Biological Forum – An International Journal **14(4): 664-669(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Physiobiochemical characterization of Wheat (*Triticum aestimum*) under the Enfluence of Salinity Stress

Jadhav U.P.[1], Folane J.N.[1]* and Etther Y.B.[1]
College of Agricultural Biotechnology,
Paithan Road, Sambhajinagar (Maharashtra), India.

(Corresponding author: Folane J.N.)*
(Received 27 August 2022, Accepted 25 October, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: A laboratory experiment was conducted to evaluate the cultivated wheat genotypes for tolerance to salinity by giving NaCl stress with concentration 50mM, 100mM, 150mM, 200mM, 250mM. During experiment observation were recorded for various biochemical and physiological parameters of the wheat varieties. According to increasing salt stress percent germination and seedling weight were decreased (except Lok-1 at 150 mM). Overall, with increased concentration of salinity stress reduction in germination percentage, shoot and root length, seedling weight were observed. Proline content was decreased during stress condition induces by NaCl. The highest proline concentration 5.10 µg/ml (50mM) and lowest 2.5 µg/ml (100mM) in Lok 1 and Ajeet 106 were recorded.

Keywords: Salt stress, NaCl, Biochemical, Proline, Physiological, Germination.

INTRODUCTION

Wheat is a stable crop for a significance proportion of the world's Population. In India production of wheat [FAO] is estimated to increase record 109.52 million tons in 2020-21 from 107.86 million tons in the previous years. Wheat is the ranked first position among all cereals, due to its contribution as primary staple food crop and its domestication (Sabagh *et al.*, 2021). The demand of wheat is increasing global due to the unique properties of adhesive gluten, viscoelastic and proteins (Day *et al.*, 2006). Wheat is important source of Starch (58%), carbohydrates(13%), protein (11%), essential vitamins and minerals(2%) such as vitamins B, Thiamin, Riboflavin, Niacin and folic Acid and E also found calcium, iron, multiple nutrients and dietary fiber (Shewry and Hey 2015).

In wheat crop about 50% production losses occurred due to salinity stress (Acquaah, 2007). For global food production salinity is a major threat and its intensity increasing because of anthropogenic activities (Seleiman *et al*, 2021). Soil salinity negatively affects the morphological traits such as germination percentage, grain per spike, plant height, grain yield and harvest index. physiological traits like relative water content, membrane stability, chlorophyll content and mineral uptake; biochemical traits like proline content, gluten content, enzymatic activity and protein synthesis involved in various metabolic processes . Salinity stress disturbs the ionic balance due to the accumulation of Na+ which reduces the mineral uptake and their translocation to grains. Salt stress also causes the production of reactive oxygen species which hampers plant growth and development. This stress disturb photosynthesis machinery, alters cell components, damage cell wall, increase production of reactive oxygen species (Hasanuzzaman *et al.*, 2014). The important osmolyte Proline, which accumulates inside plants, is known for providing cellular homeostasis during salinity stress (Ramanjulu and Sudhakar 2001). The hydrogen peroxide is a signaling molecule present in plants, during stress conditions, generates harmful hydroxyl radicals which are toxic to plant cell (Velikova *et al.*, 2000; Petrov and Van Breusegem 2012).

The outline of study was carried out to explore the effect of salinity stress on some physiological and biochemical parameters of the selected wheat genotypes. Main objective of the study is assessment of Physiological responses of different wheat genotypes under different NaCl (salt) concentrations and to explore the effect of salinity stress on biochemical parameters of wheat genotypes.

MATERIAL AND METHODS

Collection of varieties and seeds sterilization with stress treatments: The mature and healthy seeds of Wheat varieties are Ajeet-102, Ajeet-106, GW-496, Kedar, Lok1 were collected from the Krishi Vigyan Kendra, Aurangabad. The experiment was conducted in collage of Agriculture Biotechnology, Paithan road, Aurangabad. The seeds were surface Sterilized using 0.1% $HgCl_2$ for 2 min. (Singh, 2016), then washed 4-5 time with distilled water. Seed germinated on different concentration of NaCl [0mM, 50mM, 100mM, 150mM, 200mM, 250 mM] by placing germination paper in

petri plate. Seed germination percentage and different Physiological parameters at seedling stage of wheat as shoot length, Root length, Plant height, and fresh weight were measured.

