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Analysis of Two-Way ANOVA for Estimation of Chlorophyll in Under Physiological Stress

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KEYWORDS
Chlorophyll *Eleusine coracana* , variety Dapoli-3 , salt stress, drought stress, presowing salt stress

ABSTRACT:
Many plants of economic importance exhibit salt tolerance and are adapted to grow in salt-affected environments. Drought is undoubtedly one of the most important environmental stresses limiting the productivity of crop plants around the world. The assimilatory power of the plant is due to the presence of pigment called chlorophyll. One of the important factors that determines crop productivity is the chlorophyll content of the leaves. Chlorophyll was estimated from leaves of Dapoli-3 variety of *Eleusine coracana* under control plants (without any stress) as well as from plants grown under salt, drought and presowing salt treated seed stress conditions. Dapoli-3 variety of *Eleusine coracana* showed an increase in chlorophyll level at all concentrations of salt treatment w. In presowing salt treated plants, Dapoli-3, there was decrease in chlorophyll content at all salt concentration. While chlorophyll content decreased with extended duration of drought.

Introduction

The development of salt tolerant crop genotypes is a sustainable method for the productive usage of salt-affected soils (SAS), which span over 6.73 million acres of land in India [1]. degradation and declining soil fertility are both largely caused by soil salinization [2] which causes serious impact on the ecological environment, waste of resources as well as social and economic development [3]. Salinity stress has grown to be a serious problem for global agricultural productivity, especially in arid and semi-arid regions. Ion toxicity (Na+, Cl-); osmotic stress; and nutritional anomalies are used to explain the adverse effects of salinity stress on plants [4-8].

The impact of EDD (extreme temperature (>34 °C) degree days) stress is related to damages to photosynthetic cells, resulting in the reduction of chlorophyll content, photosynthetic rates and the leaf area, or even withering of the leaves [9-11]. Plant growth and productivity are limited by environmental challenges, particularly water stress [12-14]. Countries that fall under the "drought" category have water availability per year and residents that are less than 1700 m^3, especially in semi-arid areas. Arid zones are distinguished by a negative hydrological balance, mean annual rainfall of less than 800 mm, mean insolation of 2800 h/year, and mean annual temperatures of 23–27°C. Due to these factors, the region as a whole experiences significant evapotranspiration rates and a consequent water deficit [15]. The obvious reasons limiting crop productivity, especially in the southern regions marked by severe aridity, are abiotic stresses and inefficient methods of agriculture [16-18]. Undoubtedly, one of the most significant environmental factors affecting crop plant productivity worldwide is drought [19]. Due to the fact that the majority of the world's chickpea-growing regions are in arid and semi-arid regions and that around 90% of chickpea is cultivated under rain-fed circumstances, drought is also a key yield-limiting factor in the production of chickpeas (*Cicer arietinum* L.) which shows mechanisms for overcoming this condition [20]. Intermittent drought during the vegetative phase, reproductive development, or terminal drought at the conclusion of the crop cycle can all lead to yield losses [21]. Drought stress decreases the rate of photosynthesis [22]. A reduced stomatal conductance is present in plants growing in drought-stricken areas in an effort to preserve water.

Seed priming improves seedling development and establishment, enables DNA replication, boosts RNA and protein synthesis, and decreases metabolite leakage [23]. The pre-treatment with plant growth regulators is one of the efficient priming techniques. Plant stress responses are induced by growth regulators for plants such GAs and SA [24]. One of the crucial growth regulators utilized to encourage cell division and

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elongation in plants is Gibberllic acid[25].

Effect of Increasing Salt Stress on Organic Constituents Chlorophyll (Figure 1)

The assimilatory power of the plant is due to the presence of pigment called chlorophyll. One of the important factor that determines crop productivity is the chlorophyll content of the leaves. Chlorophyll was estimated from leaves of control plants as well as from plants grown under salt stress condition. These results are depicted in figures 1. Dapoli-3 variety of *Eleusine coracana* showed an increase in chlorophyll level at all concentrations of salt treatment.

