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Effects of induced potassium deficiency in groundnut and its estimation by flame photometry

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Abstract
Groundnut, an economically important oil seed crop is very sensitive in response to nutrient uptake from soil, mainly potassium; a major inorganic plant nutrient. Soil potassium analysis cannot compensate the hidden hunger of crops for potassium, thus calls for a methodology to determine the potassium level directly from plant. In the present study, groundnut was grown in nutrient free medium (marble sand) and nourished with Hoagland Nutrient Solution supplemented with variable levels of potassium, including potassium deficit as one of the treatments. The cultured plants were observed for variation at different growth stages, including plant height, leaf appearance, leaf chlorophyll content and leaf morphology for potassium deficiency symptoms. The potassium level of the leaf sap was determined by flame photometry at 30 days after sowing. Potassium deficiency below 40 mM concentration was observed in the plants at 60 days after sowing.

Keywords: Groundnut, potassium deficiency, Hoagland nutrient solution, leaf morphology, flame photometer

Introduction
Groundnut (*Arachis hypogea* L.) is an important oil seed crop of tropical and subtropical region of the world. Groundnut being prominent oilseed crop of India, it ranks first among the oil seed crops. In India, the crop occupies on area of 6.80 million hectare with the production of 5.20 mt. India is the world's largest producer of groundnut where the nutritional disorder causes yield reduction from 30-70 percent depending upon the soil types. The low productivity in groundnut attributed to many production constraints. Among these, the optimization of mineral nutrition is the key enhance the production of groundnut, as it has a very high nutrient requirement and the recently released high-yielding groundnut varieties remove still more nutrient from the soil. On contrary farmers, most part of semi-arid regions use very less nutrient fertilizer and sometimes only one or two nutrient resulting in severe mineral nutrient deficiencies due to inadequate and imbalance use of nutrients is one of the major factor responsible for low yield in groundnut (Singh, 2004) [4].

Potassium deficiency: Plants absorb potassium as the potassium ion (K+). Potassium is a highly mobile element in the plant and is translocated from the older to younger tissue. Consequently, potassium deficiency symptoms usually occur first on lower leaves of the plant and progress towards the top as the severity of the deficiency increases. One of the most common signs of potassium deficiency is yellow scorching or chlorosis along the leaf margin. In severe cases of potassium deficiency the fired margin of the leaf may fall out. However, with broadleaf crops, such as soybean and cotton, the entire leaf may shed resulting in premature defoliation of the crop. Potassium deficient crops grow slowly and have poorly developed root system. The supply of total K in soils is quite large. Yet, relatively small amounts are available for plant growth at any one time. The three forms of K (unavailable, slowly available, and readily available) exist in equilibrium in the soil system. The need for potash in a fertilizer program can be determined from plant analysis. Also soil analysis does not give the plant potassium levels and plant health related to potassium nutrient.
The present research outcome will help in determination of potassium deficiency using flame photometer from the standing crop even if the plant is not showing any visible deficiency symptoms. Potassium deficiency levels can be determined at any stage and which will help to prevent its further progress. If on determination, the plant potassium content, is found close to

the threshold deficit level or deficient; potash fertilizer which is a readily available form of potassium can be applied to the field crops to recover them from the deficiency and prevent the overall yield losses.

Materials and Methods

The pot culture medium i.e. the sand (white marble scarp) for inducing the potassium deficiency in groundnut was procured from local marble and tiles manufacturing company. The chemicals used for preparation of Hoagland Nutrient Solution (HNS) were purchased from Merck Chemicals, India. The purified water (Milli-Q, conductivity = 18.2 mS @ 25 ºC) was made available from Millipore water purification systems. The RO water (< 100 TDS) was collected from Aquagurad RO Water Purifier.

