Egypt. J. Bot. **Vol. 61**, No. 1, pp. 41-51 (2021)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Using Sugarcane Waste to Reduce Cytogenetic Impairment in *Vicia faba* Exposed to Saltiness Stress

Rasha Kamal Helmey
Botany and Microbiology Department, Faculty of Science, Minia University, El-Minia, Egypt.

SODIUM chloride is the most widely recognized salt causing saltiness stress and seriously influences plant development when become obvious in extreme concentrations in the soil or water. This study was carried out to inspect the consequence of applying sugarcane bagasse (natural and agricultural wastes) which adsorbs sodium ions on the cytogenetic responses of faba bean under salt stress *in vitro* and *in vivo* conditions. *In vitro*, plants treated with different concentrations (100, 200, 300mM) of NaCl showed cytotoxic effect reflected by the reduction of mitotic division rate in root tip cells and proliferation of different chromosomal aberrations such as chromosome stickiness, bridges and micronuclei. *In vivo*, growth performance was evaluated as plant height and leaf area which displayed a remarked reduction proportional to the increase of NaCl concentration compared to control plants. Applying sugarcane bagasse with irrigation water significantly reduce the mito-depressive effect of different concentrations of NaCl in root meristem cells and increase growth parameters in plants compared with the same concentrations without bagasse.

Keywords: Chromosomal abnormalities, Mitotic index, NaCl salt stress, Sugarcane bagasse, *Vicia faba.*

Introduction

All crop plants exhibit physiological temperance end points to salt stress. Concentration above average might lessen harvests or cause demise of cells. For example, high sodium existence (saltiness) in germ cells may prompt alterations in protein action by disturbing the hydration of nuclear contents triggering an inhibition of enzyme activity (Kim et al., 2013). Ionic pressure may be incited by sodium or potassium chloride creating explicit ionic toxicities; though, both salts also convince osmotic stress, because of the slope in salt concentration outside the cells, prompting restraint of water take-up (Claeys et al., 2014). The impact of NaCl-actuated stress has been studied generally in grains, and findings indicate that concentrations in excess of 300 mM cause severe cellular mutilation and contribute to cell death (Munns & Tester, 2008; Yumurtaci et al., 2009; Tabur & Demir, 2010). Higher concentrations of NaCl diminish root development which was further reflected in decreased root meristem size (Zadeh, 2007).

Soil saltiness has turned into a sincere natural issue which influences the development and efficiency of several crops. High salt constituent in the soil influences the soil absorbency and furthermore diminishes the water potential of the soil that outcomes in a physiological drought. High salt content likewise upsets the physiology of plants at cellular and whole plant level (Murphy & Durako, 2003). All soils comprise certain quantities of solvable salts. Many of these salts are pondered as nutrients which are needed for plant growth. Though, if the amount of salts in the soil overdoes a threshold, yield and/or quality of most crops are undesirably affected. The damage be governed by the type and amount of salts, growth phase, plant category, and further ecological aspects. Because of diminishing

Corresponding author email: rasha.galal@mu.edu.eg
Received 12/10/ 2019; Accepted 26/8/ 2020
DOI: 10.21608/ejbo.2020.18010.1366
Edited by: Prof. Dr. Soliman A. Haroun, Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt.

amounts of high-quality irrigation water, saline water is viewed as an option in contrast to freshwater in agronomy. Though, the use of low-quality irrigation water recurrently leads to soil salinization and waterlogging, consequently lessens yield on millions of hectares of cultivated land (Mohsen et al., 2013).

Recently, research labors have been heightened on the valuable usage of ecologically amicable solid phase extractors . In this regard, low cost natural polymeric wastes have gotten a lot of consideration on metal particles expulsion from polluted water (Nada et al., 2010; Ahmed, 2011). The most prevalent adsorbents among them are agricultural wastes, they are accessible in enormous amounts at a low price such as sugarcane bagasse (SCB) (Kumar et al., 2014), sugar beet pulp (BP) (Altundogan et al., 2007), and other low fee adsorbents (Luo et al., 2011). SCB and BP as natural solid phase extractors have the following compensations: (a) Low-priced (sugar cane diligence trashes) and rich in oxygen-containing functional groups; (b) Marked ability for high withdrawal of Pb and Na ions with no necessity for chemical amendment; (c) Effective dipping of the concentration of Na ions from irrigation water based on optimistic parameters used for evaluating Zea maize and wheat seed germination (Ahmed, 2015).

Beans (*Vicia faba* L.) is deliberated the first legume crop in the arable area of Egypt. Conceding the perfection of soil surface and its fruitfulness, the plant seeds are considered as a valued source for energy and proteins (National program for pulses crops- Agricultural Research Center, Giza, Egypt, 2002 (Mohsen et al., 2013). Because of its small chromosome number (n= 6) of remarkably large and small easily observed chromosomes it became an valuable source for energy and proteins ideal species for plant cytogenetics (O'Sullivan & Angra, 2016).

