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**Gaytri Soni**
Department of Agricultural Chemistry and Soil Science, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

**Dr. SG Savalia**
Department of Agricultural Chemistry and Soil Science, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

**Corresponding Author:**
**Gaytri Soni**
Department of Agricultural Chemistry and Soil Science, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

# Effect of simulated soil salinity conditions and varieties of pigeon pea (*Cajanus cajan* L.) on nutrient content, salt compositions and their ratios

**Gaytri Soni and Dr. SG Savalia**

**Abstract**
In this study, we investigated the effects of simulated saline soil on the accumulation of Na, K and Ca at 45 DAS and harvesting in shoot part is investigated in this study. The experiment was conducted at net house and the experiment constitutes five salinity levels (Control, 40, 60, 80 and 100 meq $l^{-1}$) and four pigeon pea varieties ($V_1$: GJP-1, $V_2$: Vaishali, $V_3$: BDN-2, $V_4$: AGT-2) in CRD (Factorial) replicated three times. The sodium concentration in leaves of pigeon pea observed lowest and highest potassium and calcium at 45 DAS with variety $V_4$ (AGT-2). The lowest $Na^+/K^+$ ratio and highest $Ca^{++}/Na^+$ ratio were observed significant with variety $V_4$ (AGT-2) in leaves at 45 DAS and at harvest. Primary, secondary and macro nutrients were not affected by interaction of salinity levels and varieties.

**Keywords:** Pigeon pea, salinity levels, Na, K and Ca composition, $Na^+/K^+$and $Ca^{++}/Na^+$ ratio

## Introduction
Salinity is widespread around the world and is usually a cause for concern because it affects plants negatively. Saline soils are common on Earth. Their formation can be due to natural or accelerated processes. Despite chloride being important as a micronutrient for all higher plants and sodium being important as a mineral nutrient for many halophytes and some C4 species, salt accumulation turns agricultural land into a hostile environment, reduce local biodiversity, restrict plant growth and reproduction, and are toxic in non-salt-tolerant plants, known as glycophytes (Marschner, 1995) [22]. Soil salinity is shown to increase P, Mn and Zn and drop in K and Fe (Turan *et al*., 2007a) [26] concentrations in plants. Use of saline irrigation water and fertilizers are the main factors responsible for adding salinity in soil (Epstein *et al.*, 1980) [9]. Meanwhile, climate change is causing problems for agricultural production. Increased salinity hazards are associated with climate change impacts, especially in areas below sea level. Even in inland areas, including arid and semi-arid areas, salinity stress increasingly threatens crop production as salt builds up as a result of over-irrigation with poor quality water without proper drainage.

## Materials and Methods
The experiment soil was silty clayey in texture and alkaline in reaction with pH 8.08, EC 0.30 dS $m^{-1}$, $CaCO_3$ 33.00% and CEC 35.20 cmol ($p^+$) $kg^{-1}$. This trial consists of 20 treatments combinations, including five levels of salinity and four levels of varieties under the Factorial CRD design. The soil was low in available nitrogen (180.10 kg $ha^{-1}$) and medium in phosphorus (48.00 kg $ha^{-1}$), high in available potassium (407.00 kg $ha^{-1}$), and low in available sulphur (21.38 mg $kg^{-1}$). Dried samples were utilized for determination of calcium by Versenate EDTA method (Cheng and Bray, 1951) [6] and potassium & sodium by Flame photometer (Jackson, 1974) [15]. $Na^+/K^+$ and $Ca^{++}/Na^+$ ratios were worked out by using values of content of potassium, sodium and calcium.

Chemical analysis of seed and straw were performed by taking representative samples from each pot at harvest. The samples were oven-dried at 60 °C for 24 hours and then ground with a pestle and mortar. Finally, the powdered samples were used for estimation of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, sodium, iron, zinc, manganese and copper. The nitrogen from plant samples were estimated separately by micro Kjeldahl's method as described by Kanwar and Chopra (1967) [17]. The phosphorus determined by Vanadomolybdo Phosphoric Yellow Colour method as described by Jackson (1974) [15]. The potassium and sodium by Flame Photometer as described by Jackson (1974) [15].

