



## Effect of salt and drought stress on growth related traits of maize seedling under salicylic acid applications

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### Abstract

The effect of the major abiotic stresses like salt stress, drought stress and municipal wastewater stress under salicylic acid applications were investigated in maize seedlings by measuring the growth parameters which includes: TPH, LL, LW, leaf per plant, root length and fresh root weight, dry root weight, fresh shoot weight, dry shoot weight, shoot length and root shoot length. The seeds of two varieties Yellow Pak-afghoi and Hybrid y33y87 were sowed in small size pots which were filled with sandy soil each pot had 10 seeds and experiment was designated in the greenhouse. Seeds were germinated after 7 day while and then sprayed to all plants of pots by salicylic acid of 0.5M on contracting salt NaCl ( 0.5mM, 0.25mM and 0.75mM ) and over drought stressed plants ( 25%, 50%, 75% and 100% ) and sewage water (25%, 50%, 75% and 100%) respectively. After fourth treatment T<sub>4</sub> data was recorded by the measurement of different morphological traits of maize and effects of SA on salt (NaCl), sewage water and drought stresses were also recorded and then statistically all data was analyzed for interactions, atavism analysis and all pairwise comparison test for stage, treatment and genotype. Both salt stress and drought stress were induced serious threat and deleterious effects in maize seedling by decrease the protein contents and all growth parameters measured. SA at 0.5mM concentration is more affective which helped in the best performance of maize seedling, reduced the severe impacts of saline, increasing the significant germination percentage, yields of including traits and also its impacts on drought tolerance in maize seedlings were recorded. Sewage water treatment on maize plants caused positive effects and enhanced yield, germination, all stages of growth due to presence of essential nutrients and organic matter.

**Keywords:** salicylic acid, NaCl, drought, sewage water, greenhouse, spectrophotometer

### Introduction

Maize play key role as a major cereal crop, staple food and widely-grown crop for many people and animal feed throughout the world. Maize contains proteins, sugar and fibers and use in food industries for the production of different useful products. The origin of maize (*Zea mays L.* also called as corn in North America) is not clear and most researchers agreed that it was originated in Mesoamerica before 5000BC from the family (*Gramineae*) (Brown, 1985). After that the first crop of maize was introduced in Europe around sixteenth century then it spread to Africa and Asia and now a days it spread as a worldwide crop (Leff *et al.*, 2004) <sup>[19]</sup>. In Pakistan maize is the fourth largest grown crop about one million hectares area where different types of maize are cultivates and about 3.5 million metric tons yield are obtained after rice, cotton and wheat. Improvement in maize crop growth and production relies on properly understanding the maize itself including its genome, physiology and behavior in growth and development. These factors are induced deleterious diseases in maize plants like rust disease caused by a biotrophic fungus, *Pucciniasorghii*, maize stem borer caused by insects, maize stalk caused by bacteria that cause discoloration of leaf, sheath and stalk at a node and majority of leaf in leaf blight are caused by fungi, some by bacteria and some are caused by insects (Sawada *et al.*, 2006) <sup>[27]</sup>. Environmental adverse impacts such as salinity stress, heat stress, sewage water stress and drought stress are induced very severe or deleterious effects on maize plants growth stages or agricultural productivity throughout the world (Karakas *et al.*, 1997) <sup>[18]</sup>.

SA is a derived from most diverse group of plant phenolic and soluble in water solvent. Many salicylic acid and its related compounds inducing an important and positive effects in maize seed germination, growth of including traits, interactions with other organisms and regulate the flowering stage. SA defense maize seedlings against different bacterial, fungal, insects and herbivores infections. Maize is a crop which shows sensitive to salinity because it caused many serious threats to growth of including traits in various arid areas and semi-arid areas around the whole world (Flowers and Yeo, 1995; Munns, 1993) <sup>[11, 21]</sup>. Maize is the most important cereal crop provides main purpose of food both for people and animals mostly in Africa and also use as a raw material for industry. The adverse effects of salinity are detected by measuring the including parameters like leaf width, leaf length, total plants height, root length, RFW, SFW, RDW, SDW, leaf per plant and root shoot length ratio. The significant effects of Salicylic acid on salt stress could be investigated in maize seedlings. SA increase strength of maize seedling against salt stress and enhance growth including traits and protects from pathogenic infection (Gaffney *et al.*, 1993; Raskin, 1992) <sup>[12, 25]</sup>. SA as a plant hormone regulate the efficiency of various processes such as morphological, physiological and biological in maize crop and modify or induced capability that improve plant reaction to several most severe impacts of different stresses such as drought, salinity, sewage water, ultraviolet and different climatic conditions. SA is also enhance defensive compounds like glycine and praline and improving seed germination, growth parameters, that

