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Cytogenetical and Molecular Responses of Exogenous Potassium Sulphate for Tolerance to Extreme Temperatures in *Vicia faba* L.

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Effects of potassium sulphate (K_2SO_4) on mitosis, cell cycle and chromosomes in *Vicia faba* L. seeds germinated at extreme temperatures were studied as flowcytometrically and cytogenetically. Seeds germinated at high (30°C) and low temperatures (4°C) showed a significant decrease in mitotic index as compared to those of optimum temperature conditions. 50 and 250 μM K_2SO_4 were successful in alleviating the negative effects of high and low temperature on mitotic activity and cell cycle respectively. These concentrations increased the cell division removing or decreasing the negative effects of temperature stress. Chromosomal aberrations were not observed in cells of seeds germinated in distilled water and also at any temperatures. However, the frequency of aberrations increased significantly by increasing K_2SO_4 concentration. The highest aberration frequency in all temperature degree tested was found at 1000 μM K_2SO_4 concentration.

Key words: Cell cycle progression, Chromosomal abnormalities, Flow cytometry, Potassium sulphate, Temperature stress.

Plants are often exposed to a wide range of both biotic and abiotic stresses, and they have developed intricate mechanisms to detect precise environmental changes, allowing optimal responses to adverse conditions[1]. Abiotic stresses, such as salinity, draught, and extreme temperatures cause physiological damages[2]. They elicit complex cellular responses that have been elucidated by progresses made in exploring and understanding plant abiotic responses at the whole-plant, physiological, biochemical, cellular and molecular levels[3]. Under abiotic stress, plant growth is negatively affected as a result of cell cycle inhibition. The perception of stress involves the activation of signalling cascades that result in a prolonged S-phase and delayed entry into mitosis[4].

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Temperature stress is becoming the major concern for plant scientists worldwide due to the changing climate[5]. It has devastating effects on plant growth and metabolism, as these processes have optimum temperature limits in every plant species. The ability of plants to cope with extreme temperature is a complex process and is determined by environmental factors and also by the genetic capability of the plant[6]. Global climate change is making high temperature that it is a critical factor for plant growth and productivity[7]. High temperature stress is defined as the rise in temperature beyond a critical threshold for a period of time sufficient to cause irreversible damage to plant growth and development[8]. The growth and development of plants involves a countless number of biochemical reactions, all of which are sensitive to some degree to temperature[6]. In higher plants, heat stress significantly alters cell division and cell elongation rates that affect the leaf size and weight[9]. Low temperature may affect several aspects of crop growth; survival, cell division, photosynthesis, water transport, growth, and finally crop yield[7].

Proper plant nutrition is one of the good strategies to alleviate the temperature stress. Plant nutrients play a greater role in improving the temperature stress tolerance[10]. A number of studies related to potassium role at environmental stress conditions have been previously reported[11-13].

To stabilize or remove environmental stresses, numerous researches have used plant growth regulations[14-18] and various artificial fertilizers[19,20]. However, it is still completely unknown how the proliferative activities of the meristems and the coordination between cell division and differentiation are maintained under stressful conditions.

The present study was designed to determine the effect of potassium sulphate on cell cycle and mitotic activity in the root tip meristems of *Vicia faba L.* to reveal if the cytotoxic effects on chromosomal behaviours induced by this chemical.

MATERIALS AND METHODS

6-8 uniform-sized Faba bean (*Vicia faba* L. syn. *Faba vulgaris*) seeds were put into Petri dishes covered with two sheets filter papers moistened with 40 ml of distilled water or different potassium sulphate [K_2SO_4] concentrations (10- 50- 250- 1000 µM, micromolal). Seeds were germinated in incubator for optimum and high temperature (20 and 30°C), and also in refrigerator for low temperature (4°C). When the root tips were 1–1.5 cm long, they were cut off, pre-treated with a saturated solution of paradichlorobenzene for 4 h, fixed in ethanol:acetic acid (3:1) for 24 h. The fixed root tips were hydrolysed in 1 N HCl at 60°C, stained with Feulgen for 1–1.5 h, squashed in a drop of 45% acetic acid[21]. Mitotic index, i.e. percentage of dividing cells scored was evaluated by analysing at least 10,000 cells per treatment (3,000 per slide). Chromosomal abnormalities were calculated for each concentration containing abnormal chromosomes divided by the total number of divided cells in the field of view. The abnormal chromosomes were observed at 100X objective on Olympus CX- 41 research microscope and they were photographed with C–5060 WZ camera.

