Integrated K Management Exhibit a Key Role in Potassium Uptake Transporter (ZmKUP) Expression to Improve Growth and Yield of Corn

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A B S T R A C T

Potassium contributes significantly to growth, development, yield, and quality of the crop plants. Application of unbalanced potassium fertilization leads to low productivity and quality of cereals and other crops. Hence, there is an urgent demand to find out the balanced source of potassium fertilizer through utilization of nutrient management approaches. In the current study, we applied farmyard manure (FYM) as an alternative source of potassium fertilization, combination of FYM and muriate of potash (MOP) to show their role on growth, development, yield. Expression study of potassium transporter in corn (PEHM2 variety) also carried out. A field experiment was carried out using 0, 60, 90 kg K/ha through MOP, FYM and their combinations in corn-wheat cropping system during two consecutive years 2010-11 and 2011-12. Results showed that potassium fertilization played a significant role in the enhancement in growth and development of shoot as well as root. The high expression of ZmKUP gene, associated with translocation of potassium in plants with fertilization of potassium resulted in an improvement in growth and yield which further leads to an increase in yield of corn. Application of MOP and FYM both as potassium fertilizer showed a significant improvement in growth and yield of corn through an enhancement of ZmKUP expression. So, here we are showing that FYM as an alternative source of potassium fertilization as a suitable option for sustainable yield.

Keywords
Farmyard manure (FYM), Muriate of potash (MOP), ZmKUP expression

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**Introduction**

Potassium (K) is one of the vital nutrients for growth and yield of crops. Most of the crops absorb a significant amount of potassium from the rhizospheric soil through roots (Steingrobe and Claassen, 2000). Potassium concentration in the soil varied which constitutes about 2.5% of the lithosphere. The actual concentration of K occur in soil ranged from 0.04 to 3% (Sparks and Huang, 1985). The availability of K⁺ for plant uptake depends on nutrient dynamics and total K content in the soil. The exchange of K between different pools in the soil strongly depends on the concentration of other macronutrients in the soil solution (Yanai et al., 1996).

The release of exchangeable K in soil prolonged in comparison to the rate of K⁺ acquisition by the plants (Sparks and Huang, 1985). Consequently, the availability of K⁺ in soil is declined or very low (Johnston et al., 2003). Moreover, the presence of high level of other monovalent cations such as Na⁺ and NH₄⁺ also affect K availability in the soil which ultimately interferes with K uptake (Rus et al., 2001; Qi and Spalding, 2004). Potassium plays a crucial role in antagonistic and synergistic interaction with other essential nutrients of crops (Dibb and Thompson, 1985). K improves root growth, drought resistance, maintains turgor, enhance translocation and assimilation of nutrients resulting production of starch-rich grain, enhancement of protein content in plants, and also bring retardation of crop disease (Dobermann, 2001; Polara et al., 2009; Nejad et al., 2010). Potassium deficient conditions may cause reduction of number and size of leaves; decline assimilates production and transport from leaves to the sink leads low yield and quality (Pettigrew and Meredith Jr, 1997; Jordan-Meille and Pellerin, 2004; Pettigrew, 2008).

Globally, North America is among the largest producer with a share of 49% of K fertilizers production followed by East Europe and Central Asia with a share of 39% by the end of 2018 (FAO 2015). It might be the uneconomical approach for farmers in various countries which do not have potassium reserves due to higher potassium fertilizer input index with low food output price index that raises food insecurity problems in different parts of the world (Pretty and Stangel, 1985). Hence, there is an urgent demand to find out some alternative source of potassium. Farm Yard Manure (FYM) could be a better option to overcome such problems. The FYM is cheaper, readily available, improves soil health and capable of solubilizing the native K in soil. FYM accelerates mineral weathering and aids in solubilization of plant nutrients from otherwise insoluble minerals. It also provides carbon in slow availability manner and energy source to support a large diverse, metabolically active microbial community which helps to solubilization and availability of nutrients to crop plants (Wagner and Wolf, 1999).

The present study focuses on to decipher the effect of integrated K management fertilization on its impact on growth and yield of corn under corn-wheat cropping system where FYM used as an alternative source of K fertilization to minimize the dependency on K fertilizers.

