Running Title: *Potassium fertilization to strengthen rice stem*

Potassium fertilization may improve stem strength and yield of basmati rice grown on nitrogen-fertilized soils

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Imbalance use of fertilizers is one of the important factors for lodging of rice leading to paddy-yield reduction. Potassium (K) deficiency may be the one of the contributing factors in this regard. Present study was conducted to assess the decrease in lodging losses due to use of K fertilizer in the presence of nitrogen by determining various factors contributing to stem strength. Two levels of K, one with deficient (K_d) while other with recommended (K_r) K and three levels of N *i.e.* deficient (N_d), half of recommended (N_{hr}) and recommended (N_r) were applied along with recommended dose of phosphorus and zinc (Zn) to two fine grain rice cultivars *i.e.* Super basmati and Basmati 515. The experiment was laid out under randomized complete block design in factorial arrangement with four replicates and various parameters including plant height, number of grains per panicle, paddy yield, basal internode space, cellulose, silicon and fiber contents possibly contributing to growth, yield and stem strength were determined in response to various combinations of K for N fertilizers. Results showed that application of K fertilizer significantly improved K concentration in rice stem, and paddy yield of Basmati rice. Furthermore, K fertilization improved plant parameters such as basal internode space, cellulose content and stem-fiber contents. Increase in silicon content in stem due to application of K was also observed, which may also contribute to stem strength against environmental stresses. The present study suggests further work to understand the physiological and biochemical mechanisms contributing to stem strength in response to K fertilization.

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An abstract is an outline/brief summary of your paper and your whole project. It should have an intro, body and conclusion. It is a well-developed paragraph, should be exact in wording, and must be understandable to a wide audience. Abstracts should be no more than 500 words, formatted in Microsoft Word, and single-spaced, using size 12 Times New Roman font.

Keywords: Potassium, balance-fertilization, stem strength, nitrogen, lodging

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INTRODUCTION

Rice is the second most important staple crop after wheat in Pakistan and contributes greatly to food security and foreign exchange. Area under rice cultivation in Pakistan is about 2.57 million hectares, giving 6.16 million tons annual production with an average of 2.39 tons per ha (Anonymous, 2011). Pakistan is at number eleven in total rice production (FAO, 2012) however fine-grained Basmati rice of Pakistan is famous for its quality, taste and aroma throughout the world. Therefore, rice is an important source of income for majority of the farmers in the Punjab and Sindh provinces of Pakistan. Different factors causing low rice production in Pakistan include water scarcity, high input prices, unavailability of skilled labor, less plant population, weeds and pest infestation, lodging and marketing issues (Baloch *et al*., 2004). Lodging causes severe damage to yield and quality of grain and forage in cereals especially in rice. Broken rice plant stem disturbs the vascular tissues, lowers the photosynthetic rate, increases respiration, and reduces nutrients translocation for grain filling and other plant needs (Tripathi *et al*., 2003). Lodging also increases disease attack and reduces pest resistance. Mechanical harvesting problems are also associated with lodging (Kono, 1995) and have great significance in modern agriculture systems.

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Generally, crops are more susceptible to lodging near to its maturity and is often caused by rain, wind and hailing. Its distribution may often not be uniform throughout the field, may be in spots or scattered over different field sections (Berry *et al*. 2004). Lodging reduces cereals yield especially under mechanized harvesting (Rajkumara, 2008) and restricts the opportunity of increase in rice yield even by additional fertilizer application. Imbalance and limited use of potassium (K) fertilizers may weaken the stem strength making it more susceptible to lodging (Chang, 1964).

Potassium stands on third in macronutrients after nitrogen (N) and phosphorus (P) and is the most abundant macronutrient in soil. Its availability to plants depends upon the concentration of K in soil solution and on exchange sites. Potassium is essential for enzyme activation, charge balance and osmotic regulation in plants (Wakeel *et al*., 2011; Zörb *et al*., 2014). It is present in cation form in plant cell to maintain ionic balance and up to 10 % of plant dry matter is made up of K

(Marschner, 1995). Its ease of access to plants depends upon its status in soil solution, exchangeable K^+ and rate of exchange of K^+ from exchangeable form to soil solution form (Wakeel, 2013). Specific functions that occur in cytoplasm require small amount of potassium while a major portion (90%) of K is present in vacuole where it acts as an osmoticum contributing to extension growth of plants (Wakeel *et al*., 2010, 2011).