Calcium and Magnesium content were determined by the method developed by Versenate EDTA method. Sulphur content was determined by the method developed by Williams and Steinberg's (1959) [31]. Sodium was determined by flame photometer method developed by Jackson (1974) [15]. Micronutrients *viz*., Fe, Zn, Mn and Cu were estimated by Atomic Absorption Spectrophotometer (AAS) as described by Jackson (1967) [14].

**Results**

**1. Effect of salinity and varieties on salt (Na, K and Ca) composition at 45 DAS**

Sodium content increased significantly with increasing levels of salt concentration. Significantly lower value of Na content was registered with salt concentration level S1 (Control). K and Ca content in leaves of pigeon pea decreased with increasing level of salt stress and significantly the highest values were noted with salt concentration level $S_1$.

The concentration of Na in leaves of pigeon pea at 45 DAS was significantly affected by tested varieties and the lowest content of Na recorded with variety $V_4$ (AGT-2) and the highest content was found in $V_1$ (GJP-1). Significantly the highest K and Ca content observed with variety $V_4$ (AGT-2).

Interaction effect of salinity and variety was obtained significant in K and Ca at 45 DAS. The maximum potassium content (1.88%) was perceived with treatment combination of $S_1$ (Control) x $V_4$ (AGT-2). The maximum calcium content (1.65%) was perceived with treatment combination of $S_1$ (Control) x $V_4$ (AGT-2).

**2. Effect of salinity and varieties on nutrient content at harvest (in seed and straw)**

The concentration of phosphorus, potassium and sulphur content in seed and straw of the plant did not significantly influence by different salinity levels at harvest of crop. While nitrogen content in seed and straw significantly affected by different salinity levels and the highest nitrogen content was found in $S_1$ (Control) level. At harvest of crop, the Mg content in seed and straw was significantly affected by different levels of salinity. Whereas Ca content was significantly affected by salinity levels in seed only. At harvest of crop, the concentration of Na in seed and straw of the plant were significantly influenced by different levels of saline irrigation water. The Na concentration was significantly higher found with salinity level $S_5$ (100 meq $l^{-1}$) in seed (0.101) and straw (0.099), respectively. The concentration of Mn and Zn in seed and straw of the plant did not significantly affected by different levels of saline irrigation water at harvest of crop. Whereas, Fe (seed) and Cu (seed and straw) were significantly affected by different salinity levels.

The concentration of N, P, K, S Ca, Mn and in seed and straw of the plant were not significantly influenced by different varieties of pigeon pea. Whereas, Mg and Na contenet were significantly affected in seed and straw of various varieties at harvest of crop. The Na content was observed the lowest with variety AGT-2 ($V_4$) in seed at harvest of pigeon pea crop. Fe and Cu in straw and seed respectively were not found significant. While Fe in seed and Cu in straw, were significantly influenced by different varieties of pigeon pea at harvest of crop.

**Table 1:** Effect of salinity levels and varieties on concentration (%) of Na, K and Ca content in leaves of pigeon pea at 45 DAS

| Treatments | Na | K | Ca |
|---|---|---|---|
| **Salt concentration(Salinity) (S)** | | | |
| $S_1$: Control | 0.058 | 1.68 | 1.39 |
| $S_2$: 40 meq $l^{-1}$ | 0.061 | 1.53 | 1.25 |
| $S_3$: 60 meq $l^{-1}$ | 0.064 | 1.49 | 1.23 |
| $S_4$: 80 meq $l^{-1}$ | 0.067 | 1.46 | 1.21 |
| $S_5$: 100 meq $l^{-1}$ | 0.073 | 1.39 | 1.14 |
| S.Em. ± | 0.001 | 0.02 | 0.02 |
| C.D. (P=0.05) | 0.004 | 0.06 | 0.05 |
| **Variety (V)** | | | |
| $V_1$:GJP-1 | 0.071 | 1.48 | 1.18 |
| $V_2$: Vaishali | 0.064 | 1.49 | 1.22 |
| $V_3$:BDN-2 | 0.066 | 1.50 | 1.27 |
| $V_4$: AGT-2 | 0.057 | 1.57 | 1.29 |
| S.Em. ± | 0.001 | 0.02 | 0.01 |
| C.D. (P=0.05) | 0.003 | 0.05 | 0.04 |
| **S x V Interaction** | | | |
| S.Em. ± | 0.003 | 0.04 | 0.03 |
| C.D. (P=0.05) | NS | 0.12 | 0.09 |
| C.V.% | 7.26 | 4.90 | 4.49 |