**Materials and Methods**

**Analysis of Soil properties, temperature, and rainfall during the period of the experiment**

Soil samples were collected randomly from a farm field, Indian Agricultural Research Institute, New Delhi, India situated at (28°35’ N, 77° 12’ E) and at 228.6 msl. The collected
samples were pooled and studied for pH (Jackson, 1973), organic carbon content (Walkley and Black, 1934), available N by alkaline permanganate method (Subbiah and Asija, 1956), phosphorus (Olsen et al., 1954), potassium (Stanford and English, 1949). At the initial stage, the different fraction of potassium in soil was studied such as water-soluble potassium by soil: water (1:5) extraction method (Page, 1982), exchangeable K by 1N NH₄OAC (Hanway and Heidel, 1952), and non-exchangeable K by 1N HNO₃ (Page, 1982) which presented in Table 1.

The daily temperature recorded during the growth period of corn ranged between 19.8°C and 38.5°C in the year 2010-11 whereas it ranged between 22.0 and 38.2°C during 2011-12. During 2010, the intensity of rainfall was higher, but distribution was uneven whereas during 2011 intensity was low and distribution of rainfall was even and well distributed (Fig. 1a and 1b). The total rainfall recorded during rainy season 2010 and 2011 was 763.0 and 464.8 mm with 35 and 30 rainy days respectively.

**Field Experiment conditions**

The experiment was carried out in a randomized block design (RBD) at the fixed site with three replications. Ten treatments were applied to both corn (M) in the rainy season and wheat (W) in the winter season (Table 2). Recommended doses of 150 kg N/ha and 26 kg P/ha applied to corn through urea and diammonium phosphate (DAP), respectively. The full dose of P, K and 50 kg N ha⁻¹ were given as basal and remained 100 kg N ha⁻¹ was given in two splits 50 kg N ha⁻¹ each at 30 and 60 days after sowing (DAS). Muriate of potash (MOP) and farmyard manure (FYM) were used as sources of potassium and applied as per treatments. Again recommended doses of 120 kg N ha⁻¹ through urea and 26 kg P ha⁻¹ through DAP applied in wheat. During 2010 and 2011, the amount of nitrogen, phosphorus, and potassium in FYM applied was 5 and 6 gN kg⁻¹, 4 and 4 gP kg⁻¹ and 5 and 4 gK kg⁻¹ respectively. The amount of nitrogen, phosphorus, and potassium applied through urea, DAP and FYM were adjusted in all the treatments to maintain the required nutrient combinations. The Corn hybrid variety ‘PEHM2’ a cross of CM137 X CM138 was sown at 60 cm x 20cm spacing using 20 kg seed ha⁻¹.

**Measurement of plant growth parameters and yield of corn**

Root studies were carried out at 0-15 and 15-30 cm soil depth during both the years at the silking stage. The soil adhered to the roots were washed gently according to the standard procedure (Costa et al., 2000). Root length, surface area, volume and average diameter were measured using a Hewlett Packard scanner controlled by WIN RHIZO Programme V. 2002C software (Regent Instruments Inc. Ltd. Quebec, Canada).

The greenness index was recorded using SPAD meter at 30, and 60 DAS from five plants selected randomly for dry matter studies in corn from 1 m² area.

The plant samples were separated into leaves, stem and reproductive parts (tassels/cobs/spikes) and were oven drying at 65°C till the constant weight attained and recorded for growth analysis. Leaf area index (LAI), crop growth rate (CGR), net assimilation rate (NAR) were calculated following standard procedure (Watson, 1952; Gardner et al., 2003).

Harvesting of corn and wheat was carried out in an area of 4.8 m² and 4.5 m² from the center of each plot manually. Dry weights of stems and grains were measured separately. The
grain, stover/straw, and biological yield calculated through the weight of dried plant samples.

