

**INFLUENCE OF SALT STRESS ON PEROXIDASE AND CATALASE  
ACTIVITY IN *CICER ARITINUM* L. LEAVES.**

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**ABSTRACT:**

Influence of NaCl treatment on activity of peroxidase (E. C. EC 1.11.1.7) and catalase (E. C.1.11.1.6) in the attached and detached leaf segments and leachate of *Cicer arituntum* L. cv Chafa has been studied. Sodium chloride salinity caused decline in peroxidase activity in attached and detached leaves along with leachate. Salinity also caused stimulation of catalase activity more pronounced in leaves and leachate of detached part as compared to attached leaves. But as time period extends activity goes on decreasing in both leaf and leachate. These observations indicate that membrane integrity of peroxidase shows more effect in leaching of protein in surrounding medium than catalase as there are membrane bound enzymes results in higher peroxidase activity than catalase in both cases.

**KEYWORDS:** Salinity, stress, leachate.

**1. INTRODUCTION:**

Among the environmental stresses salinisation of soils has become a problem of great concentration for the agronomist and it is most significant environmental challenges limiting plant productivity, particularly in arid and semi-arid climates ( Hussain, 2009) The *Cicer aritinum* L. is one of the important legume crop in semiarid tropical region. Changes in the activity of antioxidant enzymes in response to salinity were different in tolerant and sensitive cultivars( Meloni *et al.* 2003)

It is apparent that plant physiology should occupy a central position in research

on crop improvement. Although such consideration has been initially made in case of like rice, wheat, sugarcane, cotton and soybean. The same can not be about a crop like chick pea.

Hence we thought to probe into some of the physiological processes in this crop plant for this a promoting chick pea cultivar Chafa has been selected.

The activity of antioxidant enzymes in normal conditions is an important point because this attribute can be expressed the amount of preparation of plants for against to stress but keep up this amount in stress conditions is more important. The purpose of the present study was to contribute to a better understanding of the physiological responses of chickpea plants to salinity stress.

## 2. MATERIAL AND METHODS:

### 2.1 Plant Material

Plants of *Cicer arietinum* L. cv Chafa were raised from healthy seeds on soil bed in Botanical garden of T.C. College, Baramati. After one month of establishment, the plants were used for enzyme analysis. Leaf samples collected were healthy, of same age and fully expanded. All plant material collected in field was brought to laboratorial through chill box and subjected to stress treatments.

### 2.2 Attached leaf segment

Fully expanded leaves were collected, washed, blotted to dry. This were transfer to fresh medium containing 25mM, 50mM, 100mM, 150mM of NaCl in 9cm petridish for 24, 48, 72 hours time interval. Separate set of petridish was prepared along with control. The petridishes were kept in dark at 25° C room temperature with average humidity 60 to 70%. At the time of enzyme analysis 2ml leachate along with attached leaf was also subjected for enzyme analysis.

### 2.3 Detached leaf segment

Fully expanded leaflet were collected, washed, blotted to dry and cut into small pieces. This were transfer to fresh medium containing 25mM, 50mM, 100mM, 150mM of NaCl in 9cm petridish containing Whatman Filter Paper No.1 in addition to distilled water was applied as a control treatment. For 24, 48, 72 hours time interval. The

petridishes were kept in dark at 25 °C room temperature with average humidity 60 to 70 %. At the time of enzyme analysis 2ml leachate along with attached leaf was also subjected for enzyme analysis.

#### 2.4 Enzyme assay:

The activity of enzyme peroxidase was estimated according to the method of Maehly (1954). Enzyme activity is expressed as  $\Delta$  O.D.min<sup>-1</sup>mg<sup>-1</sup> proteins. For the study of enzyme catalase, a modified method of Herbert (1955) was adopted. Activity of enzyme is expressed as mg H<sub>2</sub>O<sub>2</sub> broken down min<sup>-1</sup>mg<sup>-1</sup> proteins. The enzyme proteins were estimated according to Lowry *et.al.*(1951).Each enzyme assay was triplicated.

