.5.5. Antioxidant enzyme activities:

Under salt stress plant cells have evolved intensive defence systems including enzymatic (superoxide dismutase (SOD), peroxidase (POD) catalase (CAT) and glutathione peroxidise (GPX) antioxidant resistance mechanisms may provide a strategy to enhance plant stress tolerance. Data presented in this study (table-4.18, 4.19, 4.20 and 4.21) showed that all the mentioned enzymatic activity

increased during salt stress. Plants possess an impressive array of defence mechanisms against oxidative stress including the enzymatic and non-enzymatic antioxidant systems. NaCl salinity generates and increases the activity of these antioxidant enzymes.

Although each antioxidant enzyme performs a specific function and its activity is assigned to a specific role in ROS detoxification, efficient antioxidant activity does not necessarily translate into the strong up-regulation of the full complement of antioxidant enzymes and vice versa **(Allakhverdiev *et al.*, 2000)**. Salinity stress could increase SOD production in plant **(Keles & Oncel, 2000)**. Modifications in antioxidants contents represent a signal of plant's tolerance to stressful environment. Thus, changes in enzymatic activities are strictly related to oxidative stress adaptation of plants **(Sudhakar *et al.*, 2001)**. CAT which is involved in the degradation of hydrogen peroxide and preventing oxidative damage **(Mittler 2002)**. Tolerance to salt-stress in higher plants correlates to the levels of antioxidant systems **(Jahnke and White, 2003)**. **Mittler (2002)** reported that changes in the content of antioxidants levels may act as a signal for ROS scavenging processes and ROS transduction.

In our results, the activity levels of antioxidant enzymes, as SOD, CAT, POD and GPX, showed progressive increases with increasing concentration of NaCl specially at high salinity level (up to 200 mM NaCl) compared to control plants. Our data agree the findings of **Lee *et al.* (2001)** which observed that salt-stress spread mitochondrial activities and chloroplastic antioxidant enzymes in leaf, offering a protection for cells against superoxide. These observations were confirmed by the correlation coefficient, which reported that CAT and POD were positively correlated with salinity. Our correlations were also confirmed by previous literature data **(Jebara *et al.*, 2005)**. Furthermore, a rise in the POD activity with salinity has also been reported in *Morus alba* **(Sudhakar *et al.*, 2001)**, *Glycine max* **(Ghorbanli *et al.*, 2004)** and *Lycopersicon esculentum* **(Rahnama and Ebrahimzadeh, 2005)**. High enzymatic activity and severe photosystem damage are simultaneously observed during the process of aging and stress as well, because the induction of common stress- and damage-induced enzymes. Moreover, the over expression of several antioxidant enzymes has been reported to improve salt tolerance **(Eltayeb *et al.*, 2007;** P**rashanth *et al.*, 2008)**. High levels of antioxidant enzymatic activities have been associated with salt sensitivity as well as salt tolerance. Thus, ROS scavenging as a whole may be relatively less important for plants that generate low ROS under conditions of salt stress **(Abogadallah, 2010).**

The resistance to environmental stress may therefore depend at least partially on the production by enhancing the antioxidant defence system **(Azevedo *et al.*, 2006)**, CAT and POD markedly increased under salt stress 40-200 mM (**Abdulwahid, 2012)** on date palm and **(Mehmet *et al.*, 2013)** on *Ctenanthe setosa*.

CAT, which is involved in the degradation of hydrogen peroxide into water and oxygen, is the most effective antioxidant enzymes in preventing oxidative damage. Increased CAT activities under salinity stress in *Cassia angustifolia* L. **(Agarwal & Pandey, 2004)**, maize **(Azevedo *et al.*, 2006)**, *Sesamum indicum* **(Koca *et al.*, 2007)** and wheat **(Hameed *et al.*, 2008)** were similar to our finding and depend on salt tolerance potential of plant's varieties. The changes in CAT activity depend on the species, the development and metabolic state of the plant, as well as on the duration and intensity of the stress **(Chaparzadeh *et al.*, 2004).**

The GPX are key enzymes of the ascorbate-glutathione cycle, the cycle may be a potential mechanism of tomato adaptation to salinity. It was reported that in the stress conditions of intense light, high temperature, flood and salinity, free radicals and reactive oxygen molecules are also formed. Therefore, a scavenging system should be very active.