There are number of reports, which state that there is an increase in chlorophyll content of the leaves when plants are raised in a saline environment [26-32]. There are equal number of reports which suggest that there is a decrease in chlorophyll content with increasing salinity [33-37]. In a study on effect of salt on horse gram seedling, it has been reported that due to NaCl and Na_2SO_4 salt concentrations there was decrease in total chlorophyll content in the leaves [38,39]. According to Bosque-Sanchez *et al*., (2003) and Eisa *et al*., (2012), variations have been identified in the photochemical phase of photosynthesis due to the effect of salinity which changes the photosynthetic rate, transpiration rate, efficiency of the use of photosynthetic water and the fluorescence activity of chlorophyll [40,41]. Perveen *et al.*, (2015) reported that, salinity causes adverse effects on crops [42]. Nusrat *et al.*, (2014) revealed that salt greatly suppresses the photosynthesis process in plants due to different factors like photosynthetic pigments, stomatal performance and generation of essential metabolites and antioxidants [43]. According to Nusrat *et al*., (2014), salt stress induces disturbance in various metabolic processes such as photosynthesis, stomatal regulation and protein synthesis [43]. And added that salt stress not only suppresses photosynthetic activity but also inhibit photosynthetic machinery of plants. Salt stress also affects the cell organelles like chloroplast and it is the site for most of photosynthetic processes (PSI and PSII) and generation of reactive oxygen species (ROS). Perveen *et al.,* (2013), observed that, plants avoid salt stress by various means like shoot damage while Kausar and Shahbaz, (2013) observed that under salt stress, stomatal aperture decreased and resulted in low CO2 availability to plants [42,44]. Shabala et al.; Rafique *et al*; Shabbir *et. al.,* noted that plants develop multigenic responses/ mechanisms for salt tolerance; these will then regulate many physiological/biochemical processes [45-47]. Srinieng *et al.,* reported that salinity disturbs uptake/distribution of essential nutrients and balanced absorption [48]. Bavei *et al.,* stated that ionic imbalance causes due to excess Na^+ in root cells competes with K^+ for uptake [49].

Waghmode and Joshi reported that the chlorophyll synthesis was stimulated with lower concentration of NaCl [50]. Mudliar and Bharati observed Lower (1 mM) KC1 concentrations resulted in increased chlorophyll synthesis, whereas higher amounts resulted in decreased chlorophyll content [51].

In contrast to salt-sensitive plants, it appears that salt-tolerant plants can accumulate chlorophyll. According to Rao and Rao, as chlorophyll is more sensitive to salinity, it gets wiped out by environments with higher salt concentrations. Higher salinity environments wipe off chlorophyll because it is more sensitive to salinity [52].

Lower chlorophyll synthesis is a recognized consequence of higher salt concentrations affecting the structure of chloroplasts and the stability of pigment protein complexes [34]. Anshuman Singh et.al., when worked on *Aegle marmelos* grown with saline stress, observed, consistent decrease in values of leaf chlorophyll (a, b and total) in *Aegle marmelos* cultivars NB-55 variety but also noted increase in salinity in *Aegle marmelos* cultivars NB-9, CB-1 and CB-2 for plants which exhibited slightly higher chlorophyll contents at moderate salinity [53]. Singh et.al., also observed significant increased membrane injury which caused reduction in relative water content in all the cultivars due to salinity [53]. Ali Y. *et.al*., (2004), while studying effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes [54]. The reduction in leaf area, yield and yield components under saline conditions were also due to reduced growth as a result of decreased water uptake, toxicity of sodium and chloride in the shoot cell as well as reduced photosynthesis. Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions. Salinity affects the strength of the forces bringing the complex pigment protein- liquid, in the chloroplast structure. As the chloroplast in membrane bound its stability is dependent on the membrane stability which under high salinity condition seldom remains intact due to which reduction

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in chlorophyll was recorded by [54].

In the present investigation Dapoli-3 cultivar seems to tolerate salinity to a greater extent as compared to HR-374 in terms of chlorophyll content which is one of the important contributory factors leading to increased crop productivity. Chlorophyll a and Chlorophyll b content of *Eleusine coracana* crop under different levels of salt and water stress and pre-sowing salt stress shows variety in results.