Biochemical and antioxidant enzyme activity measurement

Measurement of total Chlorophyll content. 100 mg of leaf tissues were crushed with 85% (v/v) aqueous acetone (Ozone International India Ltd).Chlorophyll (Chl) a, Chl b, and the total carotenoids were extracted from fifteen days old seedling and the absorbance of the extract was measured at 452, 644 and 663 nm using the spectrophotometer (Ajanta Ltd). The amount of chlorophyll was calculated according to Arnon (1949).

Proline Determination. 3% sulfosalicylic acid (1ml) use for the crushing of 100mg leaf sample. After centrifugation supernatant was added with 1 ml of Ninhydrin reagent [Nice chemical Ltd] and incubate in boiling water for 1 hr. after 5 mins and immediately absorbance were recorded at 546nm on spectrophotometer (Ajanta Ltd) (Bates *et al*., 1973). The proline content was calculated from the standard curve using L-proline as a standard and leaf proline content was expressed in unit µg/gm FW.

Carbohydrate content: Leaf samples were dried at 80°C for 48 hrs. 2 ml 1N HCl was added in dried powder samples and boiled for 1.5 hrs after cooling the sample 2 ml anthrone reagent [0.2 g anthrone, 8 ml absolute ethyl alcohol, 30 ml distilled water, and 100 ml concentrated sulfuric acid] was added. The mixture was boiled again for 7 min., cooled and O.D. was measured at 620 nm (Radwan, 2007) using Spectrophotometer. The concentration of total carbohydrate was calculated by the standard curve using Glucose was used as the standard and expressed as unit mg/gm DW.

Enzyme assay: The leaves from fifteen days old seedling of both the control and treatment were crushed in ice-cold 0.1M Tris-HCl buffer at pH-7.5 (0.1M Tris-HCl, Sucrose 5% w/v and 0.1% 2-mercaptoethanol) in 3:1 buffer volume/fresh weight. The homogenate sample was centrifuged at 4°C for 20 minutes at 10,000 rpm, after that supernatant was used for the enzyme assay (Abedi and Pakniyat 2010).

Guaiacol Peroxidase: Assays of POD (EC 1.11.1.7) activity were carried out using guaiacol as the hydrogen donor. The enzyme extract was added to the reaction mixture including Potassium-phosphate buffer 100mM at pH-7.0, 20 mM and 10mM guaiacol and H_2O_2 simultaneously, the absorbance was recorded at 436 nm using Spectrophotometer. The POD enzyme activity was calculated by the following formula (Polle *et al*., 1994).

Catalase: CAT (EC 1.11.1.6) activity was assayed based on the decomposition of H_2O_2 (Aebi H.). The enzyme extract was added to the reaction mixture having pH 7 potassium phosphate buffer of 50mM and H_2O_2 33mM and absorbance was measured at 240nm using UV Spectrophotometer [Ajanta]. Catalase activity was calculated using the following formula (Aebi, 1984).

RESULTS AND DISCUSSION

Morpho-physiological traits alternation in response to salt condition. Seed germination, shoot length, root length and seedling weight are important characteristics for wheat which could provide advantages for crop establishment. According to this experiment salt stress is significantly influenced on germination and related traits of various wheat varieties. As per the Table 1, in Ajeet-102, germination percentage significantly decreased as per increasing concentration of Salt. As compare to this, remaining wheat verities showed greater influence above 150 mM concentration. The findings of this study are in agreement with the reports of (Yadav *et al.*, 2020) that best and most suitable stress treatment for wheat seed germination is 20 to 22°C.

In Lok-1, the highest shoot length was observed 10 cm at 50mM whereas, highest fresh weight (0.580gm) recorded on 150mM concentration of NaCl. The maximum root length (7.9 cm) was observed in 50mM concentration of NaCl in Ajeet 106 variety of wheat (Table 1). Salinity effected root and shoot length also reduced growth rate same results were described by (Ali *et al*., 2012; Radi *et al*., 2013). Salinity effected varieties show a wide range of response in plants.

