INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD
ISSN(O): 2455-0620 [Impact Factor: 7.581]
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value : 86.87
Volume - 9, Issue - 10, October - 2023 Publication Date: 05/10/2023

DOIs:10.2015/IJIRMF/202310002 --:-- Research Paper / Article / Review

Effect of NPK salts on in vitro root growth of *Allium cepa* L.

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Abstract: *The three essential macronutrients commonly added to the soil to enhance the growth and yield of crop plants are Nitrogen (N), Phosphorus (P) and Potassium (K). These are usually applied to the soil in the form of inorganic and organic compounds such as phosphate and sulphate of ammonium and potassium, single superphosphate (SSP), Biochar, compost, manure and urea. In this paper the authors report the effect of various concentrations of NPK salts on growth of Allium cepa L. roots under in vitro conditions. A. cepa bulbs were placed for root growth with their basal plates in contact with solutions of NPK salts- ammonium sulphate, ammonium dihydrogen phosphate and potassium nitrate- so that the newly formed roots would be exposed to the nutrients right from their time of emergence. The roots were harvested on the sixth day and the length, number, and mitotic index of the roots as well as potential irregularities in the mitotic process were studied. The results show that ammonium dihydrogen phosphate and potassium nitrate promoted the root growth of A. cepa bulbs.*

Keywords: *Allium cepa L., NPK salts, root number, root length, mitotic index, mitotic abnormalities.*

1. INTRODUCTION:

Depletion of soil fertility is a major hindrance to sustained crop production and fertilizers play a key role in enhancing the yield of crops without being detrimental to their quality. The nutrient requirements of crops depend on their physiological needs [1]. Nitrogen (N), Phosphorus (P) and Potassium (K) are the three essential macronutrients required for plant growth and are routinely added to soils as chemical fertilizer.

Nitrogen is the element required by plants in largest amounts after carbon. N is an important structural component of nucleic acids, amino acids, proteins, chlorophyll, co-enzymes, phytohormones and secondary metabolites. The availability of N to roots is therefore very important for plant growth. N is absorbed from the soil by the roots of higher plants in the form of nitrate (NO_3^-) and ammonium (NH_4^+). Nitrate has to be first reduced to NH_4^+ before it can be incorporated into organic molecules in the plant. Ammonium is assimilated into amino acids [2]. At low concentrations, NH_4^+ promotes plant growth, whereas higher concentrations can be toxic to the cells. Ammonium can bring about rapid changes in cytosolic pH, affecting gene expression, and post-translational modifications of proteins, which can lead to acidification of apoplast, enhancing assimilation of NH_4^+ [3]. Ammonium nutrition may play a role in protection of plants from pathogen attack [4]. Ammonium also provides resistance to salt stress [5].

Phosphorus is a constituent of ATP and other nucleotides, DNA and RNA as well as the sugar phosphates which play a role in photosynthetic carbon fixation such as ribulose-1,5- bisphosphate. It is also present in cell membranes in the form of phospholipids. In large number of enzyme catalysed reactions, inorganic phosphate (Pi) is required as a substrate or is an end-product [2]. Phosphorus is also a structural component of phytin, a major form of P storage in seeds [6]. Phosphorus has been found to play a key role in leguminous plants in the process of biological nitrogen

fixation. Nodules act as a sink for P. When P supply is deficient, nodulation as well as nodule functioning are decreased, and less amount of N_2 is fixed [7,8]. This can be explained by the fact that N_2 fixation process is a highly energy-consuming process and needs a minimum of 16 moles ATP for reduction of one mole of N_2. Phosphorus deficiency also impacts reproductive growth of plants negatively. Delayed reproductive development and retarded ripening in plants may be caused by low P supply [9].

Unlike N and P which occur as constituents of biomolecules, K is found in cells as a free cation (K^+). Potassium has been reported to play a key role in several physiological functions, including photosynthesis [10], root growth and development [11], xylem-phloem transport [12], plant homoeostasis [13,14], stress responses [15], and energy transfer [16]. Potassium is essential for activity of various enzymes and osmoregulation by maintenance of turgor which is important for many plant processes, such as stomatal opening and closing [11,17]. K plays essential roles in enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance and stress resistance. K increases resistance to drought. The interaction of K with N and P has been thoroughly investigated, and is believed to be synergistic [18].

