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Comparative phosphorus uptake profile of Bt transgenic and non-transgenic cabbage (*Brassica oleraceae* L.var.capitata)

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Abstract

Plant species may differ in phosphorus uptake pattern due to genetic modification. The objective of this research was to study the comparative phosphorus uptake pattern of Bt. Transgenic cabbage and Non-transgenic cabbage (*Brassica oleraceae* L.) through radioactive phosphorus. Phosphorus content in two types of cabbage plant was more or less equal i.e. the variation was statistically insignificant. The variation in % phosphorus derived from fertilizer and percent of phosphorus utilization by transgenic cabbage was statistically higher as compared to normal cabbage and it was statistically significant. In 50 and 75 days samplings, the phosphorus content in transgenic and normal cabbage was same i.e. this variation was not statistically significant. The dry matter yield, total phosphorus uptake, percent phosphorus derived from fertilizer and percent phosphorus utilization was higher in transgenic cabbage and all these were statistically significant.

Keywords: Radioactive, ^{32}P, dry matter, isogenic

Among the vegetable Brassicas in India, cabbage is grown on large scale accounting to 10% of the world production. The global adoption rates for transgenic crops are increasing and 19% of transgenic crops grown are Bt crops. The transgenic crops are genetically modified crops and represent a promising technology that can make a vital contribution to global food, feed and fiber security. In India, crop losses due to insect pests may range from 10 to 30% annually, depending on the crop and the environment. A global adoption rate for transgenic crops was unprecedented and reflects grower's satisfaction with it as the products offer significant benefits (Dutta *et al*., 2012). The environmental impacts of genetically modified plants are still largely unknown and are often unresolved (Dunfield and Germida 1995, Hails 2000, Gray 2004 and Snow. *et. al*. 2005). Bt-transgenic plants might have direct or indirect impacts on different aspects of ecosystems. It was of interest to see whether inserting one gene in a pool of genes will cause any difference between transgenic and isogenic line of cabbage for phosphorus uptake pattern. So before releasing the Bt-transgenic cabbage in market, it was considered prudent to check whether there is any difference in the uptake of plant nutrient and nutritional quality in transgenic cabbage. In the present investigation, plant samples from transgenic cabbage (Tropical breeding line) and non-transgenic cabbage were analyzed by the radio assay method to study whether there was any difference in phosphorus uptake pattern due to variety.

Material and Methods

The experiment was conducted at Indian Agricultural Research Institute, New Delhi during Rabi season (November to March). The experiment was designed, as Completely Randomized Block Design (CRD) in pot culture by using uncontaminated surface soil. It was collected from the farm, not treated with pesticides and fertilizer. The physico-chemical properties of the soil were recorded (Table 1). The soil was dried, ground and sieved through 2 mm sieve. Ten kg of the processed soil thoroughly mixed with the required fertilizer solution tagged with ^{32}P radioactivity filled in the glazed pots of twelve kg capacity. Two cultivars of cabbage seeds, one non-transgenic Golden acre and another six lines of transgenic Golden acre namely $^{22}T_0$, $^{22.6}T_0$, $^{22.2}T_0$, $^{3}T_1$, $^{4}T_1$ and $^{11}T_1$ were sown in nursery bed in the month of November and transplanted in pots, after they were 5 to 6 cm tall, healthy plant. Three levels of phosphorous i.e. 0, 8.7 and 17.5 mg P/ kg were given to the soil in three replicates. The phosphorous was supplied through the aqueous solution of di-ammonium phosphate. A uniform dose of nitrogen through urea (30 mg N/ kg) and potassium through potassium sulphate (16.6 mg K / kg) was given and sufficient amount of water was provided to maintain soil moisture at field capacity.

Table 1 : Physico-chemical properties of experimental soil

Soil texture		
% sand	:	71.8
% silt	:	12.0
% clay	:	16.2
Textural class	:	Sandy loam
pH (1:2.5) (Soil : Water)	:	8.4
CEC (mmol +/ kg)	:	130.1
$N_{available}$ (kg/ha)	:	211.1
P_{Olsen} (kg/ha)	:	17.9
K_{exch} (kg/ha)	:	246.4
C_{org} (%)	:	0.2

The cabbage plants were collected randomly on 25, 50, and 75 days after transplanting (DAT) from both cultivars from 18 pots kept in triplicate for each. The plant samples were dried in air and kept in oven at 70°C before taking their dry weight. The determination of nutrient content in the plant material was after digestion with di-acid mixture (HNO_3: HOCl; 5:1, V/V) and heated on a hot plate. It was diluted with 6 N HCl up to 100 ml. Aliquots were collected for estimation of phosphorus.