### 3. RESULTS:

#### 3.1 Peroxidase activity-

It is clear from table 1 and figures 1,2,3,4 that as a result of increasing salinity peroxidase activity decreasing first 24 hrs.exposure and it decreasing slowly. Finally minimum was recorded at 72 hrs. in attached leaves. Similar trend was noticed in the leachate also. As peroxidase proteins located in cytoplasm, in attached leaves activity is less than leachate. Because of release of enzymes protein in surrounding medium due to increased surface for exudates leachate.

As a result of induced senescence there is breakdown of peroxidase catalyses the subsequent breakdown of H<sub>2</sub>O<sub>2</sub>. This result into at late phase of salinity and senescence very more activity is recorded. But it is more in the leaf than leachate because as a result of senescence protein degradation rate of higher than protein synthesis.

As compared to natural senescence in detached leaf similar trend is observed for enzyme degradation but due to mechanical injury activity is increased as compared to attached leaf and leachate. In attached leaves it is 28.20 while in detached leaves it is 36.14 while in leachate it increases from 34.14 to 224.20  $\Delta$  OD min<sup>-1</sup> mg<sup>-1</sup>.

Once senescence has set in peroxidase content in leaves has decreased progressively suggesting that protein degradation is completed & at the same time uptake of protein from the leachate has taken place as there is progressive decline in leachate peroxidase activity.

It is evident from table that response to mechanical injury activities of peroxidase is more than normal leaves. As a result of salinity activity is observed but

at the late phase it goes on decreasing in both leaves and leachate. This given an idea that due to injury the protein from leaf are disrupted resulting in leaching of proteins in other medium this signifies role of cell constituents in regulating the cell in tune with the surrounding.

### 3.2 Catalase activity-

From the table 2 and figure 5,6,7,8 it is evident that as a result of salinity at lower concentration catalase activity is decreased in first 24hrs and high salinity it goes on increasing from 74.70 to 95.50 in leaves while in leachate it goes on increasing at 150 mM NaCl from 110.40 to 107.30 as time period extends the activity goes on decreasing in both leaf and leachate.

As compared to attached leaves activity more pronounced in leachate and leaves of detached part. Here also at 25 mM concentration of NaCl it is increased and continues upto 100 mM concentrations. At high salinity it again decreases and continues at late phase of salinity. It decreases slowly after 48 hrs and at 72 hrs it is 74.60 to 64.55 that is, at different time intervals at low salinity catalase activity is less and high salinity enhance the activity is both detached leaf leachate.

### 4. Discussion:

The activities of peroxidase enzyme in attached & detached mature leaves of *Cicer arietinum* L. cv Chafa have been compared in order to find out whether they show a similar pattern to the one exhibited by salt stressed leaves. Salinity induces oxidative stress in plants at the subcellular level (Hernández, 2000). A salt stress condition extensively oxidizes proteins, lipids and requires enhancement in peroxidase activity to prevent those toxic substances (Apel and Hirt, 2004). Ben (2007) observed Northern cultivars registered an important increase in their peroxidases activities under moderate salt stress concentrations followed by a drastic decrease in peroxidase activity at severe stress in barley seeds during germination. Rahnama and Ebrahimsadeh (2005) studied the effect of NaCl activity of antioxidant enzymes in the seedlings of relatively salt tolerant (Agrida, Kennebec) and salt sensitive (Diamant and Ajax) potato cultivars observed similar trend of increase antioxidant enzymes. As time increased the senescence has set in attached leaves and leachate this gradually decreases in enzymes activities. Thus the metabolic reaction naturally and mechanically injured senescent under salt stress although exhibit similarly many respect, they do show difference in some important facts, this may be due to fact that in salt stressed leaves the excessive

accumulation of both sodium chloride can exert specific ion effects on the metabolism at various levels. Mittova in 2003 studied salt stress in salt-tolerant tomato plants plants suggested increased photorespiratory activity results in increases in catalase activity after an NaCl challenge,

Generally, Na<sup>+</sup> starts to inhibit most enzymes at concentration observe 100 mM concentration at which Cl<sup>-</sup> becomes toxic is even less well defined, but is probably in the same range is that for Na<sup>+</sup>. Even K<sup>+</sup> may inhibit enzymes at concentration of 100-200 mM (Greenway and Osmond 1972).

