

# Influence of Physio-chemical Changes in Different Linseed (*Linum usitatissimum* L.) Genotype Under Salinity Stress

Atul Singh<sup>1</sup>, Anu Singh<sup>2</sup>, Syed Mohamad Afrayeem<sup>3</sup>, Md. Abdul Basith<sup>4</sup>,  
Shailesh Marker<sup>5</sup> and P.W. Ramteke\*

<sup>1,4</sup> Department of Biological Sciences, <sup>3</sup> Department of Genetics and Plant Breeding,

<sup>5</sup> Directorate of Research, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India

<sup>2</sup> Centre Food Technology, University of Allahabad, Prayagraj, Uttar Pradesh India

(Received 23 July, 2020; Accepted 13 March, 2021)

## ABSTRACT

Flax is one of the oldest utilitarian plants that have been grown for fibre as well as an oilseed crop. The species has been recognized as an excellent source of micronutrients, dietary fibre, protein, vitamin B1, lignan, and essential fatty acids, namely linoleic and  $\alpha$ -linolenic acids. This study was to determine the effect of different concentrations of NaCl on Physio-Chemical parameters of different linseed genotype and the study was laid out in FRBD (4x9 Factorial), having three levels each of NaCl (100mM, 150mM and 200mM) and nine varieties viz., SHA1, SHA2, SHA3, SHA4, SHA5, SHA6, SHA7, SHA8 and SHA9, making a total of 36 treatment combinations with control, each replicated three times. NaCl affects the Chlorophyll content (mg/g), Carotenoids (mg/g), Proline ( $\mu$ g/g fresh w.t), Malondialdehyde ( $\mu$ mole/g fresh w.t). Whereas the the maximum Chlorophyll content, Carotenoids (mg/g), Proline ( $\mu$ g/g fresh w.t), is maximum was recorded in variety SHA7 Malondialdehyde ( $\mu$ mole/g fresh w.t) was found in minimum SHA3. Thus it shows that SHA7 genotype is more potential in saline soil. The research will provide useful leads in the planned development of linseed cultivars having increased salt tolerance.

**Key words :** Chlorophyll, Carotenoid, Proline, Malondialdehyde, Salinity stress.

## Introduction

Linseed also referred as flax (*Linum usitatissimum* L.), is a self-pollinated crop widely adapted to temperate climates of the world. It is an annual plant belongs to the genus *Linum* and the family *Linaceae*. In fact, the name *Linum* is originated from the Celtic word *lin* or "thread", and the name *usitatissimum* is Latin for "most useful". It is believed that flax is originated in the Middle East or Indian regions. These antiquated semantic Origins underscore the

significance of flaxseed or linseed. The terms flax and linseed have particular meanings, depending on the region. In Europe, flax refers to the seed grown for fibre (linen) production, while linseed refers to oilseed flax grown for industrial and nutritional uses. Linseed is one of the most versatile and useful crops that have been grown for thousands of years. It is cultivated as a commercial or subsistence crop in over 30 countries. Flax seeds are used for industrial, food and feed purposes. Seeds are rich source of both non-edible and edible oil. The industrial oil

is an important ingredient in the manufacture of paint, varnish and linoleum. Edible linseed oil is used for human consumption and contains  $\alpha$ -linolenic acid (ALA), a polyunsaturated fatty acid that has nutritional and health benefits (Neil and Alister, 2003). Apart from ALA, linseed is widely used as nutritional and functional food in the western world due to its high contents of therapeutic health promoting substances such as omega-3 fatty acid, soluble and insoluble fibre and lignans and its suitability to use with bread, breakfast cereals and other food products. In 2014, flax was approved by Flax Council of Canada, for a health claim to lower blood cholesterol, a major risk factor for heart disease by consuming ground or whole flaxseed. In most of the countries, linseed is cultivated mainly for its seed which is processed into oil and a high protein feed stock after oil extraction with the linseed straw generated as a by-product. Salinity is one of the major abiotic stresses, which has significant impact on plant productivity and quality (Bhargava *et al.*, 2003; Shahbaz and Ashraf, 2013). Soil salinity has significantly increased in recent years due to several factors like excessive irrigation, low precipitation, high surface evaporation, rock weathering, ion exchange and poor cultural practices (Munns and Tester, 2008; Bui, 2017). Recent reports indicate that about 20% of total cultivated and 33% of irrigated lands are afflicted by saline conditions at present, and that more than 50% of the arable land would be salinized by the year 2050 (Jamil *et al.*, 2011; Shrivastava and Kumar, 2015). Salinity stress negatively affects all growth stages of plants in various ways like reduction of plant height, deterioration of the product quality and crop yields (Shahbaz and Ashraf, 2013; Shrivastava and Kumar, 2015; Rahnesan *et al.*, 2018).