Expression analysis of potassium ion uptake transporter

Corn leaf samples at silking, milk and dough stage collected from all three potassium treatments, *i.e.*, 0, 60 and 90 kg K and immediately emerged to liquid nitrogen and stored at -80°C. Total RNA was extracted using Nucleopore RNA sure mini kit (Genetix, India) followed by DNase treatment and quantified through NanoDrop spectrophotometer (Thermo Scientific, USA). One µg total RNA used for the preparation of cDNA using Verso cDNA kit (Thermo Scientific, USA). Available coding sequences of Potassium Ion Uptake Permease (KUP) in corn, rice and Arabidopsis were retrieved from NCBI database (http://www.ncbi.nlm.nih.gov) and following gene-specific primers were designed: ZmKUPF5’-GTGGTGCGAGA ACCAAATGCAGAT-3’ and ZmKUPS’-TAGCTAACCTGCCTGCTTTGA-3’. The semi-qRT-PCR performed for expression analysis of the genes. The PCR reaction was carried out in 25 µl of reaction volume containing 2µl of cDNA as template, 2.5 µl of 10X Taq polymerase buffer, 0.5 µl of dNTPs mixture (10 mM), 1.0 µl of each primer (10 mM), 1 U of Taq DNA polymerase and rest of nuclease-free water for volume make-up. For amplification of the genes, the thermocycling conditions were followed as one cycle of the initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 45 sec., primer annealing at 60°C for 45 sec., extension at 72°C for 45 sec. also, one cycle for a final extension at 72°C for 3 min. The Actin gene of corn used as an internal control. The PCR product was checked on 1.2% agarose gel and documented using Gel Doc System of Syngene. The amplified product was purified and validated through sequencing (Chromous Biotech. Pvt. Ltd., Bangalore).

Estimation of potassium in root and shoot of the plant

Root samples and their shoot parts collected at the silking stage were oven drying at 65°C for about 48 hours till the constant weight observed. 1.0 g oven-dried samples of root and shoot were grinded and digested before chemical analysis by using 10 mL of the di-acid mixture (concentrated HNO₃ and HClO₄ in 9:4 ratios by volume) on a hot plate till the content became colorless. After digestion, a small volume of distilled water added to the digested sample. The content filter and the final volume were made up to 100 mL. Then the potassium was determined by a flame photometer (Prasad et al., 2006).

Data and statistical analysis

The experimental data were statistically analyzed using ‘Analysis of Variance’ technique for randomized block design (Gomez and Gomez, 1984). The least significant difference (LSD) at (*p*-value 0.05) was worked out for each parameter of the study. The qualitative data regarding the gel image was converted into quantitative data using AlphaView software (http://www.proteinsimple.com/software_alphaview), and the relative expression of the genes was calculated using pfaffl’s equation (Pfaffl, 2001). Correlation studies of yield and root growth parameters done by using SPSS 16.0 version.

Results and Discussion

Plant growth and yield

During 2010 and 2011, treatments with K fertilization showed significantly higher root growth over without K fertilization (control). Treatment T₄ showed the highest root length, surface area, average diameter, volume and dry weight compared to other treatments. Treatment T₃ and T₅ showed closely similar
response compare with T₄. Besides, treatment T₂, T₈, and T₉ also showed significant superiority over treatments T₁, T₆, T₇, and T₁₀ (Fig. 2).

Potassium fertilization with sole or in combination with FYM showed significant ($P=0.05$) improvement in greenness index over control at all the stages except at 30 DAS during rainy season 2011 (Fig. 3). The treatment T₄ was found significantly ($P=0.05$) superior to remaining treatments during 2010 and 2011 for greenness index. Improvement in LAI, CGR, and NAR also observed with K fertilization over control at 0 - 30 and 30 - 60 DAS during 2010 and 2011 except CGR and NAR at 30-60 DAS during 2010. The highest LAI, CGR, and NAR observed in treatment T₄. Treatments T₃ and T₅ were at par with treatments T₂, T₈, and T₉ (Table 3).

Grain, stover and biological yield of corn and wheat significantly differ due to K fertilization. All treatments with K fertilization showed higher grain yield over without K fertilization. The highest grain yield obtained with T₄ (4.44 t ha⁻¹ and 5.42 t ha⁻¹) during 2010 and 2011. This treatment (T₄) produced 27.7% and 19.4% higher yield over T₃ and T₅, in 2010 and 10.8% and 12.4%, during 2011 respectively. Stover and biological yield followed the similar trend. Although in wheat, the highest grain yield obtained with T₁₀ (5.39 t ha⁻¹ and 5.49 t ha⁻¹) during 2010-11 and 2011-12. This treatment (T₁₀) produced the highest straw yield of 8.20 t/ha and 8.98 t/ha respectively. Biological yield followed the similar trend as of grain yield (Table 4).