High K contents in plant tissue increase its resistant to sudden environmental variations like temperature stress, rain in late season, cold and frost (Chamak, 2005). Potassium accumulation in the plant tissue also decreases damage to plant in response to osmotic stress and physiological burdens (Rajkumara, 2008). There is a close interaction between K content of the lower part of culm and lodging resistance in stem. Appropriate K nutrition is closely related to lignification of sclerenchyma cells, vascular bundles and stem strength to enhance lodging resistance (Kong *et al*., 2014).

Increased use of N improves the vegetative growth and height increasing its susceptibility to lodging and decreased K fertilization weakened the plant stem. In Pakistan, use of K fertilizers is neglected; however, N application is increasing and N: K use ratio is going to increase continuously (NFDC, 2013-14). It was hypothesized that application of K fertilizer under N fertilized conditions may improve stem strength and yield of Basmati rice. This study was conducted to determine the potential role of K fertilization in stem strength, lodging resistance and paddy yield of Basmati rice in major rice growing area of Pakistan.

MATERIALS AND METHODS

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Generally, this section should include a concise description of the materials, procedures, and equipment used, including how the study was conducted, how data were collected, and what statistical and/or graphical analyses were undertaken. The materials and methods outline **WHAT WAS DONE** and **HOW IT WAS DONE**. **There should be enough detail so that someone else could repeat your study**. It should be arranged in a logical manner (i.e., keep related ideas together and write chronologically where possible). This section must be written **in paragraph form** so you must include the materials within the logical and/or chronological order of use.

Study was conducted during July-November, 2012 at Research Station of the Engro Eximp. (Private) Limited. Dera Sarawan near Sakham Muridkay, Sheikhupura. The experimental soil was clay loam and is located in rice belt of Punjab, Pakistan. Physicochemical properties (Estefane *et al*., 2013; Table 1) show that soil was deficient in K and N nutrition.

Table 1. Physicochemical properties of experimental soil.

Soil Characteristics	Values
Soil texture	Clay loam
pH	8.0
EC	0.30 dS m^{-1}
Exchangeable sodium	1.7 %
Organic matter	0.81 %
Nitrogen	0.051 %
Phosphorus (Olsen)	6 mg kg^{-1}
Exchangeable potassium	86 mg kg^{-1}
Zinc	1.08 mg kg^{-1}
Boron	0.65 mg kg^{-1}
Iron	8.75 mg kg^{-1}

***Crop husbandry*:** The study was carried out in field using randomized complete block design in factorial arrangement with four replicates. Seeds of two rice cultivars Super basmati and Basmati 515 were soaked in water for two days before nursery sowing. Seed rate was 12.5 kg for one hectare nursery sown on 15 June. Field was irrigated followed by puddling and planking and was repeated with an interval of one week to prepare field for nursery transplanting. Seedbed preparation was started keeping nursery age in view. Thirty days old nursery seedlings were transplanted manually on 15th of July. Potassium and nitrogen fertilizers were applied as deficient K (K_d) and deficient N (N_d) as (control), deficient K (K_d) and recommended N (N_r) *i.e.* 120 kg ha^{-1} N, recommended K (K_r) *i.e.* 60 kg ha^{-1} K and half recommended N (N_{hr}) i.e. 60 kg ha^{-1} N, and K_r and N_r.; whereas phosphorus was applied at the rate of 60 kg ha^{-1} at seed bed preparation in the form of single super phosphate. Zinc was applied three weeks after the nursery transplantation at 9 kg ha^{-1}. Cartap (Cartap Hydrochloride) was applied at 22.5 kg ha^{-1} on 20th August and repeated on first week of September to control insect-pests, especially the rice stem borer and rice leaf folder. In second week of September Nativo (trifoxystrobin and tebuconazol) was sprayed to cure crop from fungal attack. During 1st week of October, Actara and Score were sprayed to prevent the plant hopper.

Last irrigation was applied on 15th October 2012. Crop was harvested on 11th of November 2012 when the grain moisture content was ~19%. After manual harvesting, plant height, basal internode space, number of grains per panicle, paddy yield, acid detergent fiber concentration, cellulose concentration, and concentration of K, N and Si were determined.