Root tip cells are often the first to be subjected to chemicals spread in soil and water. Inspection of the root tip established a swift and sensitive manner for environmental checking. Cytological studies will contribute detailed data on qualitatively and quantitatively harmful possessions at the microscopic level. Genotoxicity of different chemicals can be tempered by growth hormones and bio-compounds which reduce the incidence of chromosomal aberrations (Morsi et al., 2016; Mahfouz & Rayan, 2017).

West et al., (2004) reported that quickly prevents progression in the cell cycle, averting the pass into stages where the cell is susceptible to damage, permitting the cellular defense system to be triggered. Stress-induced inhibition of cell division leads to fewer cells being produced, which consequences by lessening in meristem size (Kiełkowska, 2017a). The effect of salt stress on chromosome behavior and the cytogenetic response of cells subjected to 50 up to even 600mM of NaCl was evaluated in short time period from 0 up to 72hrs. Kiełkowska, 2017b mentioned that, the mitotic activity of cells during salt shock decreases rapidly or is totally blocked and finally cell death occurs (Tabur & Demir, 2010). The current study was designed to explore the opportunity of applying sugarcane bagasse as a natural and agricultural wastes which adsorbs sodium ions from saline irrigation water and therefore may be applied for improving plant tolerance to salt stress..

Materials and Methods:

Sugarcane bagasse that was previously prepared, as an adsorbent, by Ahmed (2015) and was obtained from Chemistry Department, Faculty of Science, Minia University, Egypt.

NaCl concentrations (0.00, 100, 200 and 300mM) were prepared using d.dH_2O. 50gm of bagasse was soaked in 5 liter of each concentration of NaCl overnight at room temperature and the filtrate was used for irrigation.

Faba bean seeds (Vicia faba CV Nobaria 3) were obtained from Seeds Center, Beni-swif, Egypt. Seeds were opted for size and shape consistency, surface sterilized (2.5% Clorox for 5min) and rinsed in distilled water. In this study seeds were divided into two groups for *in vitro* and *in vivo* experiments.

Determination of mitotic index and chromosomal aberrations

In vitro experiment: for each treatment, 5 seeds were germinated in 9cm petri dishes. Seeds were wetted (5ml daily) with different concentrations of NaCl independently or in addition to bagasse and were sampled as:

(1)- Control (H_2O) (2)- control +bagasse

(3)- 100mM NaCl (4)- 100mM NaCl + bagasse
(5)- 200mM NaCl (6)- 200mM NaCl + bagasse
(7)- 300mM NaCl (8)- 300mM NaCl + bagasse

Roots were fixed for overnight in 1:3 acetic acid/alcohol. Using (1N) HCl at 60°C for 5-6min, hydrolyzed root tips were squashed in aceto-carmine stain. Cell division was examined in approximately 5000 cells of three slides per sample and types of chromosomal abnormalities were scored. The mitotic index (MI) was calculated as the number of cells in mitosis divided by the total number of cells x 100.

Growth parameters:

For each treatment 5 seeds were sowed in pots containing garden clay soil (three replicates/sample). Pots were irrigated with different concentrations of NaCl independently or in addition to bagasse and kept at the field capacity (250ml for each pot every 3 days) and were sampled as mentioned above. Plants of faba beans in the field experiment were photographed after 30 and 45 days of planting (Fig. 1).

Growth parameters of the tested plants were measured at 60 days age (three plants/sample). Growth performance was assessed in terms of plant height and leaf area. Leaf area was measured according to a given formula (Wiersma & Bailey, 1975). Leaf area = 0.624 + 0.723 L W (Where L is the length and W is the width).

Statistical analysis

Data obtained were analyzed statistically by M STAT program to determine the degree of significance of the differences between treatments. Means were compared using Duncan's multiple-range test at the P< 0.05 levels.

Fig. 1. ***Vicia faba*** **exposed** ***in vivo*** **to different concentrations of NaCl and bagasse [(1) Control (H_2O), (2) Control+ bagasse, (3) 100mM NaCl, (4) 100mM NaCl + bagasse, (5) 200mM NaCl, (6) 200mM NaCl + bagasse, (7) 300mM NaCl, (8) 300mM NaCl + bagasse].**

Results and Discussion:

Mitotic analysis

Changes and/or inhibition in mitotic activities are generally used to measure the effects of cytotoxicity on plants under stress. Data obtained from the cytological analysis of Faba beans root tips treated with NaCl and bagasse are summarized in (Table 1 and Fig. 2). Generally, salinity inhibit cell division. The reduction of mitotic index (MI) was significantly decreased and was found to be directly proportional to the concentration of NaCl. Samples treated with 100, 200, 300mM NaCl exhibited highly significant inhibition in cell division and a decline in the mitotic index which was recorded as 14.37, 11.96 and 6.03% respectively when compared to control samples with MI value, 22.94%.

On the other hand, treatment with NaCl combined with bagasse showed strong enhancement of MI compared with the same concentrations without bagasse, as shown in Table 1. Samples treated with 100, 200 and 300mM NaCl + bagasse showed a remarked increase in MI values recorded as 18.56, 14.72 and 7.43%, respectively when compared to the same concentrations without bagasse. MI of control samples is not significantly different from the MI value of control + bagasse (22.61 %).

