



A COMPARATIVE ANALYSIS OF MORPHOLOGY AND PIGMENT CONTENTS OF A CEREAL CROP FINGER MILLET (*Eleusine coracana* L.) AND A LEGUMINOUS CROP PIGEON PEA (*Cajanus cajan* L.) WITH SALINITY (NaCl) STRESS

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ABSTRACT: The present paper aims at studying the comparative analysis the effects of NaCl on the morphology and different pigment contents of widely cultivated crop Finger millet (*Eleusine coracana* L.) and Pigeon pea (*Cajanus cajan* L.) under laboratory conditions. The effects of salinity appear to be dependent on the species and on the stage of the plant's development (such as germination or vegetative growth). A soil and sand bed in different bowls with sowing 30 number of seeds of *E.coracana* and 10 number of seeds of *C.cajan* separately at uniform distance in all the sets with test chemicals is prepared. We have selected 0mM(control),20mM,40mM,60mM for comparative analysis of both the genus. The emergence of radicle and plumule is considered as the index of germination. The seedlings are then exposed to light after germination. We also observed leaf emergence of seeds which are sown in different concentrations of NaCl, then we measured the length of shoot and root , Fresh weight of root and shoot and dry weight of root and shoot (by keeping in Oven for 15 minutes) of both the genus was taken. On 10th day, we performed pigment analysis. There is an inverse relationship between the percentage of seed germination and concentration of NaCl i.e. with increase in concentration there is decrease in germination of seeds. The morphological studies also show that, at control the length of the shoot and root is maximum, but when the concentration of the stress increases, the root and shoot length, fresh and dry weight of root and shoot decreases due to more salinity. The pigment content of *E. coracana* L. increases at 20mM but after that it decreases with increase in NaCl concentration. In *C. cajan* L. pigment content decreases with increase in NaCl concentration. The effect of NaCl (salinity) stress is found to be more in *Cajanus cajan* L. than in *Eleusine coracana* L.

KEYWORDS: Morphology, Pigment content,NaCl, *Eleusine coracana* L.,*Cajanus cajan* L.Poaceae Fabaceae.Germination,Salinity,Chlorophyll

INTRODUCTION

Finger millet (*Eleusine coracana* L.) is an annual herbaceous plant belonging to the family Poaceae, widely grown as a cereal crop in the arid and semiarid areas in Asia and Africa, also grown in worldwide. It is an important minor millet grown in India and use as a staple crop in many hill regions of the country which is highly rich in nutritive contents as it contains 9.2% of protein, 1.29% fat, 76.32% carbohydrates, 2.24% minerals and 0.33% calcium. Vitamin-A and Vitamin-B and phosphorous is also present in smaller quantities. In India it is cultivated over an area of 2.65 million hectars with total production of about 2.9 million tonnes. Pigeon pea (*Cajanus cajan* L.) is a perennial tropical legume grown mainly in India which belongs to the family Fabaceae. It uniquely combines optimal nutritional profiles and high tolerance to environmental stresses, high biomass productivity. It is the 2nd important pulse in India. Salinity is one of the most important abiotic stresses which greatly affects plants growth, survival, development and productivity through declining photosynthesis where poor-quality water is often the only available source for irrigation (Pascale et al. 2005). The negative effect of salinity is common, can be endorsed to the decline in osmotic potential of the medium and difficulty in the uptake of minerals substance of the plant / direct toxic effects on plant growth and metabolism. Imbalance of osmotic potential results in loss of turgidity, cell dehydration and ultimately, the death of cells (Ashraf 2004). Salinity decreased agricultural production up to 35% (Koyro et al.,2013), creating shortage of food for human and animal consumption and this situation is being aggravated with the passage of time. The aim of the current study is to determine the effect of NaCl stress on growth, morphology, fresh weight, dry weight and on pigment content such as chlorophyll, pheophytin and carotenoid in both finger millet and pigeon pea. Studying the mechanisms and effects involved in saline tolerance might aid in better understanding of plant growth and development under salt stress.

MATERIALS AND METHODS

Eleusine coracana L. is an important minor millet extensively grown in Odisha. The seeds of wild variety with uniform size, colour and weight were collected from OUAT extension centre, Ratanpur, Berhampur for experimental purpose.