To flowcytometric analysis, the seeds were grown up for 15- 20 day after the 1-1,5 cm root tips were cut off. When the first leaflet of *V. faba* seedling grown out, 50 mg fresh and green tissue from a coloured leaf was excised and placed in 10-mm plastic petri dish on a sterile ice The cell cycle of the each concentrations of each plants were determined using the flow cytometry protocol described by Arumuganathan and Earle[22]. The prepared material was analysed with a standard BD FACSCalibur™ model flow cytometer. The mean cell cycle were analysed 150 000 positively stained cells from ten plants for each concentrations.

All parameters were performed using SPSS programme according to Duncan's multiple range test at level of significance $P < 0.05$[23].

RESULTS

In this work, effects of the potassium sulphate and temperature stress on mitotic activity, cell cycle progression and chromosomal behaviours were investigated. The seeds of *V. faba* were germinated at low (4°C), optimum (20°C) and high (30°C) temperatures, and also different concentrations of K_2SO_4 (0.0 control and 10, 50, 250, 1000 µM K_2SO_4).

The influence of extreme temperature on mitotic activity, cell cycle and chromosomal behaviours in distilled water

Low and high temperature stress had a negative effect on mitotic activity and the cell division was repressed as compared with optimum temperature. For example, while mitotic index were 0.12 for the control group, this value was 0.09 at 4°C and 0.10 at 30°C (Table 1).

Considering all of the temperatures studied in distilled water, the most positive effect on cell cycle progression was exhibited at 20°C with the highest G2/M phase percentage (12.89 %). On the other hand, G2/M phase percentage decreased significantly with low (6.86 %) and high (4.32 %) temperature (Table 1, Fig. 1).

It was not observed chromosomal aberrations in *V. faba* seeds germinated in distilled water and the temperature degrees mentioned above. All mitotic phases were normal (Table 1).

The influence of extreme temperature on mitotic activity, cell cycle and chromosomal behaviours in the medium containing K_2SO_4

Considering all of the temperature degrees, the highest mitotic index values in seeds

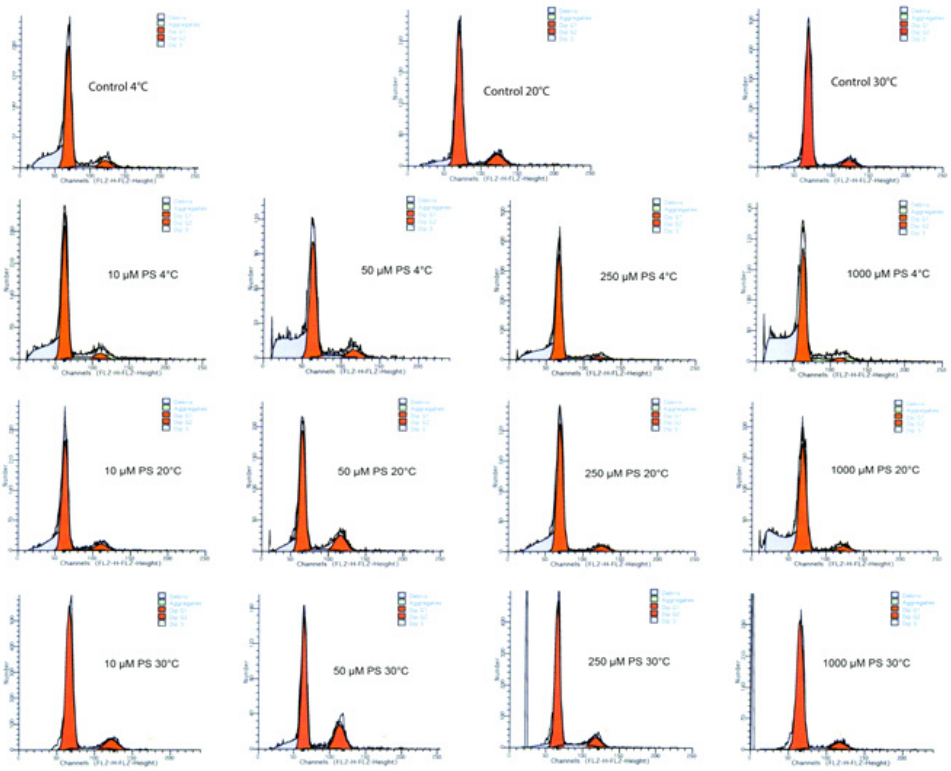

Fig. 1. Flowcytometric histograms of cell cycle progressions of *Vicia faba* germinated in distilled water and different temperature degrees (4°C, 20°C and 30°C)