One of the remarkable effects of NaCl on root tips of plants is its influence on the rate of cell division. Mitotic index was stated to be a strong predictor on the cytotoxic level, whereas chromosomal aberration was used to test mutagenicity of chemicals in cells (Leme & Marin-Morales, 2009; Akinboro et al., 2011; Mahfouz & Rayan, 2017).

In general, high salinity causes ion toxicity, nutritional disorders, water shortage and oxidative stress to plants (Sun et al., 2009). Moreover, it can destructively influence all parts of plant development and confines productivity of crop species, upsetting cell cycle progression and differentiation (Sun et al., 2004). More specifically, Cyclin Dependent Kinases (CDKs), a gathering of protein kinases that incorporate significant cell cycle controllers. They are an enormous group of serine/threonine protein kinases with a significant job in progress of cells in a deliberate manner over the various phases of cell division (Francis, 2007). Salt stress reduced the transcription levels of CDKA/CDKB and CycA/CycB subsequent by down regulation of mitotic activity in the shoot and root apex of Arabidopsis plants and cell cycle arrest. As a result, fewer cells of smaller size were produced showing a smaller meristem with limited growth (West et al., 2004). Though, CDKA and CDKB levels were slightly influenced, proposing post-translational level regulation. The time of decreased proliferative activity was viewed as versatile, then the promoter activities and transcripts of the cell cycle genes ultimately returned to their original levels (Kitsios & Doonan, 2011).

TABLE 1. Mitotic index and Chromosomal abnormalities values in *Vicia faba* root meristem cells exposed *in vitro* to different concentrations of NaCl and bagasse.

Concentrations (NaCl+Bagasse)	Mitotic index (Mean ± SE)	Chromosomal abnormalities						
		Stickiness	Dis-ordered	Lag.	Breaks	Bridge	Micro-nuclei	Total (Mean ± SE)
Control	22.94±1.8 a	0.1	0.00	0.0	0.0	0.0	0.0	0.10±0.0
Control + B	22.61±1.7 a	0.0	0.03	0.0	0.0	0.0	0.0	0.03±0.0
100	14.37±0.4 c	7.2	8.1	3.3	7.0	5.4	2.4	33.40±1.5b
100 + B	18.56±0.7 b	4.3	5.3	2.1	5.3	3.7	0.0	20.70±0.5c
200	11.96±0.5 c	10.1	9.0	4.2	8.2	7.1	1.2	39.80±2.4a
200 + B	14.72±0.8 b	8.4	9.0	4.0	5.6	3.3	0.8	31.10±1.2b
300	6.03±0.3 d	3.7	3.4	2.8	2.3	1.9	0.0	14.10±1.0d
300 + B	7.43±0.7 d	1.2	1.5	0.0	1.7	1.1	0.0	5.50±0.3e

Values with the same letter within the same column are not significant ($P < 0.05$) according to the Duncan Multiple Range Test at 5% significant level.

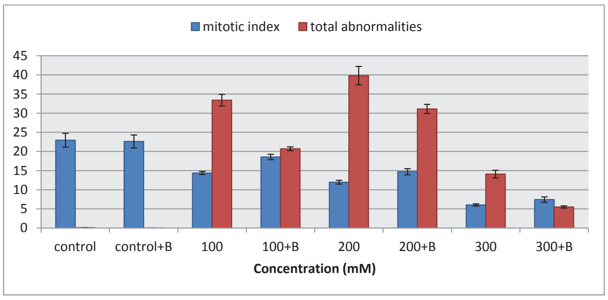

Fig. 2. Mitotic index and chromosomal abnormalities values in *Vicia faba* root meristem cells exposed *in vitro* to different concentrations of NaCl and bagasse.

Different types of chromosomal abnormalities were observed along with the depressive effect on mitotic index. Observed chromosomal aberrations were recorded as stickiness, disordered, lags, breaks, bridges and micronuclei (Table 1 and Figs. 2, 3). In control plants, most root tip cells underwent typical stages of mitosis without disorder. However, in roots of treated samples with 100 and 200 mM NaCl the total number of aberrant cells were increased as 33.4 and 39.8, respectively. While samples treated with 300mM NaCl exhibited a remarkable decrease in the total aberration value (14.1) sponsored to the reduction of cell division. Souguir et al. (2018) reported that, stickiness is known as an irreparable chromosome abnormality, an indicator of toxicity and eventually a cause of cell death. This anomaly is one of chromatid type aberrations and is credited to many factors such as the entanglement of the inter-chromosomal chromatin fibers, the degradation or depolymerization of chromosomal DNA. Several researchers consider stickiness as a clastogenic abnormality, indicating the direct damaging effect of a toxic agent on chromosomes (Renjana et al., 2013). Though, others esteem it as a result of spindle disorder (Gaulden, 1987; Khanna & Sharma, 2013). Stickiness, caused by the NaCl treatment, may be ascribed to abnormal DNA condensation, abnormal chromosome twining, entanglement of inter-chromosomal chromatin fibers or an amendment in specific proteins such as topoisomerase II and the peripheral proteins (Souguir et al., 2018).

