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Impacts of silicon and silicon nanoparticles on leaf cell ultrastructure of pea plant under salinity stress

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Abstract: Salinity is one of the most important environmental stresses that reduce plant growth and makes field crops less productive. Therefore, finding an effective, environmentally acceptable remedy for salinity stress is currently of paramount importance. There are many indications that silicon can ameliorate the injuries caused by high salinity. The current study was carried out to: (1) investigate the effect of NaCl-induced salt stress on the leaf cell ultrastructure of pea (*Pisum sativum* L.) plant, and (2) assess the possible ameliorative effects of silicon (Si) or nanosilicon (NSi) on the ultrastructural properties of pea plants grown in saline conditions. Various concentrations of NaCl (0, 100, 150, 200, and 250 mM) were used alone or in combination with Si (3 mM sodium silicate) or NSi (3 mM silicon dioxide nanoparticles) in this study. Transmission electron microscopy analysis revealed that chloroplasts underwent the most dramatic changes in leaf cell ultrastructure in response to salt stress. TEM micrographs of plants treated with different concentrations of salinity exhibited morphological deformations of chloroplasts such as swelling of thylakoids, disruption of the envelope, accumulation of plastoglobuli, and a reduction in the number of starch grains. The ultrastructural deformities of cellular organelles, especially chloroplasts, caused by salt were similarly seen to be largely repaired after treatments with Si or NSi. Ultrastructural alterations in these plants shed new light on the cellular-level effects of salt. In conclusion, we affirmed that salt stress is harmful to pea plants, and the addition of silicon showed effectiveness in mitigating the saline harmful effects.

keywords: Salinity; *Pisum sativum*; silicon; nano-silicon; ultrastructure

1.Introduction

Pisum sativum L., commonly known as pea, is a highly important legume vegetable crop that is extensively cultivated in Egypt and various other countries worldwide. Its seeds have a protein content of 20-25%, a starch content of 40-50%, and a fiber content of 10-20% [1, 2]. In Asian regions, it is consumed as a green vegetable (whole pods or immature seeds) and as a dried seed in Europe, Australia, America, and the Mediterranean area [3]. This plant is also employed for fodder production [4, 5], in cereal rotations to provide soil nitrogen, and to give disease breaks [6].

While pea has been classified as one of the most salt-tolerant legumes due to a 50% yield loss at 100 mM NaCl [7], its productivity was significantly reduced at higher salinity levels [8]. Salinity is one of the most severe environmental limitations affecting agricultural output, and it is caused by the presence of too much salt in both soil and irrigation water [9]. The reduction in plant growth and productivity resulting from salinity is associated with various factors, including osmotic stress, specific ion toxicity, imbalanced nutrition (primarily due to heightened Na^+ uptake at the cost of K^+), and oxidative harm [10]. The generation of reactive oxygen species (ROS) is associated with the adverse impact of salt stress on plants [11]. ROS includes free radicals such

as superoxide anions ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$) as well as non-radical molecules like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) [12]. To increase pea output in adverse conditions, it is crucial to discover effective and environmentally safe salt stress alleviators. Silicon (Si) is widely acknowledged as a highly beneficial nutritional element for the growth and development of plant organisms [13]. The literature indicates that Si has the potential to mitigate the deleterious impacts of salt stress on plant growth and development [14]. Numerous research studies have reported that silicon reduces the negative effects of salinity in wheat [15], barley [16-18], rice [19], cucumber [20], tomato (*Solanum lycopersicum* L. cv. Hong mei) [21], spinach and tomato [22], canola [23], and maize [24]. There have been reports that treating plants with Si increases growth and yield via altering the ultrastructure of leaf organelles, triggering plant defense systems, and attenuating the effects of certain ions. [24].

The unique physicochemical properties of nanoparticles compared to bulk particles have piqued the curiosity of scientists [25]. The unique characteristics of nanomaterials come from their little size. Their solubility and surface reactivity are enhanced because their surface area is greater than that of bulk materials [26]. One of the helpful nanomaterials that has been reported to have a good effect on current agriculture is nano-silicon (NSi) [27]. Because of studies of the potential of NSi to reverse the harmful effects of salt on plant development rates, the function of NSi in the alleviation of salt stress has recently attracted widespread attention.

Damage to cellular structures and disruption of normal physiological processes may result from exposure to abiotic stress. It would suggest that the chloroplast is the principal target of the stress [28]. Because salt damage is caused mostly by Na^+ accumulation in cells and cellular alterations of organelles under salt stress [29], transmission electron microscopy (TEM) is a common method for visualizing cellular structures with nanometer precision.

In view of the importance of Si in salt tolerance, it is hypothesized that Si can protect plants against the effects of salinity stress. Therefore, the main objectives of this study are to investigate (1) the effect of salt stress on the leaf ultrastructure of pea plants and (2) the impact of Si or NSi on the ultrastructure properties of pea plants under salinity stress conditions.

