Research Article

Jabeen Farheen* and Simeen Mansoor

# Cytogenetic impact of sodium chloride stress on root cells of Vigna radiata L. seedlings

# Sodyum klorür stresinin Vigna radiata L. fidelerinin kök hücreleri üzerindeki sitogenetik etkisi

https://doi.org/10.1515/tjb-2018-0352
Received August 29, 2018; accepted January 17, 2019

**Abstract**

**Objectives:** The high salinization stress to seedling is the substantial ecological problem in the ongoing era. It negatively influences the growth that retard mitotic division by enhancing aberrations in nuclear chromatin. In the light of these views, the current work was designed to investigate the response of Vigna seedlings root tip cells to the presence of NaCl ions.

**Materials and methods:** NM-92 and NM19-19 seeds were imbibed separately in distilled water for 24 h and allowed to grow into 0, 50, 150, 250, and 350 mM NaCl solution for 24 h. Excised root tips were stained, and slides were scored at 100× objective for the mitotic index (MI) and chromosomal aberrations.

**Results:** Our data demonstrated that as NaCl molarity increased, the MI was declined along with various chromatin abnormalities. The 150 mM of NaCl showed more lagging (69%) of chromosomes during anaphase in NM19-19. The highest stickiness at metaphase stage (68%) was found in 250 mM NaCl in variety NM19-19. However, both varieties were differed non-significantly for c-mitosis that was recorded 99% at 350 mM NaCl concentration.

**Conclusions:** The NaCl ions toxicity induced various cytological anomalies in seedling roots that adversely affect the growth of Vigna seedlings.

**Keywords:** Chromosomal aberration; Genotoxicity; Mitotic index; Mungbean; Salt stress.

**Öz**

**Amaç:** Fide üzerindeki yüksek tuz stresi, devam eden çağdaki önemli bir ekolojik sorundur. Büyümeyi olumsuz yönde etkiler, nüklear kromatin içindeki sapmaları artırarak mitotik bölünmeyi geciktirir. Bu görüşlerin ışığında, mevcut çalışma Vigna fidelerinin kök ucu hücrelerinin NaCl iyonlarının varlığına tepkisini araştırmak için tasarlanmıştır.

**Gereç ve Yöntem:** NM-92 ve NM19-19 tohumları, 24 saat boyunca distile su içinde ayrı ayrı emdirildi ve 24 saat boyunca 0, 50, 150, 250 ve 350 mM NaCl çözeltisi içinde büyümelerine izin verildi. Kesilmiş kök ucu boyandı ve mitotik indeks (MI) ve kromozomal sapmalar için 100× objektifte slaytlar puanlandı.

**Bulgular:** Verilerimiz, NaCl molaritesi arttıkça, MI'nin çeşitli kromatin anormallikleri ile birlikte azaldığını göstermiştir. 150 mM NaCl, NM19-19'da anafaz sırasında daha gecikmeli (% 69) kromozomlar gösterdi. Metafaz aşamasında en yüksek yapışkanlık (% 68), NM19-19 çeşitlerinde 250 mM NaCl'de bulunmuştur. Bununla birlikte, her iki çeşit de 350 mM NaCl konsantrasyonunda % 99 olarak kaydedilen c-mitoz için anlamlı şekilde farklı değildi.

**Sonuç:** NaCl iyonlarının toksisitesi, fide köklerinde Vigna fidelerinin büyümesini olumsuz yönde etkileyen çeşitli sitolojik anomalilere neden olmuştur.