<u>Scientific Classification:</u>	<u>Scientific Classification:</u>
Kingdom: Plantae	Kingdom: Plantae
Order: Poales	Order: Fabales
Family: Poaceae	Family: Fabaceae
Genus: <i>Eleusine</i>	Genus: <i>Cajanus</i>
Species: <i>coracana</i>	Species: <i>cajan</i>
Binomial Name: <i>Eleusine coracana</i> L.	Binomial Name: <i>Cajanus cajan</i> L.

Cajanus cajan L. is a common pulse crop which is widely cultivated by the local farmers in Odisha. The seeds of pigeon pea (*Cajanus cajan* L.) of variety Manak, are procured from the Pulse Research Institute (CPRI) Ratanpur, Berhampur. The seeds with uniform size, colour and weight are taken for experiment.

Chemical Test: First stock solution is prepared by dissolving 58.442g of test chemical in 1L distilled water. Different concentration of stress (NaCl) are prepared by using distilled water as the solvent from the above stock solution. For *Cajanus cajan* 5mM, 10mM, 20mM, 40mM, 60mM and control & for *Eleusine coracana* 20mM, 40mM, 60mM, 80mM, 100mM, 200mM, 400mM and control are prepared by proportional dilution with distilled water.

Germination Studies: The seeds of *Eleusine coracana* and *Cajanus cajan* show 90% germination in 2-3 days. For germination studies, plastic bowls are used for the study by making holes at the bottom. Then surface sterilized 200 gm of soil is added to 3/4th volume of bowls. 30 number of seeds of *Eleusine coracana* and 10

number of seeds of *Cajanus cajan* are sown separately in each bowl at uniform distance in all the sets. Respective concentrations of the test chemical 5mM, 10mM, 20mM, 40mM, 60mM, 80mM, 100mM, 200mM,400mM and control is poured in the respective bowls after sowing seed into the soil. For further study we have selected concentrations (0mM(control), 20mM,40mM,60mM) of both the genera *Eleusine coracana* & *Cajanus cajan* for comparative analysis. The bowls are incubated in the dark at room temperature and kept under the light bulb. The emergence of radicle / plumule was considered as an index of germination. Better sprouted and healthy seedlings of 10 days old are used as experimental material.

Morphological Studies: The growth of seedlings of *Cajanus cajan* & *Eleusine coracana* is evaluated by measuring the shoot and root length of seedling on the 10th day. 15cm, scale is used for the experiment of the shoot and root length. Seedlings of each replicate are taken, shoots are separated from roots and are washed thoroughly, surface dried by means of blotting paper and then fresh weight of roots and shoots are taken separately by a single pan in electronic balance. The weighed materials are kept in an oven for 15mins at a temperature of 150⁰C and their dry weights are recorded.

Estimation of Pigments: The fresh samples of shoot materials of the 10 days old seedlings of *Cajanus cajan* & *Eleusine coracana* are collected. Care is taken for separation of each control and treated species. A known quantity of about 100 mg of samples of weighed shoot material is taken in mortar and pestle and macerated to a paste by adding 80% acetone, stirred thoroughly and again centrifuged (10min). The pellet is discarded and the supernatant was kept for chlorophyll estimation. The absorbance of each extract is determined in a spectrophotometer at a wave length of 645nm and 663nm. The total chlorophyll, chl-a and chl-b content is measured by recording the absorbance of the extract at 645nm and 663nm wave length and the values are calculated by using the formula given by Arnon (1949).

$$\text{Chl a} = (12.7 \times \text{OD at } 663\text{nm}) - (2.63 \times \text{OD at } 645\text{nm})$$

$$\text{Chl b} = (22.9 \times \text{OD at } 645\text{nm}) - (4.68 \times \text{OD at } 663\text{nm})$$

$$\text{Total chlorophyll} = (20.2 \times \text{OD at } 645\text{nm}) + (8.02 \times \text{OD at } 663\text{nm})$$

