

Short communication

## Detection and quantification of phytic acid in black pepper variety Panniyur-1 using Polyacrylamide Gel Electrophoresis

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### Abstract

Inositol hexakisphosphate (InsP6), commonly called phytic acid is a major phosphorus store in plants. It has important functions in germination and growth of seedlings and also help in plant defence against various biotic as well as abiotic stresses. The quantity of InsP6 is estimated commonly by tedious procedures like HPLC, NMR spectroscopy, TLC etc. Some of these methods also involve the use of harmful radioactive elements. Polyacrylamide gel electrophoresis (PAGE) is a rather easier and simpler method for the estimation of phytate in plant sample. PAGE was used to detect and quantify InsP6 in leaf tissues of black pepper variety Panniyur1. Crude extract of Panniyur1 leaf tissue and different concentrations of the commercially available phytate standards were prepared and analysed using PAGE. Standard curve was plotted using the band intensities (volume) (y-axis) and concentrations of phytate standard (x-axis). The estimated quantity of phytate in the sample based on standard curve was 620 nmolesg<sup>-1</sup> fresh weight of leaf tissue.

**Keywords:** Black pepper, Inositol hexakisphosphate (InsP6), Phytic acid, Polyacrylamide gel electrophoresis

Black pepper (*Piper nigrum*), belonging to the Piperaceae family is one of the major spice crops of Kerala. It is renowned as the “king of spices” owing to its enormous medicinal and other uses, and “black gold” as it fetches the highest return from international trade. Apart from the use as a spice, black pepper has many other uses like dietary, medicinal, perfumery and preservative. The crop has an antioxidant potential, anti-inflammatory potential, anti-microbial property, anti-cancerous property and neuro protectant activity.

*Myo*-inositol hexakisphosphate (InsP6), also called phytic acid, is an important phosphorus storage compound found in eukaryotic cells. It is commonly seen in plant parts including storage organs like seeds, roots and tubers. It also helps in germination and growth of seedlings. Studies have shown that

InsP6 performs a key role in plant defence mechanism, as plants defective in InsP6 are found more susceptible to diseases including viral, bacterial and fungal pathogens (Murphy et al., 2008). In guard cells, InsP6 acts as a key signalling molecule produced in response to drought stress hormone abscisic acid and triggers the release of endomembrane stored calcium ions (Lemtiri-Chlien et al., 2003). Phytic acid also plays a role as a co-factor in auxin mediated gene expression of the auxin receptor, Transport Inhibitor Response 1 (TIR1) (Tan et al., 2007). In eukaryotic cells, it is found to help mRNA export and repair of DNA double strand breaks.

The compound InsP6, although it has got important roles in vital physiological functions of plant and animal systems, has an anti-nutritional effect as it

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chelates with various mineral cations like  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{K}^{+}$  etc, making these elements unavailable to animals. The undigested InsP6 also gets accumulated in waterbodies leading to eutrophication. Therefore quantification of phytate in food crops is very essential. There are various methods of detecting phytate in the plant samples, the common over being HPLC and NMR spectroscopy. TLC is also used for estimation of phytate. But all these methods are labour intensive and involve complex procedures, including the use of radio labelled probes. Poly Acrylamide Gel Electrophoresis is a rather simpler method for estimating the amount of phytate in plant samples (Losito et al., 2009; Alimohammadi et al., 2013).

Hence, realizing the enormous potential of the crop in the medicinal and dietary fields, its high commercial value in agricultural sector, and identification of the importance of the biomolecule inositol hexakisphosphate in plant and animal kingdoms, the study was undertaken to detect and quantify InsP6 in black pepper.

Quantity of InsP6 in sample was estimated using the method suggested by Alimohammadi et al. (2013). Leaf sample (200 mg) from variety Panniyur1 was weighed and the tissue was homogenized using 500  $\mu\text{L}$  of 0.4 M HCl in liquid nitrogen. The homogenate was boiled for 5 minutes. The denatured sample was briefly vortexed and centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to a new microfuge tube and sample was snap frozen in liquid nitrogen and thawed at room temperature. This was centrifuged again at 12,000 rpm for 5 minutes and the supernatant was transferred to a fresh microfuge tube and stored at  $-20\text{ }^{\circ}\text{C}$ .