Induction of Potassium Deficiency in Model Plant-Groundnut: In order to induce the potassium (mineral) nutrient deficiency in groundnut plant, a sand culture medium was used. A special kind of sand i.e. coarse, scrap materials of white marble collected from local marble and tiles manufacturing company was used as culture medium for the pot study. As soil has variable and complex form of nutrient composition and the level of potassium induction cannot be controlled. Before initiating the pot study and induction of potassium mineral deficiency, the initial nutrient content in the form of physico-chemical parameters such as Nitrogen (N), Phosphorus (P), Potassium (K), Electrical Conductivity (EC) and pH were determined.

The estimation of Nitrogen (N), Phosphorus (P), Potassium (K), from sand was performed by methods described by Jackson, (1958) [2]. Electrical Conductivity (EC) and pH were measured by soaking the sand in MilliQ water incubated at room temperature overnight, next day the conductivity and pH of the water was measured using pre-calibrated conductivity meter and pH meter respectively.

Potassium deficiency medium: The full strength Hoagland Nutrient Solution (HNS) as described by Hoagland and Aron (1950) was used as the nutritional source for the pot study. To demonstrate the potassium mineral deficiency, the pot study experiment was designed with variable treatments (Control + 4 treatments), where the HNS was modified as per treatments. The control (C) HNS was considered as 100% potassium (K) while, the potassium deficiency induced by HNS was decreased in order of 50% to 0% in T3 to T1 and T4 was induced with double concentration of potassium as of C i.e. 200% K

Table 1: Details of experiment design for pot study

Plant variety:	Groundnut- GG2
Pot weight:	8 Kg
Seeds per pot:	4 seeds
Number of Replications:	5 replicates
Statistical Design:	CRD
Treatments:	C - Control (Full strength HNS- considered as 100% K)
	T1 - 0% Potassium (HNS – K (*absent*))
	T2 - 25% Potassium (HNS with 0.25 X- K of Control)
	T3 - 50% Potassium (HNS with 0.50 X- K of Control)
	T4 - 200% Potassium (HNS with 2.0 X- K of Control)
Dosage of HNS:	10 ml/0.2 L of RO water per pot for each treatment thrice a week.

Growth and Development of Groundnut- Observations: The growth stages of groundnut plants based on visual observation of vegetative and reproductive growth have been described and defined by Boote (1982) [1]. This widely adopted system describes a series of vegetative (V) and reproductive stages (R) details given in table-2.

Table 2: Growth stage descriptor for groundnut (Boote, 1982) [1]

Stage	Stage Title	Description
Vegetative Stages		
VE	Emergence	Cotyledons near the soil surface with seedlings partly visible
V0		Cotyledons are flat and opens at or below the soil surface
V1	First trifoliolate	One of n developed nodes on the main axis, a node is counted when its trifoliolate is unfolded and its leaflets are flat
Reproductive Stages		
R1	Beginning bloom	One open flower at any node
R2	Beginning peg	One elongated peg (gynophores)
R3	Harvest maturity	66-75% of all developed pods have testa or pericarp coloration

The observations for each stage mentioned in table-2 were recorded for each treatments and corresponding replications in terms of DAS (Days after Sowing). Also morphological parameters such as plant height, leaf appearance, leaf chlorophyll content were also determined groundnut plants.

Sampling and analysis of leaf sap for potassium content: Leaf samples were collected from each treatment and corresponding replicate on a duration of 30 DAS, 60 DAS.

The first fully open leaf sample was crushed in mortar-pestle at concentration of 1gm/10 ml MilliQ water. The crushed sample was centrifuged at 8000 rpm for 10 min at 20ºC. The supernatant i.e. the water extract was collected and was directly used estimation of potassium concentration by Flame Photometer, which was pre-calibrated at 100 mM, 50 mM and 10 mM of potassium chloride (KCl) standard solution prepared in MilliQ water.