Similarly, pheophytin content (666nm & 665nm) and carotenoid content (475nm) was measured by recording the absorbance of the extract at the wave lengths and the values were calculated by using the following formulas

$$\text{Pheophytin} = (675 \times \text{OD at } 666\text{nm}) + (26.03 \times \text{OD at } 665\text{nm})$$

$$\text{Carotenoid} = \text{DVK}/2500 \quad [\because \text{D}=\text{OD at } 475\text{nm}]$$

$$\text{V} = \text{Volume}$$

$$\text{K} = 1 \text{ Dilution Factor}]$$

TABULATION

Table-1: Comparative analysis of effect of different concentrations of NaCl (in mM) on the seed germination and leaf emergence of *Eleusine coracana* L. and *Cajanus cajan* L. noted after 48hrs. of sowing.

Concentration of NaCl (in mM.)	<i>Eleusine coracana</i> L.		<i>Cajanus cajan</i> L.	
	% of seed germination	% of leaf emergence	% of seed germination	% of leaf emergence
0(control)	100	100	100	100
20	90	87	70	35
40	80	80	65	20
60	75	74	40	10

Graph-1: A clustered column chart showing comparative analysis of percentage of change in seed germination and leaf emergence of *Eleusine coracana* L. and *Cajanus cajan* L. seeds treated with different concentration of NaCl(in mM).

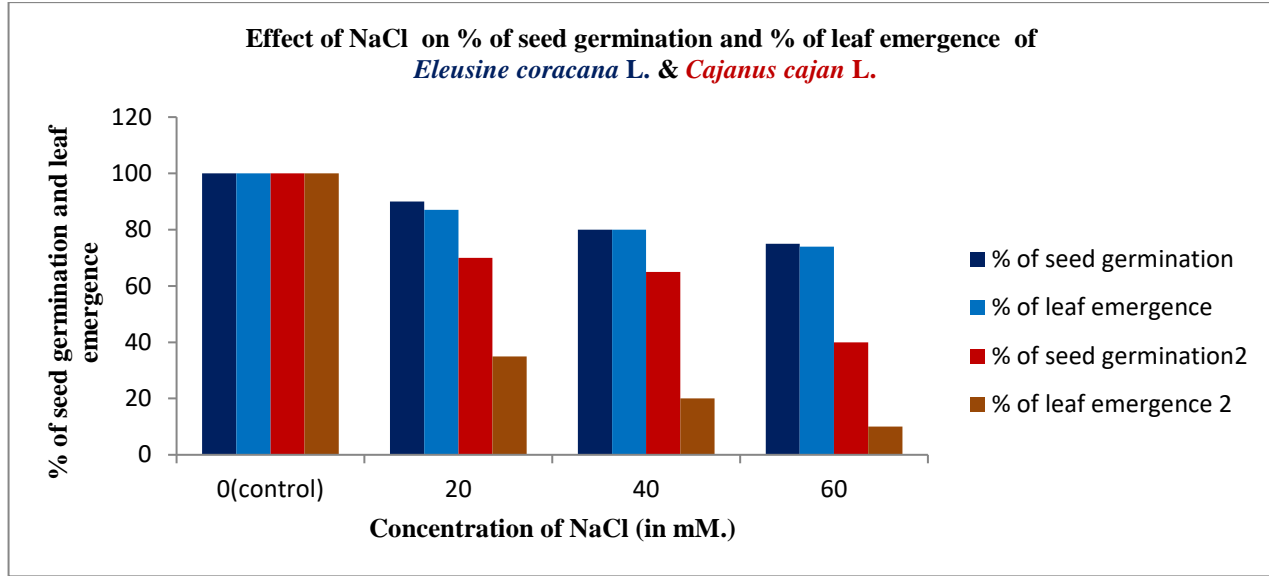


Table-2: Comparative analysis of effect of different concentrations of NaCl (in mM) on root length, shoot length and R/S ratio of *Eleusine coracana* L. and *Cajanus cajan* L. of 10 days old seedlings.