Effect of Pre-sowing Salt Treatment on Organic Constituents Chlorophyll (Figure 2)

The chlorophyll content of the leaves from both the cultivars, Dapoli-3 of *Eleusine coracana* were estimated. The crop was raised after giving salt presowing treatment to the seeds of both the cultivars and results obtained are represented graphically in figure. There was an increase in the chlorophyll content of the leaves when the salt concentration was 8 and 30 mM however, any further increase was not favourable resulting in 60 % decrease in the chlorophyll content. In Dapoli-3, there was decrease in chlorophyll content at all salt concentration.

There are reports regarding the influence of pretreatment of growth regulators on chlorophyll content in plant leaves. Nimbalkar and Joshi found that, pre-planting treatment with GA, kinetin, and ascorbic acid, there was a reduction in chlorophyll content in all above pretreatments but more reduction was observed due to GA pretreated leaves of sugarcane variety Co-740 [37]. Saran (1991) observed that seed soaking in 50 ppm IAA for 24 hrs increased chlorophyll content but lowered chlorophyll a/b ratio in mustard [55]. However, reports on the effect of pretreatment of seeds with salt are negligible. Kausar Farhana and Shahbaz Muhammad, experimented with pre-sowing seed treatment with strigolactone (GR24) on two wheat cultivars and observed an increasing response towards chlorophyll fluorescence [56]. Pre-sowing seed treatments with chemicals like GA3, Thiourea, KNO3, and NAA influence the duration of germination, percent seed germination, seedling height, and number of branches and roots [57-60]. Barley seed treatment with preparations based on iron, silicon, molybdenum, bogum, and molybdenum nanoparticles stimulates an increase in the level of photosynthetic pigments and the activity of antioxidant enzymes in plants [61]. While Fatemeh Jamal *et al.*, (2011), examined the impact of priming on the growth and chemical properties of the green leaf of wheat and stated that chlorophyll b had been significantly increased by seed priming [62].

Effect of Increasing Water Stress on Organic Constituents Chlorophyll (Figure 3)

It is evident from these results that the chlorophyll content decreased with extended duration of drought. In the Dapoli-3 variety, this decrease was more as compared to that of the control.

Reduction in chlorophyll content due to drought has been reported by many workers during a study on biochemical changes in *Oryza sativa* under water stress noticed a decrease in chlorophyll level [63,64]. Albert *et al.*, (1977), showed that in corn leaves the chlorophyll content declined after 8 days of water stress [63]. Sanchez R.A., et.al., observed reduced chlorophyll levels, stomatal conductance, and photosynthesis due to water stress [65]. Mafakheri A. *et.al.*, when working on Chickpea Cultivars, revealed that due to drought stress all physiological parameters get affected while due to drought, vegetative growth or anthesis significantly decreased chlorophyll a, chlorophyll b and total chlorophyll content [66].

González *et al.*, when studied on Chenopodium quinoa, reported that the reduction in soil moisture leads to a decrease in the relative water content of the leaves, which causes the stomata to close impacting the exchange of gases [13]. This affects the reduction in the rate of photosynthesis, photorespiration, transpiration, and absorption of nutrients, along with a decrease in the rate of consumption of ATP and NADPH for CO2 assimilation. Fghire *et al*., (2015); Killi and Haworth, (2017), revealed that water stress affects the balance between photochemical activity in the FSII and the demand for electrons for photosynthesis, generating an over-excitation in the photosynthetic system and photo-inhibitory damage in the reaction centres [67,68]. Garcia-Parra M *et al.* and Hinojosa *et al*. noted that in drought conditions, chlorophyll fluorescence analyses provide extensive information on the structure and function of the photosynthetic machinery [69,70]. Thakur, J., Shinde, B.P., while studying on effect of water stress and AM fungi on the growth performance of pea plants revealed a decrease in the chlorophyll contents in the three ascensions during the period of stress due to the increased catalytic activity of chlorophylls and degradation of photosynthetic pigments and this process is also the result of not providing the necessary factors

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for the synthesis of chlorophyll and the destruction of its structure under stress conditions [71].