Result and Discussions

Nutritional composition of culture medium (sand): The physico-chemical parameter of the sand used for pot study was determined and was found to be almost neutral in terms of nutrient composition (table-3); where the nitrogen was not detected, the content of phosphorus was around 6 ppm while that of potassium was 5 ppm both amounts were assumed to be negligible in terms of plant requirements. The EC of the sand was measured and found to be below 200 µS, this proved that the sand was free from salts and the pH was near neutral i.e. 7.05. Thus was used as the culture medium for groundnut to induction of potassium deficiency.

This neutral sand was further used filling the pots and later the groundnut seeds were sown as mentioned in table-1.

Table 3: Physico-chemical content of sand

Parameter	Content
Nitrogen (N)	Nil (ND)
Phosphorus (P)	5.89 ppm
Potassium (K)	5.00 ppm
Salt content (EC)	190 µS
pH	7.05

Pot Study-Induced Potassium Deficiency: For sand culture of groundnut, 4 seeds per 8 kg pot were sown as mentioned in table-1 and is shown in figure-1(A). Figure-1(B) shows, in total 25 pots were sown each with 4 seeds; corresponding to 5 treatment x 5 replications.

Fig 1: A) Pot filled with sand (8 kg) showing 4 seeds of groundnut seeds. B) 25 pots [5 treatment x 5 replications]

The seeds sown in the pots were nourished with HNS (10 ml/0.2L in RO water) thrice a weeks and monitored for different growth stages of groundnut as mentioned in table-2. The pots were kept in isolated shade with sufficient sunlight and air. The study was initiated during the month of March, that is the initiation of summer season in Gujarat; so the overall temperature of the shade was maintained below 30 ºC by water foggers during the day time when the temperature has elevated to about 38-40 ºC.

Vegetative stage-VE: The emergence of cotyledons near the sand surface was observed in mostly all of the treatments including their corresponding replicates at 5 DAS (table-4). This shows that during the first few days the developing seedlings are dependent on assimilates stored in cotyledons.

Vegetative stage-V0: After 5-8 days, depending on the environmental conditions, the seedlings grow autotrophically and are capable of absorbing minerals via roots while the epicotyls is exposed to light and capable of photosynthesis. Here, in the study; at 7 DAS (table-4) all the seeds for all the treatments were fully germinated and the cotyledons were flat and open to sand surface.

Vegetative stage-V1: In the experiment, first trifoliolate opening was observed at 10 DAS and there was variation corresponding to the treatments (C, T1, T2, T3 and T4) of potassium level by HNS supplementation. Table-4 shows the unfolding of first trifoliolate at 10 DAS. The values in the replications corresponds to the number of seedlings per pot showing opening of first trifoliolate, which shows that treatment (T4) showed highest first trifoliolate opening as (4 ± 0.45) followed by control (C) treatment with (3 ± 0.55), T2 and T3 with (3 ± 0.45) and least opening was observed in T1 with (2 ± 0.55).

Reproductive stage-R1: The first reproductive stage of beginning bloom as one open flower at any node was monitored regularly. Table-4 depicts the day of first flower blooming corresponding to treatments and its replications. The earliest first flowering for all the treatments was observed in control (C) treatment at 25 ± 0.89 DAS, followed by T4 treatment at 26 ±0.55 DAS, then T3 and T2 at 28 ±0.55 and 30 ±0.84 DAS respectively and lastly T1 treatment showed longer time for first blooming at 32 ±0.55 DAS.

Reproductive stage-R2: This stage of groundnut growth represents the day of first pegging and it was monitored in similar fashion as of stage R1 for all the treatments and corresponding replications. Table-4 shows the DAS of pegging i.e. formation first gynophores for all the treatments. The initiation of pegging for control (C) treatment was observed earlier at 31±0.45 DAS compared to T1 treatment at 41±0.89 DAS. The pegging in T4, T3, and T2 was observed at incremental delay at 32±0.84, 34±0.55 and 37±0.55 DAS responsive to decreasing concentration of potassium mineral nutrient.