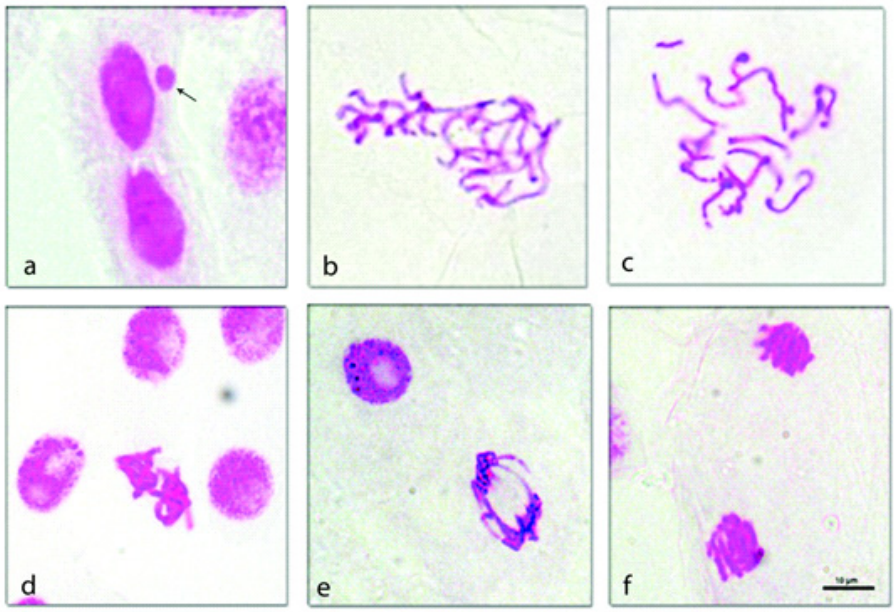

Fig. 2. Chromosomal aberrations of *Vicia faba* seeds germinated in different temperature degrees and various ammonium sulphate concentrations. a, micronucleus (arrow); b,c, uncoiling chromosomes; d, sticky chromosomes; e, anaphase bridge; f, fault polarization, respectively. Scale Bar = 10 µm

Table 1. Mitotic index, cell cycle progression and chromosomal aberration frequencies in *Vicia faba* exposed different temperature degrees and various potassium sulphate (K_2SO_4) concentrations

Temperature (°C)	Potassium sulphate (µM)	Cell Cycle Phases			Mitotic Index (MI)	Chromosome Aberrations (CA)
		G0/G1 (%)	S (%)	G2/M (%)		
4°C	0 (Control)	*85,42±2,71^{bcd}	7,71± 1,21^{abc}	6,86± 2,67^{abc}	0,09±0,02^{ab}	0,00±0,00^{a}
	10	89,50± 2,19^{d}	4,88± 0,71^{ab}	5,63± 1,47^{abc}	0,07±0,01^{a}	0,48±0,11^{bcd}
	50	81,96± 0,01^{bcd}	6,31± 0,01^{abc}	11,74± 0,02^{abc}	0,08±0.01^{a}	0,20±0,05^{a}
	250	79,50± 2,09^{abc}	5,18± 1,20^{ab}	15,32± 0,29^{d}	0,09±0,02^{ab}	0,41±0,01^{bc}
	1000	86,12± 2,96^{bcd}	4,50± 2,34^{ab}	9,38± 1,43^{abc}	0,07±0,08^{a}	0,56±0,03^{cd}
20°C	0 (Control)	83,27± 0,35^{bcd}	2,83± 0,14^{a}	12,89± 0,52^{bc}	0,12±0,01^{bc}	0,00±0,00^{a}
	10	88,98± 0,98^{cd}	6,09± 1,41^{abc}	4,93± 1,63^{ab}	0,09±0,01^{ab}	0,39±0,05^{bc}
	50	78,92± 2,68^{ab}	11,21± 0,05^{d}	9,88± 0,70^{abc}	0,11±0,09^{bc}	0,41±0,04^{bcd}
	250	91,67± 1,50^{d}	5,71± 0,86^{ab}	2,61± 0,06^{a}	0,09±0,02^{ab}	0,44±0,05^{bcd}
	1000	88,58± 0,01^{bcd}	2,66± 0,02^{a}	8,76± 0,01^{abc}	0,16±0,01^{c}	0,50±0,02^{cd}
30°C	0 (Control)	86,55± 0,03^{bcd}	9,12± 0,33^{abc}	4,32± 0,31^{ab}	0,10±0,01^{ab}	0,00±0,00^{a}
	10	87,60± 2,94^{bcd}	3,87± 1,32^{ab}	8,53± 1,70^{abc}	0,06±0,01^{a}	0,22±0,04^{a}
	50	72,28± 0,72^{a}	4,72± 1,65^{ab}	22,99± 0,39^{d}	0,16±0,01^{c}	0,37±0,03^{ab}
	250	79,50± 0,01^{abc}	9,19± 0,02^{bc}	11,31± 0,03^{abc}	0,12±0,01^{bc}	0,50±0,10^{bcd}
	1000	88,02± 1,58^{bcd}	4,42± 0,29^{ab}	7,55± 1,12^{abc}	0,07±0,01^{a}	0,60±0,02^{d}