In corn during 2010, significant and positive correlations (Supplementary Table 1) were observed ($P=0.01$) between yield with root length ($r=0.992$), root surface area ($r=0.985$), root diameter ($r=0.959$) and root volume ($r=0.944$) at 0-15 cm soil depth. The root length ($r=0.995$), root surface area ($r=0.993$), root diameter ($r=0.979$) and root volume ($r=0.997$) at 15-30 cm soil during both the years shows significant and positive among them (Supplementary Table 2).

### Table 1. Analysis of various parameters in soil

| Parameters                  | Properties     |
|-----------------------------|----------------|
| **Soil Type**               | Sandy loam     |
| Sand (%)                    | 51.5           |
| Silt (%)                    | 23.0           |
| Clay (%)                    | 25.5           |
| pH                          | 8.0            |
| EC (dS m⁻¹)                 | 0.43           |
| Organic C (%)               | 0.4            |
| Available N (mg kg⁻¹)       | 77.32          |
| Available P (mg kg⁻¹)       | 6.16           |
| Water soluble K (mg kg⁻¹)   | 19.3           |
| Exchangeable K (mg kg⁻¹)    | 99.3           |
| Non-Exchangeable K (mg kg⁻¹)| 850.7          |
Table 2: Designing of various treatments for integrated potassium fertilization under Corn-wheat cropping system

| Treatment Symbol | Treatment detail | Crop     | Amount of nutrient applied (kg ha\(^{-1}\)) |
|------------------|------------------|----------|------------------------------------------|
|                  |                  |          | N  | P  | K  |
| T₁               | K₀ (M) – K₀ (W)  | Corn     | 150| 26 | 0  |
|                  |                  | Wheat    | 120| 26 | 0  |
| T₂               | MOP\(_{60}\) (M) – K₀(W) | Corn     | 150| 26 | 60 |
|                  |                  | Wheat    | 120| 26 | 0  |
| T₃               | MOP\(_{30}\)+FYM\(_{30}\)(M)–MOP\(_{60}\)(W) | Corn     | 150| 26 | 60 |
|                  |                  | Wheat    | 120| 26 | 0  |
| T₄               | MOP\(_{60}\)+FYM\(_{30}\)(M) – K₀(W) | Corn     | 150| 26 | 90 |
|                  |                  | Wheat    | 120| 26 | 0  |
| T₅               | MOP\(_{30}\)+FYM\(_{30}\)(M) – K₀(W) | Corn     | 150| 26 | 60 |
|                  |                  | Wheat    | 120| 26 | 0  |
| T₆               | K₀(M)–MOP\(_{60}\) (W) | Corn     | 150| 26 | 0  |
|                  |                  | Wheat    | 120| 26 | 60 |
| T₇               | K₀(M)–MOP\(_{30}\)+FYM\(_{30}\)(W) | Corn     | 150| 26 | 0  |
|                  |                  | Wheat    | 120| 26 | 60 |
| T₈               | MOP\(_{60}\) (M)– MOP\(_{30}\)+ FYM\(_{30}\) (W) | Corn     | 150| 26 | 60 |
|                  |                  | Wheat    | 120| 26 | 60 |
| T₉               | MOP\(_{60}\) (M)– MOP\(_{60}\) (W) | Corn     | 150| 26 | 60 |
|                  |                  | Wheat    | 120| 26 | 60 |
| T₁₀              | K₀ (M)–MOP\(_{60}\) +FYM\(_{30}\) (W) | Corn     | 150| 26 | 0  |
|                  |                  | Wheat    | 120| 26 | 90 |
Table 3 Effect of potassium fertilization on growth attributes of corn during 2010 and 2011