Reproductive stage-R3: During this stage the groundnut plant has 66-75% of all developed pods, which are having coloration in testa or pericarp and is designated as harvest maturity. This stage is achieved at around 90-100 DAS in typical groundnut plants and following this stage the pods get mature and can be harvested. In the present study of potassium mineral deficiency induction, the plants were harvested at 95 DAS and the yield in terms of number of pods per pot was calculated. Table-4 shows the yield obtained in all the treatments as is summarized as average of all the replications. T4 treatment showed maximum yield as 64 ± 1.64 pods per pot followed by control (C) treatment as 59 ± 2.77 pods per pot, T3 as 26 ± 1.14 pods per pot, T2 having yield of 23 ± 2.39 pods per pot and least yield was obtained in T1 treatment as 14 ± 2.07 pods per pot.

This clearly shows that potassium deficiency has detrimental effect on plant growth and lead to high yield losses.

Table 4: Observations of growth stages for pot study

Growth Stages	Vegetative stage			Reproductive stage		
	VE*	V0*	V1*	R1*	R2*	R3*
Treatments	Emergence of Cotyledons	Cotyledons are flat and opens	1st trifoliolate opening	Beginning bloom	Beginning peg	Harvest maturity
Control	5 DAS	7 DAS	3 ± 0.55	25 ± 0.89	31±0.45	59 ± 2.77
T1	5 DAS	7 DAS	2 ± 0.55	32 ±0.55	41±0.89	14 ± 2.07
T2	5 DAS	7 DAS	3 ± 0.45	30 ±0.84	37±0.55	23 ± 2.39
T3	5 DAS	7 DAS	3 ± 0.45	28 ±0.55	34±0.55	26 ± 1.14
T4	5 DAS	7 DAS	4 ± 0.45	26 ±0.55	32±0.84	64 ± 1.64

*n=5; DAS= Days after Sowing, values for V1, R1, R2 & R3 are number (counts)

Plant Height, Leaf Appearance and Leaf Chlorophyll content: Groundnut crop responds well for potassium (K) application and addition of K increased its concentration at all stages in groundnut crop. The concentration of K was high in initial stages and declined in the later stage, indicating that groundnut absorbs K rapidly in early stages (Madkour *et al*., 1992) [3]. Potassium is also required in large amount by oil seed crop (Singh, 2004) [4]. Application of potassium at 100 kg ha-1 significantly increased the plant height, nodule weight, pod number, pod and haulm yields of groundnut (Singh and Chaudhari, 1996) [5].

In the present study, the groundnut plants are induced with variable concentration of potassium nutrient via HNS. The characterization of induced plants for plant height, leaf appearance in terms of potassium deficiency symptoms (figure-3) and corresponding leaf chlorophyll content as described by Sadasivam and Manickam (1992) [6] as shown in table-5 was done at 30 DAS and 60 DAS.

Plant height: The height of groundnut plants grown in sand culture was measured on centimeter scale from the surface of the sand to the top most index leaf of the plant. All the four plantlet from each pot corresponding to different treatment and replication were measured at 30 DAS and 60 DAS and the average results for each treatment are summarized in table-5. At 30 DAS the height of the plants were almost similar while the effect of variable induction of potassium concentration was clearly observed at 60 DAS and is depicted in figure-2; as plant height increased with increasing concentration of potassium nutrient.

Table 5: Variation in plant height against variable potassium levels (30 and 60 DAS)

Treatments	Plant Height (cm)	
	30 DAS*	60 DAS*
Control	13.20 ± 0.82	36.46 ± 2.51
T1	10.86 ± 1.04	22.56 ± 0.61
T2	12.00 ± 0.29	29.80 ± 0.91
T3	12.76 ± 1.36	30.32 ± 0.61
T4	13.52 ± 0.75	39.38 ± 2.18