Concentration of NaCl (in mM.)	<i>Eleusine coracana</i> L.			<i>Cajanus cajan</i> L.		
	root length(in cm.)	shoot length (in cm.)	R/S Ratio	root length(in cm.)	shoot length (in cm.)	R/S Ratio
0(control)	3.7	5.6	0.66	9.5	16.5	0.57
20	2.2	5.4	0.4	5.4	11.7	0.46
40	2.6	4.2	0.61	4.5	8	0.56
60	3.1	3.9	0.79	3.3	5	0.66

Graph-2: A clustered column chart showing comparative analysis of effect of different concentrations of NaCl (in mM) on root length, shoot length & R/S ratio of seeds of *Eleusine coracana* L. and *Cajanus cajan* L. of 10 days old seedlings.

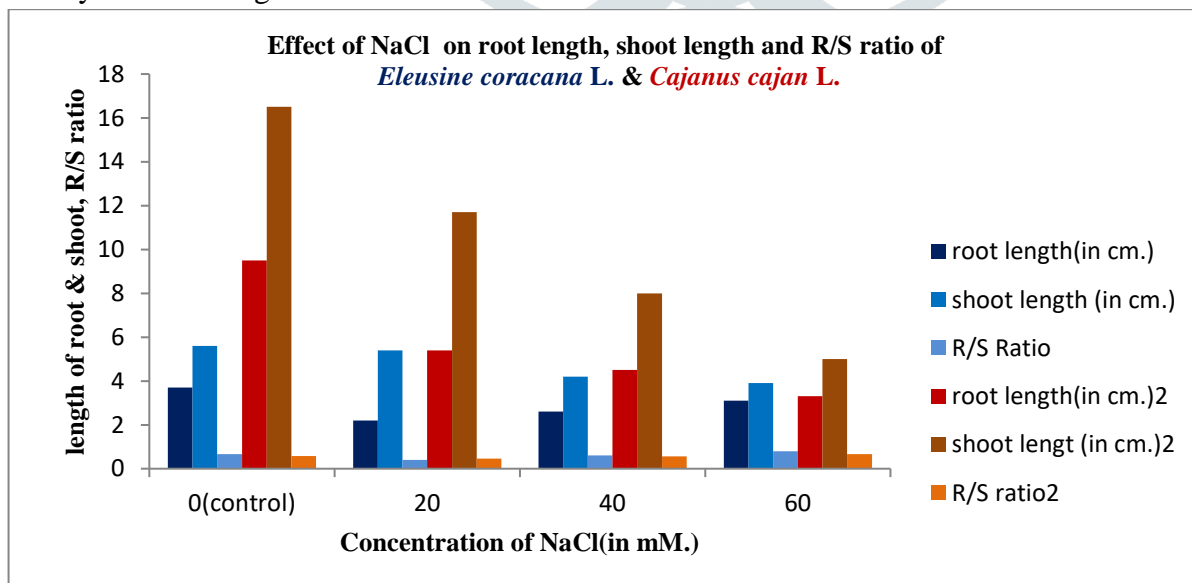


Table-3: Comparative analysis of effect of different concentrations of NaCl (in mM) on the Fresh weight (F.W.) and Dry weight (D.W.) of root and shoot of *Eleusine coracana* L. and *Cajanus cajan* L. seedlings of 10 days old and percentage change with control. Figures in parenthesis show the % change (+/-) from the control value.

Concentration of NaCl (in mM.)	<i>Eleusine coracana</i> L.				<i>Cajanus cajan</i> L.			
	% F.W. root	% F.W.shoot	% D.W. root	% D.W. shoot	% F.W. root	% F.W.shoot	% D.W. root	% D.W. shoot
0(control)	100	100	100	100	100	100	100	100
20	97.27	98.14	93.33	92.18	82.52	78.48	73.33	59.09
40	93.31	96.59	82.7	89.16	75.72	57.37	66.66	53.03
60	84.76	92.29	81	83.78	57.28	45.01	60	19.69

Graph-3: Clustered column chart showing the percentage of change in Fresh Weight (F.W.) & Dry Weight (D.W.) of root and shoot of 10 days old seedling of *Eleusine coracana* L. and *Cajanus cajan* L. treated with different concentrations of NaCl (in mM)