2. Materials and methods

2.1. Plant materials and growth conditions

Pea (*Pisum sativum* L.) seeds of the pure strain Intisar 1 were provided by the Central Administration for Seed Production, Horticulture Research Institute, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Sodium silicate (which may be purchased) was employed as the silicon source. NanoTech Egypt supplied the powdered SiO_2 nanoparticles used in Photo-Electronics. From November 2018 to February 2019, researchers from Mansoura University's Faculty of Agriculture performed an outdoor pot experiment at the university's Botanic Garden. Pea seeds that were selected because they were healthy and uniform in size were soaked for three hours in tap water after being rinsed with a 0.001 M mercuric chloride ($HgCl_2$) solution for one minute. The seeds were planted in sterilized plastic pots (22 cm in diameter) filled with a soil mixture of 2:1 clay to sand by volume. Ten seeds were sown in a container with uniform soil weighing 7 kg. There were three distinct piles of pots. The first group served as the unmodified control. The Si treatment group was the second. The NSi group made up the last category. Nanoparticles of silicon and silica (silicon dioxide) were spread out across the ground. When the seedlings had grown two leaves, or two weeks after sowing, in the first group, water was used to irrigate the soil to field capacity (500 ml), in the second group, sodium silicate solution was used, and in the third group, a nano- silica suspension was used. For two weeks (every five days), the therapies were administered. At the sixth-leaf stage, 5 days after the final treatment, each group was split into five subgroups based on the salt irrigation they received (0 mM, 100 mM, 150 mM, 200 mM, and 250 mM NaCl solution). Therefore, in a very randomized design, all fifteen treatments were intended uses of penta-replicated. There were five sets (one pot of six plants per replication) for each treatment. A salt treatment of 250 cc NaCl

solution was applied to the soil of 30-day-old seedlings. Two weeks at 5-day intervals of salt irrigation was used. After 15 days under the salt treatment, the plants were harvested in their vegetative state and samples were taken for transmission electron microscopy.

2.2. Transmission Electron Microscopy

The fifth fully expanded leaves, numbered basipetally, were sampled for ultramicroscopic examination. The freshly excised leaves of control and treated plants were submerged in a modified solution (2.5% buffered glutaraldehyde and 2 % paraformaldehyde in 0.1 M Sorensen sodium phosphate buffer pH 7.4), cut into small pieces (about 1-2 mm in diameter) and fixed immediately in the same solution for 24 h at 4° C [30]. The samples were washed in three changes each for15 minutes with the same phosphate buffer and 0.1 M Sucrose then post-fixed in 2 % sodium phosphate buffered osmium tetroxide OsO_4 pH 7.4 for another 90 min. at room temperature. After post fixation, samples were rinsed three times each for 15 min. with 0.1 M sodium phosphate buffer pH 7.4. Samples were then dehydrated two times each for 15 min. using 50 % ethanol and stained overnight using 70 % acetone + 0.5 % (w/v) uranyl acetate + 1% phosphotungstic acid at 4° C. Complete dehydration was achieved by subjecting the specimens to a succession of ethanol concentrations ranging from 30% to 100% at room temperature, followed by a 15-20 minute soak in absolute acetone. The samples were dehydrated, and then Epon resin was used to permanently embed them. Polymerization was place for three days at 40 °C, one day at 45 °C, and three days at 60 °C after the specimens were placed into freshly made Epon solution and inserted in gelatin capsules.

Using an ultramicrotome, 1 m thick semithin slices were cut, mounted on slides, and stained with toluidine blue and azure II for 1–2 minutes each before being dried on a hot plate for 5 minutes. The semithin slices were mounted with Entellan and analysed under an Olympus photomicroscope. Select regions of mesophyll tissues were cut off of blocks.

Ultra-thin slices of 150-300 A (15-30 nm) were produced from the designated areas and put on film-coated 100 or 200-mesh copper grids using an ultramicrotome (Reichert Supernova, Germany). Before being kept in a grid box, ultrathin sections were post-stained with 8% uranylacetate for 10 minutes, followed by 0.7% lead citrate and 0.9% sodium citrate for 5 minutes [31]. Electron micrographs were acquired at 80 kV using a JEOL JEM -2100 electron microscope at EM Unit, Mansoura University, Egypt to investigate materials on grids.

3. Results and Discussion

The leaves are responsible for the bulk of plant essential functions such as dry matter generation, respiration, and transpiration [32]. Because the leaf is the organ that may change the most in reaction to its environment [33, 34], its structure demonstrates the impacts of stress more vividly than the stems or roots. The effects of varying salinity concentrations, either alone or in combination with Si or NSi, on the ultrastructure of pea leaves were examined using transmission electron microscopy in this work.

