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EFFECTS OF SODIUM CHLORIDE STRESS ON THE MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS OF *BRASSICA CAMPESTRIS* L. CULTIVARS

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Abstract: In present experiment the effect of salt stress (0, 150, 300, 600 mM NaCl) on three cultivars of *B. campestris* L. like KOS-1, AGRANI and NRCYS-05-02 were studied. The high salt stress significantly decreased both morphological and physiological attributes like shoot length, root length, shoot fresh weight, shoot dry weight, and root fresh weight and root dry weight, leaf number, leaf length and leaf width of all three cultivars. The RWC values and chlorophyll a, b and a+b contents were decreased up to several fold at high salt levels (300 and 600 mM), while proline contents increased at these concentrations. Maximum RWC values in AGRANI and maximum proline contents were recorded in NRCYS-05-02 while chlorophyll a, b, a+b and carotenoid contents were higher in KOS-1 followed by AGRANI. The results demonstrated that KOS-1 exhibits better performance than AGRANI and NRCYS-05-02 in both control and salinity treatment and can be a good option for cultivation in salt affected areas.

Keywords: Ayodhya, *Brassica campestris*, Chlorophyll, NaCl induced salt stress, Proline.

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INTRODUCTION

Rapeseed-mustard is the most economically important agricultural crops, and it is the third major oilseed crops after soyabean and groundnut. Rapeseed- mustard with a production of 72.41 million metric tonnes (MMT) contributed 12.1% to the global oil seed production (597.27 MMT) during 2018-19 (Anonymous, 2020). India ranked third after Canada and China; that sharing around 10.38% of the global rapeseed-mustard production (76.24 million tonnes). The crop was sown in 6.23 million hectare land with production of 9.34 million tonnes of oilseeds during 2018-19 (Anonymous, 2019). In provision of foodstuff, Rapeseed family generally found at level 3rd after cereals and pulses. Seeds of *Brassica campestris* L. are crushed for extraction of oil, meal residue obtained after crushing the seeds is utilized as major animal fodder resources. Rapeseeds are grown for oil and it tolerant to some extent at various levels of salinity. Rapeseed varieties shows differential capability to sustain their metabolic activities under saline conditions, but germination and some stages of growth are more susceptible to different levels of salinity (Schillinger and Paulitz, 2018; Rasel *et al.*, 2020).

Salt stress affects both soil composition and quality (Nakashima *et al.,* 2000). Excess amount of salt (NaCl) in soil retard the normal growth, development and other physiological functions of plant body by excessive accumulation of Na^+ and Cl^- and nutrient deficiency (Narusaka *et al.,* 2003; Ashraf and Harris, 2004; Garthwaite *et al.,* 2005). Calcium also plays a key role (Singh, 2020). Salt stress is the main restrictions to crop harvest in arid and semiarid regions of the world. As it is growing about 10% annually, it is assumed that 50% arable land will be wasted due to salinity up to the mid of 21st century (Moghadam *et al.,* 2020; Akhter *et al.,* 2021a).

At present, agricultural production is disturbed by increasing soil salinity worldwide (Zhang *et al.,* 2001; Bybordi *et al.,* 2010; Moghadam *et al.,* 2020). Over 50% of yield of different crops is decrease in infertile and partially fertile lands exhibiting salt stress (Bray *et al.,* 2000). Salt stress can cause the decrease in crop yield by means of disturbing nutritional and water equilibrium of the crop plants (Belouchrani *et al.,* 2021). Salt tolerance in plants is a very complex event that depends on multiple associated factors including on morphological, physiological and biochemical processes. Na^+ and Cl^- ions toxicity causes high osmotic potential that results in inadequate supply of water and nutrients to plant roots (Bybordi *et al.,* 2010; Aqeel *et al.,* 2021). The present experiment was carried out to see the effects of different grade of salt stress (NaCl) on the morphological and physiological attributes in cultivars of *Brassica campestris* L.