Onion is considered to be very inefficient in the uptake of water and nutrients because of its shallow unbranched root system lacking root hairs. This has led to the use of large amounts of chemical fertilizers for onion cultivation [19, 20]. In the present investigation, the effect of different concentrations of NPK salts on in vitro growth of roots of *Allium cepa* was studied. An experiment was set up using *A. cepa* bulbs to examine how NPK salts affect the length, number, and mitotic index of the roots as well as potential irregularities in the mitotic process. *A. cepa* was chosen because its roots grow quickly and it has just 8 pairs (2n = 2x = 16) of chromosomes, which are relatively large and easy to observe. *A. cepa* is a species of one of the largest plant genera, *Allium*, which belongs to the Amaryllidaceae family.

2. MATERIALS AND METHODS:

2.1 Preparation of solutions

Aqueous solutions of NPK salts – ammonium sulphate [$(NH_4)_2SO_4$], ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and potassium nitrate (KNO_3) – with concentrations 10 μM, 50 μM, 100 μM, 200 μM, respectively, of each salt and another solution containing mixture of all the three salts at a concentration of 200 μM were prepared.

2.2 Experimental setup and treatment

Medium sized onion bulbs were taken. Their roots and basal plate were scraped off carefully so as to expose the root primordia. The solutions of all concentrations of each salt and distilled water (control) were filled up in a set of Coplin jars/ wide mouthed glass bottles/ beakers and onion bulbs placed on them with only their basal plates dipped in the solutions.

The set up was kept at in an incubator at 26°C for 5 days and the solution was topped up every day in each jar to compensate for any loss of solution by evaporation ensuring that the roots did not dry up. Three replicates of the experiment were set up.

On the sixth day, the roots were harvested after counting the number of roots grown per onion and measuring the length of each root. The harvested roots were then fixed in acetic alcohol (45% acetic acid: ethanol, 1:3). After 24 hours the roots were transferred into 70% alcohol from acetic alcohol for cytological studies.

2.3 Cytological analysis

Roots treated with each salt [KNO_3, $NH_4H_2PO_4$, $(NH4)_2SO_4$, and salt mixture] and control were used to make squash preparations of root tips either stained with 2% Acetoorcein or Feulgen stain as suggested by Sharma & Sharma [21]. The slides were observed under the microscope to study different stages of mitosis and to screen for chromosomal abnormalities in the divisional stages. Mitotic index (MI) for all treatments was also calculated as given below.

$$\text{MI} = \frac{\text{number of dividing cells}}{\text{total number of cells}}$$

3. RESULTS AND DISCUSSION:

Table 1. Average values of root number, root length and mitotic index for the three replicates of the experimental setup

S. No.	Treatment Salt	Salt Concentration (μM)	Number of Roots per Onion	Average Root Length (mm)	MI
1	Control	0	13.58	24.57	0.045
2	KNO_3	10	47.67	13.67	0
		50	56.67	22.07	0.004
		100	47.33	21.38	0.118
		200	60.67	37.57	0.06
3	$NH_4H_2PO_4$	10	23.50	18.66	0.146
		50	33.92	14.57	0.153
		100	32.00	31.64	0.182
		200	44.00	14.75	0.198
4	$(NH_4)_2SO_4$	10	18.00	15.02	0.16
		50	13.00	8.66	0.003
		100	22.67	8.32	0.078
		200	19.33	10.52	0.07
5	Mixture of KNO_3, $NH_4H_2PO_4$ & $(NH_4)_2SO_4$	200	23.67	28.58	0.068