**Anahtar Kelimeler:** Kromozomal sapma; Genotoksisite; Mitotik indeks; Taze fasülye; Tuz stresi.

***Corresponding author: Jabeen Farheen,** Department of Genetics, Faculty of Science, University of Karachi, Karachi 75270, Pakistan, e-mail: farheenj.uok@gmail.com. https://orcid.org/0000-0002-0244-7856

**Simeen Mansoor:** Department of Genetics, Faculty of Science, University of Karachi, Karachi, Pakistan

Published online April 28, 2020

# Introduction

The soil salinization is the most important abiotic stress in the barren zone of land [1]. Due to which there is an excess accumulation of salts in the rooting region ensured complete or limited loss of soil structure and fertility [2]. Salty soils consist of carbonate, magnesium, potassium, sodium, calcium, sulfate, chloride, nitrate and borate. In which sodium chloride will be standout amongst the salts in nature and has a dangerous impact due to its superior solubility [1]. It badly devastates seedlings biochemistry and inducing ionic stress [3, 4] by storing $Na^+$ & $Cl^-$ ions in the cytosol. These ionic toxicities distort the double helix structure of DNA and orientation of chromosome at metaphase that caused chromosome abnormalities like stickiness in chromosomes, C-mitosis, lagged and disturbed anaphase [3, 5]. The decline in mitotic index usually down-regulated the development of seedlings [2] which hampered agrarian productivity [5]. Over fact, no hazardous compound hinders the development of crops besides salt stress on the earth scale [6].

As a matter of fact, the presence of excessive amount of salts in the soil is hazardous to most of the comestible species like Vigna radiata. The Vigna genus is comprised of about 150 species mainly belong to Asia and Africa [7]. Vigna radiata ($2n = 2\times = 22$) is one of its cultivated species grown in the tropic locale of Pakistan, India, Bangladesh, Central Asia, Sri Lanka, and South China [7, 8]. It is generally known as mungbean or green or black gram [9]. In Pakistan, beans are cultivated around 950 hectares of lands that yield 0.715 tons per hectare [10]. Its seedling has high nutritive value but very sensitive for saline stress [11]. It was reported that salt alters double helix of DNA and become a root cause of the chromosomal aberration in onion [12], barley [13], and wheat [5]. However, no precise research on high salt (350 mM NaCl) induced Vigna radiata seedlings roots damage, and chromosomal aberrations have been reported during last few decades. Therefore, the aim of this research is to evaluate the impact of salt stress over root cells through the study of mitotic index (MI), and distinct chromosomal anomalies, utilizing root tip cells from NM-92 and NM19-19 Vigna radiata varieties. As suggested by Çavuşoğlu et al. [14] and Singh and Roy [15], it is conceivable to assess the cytotoxicity of tested chemical utilizing above-mentioned criterion. Thus, the cytogenetic investigation of Vigna radiata may provide valuable information about the impact of high salinization of NaCl at seedlings stage.

# Materials and methods

## Experimental design

Mungbean variety NM-92 and NM19-19 were collected from National Agricultural Research Center, (NARC) Islamabad, Pakistan. The whole study was conducted during the winter season of the year 2016 in the laboratory of Genetics Department, University of Karachi, Pakistan. Oxidative stress was given through 0, 50, 150, 250 and 350 mM concentrations of sodium chloride salt (Sigma-Aldrich). From each variety approximately 150 sterilized seeds [11] were first imbibed for 24 h in pure water, then same sized imbibed seeds (20 seeds/conc./variety) were treated in 50 mL NaCl solutions containing beaker for 24 h. Root tips of about 8–9 mm in length were excised from each treatment as well as control.

## Slid perminization method

The root tips were fixed in "Farmer fixative" (absolute alcohol: acetic acid glacial pure in 3:1 ratio) for 24 h. After then root tips were stained with 1.8% Aceto-orcein stain for 72 h [16]. The aceto-orcein stain was prepared by stirring 1.8 grams aceto-orcein in 100 mL of 45% acetic acid solution, followed by boiled in a water bath for 6–7 h on Magnetic stirrer & hot plate (M6/1, Germany), thereat filtered and stored at room temperature. The slides were prepared by heating tips in 45% acetic acid solution for few second then squashed [17]. Spread cells were made permanent by immersing in Farmer's fixative filled container until its coverslip became separated. Thereat air dried and again submerged in absolute alcohol for 1 min. A drop of Canada balsam was placed over the air-dried specimen covered with new glass coverslip and dried for a week.