Soil salinity is also known to affect different physiological and metabolic processes of plants such as reduction in water uptake, chlorophyll content, photosynthesis, transpiration rate, nutrient availability, stomata conductance and root hydraulic conductance (Khataar *et al.*, 2018; Hernandez, 2019). Proline is an important amino acid and believed to be a single molecule, which helps in activating many physiological and molecular responses in the plants even in stress conditions. It was observed that, when plants undergo Cu stress condition, they accumulate high contents of proline in their tissue to overcome metal stress (Szabados and Saviouré 2010; Ku *et al.* 2012). The Malondialdehyde rate increases and it

reaches its maximum after 48 hours after stress application. The Malondialdehyde components show the cellular damage rate in the plant. The salinity stress in Flax leads to an increase in Malondialdehyde rate and as a result, the increase in the cellular damage in the studied cultivars.

## Materials and Method

The present study entitled "Estimation of heterosis and physiological variation in some economic Traits in elite Linseed (*Linum usitatissimum* L.) genotypes under salinity stress", was conducted in the Sam Higginbottom University of Agriculture, Technology and Sciences, Naini, Prayagraj. The climate of Prayagraj city is subtropical. The winter season is very cold (temperature reaching as low as 2.5 °C and in summer season temperature reaching up to 48 °C. The study was laid out in FRBD (4x9 Factorial), having three levels each of NaCl(100mM, 150mM and 200mM) and nine different genotypes viz., SHA1, SHA2, SHA3, SHA4, SHA5, SHA6, SHA7, SHA8 and SHA9, making a total of 36 treatment combinations with control, each replicated three times. Observations for all the traits were recorded on five randomly selected plants for each replication and for each plot. The observations were taken on different plant characters viz., Chlorophyll content (mg/g), Carotenoids (mg/g), Proline ( $\mu$ g/g fresh w.t), Malondialdehyde ( $\mu$ mole/g fresh w.t) of linseed. Chlorophyll content and carotenoids was measured according to method described by Arnon, (1949). Proline content was measured according to Bates *et al.*, (1973) and the level of lipid peroxidation was measured in terms of Malondialdehyde (MDA) content using the method of George, (1968). The MDA content was calculated using its absorption coefficient of 155 n mol<sup>-1</sup> cm<sup>-1</sup> and expressed as n mol (MDA) g<sup>-1</sup>FW. The analysis of variance was worked out to test the significance of F tests. It was carried out according to the procedure of factorial randomized block design for each character as per methodology advocated (Fisher, 1936).

## Results and Discussion

### Effect of different levels of NaCl on growth and yield of Linseed

Observations regarding the response of different levels of NaCl on Chlorophyll content (mg/g), Caro-

tenoids (mg/g), Proline ( $\mu\text{g/g}$  fresh w.t), Malondialdehyde ( $\mu\text{mole/g}$  fresh w.t) of linseed as influenced by different levels of NaCl and different nine varieties and their interaction is presented in Table 1. The maximum Chlorophyll content (1.50 mg/g), Carotenoids (0.710mg/g), in SHA 7 was recorded in control and Proline (15.580 $\mu\text{mole/g}$  fresh w.t), Malondialdehyde (6.370 $\mu\text{mole/g}$  fresh w.t), was founded in 200Mmand The minimumChlorophyll content (1.153mg/g), Carotenoids (0.473mg/g), was found in 200mM in SHA3 andProline (3.813 $\mu\text{mole/g}$  fresh w.t)SHA3, Malondialdehyde (3.310 $\mu\text{mole/g}$  fresh w.t)SHA7, was recorded in control in linseed crop. The presence of NaCl caused a significant decrease in chlorophyll content compared to the control. However, the decrease was more pronounced during NaCl stress. Generally lowest reduction in the carotenoid content was witnessed in both the experimental years during salt stress (Table 1) (Taïbi *et al.*, 2016), (Rahman *et al.*, 2017) Lipid peroxidation in term of Malondialdehyde (MDA) contents was also determined from the leaves of linseed. It was noticed that increased MDA contents from control to 200Mm in leaves of linseed, which is the indica-