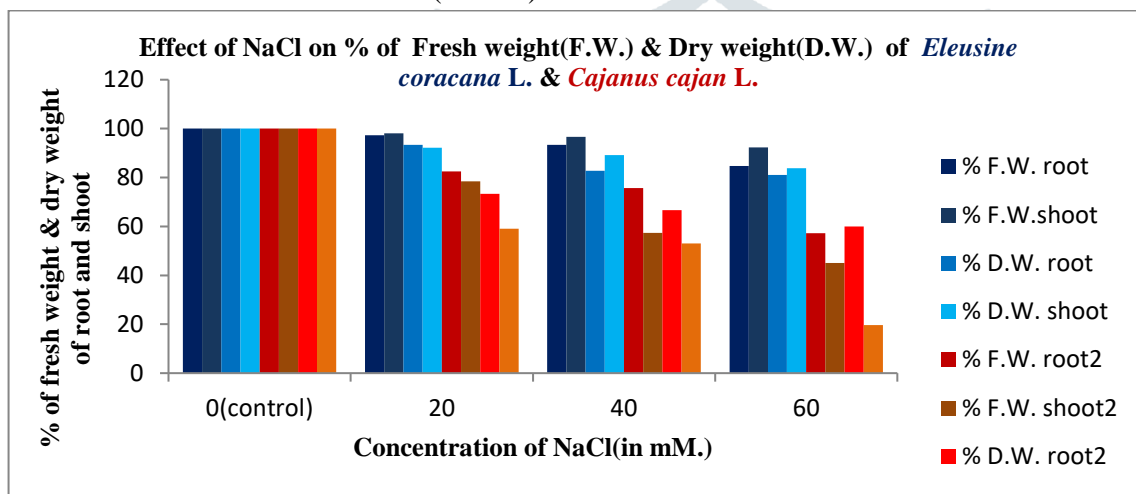
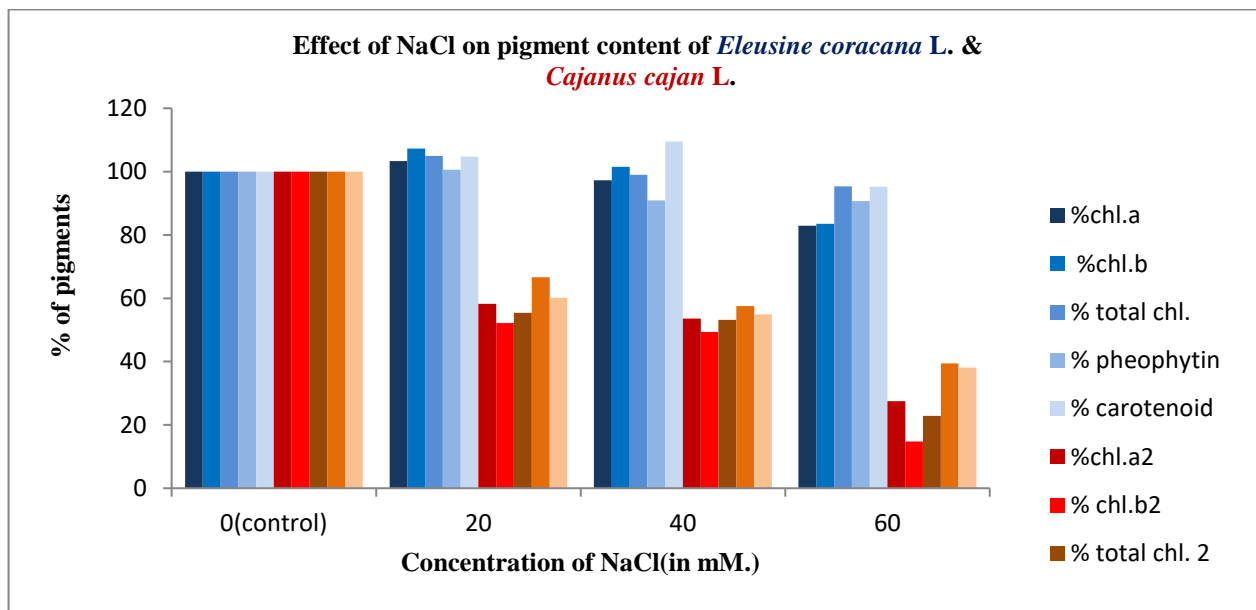


Table-4: Comparative analysis of effect of different concentrations of NaCl (in mM) on different pigments of *Eleusine coracana* L. & *Cajanus cajan* L. seedlings of 10 days old and percentage change with the control. Figures in parenthesis show the % change (+/-) from the control value.