## Cytogenetic assay

The cytogenetic investigation was done by examining at least 1000 cells from each salt treatment under a 100× lens of Nikon DS-Fi 1 Japan. Photographs were taken by Nikon Eclipse E400 Japan. Mitotic index & percent aberrations were calculated from following formulae [18].

$$\text{Mitotic Index } \% = \frac{\text{No. of dividing cells}}{\text{Total no. of cells}} \times 100$$

$$\text{Mitotic inhibition \%} = \frac{\text{Mitotic index of Control} - \text{Mitotic index of Treatment}}{\text{Mitotic index of Control}} \times 100$$

$$\text{Total Chromosomal Aberrations \%} = \frac{\text{Total no. of Aberrated cells}}{\text{Total no. of cells}} \times 100$$

$$\text{Aberrations \%} = \frac{\text{Total no. of certain type of aberrated cells}}{\text{Total no. of Aberrated cells}} \times 100$$

The aberration % formula was mentioned above as general formula for calculating C-mitosis, stickiness, lagged anaphase, spiralization, bridge anaphase, and disturbed anaphase.

### Statistical analysis

Experiment was set in beakers as CRD in Factorial with four replications to authenticate our results [19]. Three slides / treatments were made to score 1000 cells per treatment. Collected data for mitotic index and chromosomal aberrations were subjected to univariate analysis of variance on IBM SPSS version 19. The Duncan multiple range test was utilized for the scrutiny of significant difference among mean values at $p \leq 0.01$ level. Data of mitotic index and chromosomal aberrations were mean ± SE (n = 4).

## Results and discussion

Table 1 of ANOVA showed that two Vigna varieties NM-92 and NM19-19 performed differently for mitotic index (MI), stickiness, spiralization, lagged, bridge and disturbed anaphase. However, NM-92 and NM19-19 differed non-significantly for total chromosomal aberrations and C-mitosis. The effect of five levels of NaCl stress were statistically different for all inspected cytogenetic parameters. The interaction of Vigna varieties with five levels of NaCl stress on lagged anaphase, spiralization, bridge and disturbed anaphase was highly significant at 1% probability level. In contrast, the impact of interaction between five concentrations of NaCl on two Vigna varieties showed non-significant difference for mitotic index, total chromosomal aberration, C-mitosis, and stickiness of chromosomes. Moreover, the coefficient of variation found maximum in C-mitosis and minimum in mitotic index that were 73% and 9%, respectively (Table 1).

Salts generated ionic stress on seedlings morpho-physiology and biochemistry have been studied [20, 21] While, the impact of NaCl stress at cytological level is not well examined [5]. According to Lubini et al. [22], cytotoxicity of any chemical could be determined through promotion or inhibition in mitotic index. The MI revealed cell division and proliferation frequency and rate of meristem growth [23]. Here, it is utilized to investigate the impact of NaCl stress over Vigna seedling root cells. It was seen that varying levels of NaCl imposed the deleterious effect on root cells. The lower level of NaCl stress inhibited 7% cell division while 350 mM manifested 20% inhibition (Figure 1A). Further, the severe NaCl stress caused the highest level of mitotic aberrations that was 75% than $H_2O$ control cells (Figure 1B). Moreover, various NaCl levels induced number of chromatin (C-mitosis, and stickiness of chromosomes) and spindle fiber associated abnormalities (lagged anaphase, spiralization, bridge anaphase, and disturbed anaphase) in Vigna root cells.

**Table 1:** Mean sum of square table of five investigated 0–350 mM NaCl stressed parameters of two Vigna radiate varieties NM-92 and NM19-19.

| S.V. | Df | Mean Squares of Cyto-genetic parameters under 0–350 mM salt stress | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | MI % | TCA % | C-M % | Stick % | LA % | Sp % | BA % | DA % |
| Var | 1 | 2.0[a] | 8.1[ns] | 0.1[ns] | 3.6[a] | 809.1[a] | 112.2[a] | 52.9[a] | 27.2[a] |
| Treat | 4 | 498.5[a] | 6349.1[a] | 13889.9[a] | 7761.4[a] | 4230.9[a] | 54.0[a] | 33.3[a] | 161.2[a] |
| T*V | 4 | 2.2[ns] | 0.5[ns] | 1.4[ns] | 0.6[ns] | 881.2[a] | 37.7[a] | 8.7[a] | 29.9[a] |
| Error | 27 | 3.5 | 37.2 | 2.2 | 1.0 | 950.7 | 0.4 | 0.3 | 0.4 |
| C.V. | | 9% | 68% | 73% | 63% | 48% | 14% | 11% | 20% |