The parameters studied in the present investigation were average number of roots per onion bulb, average root length and mitotic index. Treatment with NPK containing inorganic salts significantly increased the average number of roots per plant, relative to control, at all concentrations used except in case of 50 μM $(NH_4)_2SO_4$. It is apparent that out of the three salts used, viz. KNO_3, $NH_4H_2PO_4$ and $(NH4)_2SO_4$, the highest average number of roots resulted after KNO_3 treatment in comparison to control. This was followed by $NH_4H_2PO_4$ treatment and the least average number of roots was seen after treatment with $(NH4)_2SO_4$. Out of the four concentrations of KNO_3 tested, maximum average number of roots grew after 200 μM treatment while the least number resulted after 10 μM and 100 μM treatment. For $(NH_4)_2SO_4$ treatment the highest value was observed after 100 μM treatment. When mixture of KNO_3, $NH_4H_2PO_4$ and $(NH_4)_2SO_4$ was tested at a concentration of 200 μM, the average number of roots was closer to that obtained for $NH_4H_2PO_4$ and $(NH_4)_2SO_4$ treatments which was much less compared to the value observed with KNO_3 treatment (Table 1, Fig.1).

The average root length was greatest after KNO_3 treatment in comparison to control followed by $NH_4H_2PO_4$ and $(NH_4)_2SO_4$ treatments in that order. The average root length in $(NH_4)_2SO_4$ treated roots was found to be much lower than that of control. The average root length obtained after treatment with mixture of KNO_3, $NH_4H_2PO_4$ and $(NH_4)_2SO_4$ was higher than for $NH_4H_2PO_4$ and $(NH_4)_2SO_4$ treatments but lower than that for 200 μM KNO_3 treatment. For KNO_3 treatment, 200 μM concentration resulted in the maximum average root length whereas 10 μM treatment produced the least average root length (Table 1, Fig. 2). Akhtar et al. [22] also reported that when effect of two fertilizer treatments consisting of N and P, with and without K, was studied on *A. cepa*, the yield was considerably enhanced in presence of K. Similarly, an increase in yield of *A. cepa* after K application has been reported by other investigators [23,24].

The cytological studies showed that unlike average root number and average root length, the mitotic index was highest in case of $NH_4H_2PO_4$ treated roots and an increase was observed with increase in concentration. The mitotic index values for KNO_3 and $(NH_4)_2SO_4$ treatment, which were lower than $NH_4H_2PO_4$, were comparable to the control.

The mitotic index value obtained after treatment with the mixture of the three salts was slightly higher than control and equivalent to the value for 200 µM $(NH_4)_2SO_4$ treatment (Table 1).

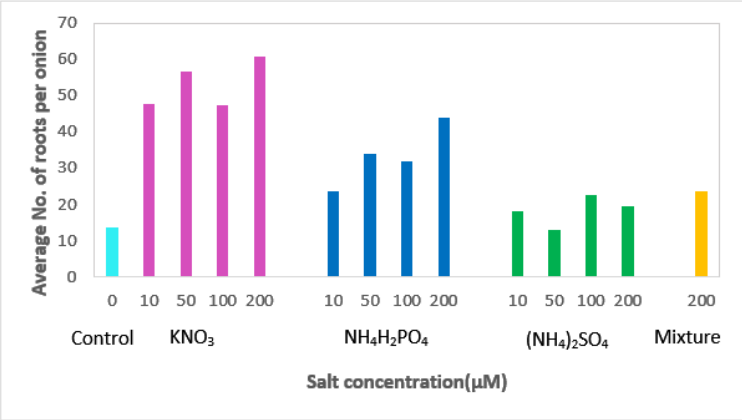

Fig. 1. Effect of different concentrations of NPK salts on average number of roots.

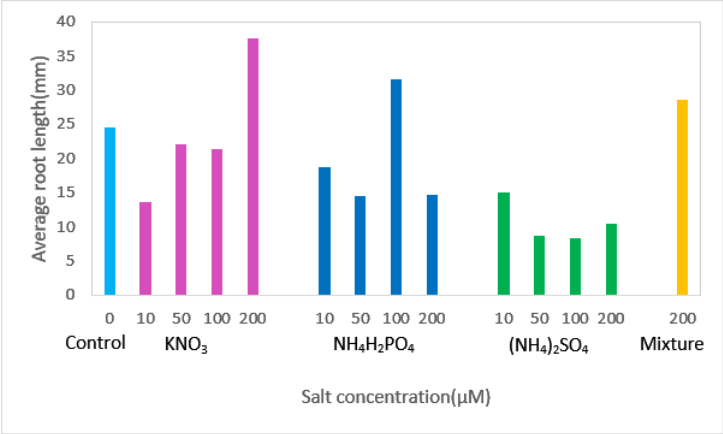

Fig. 2. Effect of different concentrations of NPK salts on root length.