The environmental or manmade stresses have been reported to lead to the production of reactive oxygen species (ROS) that cause oxidative damage (Smionoff, 1993). Plants possess efficient systems for scavenging active oxygen species that protect that from destructive oxidative reaction (Foyer *et al.*, 1994). As part of this system, antioxidant enzymes are key elements in the defense mechanism. Catalase accelerates peroxidase and a variety of peroxidase catalyses the subsequent break down of H<sub>2</sub>O<sub>2</sub> to water and oxygen (Chang *et al.* 1984). Noel *et al.* (2007) shows inhibition of catalase activity in *Phaseolus vulgaris* and *Medicago Sativa* by NaCl. The oxidase activity usually decreases during dark induced senescence (Dhindsa *et al.*, 1981).

Garratt *et al.* (2002) reported an increase in activity of antioxidative enzymes in plant under salt stress. They found a correlation between these enzymes levels & salt tolerance. Similarly many change have been detected in the activity of anti-oxidant enzyme was reported to increase under saline condition in shoot cultures of Rice (Fadzilla *et al.*, 1997). But decreased in wheat (Meneguzzo *et al.* 1999). The variations in these observations may be due to the fact that the effect of salinity depend on number of factors for example salt type, their concentration plant genotype, growth stage & an environmental conditions (Shannon *et al.*, 1994).

Jose (2017) in his studies on plant responses to salt stress adoptive mechanism concluded that there is coordinated up-regulation of the antioxidative machinery as one of the mechanisms involved in the salt tolerance response whereas in contrast, salt-sensitive species show an unchanged response or decrease in antioxidant defences shows lower constitutive antioxidant enzyme levels than salt-tolerant species

In *Cicer arietinum* L. cv Chafa both peroxidase and catalase activities are more

in detached leaves than attached. As Proteins are released in surrounding medium in response of natural and mechanical senescence. High protein content in treated tissue correlated with a lower rate of proteolysis. The higher protein content in treated tissue could possibly be attributed to a test compound induced decrease in free radical and particularly in  $H_2O_2$  accumulation result in decline of activity.

Both enzymes play a role in detoxifying  $H_2O_2$  but their behavior suggests quite different roles in senescence. The observed changes in the activities of peroxidase and catalase showed a similar trend in our experiment with some exceptions.

#### ACKNOWLEDGEMENTS:

Authors are very much grateful to Anekant Education Society for providing laboratory facilities during completion of research work.

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**Table 1-** Effect of salinity on Peroxidase activity in *in-vitro* condition in attached and detached leaves and leachate of *Cicer arietinum* L. cv Chafa cultivar.

Time in Hrs.	NaCl Conc (mM)	Peroxidase ( $\Delta$ O.D.min <sup>-1</sup> $\mu$ g <sup>-1</sup> proteins)			
		Attached		Detached	
		leaf	Leachate	Leaf	Leachate
	Control	28.20 $\pm$ 0.50	34.14 $\pm$ 0.01	36.14 $\pm$ 2.10	224.20 $\pm$ 0.04
24	25	24.12 $\pm$ 0.00	28.12 $\pm$ 0.00	24.10 $\pm$ 0.03	188.81 $\pm$ 0.00
	50	12.00 $\pm$ 0.60	18.10 $\pm$ 0.01	24.00 $\pm$ 1.42	96.32 $\pm$ 0.16
	100	10.20 $\pm$ 0.00	14.12 $\pm$ 0.62	9.16 $\pm$ 0.03	94.10 $\pm$ 0.09
	150	6.18 $\pm$ 0.09	9.63 $\pm$ 0.00	8.14 $\pm$ 0.40	70.94 $\pm$ 0.01
48	25	4.16 $\pm$ 0.00	8.51 $\pm$ 0.00	7.13 $\pm$ 0.10	70.31 $\pm$ 0.54
	50	3.14 $\pm$ 0.01	4.52 $\pm$ 0.15	4.61 $\pm$ 0.00	70.00 $\pm$ 0.05