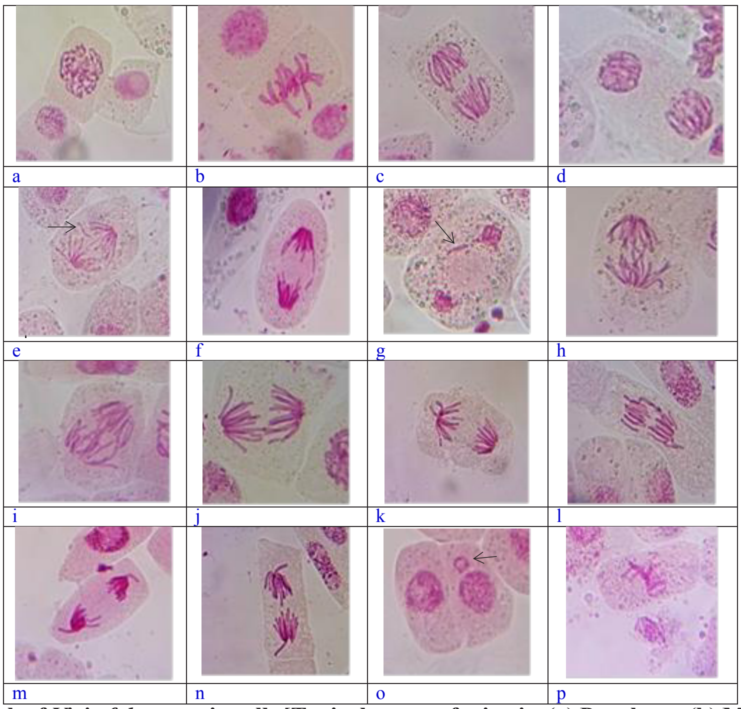

Fig. 3. Micrograph of *Vicia faba* root tip cells [Typical stages of mitosis: (a) Prophase; (b) Metaphase; (c) Anaphase; (d) Telophase], [Chromosomal aberrations after NaCl treatment: (e, f, g) Laggards; (h, i, j) Chromosome bridge; (k, l, m, n) Chromosome disorders; (o) Micronuclei; (p) Stickiness (Scale bar = 10µm).

Chromosomal aberrations include structural aberrations (clastogenesis) and spindle malfunction which affects chromosome number (aneugenesis). Kiełkowska (2017a) reported that, observed chromosome breaks state clastogenic action, while chromosome stickiness may be a consequence of inter-chromosomal linkages combined with excessive formation of nucleoproteins (Leme & Marin-Morales, 2009). Bridges impelled from dicentric chromosomes caused by botched repairs of the double strand DNA breaks or the fusion of telomere ends.

Lagging chromosomes resulted most probable from the disappointment in assembly of spindle apparatus (Tabur & Demir, 2009; Utani et al., 2010).

Sodium chloride not only causes structural aberrations, it also alters the functional properties of the mitotic spindle in dividing cells and prompts chromosome disorders. NaCl may bind onto tubulin and may either inhibit tubulin assemblage or cause the depolymerization of the previously gathered microtubules. As a consequence of microtubule disturbance, the mitotic spindle does not form, instigating a misalignment and chromosome non-disjunction during mitosis (Souguir et al., 2018).

The observed micronuclei in this study were previously recorded in Vicia faba cells by Souguir et al. (2018). They studied the nuclear abnormalities induced by NaCl and reported that, micronuclei mainly originate from centric chromosome fragments, centric chromatid fragments, or entire chromosomes failing to be included in the daughter nuclei at the end of the telophase. This was formerly described by Khanna & Sharma (2013), who found out that micronuclei can produced due to the development of an sequestered chromosome from unequal dissemination of genetic material.

Salt stress enhances an overproduction of reactive oxygen species (ROS), and this irregularity in ROS causes a remarkable delay in the switch from prophase to pro- metaphase, which influences chromosome separation in anaphase, chromosomal development, and nuclear dynamics (Foreman et al., 2003; Teixeira, 2018). Some reports demonstrated an overproduction of ROS in plants under brackish conditions (Souguir et al., 2015; Wang et al., 2015) which triggered harm cell structures and macromolecules, particularly DNA. Sobieh et al. (2019) reported that, with the increase of salt stress there was a significant accumulation of osmoprotectants (proline) and induction of DNA reparations.

Results presented in Table 1 showed that, bagasse successfully alleviated the frequency of chromosomal aberrations in all salt concentrations tested. Total number of aberrations in samples treated with 100, 200 and 300mM NaCl + bagasse was decreased to 20.7, 31.1 and 5.5, respectively, compared to the same concentrations without bagasse.