* Values with insignificant difference (P<0.05) for each column are indicated with same letters (means ± SD)

treated with K_2SO_4 were observed in 250 µM at 4°C (0.09), 1000 µM at 20°C (0.16), 50 µM at 30°C (0.16). 50 and 250 µM concentrations of K_2SO_4 showed the most successful performance on mitotic index at high and low temperatures. The lowest mitotic index values were determined at 10 µM K_2SO_4 concentrations for all temperature degrees (Table 1).

Flow cytometry histograms of cell cycle progression of *V. faba* seeds germinated in media containing different concentrations of K_2SO_4 (10, 50, 250 and 1000 µM) and various temperature degrees are indicated in Figure 1. G2/M phase of 50 and 250 µM K_2SO_4 concentrations showed a perfect success on cell cycle in stress conditions as compared with own control groups. 50 µM K_2SO_4 concentration at 30°C with 22.99 G2/M phase percentage stimulated excessively cell division (Table 1).

Scores of chromosomal aberrations in root tips meristems of *V. faba* exposed to different concentrations of K_2SO_4 and the temperature degrees mentioned above are given in Table 1. The frequency of chromosomal aberrations excessively increased in parallel with K_2SO_4 concentration rise. That is, seeds treated with 1000 µM K_2SO_4 caused the highest frequency of mitotic abnormalities in all temperature degrees. Considering all of the temperature degrees, the highest chromosomal aberrations were observed at 30°C and 1000 µM (0.60). The most prominent aberrations were the micronucleus, uncoiling chromosomes, sticky chromosomes, anaphase bridges and fault polarization in telophase (Fig. 2).

DISCUSSION

Extreme temperatures are the major environmental factors affecting plant growth and development. The high and low temperature stresses induce morphological, physiological and biochemical changes in plants[24-26]. Mineral nutrition of plants plays a critical role in increasing plant resistance to environmental stresses[27]. Among the mineral nutrients, potassium (K) is an important mineral for survival of crop plants under environmental stress conditions. It is essential for many physiological processes, such as photosynthesis, translocation of photosynthesis into sink organs, activation of enzymes under stress conditions[27,28]. Therefore, a number of reports have been published on the effect of artificial fertilizers-especially potassium fertilizers, on growth, development and yield quality in plants[10,29,30].

However, there is only limited information on the effect of these fertilizers on mitotic activity and chromosomal aberrations[20]. The effects of artificial fertilizers on mitotic activity, cell cycle and chromosomal aberrations under abiotic stresses have not yet been explained precisely.

This study was performed to investigate effects of artificial fertilizers on mitotic index, cell cycle and chromosomal aberrations under both low and high temperature stresses.

Mitotic index were 0.12 in seeds germinated at 20°C and in distilled water, whereas seeds germinated at low and high temperature could not reached to this value (see Table 1). In other words, mitotic index decreased with low and high temperature stresses. The inhibitory effects of water stress depend on the high temperature on mitotic activity are known for a long time[26,31,32]. However, there is only a few literature data relating to the influence of the low temperature stress on this parameter[33,34]. In our study, we determined that mitotic activity was highly decreased by the low temperature as compared to optimal temperature (20°C). On the other hand, application of K_2SO_4 50 µM at 30°C and 1000 µM at 20°C was successful to alleviation the inhibitory effects of temperature stress on mitotic activity. Meanwhile, effectiveness of certain concentrations of K_2SO_4 on mitotic index, cell cycle and chromosome aberrations has been presented for the first time in this study.