**Table 2:** Interaction effect of salinity levels and varieties on concentration (%) of K content in leaves of pigeon pea at 45 DAS

| | **$S_1$: Control** | **$S_2$: 40 meq $l^{-1}$** | **$S_3$: 60 meq $l^{-1}$** | **$S_4$: 80meq $l^{-1}$** | **$S_5$: 100 meq $l^{-1}$** | **Mean** |
|---|---|---|---|---|---|---|
| $V_1$:GJP-1 | 1.66 | 1.49 | 1.47 | 1.44 | 1.32 | 1.48 |
| $V_2$: Vaishali | 1.55 | 1.53 | 1.49 | 1.47 | 1.42 | 1.49 |
| $V_3$:BDN-2 | 1.62 | 1.50 | 1.48 | 1.45 | 1.42 | 1.50 |
| $V_4$: AGT-2 | 1.88 | 1.59 | 1.51 | 1.47 | 1.39 | 1.57 |
| Mean | 1.68 | 1.53 | 1.49 | 1.46 | 1.39 | |
| S.Em. ± | 0.04 | | C.D. (P=0.05) | | 0.12 | |

**Table 3:** Interaction effect of salinity levels and varieties on concentration (%) of Ca content in leaves of pigeon pea at 45 DAS

| | **$S_1$: Control** | **$S_2$: 40 meq $l^{-1}$** | **$S_3$: 60 meq $l^{-1}$** | **$S_4$: 80 meq $l^{-1}$** | **$S_5$: 100 meq $l^{-1}$** | **Mean** |
|---|---|---|---|---|---|---|
| $V_1$:GJP-1 | 1.30 | 1.21 | 1.19 | 1.17 | 1.05 | 1.18 |
| $V_2$: Vaishali | 1.30 | 1.25 | 1.22 | 1.19 | 1.15 | 1.22 |
| $V_3$:BDN-2 | 1.31 | 1.29 | 1.28 | 1.25 | 1.24 | 1.27 |
| $V_4$: AGT-2 | 1.65 | 1.25 | 1.23 | 1.22 | 1.12 | 1.29 |
| Mean | 1.39 | 1.25 | 1.23 | 1.21 | 1.14 | |
| S.Em. ± | 0.03 | | C.D. (P=0.05) | | 0.092 | |

The interaction effect of salinity levels and varieties for all these nutrients were found non-significant.

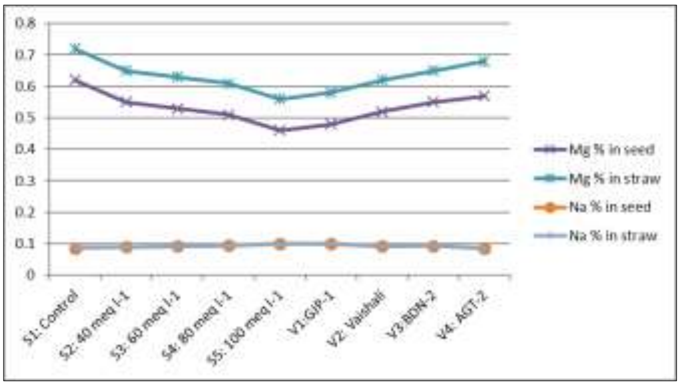


**Fig 1:** Effect of salinity levels and varieties on Mg and Na (%) content in seed and straw pigeon pea at harvest

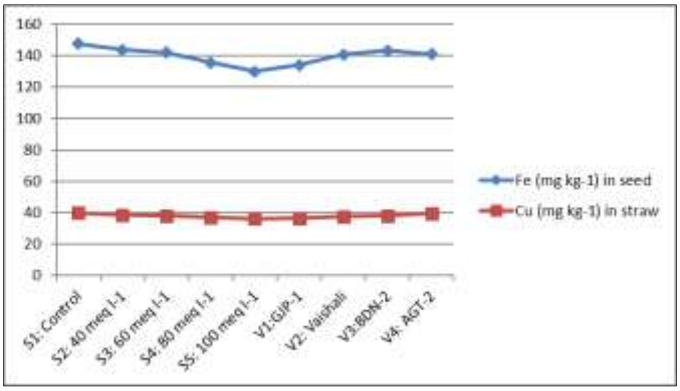


**Fig 2:** Effect of salinity levels and varieties on micronutrient (mg kg$^{-1}$) content in seed and straw pigeon pea at harvest