Response of Salt condition to total chlorophyll content. The total carotenoid degradation in seedlings at salt levels up to 250mM were found in order Ajeet 102 >Kedar> Lok1 >Ajeet 106 >Gw 496 (Fig. 1).

Our results show an inverse relationship between salinity and chlorophyll content. As per Fig.1, the highest chl a content in Ajeet 106 (50mM) and indicate lowest chla content under severe stress condition (100mM) in Ajeet 102. The highest chl b was observed in Ajeet 106 (100mM) and lowest was observed in Gw 496 at higher stress. Ajeet 102 variety recorded highest carotenoid (7.05%) at 50mM and lowest (1.55%) on 100mM of NaCl stress.

Similler findings were agreement with previous results of Khaled *et al.* (2020) on *Phaseolus vulgaris* L. and Taffouo *et al*. (2010) on *Vigna subterranean* L. Salinity induced decline in carotenoid contents in all wheat genotypes. These results concur with those found by Khaled *et al*. (2020) on *P. vulgaris* L. and Singh *et al.* (2008) in maize and wheat genotypes.

Estimation of Proline content under salt stress. In present work, the sharp decreased in Proline content might theoretically and degradation of Proline which are strongly affect on high drought stress. It might be supply energy for growth, survival and help the plant to tolerate stress (Sankar *et al*., 2007). We observe the highest proline concentration 5.10 µg/ml (50mM) and lowest 2.5 µg/ml (100mM) in Lok 1 and Ajeet 106 simultaneously (Fig. 2). These results suggest that Proline is not directly involved in the drought resistance and is not essential for improved resistance.

Table 1.

Varieties name	Treatments	% of germination	Shoot length (cm)	Root length(cm)	Seedlings weight (mg)
Ajeet 102	Control, 0mM	100%	9.21	8.99	0.201
	50mM	90%	8.23	7.5	0.198
	100mM	90%	5.95	5.4	0.266
	150mM	90%	3.2	3.9	0.130
	200mM	80%	3.1	6.2	0.17
	250mM	0%	0	0	0
Ajeet 106	Control,0mM	100%	9.27	9.82	0.193
	50mM	100%	8.3	**7.9**	0.148
	100mM	100%	6.23	5.5	0.198
	150mM	80%	4.65	3.75	0.158
	200mM	70%	3.2	3.28	0.130
	250mM	70%	**1.95**	0.4	**0.066**
GW 496	Control,0mM	100%	10.1	4.7	0.153
	50mM	100%	7.9	4.1	0.154
	100mM	100%	8.1	3.7	0.132
	150mM	100%	7.5	3.5	0.093
	200mM	100%	6	2.9	0.085
	250mM	90%	3	2.2	**0.066**
Lok1	Control,0mM	100%	10.2	6.2	0.210
	50mM	100%	**10**	6	0.130
	100mM	100%	7.2	6.5	0.395
	150mM	100%	4.13	4.85	**0.580**
	200mM	90%	3.12	4.05	0.300
	250mM	80%	3.1	3.1	0.220
Kedar	Control,0mM	100%	9.2	5.2	0.250
	50mM	100%	7.2	4.7	0.24
	100mM	100%	5	4.5	0.20
	150mM	100%	4.9	4.1	0.19
	200mM	100%	3.9	3.7	0.15
	250mM	90%	3.2	2.7	**0.09**