Mitosis was completely normal in control roots (Fig. 3). Surprisingly, the meristematic cells of roots subjected to NPK treatment showed various mitotic abnormalities. The cells of the tip region of roots treated with 50 µM KNO_3 as well as 10 µM and 50 µM $NH_4H_2PO_4$ solution contained unusually elongated nuclei in addition to dividing cells. The cells of root tips treated with 50 µM $NH_4H_2PO_4$ also showed disorderly prophase. Irregular anaphase due to vagrant chromosomes was observed in cells of roots treated with 10 µM KNO_3 as well as 10 µM, 50 µM and 100 µM $NH_4H_2PO_4$. In cells of root tips treated with 100 µM $NH_4H_2PO_4$, chromosomal bridge could be seen. After treatment with both 100 µM $NH_4H_2PO_4$ and mixture containing all three salts, sticky chromosomes were noted in a few cells (Fig.4).

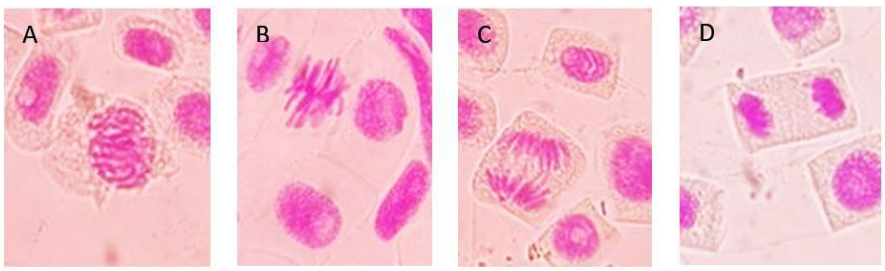

Fig. 3. Squash preparations of root tip cells of *Allium cepa* L. showing normal mitotic stages. A. Prophase; B. Metaphase; C. Anaphase; and D. Telophase