## 3. $Na^+/K^+$ and $Ca^{++}/Na^+$ Ratio

### 3.1 $Na^+/K^+$ and $Ca^{++}/Na^+$ ratio in leaves at 45 DAS

Various salinity levels produced significant result on $Na^+/K^+$ ratio and $Ca^{++}/Na^+$ ratio. The lowest value of $Na^+/K^+$ ratio (0.033) recorded under $S_1$ (Control) in leaves of pigeon pea at 45 DAS. While $Ca^{++}/Na^+$ ratio found significant by different varieties and salinity levels. The Highest $Ca^{++}/Na^+$ ratio (24.27) observed at $S_1$ (Control) level of irrigation in pigeon pea leaves at 45 DAS.

Different varieties of pigeon pea tested in experiment are produce significant effect on both $Na^+/K^+$ and $Ca^{++}/Na^+$ ratio at 45 DAS. Significantly the lowest value of $Na^+/K^+$ ratio (0.037) and the highest value of $Ca^{++}/Na^+$ ratio (23.27) were recorded with the same variety $V_4$ (AGT-2).

Significant effect was observed in context to combined effect of salinity levels and varieties on $Na^+/K^+$ and $Ca^{++}/Na^+$. The minimum value of $Na^+/K^+$ ratio (0.024) and the highest value of $Ca^{++}/Na^+$ (32.93) were found in salt concentration level $S_1$ (Control) in variety $V_4$ (AGT-2).

### 3.2 $Na^+/K^+$ and $Ca^{++}/Na^+$ ratio in Seed and Straw at harvest

The results data depicted that increased trend was noted with respect to $Na^+/K^+$ ratio with increasing salt concentrations. On other hand, $Ca^{++}/Na^+$ ratio was vice-versa of $Na^+/K^+$ ratio. At the same level of salinity $S_1$ (Control), the lowest value of $Na^+/K^+$ ratio in seed (0.067) and straw (0.211). Whereas, the highest value of $Ca^{++}/Na^+$ ratio in seed (16.35) and straw (40.18) were recorded at salt concentration level of $S_1$ *i.e.* Control. $Ca^{++}/Na^+$ ratio in straw remains statistically at par with $S_1$ (40 meq l$^{-1}$).

Different varieties of pigeon pea significantly influenced $Na^+/K^+$ ratio and $Ca^{++}/Na^+$ ratio in seed and straw of pigeon pea at harvest. The significantly lower value of $Na^+/K^+$ ratio was (0.068 and 0.201) and significantly the highest of $Ca^{++}/Na^+$ ratio was (15.45 and 40.88) in seed and straw were observed with variety $V_4$ (AGT-2), respectively.

The combined effect of salinity levels and varieties was found non-significant on $Na^+/K^+$ and $Ca^{++}/Na^+$ ratio at harvest in seed and straw of pigeon pea.

**Table 4:** Effect of salinity levels and varieties on $Na^+/K^+$ and $Ca^{++}/Na^+$ ratio in leaves of pigeon pea at 45 DAS

| Treatments | $Na^+/K^+$ | $Ca^{++}/Na^+$ |
|---|---|---|
| **Salt concentration (Salinity) (S)** | | |
| $S_1$: Control | 0.033 | 24.27 |
| $S_2$: 40 meq l$^{-1}$ | 0.041 | 20.82 |
| $S_3$: 60 meq l$^{-1}$ | 0.043 | 19.44 |
| $S_4$: 80 meq l$^{-1}$ | 0.046 | 18.35 |
| $S_5$: 100 meq l$^{-1}$ | 0.052 | 15.83 |
| S.Em. ± | 0.001 | 0.32 |
| C.D. (P=0.05) | 0.002 | 0.92 |
| **Variety (V)** | | |
| $V_1$: GJP-1 | 0.049 | 16.87 |
| $V_2$: Vaishali | 0.043 | 19.25 |
| $V_3$:BDN-2 | 0.042 | 19.57 |
| $V_4$: AGT-2 | 0.037 | 23.27 |
| S.Em. ± | 0.001 | 0.29 |
| C.D. (P=0.05) | 0.002 | 0.82 |
| **S x V Interaction** | | |
| S.Em. ± | 0.002 | 0.64 |
| C.D. (P=0.05) | 0.005 | 1.84 |
| C.V.% | 6.61 | 5.64 |