Cytological impact of 0, 50, 150, 250, and 350 millimolar NaCl concentration on two Vigna radiata varieties seedlings root tip cells and five salt concentrations x varieties interaction were assessed. This examination was the outcome of four replicates. ANOVA was conducted at $p \leq 0.01$ level. The data expressed in percentage where, [a]indicated significant difference at 0.01 level, ns stands for non-significant difference, C.V., coefficient of variation; MI, Mitotic index; TCA, Total Chromosomal aberration; C-M, C-Mitosis; Stick: Stickiness; LA, Lagged anaphase; Sp, Spiralization; BA, Bridge anaphase; DA, Disturbed anaphase.


**Figure 1:** Cytological impact of 0, 50, 150, 250, and 350 millimolar NaCl concentration on seedlings root tip cells of *Vigna radiata* varieties. Above examination was the outcome of four replicates. Post hoc test for means comparison was conducted at $p \leq 0.01$ level. (A) mitotic index; (B) total chromosomal aberrations; (C) C-mitosis; (D) chromosome stickiness; and (E) lagged-anaphase. The data expressed in percentage while, trendline showed promotion and inhibition pattern in the graphs.

The least inhibition in mitotic index at 50 mM NaCl concentration indicated an active division of root meristem cells. In contrast, maximum decrease in MI at 350 mM salt exhibited extreme stress over biochemical mechanism which caused decline in cell division that retard Vigna seedling growth. However, cell division in apical meristem became the cause of root growth that is directly related to the mitosis duration. The greater mitotic index in NM-92 under each concentration showed salt stress tolerance whereas lower MI in NM19-19 indicated genotype sensitivity against salinity stress. These findings are well in conformity with Kielkowska [12] and Çavuşoğlu et al. [2] in onion. Marakli et al. [13] tested 150 and 250 mM NaCl impact over barley seedlings. Both concentrations

caused the decrease in mitotic index and promoted disorganized prophase, metaphase, and anaphase and bridge in anaphase. However, the total absence of mitotic activity was seen from 300 to 600 mM of NaCl stress [5, 12]. In wheat, 250 mM NaCl reduced mitotic activity and induced chromosomal anomalies, for instance, irregular prophase, stickiness, C-mitosis, bridges, and multipolarity [5]. In another study 300, 350, 400 and 450 mM saline stress inhibited mitotic index of barley (cv. 'Bulbul 89') and the higher number of chromosomal aberrations as salt concentration increase [1]. These findings support our results (Figure 2).

The server level of NaCl (350 mM) stress showed 99% C-mitosis in both examine varieties (Figure 1C). However, 250 mM NaCl stress exhibited highest (68%) stickiness of chromosome at metaphase in variety NM19-19 and lower (40%) in NM-92 (Figure 1D). Similarly, lagged anaphase was found higher (69%) in NM19-19 and lesser (44%) in NM-92 at 150 mM salt concentration (Figure 1E). The reason for the occurrence of C-mitosis and lagged anaphase may