***Biochemical analysis*:** Plant samples were digested following McGill and Figueiredo (1993). Half gram of dried and ground rice shoot sample was taken in each of 100 mL Pyrex digestion tube. Five milliliters of concentrated H_2SO_4 were added and allowed to predigest overnight in a fume hood. Next day, the contents of digestion tube were swirled; 2 mL of 35 % H_2O_2 was added in each tube and allowed to cool. Cooled tubes were heated on block digester at 150^{o}C. Cooled the tubes again and 2 mL of 35 % H_2O_2 was added. Temperature was raised to 380^{o}C and samples were digested for 10 min. The above step was repeated until digest remained clear / transparent. The digested samples were diluted to 50 mL volume and stored in stoppered plastic bottles for the estimation of N, P and K concentration.

***Nitrogen determination*:** Nitrogen contents were measured in this digest by distillation method as described by Bremner and Mulvaney (1982). Ten mL of aliquot were pipetted out into a 100 mL distillation flask and distilled for 90 minutes with 10 mL of 12 *N* NaOH. The distillate was collected in receiver flask containing 5 mL of mixed indicator 2% boric acid solution. The distillation time of 90 sec. allowed 35 mL distillate collection. The distillate was titrated against 0.01 *N* H_2SO_4 and nitrogen was calculated as follow;

$$N (\%) = \frac{(V - B) \times N \times R \times 14.01 \times 100}{W_t \times 1000}$$

Where, R = Ratio between total digest volume and distillation volume, B = Digested blank V= Titration volume (mL), W_t = Mass of dry plant (g), 14.01= Constant

***Potassium determination*:** Potassium was determined by flame photometer according to the method described by Chapman and Pratt (1961). The instrument was calibrated using a series of K standard solutions and emission of unknown sample was taken. The concentration of K in test samples was determined using regression equation drawn between standard solution concentrations and their emission. The corrected K concentration was calculated as follow;

K (ppm) = (K in extract- K in blank) × A/W_t

Where, A = Total volume of the digest (mL), W_t = Mass of plant material taken for digestion (g)

***Acid detergent fiber (ADF) and cellulose determination*:** Acid detergent fiber, cellulose, fiber and silica determination is done by sequence wise procedure which is called acid detergent fiber method and is proposed by Goering and Soest (1970). By this method in first step, we find acid detergent fiber which is constituent of cellulose, lignin and finally silica and then from this material cellulose, lignin and finally silica is determined. One gram rice plant sample was taken in 250 mL conical flask. One hundred mL acid detergent was added in flask containing sample. The flask was heated on hot plate for one hour and filtered it giving two washings with hot boiling water followed by one washing of acetone. Residue was collected in previously tarred dry crucible. Then the residue was dried for 1 hour at 100-105 °C for constant weight.

ADF (%) = Loss in weight after acid detergent treatment/Weight of sample × 100

Five mL 72 % H_2SO_4 was added in crucible containing ADF. Paste was made of this material and then crucible was covered and placed for one hour. After one hour again 5 mL 72 % H_2SO_4 was added and stirred thoroughly and let the crucible again for one hour. 72 % H_2SO_4 was added again, stirred and let it for one hour. Material was filtered giving two washings distilled water to make material acid free. Checked the material by phenolphthaline and NaOH solution either it is acid free or not. Final washing was done by acetone to make it water free. Residue was taken in china dish, placed china dish in oven at 100 °C for one hour. Cooled and weighed the china dish, the loss in mass was cellulose.

Cellulose (%) = Weight loss by digestion/Mass of sample × 100

***Statistical analysis*:** Means of four replications were taken for each treatment and statistical significance was calculated by ANOVA using Statistix 8.1 (Analytical Software, 2005) and linear correlation was plotted using MSTAT.

RESULTS

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- Some of the common reasons the results and discussion sections might cause reviewers to reject a manuscript are;
- confusing tables or figures
- inconsistent or inaccurate data
- potential variables that are not reported
- over interpretation/under interpretation of the results

***Plant height and number of grains per panicle*:** Basmati 515 genetically is high stature than the Super basmati, and N fertilization further increased plant height of both genotypes but more in Basmati 515 (Table 2). Maximum (130.2 cm) was in N_r K_r in Basmati 515, whereas maximum plant height in Super basmati was 110 cm. Number of grains per panicle has significant contribution towards paddy yield. More the number of grains panicle^{-1}, more will be the paddy yield if the grain weight is similar. Fertilizer treatments and cultivars showed significant difference in number of grains per panicle. More number of grains (175 per panicle) was produced with N_r K_r than the other treatments, while Basmati 515 produced a greater number of grains per panicles than Super basmati (Table 2). Number of grains per panicle ranged between 110 to 134 and 127 to 175 in Super basmati and Basmati 515, respectively.