MATERIALS AND METHODS

1. Experimental design

A pot experiment was conducted in experimental area of Department of Botany, K.S. Saket P.G. College Ayodhya, India to see the effect of NaCl stress on three cultivars of rapeseed during 2022-2023. Stone free, clean well mixed loamy soil was fertilized to ensure healthy growth of seeds and seedlings. Seeds of three rapeseed cultivars namely KOS-1 (brown sarson), AGRANI (toria) and NRCYS-05-02 (yellow sarson) were obtained from DRMR Bharatpur, Rajasthan and seeds were first sterilized by distilled water for about 8-10 min and then treated with 10% hypochlorite solution for 11-15 min. After sterilization seeds were dried with the help of filter paper. Pots were arranged as control and different salinity levels. Seeds were sown in total thirty six plastic pots, each pot containing 4.5 Kg of well mixed soil. Pots filled with fertilized soil were watered in alternative days. Seeds of all three cultivars were subjected to four NaCl stress levels (0, 150, 300, 600 mM). The experiment was designed in CRD and run with three replications. The salt stress was applied after 14 days of seed germination and retained in uninterrupted periods till crop maturity.

2. Plant sampling

Two plants from each pot were selected for measurement of morphological characters like Root/ Shoot length, Root/Shoot fresh and dry weights, Number of leaves, leaf length and width and Plant height. The length of root, shoot, leaves (length and width) and plant height was measured in centimeter by using meter rod; Number of leaves was counted manually while shoot/root fresh and dry weights (g) and seeds weight (g) were determined with the help of electrical balance.

3. Plant pigments contents

Chlorophyll analysis was done on the fresh leaves. The estimation of pigments was done by using methods of Arnon (1949).

4. Measurement of relative water content (RWC):

The percent leaf relative water content (RWC %) was measured by using protocol of Barrs and Weatherly (1962).

5. Proline:

The content of proline (mmol g^{-1} FW) was measured by using established methods of Bates *et al.* (1973). The proline is a non-essential amino acid (Verma, 2007).

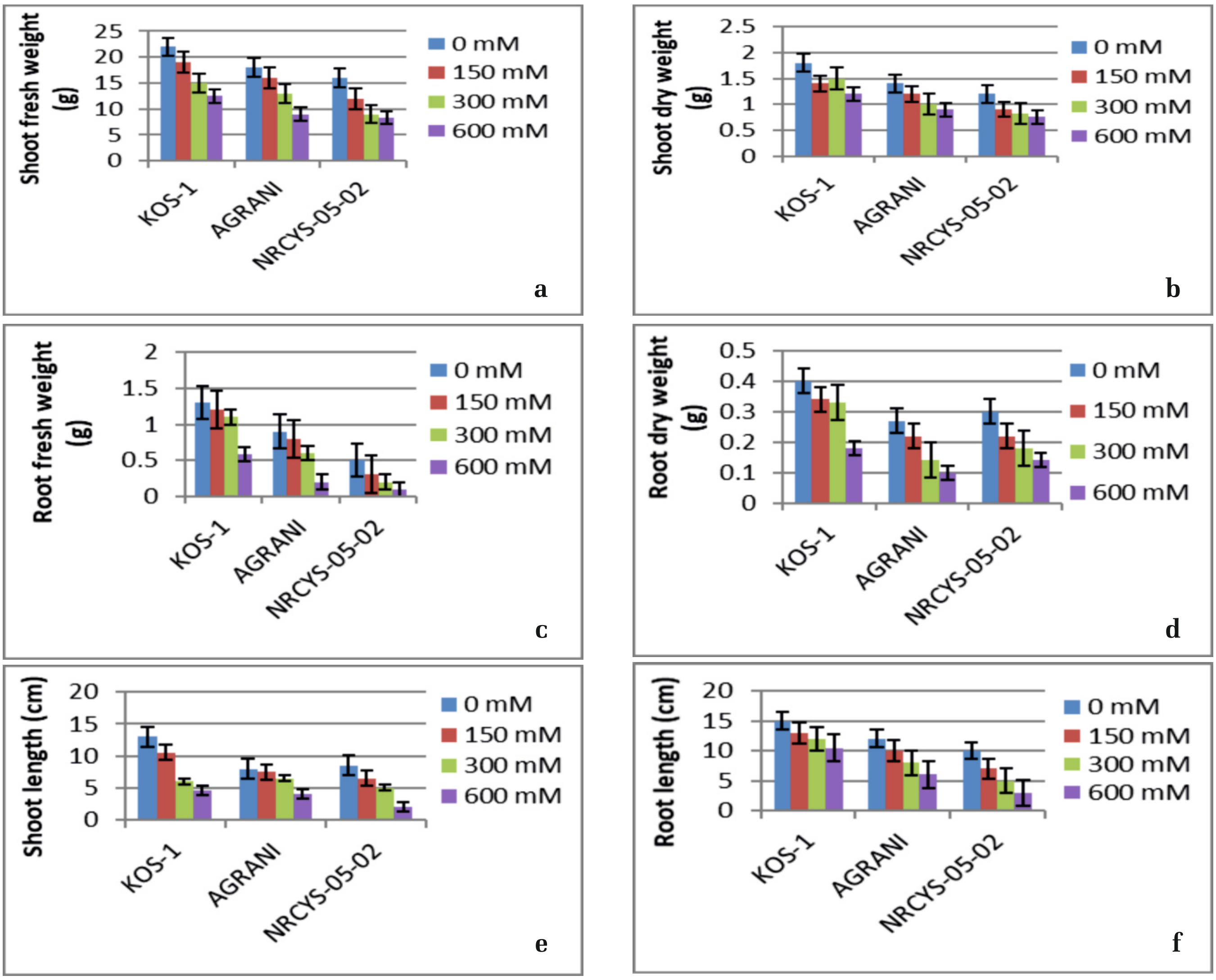
6. Statistical analysis:

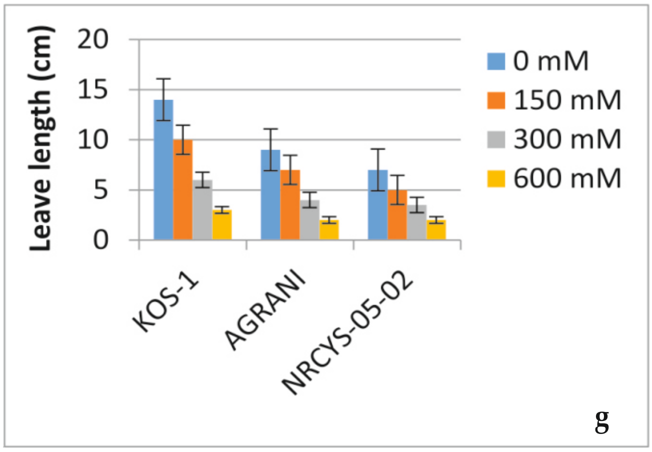
Recorded data of two factors with CRD was analyzed statistically by running Co-stat software with 5% probability level to compare treatment means (Steel *et al.*, 1997).

RESULTS

1. Morphological parameters:

Salt stress mainly NaCl critically affected all the three cultivars of *Brassica campestris* L. i.e., KOS-1, AGRANI, NRCYS-05-02. Salinity reduced the shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, shoot / root length (cm), leave length and width (cm) and number of leaves (Fig. 1: a-i). The maximum reduction of 70% was documented in root length of NRCYS-05-02. The KOS-1 cultivars behave as tolerant with minimum reduction of 30% in root length. Similar behavior was observed for the shoot length where the maximum decrease of 75% was recorded in NRCYS-05-02 while minimum reduction (35%) was observed in KOS-1. For root and shoot fresh weight 55.38% and 43% decrease was observed in KOS-1 while the reduction percentage for the NRCYS-05-02 remained 76% and 48% for RFW and SFW respectively. The RDW and SDW data also proved to be significantly different; the percentage decrease for these parameters was recorded to be 58% and 36% for the susceptible NRCYS-05-02. The decrease in value of RDW and SDW for KOS-1 is 50% and 33% respectively. The