| Treatment | LAI | 0-30 DAS | 30-60 DAS | CGR (g m⁻² (land area) day⁻¹) | 0-30 DAS | 30-60 DAS | 0-30 DAS | 30-60 DAS | NAR (g m⁻² (leaf area) day⁻¹) | 0-30 DAS | 30-60 DAS |
|-----------|-----|----------|-----------|-------------------------------|----------|-----------|----------|-----------|-------------------------------|----------|-----------|
|           |     | 2010     | 2011      | 2010                          | 2011     | 2010      | 2011     | 2010      | 2011                          | 2010     | 2011      |
| T₁        | 1.73| 3.00     | 4.06      | 4.63                          | 4.75     | 5.46      | 13.6     | 14.3      | 1.51                          | 2.00     | 4.95      | 3.81      |
| T₂        | 1.91| 4.00     | 4.86      | 5.62                          | 5.35     | 7.96      | 18.3     | 23.5      | 1.81                          | 2.76     | 5.78      | 4.92      |
| T₃        | 2.04| 4.17     | 4.91      | 5.67                          | 5.58     | 10.05     | 21.4     | 25.0      | 1.94                          | 3.44     | 6.59      | 5.13      |
| T₄        | 2.24| 4.27     | 5.47      | 5.90                          | 5.95     | 11.12     | 25.8     | 27.4      | 2.14                          | 3.77     | 7.29      | 5.45      |
| T₅        | 2.01| 4.14     | 4.87      | 5.61                          | 5.59     | 9.15      | 21.7     | 24.6      | 1.94                          | 3.14     | 6.73      | 5.07      |
| T₆        | 1.71| 3.29     | 4.11      | 4.91                          | 4.79     | 6.08      | 13.1     | 16.5      | 1.50                          | 2.20     | 4.80      | 4.11      |
| T₇        | 1.74| 3.28     | 4.17      | 4.94                          | 4.77     | 6.27      | 13.5     | 16.6      | 1.51                          | 2.26     | 4.85      | 4.12      |
| T₈        | 1.90| 4.10     | 4.91      | 5.58                          | 5.40     | 8.07      | 17.7     | 24.4      | 1.82                          | 2.78     | 5.58      | 5.07      |
| T₉        | 1.91| 4.05     | 4.86      | 5.60                          | 5.35     | 7.86      | 18.6     | 24.1      | 1.81                          | 2.71     | 5.91      | 5.05      |
| T₁₀       | 1.74| 3.39     | 3.49      | 4.93                          | 4.71     | 6.39      | 13.7     | 17.5      | 1.49                          | 2.30     | 5.45      | 4.26      |

LSD (P=0.05) 0.17 0.41 0.69 0.58 0.53 0.96 NS 4.37 0.19 0.33 NS 0.99

DAS: Days after sowing
Table 4: Effect of integrated potassium fertilization on yield (t ha\(^{-1}\)) of corn and wheat

| Treatment | Corn       |          | Biological |          | Wheat       |          | Biological |
|-----------|------------|----------|------------|----------|------------|----------|------------|
|           | Grain 2010 | 2010     | Stover 2011| 2011     | Biological 2010-11 | 2011     | 2010-11 2011-12 | 2010-11 2011-12 | 2010-11 2011-12 |
| T\(_1\)   | 2.21       | 2.72     | 4.48       | 5.26     | 6.69       | 7.98     | 3.80       | 3.89     | 7.30       | 7.59  | 11.1  | 11.5   |
| T\(_2\)   | 3.06       | 4.07     | 5.07       | 6.33     | 8.13       | 10.40    | 4.10       | 4.18     | 7.75       | 8.06  | 11.8  | 12.2   |
| T\(_3\)   | 3.68       | 4.89     | 5.90       | 6.75     | 9.59       | 11.64    | 4.94       | 5.05     | 8.13       | 8.53  | 13.1  | 13.6   |
| T\(_4\)   | 4.44       | 5.42     | 6.53       | 7.03     | 10.97      | 12.45    | 4.22       | 4.31     | 7.78       | 8.09  | 12.0  | 12.4   |
| T\(_5\)   | 3.72       | 4.82     | 5.83       | 6.62     | 9.55       | 11.25    | 4.10       | 4.19     | 7.76       | 8.07  | 11.9  | 12.3   |
| T\(_6\)   | 2.21       | 2.96     | 4.54       | 5.64     | 6.75       | 8.60     | 4.78       | 4.95     | 7.86       | 8.17  | 12.6  | 13.1   |
| T\(_7\)   | 2.21       | 3.02     | 4.39       | 5.72     | 6.60       | 8.74     | 5.15       | 5.25     | 8.18       | 8.66  | 13.3  | 13.9   |
| T\(_8\)   | 2.99       | 4.30     | 5.14       | 6.53     | 8.13       | 10.92    | 5.05       | 5.16     | 8.14       | 8.77  | 13.2  | 13.9   |
| T\(_9\)   | 3.02       | 4.21     | 5.21       | 6.43     | 8.23       | 10.74    | 4.81       | 5.01     | 7.93       | 8.24  | 12.7  | 13.3   |
| T\(_{10}\)| 2.21       | 3.20     | 4.47       | 5.83     | 6.68       | 9.03     | 5.39       | 5.49     | 8.20       | 8.98  | 13.6  | 14.5   |
| LSD \((P=0.05)\) | 0.46     | 0.51     | 0.44       | 0.71     | 0.8        | 0.90     | 0.5        | 0.6      | NS         | NS   | 1.4   | 1.3    |
Table 5: Effect of integrated potassium fertilization on K uptake in corn under corn–wheat cropping system