***Basal internode space and paddy yield*:** There was no significant difference between Super basmati and Basmati 515 regarding basal internode spaces and minimum basal internode space (10.77, 10.68 cm) was recorded in N_r K_r in both genotypes while maximum basal internode length (8.47 cm) was recorded with N_d K_d in Super Basmati (Fig 1A).

The ultimate objective of rice crop is to get paddy yield. Super basmati produced paddy better than basmati 515 because of a greater number of tillers in super basmati. Maximum 5.45 tons ha^{-1} and minimum 4.31 tons ha^{-1} paddy yield were recorded in N_r K_r and N_d K_d, respectively (Fig. 1B).

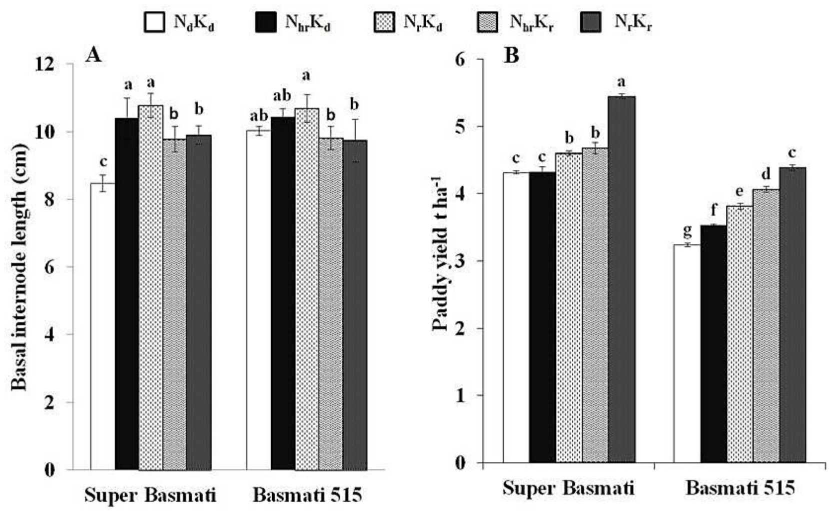

Figure 1. Effect of N and K fertilization on (A.) Internode space of two rice genotypes (B.) paddy yield per hectares. Five treatments $N_d K_d$ = no N and no K, $N_{hr} K_d$ = 60 kg ha^{-1} N and no K, $N_r K_d$ = 120 kg ha^{-1} N and no K, $N_{hr} K_r$ = 60 kg ha^{-1} N and 60 kg ha^{-1} K, $N_r K_r$ = 120 kg ha^{-1} N and 60 kg ha^{-1} K. Column shows mean values of four replicates and bar shows the standard error of means. Column labeled with different alphabets show significant difference according to LSD test at 5% probability. (LSD Value A=3.4, B=1.2)

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Table 2. Effect of N and K fertilization on plant height, number of grains panicle^{-1}, shoot-ADF concentration, and shoot-cellulose concentration of two rice genotypes.

Treatment	Plant height (cm)		Grains panicle^{-1}		ADF (%)		Cellulose (%)	
	Super Basmati	Basmati 515	Super Basmati	Basmati 515	Super Basmati	Basmati 515	Super Basmati	Basmati 515
N_dK_d	96.52±1.2f	105.62±1.2e	110.50±4.8f	127±1.9cde	42.26±1.16cd	37.16±2.9d	34.25±1.54bc	32.85±1.2
N_{hr} K_d	104.33±1.9e	115.08±1.9bed	117± 2.4ef	132.7±2.9bc	49.11±1.8bc	46.16±3.3c	39.36±1.5abc	38.14±2.2abc
N_rK_d	110.33±1.3cde	121.08±1.3b	125.75±2.6def	140±3.4b	50.04±2.bc	44.56±2.1cd	41.19±2.7ab	37.10±1.2abc
$N_{hr}K_r$	107.08±2.33e	117.25±2.33bc	131±1.7def	153±5.7b	44.58±1.70cd	46.71±2.81c	34.92±1.5bc	39.87±1.8abc
N_rK_r	108.75±3.14de	130.92±3.14a	134.5±4.09bcd	175±5.4a	57.65±2.8a	55.96±3.8ab	43.84±2.3a	44.45±4.1a