data collected for leaf length, leaf width and number of leaves (Fig. 1: i) revealed a significant ($P \leq 0.05$) decrease was observed when the increasing amount of salt stress. The behavior of KOS-1 showed a tolerance to salt stress as compared to other cultivars. All these parameters showed maximum significant ($P \leq 0.05$) reduction at 600 mM salt stress level. The KOS-1 cultivars are least susceptible to salt stress as compared to NRCYS-05-02. KOS-1 cultivars showed highest growth under controlled environment. Maximum decrease in plant height (69.23%) was displayed by NRCYS-05-02, while KOS-1 displayed minimum decline (35%). However, the behavior of cultivars of *Brassica campestris* L. under control environment was better than under NaCl induced stress environment.





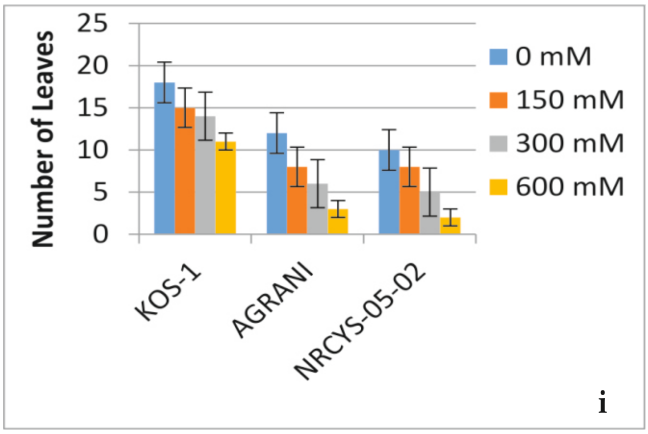
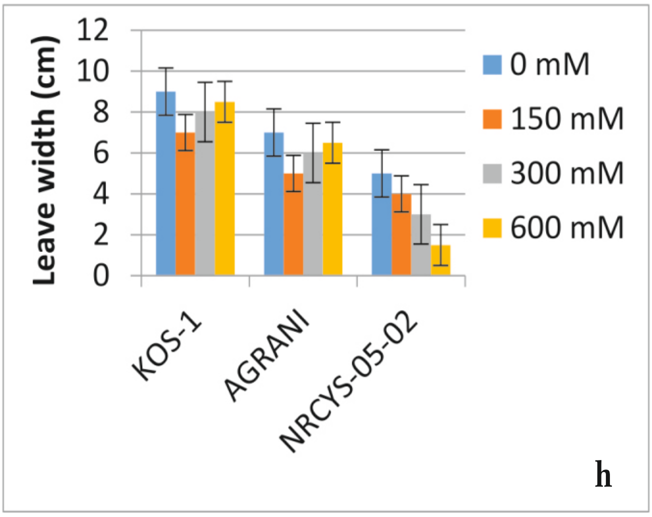


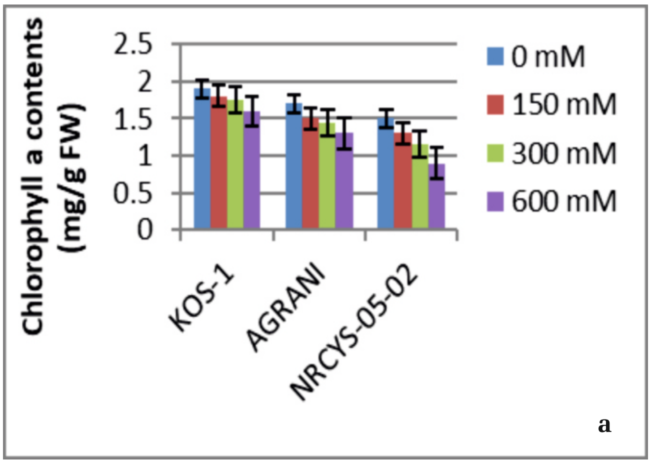
Fig.1: (a) Shoot fresh weight (g), (b) Shoot dry weight (g), (c) Root Fresh weight (g), (d) root dry weight (g), (e) shoot length (cm), (f) root length (cm), (g) leave length (cm) (h) leave width (cm) (i) number of leaves of three *Brassica campestris* L. cultivars treated with 0, 150, 300, 600 mM NaCl stress. Vertical Bars indicate Mean ± SE (n = 2), significant P ≤ 0.05.