Present study showed that genotype H-8878-1 possessed an effective superoxide dismutase/ascorbate-glutathione cycle under high salinity (200 mM). The major function of GPX in plants appears to be the scavenging of phospholipid hydroperoxides and thereby the protection of cell membranes from peroxidative damage **(Gueta-Dahan *et al.*, 1997).** The expression of many GPX is enhanced in response to abiotic and biotic stresses, including salinity, heavy metal toxicity and infection with bacterial or viral pathogens **(Avsian-Kretchmer *et al.*, 2004).**

The activities of SOD, CAT, POD and GPX showed progressively increased with increasing salinity level, Salicylic acid treatment induced significant decreases in the activities of SOD, CAT, POD, and GPX compared with those of the reference control.

4.5.6. Yield parameters: The fruit-yield related parameters as number of fruits per plant, average fruit-diameter and fruit-yield per plant showed the gradual decrease in co-relation with gradual increase in salt stress level (table-4.22, 4.23 and 4.24). One of the most widely used agricultural indices to define stress tolerance is data for plant biomass and yield **(Juan *et al.*, 2005; Sairam *et al.*, 2002)**. In this study and in agreement with previous studies, salinity reduced plant height **(Achilea, 2002; Agong *et al.*, 2004 and Hajer *et al.*, 2006)** , fresh weight **(Hassan, 1999; Li, 2000; Sonneveld, 2000; Amico *et al.*, 2003 and Hajer *et al.*, 2006)** as well as dry weight **(Li, 2000; and Yurtseven *et al.*, 2003).** Tomato treated with salicylic acid had higher fruit yield compared to non-treated plants due to an increase in number of cluster per plant **(Javaheri *et al.*, 2012).**

Chapter Five

Summary and Conclusion

SUMMARY AND CONCLUSION

Among abiotic stresses, drought and salinity cause a reduction in hydraulic conductivity in plants. The stress imposed by salts excess is an important restriction for the productive use of lands as it reduces plant growth and productivity at a soil conductivity over 4.5 dS/m (50 mM). High salt content, especially chloride and sodium sulphates, affects plant growth by modifying their morphological, anatomical and physiological traits. Such growth impairment is due to osmotic effects and ionic imbalances affecting plant metabolism.

As some morphological parameters were negatively affected by salinity, some anatomical variables were diminished as well.

Salinity is the most serious growth limiting factor and evolution of crop varieties suitable to salt stress situations, therefore, no longer be ignored. Hence, the present investigation was carried out at the Department of Biological Sciences, SHUATS, Allahabad with the main objective of physiological, biochemical and molecular characterization of selected tomato genotypes for salinity tolerance.

The material for the present investigation consisted of twenty tomato genotypes *viz*. H-8878-1, WIR-13706, Anigoarlentha, Ec-521080, Arka marginal, Ec-520079, WIR-3957, P-6 chhu chara, VRT-2, H-24, Roma, Ec-520078, T-Loeal, Arka Saurabh, H-86, Ec-520061, DT-10, Agata-30, WIR-13708 and WIR-3928.

These genotypes were evaluated in laboratory and pot culture conditions and observations were recorded on different parameters.

Data on these traits were subjected to statistical analysis. The salient features of the findings from the investigations are summarized below.

