Protoplasma (2020) 257:319–330
https://doi.org/10.1007/s00709-019-01424-9

NEW METHODS IN CELL BIOLOGY

Specific H^+ level is crucial for accurate phosphate quantification using ascorbate as a reductant

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Received: 14 May 2019 / Accepted: 19 July 2019 / Published online: 29 July 2019

Abstract

Owing to its essentiality for cellular metabolism, phosphate (PO_4^{3-}) plays a pivotal role in ecosystem dynamics. Frequent testing of phosphate levels is necessary to monitor ecosystem health. Present investigations were aimed to identify the key factors that are essential for proper quantification of PO_4^{3-}. Primarily, H^+ levels played a critical role in the development of molybdenum blue complex by ammonium molybdate and PO_4^{3-} with ascorbic acid as a reductant. Molybdenum blue complex formed in the presence of 8 to 12 mmol of H^+ in 3 ml reaction mixture remained stable even after 72 h. Of different concentrations of ammonium molybdate and ascorbic acid tested, best molybdenum blue complex was formed when their concentrations were 24.3 and 5.68 μmol, respectively. More or less similar intensity of molybdenum blue complex (due to reduction of phosphomolybdic acid and not molybdic acid) was formed in the presence of H^+ at levels ranging from 8 to 10 mmol in 3 ml reaction mixture. Our findings unequivocally demonstrated that (i) the reaction mixture containing 3% ammonium molybdate, 0.1% ascorbic acid and 5 M H_2SO_4 in the ratio of 1:1:1 is ideal for PO_4^{3-} quantification; (ii) antimony (Sb) significantly curbs the formation of molybdenum blue under these ideal conditions; (iii) this fine-tuned protocol for PO_4^{3-} quantification could be extended without any problem for determining the level of PO_4^{3-} both in plant as well as soil samples; and (iv) *Azotobacter* possesses potential to enhance levels of total PO_4^{3-} in leaves and grains and soluble/active PO_4^{3-} in rhizosphere soils of wheat.

Keywords Phosphate quantification · Ammonium molybdate · Ascorbic acid · H^+ levels · Molybdenum blue complex

Introduction

Phosphorus, the eleventh most abundant element in the Earth's crust, is pivotal for life and the ecosystem dynamics. Phosphorous plays an essential role in organisms belonging to all kingdoms of life. It is a critical part of the basic backbone of nucleic acids (both DNA and RNA) imparting desired net negative charge. Besides playing a central role in cell signalling, phosphate (PO_4^{3-}) plays a pivotal role in regulating (i) general cell energy dynamics as a part of ATP, ADP, AMP, PPi, and $NADP^+$; (ii) cell membrane structure and function; (iii) activities of several enzymes; and (iv) synthesis of all cell polymers (Chiou and Lin 2011; Fettke et al. 2011; Fernández-García et al. 2017; Kunz et al. 2010).

Owing to its essentiality for almost all cellular metabolic events, scantiness of soluble phosphate in agricultural ecosystems acts as a vital limiting factor that severely influence primary productivity (Worsfold et al. 2016) and hence crop yield. Therefore, soluble/active phosphate is invariably applied along with nitrogen and potassium fertilisers in agricultural fields for optimal yield of both food and non-food products (Julia et al. 2016). Phosphate is commonly absorbed by plants in the form of $H_2PO_4^-$ and HPO_4^{2-} (Raghothama and Karthikeyan 2005; Sentenac and Grignon 1985). However, excessive use of fertilisers often results in disproportionate loading of phosphate in many freshwater ecosystems, through agricultural runoff. Similarly, other anthropogenic activities

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