tion of oxidative damage occur in the plants due to phytotoxicity of Cu concentration. Similarly, proline contents were also increased in the plants as concentration of proline contents increased in leaf compared with the control to 200Mm in linseed.

#### NaCl and varieties effect on different genotype with respect of Physio-Chemical properties of Linseed

The NaCl is found to influence the Chlorophyll Content, Carotenoids, Proline Content and Malonyldehyde (MDA) which is directly related to the yield of the crop. The maximum Chlorophyll content (1.385 mg/g), Carotenoids (0.629 mg/g), was recorded in control and Proline (14.650  $\mu\text{mole/g}$  fresh w.t), Malondialdehyde (5.404  $\mu\text{mole/g}$  fresh w.t), was founded in 200Mm and The minimum Chlorophyll content (1.153mg/g), Carotenoids (0.552 mg/g), was found in 200Mm and Proline (4.746  $\mu\text{mole/g}$  fresh w.t), Malondialdehyde (3.647 $\mu\text{mole/g}$  fresh w.t), was recorded in control in linseed crop (Table 1) and the varieties effect on The maximum Chlorophyll content (1.477mg/g), Carotenoids (0.685 mg/g), and Proline (10.064 $\mu\text{mole/g}$  fresh w.t), was recorded in SHA7

**Table 1.** Effect of NaCl, varieties and its interaction on Physio-Chemical of linseed (*Linum usitatissimum* L.)

Treatments	Parameters			
	Chlorophyll Content (Mg/g)	Carotenoids (Mg/g)	Proline Content ( $\mu\text{mole/g}$ fresh w.t)	Malondialdehyde ( $\mu\text{mole/g}$ fresh w.t)
Level of NaCl				
Control	1.385	0.629	4.746	3.647
100mM	1.370	0.601	6.684	3.938
150mM	1.316	0.581	10.547	4.204
200mM	1.280	0.552	14.650	5.404
F-test	S	S	S	S
C.D. at 0.5%	0.004	0.007	0.009	0.053
S.Ed. ( $\pm$ )	0.002	0.003	0.005	0.027
			Varieties	
SHA1	1.248	0.535	8.450	4.680
SHA2	1.283	0.565	9.018	4.386
SHA3	1.238	0.515	8.172	4.860
SHA4	1.335	0.573	8.858	4.286
SHA5	1.356	0.608	9.447	4.192
SHA6	1.272	0.542	8.883	4.506
SHA7	1.477	0.685	10.064	3.864
SHA8	1.427	0.658	9.905	3.940
SHA9	1.404	0.638	9.613	3.970
F-test	S	S	S	S
C.D. at 0.5%	0.006	0.010	0.014	0.080
S.Ed. ( $\pm$ )	0.003	0.005	0.007	0.040

Table 1. Continued ...