The cell cycle is an intrinsic part of plant growth and development. However, less is known about how the cell cycle may be affected upon cold stress and how this may affect the plant's survival. Evidence suggests that cell cycle activities are involved in the stress response mediated by transcription factors[35,36]. Xia *et al*.,[37] found that plant growth was inhibited by a prolonged duration of cell cycle under constant 4°C conditions. Low temperature causes a disproportionate lengthening of the G1 phase. Likewise, also in our study, the number of cells in G0/G1 phase was remarkably higher than those of at low temperature as compared with optimum temperature. This also shows that duration of the G0/G1 phase have prolonged under low temperature stress (see Table 1). Furthermore, it was remarked that the temperature at which the cell cycle had a minimum duration closed to 30°C in many species and the rate of DNA replication increased when the temperature was raised[38]. Reduction in the mitotic activity due to slow down the cell cycle could explainable with low S and G2/M phase and high G0/G1 phase. In our study, G2/M phase (12.89) of seeds germinated at optimum temperature was found higher according to those of low (6.86) and high (4.32) temperatures. Decline of cell division rate at low and high temperature was suggested to be related to the activity of the enzyme responsible to mitosis[39]. In all temperature degrees studied, application of K_2SO_4 was lead to changes in cell division rate. The highest G2/M phase percentage in all temperatures studied was observed at 50 and 250 µM K_2SO_4 concentrations as compared to own control groups (see Table 1).

Many studies have been carried out on the effect of temperature, particularly high temperature on cell division and chromosome behaviours[31,32,39-41]. It was reported that root meristems of some crop varieties exposed to high temperature exhibited various chromosome abnormalities such as micronuclei, disturbed metaphase, anaphase bridges, lagging chromosome and sticky chromosomes[32,39]. However, in our work, chromosomal aberrations were not observed in root tip meristems of seeds germinated at low and high temperatures. The reason for this is that upper and lower threshold temperatures may be different for different plant species, and also genotypes within species[8]. According to data in the present study, the frequency of chromosomal aberrations considerably increased with increasing K_2SO_4 concentrations. The highest chromosomal aberration frequencies were detected at 1000 µM K_2SO_4 concentrations in all temperatures studied. In the light of this study, we conclude that K_2SO_4 may be harmful unless are used by suitable doses. Although there are limited investigations related to mentioned fertilizer on chromosomal behaviours[20], it is well known effects of mutagenic environmental pollutants (chemical fertilizers, insecticide, etc.) on chromosome abnormalities[42,43]. Ruan *et al*.[44] noticed that bridges, lagging chromosome and micronuclei were recorded and these aberrations may lead to loss of genetic material. In our study, we identified lots of chromosomal aberrations such as micronucleus, uncoiling chromosome, sticky chromosome,

anaphase bridges, fault polarization in cells of seeds germinated in the medium containing K_2SO_4 (Fig. 2).

Mitotic irregularities such as alignment anaphase and bridge may be mainly the result of spindle dysfunction and constitute a significant portion of chromosomal aberrations. The formations of micronucleus are likely the consequence of vagrant chromosomes and fragments[45]. Chromosome stickiness may result from improper folding of the chromatin fibres[46]. Some researchers reported that the stickiness reflects highly toxic effect on chromatin[47] and chromosome fragments might lead to clastogenic effect and possible mutagenicity[48]. Also, it was thought that anaphase bridges could be the result of inversions[17].

CONCLUSIONS

The outcomes of the study revealed that application of K_2SO_4 in convenient concentrations could be successful in ameliorating of the negative effect of temperature stress by increasing the activity of the enzyme responsible for cell division and cell cycle progression. Furthermore, it will help to clarify the issue to investigate whether this chemical fertilizer directly or indirectly is effective in fundamental metabolic events such as metabolisms of nucleic acid and synthesis of enzyme and protein or not. Our results provide basic data for future research on low and high temperature stress responses. After all, further work is needed to understand the tolerance mechanisms, research the relationship among various types of stress including low and high temperature stress, identify more functional proteins, and validate the cell cycle progressions utilizing molecular biological methods. We hope that our study will encourage future studies, and contribute to improving the survival of many plants challenged by intense heat and cold.

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