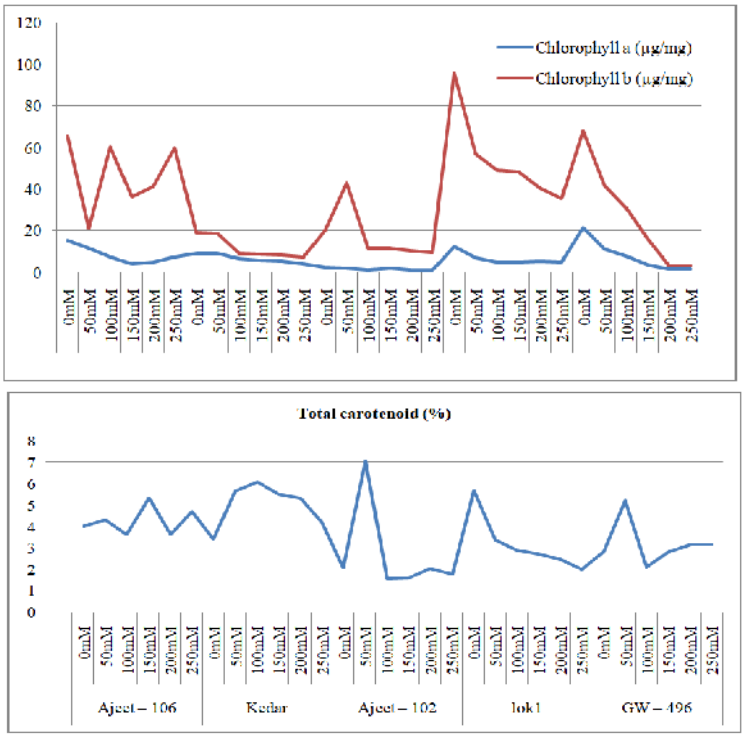

Fig.1. Estimation of chlorophyll contain in wheat varieties at seedling stage.

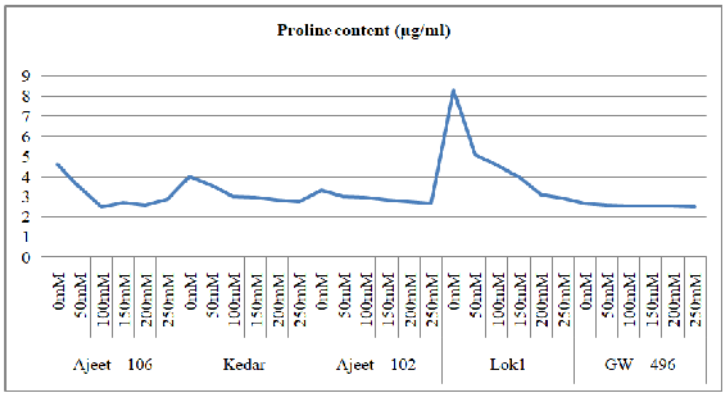

Fig. 2. Effect of salt stress on proline contain in wheat varieties.

Similar findings have been observed in number of plant species such as *Paspalum vaginatum* (Lee *et al*., 2008). Under these conditions, proline could be considered as an osmoregulator. Hussain *et al.* (2021) also reported that, in salt stress condition, proline accumulation was significantly increases in leaves of all wheat varieties.

Carbohydrates content estimation. Leaf carbohydrate compositions were differently altered by salinity stress. The carbohydrates concentration were simultaneously decreased according to raise in NaCl stress, but only Ajeet 106 showed highest percent (6.73) whereas the lowest percent (2.39) was recorded in Kedar at 100mM NaCl concentration (Fig. 3). Due to salt stress in wheat varieties cause an increase in degradation of starch or polysaccharides if increase in total soluble sugars (Athar *et al*., 2015; Ashraf *et al*., 2008).

Five varietes exhibited a significant increase in catalase (CAT), Guaiacolperoxidase (POD) activities with increasing NaCl concentrations in the growth medium. Striking differences in the antioxidant enzyme activities between the three varieties (except Lok-1 and GW 496) with increasing NaCl stress. One unit of POD activity is the amount of enzyme, which causes the decomposition of 1 µg substrate per minute in 1 mg fresh sample at 37°C. Highest POD level was observed in Ajeet 102 (3.03 µmol/min) at 100mM and lower (0.60 µmol/min)in Kedar at 50mM. Under stress condition POD affect plant process therefore have impact on plant health.