The numerous defects and alterations in organelles of pea mesophyll cells were detected using transmission electron microscopy. The principal alternations detected in different treatments were discovered in the chloroplasts, according to TEM micrographs of pea leaves treated with varied salt concentrations. Chloroplasts are the primary site of photosynthesis, where both light and dark reactions occur. Chloroplasts are crucial in studying plant responses to many stressors, including salt. Alterations in the ultrastructure of plant cells are frequently the first observable signs of stress-induced damage [35].

Transmission electron microscopy of control pea leaves (Figure 1, A and B) revealed normal mesophyll cells surrounded by a plasma membrane and cell wall with integral cytoplasmic contents. The cytoplasm contained normal chloroplasts with starch grains, mitochondria, vacuole and nucleus having nucleolus. The mitochondria are small and round. Chloroplasts ultrastructure showed the normal appearance of typical chloroplast structure; it was mainly ellipsoidal-shaped, and their length exceeded at least twice the width. They possessed clearly defined envelope membranes and a well-developed internal

membrane system composed of thylakoid membranes organized in grana and interconnecting stromal lamellae aligned in parallel with the chloroplast axis. Grana were distributed in a regular manner throughout the sectioned areas of chloroplasts, and granal thylakoids were well-developed and packed closely together. The elongated oval-shaped chloroplasts exhibited condensed stroma matrix and were evenly distributed around the periphery of mesophyll cells (Figure 1, A and B). Starch grains and very small amounts of darkly stained plastoglobuli were scattered in the stroma of these healthy chloroplasts.

The ultrastructural analysis of pea leaves treated with Si alone (Figure 1, C and D) showed no changes compared with the control except that the number of starch grains in the chloroplast was increased compared with the control. In addition, ultrastructural analysis of pea leaves treated with NSi alone (Figure 1, E and F) showed no changes compared with the control except for the number of starch grains in chloroplasts and the number of mitochondria in the cytoplasm, which were increased compared with the control.

In contrast, ultrastructural alternations were observed in leaf mesophyll cells after treating pea plants with different concentrations of salinity. Salinity stress elicited changes in the quantity, size, shape, and ultrastructure of chloroplasts in leaf cells exposed to varying salinity concentrations (Figures 2- 5, A and B). Leaves of NaCl-treated plants, as shown in transmission electron micrographs, had fewer chloroplasts per cell than controls. Similar to our findings, others have found that during salt stress, the number of chloroplasts per cell in aubergine decreases, and the chloroplasts become larger and more spherical due to damage to the cell membrane [36]. The number of chloroplasts in potato (*Solanum tuberosum* L.) plantlets reduced significantly as the external NaCl concentration and treatment period increased [37].

By disrupting the chloroplast organization, salt stress can directly affect photosynthesis in plants [38]. Salinity stress produced morphological deformations of chloroplasts in pea leaf mesophyll cells, such as enlargement of thylakoids, loss of grana, rupture of chloroplast membrane, and increased abundance of plastoglobuli, according to the TEM data. As was previously demonstrated in salt stressed *Cucumis sativus* L., this chloroplast modification represents a typical salinity response [39]. Under salt stress, similar outcomes were reported in quinoa [40], potato plantlets [37], cotton [41], and barley [42]. It was discovered in the plant *Thellungiella salsuginea* [43] that high salinity (400 mM NaCl) has a strong effect on the ultrastructure of the chloroplasts, with pronounced swelling of thylakoids, most likely due to an imbalance in the osmotic equilibrium of organelles, as previously demonstrated [37, 44]. It is possible that photooxidative damage brought on by an accumulation of reactive oxygen species (ROS) disintegrated the chloroplast grana more readily than the stroma [45]. NaCl stress causes damage to the thylakoidal structure, and Hernandez et al. [46] hypothesised that reactive oxygen species had a role in this damage. Thylakoid expansion appears to be caused mostly by reactive oxygen species (ROS), namely the hydroxyl radical and hydrogen peroxide [47]. Long-term (30-day) NaCl treatments were also found to alter the ultrastructure of the chloroplasts in potato (*Solanum tuberosum* cv. Désirée) leaves, leading the researchers to conclude that oxidative salt stress was likely to blame for the structural change in the photosynthetic apparatus. This is because thylakoid membrane damage is a commonly reported symptom of oxidative stress [48]. Concurrently, grana degradation was linked to an increase in the number of plastoglobuli (lipid droplets) in chloroplast stroma, suggesting lipid breakdown in the thylakoid membrane [49]. In the current work, TEM revealed an increase in the quantity and size of plastoglobuli (lipid droplets) in salt-treated pea leaves. Plastoglobuli, which were numerous and large in chloroplasts from salt-stressed plant leaves, might have been produced by lipid release after thylakoid death under salt stress [50] or by early cellular ageing and senescence [51]. There was an increase in the number of plastoglobuli in the chloroplasts of salt-treated barley [42], *Kandelia candel* [52], and *Sulla carnosa* [53]. Changes in chloroplast ultrastructure seen in salt-treated plant mesophyll cells are indicative of chloroplast

metabolic disorders. Salt-sensitive enzymes in the photosynthesis carbon reduction cycle may have been inhibited, leading to a decline in photosynthesis and growth [54].