*n=5; DAS= Days after Sowing

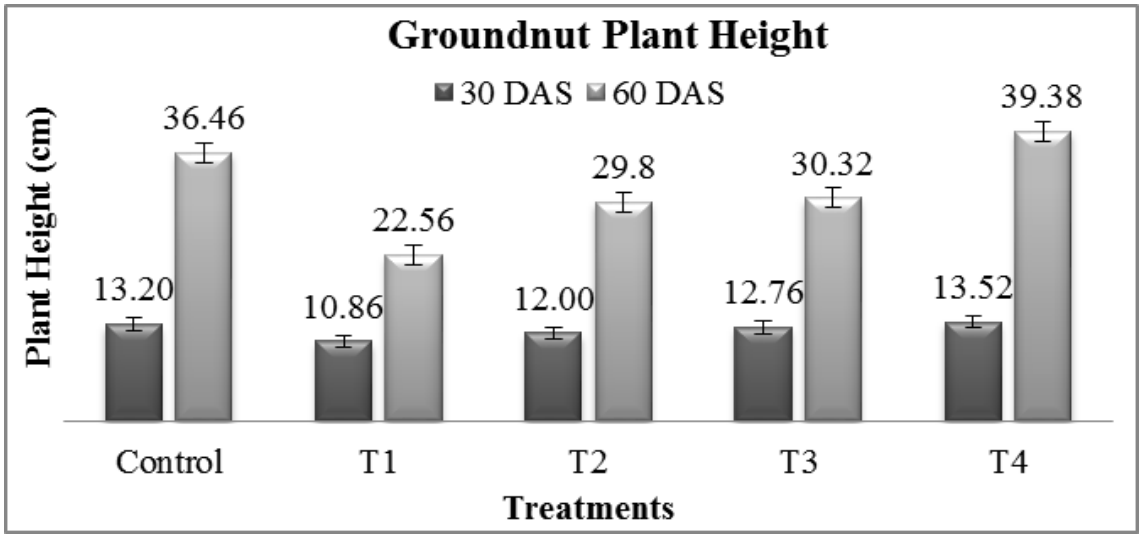

Fig 2: Effect of potassium concentration on plant height

Leaf appearance in terms of potassium deficiency symptoms: The morphological effect of potassium deficiency in most plant is observed as chlorosis of leaf margins and progressing towards the midrib of the leaf. Figure-3 shows the leaf morphology at 30 DAS and 60 DAS. At 30 DAS the initiation of potassium deficiency is observed in terms of depigmentation of leaf margins. The variation corresponding to potassium level is clearly observed at 60 DAS.