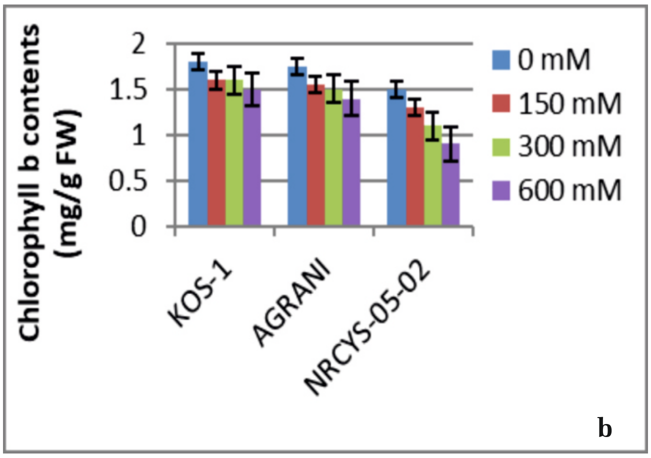
2. Photosynthetic pigments:

The physiological characteristics such as photosynthetic pigments are evaluated, which are essential in respect to increase plant productivity under NaCl salinity in various crop cultivars. Total amount and activity of photosynthetic pigments played very important role in plant growth and productivity. In present trial, treatment of 150 mM, 300 mM and 600mM salt stress noticeably ($P \leq 0.05$) decreased the chlorophyll pigments i.e., chlorophyll 'a', chlorophyll 'b', carotenoids and total chlorophyll contents in all three rapeseed cultivars (Fig. 2: a-d). For this pigments the behavior of all the three cultivars were observed to follow the same pattern as in their morphological traits. The values for chlorophylls were maximum in KOS-1 under controlled conditions. These cultivars also behaved better under salt stress.



The AGRANI showed an intermediate behavior while the response of NRCYS-05-02 (39.37% decrease) observed to be poor when subjected to salt stress. Increasing the salt concentration caused a gradual decrease in chlorophyll a contents. The chlorophyll b and total chlorophyll contents also show similar results. The carotenoids contents were observed to be least in NRCYS-05-02 where as the KOS-1 showed a minimum decline (22.85%) in carotenoids contents under 600 mM salt stress.