The consequences of salinity stress are both ionic and osmotic [55]. Yamane et al. [56] treated hydroponic rice plants with NaCl and polyethylene glycol (PEG) for three days at a water potential of -1.0 MPa. NaCl caused thylakoids to expand and the chloroplast membrane to be somewhat destroyed. The chloroplast envelope was severely damaged by PEG, but the thylakoids did not enlarge. They proposed that the ionic effects of NaCl caused thylakoids to enlarge, while the osmotic impacts destroyed the chloroplast membrane [56].

The number of starch grains per chloroplast was likewise reduced in leaves from salt stressed pea plants compared to control plants grown under normal conditions, and the magnitude of the loss was depending on the salt concentration. This reaction is consistent with ultrastructural studies of NaCl-treated pea and sweet potato plants [46, 57]. Fidalgo et al. [48] discovered a reduction in carbohydrate concentration in potato chloroplasts after treatment with 200 mM NaCl. Under high salt stress (200 mM NaCl), Yue et al. [58] found that starch granules in black locust were smaller and less well defined. Starch granules were observed to grow in the chloroplasts of potato, quinoa, and eggplant plants in response to salt, which ran counter to the results of [37], [40], and [59]. Plants may alter their ratio of soluble sugars to starch in response to salt, which increases their osmotic potential. Sugar accumulation and starch depletion were also demonstrated by other researches [60]. The current investigation demonstrated that chloroplasts were progressively degraded to the point of full disorganization when NaCl concentration was increased. Enhanced concentrations of Na+, which was likely transported into the cytosol via non-selective cation channels (NSCCs), high-affinity K^+ transporters (HKTs, likely HKT1;2; HKT1; 4; HKT1; 5 and HKT 2;1), and Cl, which was likely transported via cation-Cl cotransporter (CCC) [61-68], may have induced the aforementioned ultrastructural changes.

Use of Si or NSi, on the other hand, mitigated the negative effect of salinity on chloroplast ultrastructure. Si or NSi treatment retained the internal lamellar system in the chloroplasts of salt-stressed pea leaves, and the chloroplasts had less osmiophilic plastoglobuli (Figures 1-5). Gengmao et al. [69] found that exogenous K_2SiO_3 assisted in the maintenance of chloroplast lamellae in a salt stressed Japanese honeysuckle plant. El-khawaga [42] discovered that exogenous remission of K_2SiO_3.nH2O reduced the structural alterations of chloroplasts caused by salt stress and retained a well-preserved internal lamellar system in the chloroplasts of salt-stressed leaves, as well as fewer osmiophilic plastoglobuli. Si or NSi treatment also enhanced the quantity of starch grains per chloroplast. This increase in starch grains appears to be due to enhanced net photosynthesis. It was hypothesized that starch production helps to reduce hyperosmotic stress as osmoticum [37]. Goussi et al. [43] investigated the effects of salt stress on the chloroplast ultrastructure in the model halophyte *Thellungiella salsuginea* and found that resistance to mild salinity included starch accumulation as an osmoticum in maintaining chloroplast structural integrity.

The findings of the current study revealed that using Si or NSi assisted to maintain the integrity of the chloroplast ultrastructure, allowing the plant to perform normal physiological processes when under salt stress. The conversion of light energy for photosynthesis requires the structural integrity and orderliness of chloroplasts [42]. Consequently, our findings indicated that silicon was engaged in the protection of the photosynthetic machinery and clearly underlined the role of Si in shielding the plant from the harmful effects of salt.

In conclusion, the data presented here suggest that the salt stress imposed in this study distorted the cellular architecture. The findings also showed that pretreatment of the pea plant with Si or NSi solutions reduced the ultrastructural alterations of the mesophyll cells brought on by salt stress. NSi, in particular, was found to be superior to Si in mitigating negative effects of salt stress.

