ISBN 978-81-926339-06

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

Madhuri Patil, Supriya Mahadik, Chandrashekhar Murumkar

Post Graduate Research Centre, Department of Botany, Tuljaram Chaturchand College, Baramati,413102, Maharashtra, India

patilmadhuri352@gmail.com

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 ariuntium* 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 pare 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 arititnum* 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

ISBN 978-81-926339-06

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 beenselected.

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 arietium* 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^{0} C room temperature with average humidity 60 to70%. 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

ISBN 978-81-926339-06

petridishes were kept in dark at 25 ^{0}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 Δ O.D.min^{-1}mg^{-1} proteins. For the study of enzyme catalase, a modified method of Herbert (1955) was adopted. Activity of enzyme is expressed as mg H_2O_2 broken down min^{-1}mg^{-1} 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_2O_2. 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 Δ OD min^{-1} mg^{-1}.

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

ISBN 978-81-926339-06

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 arietium* 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 (Agria, 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

ISBN 978-81-926339-06

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^+ starts to inhibit most enzymes at concentration observe100 mM concentration at which Cl^- becomes toxic is even less well defined, but is probably in the same range is that for Na^+. Even K^+ 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 posses 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_2O_2 to water and oxygen (Chang *et al.* 1984). Noel *et al.* (2007) shows inhibition of catalase activity in *Phaseolus valgaris* and Medicago *Sativa* by NaCl. The oxidase activity usually decreases during dark induced senesance (Dhinsda *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 arittinum* 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 possible to attributed to a test compounds induced decreased 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 role 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.

REFERENCES:

1 Apel, K. and H. Hirt, (2004). Reactive oxygen species: Metabolism, oxidative stress and signal transduction. Annu. Rev. Plant Biol., 55: 373-399.

2 Ben Naceur M'barek, Cheick-M'hamed Hatem, Abdellaoui Raoudha and Bettaib-Kaab Leila, (2007). Relationship Between Peroxidase Activity and Salt Tolerance During Barley Seed Germination. *Journal of Agronomy, 6: 433-438.*

3 Chang, H.B, Singel & S.M Siegel, (1984).Salinity Induced change in isoperoxides in taro ,*Colocata ecullenta*, Phytochemitery 23,233-235

4 Dhindsa,R.S Plumb –Dhindsa ,T.A Theraoe, (1981),Leaf senescence correlated with increased level of membrane permeability & lipid peroxidatoin &decreased level of SOD & catalase ,J. Exp Bot., 10, 32 -93.

5 Fadzilla –N.M.R.P Finch &H.B.Roy; (1997) Salinity oxidative stress & antioxidant responses in shoot culture of rice J Exp. Bot.48 325 -331

6 Foyer C. H. P Desourvieres & K. J Kunett, (1994), Protection against oxygen radicals An important defense mechanism .studied in transgenic plant, Plant cell environment.17,507-523

7 Garratt .L.C., B. S.Janagounder ,K .Lowe .P .Anthony,J.B Power & M.K Davey; (2002).Salinity tolerance & antioxidant status in cotton culture, Free Radicle Biol .Medicine,33: 502 -511

ISBN 978-81-926339-06

8 Greeway H and Osmond, C. B. (1972), Salt response of enzymes from species differing in salt tolerance .Plant physiology 49,256-259

9 Hernández, J.A.; Jiménez, A.; Mullineaux, P.M.; Sevilla, F. (2000), Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. Plant Cell Environ. 23, 853–862. [CrossRef]

10 Hussain, K.; Majeed, A.; Nawaz, K.; Khizar, H.B.; Nisar, M.F. (2009), Effect of different levels of salinity on growth and ion contents of black seeds (Nigella sativa L.). Curr. Res. J. Biol. Sci., 1, 135–138

11 Herbert D (1955) Catalase from bacteria In "Methods in Enzymology" Vol II Eds Colowick SP and Kaplan NO Academic Press Inc Publishers New York pp784-786.

12 Jose Ramón Acosta-Motos, Maria Fernanda Ortuño, Agustina Bernal-Vicente, Pedro Diaz-Vivancos, Maria Jesus Sanchez-Blanco and Jose Antonio Hernandez , (2017), Plant Responses to Salt Stress: Adaptive Mechanisms, Agronomy,7:18.

13 Lowry OH Rosebrough NT Farr AI and Randall RJ (1951) Protein measurement with folin phenol reagent J Biol Chem 193: 262-275

14 Maehly AC Chance B (1954) The assay of catalase and peroxidase Method Biochem Anal 1:357–424

15 Meloni, D.A., M.A. Oliva, C.A. Martinez and J. Cambraia,(2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot., 49: 69-76.

16 Meneguzzo,S,F, Navari lzzo & R. lzzo,; (1999), Antioxidative response of shoot root on wheat to increasing NaCl concentration; J Plant physiology L.55; 274 -280.

17 Mittova,V.M Tal .M.Valokita &M. Guy, (2003), UP regulation of the leaf mitochondrial & peroxisomal antioxidative systems in response to salt tolerant tomato species lycopersion pennellii .plant cell Environ ,26 845-856

18 Noel A Tejara Caraia, Germen Iribaonne, Frencisco, Palma ,Carmen ; (2007).Inhibition of the catalase activity from *Phaseolus vulgaris* &*Medicago sativa* by sodium chloride; Plant physiology &Biochemistry ,Vol 45(8) ,535-554.

19 Rahnama H and Ebrahimzadeh H, (2005),The effect of NaCl on antioxidant enzyme activities in potato seedlings, Biologia Plantarum, 49, pp. 93–97

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

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

ISBN 978-81-926339-06

	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 arietium* L cv Chafa cultivar.

Time in Hrs.	NaCl Conc (mM)	Catalase (mg H_2O_2 broken down $min^{-1}mg^{-1}$ proteins)			
		Attached		Detached	
		Leaf	Leachate	Leaf	Leachate
	Control	74.70.±0.14	110.40±0.14	95.50±0.42	107.30±0.21
24	25	59.65±0.21	107.30±0.49	127.40±0.14	74.50±0.28
	50	66.40±0.14	112.55±0.35	124.30±0.28	86.55±0.24
	100	83.35±0.07	113.70±0.28	113.70±0.26	108.50±0.28
	150	91.70±0.14	119.70±0.2	90.25±0.21	110.30±0.4

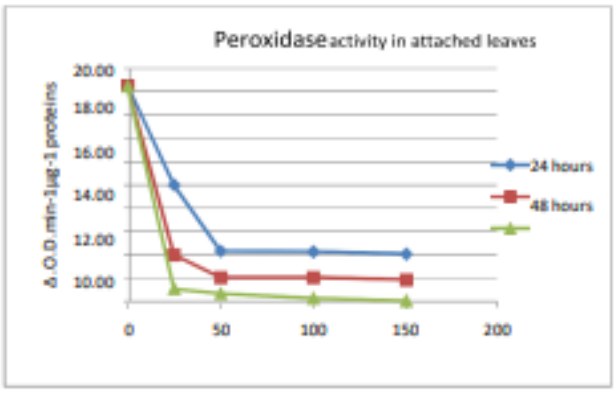
ISBN 978-81-926339-06

			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±0.42
72	25	69.60±0.14	87.55±0.07	91.65±0.28	102.25±0.42
	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 arietium* L cv Chafa cultivar.**



ISBN 978-81-926339-06

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