	100	3.09±0.56	4.32±0.00	4.34±0.04	65.13±0.50
	150	3.00±0.00	4.19±0.00	3.45±0.00	60.94±0.70
72	25	2.80±0.07	4.15±0.00	2.89±0.12	46.34±0.04
	50	2.60±0.00	3.16±0.90	2.36±0.00	40.09±0.08
	100	2.16±0.13	2.46±0.00	1.61±0.00	31.51±0.05
	150	2.09±0.00	2.13±0.00	1.49±0.01	30.93±0.92

Values are means of three determinations

± indicates standard deviation.

Table 2 - Effect of salinity on catalase activity in *in-vitro* condition in attached and detached leaves and leachate of *Cicer arietinum* L. cv Chafa cultivar.

Time in Hrs.	NaCl Conc (mM)	Catalase (mg H <sub>2</sub> O <sub>2</sub> broken down min <sup>-1</sup> mg <sup>-1</sup> proteins)			
		Attached		Detached	
		Leaf	Leachate	Leaf	Leachate
	Control	74.70±0.1 4	110.40±0.1 4	95.50±0.42	107.30±0.2 1
24	25	59.65±0.21	107.30±0.4 9	127.40±0.1 4	74.50±0.28
	50	66.40±0.14	112.55±0.3 5	124.30±0.2 8	86.55±0.24
	100	83.35±0.07	113.70±0.2 8	113.70±0.2 6	108.50±0.2 8
	150	91.70±0.14	119.70±0.2	90.25±0.21	110.30±0.4

			8		2
48	25	73.15 ±0.12	96.65±0.35	73.30±0.28	81.40±0.21
	50	72.40±0.07	95.25±0.21	74.65±0.14	91.60±0.28
	100	71.45±0.05	88.25±0.21	79.55±0.21	95.35±0.21
	150	69.60±0.14	87.55±0.07	91.65±0.28	102.25±0.4 2
72	25	69.60±0.14	87.55±0.07	91.65±0.28	102.25±0.4 2
	50	74.60 ±0.13	86.10±0.28	64.80±0.93	68.25±0.21
	100	73.30 ±0.05	85.40±0.00	71.50±0.21	69.50±0.28
	150	66.40±0.28	73.70±0.28	73.25±0.12	74.65±0.25

Values are means of three determinations

± indicates standard deviation.

Fig.1: Effect of salinity on Peroxidase activity in *in-vitro* condition in attached leaves of *Cicer arietinum* L. cv Chafa cultivar.

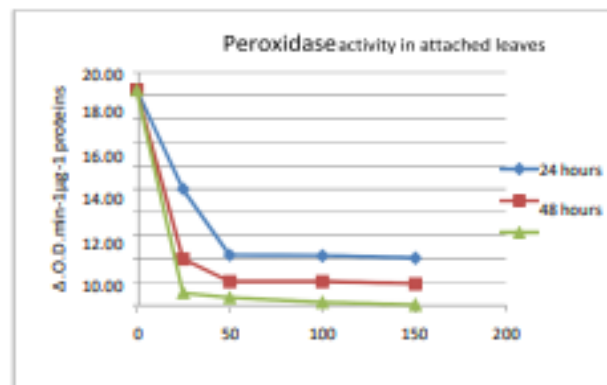


Fig.8 - Effect of salinity on catalase activity in *in-vitro* condition in detached leachate of *Cicer arietinum* L. cv Chafa cultivar.