Five treatments (N_d K_d = no N and no K, N_{hr} K_d = 60 kg ha^{-1} N and no K, N_r K_d = 120 kg ha^{-1} N and no K, N_{hr} K_r = 60 kg ha^{-1} N and 60 kg ha^{-1} K, N_r K_r = 120 kg ha^{-1} N and 60 kg ha^{-1} K)

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Tables are a concise and effective way to present large amounts of data. You should design them carefully so that you clearly communicate your results .
The following is an example of a well-designed table:
- Clear and concise legend/caption
- Data divided into categories for clarity
- Sufficient spacing between columns and rows
- Units are provided
- Font type and size are legible

Acid detergent fiber and cellulose concentrations: Cellulose is high molecular weight compound in plant stem and it plays important role in stem strengthening. It has largest proportion in acid detergent fiber than that of silicon and lignin. Maximum cellulose concentration (44.1%) was determined in $N_r K_r$ while minimum (33.5%) was with $N_d K_d$ (Table 2). Cellulose content of rice plant was increased with increasing the dose of K and N fertilizers. However, when N was applied without K the cellulose concentration was less. Maximum acid detergent fiber content (56.5%) was produced with $N_r K_r$ while minimum (40%) was at $N_d K_d$ (Table 2). Lignin concentration in shoot was not significantly different in response to K or/and N fertilization (data not shown).

Shoot N, Si and K concentrations: Maximum N concentration (0.98 mg g^{-1} dry weight) was recorded in $N_r K_r$. Super Basmati showed more N concentration than the Basmati 515. Potassium fertilization has also synergistic effect on N uptake by rice but major effect of increased N concentration was due to N fertilization.

Generally, silicon (Si) is non-essential element for plants but it is beneficial for plants and play role in many important functions including stem strengthening. Maximum Si concentration (60.1 mg g^{-1}) was recorded in $N_r K_r$ for Basmati 515 and minimum (21.9 mg g^{-1}) was in $N_d K_d$ while in Super basmati maximum Si was observed in $N_{hr} K_r$ treatment *i.e.* 57.6 mg g^{-1}. Maximum K ion concentration was determined in shoot at treatment $N_r K_r$ which may show synergistic effect of K on Si uptake by plant in case of Basmati 515.

DISCUSSION

Application of K fertilizer plays role in osmoregulation, maintaining turgor pressure in cell, cell elongation, and growth consequently (Marschner, 1995). In our results, the application of potassium and nitrogen increased plant height. The increase in plant height may be due to the increased amount of N fertilizer because it increases the plant vegetative growth by enhancing cell division (Haghighi *et al*., 2011). Potassium fertilization also improve the plant height by improving its metabolism, however K reduced the growth rate and strengthen the stem. Increase in height by application of N as well as K fertilizers (Table 2) is similar to Shakouri *et al*. (2012) who reported that higher fertilizer rate increases plant height.

Nitrogen and K fertilizer increased grain yield significantly by improving photosynthetic rate and grain health (Bahmaniar and Ranjbar, 2007). Application of K with N plays a supportive role for

N functioning and causes increase in yield production (Usherwood and Segars, 2001). The increase in yield of rice by application N and then further increase by application of K fertilizer may be due to the positive interaction of nitrogen and potassium as described by Usherwood and Segars (2001). Application of K fertilizer increased the number of grains per panicle due to increased fertilization (Bahmaniar and Ranjbar, 2007) and also due to healthy grains i.e. 1000 grains mass (data not shown) may be due to efficient translocation of sugars to grains. Application of N and K in combination produced maximum number of grains per panicle (Bahmaniar and Ranjbar, 2007) and overall performance of crop was better than N application only (Uddin *et al*., 2013). Application of N fertilizer with deficient K conditions may increase the vegetative growth but may not produce more and healthy grains due to inefficient fertilization as well as sugar translocation to grains. Qiangsheng *et al*., (2004) reported increase in yield with recommended K fertilizer application and positive effect of combined application of N and K on paddy yield (Bahmaniar *et al*., 2007; Fig. 1B).