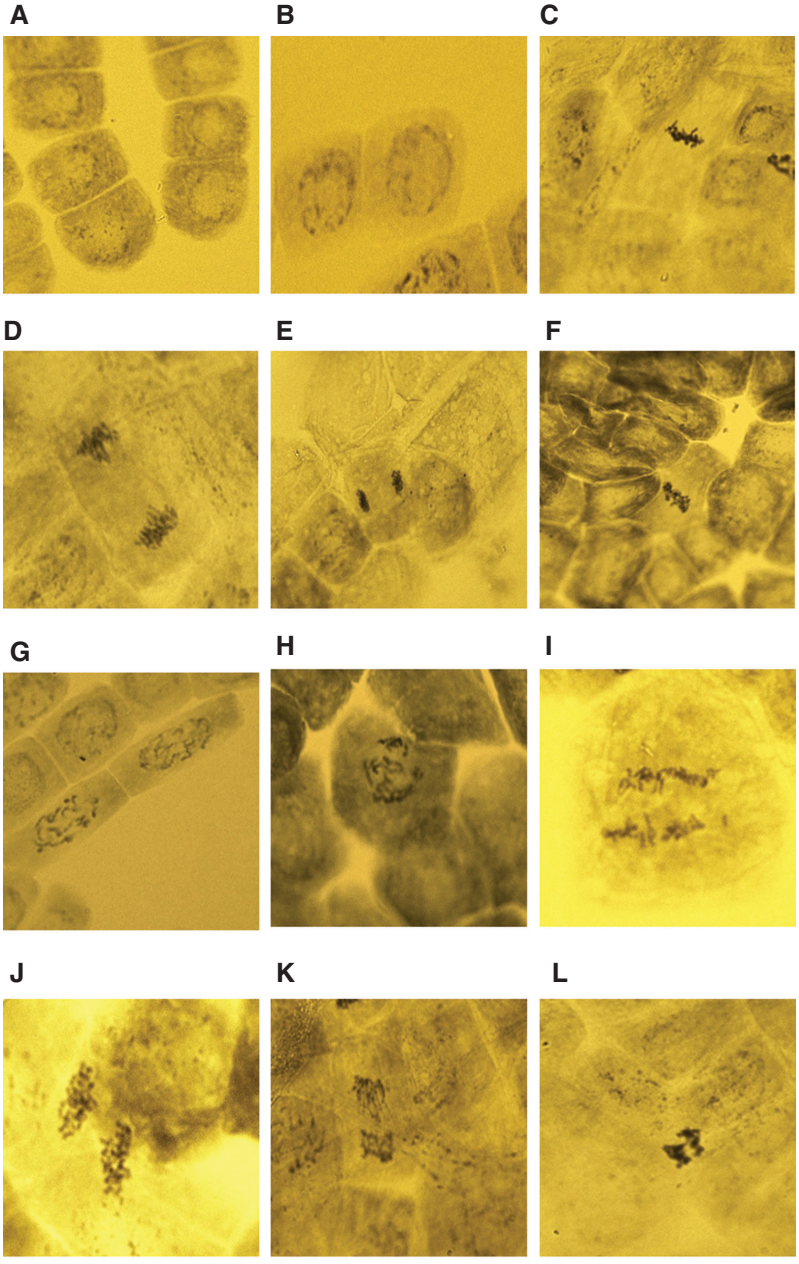


**Figure 2:** Cytological impact of 0, 50, 150, 250, and 350 millimolar NaCl stress for 24 h on seedlings root tip cells of two *Vigna radiata* varieties. Above examination was the outcome of four replicates. (A) normal interphase; (B) normal prophase; (C) normal metaphase; (D) normal anaphase; (E) normal telophase; (F) sticky metaphase; (G) spiralization at metaphase; (H) c-mitosis; (I) lagged anaphase; (J) disturbed anaphase; (K) spiralization at anaphase; and (L) bridge anaphase.

result in spindle formation failure due to sever salt stress [24, 25]. These observed chromosome fragmentation and breaks showed clastogenic action [26] while, stickiness in chromosomes may be due to inter-chromosomal linkages coupled with the excessive formation of nucleoproteins that caused stickiness in chromosomes [24]. These nucleoproteins production and inter-chromosomal linkage may be because of unwanted $Na^+$ and $Cl^-$ ions accumulation in the cytosol and vacuole of the cell of Vigna seedlings.

