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 adsorb 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
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 (Kielkowska, 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. Kielkowska, 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.dH₂O. 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 (H₂O)  (2)- control +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 (H₂O), (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.

<table>
<thead>
<tr>
<th>Concentrations (NaCl+Bagasse)</th>
<th>Mitotic index (Mean ± SE)</th>
<th>Chromosomal abnormalities</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stickiness</td>
<td>Disordered</td>
<td>Lag.</td>
<td>Breaks</td>
<td>Bridge</td>
<td>Micro-nuclei</td>
</tr>
<tr>
<td>Control</td>
<td>22.94±1.8 a</td>
<td>0.1</td>
<td>0.00</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Control + B</td>
<td>22.61±1.7 a</td>
<td>0.0</td>
<td>0.03</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>100</td>
<td>14.37±0.4 c</td>
<td>7.2</td>
<td>8.1</td>
<td>3.3</td>
<td>7.0</td>
<td>5.4</td>
</tr>
<tr>
<td>100 + B</td>
<td>18.56±0.7 b</td>
<td>4.3</td>
<td>5.3</td>
<td>2.1</td>
<td>5.3</td>
<td>3.7</td>
</tr>
<tr>
<td>200</td>
<td>11.96±0.5 c</td>
<td>10.1</td>
<td>9.0</td>
<td>4.2</td>
<td>8.2</td>
<td>7.1</td>
</tr>
<tr>
<td>200 + B</td>
<td>14.72±0.8 b</td>
<td>8.4</td>
<td>9.0</td>
<td>4.0</td>
<td>5.6</td>
<td>3.3</td>
</tr>
<tr>
<td>300</td>
<td>6.03±0.3 d</td>
<td>3.7</td>
<td>3.4</td>
<td>2.8</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>300 + B</td>
<td>7.43±0.7 d</td>
<td>1.2</td>
<td>1.5</td>
<td>0.0</td>
<td>1.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

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

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 300 mM 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 12.7cm², respectively.
TABLE 2. Plant height (cm) and leaf area (cm²) values in *Vicia faba* plants exposed to different concentrations of NaCl and bagasse.

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

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**


southern Australia. *Aquatic Botany, 111*, 81-88.


organization in potato (Solanum tuberosum L.).


استخدام المخلفات الطبيعية للحد من الضعف الوراثي الخلوي في نباتات القول 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 إجراء دراسات لكشف تأثير استخدام قصب السكر كأحد المخلفات الطبيعية الزراعية والذي يتميز بخصائصه الإزاحية للأيونات الصوديوم على سطحه وبالتالي تخفيف مستوي الملوحة في الماء. وقد تم اختيار مدى استجابات نباتات القول المجهدة بالملوحة وتأثير ذلك على معدل الانقسام الخلوي والتشوهات الكروموسومية وبعض مظاهر نمو وحيوية النباتات مثل طول النبات ومساحة الورقة.

أظهرت النباتات التي عولجت بتكرمات مختلفة (100، 200، 300 ملي مول) من كلوريد الصوديوم تأثيره السام على الخلايا والذي يعكس على الحد من نشاط الانقسام الميتوزى في خلايا القمة النامية للجذر وزيادة تشتت الكروموسومات مثل تلزن الكروموسومات، الكسور الكروموسومية، الجسور والأنوية الصغيرة. كما أظهرت النباتات المجهدة بالملوحة انخفاضًا ملحوظًا في الطول ومساحة الورقة بتناسب مع زيادة تركيز كلوريد الصوديوم مقارنة بالنباتات المجهدة (الكنترول). وعند إضافة الملح، وحالة لوحظ أنها تقلل بشكل كبير من التأثير السلبي للتركيزات المختلفة من كلوريد الصوديوم على معدل الانقسام الميتوزى في خلايا الجذر ويزيد من معايير النمو في النباتات مقارنة بنفس الظروف دون إضافة الملح.