severely limit the agricultural development in several arid and semi-arid areas (Skhabutdinova, 2003) [26]. The impacts of environmental stresses such as drought, salt and sewage water on maize seedling are very dangerous and adversely effects growth physiology, morphology and yield production. The most important objectives of this work were; 1) to access effects of salt, drought and sewage water on maize seedling under salicylic acid application, 2) to investigate effects of SA that ameliorates the influence of salt stress and increasing salt tolerance in maize plants, 3) to evaluate the role of SA applications which improving maize drought tolerance and assessing yield components and some morphological traits, 4) to access the suitability of sewage water treatment as irrigation water.

## Materials and Methods

### Pots of salt stress

Pot no.1 control plants	
Pot no. 2 sodium chloride 1mM + SA 0.5M	Apply 10ml + apply 10ml
Pot no.3 sodium chloride 0.5mM+ SA 0.5M	Apply 10ml + apply 10ml
Pot no.4 sodium chloride 0.25mM+ SA0.5M	apply 10ml + apply 10ml
Pot no.5 sodium chloride 0.75mM+ SA 0.5M	Apply 10ml + apply 10ml

### Pots of drought stress

Pot no.1 control plants	
Pot no.2 drought + SA 0.5M	Apply 25% + apply 10ml
Pot no.3 drought + SA 0.5M	Apply 50% + apply 10ml
Pot no.4 drought + SA 0.5M	Apply 75% + apply 10ml
Pot no.5 drought + SA 0.5M	Apply 100% + apply 10ml

### Pots of sewage water stress

Pot no.1 control plants	
Pot no.2 sewage water + SA 0.5M	Apply 25% + apply 10ml
Pot no.3 sewage water + SA 0.5M	Apply 50% + apply 10ml
Pot no.4 sewage water + SA 0.5M	Apply 75% + apply 10ml
Pot no.5 sewage water + SA 0.5M	Apply 100% + apply 10ml

Seeds of two different varieties of maize (Yellow Pak-afghoi and Hybrid y33y87) were used to investigate the impacts of environmental stresses like (salt, drought and sewage water) on maize seedlings. The experiment was designed in the greenhouse. 30 pots of small size were filled with sandy soil. Each treatment has 10 pots for two varieties of maize seeds (1 for control and 4 for NaCl of yellow pak-afghoi and 1 for control and 4 for NaCl of hybridy33y87). Seeds of similar size and weight were selected. 10 seeds without of any treatment were sowed in each pots. Pots were labeled with the help of permanent color marker according to their treatments. Water was added in pots according to the requirements. After sowing the seeds treatments of NaCl (0mM, 1mM, 0.5mM, 0.25mM and 0.75mM), drought (25%, 50%, 75% and 100%) and sewage water (25%, 50%, 75% and 100%) were applied. Salicylic acid (0.5M) were sprayed over all stresses with help of simple saver bottle. After one week seeds were germinated. Two plants from each pot were selected and data was recorded. In a similar way 4 treatments were applied and data was recorded (total plants height, leaves length, leaves width, RL, RFW, RDW, SFW, SDW, root shoot length ratio and leaf per plants).

### Absolute growth studies

Only five plants from each treated pots and untreated pots will be selection of each stress for absolute growth studies. For this purpose following method will be adopted.

- The leaf length will be measured in (cm) by using the scale.
- The width of leaf will be measured in (cm) by using scale.
- The root or shoot length measure in (cm) with the help of scale.
- The fresh mass of root or shoot measured in (g) by using electrical balance.
- The root or shoot dry weight recorded in (g) with the help of electric balance.
- The total plant height will be measured by using the centimeter scale.
- Protein contents through use of spectrophotometer.