| Treatment | Corn 2010 |  | Corn 2011 |  |
|-----------|-----------|---|-----------|---|
|            | Shoot (g plant⁻¹) | Root (mg g⁻¹ dry wt.) | Shoot (g plant⁻¹) | Root (mg g⁻¹ dry wt.) |
| T1        | 0.84      | 8.90 | 1.01      | 9.81     |
| T2        | 1.26      | 46.17| 1.96      | 49.40    |
| T3        | 1.65      | 67.82| 2.36      | 83.17    |
| T4        | 2.09      | 115.58| 2.84     | 131.34   |
| T5        | 1.66      | 72.76| 2.14      | 75.28    |
| T6        | 0.80      | 8.35 | 1.20      | 11.97    |
| T7        | 0.85      | 8.47 | 1.25      | 13.31    |
| T8        | 1.22      | 45.52| 2.18      | 55.74    |
| T9        | 1.30      | 46.71| 2.06      | 51.57    |
| T10       | 0.85      | 8.82 | 1.34      | 13.69    |
| LSD (P=0.05) | 0.22 | 5.44 | 0.30 | 8.88 |
**Supplementary Table.1** Pearson’s correlations matrix between yield and root growth parameters at 0-15 and 15-30 cm soil depth 2010

| Parameter                          | Grain yield (t/ha) | Length (cm) | Surface area (cm²) | Average Diameter (mm) | Volume (cm³) |
|-----------------------------------|--------------------|-------------|--------------------|-----------------------|--------------|
| **0-15 cm depth**                 |                    |             |                    |                       |              |
| Grain yield (t/ha)                | 1                  | 0.992**     | 0.985**            | 0.959**               | 0.944**      |
| Length (cm)                       |                    | 1           | 0.993**            | 0.922**               | 0.968**      |
| Surface area (cm²)                |                    |             | 1                  | 0.900**               | 0.986**      |
| Average Diameter (mm)             |                    |             |                    |                       |              |
| Volume (cm³)                      |                    |             |                    |                       |              |
| **15-30 cm depth**                |                    |             |                    |                       |              |
| Grain Yield (t/ha)                | 1                  | 0.995       | 0.993              | 0.979                 | 0.997        |
| Length (cm)                       |                    | 1           | 0.987              | 0.973                 | 0.993        |
| Surface area (cm²)                |                    |             | 1                  | 0.964                 | 0.992        |
| Average Diameter (mm)             |                    |             |                    | 1                     | 0.969        |
| Volume (cm³)                      |                    |             |                    |                       | 1            |