**Table 5:** Interaction effect of salinity levels and varieties on $Na^+/K^+$ ratio in leaves of pigeon pea at 45 DAS

| | $S_1$: Control | $S_2$: 40 meq $l^{-1}$ | $S_3$: 60 meq $l^{-1}$ | $S_4$: 80meq $l^{-1}$ | $S_5$: 100 meq $l^{-1}$ | Mean |
|---|---|---|---|---|---|---|
| $V_1$: GJP-1 | 0.039 | 0.045 | 0.047 | 0.052 | 0.061 | 0.049 |
| $V_2$: Vaishali | 0.040 | 0.041 | 0.043 | 0.045 | 0.049 | 0.043 |
| $V_3$: BDN-2 | 0.028 | 0.043 | 0.044 | 0.046 | 0.052 | 0.042 |
| $V_4$: AGT-2 | 0.024 | 0.033 | 0.039 | 0.041 | 0.047 | 0.037 |
| Mean | 0.033 | 0.041 | 0.043 | 0.046 | 0.052 | |
| S.Em. ± | 0.002 | | C.D. (P=0.05) | | 0.004 | |

**Table 6:** Interaction effect of salinity levels and varieties on $Ca^{++}/Na^+$ ratio in leaves of pigeon pea at 45 DAS

| | $S_1$: Control | $S_2$: 40 meq $l^{-1}$ | $S_3$: 60 meq $l^{-1}$ | $S_4$: 80meq $l^{-1}$ | $S_5$: 100 meq $l^{-1}$ | Mean |
|---|---|---|---|---|---|---|
| $V_1$: GJP-1 | 20.26 | 18.06 | 17.25 | 15.81 | 12.96 | 16.87 |
| $V_2$: Vaishali | 21.67 | 20.56 | 19.27 | 18.35 | 16.43 | 19.25 |
| $V_3$: BDN-2 | 22.20 | 20.38 | 19.69 | 18.89 | 16.71 | 19.57 |
| $V_4$: AGT-2 | 32.93 | 24.28 | 21.54 | 20.34 | 17.23 | 23.27 |
| Mean | 24.27 | 20.82 | 19.44 | 18.35 | 15.83 | |
| S.Em. ± | 0.64 | | C.D. (P=0.05) | | 1.84 | |

**Table 7:** Effect of salinity levels and varieties on $Na^+/K^+$ and $Ca^{++}/Na^+$ ratio in seed and straw of pigeon pea at harvest

| Treatments | $Na^+/K^+$ | | $Ca^{++}/Na^+$ | |
|---|---|---|---|---|
| | Seed | Straw | Seed | Straw |
| **Salinity (Salinity) (S)** | | | | |
| $S_1$: Control | 0.067 | 0.211 | 16.35 | 40.18 |
| $S_2$: 40 meq $l^{-1}$ | 0.070 | 0.213 | 15.01 | 38.57 |
| $S_3$: 60 meq $l^{-1}$ | 0.072 | 0.220 | 14.37 | 37.22 |
| $S_4$: 80 meq $l^{-1}$ | 0.075 | 0.230 | 13.75 | 36.07 |
| $S_5$: 100 meq $l^{-1}$ | 0.081 | 0.253 | 12.44 | 34.37 |
| S.Em. ± | 0.001 | 0.006 | 0.31 | 0.63 |
| C.D. (P=0.05) | 0.003 | 0.016 | 0.90 | 1.79 |
| **Variety (V)** | | | | |
| $V_1$: GJP-1 | 0.078 | 0.254 | 12.93 | 33.55 |
| $V_2$: Vaishali | 0.073 | 0.222 | 14.26 | 37.14 |
| $V_3$:BDN-2 | 0.074 | 0.225 | 14.90 | 37.55 |
| $V_4$: AGT-2 | 0.068 | 0.201 | 15.45 | 40.88 |
| S.Em. ± | 0.001 | 0.005 | 0.28 | 0.56 |
| C.D. (P=0.05) | 0.003 | 0.014 | 0.80 | 1.60 |
| **S x V Interaction** | | | | |
| S.Em. ± | 0.002 | 0.011 | 0.63 | 1.25 |
| C.D. (P=0.05) | NS | NS | NS | NS |
| C.V.% | 4.60 | 8.56 | 7.57 | 5.81 |