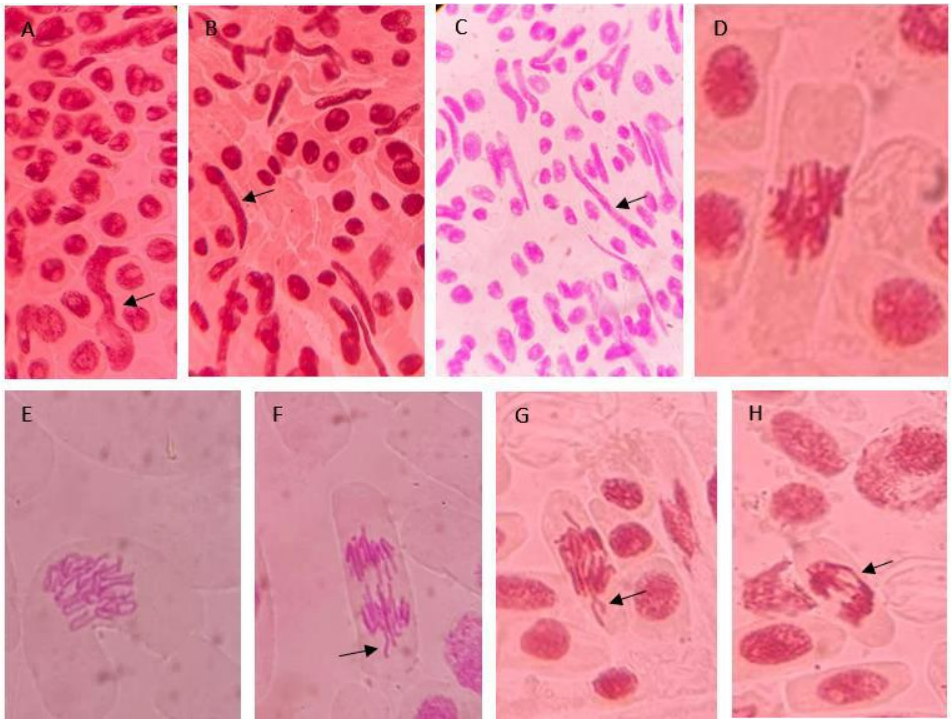

Fig. 4. Squash preparations of root tip cells showing mitotic abnormalities. Elongated nuclei (A-C) A. 50 µM KNO_3, B and C. 10 µM and 50 µM $NH_4H_2PO_4$, respectively; D. Sticky chromosomes at 100 µM $NH_4H_2PO_4$; E. Abnormal prophase at 50 µM $NH_4H_2PO_4$; F and G. Irregular anaphase due to vagrant chromosomes at 50 µM and 100 µM $NH_4H_2PO_4$, respectively and H. Chromosomal bridge at 100 µM $NH_4H_2PO_4$.

Many investigators have reported enhanced growth parameters in *A. cepa* L. by addition of NP [19,25] or NK [26] or K [27-29] or NPK fertilizer to the soil [30-39]. An increase in number of leaves per plant, length and width of leaves, height of plant, neck thickness and biomass per plant with increasing amount of K fertilizer were reported in *A. cepa* L. [29]. On the other hand, Messele [40] found that while application of N showed significant increase in growth parameters of *A. cepa* L., the use of P alone or in combination with N did not.

Comparable investigations have also been carried out on another species of *Allium*, *A. sativum* L. (garlic). Nouri et al. [41] observed that parameters such as weight, length and diameter of garlic, length of leaf and yield in *A. sativum* L. increased on application of 200 Kg per hectare of N fertilizer but decreased when amount of fertilizer was raised to 300 Kg per hectare. A similar trend of increased growth parameters was described in *A. sativum* L. [1,42]. The poor response of onion bulbs to $(NH_4)_2SO_4$ in comparison to KNO_3 and $NH_4H_2PO_4$ may be due to NH_4^+ toxicity rather than SO_4^- as the latter has been shown to enhance all growth parameters in *A. cepa* [43] whereas high concentration of former is known to be toxic [3]. The higher values for average number of roots per onion and average root length after treatment with $NH_4H_2PO_4$ in comparison with $(NH_4)_2SO_4$ may be due to the effect of P rather than NH_4^+.

Although, there are many studies on the effect of NPK fertilizer on growth parameters of bulbs and foliage of *Allium* species, very few on the effect of NPK fertilizer on root growth have been undertaken. According to Garde et al. [44] who studied the effect of inorganic NPK fertilizers along with organic sources on roots and bulbs of field grown onion (*A. cepa* L.), numbers of roots per bulb and root length was higher with higher concentration of fertilizer. They attributed the stimulation of root growth to the application of nitrogen and phosphorus. A positive correlation between increasing amount of fertilizer and root length of green onion also has been observed [45].

Mitotic abnormalities have been reported to be caused by salinity stress in *A. cepa* L. root meristematic cells [46]. Vagrant chromosomes and chromosomal bridge in anaphase may be caused by spindle dysfunction. Spindle dysfunction may result in defective interaction between microtubule and kinetochore leading to abnormal segregation of chromosomes [47]. Stickiness of chromosomes reflects improper folding of chromatin fibres [48] or effect of toxicity on chromatin [49]. Also, anaphase bridges could be the outcome of inversions [47].

4. CONCLUSION:

All three salts, viz. KNO_3, $NH_4H_2PO_4$ and $(NH_4)_2SO_4$, were enhancers of root growth in terms of root number and root length. Mitotic index values indicate that except for lower concentration of KNO_3 and $(NH_4)_2SO_4$ (which needs to be studied further), all salts promoted cell division also. Thus, in addition to increasing bulb and foliage growth parameters as reported by many investigators, NPK salts also increase root growth parameters. The incidence of mitotic abnormalities suggests genotoxicity of the NPK salts which too requires further investigation.

Acknowledgements

The authors sincerely thank Professor Bijayalaxmi Nanda, Principal, Miranda House, and Professor Monika Tomar, Coordinator, DS Kothari Centre for Research and Innovation in Science Education, Miranda House, for encouraging students to undertake short-term research projects. The authors also thank the laboratory staff of the Department of Botany for their support.

Conflict of Interest

The authors declare that there is no conflict of interest.

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