Preparation of labeled Di-ammonium phosphate solution (DAP) solution

Labeled DAP was prepared by dissolving the required amount of DAP (Laboratory reagent) in minimum quantity of distilled water in a volumetric flask to which carrier free $H_3P^{32}O_4$ in dilute HCl, supplied by BARC, Trombay, was added to give a specific activity of 0.4 mci/ g P and mixed well and the volume was made up.

Estimation of phosphorus

The P_{tot} content of the aliquot was determined calorimetrically by vanado molybdate method in nitric acid medium (Koenig and Johnson, 1942).

Estimation of radioactive phosphorus

The radio assay method followed was the same as described by Mackenzie and Dean (1948). Counting measurements were made in an end window Geiger counter (Window thickness of 2.5 mg/cm²). Counts per minute (CPM) is a measure of radioactivity. It is the number of atoms in a given quantity of radioactive material that is detected to have decayed in one min. From the counts of the sample, specific activity of the plant material was calculated.

Calculation of per cent Pdff, fertilizer phosphorus uptake and percent utilization of applied fertilizer

Corrections for radioactive decay were noted and applied to calculate the specific activity of the sample using the formula given below:

$$\text{a. Specific activity} = \frac{P^{32}}{P^{31} + P^{32}}$$

$$= \frac{\text{CPM of the sample - CPM of the background}}{\text{mg of P in the plant sample}}$$

b. Per cent phosphorus in the plant derived from tagged fertilizer (% Pdff)

$$= \frac{\text{Specific activity of plant sample}}{\text{Specific activity of the tagged source}}$$

c. Fertilizer phosphorus uptake by plant (mg/plant)

$$= \frac{\text{\% Pdff x total P uptake (mg/ plant)}}{100}$$

d. Per cent utilization of applied fertilizer phosphorus

$$= \frac{\text{Fertilizer P uptake (mg / plant)}}{\text{Fertilizer P applied to the soil (mg/ pot)}} \text{ x100}$$

Statistical Analysis

The data was subjected to statistical analysis using SPSS-10. Raw data was fed in Excel format and ANOVA was done to find whether there was any difference in the quantity of the desired element present in transgenic and non-transgenic cabbage.

Results and Disscussion

The uptake Phosphorus was carridout by transganic grown transgenic cabbage. The experimental soil was treated at the rate of 0 kg, 17.5 kg and 34.9 kg P/ ha by using radio labeled phosphate fertilizer. The sample was taken at 25, 50 and 70 days after transplanting (DAT) to study, whether there is any difference between these two types of cabbage plant. Dry matter yield (Table 2) at 25 DAT of the transgenic plant and non transgenic plant were significantly different from each other and they were no significant difference based on phosphorus content in these two types of cabbage. It was also found that the response due to fertilizer treatment was also statistically significant. The phosphorus uptake pattern was same for the transgenic and isogenic cabbage with the increase in phosphorus dose. The dry matter yield of transgenic plant was higher than the dry matter yield of non transgenic plant. Due to this fact, the total phosphorus uptake was also high. At 17.5 kg P/ ha the total phosphorus uptake by transgenic cabbage was 1.9 mg/ plant whereas 1.4 mg/ plant by non transgenic plant. The same trend was found at 34.9 kg P/ ha treated pot, where the total phosphorus uptake was 2.5 mg/ plant and 1.7 mg /plant by transgenic and non transgenic cabbage, respectively. At 25 days sampling, percentage (%) phosphorus derived from fertilizer by transgenic and non transgenic cabbage, though appeared to vary, but was not statistically significant. Fertilizer phosphorus uptake by transgenic and non-transgenic cabbage was statistically significant. Fertilizer phosphorus uptake by transgenic and non transgenic cabbage was 0.9 mg/ plant and 0.6 mg/ plant, when the soil was treated at the rate of 17.5 kg P/ ha. The fertilizer phosphorus uptake was 1.4 mg/ plant and 0.8 mg/ plant by transgenic and non transgenic cabbage crop, respectively, when the pot soil was treated at the rate of 34.9 kg P/ ha. It was found that % of phosphorus utilization pattern was comparatively higher in 25 days sample. It was 2.0% and 1.5% by transgenic and non transgenic plant respectively in pot treated @ 17.5 kg P/ ha. But it was noticed that at higher dose of phosphate application, the % phosphorus utilization pattern was reduced in both the cases namely transgenic and non transgenic plant, implying that higher application of P is not useful and is rather wasted. The results are suggestive in nature and are needed to be validated by field trials.