Interaction (NaCl x Varieties)					
S. No.	Treatment	Chlorophyll Content(Mg/g)	Carotenoids (Mg/g)	Proline Content ( $\mu\text{mole/g}$ fresh w.t)	Malondialdehyde ( $\mu\text{mole/g}$ fresh w.t)
T1	SHA1+ (Untreated)	1.307	0.577	4.017	3.843
T2	SHA2+ (Untreated)	1.353	0.630	4.273	3.663
T3	SHA3+ (Untreated)	1.300	0.557	3.813	4.223
T4	SHA4+ (Untreated)	1.380	0.617	4.813	3.610
T5	SHA5+ (Untreated)	1.403	0.640	5.117	3.567
T6	SHA6+ (Untreated)	1.333	0.587	4.157	3.737
T7	SHA7+ (Untreated)	1.500	0.710	5.837	3.310
T8	SHA8+ (Untreated)	1.453	0.687	5.360	3.417
T9	SHA9+ (Untreated)	1.433	0.547	5.323	3.453
T10	SHA1 +100Mm	1.293	0.567	5.903	4.090
T11	SHA2+100Mm	1.320	0.523	6.320	3.910
T12	SHA3+100Mm	1.283	0.583	5.607	4.180
T13	SHA4+100mM	1.370	0.617	6.900	4.157
T14	SHA5+100mM	1.397	0.550	7.137	3.897
T15	SHA6+100mM	1.317	0.700	6.193	4.000
T16	SHA7+100mM	1.490	0.670	7.433	3.690
T17	SHA8+100mM	1.443	0.650	7.400	3.787
T18	SHA9+100mM	1.413	0.547	7.260	3.733
T19	SHA1+150mM	1.223	0.527	9.577	4.457
T20	SHA2+150mM	1.247	0.547	10.277	4.150
T21	SHA3+150mM	1.217	0.507	9.377	4.667
T22	SHA4+150mM	1.310	0.560	10.417	4.127
T23	SHA5+150mM	1.330	0.603	11.137	4.077
T24	SHA6+150mM	1.233	0.530	10.077	4.267
T25	SHA7+150mM	1.470	0.680	11.407	3.990
T26	SHA8+150mM	1.417	0.650	11.383	4.033
T27	SHA9+150mM	1.397	0.630	11.270	4.067
T28	SHA1+200nM	1.167	0.490	14.303	6.330
T29	SHA2+200nM	1.210	0.517	15.200	5.820
T30	SHA3+200nM	1.153	0.473	13.890	6.370
T31	SHA4+200nM	1.280	0.530	13.303	5.250
T32	SHA5+200nM	1.293	0.573	14.397	5.227
T33	SHA6+200nM	1.203	0.500	15.107	6.020
T34	SHA7+200nM	1.447	0.650	15.580	4.467
T35	SHA8+200nM	1.393	0.623	15.477	4.523
T36	SHA9+200nM	1.373	0.610	14.597	4.627
	F-test	S	S	S	S
	C.D. at 0.5%	0.013	0.020	0.028	0.160
	S.Ed. ( $\pm$ )	0.006	0.010	0.014	0.080

Malondialdehyde ( $4.860\mu\text{mole/g}$  fresh w.t), was founded in SHA3 and The minimum Chlorophyll content ( $1.238\text{mg/g}$ ), Carotenoids ( $0.515\text{mg/g}$ ), and Proline ( $8.172\mu\text{mole/g}$  fresh w.t), was recorded in SHA3 Malondialdehyde ( $3.864\mu\text{mole/g}$  fresh w.t), was founded in SHA7 in linseed crop (Table 1). Availability of free  $\text{Ca}^{2+}$  is necessary to maintain membrane stability, participating in signaling and

this is needed for synthesis of cell walls. Meanwhile,  $\text{Mg}^{2+}$  is an important component of chlorophyll, Carotenoids and  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  play important roles in various physiological and biochemical processes (Knight *et al.*, 1997; Mori, 1999; Parida and Das, 2005). Proline and betaine are known to play important roles in osmotic adjustment (Stewart and Lee, 1974; Rhodes and Hanson, 1993). We found that

proline accumulated in shoots of linseed seedlings in response to Salinity stress and high NaCl concentration in the soil causes oxidative stress by increasing MDA contents, and, due to generation of ROS.

## Conclusion

The current investigation is carried out to assess nine genotype of linseed for salt stress tolerance. The investigation showed a gradual increase in the amplitude of salt stress exert differential response in all the nine linseed genotypes. Findings of the present investigation suggest that SHA7 genotype showed a higher level of salt tolerance, and it might be attributed through the increased potential of Chlorophyll content, Carotenoids, Proline, Malondialdehyde. Moreover, it could be hypothesized that SHA7 cultivar of linseed could be used to minimize the yield loss of linseed crop due to salt stress. However, future research is needed on the effects of Na<sup>+</sup> and Cl<sup>-</sup> on linseed (*Linum usitatissimum* L.) crop separately. Moreover, potential for *L. usitatissimum* in remediation of soils polluted with heavy metals should be tested under field conditions.