In addition, various other aberrations like spiralization, bridge-anaphase, and disturbed anaphase were also noticed under salt stress (Figure 3). The maximum 12% spiralization, and 15% disturbed anaphase in variety NM19-19 while, 1.5% spiralization and 6% disturbed anaphase were observed in NM-92 at 250 mM salt stress (Figure 3A,B). Whereas, 7% bridges at anaphase was noted in NM19-19 and 3.5% in NM-92 under 150 mM salt level (Figure 3C). The spiralization, disorder, and bridges at anaphase could be as a result of inversions or unequal allocation of nuclear chromatin and spindle dysfunction. Spindle dysfunction may induce aberrant segregation of chromosomes because of faulty kinetochore and microtubule synergy which provokes bridges creation [1] at anaphase. In short, nuclear chromatin degradation is the consequence of NaCl ions toxicity [27], that was responsible for root and shoot growth retardation in Vigna seedlings. Also, it was observed that the maximum types of aberrations found at 150 and 250 mM concentration of sodium chloride though, 350 mM salinity level was lethal for both varieties seedling's cell division and because of this single type of aberration found. However, the higher percentage of stickiness, spiralization, lagged, bridge and disturbed anaphase aberrations in NM19-19 at each level of NaCl stress showed genetic inability to resist salt stress. While in the current examination, NM-92 proved as salt tolerant variety due to exhibiting less percentage of chromosomal aberrations at all concentration of salt.

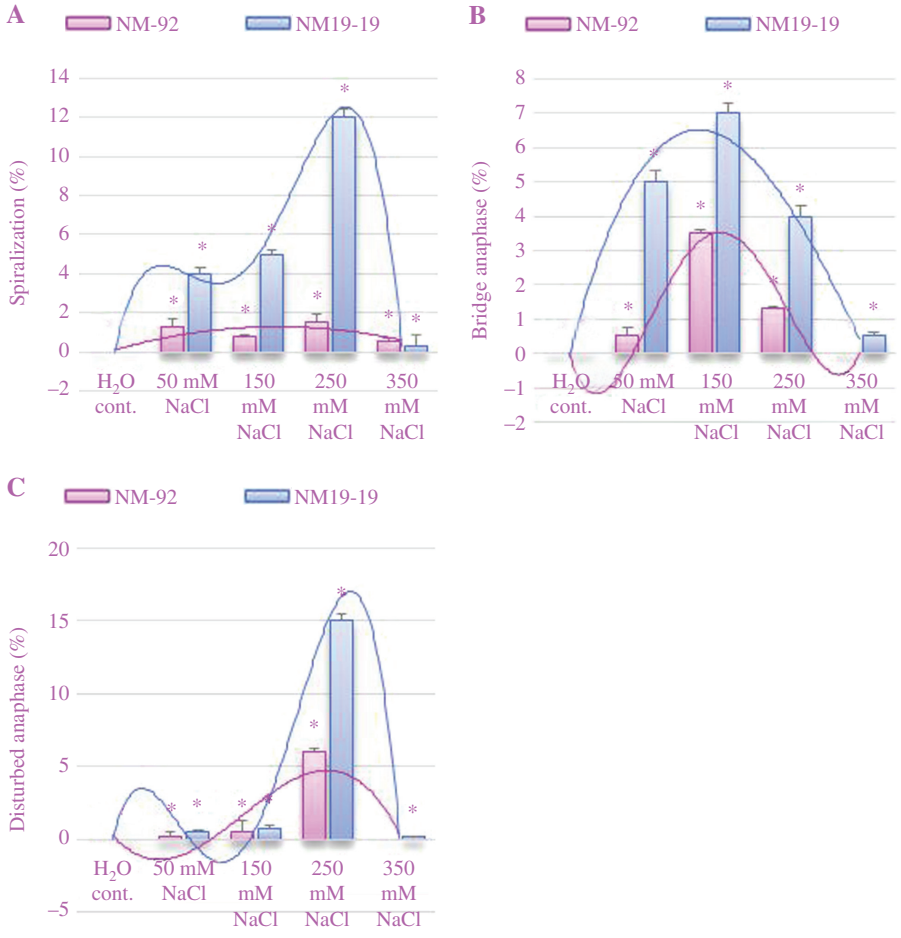


**Figure 3:** Cytological impact of 0, 50, 150, 250, and 350 millimolar NaCl concentration on seedlings root tip cells of *Vigna radiata* varieties. Above examination was the outcome of four replicates. Post hoc test for means comparison was conducted at $p \leq 0.01$ level. (A) spiralization at meta and anaphase; (B) bridge-anaphase; and (C) disturbed-anaphase. The data expressed in percentage while, trendline showed promotion and inhibition pattern in the graphs.