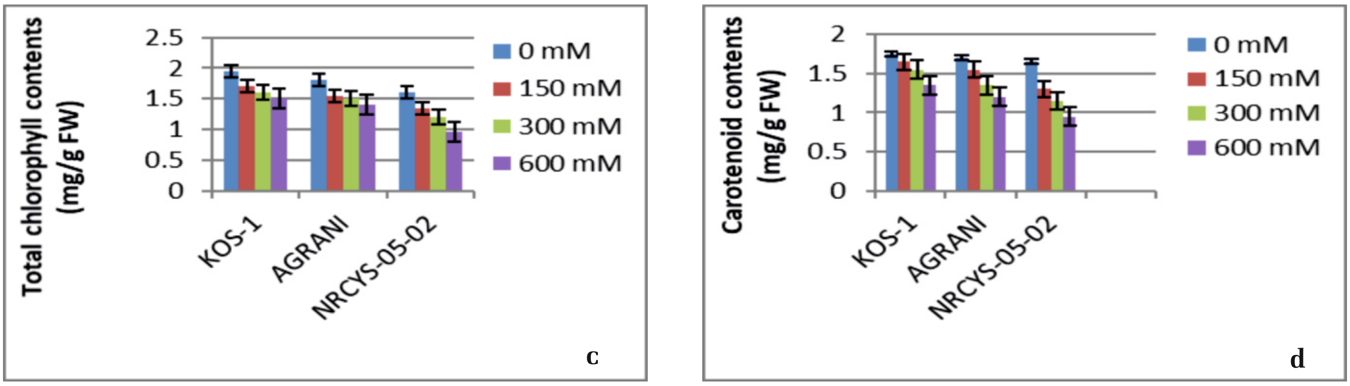


Fig. 2: (a) Chlorophyll a contents (mg/g), (b) Chlorophyll b contents (mg/g), (c) Total chlorophyll contents (mg/g), (d) Carotenoid contents (mg/g) of three *Brassica campestris* L. cultivars treated with 0, 150, 300, 600 mM NaCl stress. Vertical Bars indicate Mean ± SE (n = 2), significant P ≤ 0.05.

3. Leaf relative water content:

The leaf Relative Water Content (RWC) was significantly decreased with the increase of salt concentrations as compared to controlled condition in all tested genotypes. At 150 mM salt level maximum relative water content of 78% was noted from genotype AGRANI followed by 70% in KOS-1. The maximum RWC values of 60% and 45% at 300 and 600 mM salt stress levels were calculated in AGRANI. The Leaf relative water content was lowest in genotype NRCYS-05-02.

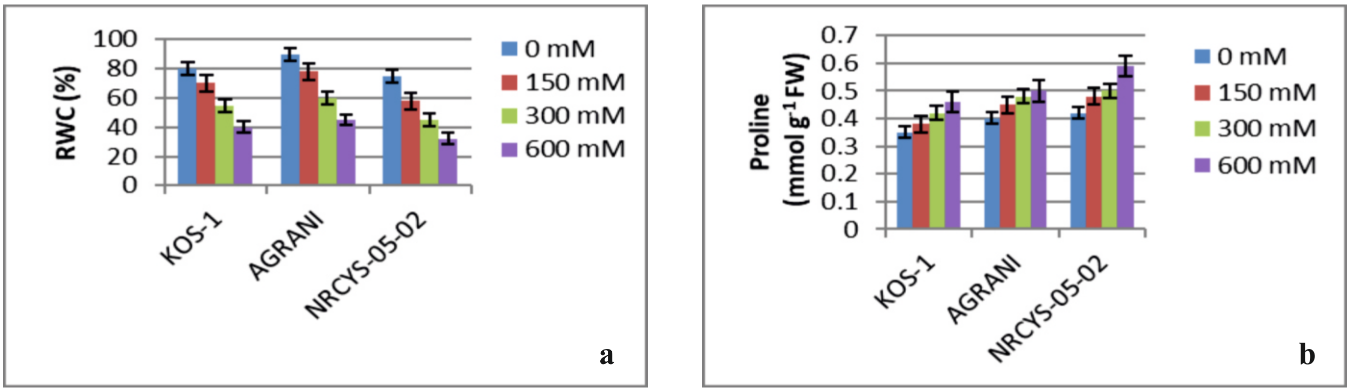


Fig.3: (a) Leaf relative water content (RWC %), (b) Proline contents (mmolg^{-1}FW) of three *Brassica campestris* L. cultivars treated with 0, 150, 300, 600 mM NaCl stress. Vertical Bars indicate Mean ± SE (n = 2), significant P ≤ 0.05.

4. Proline

It was estimated that proline contents and salt stress are directly proportional to each other, as salt stress increases then proline value are also increases, Proline contents were lesser in *Brassica campestris* L. cultivars treated with 150 mM or 300 mM than in 600 mM salt stress (Fig. 3). It is considered as basic reaction of rapeseed plants under NaCl stress to save compartmental injury. The maximum increase (40.47%) in proline contents was observed in NRCYS-05-02 cultivar.