With the increase in the number of days, the dry matter of the plant increased for both types of plants. For control pot, the dry matter of the cabbage plant became 3.2 g which was earlier 0.2 g in case of transgenic plant (Table 3). In case of non transgenic plant of control pot, the dry matter yield was 1.9 g/ plant in 50 DAT. The phosphorus content was more or less equal as it was observed in the earlier case, although there was a slight decline. Statistically the dry matter content in these two varieties was not similar but there was no significant difference in their phosphorus content. Due to the higher dry matter yield, total phosphorus uptake by transgenic plant was also higher than that of non transgenic plant and it was statistically significant in 50 DAT. Phosphorus derived from fertilizer was higher by transgenic plant as compared to the non transgenic plant. At 50 DAT, the phosphorus derived from fertilizer was statistically significant when it was compared with the non transgenic plant. It indicates that there was statistically significant difference due to variety but not due to treatment. Fertilizer phosphorus uptake by both types of plant was also statistically significant and fertilizer phosphorus uptake was higher in case of transgenic plant when compared to the non-transgenic plant. It was also found that there was significant difference due to P treatment and variety both, at this stage. Percent phosphorus utilization pattern was also statistically significant due to transgenic and non-transgenic factor (Table 3). In every case higher value was obtained in case of transgenic plant. But one important thing was found that with the increase of the fertilizer dose the utilization pattern decreased gradually. At the final stage of observation, the dry matter gradually increased and the dry matter produced by transgenic plants was higher when compared to the non transgenic plant and it was statistically

Table2: Phosphorus utilization pattern by 25 days old Transgenic and Normal Cabbage

Transgenic Cabbage	Dry matter yield Average ± SD (g/Plant)	Phosphorus Content Average ± SD (mg/plant)	Total Phosphorus uptake Average ± SD (mg/plant)	% Phosphorus derived from fertilizer Average ± SD	Fertilizer Phosphorus Average ± SD uptake (mg/plant)	% Phosphorus utilization Average ± SD
Treatment						
Control	0.2 (0.0)	4.3 (2.6)	1.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0(0.0)
17.5 kg P	0.3 (0.0)	5.7 (0.4)	1.9 (0.1)	48.2(3.5)	0.9 (0.1)	2.0(0.1)
34.9 kg P	0.4 (0.0)	6.0 (0.5)	2.5(0.1)	56.3 (5.9)	1.4 (0.1)	1.3(0.1)
Normal Cabbage						
Treatment						
Control	0.2 (0.0)	3.9 (0.8)	0.8(0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
17.5 kg P	0.3(0.0)	5.4 (0.5)	1.4(0.2)	42.2(4.1)	0.6 (0.0)	1.5 (0.3)
34.9 kg P	0.3(0.0)	5.9 (0.2)	1.7(0.3)	47.9(4.9)	0.8 (0.1)	0.9 (0.1)

Table 3: Phosphorus utilization pattern by 50 days old Transgenic and Normal Cabbage

Transgenic Cabbage	Dry matter yield Average ± SD (g/Plant)	Phosphorus Content Average ± SD (mg/plant)	Total Phosphorus uptake Average ± SD (mg/plant)	% Phosphorus derived from fertilizer Average ± SD	Fertilizer Phosphorus Average ± SD uptake (mg/plant)	% Phosphorus utilization Average ± SD
Treatment						
Control	3.2(0.5)	4.0(0.9)	12.5(1.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
17.5 kg P	5.8(0.7)	5.2(0.2)	30.7(5.0)	41.1(3.8)	5.1(0.2)	32.8(5.3)
34.9 kg P	6.2(0.8)	5.7(0.2)	35.4(5.9)	43.9(4.2)	16.6(1.9)	18.9(3.2)
Normal Cabbage						
Treatment						
Control	1.9(0.4)	3.8(0.6)	7.1(1.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
17.5 kg P	3.3 (0.7)	5.0 (0.2)	16.9(3.9)	35.6(4.3)	2.5(0.4)	18.0 (4.2)
34.9 kg P	4.9(0.9)	5.5 (0.4)	26.7 (5.2)	37.8(2.5)	10.0(1.7)	14.3(2.8)

Table 4: Phosphorus utilization pattern by 75 days old Transgenic and Normal Cabbage