- There was decrease in plant-height with an increase in the levels of salinity. The genotypes WIR-13708 followed by WIR-3928, Agata-30 and H-8878-1 at the highest level of NaCl showed maximum height, was found to be tolerant with lower reduction in plant-height whereas, VRT-2 followed by H-24, Ec-520061, H-24 and WIR-3957, had comparatively higher reduction showing the susceptible nature of the genotypes.
- All the morphological parameters showed significant reduction under the stressed situation compared to control. In general, the no of leaves per plant, no of branches per plant, no f fruits per plant and also the average fruit diameter and finally the yield per plant decreased under the salinity stress

- The tolerant genotypes H-8878-1 and showed higher level of SOD activity at all salinity levels. However, the genotypes WIR-3928 showed decreased at higher salinity levels showing sensitivity of genotypes at higher salinity levels.
- Arka Saurabh and Arka marginal genotypes showed maximum electrolyte leakage at 200mM NaCl concentration while the tomato genotypes WIR-13708 and VRT-2 showed minimum electrolytic leakage at highest salinity level. Arka Saurabh and T-Loeal tomato genotypes showed maximum value of relative water content at highest salinity level. DT-10 and Agata-30 showed minimum value of that.
- Proline and protein content also had to assume the tolerance level of tomato varieties. The tomato genotype WIR-13706 and DT-10 showed highest level of protein at the salinity level 200mM concentration and the genotype VRT-2 followed by H-24 showed minimum protein content at the same salinity level.

Agata-30 followed by Arka Saurabh showed tolerant nature of genotype, had maximum proline content at highest salinity level while at the same salinity level i. e. 200mM NaCl concentration, the genotype H-8878-1 and WIR-3957showed the minimum value of proline content.

- The catalase activity in tolerant genotypes increased with increase in the salinity levels. Whereas, in susceptible genotypes WIR-13708 also showed enhancement of enzyme activity, but it was relatively less when compared to tolerant genotypes and genotype WIR-3928, DT-10, Arka Saurabh and Arka marginal showed decreased enzyme activity at higher salinity level.
- The genotypes Arka marginal showed maximum peroxidase activity at highest salinity level, while DT-10 showed the minimum enzyme activity at highest salinity level.
- The genotypes representing all the categories of tolerance level showed different pattern of catalase peroxidise, glutathione peroxidase and superoxide dismutase activity with higher activity under salinity stress over control. In general activity of these enzymes was higher in tolerant genotypes than the susceptible ones.
- The application of salicylic acid alone to the tomato genotypes enhanced all the morphological, physiological as well as the biochemical parameters of tomato plants. Present study also showed that the application of salicylic acid in the combination with different levels of salt stress minimized the effect of salt stress at all the salinity level and finally showed the increased yield.

It can be concluded that genotypes differed widely in their response to salinity and plants possess different adaptation traits to cope up salinity stress. Based on this study, the genotypes H-8878-1 was found to be physiologically and biochemically efficient in salt stressed environments.

Application of salicylic acid (SA) with 25 micro molar concentration could enhance quantity and quality of tomato fruits. SA could be attributed to an increased tomato fruit number and quality due to Application of SA from planting up to harvesting, each 15 days, potentially impacted on physiological enhanced photosynthetic rate via addition leaf number. and biochemical characteristics as fruit yield.

In the light of this experiment, it is concluded that:

1) Plants grown at high salinity exhibited reduced plant-height, number of leaves, number of branches, number of fruits per plant, fruit-diameter, chlorophyll content, relative water content, and increased electrolyte leakage, proline and protein content, antioxidant enzymes (SOD, CAT, POX, GPOX) compared to control plants.

2) Application of salicylic acid alleviated the adverse effects of high salinity on plants and improved all parameters mentioned above.

According to the results, SA had a positive effect in improving plants performance in salinity condition. It is concluded that genotypes differed widely in their response to salinity and plants possess different adaptation traits to cope up salinity stress. Based on this study, the genotypes H-8878-1 was found to be physiologically and biochemically efficient in salt stressed environments.

Results showed that exogenous application of salicylic acid minimizes the negative effects of salt stress with evidence of increasing the growth and productivity of tomato plants which were in parallel line with enhancing finally the yield of salt stressed tomato varieties

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