The state of plant plastid apparatus under the conditions of water stress has been studied. It has been observed that drought conditions affect to a lesser degree, the quantity of segments strongly bound with the lipoprotein complex, particularly chlorophyll-b. According to a study, drought-resistant plants are characterised by the least changes in pigment content, but the nitrogen content of the leaves is not affected. Gill studied ultra-structural changes in the mesophyll and bundle sheath cells of maize leaves in response to water stress [72]. He found that mesophyll cells are more sensitive to water stress than bundle sheath cells. It has been seen that; rapid leaf dehydration causes profound changes in the photochemical activity of thylakoid membranes in detached tobacco leaves. It consequently shows that, in most plant species, water deficit has a profound influence on the photosynthetic apparatus and more specifically on the photosynthetic pigment system. In most cases, the chloroplast membrane is disrupted and degradation of chlorophyll takes place.

Table 1: Each value is the mean and SD of three replicates

Treatment		**Chlorophyll a**	**Chlorophyll b**	**Total Chlorophyll**
Salt Stress	Control	155.55±1.00	113.85±2.00	273.73±3.06
	8mM	136.07±2.00	86.79±1.53	217.75±1.53
	30mM	127.91±1.53	101.40±2.21	233.97±3.06
	80mM	129.24±3.00	54.69±2.52	113.51±3.00
	150mM	152.53±2.08	97.28±2.58	247.14±3.12
Pre-sowing salt treated salt stress.	Control	73.21±1.53	51.51±1.53	103.71±2.12
	8mM	193.87±2.52	143.38±2.65	334.26±3.16
	30mM	88.10±1.73	66.20±1.75	154.97±1.15
	80mM	57.46±1.15	32.65±2.31	88.44±0.58
	150mM	13.99±0.58	17.80±1.15	33.13±1.73
Water stress	Control	131.74±2.15	85.68±1.15	237.08±1.70
	2 Days	127.72±1.15	82.93±1.73	211.64±2.39
	4 Days	49.88±1.18	41.57±2.31	92.12±2.23
	6 Days	57.52±1.20	47.20±1.79	104.38±2.29
	8 Days	56.64±2.31	49.21±2.89	104.17±2.22

Table 2: Two-way ANOVA showing variation Chlorophyll a, Chlorophyll b content Dapoli crop Dapoli-3 variety of *Eleusine coracana* under different levels of salt stress, water stress, and pre-sowing salt stress.

	Source	df	F	P value
Chlorophyll a,	Stress Treatment	2	5125.567	.000
	Concentration	4	3204.208	.000

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	Stress Treatment * Concentration	8	1581.287	.000
Chlorophyll a,	Stress Treatment	2	1001.175	.000
	Concentration	4	1190.469	.000
	Stress Treatment * Concentration	8	598.233	.000
Total Chlorophyll	Stress Treatment	2	4641.015	.000
	Concentration	4	6122.841	.000
	Stress Treatment * Concentration	8	2955.302	.000

Inference: Chlorophyll a, Chlorophyll b content Dapoli crop under different levels of salt and water stress is significantly affected by different stress levels. There was a significant interaction effect noticed among different levels of Treatment stress (table 1 and table 2).

Fig-1Effect of Increasing Salt Stress on Chlorophyll in Dapoli-3 variety of *Eleusine coracana*