## Acknowledgements

The authors express their gratitude to the Department of Biological Sciences SHUATS Prayagraj for providing lab facilities to research work and Thanks to DST Inspire for financial support for research work.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24: 1–15.
- Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil.* 39 : 205–207.
- Bhargava, A., Shukla, S., Katiyar, R.S. and Ohri, D. 2003. Selection parameters for genetic improvement in Chenopodium grain in sodic soil. *Journal of Applied Horticulture.* 5 : 45–48.
- Bui, E.N. 2017. Causes of soil salinization, sodification, and alkalization. Oxford Research *Encyclopedia of Environmental Science.*
- Fisher, R. A. and Yates, F. 1938. Correlation between relatives on the supposition of Mendelian inheritance. France Royal Soc., *Edinburg.* 52 : 399–433.
- George, W. O. and Mansell, V. G. Nuclear magnetic resonance spectra of acetylacetaldehyde and Malondialdehyde. *J. Chem. Soc. B,* 132â 134, 1968.
- Hernández, J.A. 2019. Salinity Tolerance in Plants: Trends and Perspectives. *International Journal of Molecular Sciences.* 20 : 2408.
- Jamil, A., Riaz, S., Ashraf, M. and Foolad, M.R. 2011. Gene expression profiling of plants under salt stress. *Critical Reviews in Plant Science.* 30 : 435–458.
- Khataar, M., Mohammadi, M. and Shabani, F. 2018. Soil salinity and matric potential interaction on water use, water use efficiency and yield response factor of bean and wheat. *Scientific Reports.* 8 : 2679.
- Knight, H., Trewavas, A.J. and Knight, M.R. 1997. Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity. *Plant J.* 12 : 1067–1078.
- Ku, H. M., Tan, C. W., Su, Y. S., Chiu, C. Y., Chen, C. T. and Jan, F. J. 2012 The effect of water deficit and excess copper on proline metabolism in *Nicotiana benthamiana*. *Biol Plant.* 56 : 337–343.
- Mori, S. 1999. Iron acquisition by plants. *Curr. Opin. Plant Biol.* 2 : 250–253.
- Munns, R. and Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology.* 59 : 651–681.
- Neil, D., Westcott, D. and Alister, D. and Muir. 2003. Flax seed lignan in disease prevention and health promotion. *Phytochemistry Reviews.* 2 : 401–417.
- Parida, A.K. and Das, A.B. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Saf.* 60 : 324–349.
- Rahman, M.M., Rahman, M.A., Miah, M.G., Saha, S.R., Karim, M.A. and Mostofa, M.G. 2017. Mechanistic insight into salt tolerance of *Acacia auriculiformis*: the importance of ion selectivity, osmoprotection, tissue tolerance, and Na<sup>+</sup> exclusion. *Frontiers in Plant Science.* 8 : 155.
- Rahneshan, Z., Nasibi, F. and Moghadam, A.A. 2018. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. *Journal of Plant Interactions.* 13 : 73–82.
- Rhodes, D. and Hanson, A.D. 1993. Quaternary ammonium and tertiary sulphonium compounds in higher plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 44 : 357–383.
- Shahbaz, M. and Ashraf, M. 2013. Improving salinity tolerance in cereals. *Critical Reviews in Plant Sciences.* 32: 237–249.
- Szabados, L. and Saviouré, A. 2010. Proline: a multifunctional amino acid. *Trends Plant Sci.* 15 : 89–97.
- Shrivastava, P. and Kumar, R. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Science.* 22 : 123–131.

- Shah, S.H., Houborg, R. and McCabe, M.F. 2017. Response of chlorophyll, carotenoid and spad-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy*. 7 : 61.
- Stewart, G.R. and Lee, J.A. 1974. The role of proline accumulation in halophytes. *Planta*. 120 : 279-289.
- Taïbi, K., Taïbi, F., Abderrahim, L.A., Ennajah, A., Belkhouja, M. and Mulet, J.M. 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. *South African Journal of Botany*. 105 : 306-312.