Growth parameters

Plant height

Plant height is an essential parameter for plant development assurance. The effect of salinity levels on plant height were presented in Table 2 and Fig.4 . The highest value of plant height (46.8cm) was obtained from control plants, while plants under salt stress revealed a remarked lessening in plant height proportional to the increase of NaCl concentration (100, 200 and 300mM NaCl) recorded as 40.6, 38.2 and 28.7cm, respectively. Samples irrigated with 100, 200 and 300mM NaCl+ bagasse showed plant height values of 45.8, 39.5 and 34.2cm, respectively which were higher than the same concentrations without bagasse.

These results were supported by many preceding studies on plants which informed that root and shoot length were affected undesirably by salt stress (Gama et al., 2007; Kaymakanova et al., 2008; Abdul Qados, 2011; Hasanuzzaman et al., 2012; Mahdi, 2016; Puvanitha & Mahendran, 2017). Köksal et al. (2015) reported that, cytokinesis and cell development are inhibited as a harmful impact of salts. In addition, the increase in osmotic pressure nearby the roots as a result of saline atmosphere can also avert water uptake by roots, causing shorter root length and plant height (Mensah et al., 2006; Sadat-Noori et al., 2008).

Leaf area

Leaf area speaks to a proportion of plant development, which can be influenced by various anxieties, comprising salt stress. The findings recorded in Table 2 established the response of leaves to salt stress. Generally, the results revealed a reduction in leaf area with cumulative salinity. In comparison to control plants, leaf area values for the 100, 200 and 300mM NaCl treatments decreased to 20.67, 16.2 and 12.7cm^2, respectively.

TABLE 2. Plant height (cm) and leaf area (cm²) values in *Vicia faba* plants exposed to different concentrations of NaCl and bagasse.

Concentrations (NaCl +Bagasse)	Plant height (Mean ± SE)	Leaf area (Mean ± SE)
Control	46.8 ± 1.2ab	25.13 ±0.4a
Control + B	46.9 ± 0.9a	24.80 ±0.6a
100	40.6 ± 0.5c	20.67 ±0.5b
100 + B	45.8 ± 0.5b	23.70 ±0.2a
200	38.2 ± 0.9c	16.20 ±0.3c
200 + B	39.5 ± 0.4c	20.59 ±0.2b
300	28.7 ± 0.2d	12.70 ±0.9d
300 + B	34.2 ± 1.5c	16.40 ±0.8c

Values with the same letter within the same column are not significant (P< 0.05) according to the Duncan Multiple Range Test at 5% significant level.

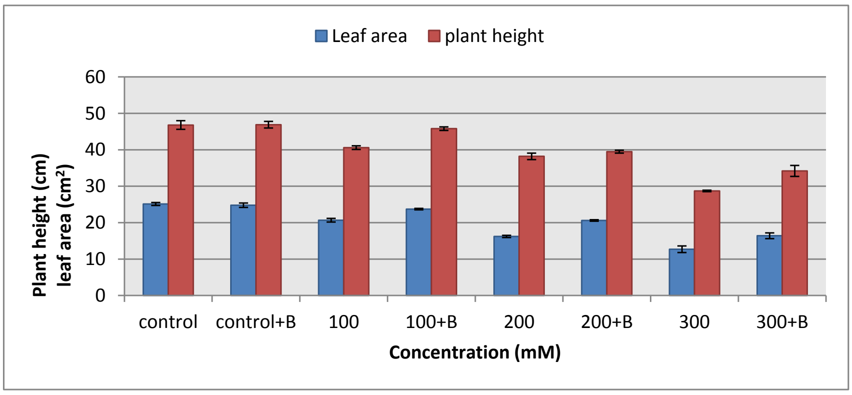

Fig. 4. Plant height (cm) and leaf area (cm²) values in *Vicia faba* plants exposed to different concentrations of NaCl and bagasse.

Applying bagasse to the irrigation water showed that, samples irrigated with 100, 200 and 300mM NaCl + bagasse recorded leaf area values of 23.7, 20.59 and 16.4cm², respectively which was higher than the same concentrations without bagasse (Table 2 and Fig. 4).

Decreases in leaf area possibly perform a result of the harmful effect of stress on plant cell growth and division due to turgor defeat in prolonged cells. Reductions in leaf growth rates can be recognized to hasty leaf senescence and defoliation enhanced due to imperfect water uptake by roots, as reported by Passioura & Angus (2010).

Results in the present study come to an agreement with Mathur et al. (2006). He reported that, stress of the moth bean plant (*Vigna aconitifolia* L.) with expanding merging of sodium chloride, provoked a lessening in leaf area. This diminution was inversely proportional to the concentrations. Also, a noticed decrease in leaf area of cabbage in response to salt stress using different concentrations (0.0, 50, 100, 150mM) of sodium chloride, has been reported (Jamil et al., 2007). Other supporting results embrace those of Zhao et al. (2007) by way of their study on oat (*Avena sativa* L.). They found that, the revelation to saltiness by NaCl diminished leaf area. This remarkable decrease in leaf area recorded in this study as a result of the treatment with augmented concentrations of sodium chloride, could be explained by the adverse effect of salt on photosynthesis that leads to the reduction of plant growth, leaf development, and chlorophyll content (Netondo et al., 2004).