Lodging is among the major factor responsible for growth and yield reduction in rice, especially. Improvement in stem strength will definitely reduce the lodging losses. A number of factors are responsible for stem resistance against lodging which includes plant height, basal internode space, fiber content *i.e.* cellulose, lignin (Islam *et al*., 2007). Potassium plays role in hormonal balance and may increase the number of internode so as the decrease in first internode space (Beringer, 1980). Present study has clearly shown that plant height was not decreased by application of K even was increased in basmati 515; however significant decrease in basal internode space may contribute stem strength to resist against lodging (Fig. 1A). Similar results has been shown earlier by Wakhloo, (1975). Furthermore, Rahman *et al*., (2007) noticed that high N dose produced more vegetative growth and increased plant height which may increase length of basal internode. It was further explained that heavy N application decreases upper internode length, stem thickness and diameter, and increased lower internode length. High N dose causes reduced stem base strength, stem wall thickness and stem diameter (Hobbs *et al*., 1998). Application of N at transplanting causes lodging while fertilization at early booting stage is ideal causing lower lodging percentage. Generally, N has less effect on root growth as compared to shoot growth which increases root: shoot ratio making the plant more vulnerable to lodging (Pinthus, 1973). Basal internode space in comparison with stem K contents has significant results that show a positive relation between them (Fig. 2A).

Concentrations of stem strengthening components increased with application of K nutrition. Plants with sufficient K synthesize high molecular weight compounds like cellulose (Marschner, 2012). Increase in cellulose concentration by K application along N fertilization was also observed by Feng-zhuan *et al*., (2010) and Zhou *et al*., (2012). ShangLian *et al*., (2009) also observed that cellulose concentration increased with combined application of N and K. Silicon concentration of plant parts differ from each and generally Si accumulation decreased with increased N application which may reduce stem strength. Application of K along with N fertilizer increased Si concentration in rice shoot which is clear indication of stem strengthening by K application. Stem K contents have positive correlation with both stem strengthening components silicon (R= 0.75) and cellulose (R= 0.35) and may reduce rice lodging

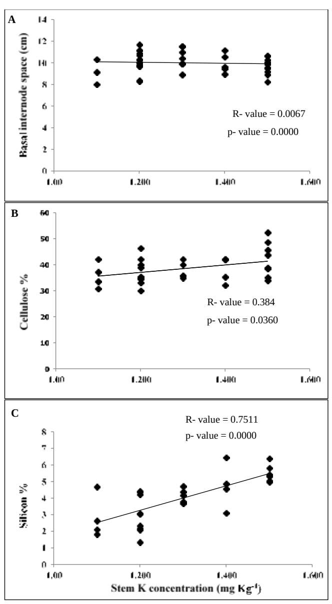

Figure 2. Correlation graphs between K concentration and (A) basal internode space (B) cellulose % (C) silicon %. Five treatments N_d K_d = no N and no K, $N_{hr}K_d$ = 60 kg ha^{-1} N and no K, N_r K_d = 120 kg ha^{-1} N and no K, N_{hr} K_r = 60 kg ha^{-1} N and 60 kg ha^{-1} K, N_rK_r = 120 kg ha^{-1} N and 60 kg ha^{-1} K

(Fig. 2B, 2C). Silicon accumulation in shoot is beneficial for plant due to the formation of silica gel that is accumulated on leaf surface, stems and plants other organs (Ma *et al*., 2001). A number of researchers have already reported that application of K increases the Si concentration in plant shoot as well as K concentration (Qiangsheng *et al*., 2004; Bahmaniar *et al*., 2007). Potassium fertilization affect the plants K concentration, K fertilized plants contained more K than the unfertilized plants (Sulok-kevin *et al*., 2007). Early-season plant K fertilization increases plant K uptake than the late season application (Slaton *et al*., 2004). More K content of plant of fertilized plots was due to the application of K fertilizer than the un-fertilized plots and more K in super basmati may be due to its specific genotypic character. Increase in N concentration by application of higher dose of K may show the synergistic effect of K fertilization for N uptake by rice plants.

***Conclusions*:** It may be concluded from that application of K fertilizer in combination with N fertilization increased paddy yield owing to increased number of grains panicle^{-1} as well as improved grain health. Increased concentration of acid detergent fiber in shoot and cellulose by application of K along with N was also observed that might have contributed to stem strength. Positive correlation of shoot-K concentration with shoot-cellulose and Si and negative correlation with basal internode space conclude that K might has contribute significantly to stem strength of rice plants to decrease lodging losses due to environmental stresses. Response of K fertilization on rice growth and yield suggest K fertilization in rice growing area of Pakistan, however, further studies are required to investigate the exact role of K in stem-strengthening.

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***Conflict of Interest*:** The Authors declare that there is no conflict of interest

***Authors' Contribution Statements*:** UZ, ZA and MA executed the field research, SS few laboratory analyses, whereas AW and MF conceived the idea and supervised the work.

Acknowledgement:

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