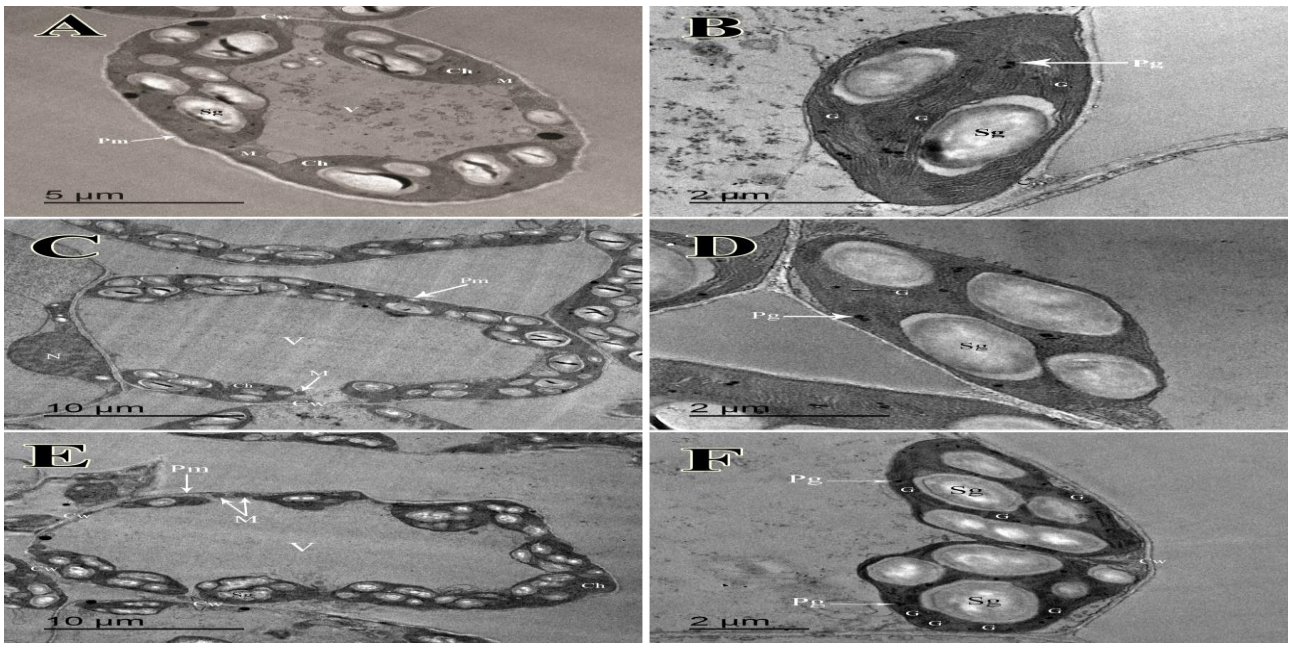

Figure 1: TEM micrographs of *Pisum sativum* leaves of control (not treated) (A, B), treated with Si alone (C, D) and treated with NSi alone (E, F). A. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg), mitochondria (M), vacuole (V), cell wall (Cw) and plasma membrane (Pm). B. A magnified chloroplast with an organized membrane system. G: grana; Pg: plastoglobule. C. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg), mitochondria (M), nucleus (N), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number of chloroplast and an increase in number of starch grains. D. showing part of a mesophyll cell containing chloroplasts with an organized membrane system, numerous big starch grains and plastoglobuli (Pg). G: grana. E. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg), mitochondria (M), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number of chloroplast and an increase in number of starch grains. F. showing part of a mesophyll cell containing chloroplasts with an organized membrane system, numerous big starch grains and plastoglobuli (Pg). G: grana.