**. Correlation is significant at the 0.01 level (2-tailed).
Supplementary Table 2 Pearson’s correlations matrix between yield and root growth parameters at 0-15 and 15-30 cm soil depth during 2011

| Parameter               | 0-15 cm depth | 15-30 cm depth |
|-------------------------|---------------|----------------|
|                         | Grain yield (t/ha) | Length (cm) | Surface area (cm²) | Average Diameter (mm) | Volume (cm³) | Grain Yield (t/ha) | Length (cm) | Surface area (cm²) | Average Diameter (mm) | Volume (cm³) |
| Grain yield (t/ha)      | 1             | 0.966**       | 0.997**         | 0.988**               | 0.992**      | 1               | 0.919        | 0.995           | 0.996               | 0.981        |
| Length (cm)             |               | 1             | 0.981**         | 0.938**               | 0.935**      |                 | 1            | 0.899           | 0.945               | 0.958        |
| Surface area (cm²)      |               |               | 1               | 0.983**               | 0.984**      |                 |              | 1               | 0.989               | 0.972        |
| Average Diameter (mm)   |               |               |                 |                       |              |                 |              | 1               | 0.989               | 0.972        |
| Volume (cm³)            |               |               |                 |                       |              |                 |              |                 | 1                   |              |

**. Correlation is significant at the 0.01 level (2-tailed).
Fig. 1a (top) and 1b (bottom) Weather conditions during the crop growing period 2010 (top) and 2011 (bottom)
**Fig. 2** Effect of integrated potassium fertilization on root: 0-15 cm (top) and 15-30 cm (bottom) soil depth.
Fig. 3 Effect of integrated potassium fertilization on greenness index in corn during 2010 (top) and 2011 (bottom)

* Error bar denotes the LSD value
** Absence of error bar denotes treatment means non-significant
Fig. 4 Expression analysis of ZmKUP gene under three different external application of potassium (K) in field conditions of corn cv. PEHM2 at silking, milk and dough stage. Bar K₀ indicates 0 kg K/ha, K₆₀ indicates 60 kg K/ha and K₉₀ indicates 90 kg K/ha.
**Fig.5** Gene expression correlation with growth stages at different K levels and effect on yield
Potassium content in root and shoot of corn

Application of potassium significantly affects K content in root and shoot of corn crop during both the years (2010 and 2011) of experimentation (Table 5). The highest uptake of potassium observed in treatment T4, applied with 30 kg K through MOP and 30 kg K through FYM followed by treatment T3 and T5 applied with 60 kg K through MOP and 30 kg K through FYM. The least uptake of potassium observed in Treatment T1, T6, T7, and T10 where no potassium applied during both the years.

Transcript expression profiling of ZmKUP in Corn

The expression analysis of Corn Potassium ion Uptake Permease (ZmKUP) was carried out at three different stages with three K fertilization (0, 60 and 90 kg/ha). Plant samples without K fertilization used as a control. Fertilization of K (90 kg/ha) showed an induced expression of ZmKUP gene at silking, milk and dough stage. At the milk stage, ZmKUP gene showed comparatively higher expression followed by silking and dough stage. However, there is no expression of ZmKUP gene was exhibited with 60 kg/ha fertilization of K in all stages. The relative expression analysis showed 2.1, 2.3 and 2.1 fold-changes in expression in silking, milk and dough stage respectively as compared to the control (Fig. 4).

Data revealed (Fig. 2) that the treatments with K fertilization have significantly better for root growth parameters compared to without K fertilization. The treatment without K fertilization resulted low root growth which may be due to less transport of photosynthetic assimilates away from the source to sink under extreme K deficiency conditions (Ashley and Goodson, 1972; Mengel and Viro, 1974; Mengel and Haeder, 1977). This restriction on the transport of photosynthates can lead to an accumulation of sugars in leaf tissues of K+ deficient plants resulting in inhibition of root growth (Pettigrew, 1999; Zhao et al., 2001). Potassium fertilization results in an enhanced translocation of photosynthetic assimilate from source to sink leads to better root growth (Polara et al., 2009; Roshani and Narayanasamy, 2010).

Data represented in Figure 3 and Table 3 showed a significant and positive effect of potassium fertilization on greenness index, LAI, CGR and NAR in corn during 2010 and 2011. Potassium plays a significant role in nutrient transportation in plants. The increase in greenness index under T4 was due to the synergistic effect of K over other nutrients. Although, the uptake of N, P and K was increased with increasing levels of K in plants (Baque et al., 2006). As potassium plays a vital role in promoting photosynthesis, cell expansion by regulating solute potential which increases the rate of leaf expansion and the leaf area may also result into better LAI, CGR and NAR in treatment with K fertilization (Rao, 1983; Yahiya et al., 1996).