### Statistical analysis

After recording data of all growth related traits and investigating the impacts of all applied stresses on maize seedlings the data will be analyzed statistically using the least significant differences (LSD) test as described by (Steel *et al.*, 1997) [30].

### Results and discussions

Results of table 1 showed the higher, constructive or important associations were present among different morphological traits (DRW, RL, DSW, SFW, SL, LL, LPP and root shoot length ratio under (salicylic acid, salt, sewage water and drought stress) different treatments or stress and negative and important associations were presents among RFW, TPH and LW. The higher and significant correlation of DRW with DSW showed the higher increased of essential organic compound in maize seedlings or dry root weight under different treatments like salt (1mM, 0.5mM, 0.25mM and 0.75mM of NaCl), sewage water (25%, 50%, 75% and 100%) and drought (25%, 50%, 75% and 100%) under 0.5M of salicylic acid. The higher accumulation of essential minerals, organic and inorganic compounds were increased the growth and development of maize seedlings and photosynthetic rate and salicylic acid induced more tolerance and resistance in maize genotypes for heavy metals, saline and drought stress (Abbas *et al.*, 2016; Hafeez *et al.*, 2015; Wang *et al.*, 2007) [3, 15, 31].

The results for analysis of multiple linear regression for plant parameters or seedling length under different treatments of salt, drought, sewage and salicylic acid given in table 2 showed the highest and positive dispensation of dry root weight (0.031) for seedling length under accumulative effects of saline, drought and sewage water. The accumulative coefficient of determination or  $R^2$  was found plant length while  $R^2$  (1.5%) was recorded for dry root weight. The regression equation was predicted as,  $Y = 7.216 + 0.031 (DRW) - 0.599 (DSW) + 0.031 (FRW) - 0.047(FSW) + 0.036(LL) + 0.011(LPP) - 0.058(LW) + 0.536 (RL) - 3.215(RSLR) - 0.045(TPH)$ . The doses of salicylic acid which enhanced plant height in drought and normal conditions and induced more resistance and tolerance in maize genotypes may be used as growth

enhancer in maize plant (Abbas *et al.*, 2015; Hafeez *et al.*, 2019; Mahmood *et al.*, 2019; Naseem *et al.*, 2015ab; Shalaby, 2006) [2, 15, 20, 22-23, 29].

Results that given in table 1 showed the high, positive and important associations were presents among different morphological parameters (DRW, RL, SDW, SFW, shoot length, leaf length, leaf per plant, RSLR under (salicylic acid, salt, sewage water and drought stress) different treatments or stress and the negative or important correlations were presents among FRW, TPH and LW. The higher and important associations of dry SDW with FRW showed that there were highly increased of essential organic compound in maize seedlings or dry root weight under different treatments like salt (1mM, 0.5mM, 0.25mM and 0.75mM of NaCl), sewage water (25%, 50%, 75% and 100%) and drought (25%, 50%, 75% and 100%) under 0.5M of salicylic acid. The higher accumulation of essential minerals, organic and inorganic compounds were increased the growth and development of maize seedlings and photosynthetic rate and salicylic acid induced more tolerance and resistance in maize genotypes for heavy metals, saline and drought stress (Ali *et al.*, 2013; Ali *et al.*, 2014; Kanwal *et al.*, 2019; Bibi *et al.*, 2018; Shakirova *et al.*, 2003) [4, 5, 17, 8, 28]. The results for analysis of multiple linear regression for plant parameters or seedling length under different treatments of salt, drought, sewage and salicylic acid given the table 2 showed the high and negative dispensation of dry shoot weight (-0.599) for seedling length under accumulative effects of saline, drought and sewage water. The accumulative coefficient of determination or R<sup>2</sup> was found plant length while R<sup>2</sup> (1.1%) was recorded for dry shoot weight. The doses of salicylic acid which enhanced plant height in drought and normal conditions and induced more resistance and tolerance in maize genotypes may be used for improving stress tolerance in maize (Ali *et al.*, 2016; Ali *et al.*, 2017; Pollice *et al.*, 2004) [7, 6, 24].