Conc. of NaCl (in mM.)	<i>Eleusine coracana</i> L.					<i>Cajanus cajan</i> L.				
	%chl.a	%chl.b	% total chl.	% pheophytin	% carotenoid	%chl.a	%chl.b	% total chl.	% pheophytin	% carotenoid
0(control)	100	100	100	100	100	100	100	100	100	100
20	103.3	107.31	104.93	100.57	104.76	58.28	52.14	55.42	66.66	60.16
40	97.21	101.48	98.96	90.9	109.52	53.61	49.35	53.16	57.57	54.92
60	82.9	83.46	95.35	90.7	95.23	27.48	14.8	22.87	39.39	38.16

Graph-4: Clustered column chart showing the percentage of change in pigment content (chl.a, chl.b, total chl., pheophytin, carotenoid) of *Eleusine coracana* L. & *Cajanus cajan* L. seeds treated with different concentrations of NaCl (in mM) of 10 days old seedlings.



RESULT

This experiment is studied under the different concentration of NaCl treatment on *Eleusine coracana* L. and *Cajanus cajan* L. under laboratory conditions. The index of germination and pigment analysis are observed in different bowl having 200gm of soil and sand (3:1) on 10th day of sowing seeds. The percentage of germination and leaf emergence in different concentrations of NaCl along with control is shown in Table-1 and Graph-1. Concentration of NaCl shows an inverse relationship with the percentage of seed germination and leaf emergence. In case of *Eleusine coracana* L. seed germination is assumed as 100% in control (no NaCl) which gradually decreases with increase in concentration of chemical. i.e., 20mM is 90% > 40mM is 80% > 60 mM is 75%. The percentage of leaf emergence decreases with increase in concentration of NaCl. i.e., 20mM: 87% > 40mM: 80% > 60mM: 74%. In *Cajanus cajan* L. at control the rate of seed germination is 100%, it gradually decreases with increase in concentration. i.e., 20mM: 70% > 40mM: 65% > 60mM: 40%, The rate of leaf emergence is 100% and sharp decrease in percentage of leaf emergence with increase in concentration of NaCl. i.e., 20mM: 35% > 40mM: 20% > 60mM: 10%. The effect of NaCl stress is found to be more in *Cajanus cajan* L. than in *Eleusine coracana* L. Root and shoot length are measured after 10 days of exposure to the tested chemical (NaCl). Data with respect to root and shoot lengths and R/S ratio are presented in Table-2 & Graph-2. Data clearly shows that there is decline in percentage change in root and shoot length of seedlings. The roots and shoots of *Eleusine coracana* L. are shorter as compared to *Cajanus cajan* L. However, length of root and shoot of both the genus are decreased with increase in NaCl concentration. The ratio of root and shoot length is found to be random. Effect of NaCl stress on the fresh weight and dry weight of *Eleusine coracana* L. and *Cajanus cajan* L. are shown in Table-3 and Graph-3. The fresh weight as well as the dry weight of both shoot and root of both genera have decreased from control to 60 mM of NaCl treated seedlings. In case of *Eleusine coracana* L. fresh weight of root and shoot are 100% > 20mM: 97.27% >> 40mM: 93.31% > 60mM: 84.76%. 100%. 20mM: 98.14%, >40mM: 96.59% > 60mM: 92.29%. Likewise, the dry weight of both shoot and root show a decreasing trend 100% >> 60mM: 81% root and that of shoot is 83.78%. In *Cajanus cajan* L. also, fresh weight of root and shoot are gradually decreased 100% > 20mM: 82.52% > 40mM: 75.72% > 60mM: 57.28%. Similarly, fresh weight of shoot has shown a decreasing trend. 100% > 20mM: 78.48% > 40mM: 57.37% > 60mM: 45.01%. Likewise, the dry weight of both shoot and root has shown a decreasing trend i.e., 100% >>> 60mM. root is 60% and shoot is 19.69%. The effect of NaCl on different pigment contents against the control is presented in Table-4 and Graph-4. The effect of NaCl on different pigment contents against the control is presented in Table-4 and Graph-4. In case of *Eleusine coracana* L. chlorophyll-a content increases at 20mM: 103.30% then decreases at 40mM: 97.21% >