Plants exposed to high saline conditions can't engross sufficient water for metabolic activities or keep up turgidity because of the low osmotic potential in the media. Concurrently, plants absorb impairing amounts of Na^+ and Cl^-. Na^+ is the essential driver of ion explicit damage, upcoming due to a series of complaints in catalyst initiation and protein synthesis (Tester & Davenport, 2003; Shawquat et al., 2014).

Nessem & Kasim (2019) mentioned that among the more deleterious effects of salinity stress on plants are the significant reductions in various growth parameters (Rahneshan et al., 2018).

Conclusion:

Bagasse as a natural solid phase extractor with some advantages: (a) Inexpensive (sugar cane as an industry wastes) (b) Marked capability for high extraction of sodium ions with no need for chemical modification. The present work suggests that, adequate supply of bagasse to the saline irrigation water can mitigate plant growth and development by suppressing cellular damage induced under salt stress.

Ethical approval: Not applicable.

References

Abdul Qados, A.M. (2011) Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences*, **10**, 7-15.

Ahmed, S.A. (2011) Batch and fixed-bed column techniques for removal of Cu(II) and Fe(III) using carbohydrate natural polymer modified complexing agents. *Carbohydrate Polymers*, **83**, 1470-1478.

Ahmed, S.A. (2015) Removal of lead and sodium ions from aqueous media using natural wastes for desalination and water purification. *Desalination and Water Treatment*, **57**(19), 8911-8926.

Akinboro, A., Mohamed, K. B., Asmawi, M.Z., Sofiman, O. A. (2011) Mutagenic and antimutagenic potentials of fruit juices of five medicinal plants in *Allium cepa* L.: Possible influence of DPPH free radical scavengers. *African Journal of Biotechnology*, **10**, 10520- 10529.

Altundogan, H.S., Arslan, N.E., Tumen, F. (2007) Copper removal from aqueous solutions by sugar beet pulp treated by NaOH and citric acid. *Journal of Hazardous Materials*, **149**, 432-439.

Claeys, H., Landeghem, S.V., Dubois, M., Maleux, K.and Inzé, D. (2014) What is stress? Dose-response effects in commonly used *in vitro* stress assays. *Plant Physiology*, **165**, 519-527.

Foreman, J,. Demidchik, V., Bothwell, J.H., Mylona, P., Miedema, H., Torres, M.A., et al. (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature*, **422**(6930), 442-6.

Francis, D. (2007) The plant cell cycle—15 years on. *New Phytologist*, **174**, 261-278.

Gama, P.B.S., Inanaga, S., Tanaka, K., Nakazawa, R. (2007) Physiological response of common bean (Phaseolus vulgaris L.) seedlings to salinity stress. *African Journal of Biotechnology*, **6**(2), 79-88.

Gaulden, M.E. (1987) Hypothesis: Some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. *Mutagenesis*, **2**, 357-365.

Hasanuzzaman, M., Hossain, M.A., da Silva, J.A., Fujita, M. (2012) Plant response and tolerance to abiotic oxidative stress: Antioxidant defense is a key factor. In: "*Crop Stress and its Management: Perspectives and Strategies*", pp. 261-315.

Jamil, M., Rehman, S., Rha, E.S. (2007) Salinity effect on plant growth, ps11 photochemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea capitata* L.). *Pakistan Journal of Botany*, **39**(3), 753-760.

Khanna, N., Sharma, S. (2013) *Allium cepa* root chromosomal aberration assay: A review. *Indian Journal of Pharmaceutical and Biological Research*, **1**(3), 105-119.

Kaymakanova, M., Stoeva, N., Mincheva, T. (2008) Salinity and its effect on physiological response of bean (*Phaseolus vulgares* L.). *Journal of Central European Agriculture*, **9**(4), 749-756.

Kiełkowska, A. (2017a) *Allium cepa* root meristem cells under osmotic (sorbitol) and salt (NaCl) stress *in vitro*.*Acta Botanica Croatic*a, **76**(2), 146-153.

Kiełkowska, A. (2017b). Cytogenetic effect of prolonged in vitro exposure of *Allium cepa* L. root meristem cells to salt stress. *Cytology and Genetics* **51**, 478-484.

Kim, D.H., Aldridge, K.T., Brookes, J.D., Ganf, G.G. (2013) The effect of salinity on the germination of Ruppia tuberosa and Ruppia megacarpa and implications for the Coorong: A coastal lagoon of southern Australia. *Aquatic Botany*, **111**, 81-88.

Kitsios, G., Doonan, J.H. (2011) Cyclin dependent protein kinases and stress responses in plants. *Plant Signaling & Behavior*, **6**, 204-209.

Köksal A., Dursun B., Nazmi D., Cihan K. (2015) Impact of salinity stress on growing, seedling development and water consumption of peanut (Arachis hypogaea cv. NC-7). *Akdeniz Üniversitesi Ziraat Fakultesi Dergisi*, **28**, 77-84.