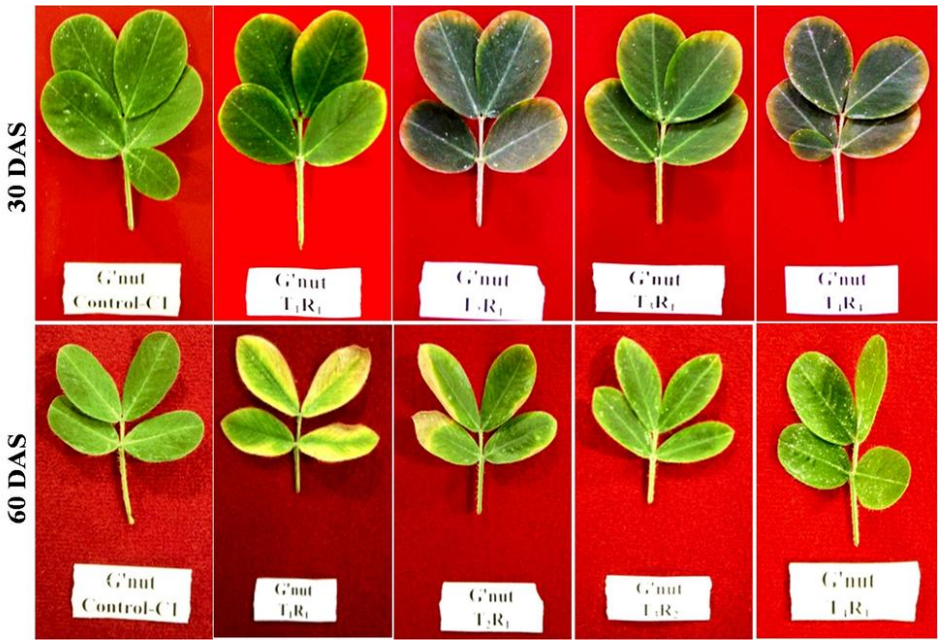

Fig 3: Leaf morphology against variable potassium treatments (C, T1, T2, T3 & T4) at 30 DAS and 60 DAS.

Leaf Chlorophyll content: While potassium is not a constituent of any plant structures or compounds, it plays apart in many important regulatory roles in the plant. It is essential in nearly all processes needed to sustain plant growth and reproduction. Potassium plays a vital role in; photosynthesis, translocation of photosynthates, protein synthesis, control of ionic balance, regulation of plant stomata and water use, activation of plant enzymes, and many other processes directly affecting plant quality, plant health and ultimately the crop yield (Hopkins and Hunter, 2004) [7].

The leaf chlorophyll content of the groundnut plants in the present study was determined at 30 DAS and 60 DAS (table-6) which could be directly correlated to the leaf morphology and potassium deficiency levels and is represented in figure-4.

Table 6: Leaf chlorophyll content

Treatments	Chlorophyll content (mg/gm)	
	30 DAS	60 DAS
	*Mean ± SD	*Mean ± SD
Control	1.45 ± 0.04	1.75 ±0.04
T1	1.39 ± 0.02	0.32 ±0.04
T2	1.40 ± 0.03	0.36 ±0.06
T3	1.39 ± 0.03	0.44 ±0.04
T4	1.46 ± 0.03	1.54 ±0.09

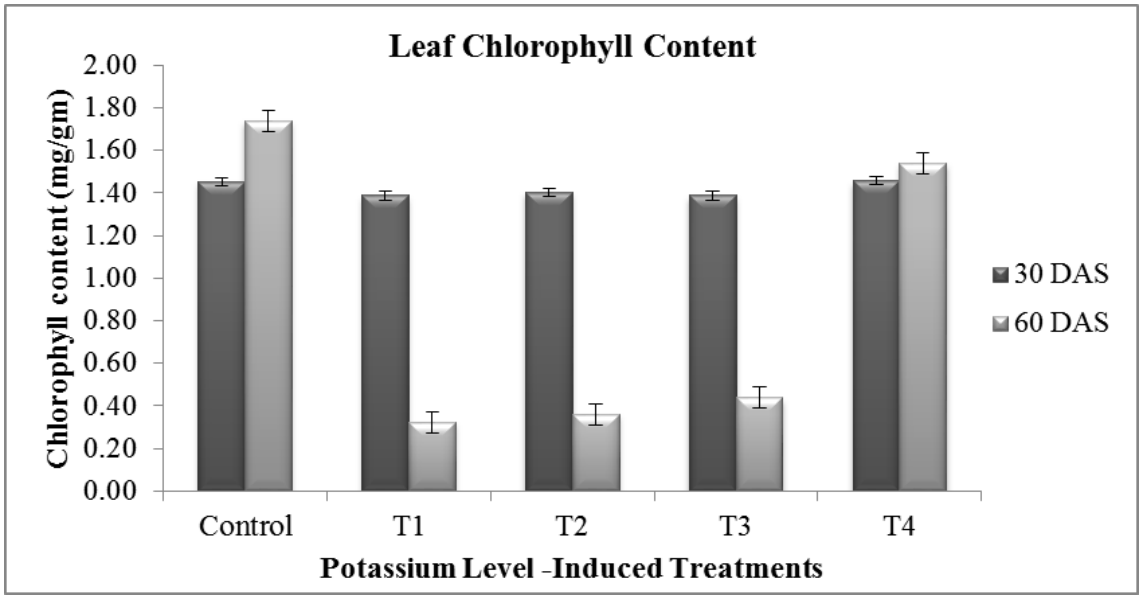

Fig 4: Variation in leaf chlorophyll content at 30 and 60 DAS of groundnut plant induced with variable concentration of potassium nutrient