Significant higher amount of K content was observed under K fertilized plots in both the years of experimentation (Table 5). Lower K content in root and shoot observed under the no K applied treatments. Rashid et al., (2001) reported the significant increase in potassium concentration with the application of potassium and uptake of potassium in no K treatment attributed to an interactive effect of N and K. The higher K uptake recorded when K supplemented through FYM along with K added through MOP in comparison with K application through MOP alone. These results could be associated with an increased K uptake due to other nutrients available in the manure in equal proportion (Subba Rao et al., 1993; Kumar et al., 2015).
The yields of K fertilized plots were higher compared to no K fertilized plots (Table 4). It may be attributed to higher concentration K content in root and shoot in plots fertilized by K. The K fertilization is vital to many plant processes including photosynthesis, photosynthates translocation, protein synthesis, activation of plant enzymes. Integrated K management involves FYM supplies N, P, and K in available forms to the plants through biological decomposition along with micronutrients. Due to this addition benefits of FYM application higher yield was obtained in treatments applied with FYM compared to yields from plot fertilized through MOP alone or no K. Integrated nutrient management (NPK and FYM) recorded 19% higher yield of corn compared to the treatment applied with NPK only (Jiang et al., 2006). Rehman et al. (2008) reported that different levels of NPK and FYM alone or in combination had the significant effect on grain and biological yield. Inorganic NPK fertilization significantly increased grain yields of wheat (21%) and corn (16 % -72%) compared to inorganic nitrogen and phosphorus fertilization (Zhang et al., 2011). Correlation studies have shown the significant relationship between yield and different root growth parameters at 0-15 and 15-30 cm depth in soil with integrated fertilization of K and MOP alone. Yield and root growth parameters were found significantly correlated with each other indicating that these have a significant role in improving the yield of the crop. The positive correlation showed that fertilization of K improved root development, which results in the better yield of the corn crop.

Expression profiling of corn potassium ion uptake permease (ZmKUP) was carried out to decipher the role of integrated potassium fertilization on K transport across the plant system from roots. In the present study induction of KUP1 expression at silking, milk and dough stage in corn leaves increased 2 fold with fertilization of 90 kg K ha⁻¹. This induced expression of K transporter could attribute to K at higher available concentration transported from roots to various developmental parts including leaves. The correlation of gene expression with yield showed a direct correlation between gene expression and yield (Fig. 5), which inference that whenever the expression level of the gene is high, then the yield will increase. The other possibility of less expression of ZmKUP in treatment applied with 0 Kg K ha⁻¹ and 60 kg K ha⁻¹ might be due to expression was studied only in leaves but not in roots where uptakes of minerals take place from the soil. When potassium content in roots reduced by approximately 60%, AtHAK5, a potassium transporter gene from the KUP/HAK/KT family, was most consistently and strongly up-regulated in its expression level across 48-h, 96-h, and 7-d potassium deprivation experiments (Gierth et al., 2005). ZmHAK5 member besides mediating initial K uptake from soil transporters might also be involved in other regulatory and developmental processes. The expression of ZmHAK5 increased by increasing concentration of K. Transporter gene from the KUP/HAK/KT family, was most consistent and not strongly up-regulated in its expression level across the different concentration of potassium (Gupta et al., 2008).

MOP and FYM were used as sources of K fertilization to study their impact on growth, yield, and expression of potassium uptake transporter of corn in the corn-wheat cropping system. Current study suggested that transporter gene from the KUP/HAK/KT family was most consistent and not strongly up-regulated in its expression level across the different concentration of potassium. The consistency in the expression of potassium uptake transporter leads to better root system by increasing root length, surface area, roots
diameter, root volume of root under treatment applied with integrated fertilization of potassium over the treatment designed with MOP fertilizer alone or control. The better root system resulted into better greenness index, LAI, CGR and NAR and yield of the corn crop. The Positive correlations observed between root growth and crop yield.

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