In short, the salinization has chromo toxic effect over seedling cells that inhibits DNA, nuclear proteins synthesis, compatible osmolytes, antioxidant defense enzymes, polyamines and restrict water availability [28]. Other than these, it originates responsive oxygen species, ion toxicity, enhance alkalinity and osmotic pressure, disturb metabolic mechanism, cellular physiology, and ion transport that notable raises altogether types of chromatin anomalies in radical cells [12, 14, 24] which leads to fewer cells production cause reduction in meristem size [12]. While concentrations greater than 300 mM cause severe damage at the cellular level and lead to cell death [1].

The impact of stress over seedlings depends on the chemical and physiological nature of stress-inducing compound such as NaCl. Sodium chloride is an ionic compound induce ion toxicity in the cytosol of the cell. It could be concluded that chloride and sodium ions toxicity induced various cytological anomalies in seedlings roots that adversely affect the growth of Vigna radiata. Hence, cytological examination of Vigna species may play a key role in understanding the impact of salinization over seedling growth.

**Acknowledgments:** The author wants to special thanks to Dr. Gul Sanat Shah (Deputy Chief Scientist, Nuclear Institute for Food and Agriculture, Peshawar, Pakistan) for providing two varieties of Vigna radiata of NIAB from his stock. Authors also extend their thanks to Professor Dr. Shahina Fayyaz (Director, National Nematological Research Center, University of Karachi, Pakistan), and Dr. Tabussum Ara Khanum (Senior Scientific Officer, National Nematological Research Center, University of Karachi, Pakistan) for taking photographs of slides for this research.