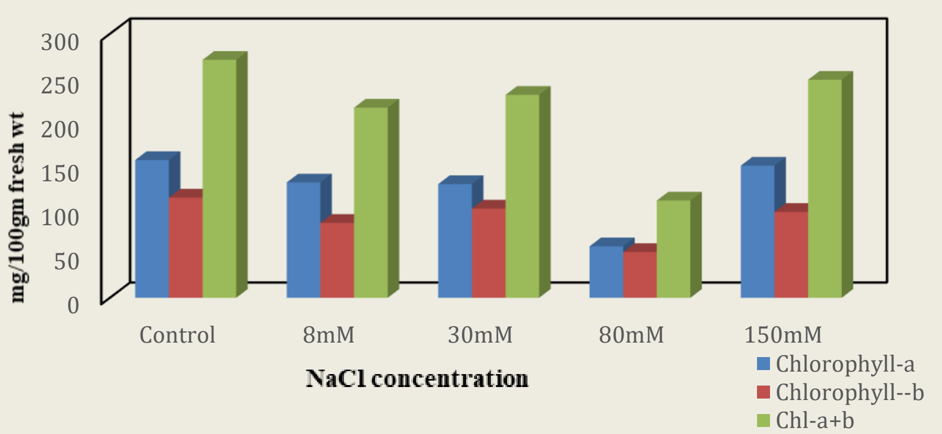

Fig-2 Effect of presowing Salt Stress on Chlorophyll in Dapoli-3 variety of *Eleusine coracana*

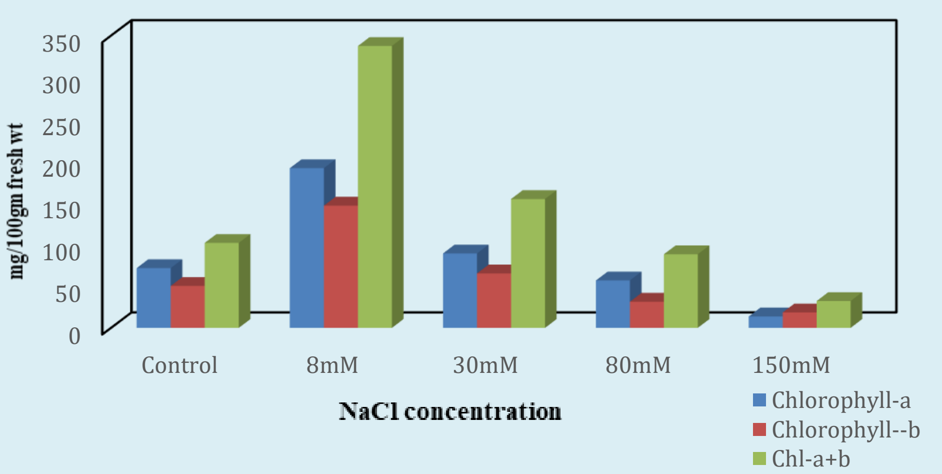

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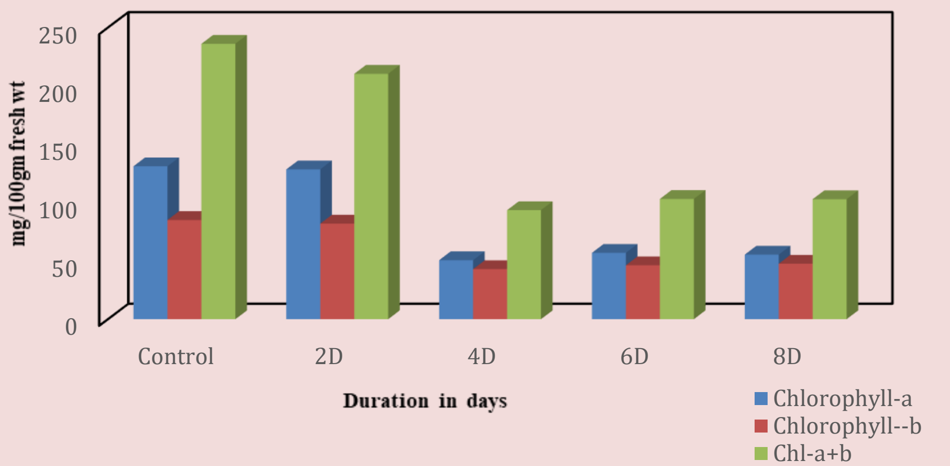
Fig-3 Effect of Increasing water Stress on Chlorophyll in Dapoli-3 variety of *Eleusine coracana*



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