The resolving gel (33.3%) was prepared in TBE with a total volume of 5 ml and the components involved 3.7 ml of 40% acrylamide/bisacrylamide (19:1), 0.5 ml of 10X TBE buffer (pH 8), 0.3 mL  $\text{ddH}_2\text{O}$ , 32  $\mu\text{L}$  10% APS and 3.5  $\mu\text{L}$  TEMED. The stacking gel topped on the resolving gel had the

composition of 1 mL 16% acrylamide/bisacrylamide (19:1), 120  $\mu\text{L}$  5X TBE (pH 6), 69.5  $\mu\text{L}$   $\text{ddH}_2\text{O}$ , 8.5  $\mu\text{L}$  10% APS and 1  $\mu\text{L}$  TEMED. The gel was mounted on the electrophoresis unit and pre-run was performed for 20 mins at 100 V/10 mA. 20  $\mu\text{L}$  of phytate extract and phytate standards (1-5 n moles of phytic acid dipotassium salt commercially available from Sigma Aldrich) were loaded on to the wells after mixing with 5  $\mu\text{L}$  of Orange G loading dye. The gel was run at 100 V/10 mA until the dye front reached the bottom. The gel was then stained with toluidine blue (Sigma Aldrich) staining solution (20% methanol, 2% glycerol, 0.05% toluidine blue) for 30 minutes and then de-stained overnight using the de-staining solution with the same components except dye for over night in a shaker after several changes of the solution. The de-stained gel was visualized and the image was captured using gel imaging system (Figure 1).

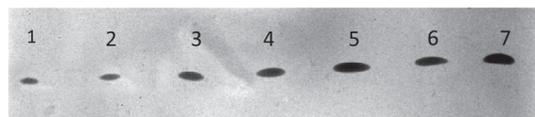


Figure 1. Band intensities of different phytate standards and samples loaded on the gel.

Lane 1- 5 represent the band intensities of phytate standards (1-5 nmoles). Lanes 6 and 7 band intensities of 10  $\mu\text{L}$  and 20  $\mu\text{L}$  sample.

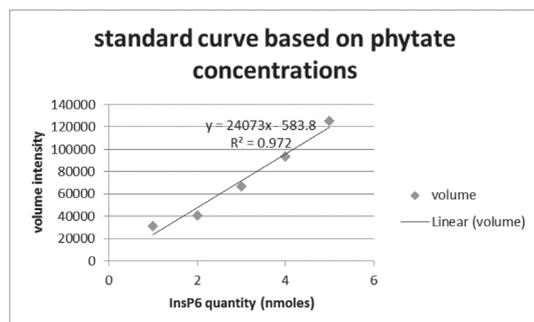


Figure 2. Standard curve plotted against different concentrations of phytate standard on x-axis and corresponding band intensities (volume intensity) on y-axis.

Band intensities of the gel image was quantified using the software GelQuant.NET (Biochemlabsolutions.com). Quantity of phytate in the sample was calculated based on the standard curve obtained by plotting a graph corresponding to band intensities of different concentrations of phytate standards loaded on the gel. Standard curve was drawn with quantity of phytate standards (n moles) on x axis and volume intensity on y axis. The quantity of phytate in sample was estimated from the standard curve (Figure 2) using the equation,  $\text{Volume intensity} + 583.8 = 24073 * \text{quantity of InsP6}$ . The values were then converted to  $\text{nmol g}^{-1}$  fresh weight of tissue.

The quantity of phytate in sample was estimated to be  $620 \text{ nmol g}^{-1}$  fresh weight of leaf tissue based on the standard curve. To our knowledge no other work detecting the presence of the compound in black pepper has been reported and hence this study has given a primary evidence of the presence of the biomolecule in the plant. Similar results were found in tomato, rice and tobacco with  $100 \text{ nmol g}^{-1}$ ,  $450 \text{ nmol g}^{-1}$  and  $200 \text{ nmol g}^{-1}$  respectively (Alimohammadi et al., 2013). The data shows that quantity of phytate in different plant samples may vary widely. This method has the advantage of avoiding complex analytical procedures using harmful radioactive elements as is the case in the most commonly used techniques.

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