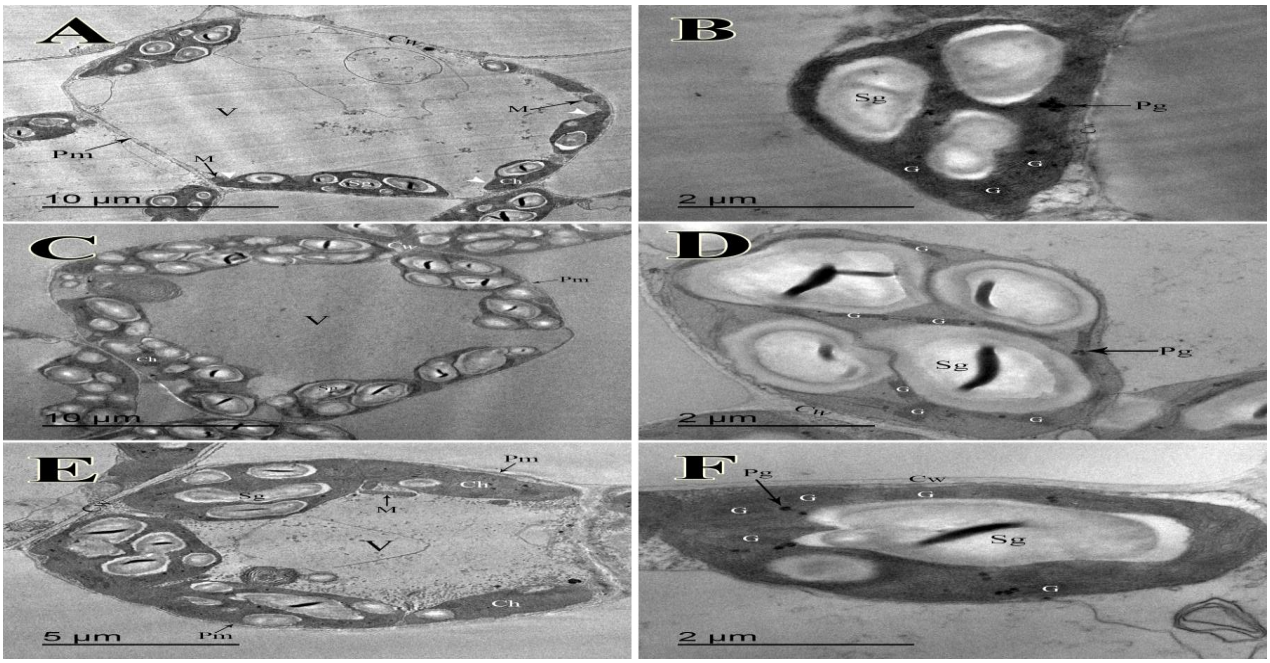

Figure 2: TEM micrographs of *Pisum sativum* leaves treated with 100 mM NaCl alone (A, B), with 100 mM NaCl +Si (C, D) and treated with 100 mM NaCl + NSi (E, F). A. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg),

mitochondria (M), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: decrease in number and size of chloroplast, decrease in number and size of starch grain and tailed chloroplasts (white arrowheads). B. illustrating part of a mesophyll cell containing chloroplasts with starch grains and plastoglobuli (Pg). G: grana. Note: an increase in number of plastoglobule. C. showing a complete mesophyll cell with irregular shaped-chloroplasts (Ch) including starch grains (Sg), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number and size of chloroplast, an increase in number and size of starch grains. D. showing part of a mesophyll cell containing irregular shaped chloroplasts with numerous big starch grains and plastoglobuli (Pg). G: grana. Note: a decrease in number and size of plastoglobule. E. showing a complete mesophyll cell with irregular shaped-chloroplasts (Ch) including starch grains (Sg), mitochondria (M), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number and size of chloroplast, an increase in number and size of starch grains. F. showing part of a mesophyll cell containing irregular shaped chloroplasts with numerous big starch grains and plastoglobuli (Pg). G: grana. Note: a decrease in number and size of plastoglobule.