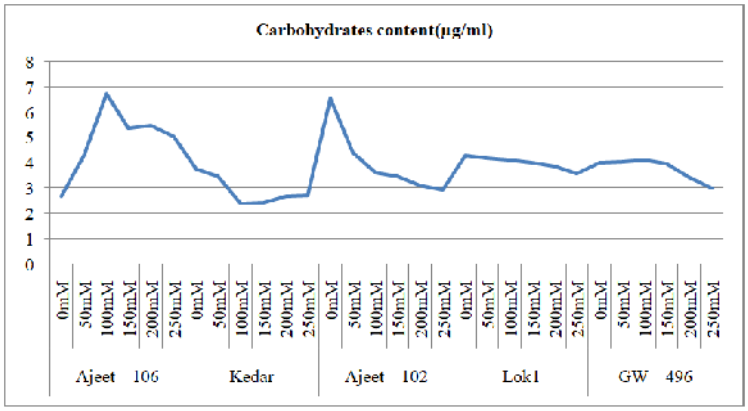

Fig. 3. Effect of NaCl treatment on carbohydrates content in wheat seedling.

Analysis of Enzyme activity under salt stress. One unit of CAT activity is the amount of enzyme which causes the decomposition of 1 µmol H_2O_2 per minute in 1 mg fresh sample at 37°C (Beers and Sizer 1952). According to Fig. 4, Ajeet 106 recorded highest 10.03 µmol/min at (50mM) whereas Kedar shows 1.62 µmol/min lowest CAT activity at (100mM) salt condition. Ajeet 106 recorded highest 10.03 µmol/min at (50mM) whereas Kedar shows 1.62 µmol/min lowest CAT activity at (100mM) salt condition. Previous researches reported the higher activities of CAT and POD in response to salinity in tomato, cabbage and wheat (Li, 2009; Ali *et al*., 2017; Sarker and Oba 2020b).

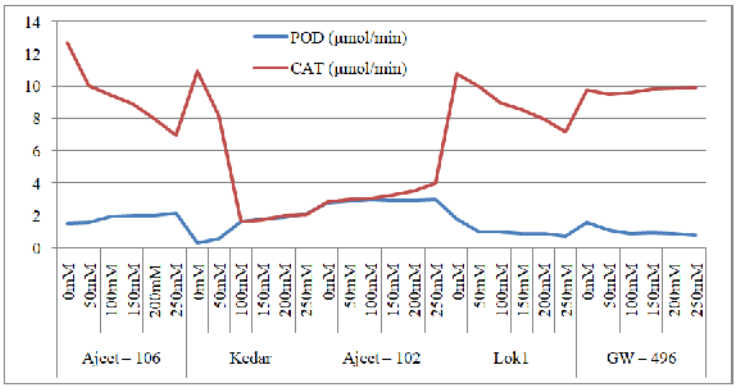

Fig. 4. Analysis of Enzyme activity under saline condition in wheat varieties.

CONCLUSION

The results of study showed that there was considerable difference between most differential factor is the enzyme activity, carbohydrate, proline, chlorophyll content and physiological damage. This experiment indicates the difference between varieties is adequately depend on the salt stress condition.

Based upon the observed physiological and biochemical data for the wheat varieties salinity tolerance was found in the order Lok 1 > GW 496 >Ajeet 106 >Ajeet 102 >Kedar. A significant correlation was seen among the studied parameters with increasing salinity levels.

The main aim of this study is in plant breeding strategies for developing new genotypes with increased yield upon salt stress. Plant breeder may focus on selecting plants with high antioxidant activity, except plants able to accumulate more salt.

Acknowledgement. Authors thankful to SDMVM's college of Agricultural biotechnology affiliated to VNMKV, Parbhani (MH) for providing laboratory facility for this research work and also thankful to KVK, Aurangabad (MH) for giving seed material of wheat varieties.

Conflict of Interest. None.

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How to cite this article: Jadhav U.P., Folane J.N. and Etther Y.B. (2022). Physiobiochemical characterization of Wheat (*Triticum aestimum*) under the Enfluence of Salinity Stress. *Biological Forum – An International Journal, 14*(4): 664-669.