Kumar, R., Barakat, M.A., Soliman, E.M. (2014) Removal of tannic acid from aqueous solution by magnetic carbohydrate natural polymer. *Journal of Industrial and Engineering Chemistry*, **20**, 2992-2997.

Leme, D.M., Marin-Morales, M.A. (2009) *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research*, **682**, 71-81.

Luo, X., Deng, Z., Lin, X., Zhang, C. (2011) Fixed-bed column study for Cu2+ removal from solution using expanding rice husk. *Journal of Hazardous Materials*, **187**, 182-189.

Mahdi, A.H.A. (2016) Improvement of salt tolerance in *Vicia faba* (L.) plants by exogenous application of polyamines. *Egyptian Journal of Agronomy*, **38**(1), 1-21.

Mahfouz, H., Rayan, W.A. (2017) Antimutagenics effects of stigmasterol on two salt stressed *Lupinus termis* cultivars. *Egyptian Journal of Genetics and Cytology*, **46**, 253-272.

Mathur, N., Singh, J., Bohra, S., Bohra, A., Vyas, A. (2006) Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. *American Journal of Plant Physiology*, **1**(2), 210-213.

Mensah, J.K., Akomeah, P.A., Ikhajiagbe, B., Ekpekurede, E.O. (2006) Effects of salinity on germination, growth and yield of five groundnut genotypes. *African Journal of Biotechnology*, **5**(20), 1973-1979.

Mohsen, A.A., Ebrahim, M.K.H., Ghoraba, W.F.S. (2013) Effect of salinity stress on *Vicia faba* productivity with respect to ascorbic acid treatment. *Iranian Journal of Plant Physiology*, **3**(3), 725-736.

Morsi, M.M., Hammad, D.M., Rashwan, R.S. (2016) Efficiency of three bio-components against broad bean beetle (*Bruchidius incarnates*) and their effect on germination, seedling growth and cytogenetic changes of Vicia faba L. plants. Res. *Journal of Pharmaceutical, Chemical and Biological Sciences*, **7**, 1833-1847.

Munns, R., Tester, M. (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, **59**, 651-681.

Murphy, K.S.T., Durako, M.J. (2003) Physiological effect of short term salinity changes on *Ruppia maritime*. *Aquatic Botany*, **75**, 293-309.

Nada, A.M.A., El-Gendy, A.A., Mohamed, S.H. (2010) Banana leaves as adsorbents for removal of metal ions from waste water. *Carbohydrate Polymers*, **82**, 1025-1030.

Netondo, G.W., Onyango, J.C., Beck, E. (2004) Crop physiology and metabolism Sorghum and salinity II – gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Science*, **44**(3), 806-811.

O'Sullivan, D.M., Angra, D. (2016) Advances in faba bean genetics and genomics. *Frontiers in Genetics*, **7**, 1-12.

Passioura, J.B., Angus, J.F. (2010) Improving productivity of crops in water-limited environments. *Advances in Agronomy*, **106**, 37-75.

Puvanitha, S., Mahendran, S. (2017) Effect of salinity on plant height, shoot and root dry weight of selected rice cultivars. *Scholars Journal of Agriculture and Veterinary Sciences*, **4**(4), 126-131.

Renjana, P.K., Anjana, S., Thoppil, J.E. (2013) Evaluation of genotoxic effects of baking powder and monosodium glutamate using Allium cepa assay. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5**, 311-315.

Sadat-Noori, S.A., Mot,taghi, S., Lotfifar, O. (2008) Salinity tolerance of maize in embryo and adult stage. *American-Eurasian Journal of Agricultural & Environmental Sciences*, **3**(5), 717-725.

Sobieh, S.S., Mohamed, T.R., Adam, Z.M., El-Fiki, A., Awad, A.S. (2019) Salt stress induces changes in genetic composition, proline content and subcellular organization in potato (*Solanum tuberosum* L.). *Egyptian Journal of Botany*, **59**(1), 269-282.

Nessem, A., Kasim, W.A. (2019) Physiological impact of seed priming with $CaCl_2$ or carrot root extract on *Lupinus termis* plants fully grown under salinity stress. *Egyptian Journal of Botany*, **59**(3), 763-777.

Rahneshana, Z., Nasibia, F., Moghadama, A.A. (2018) Effects of salinity stress on some growth physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. *Journal of Plant Interactions*, **13**(1), 73-82.

Shawquat, A.K., Mamun, A.A., Mahmud, A.A., Bazzaz, M., Hossain, A., Alam, S., Shamimuzzaman, Karim A. (2014) Effects of salt and water stress on leaf production, sodium and potassium ion accumulation in soybean. *Journal of Plant Sciences,* **2**(5), 209-214.

Souguir, D., Abd-Alla, H.I., El Ferjani, E., Khouja, M.L., Hachicha, M. (2015) *Aloe vera* long-term saline irrigation increases contents of hydrogen peroxide, lipid peroxidation and phenolic compounds. *Acta Agriculturae Scandinavica, Section B*, **65**, 688-696.