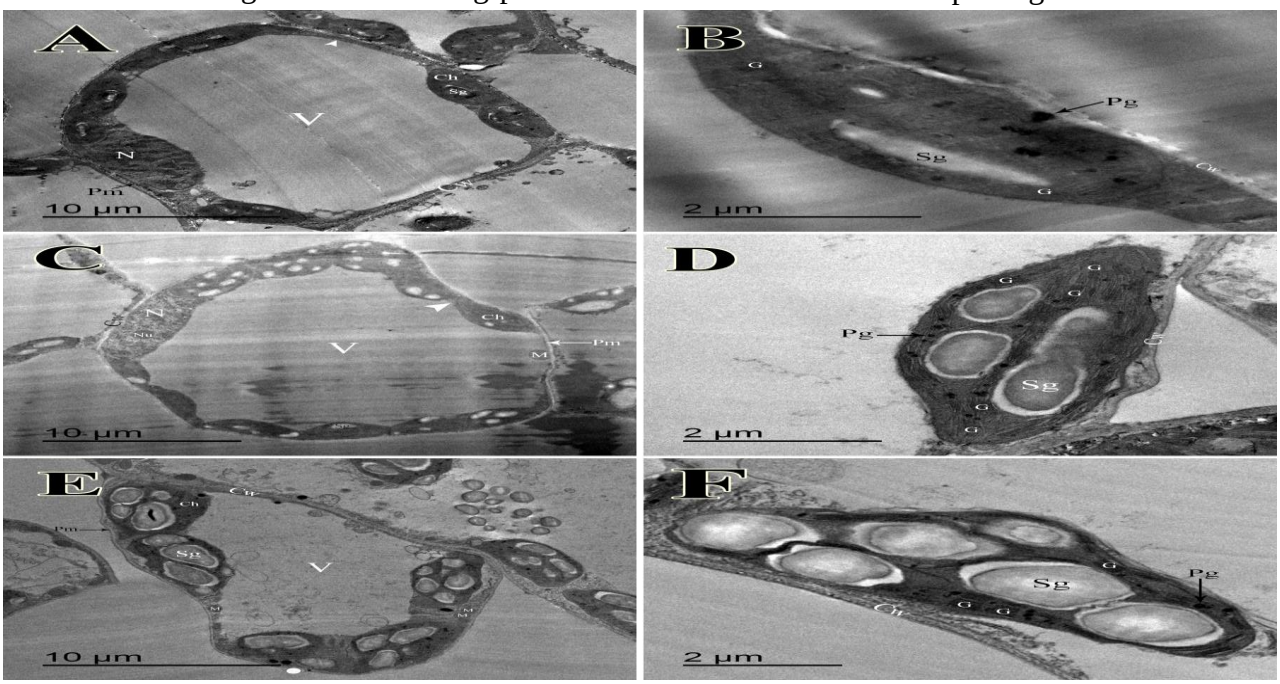

Figure 3: TEM micrographs of *Pisum sativum* leaves treated with 150 mM NaCl alone (A, B), treated with 150 mM NaCl+Si (C, D) and treated with 150 mM NaCl+NSi (E, F). A. showing a complete mesophyll cell with chloroplasts (Ch) including starch grains (Sg), nucleus (N), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: decrease in number and size of chloroplast, decrease in number and size of starch grain and tailed chloroplasts (white arrowheads). Note also unclear membrane system of chloroplast. B. illustrating part of a mesophyll cell containing chloroplasts with starch grains and plastoglobuli (Pg). G: grana. Note: an increase in number and size of plastoglobule. C. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg), mitochondria (M), nucleus (N) with nucleolus (Nu), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number and size of chloroplast and starch grains. D. showing part of a mesophyll cell containing chloroplasts with numerous starch grains and plastoglobuli (Pg). G: grana. Note: clearly arranged thylakoid membranes and decrease in number and size of plastoglobule. E. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg), mitochondria (M), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number and size of chloroplast and starch grains. F. showing part of a mesophyll cell containing chloroplasts with numerous large starch grains and plastoglobuli (Pg). G: grana. Note: clearly arranged thylakoid membranes and decrease in number and size of plastoglobule.

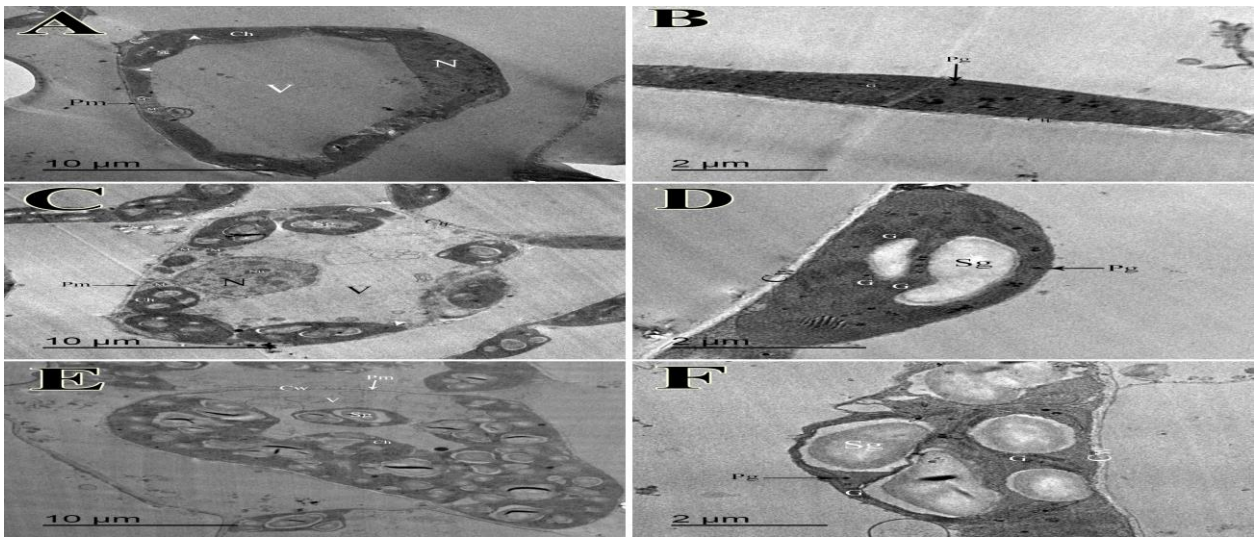

Figure 4: TEM micrographs of *Pisum sativum* leaves treated with 200 mM NaCl alone (A, B), treated with 200 mM NaCl+ Si (C, D) and treated with mM NaCl+ NSi (E, F). A. showing a complete mesophyll cell with chloroplasts (Ch) including starch grains (Sg), nucleus (N), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: deformed membrane system. Note also decrease in number and size of chloroplast, decrease in number and size of starch grain. B. illustrating part of a mesophyll cell containing chloroplasts with disorganized membrane system and totally devoid of starch grains. Pg: plastoglobuli. G: grana. Note: tailed chloroplasts (white arrowheads) and an increase in number and size of plastoglobule. C. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg), mitochondria (M), nucleus (N) with nucleolus (Nu), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number and size of chloroplast and starch grains. D. showing part of a mesophyll cell containing chloroplasts with numerous large starch grains and plastoglobuli (Pg). G: grana. Note: clearly arranged thylakoid membranes and decrease in number and size of plastoglobule. E. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number and size of chloroplast and starch grains. F. showing part of a mesophyll cell containing chloroplasts with numerous large starch grains and plastoglobuli (Pg). G: grana. Note: clearly arranged thylakoid membranes and decrease in number and size of plastoglobule.