The results from tables 3-5 showed the difference between maize varieties and treatments of salt, drought and sewage

water under salicylic acid application. There was higher protein concentration in leaves, roots and shoots of maize under different treatments salt NaCl (1mM, 0.5mM, 0.25mM and 0.75mM under 0.5 M salicylic acid), sewage water (25%, 50%, 75% and 100% under 0.5M salicylic acid) and drought (25%, 50%, 75% and 100%) under 0.5M salicylic acid. The highest protein was found in leaves of maize both genotypes (Hybrid y33Y87 and yellow PAK-Afghoi) under sewage water + salicylic acid. It has been revealed from results given in table 4.14a the highest proteins and carotenoids were found in leaves under 0.5M of salicylic acid and reduced the adverse effects of different stress like saline, sewage water and drought. The high protein concentration in shoots and lowest protein concentration in roots of maize both genotypes (Hybrid y33Y87 and yellow PAK-Afghoi) was found under treatments of salt NaCl (1mM, 0.5mM, 0.25mM and 0.75mM under 0.5M salicylic acid), sewage water (25%, 50%, 75% and 100% under 0.5M salicylic acid) and drought (25%, 50%, 75% and 100%) under 0.5M salicylic acid. The highest protein concentration under 0.5M of salicylic acid indicated that salicylic acid increased the protein concentration under salt stress (1mM, 0.5mM, 0.25mM and 0.75mM and 0.5 M salicylic acid), sewage water stress (25%, 50%, 75% and 100% and 0.5M salicylic acid) and drought stress (25%, 50%, 75% and 100% and 0.5M SA). Results of table 6 showed that protein concentration in control plant's root, shoot and leave samples. The highest protein concentration was found under treatment of control plant's leaves, root and shoot samples as compared to the stressed plant's root, shoot and leave samples from maize genotypes. Results of table 4.16c showed the high protein concentration in leaves for control plant and lower protein concentration in root for control plant. SA application significantly increased photosynthesis rate and therefore the leaf areas was increased (Abbas *et al.*, 2016; Hafeez *et al.*, 2015; Chen *et al.*, 2007; Hu *et al.*, 2007; Golbashy *et al.*, 2010) [3, 15, 10, 16, 13].

**Table 1:** Pooled correlation among different morphological traits of maize seedling under salicylic acid, salt, sewage water and drought stress or treatments

Traits	DRW	DSW	FRW	FSW	LL	LPP	LW	RL	RSLR	SL
DSW	0.1755									
FRW	-0.0199	0.1151								
FSW	0.3083	0.254	0.2229							
LL	0.1137	0.2252	0.2963	0.4875						
LPP	0.4393	0.264	0.3976	0.6316	0.4103					
LW	0.2834	0.2162	0.1328	0.6458	0.5453	0.5619				
RL	0.0666	0.2161	0.1264	0.4636	0.4078	0.3481	0.4283			
RSLR	0.0049	0.0944	-0.1535	0.0497	0.0677	0.0068	-0.0483	0.3558		
SL	0.0407	0.0372	0.2439	0.2713	0.2546	0.2412	0.3403	0.3824	-0.6855	
TPH	0.1779	0.2149	0.2734	0.6537	0.6042	0.6331	0.7246	0.4971	-0.0095	0.3447

DSW = dry shoot weight, FRW = Fresh root weight, FSW = Fresh shoot weight, LL = Leaf length, LPP = Leaves per plant, LW = Leaf width, RL = Root length, RSLR = Root shoot length ratio, SL = Shoot length, TPH = Total plant height

**Table 2:** Pooled multiple linear regression for plant parameters or seedling length under different treatments of salt, drought, sewage and salicylic acid

Variable	Coefficient	Std Error	T	P	R <sup>2</sup>
DRW	0.031	0.167	0.190	0.851	1.5
DSW	-0.599	0.306	-1.960	0.052	1.1
FRW	0.031	0.100	0.310	0.758	1.3
FSW	-0.047	0.581	-0.080	0.936	2.1
LL	0.036	0.022	1.610	0.109	1.5
LPP	0.011	0.063	0.180	0.858	2.5
LW	-0.058	0.059	-0.980	0.330	2