DISCUSSION

The salt stress caused by NaCl critically affected all the three cultivars of *Brassica campestris* L. *i.e.* KOS-1, AGRANI and NRCYS-05-02. Salinity reduced the shoot/root length, shoot/root fresh and dry weights, leaf length and width, number of leaves. All these parameters showed maximum significant (P ≤ 0.05) reduction at 600 mM salt stress level. These results are in accordance with the past research of (Munns and Tester, 2008; Shahbazi *et al.*, 2011; Umar *et al.*, 2011). This reduction takes place due to disruption in metabolism of ion exchange, lower vital nutrients concentration or by the accumulation of toxic substances in plants under stress (Vital *et al.*, 2008). Under high osmotic stress large amount of ROS production occurs that causes the decrease in all growth attributes (Hasanuzzaman *et al.*, 2013; Akhter *et al.*, 2021b). The KOS-1 is less susceptible cultivars to salt stress as compared to NRCYS-05-02. KOS-1 cultivars showed highest growth under controlled environment.

The plant photosynthetic pigments are evaluated that are essential in respect to increase plant productivity under NaCl salinity in various crop cultivars (Hasanuzzaman *et al.,* 2013; Akhter *et al.,* 2021b). During observation it was found and hence proved in many studies that the reduction in photosynthetic attributes consequently decreased the morphological parameters, overall growth, hence yield of many crops are also decreases. Many metabolic disorders developed in *Brassica napus* L. seedlings when exposed to relative salt (Kumar, 1995). Application of NaCl stress at early stages of *Brassica napus* L. cultivars have more negative impact on some biochemical attributes, caused chlorophyll contents to decline (Su *et al.,* 2013).

Total quantity and activity of photosynthetic pigments played a vital role in plant growth and development. In present study, treatment of 150 mM, 300 mM, 600 mM salt stress noticeably ($P \leq 0.05$) decreased the chlorophyll pigments *i.e.,* chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents in all rapeseed cultivars. Formation of reactive oxygen species (ROS) under osmotic stress (Singh, 2019) caused by salt stress (NaCl) start the breakdown of chloroplast that ultimately decreases its total amount as studied in maize (Ahmad *et al.,* 2021) and tomato (Ahanger *et al.,* 2019). Increase in Na^+ & Cl^- ions in leaves also caused reduction of Chl-b, Chl-a and total chlorophyll contents, because in the presence of these ions different enzymes function gets disturbed that are participating in chlorophyll formation (Ahanger *et al.,* 2019; Ahmad *et al.,* 2021). The proline is an osmolyte that contains antioxidant properties also involves in signal transformation metabolism (Dar *et al.,* 2021).

In this research high proline value was observed in NaCl stressed plants than the control ones. It was found that proline contents and salt stress are directly proportional to each other, as salt stress increases then proline value are also increases; these results are similar with previous studies (Nounjan and Theerakulpisut, 2012). Hence, found that most compatible osmoprotectant is proline under NaCl affected cultivars. Proline contents were lesser in *Brassica campestris* L. cultivars treated with 150 mM and 300 mM than in 600 mM salt stress.

CONCLUSION

The salt stress induced by NaCl adversely affected all three cultivars of rapeseed (*Brassica campestris* L.). Salinity decreased all morphological and physiological parameters. However, among the three cultivars, KOS-1 proved to be less affected by NaCl salinity stress, these cultivars showed lesser reduction in all parameters, whereas NRCYS-05-02 were more affected to salinity because these displayed greater reduction in all growth and physiological attributes. However, proline contents tend to increase under salt stress in all three cultivars. 150 mM salt stress concentrations were proved to be less harmful whereas 300 mM and 600 mM NaCl concentrations were very harmful for all three cultivars. All three cultivars showed adverse symptoms and growth under 600 mM NaCl salt stress. KOS-1 rapeseed performed better under control conditions as well as under salinity environment among all three cultivars of *Brassica campestris* L.

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