**Conflicts of interest:** Both authors have no conflicts of interest regarding current research paper.

## References

1. Tabur S, Demir K. Protective roles of exogenous polyamines on chromosomal aberrations in Hordeum vulgare exposed to salinity. Biologia 2010;65:947–53.
2. Çavuşoğlu D, Tabur S, Çavuşoğlu K. Role of Ginkgo biloba L. leaf extract on some physiological and cytogenetical parameters in Allium cepa L. seeds exposed to salt stress. Cytologia 2016;81:207–13.
3. Nasri N, Saïdi I, Kaddour R, Lachaâl M. Effect of salinity on germination, seedling growth and acid phosphatase activity in lettuce. Am J Plant Sci 2015;6:57–63.
4. Chauhan A, Rajput N, Kumar D, Kumar A, Chaudhry AK. Effect of different salt concentration on seed germination and seedling growth of different varieties of oat (Avena sativa L.). Int J Inf Res Rev 2016;3:2627–32.
5. Pekol S, Baloğlu MC, Çelik Altunoğlu Y. Evaluation of genotoxic and cytologic effects of environmental stress in wheat species with different ploidy levels. Turk J Biol 2016;40:580–8.
6. Jones MB, Finnan J, Hodkinson TR. Morphological and physiological traits for higher biomass production in perennial rhizomatous grasses grown on marginal land. GCB Bioenergy 2015;7:375–85.
7. Dikshit HK, Singh D, Singh A, Jain N, Kumari J, Sharma TR. Utility of adzuki bean [Vigna angularis (Willd.) Ohwi & Ohashi] simple sequence repeat (SSR) markers in genetic analysis of mungbean and related Vigna spp. Afr J Biotechnol 2012;11:13261–8.
8. Sony SK, Habib A, Islam MN. Genetic diversity analysis of thirteen mungbean (Vigna radiata (L.) Wilczek) cultivars under RAPD markers. Bangl J Bot 2012;41:169–75.
9. Taunk J, Yadav NR, Yadav RC, Kumar R. Genetic diversity among green gram [Vigna radiata (L.) Wilczek] genotypes varying in micronutrient (Fe and Zn) content using RAPD markers. Indian J Biotechnol 2012;11:48–53.
10. Government of Pakistan (2015–2016). Ministry of national food security and research (NFS & R), 2015–2016. FCA Working Paper Draft 27 April 15 Final.pdf.
11. Farheen J, Mansoor S, Abideen Z. Application of salicylic acid improved growth, photosynthesis pigments and oxidative stability in mungbean seedlings at salt stress. Pak J Bot 2018;50:901–12.
12. Kiełkowska A. Allium cepa root meristem cells under osmotic (sorbitol) and salt (NaCl) stress in vitro. Acta Bot Croat 2017;76:146–53.
13. Marakli S, Temel A, Gozukirmizi N. Salt stress and homobrassinosteroid interactions during germination in barley roots. Not Bot Horti Agrobo 2014;42:446–52.
14. Çavuşoğlu K, Cadıl S, Çavuşoğlu D. Role of potassium nitrate (KNO3) in alleviation of detrimental effects of salt stress on some physiological and cytogenetical parameters in Allium cepa L. Cytologia 2017;82:279–86.
15. Singh D, Roy BK. Salt stress affects mitotic activity and modulates antioxidant systems in onion roots. Braz J Bot 2016;39:67–76.
16. Kaur M, Soodan RK, Katnoria JK, Bhardwaj R, Pakade YB, Nagpal AK. Analysis of physico-chemical parameters, genotoxicity and oxidative stress inducing potential of soils of some agricultural fields under rice cultivation. Trop Plant Res 2014;1:49–61.
17. Sharma AK, Sharma A. Chromosome technique a manual. Chur, Switzerland: Harwood Academic, 1994;305–6.
18. Jairajpuri M, Raval R, Patel K. Chromosomal aberrations in root meristems of Allium cepa L. induced by dyeing industrial effluent. Int J Multidiscip Res Dev 2016;3:272–5.
19. Steel RG, Torrie JH. Principles and procedures of statistics: a biometrical approach. 2nd ed. Singapore: McGraw Hill Book Co., 1984.
20. Khan MA, Shirazi MU, Shereen A, Mujtaba SM, Khan MA, Mumtaz S, et al. Identification of some wheat (Triticum aestivum L.) lines for salt tolerance on the basis of growth and physiological characters. Pak J Bot 2017;49:397–403.
21. Stavridou E, Hastings A, Webster RJ, Robson PR. The impact of soil salinity on the yield, composition and physiology of the bioenergy grass Miscanthus X giganteus. GCB Bioenergy 2017;9:92–104.

22. Lubini G, Fachinetto JM, Laughinghouse HD, Paranhos JT, Silva AC, Tedesco SB. Extracts affecting mitotic division in root-tip meristematic cells. Biologia 2008;63:647–51.
23. Jităreanu A, Pădureanu S, Tătăringă G, Tuchiluş C, Stănescu U. Evaluation of phytotoxic and mutagenic effects of some cinnamic acid derivatives using the Triticum test. Turk J Biol 2013;37:748–56.
24. Tabur S, Demir K. Cytogenetic response of 24-epibrassinolide on the root meristem cells of barley seeds under salinity. Plant Growth Regul 2009;58:119–23.
25. Utani K, Kohno Y, Okamoto A, Shimizu N. Emergence of micronuclei and their effects on the fate of cells under replication stress. PLoS One 2010;5:e10089.
26. Leme DM, Marin-Morales MA. Allium cepa test in environmental monitoring: a review on its application. Mutat Res 2009;682:71–81.
27. Yazdani M, Mahdieh M. Salinity induced apoptosis in root meristematic cells of rice. Int J Biosci Biochem Bioinforma 2012;2:40–3.
28. Mudgal M, Madaan N, Mudgal A. Biochemical mechanisms of salt tolerance in plants: a review. Int J Bot 2010;6:136–43.