60mM:82.9%. chlorophyll-b content first increases at 20mM:107.31% then decreases at 40mM:101.48% > 60mM :83.46%. Total chlorophyll content increases at 20 mM:104.93% then decreases at 40mM:98.96% > 60mM:95.35%. Pheophytin content increases at 20mM:100.57% then decreases 40mM :90.9% > 60mM :90.7%. Carotenoid content increases at 20Mm:104.76% then decreases at 40mM:109.52% > 60mM:95.23%. In *Cajanus cajan* Chlorophyll-a content decreases 100%> 20mM:58.28% > 40mM:53.61% > 60mM:27.48%. Rate of chlorophyll-b content decreases 100% > 20mM:52.14% > 40mM:49.35% > 60mM:14.8%. total chlorophyll content decreases 100% > 20mM:55.42% > 40mM: 53.16% > 60mM:22.87%, pheophytin content decreases 100% > 20mM: 66.66% > 40mM:57.57% > 60mM:39.39%. carotenoid content decreases 100% > 20mM:60.16% > 40mM:54.92% > 60mM:38.16%. Due to salinity the percentage of pigment content increased at 20mM and then it decreases in *Eleusine coracana* L..The pigment content of *Cajanus cajan* L. shows a gradual decreasing rate across higher concentrations of NaCl. In *Cajanus cajan* L. NaCl stress is found to be more effective.

DISCUSSION

Salinity is one of the most important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation may be insufficient for leaching (Zhao *et al.*, 2007). Soil salinity can be defined as the concentration of dissolved mineral salts in soil solution as a unit of volume or weight basis (Ghassemi *et al.*, 1995). Salt stress has various damaging effects on plant physiological processes such as increased respiration rate, ion toxicity (Sudhir and Murthy, 2004), changes in C and N metabolism, mineral distribution, membrane instability, membrane permeability (Gupta *et al.*, 2002) and decreased efficiency of photosynthesis, reduced leaf area, dry mass and stomatal conductance. Processes such as seed germination, seedling growth and vigour, vegetative growth, are adversely affected by high salt concentration, that ultimately cause poor plant growth (Sairam and Tyagi, 2004). Increased salinity reduces vegetative growth, which eventually causes crop production loss, even in low saline concentrations, agreeing with previous stress related studies in sunflower (Shi and Sheng 2005), common reed (Gorai et al. 2010). Parida and Das (2005) reported that reduction of chlorophyll content in response to salinity stress is a common phenomenon, triggered by disordering chlorophyll synthesis and causing chlorosis in plants. Cell viability is difficult to evaluate at the entire plant level since the various organs contain several cell types and ages revealing different sensitivities to NaCl stress (Lutts et al. 2004).

CONCLUSION

The present study is a comparative analysis of the effect of salinity on morphology and pigment content of *Eleusine coracana* L. and *Cajanus cajan* L. In both cases germination of seed decreases with increase in concentration of the NaCl. The morphological studies also showed that at control the length of the shoot and root is maximum, in pigeon pea, when the concentration of NaCl increases the length decreases due to more salinity. However, in Finger millet root length showed variation & increased from 20mM to 60mM. Dry weight and fresh weight of both root and shoot of both the genera gradually decrease with increase concentration. In Finger millet along the concentration gradient chlorophyll-a, chlorophyll-b and total chlorophyll increases at 20mM and then decreases with increase in concentration. However, carotenoid content increases up to 40mM concentration and then decreases. In Pigeon pea all the pigment content decreases along the concentration gradient. Conclusively, our results show that at higher concentration of NaCl seed germination, morphological parameters such as leaf emergence, root and shoot length and all the pigment content decreases.

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