## Discussion

Sodium is not considered an essential element for plants and plants accumulate $Na^+$ at the expense of $Ca^{++}$ and $K^+$ under saline conditions (Kuiper, 1984) [19]. According to Ioneva (1988) [13], the increased in $Na^+$ contents, decreased in $K^+$ contents and $K^+/Na^+$ ratios in plant leaves are attributed to the effects of competition between $Na^+$ and $K^+$ ions at absorption sites in plant roots. Mahmood *et al.* (2008) [21] also reported that with the addition of salt in the medium, Na content of shoot increased. Patel *et al.* (2010) [23] reported that $Na^+$ increases with increasing salinity. According to Greenway and Munns (1980) [10], lower $K^+$ concentrations may inhibit growth by reducing the capability to regulate osmotic pressure and maintain turgor pressure, and may negatively affect metabolic function. Mahmood *et al.* (2008) [21] also reported that K content dropped with the addition of salt to the medium. Essa (2002) reported that the main response of the plant to salt stress is a change in $Ca^{++}$ homeostasis and attributed that the salt tolerance of plants is their ability to avoid $Na^+$ toxicity and to maintain $Ca^{++}$ and $K^+$ concentrations.

Similar results were found in studies done by Kurban *et al.* (1999) [20], Brown *et al.* (2006) [4], Waheed *et al.* (2006) [30], Alam *et al.* (2004) [1] and Turan *et al.* (2007b) [27] for N content. This result is consistent with Chakrabarti and Mukherji (2002) [5], who found that K decreased with increasing salinity. Turan *et al.* (2009) [28] reported that the application of NaCl to soil reduced the K content of plants. The decrease in K is thought to be due to its antagonistic relationship with Na. These investigators reported that high salt (NaCl) intake competes with the intake of other nutrient ions, especially $K^+$, leading to depletion of $K^+$ and other ions, as reported by Shokri and Maadi (2009) [25]. Antagonistic relation between Na and K may reduce the K concentration. This result is consistent with Chakrabarti and Mukherji (2002) [5] that Mg decreases with increasing salinity. As reported by Turan *et al.* (2009) [28], increasing NaCl levels increased Na uptake in plants. $Na^+$ is also thought to maintain turgor, but cannot replace certain functions of $Ca^{++}$ and $K^+$ (Patel *et al.* 2010) [23].

These results were supported by the study of Brown *et al.* (2006) [4], Turan *et al.* (2007b) [27], Hasan and Miyake (2017) [12] and Rodda (2018) [24] for Fe content. Similar result was found in the studies of Rodda (2018) [24] for Mn and Zn content.

The $K^+/Na^+$ ratios in plant leaves is attributed to the effect of competition between $Na^+$ and $K^+$ ions at the absorption sites in plant roots. As Patel *et al.* (2010) [23] reported increasing soil salinity decreased the $K^+/Na^+$ ratio. The result is in agreement with the studies of Waheed *et al.* (2006) [30] and Kholovo *et al* (2010) [18].

High salt accumulation can disrupt the normal balance of plant physiological processes and lead to starvation. These results are in consistent with work of Essa (2002) [8], Dogan (2011) [7] and Bazrafshan and Ehsanzadeh (2014) [3]. These results are consistent with those reported by Jat *et al.* (2011) [16], Uddin *et al.* (2011) [29], Babu *et al.* (2012) [2] and Hakim *et al.* (2014) [11] for $Na^+/K^+$ and $Ca^{++}/Na^+$ ratios at harvest in seed and straw.

## Conclusion

Overall, variety AGT-2 showed lower values of $Na^+/K^+$ ratio and higher $Ca^{++}/Na^+$ ratios in leaves at 45 DAS as well as in seed and straw at harvest. Therefore, AGT-2 was shown to be resistant to salt stress. The $K^+/Na^+$ ratio decreased in the straw and seeds of the tested cultivars, whereas the Na content increased under saline conditions. Higher $Ca^{++}/Na^+$ ratios at higher EC values indicated greater tolerance to salinity than lower $Ca^{++}/Na^+$ ratios at lower levels of EC.

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