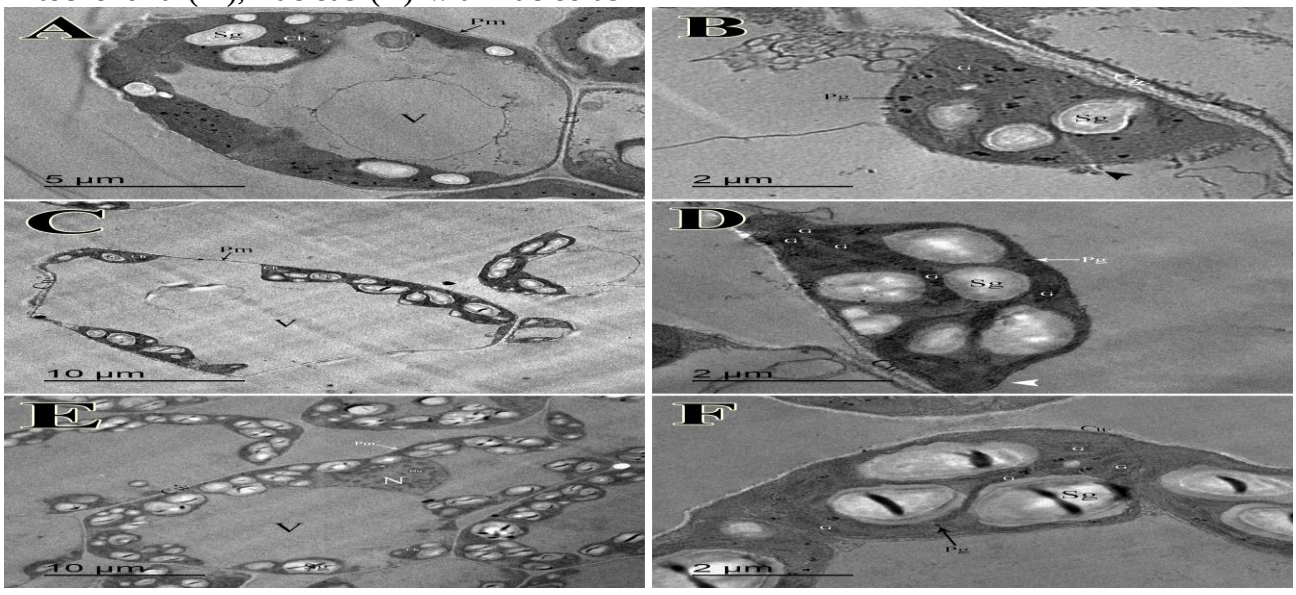

Figure 5: TEM micrographs of *Pisum sativum* leaves treated with 250 mM NaCl alone (A, B), treated with 250 mM NaCl+ Si (C, D) and treated with 250 mM NaCl+ NSi (E, F). A.

showing abnormal mesophyll cell with irregular shape and damaged contents. These cells contain chloroplasts (Ch) including starch grains (Sg), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: decrease in number and size of chloroplast, decrease in number and size of starch grain. B. illustrating part of a mesophyll cell containing chloroplasts with disorganized membrane system. Pg: plastoglobuli. G: grana. Note: tailed chloroplasts (white arrowheads), disintegration of chloroplast envelope (black arrowhead) and decrease in number and size of starch grain. Note also an increase in number and size of plastoglobule. C. showing a complete mesophyll cell with normal chloroplasts (Ch) including starch grains (Sg), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number of chloroplast and starch grains. D. showing part of a mesophyll cell containing chloroplasts with numerous starch grains and plastoglobuli (Pg). G: grana. Note: clearly arranged thylakoid membranes and decrease in number and size of plastoglobule. Note also tailed chloroplast (white arrowhead). E. showing a complete mesophyll cell with irregular shaped-chloroplasts (Ch) including starch grains (Sg), nucleus (N), vacuole (V), cell wall (Cw) and plasma membrane (Pm). Note: an increase in number and size of chloroplast, an increase in number and size of starch grains starch grains. F. showing part of a mesophyll cell containing chloroplasts with numerous large starch grains and plastoglobuli (Pg). G: grana. Note: clearly arranged thylakoid membranes and decrease in number and size of plastoglobule

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