RL	0.536	0.016	33.780	0.000	1.3
RSLR	-3.215	0.065	-49.800	0.000	1.2
TPH	-0.045	0.017	-2.660	0.008	2.3

Y = 7.216, R Squared 0.9394 Adjusted R Sq 0.9364 SD 0.75999

DSW = dry shoot weight, FRW = Fresh root weight, FSW = Fresh shoot weight, LL = Leaf length, LPP = Leaves per plant, LW = Leaf width, RL = Root length, RSLR = Root shoot length ratio, SL = Shoot length, TPH = Total plant height

**Table 3:** Results of leaf, shoot and root for protein contents of 0.5M conc. and salt NaCl concentrations

Genotypes	Treatments	Plant part		
		Leaf	Shoot	Root
Hybrid y33Y87	1mM NaCl+0.5M SA	0.546	0.078	0.021
Hybrid y33Y87	0.5mM NaCl+0.5M SA	0.476	0.087	0.025
Hybrid y33Y87	0.25mM NaCl+0.5M SA	0.654	0.069	0.027
Hybrid y33Y87	0.75mM NaCl+ 0.5M SA	0.531	0.084	0.028
yellow PAK-Afghoi	1mM NaCl+0.5M SA	0.643	0.077	0.031
yellow PAK-Afghoi	0.5mM NaCl+0.5M SA	0.432	0.073	0.029
yellow PAK-Afghoi	0.25mM NaCl+0.5M SA	0.543	0.069	0.027
yellow PAK-Afghoi	0.75mM NaCl+ 0.5M SA	0.624	0.087	0.033

**Table 4:** Results of leaf, shoot and root for protein contents of 0.5M conc. and drought

Genotypes	Treatments	Plant part		
		Leaf	Shoot	Root
Hybrid y33Y87	25%Drought+0.5M SA	0.546	0.068	0.025
Hybrid y33Y87	50%Drought+0.5M SA	0.476	0.081	0.022
Hybrid y33Y87	75%Drought+0.5M SA	0.654	0.056	0.029
Hybrid y33Y87	100%Drought+0.5M SA	0.531	0.074	0.027
yellow PAK-Afghoi	25%Drought+0.5M SA	0.643	0.067	0.032
yellow PAK-Afghoi	50%Drought+0.5M SA	0.432	0.083	0.025
yellow PAK-Afghoi	75%Drought+0.5M SA	0.543	0.068	0.029
yellow PAK-Afghoi	100%Drought+0.5M SA	0.624	0.077	0.023

**Table 5:** Results of leaf, shoot and root for protein contents of 0.5M conc. and sewage water

Genotypes	Treatments	Plant part		
		Leaf	Shoot	Root
Hybrid y33Y87	25%Sewage watert+0.5M SA	0.648	0.078	0.035
Hybrid y33Y87	50%Sewage water+0.5M SA	0.776	0.083	0.024
Hybrid y33Y87	75%Sewage water+0.5M SA	0.594	0.066	0.029
Hybrid y33Y87	100%Sewage water+0.5M SA	0.731	0.073	0.037
yellow PAK-Afghoi	25%Sewage watert+0.5M SA	0.693	0.069	0.031
yellow PAK-Afghoi	50%Sewage water+0.5M SA	0.832	0.079	0.025
yellow PAK-Afghoi	75%Sewage water+0.5M SA	0.843	0.088	0.028
yellow PAK-Afghoi	100%Sewage water+0.5M SA	0.724	0.078	0.023

**Table 6:** Results of control plant sample reading for proteins

Genotypes	Treatments	Plant parts		
		Leaf	Shoot	Root
Hybrid y33Y87	0	0.896	0.077	0.067
yellow PAK-Afghoi	0	0.971	0.123	0.072
Hybrid y33Y87	0	1.172	0.095	0.063
yellow PAK-Afghoi	0	0.879	0.221	0.075
Hybrid y33Y87	0	0.919	0.093	0.059
yellow PAK-Afghoi	0	1.268	0.129	0.069

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