Transgenic Cabbage	Dry matter yield Average ± SD (g/Plant)	Phosphorus Content Average ± SD (mg/plant)	Total Phosphorus uptake Average ± SD (mg/plant)	% Phosphorus derived from fertilizer Average ± SD	Fertilizer Phosphorus Average ± SD uptake (mg/plant)	% Phosphorus utilization Average ± SD
Treatment						
Control	15.7(2.0)	2.5(0.3)	38.8(3.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
17.5 kg P	20.3(1.5)	3.7(0.3)	74.3(2.8)	21.2(3.1)	19.5 (4.5)	79.4(3.0)
34.9 kg P	26.0 (1.5)	4.1(0.6)	105.4(10.4)	28.0(3.1)	32.2(7.5)	56.3(5.6)
Normal Cabbage						
Treatment						
Control	13.6(3.2)	2.4 (0.3)	31.7(4.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
17.5 kg P	16.9(1.4)	3.40.8	54.3 (8.3)	14.6 (3.3)	7.1(2.5)	58.0 (8.9)
34.9 kg P	20.41(2.5)	3.6(0.5)	71.7(4.4)	26.2(4.1)	18.8 (3.8)	38.3(2.3)

significant and the dry matter increase due to treatment was also statistically significant (Table 4). The phosphorus content at 75 DAT was statistically significant due to the treatment but the difference due to variety was not statistically significant. Total phosphorus uptake by both the plants was statistically significant due to treatment and variety (Table 5). Phosphorus derived from fertilizer by transgenic cabbage was significantly different from the non transgenic cabbage. The difference in phosphorus derived from fertilizer by both the cabbage was not statistically different due to variety and treatment, but the fertilizer phosphorus uptake and % phosphorus utilization was statistically different due to both variety and treatment. The fertilizer phosphorus uptake and % phosphorus utilization was higher in case of transgenic plant as compared to the non transgenic plant .Considering that P is an essential and often limiting nutrient for plant growth, it is surprising that many aspects of P uptake and transport in plants are not thoroughly understood. P is an important plant macronutrient, making up about 0.2% of a plant's dry weight. It is a component of key molecules such as nucleic acids, phospholipids, and ATP, and, consequently, plants cannot grow without a reliable supply of this nutrient (Schachtman et.al . 1993**).**P is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993). After N, P is the second most frequently limiting macronutrient for plant growth. This update focuses on P in soil and its uptake by plants, transport across cell membranes, and compartmentation and redistribution within the plant. In this context it is found that phosphorus utilization is better by the Bt-cabbage plant when it is applied at recommended dose. It was also found that comparatively the transgenic plant utilized more phosphorus in other words the phosphorus utilization efficiencies of transgenic is higher as compared to the non transgenic plant. Though the transgenic plant was made from the same non transgenic cabbage i.e. isogenic line but when one foreign (Bt) gene was inserted into the transgenic cabbage plant, the gene might have got inserted in the vicinity of a gene which may be responsible for the phosphorus uptake by the cabbage plant. Molecular studies have confirmed the presence of multiple genes encoding phosphate transporters that are differentially expressed (Schachtman et.al . 1993).. This Bt gene in this vicinity of uptake gene might have enhanced the activity of latter gene. So, the transgenic cabbage utilizes phosphorus more efficiently than isogenic line of the cabbage plant. Plant root geometry and morphology are important for maximizing P uptake, because root systems that have higher ratios of surface area to volume will more effectively explore a larger volume of soil (Lynch, 1995). It was also found that this transgenic plant is very much responsive to the fertilizer phosphate than the native one. Example of hybrid vigor is known but the Bt gene may act as a marker for locating line to unknown P uptake gene for future exploitation.

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References

Dunfield K.E., Germida J.J. 1995. Impact of genetically modified crops on soil- and plant associated microbial communities. *Journal of Environmental Quality*, **33:** 806-815.

Dutta, D., Gopal, M., Shukla, L., Mahajan V.K. 2012. Comparative study of Nematode Population in the Rhizosphere of Bt-Transgenic Cabbage and Non-transgenic Cabbage (*Brassica oleracea* var. Capitata). *International Journal of Agricultural Environment and Biotechnology*. **5**(2):151-155.

Gray, A.J. 2004. Ecology and government policies: the GM crop debate. *Journal of Appliances Ecology*, **41:** 1-10.

Hails R.S. 2000. Genetically modified plants - the debate continues. *Trends in Ecology and Evolution*, **15**:14-18.

Koening, H.A., Johnson, C.R. 1942. Colorimetric determination of phosphorus in geological materials. *Industrial & Engineering Chemistry*. **14**: 155-156.

Lynch, J. 1995. Root architecture and plant productivity. *Plant Physiology*, **109:**7–13.

Mackenzie, A.J., Dean L.A. 1948. Procedure for measurement of P^{31} and P^{32} in plant material. *Analytical Chemistry.* **20**:559-560.

Schachtman, D.P., Reid, R.J., Ayling, S.M. 1998. Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiology*, **116**: 447–453

Snow, A.A., Andow, D.A., Gepts, P., Hallerman, E.M., Power A., Tiedje J.M., Wolfenbarger L.L.2005.Genetically engineered organisms and the environment: Current status and recommendations. *Ecology Appliances*, **15**: 377-404.

Theodorou, M.E., Plaxton, W.C. 1993. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. *Plant Physiology*, **101:**339–344.