Souguir, D., Abd-Alla, H.I., Hormann, G., Hachicha, M. (2018) Chromosomal and nuclear alterations in the root tip cells of *Vicia faba* induced by sodium chloride. *Water Environment Resarch*, **90**(2), 164-171.

Sun, J,. Chen, S.L., Dai, S.X., Wang, R.G., Li, N.Y., Shen, X., et al. (2009) Ion flux profiles and plant ion homeostasis control under salt stress. *Plant Signaling & Behavior*, **4**, 261-4.

Sun, K., Hunt, K., Hauser, B.A. (2004) Ovule abortion in Arabidopsis triggered by stress. *Plant Physiology*, **135**, 2358-67.

Tabur, S., Demir, K. (2009) Cytogenetic response of 24-epibrassinolide on the root meristem cells of barley seeds under salinity. *Plant Growth Regulation*, **58**, 119-123.

Tabur, S., Demir, K. (2010) Role of some growth regulators on cytogenetic activity of barley under salt stress. *Plant Growth Regulation*, **60**, 99-104. DOI 10.1007/s10725-009-9424-6

Teixeira, B., Pires, S.N., Ávila, G.E., et al. (2018) Cytogenetic activity of root meristems of rice in response to conditioning in carrot extract and salinity. *Journal of Experimental Agriculture International*, **24**(5), 1-12.

Tester, M., Davenport, R. (2003) Na^+ resistance and Na^+ transport in higher plants. *Annals of Botany*, **91**, 1-25.

Utani, K., Kohno, Y., Okamoto, A., Shimizu, N. (2010) Emergence of micronuclei and their effects on the fate of cells under replication stress. *PLoS One*, **5**(4), e10089.

Wang, Y., Shen, W., Chan, Z., Wu, Y. (2015) Endogenous cytokinin overproduction modulates ROS homeostasis and decreases salt stress resistance in Arabidopsis thaliana. *Frontiers in Plant Science*, **6**, 112-119.

West, G., Inze, D., Gerrit, T.S., Beemster, G.T.S. (2004) Cell cycle modulation in the response of the primary root of Arabidopsis to salt stress. *Plant Physiology*, **135**, 1050-1058.

Wiersma, J.V., Bailey, T.B. (1975) Estimation of leaflet, trifoliate and total leaf area of soybean. *Agronomy Journal*, **67**, 26-30.

Yumurtaci, A., Aydin, Y., Uncuoglu, A.A. (2009) Cytological changes in Turkish durum and bread wheat genotypes in response to salt stress. *Acta Biologica Hungarica,* **60**, 221-232.

Zadeh, H.M. (2007) Root apical meristem characteristics of canola (*Brassica napus* L.) in response to salt stress. *Journal of Biological Sciences*, **7**, 1258-1261.

Zhao, G.Q., Ma, B.L., Ren, C.Z. (2007) Growth, gas exchange,chlorophyll fluorescence and ion content of naked oat in response to salinity. *Crop Science*, **47**(1), 123-131.

استخدام المخلفات الطبيعية للحد من الضعف الوراثي الخلوي في نباتات الفول المعرضة لإجهاد الملوحة

رشا كمال حلمي
قسم النبات و الميكروبيولوجي – كلية العلوم – جامعة المنيا - المنيا - مصر.

كلوريد الصوديوم هو الملح الأكثر شيوعًا الذي يسبب إجهاد الملوحة ويؤثر سلبا على نمو النبات عند وجوده بتركيزات زائدة في التربة أو الماء. تم اجراء دراسات للكشف عن تأثير استخدام قصب السكر كأحد المخلفات الطبيعية الزراعية والذي يتميز بخاصية ادمصاص أيونات الصوديوم على سطحه وبالتالى يخفض مستوى الملوحة في الماء. وقد تم اختبار مدى استجابة نباتات الفول المجهدة بالملوحة وتأثير ذلك على معدل الانقسام الخلوي والتشوهات الكروموسومية وبعض مظاهر نمو وحيوية النباتات مثل طول النبات ومساحة الورقة. أظهرت النباتات التي عولجت بتركيزات مختلفة (100، 200، 300 ملى مول) من كلوريد الصوديوم تأثيره السام على الخلايا والذى ينعكس على الحد من نشاط الأنقسام الميتوزى في خلايا القمة النامية للجذر وزيادة تشوهات الكروموسومات مثل تلزن الكروموسومات، الكسور الكروموسومية، الجسور والأنوية الصغيرة. كما أظهرت النباتات المجهدة بالملوحة انخفاضًا ملحوظًا في الطول و مساحة الورقة يتناسب مع زيادة تركيز كلوريد الصوديوم مقارنةً بالنباتات الغير مجهدة (الكنترول). وعند اضافة (bagasse) قصب السكر إلى مياه الري المالحة لوحظ أنه يقلل بشكل كبير من التأثير السلبى للتركيزات المختلفة من كلوريد الصوديوم على معدل الانقسام الميتوزى في خلايا الجذر ويزيد من معايير النمو في النباتات مقارنة بنفس التركيزات دون اضافة (bagasse).