Estimation of leaf sap potassium content by flame-photometry:

The potassium level from the groundnut plant induced with variable concentration of potassium mineral via HNS was estimated at 30 and 60 DAS against the visual symptoms of potassium deficiency. Figure-5 and 6 shows the content of potassium mineral from leaf samples collected at 30 DAS and 60 DAS. As described by Oosterhuis *et.al* (2013) [8], K is a dominant cation and is commonly found to in concentration ranging from 80 mM to 150 mM on dry basis. Thus, the groundnut plant tends to enter the deficiency level starts below 40 mM (considering 50% moisture content of leaves), which can be observed in results obtained by the leaf sap potassium after 60 DAS (Table-7).

Table 7: Leaf chlorophyll content

Treatment	Leaf Sap Potassium (mM)	
	30 DAS	60 DAS
C (100% K)	113.85 ± 6.69[d]	49.49 ± 3.69[b]
T1 (0% K)	143.59 ± 8.11[a]	32.15 ± 4.38[e]
T2 (25 % K)	140.24 ± 8.44[b]	41.90 ± 3.48[d]
T3 (50% K)	107.69 ± 3.63[e]	46.97 ± 2.08[c]
T4 (200%K)	126.15 ± 2.97[c]	51.74 ± 1.89[a]

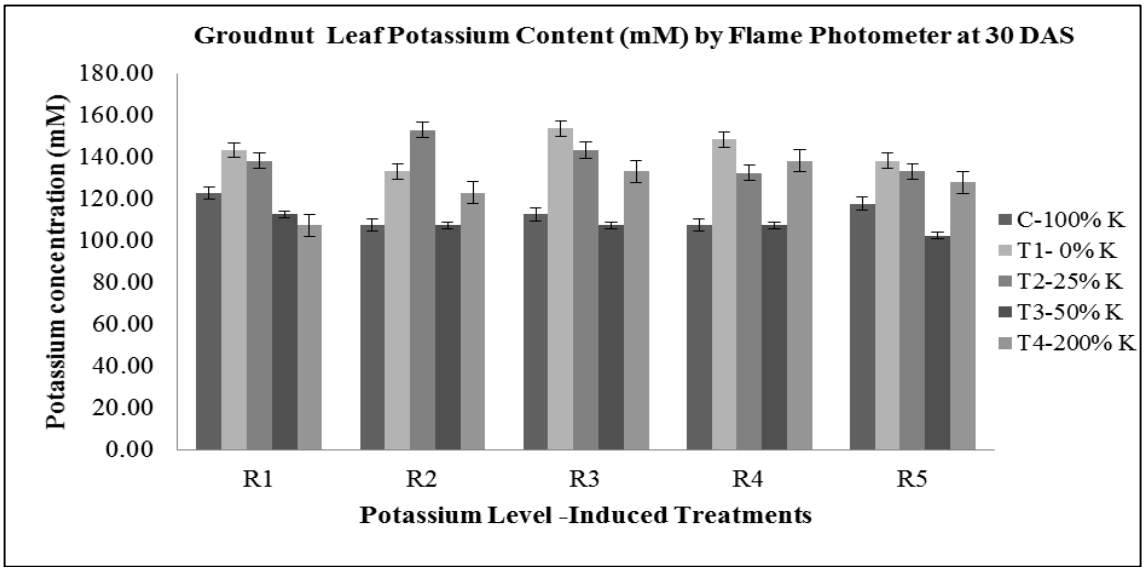

Fig 5: Potassium estimation from leaf samples at 30 DAS by flame photometry

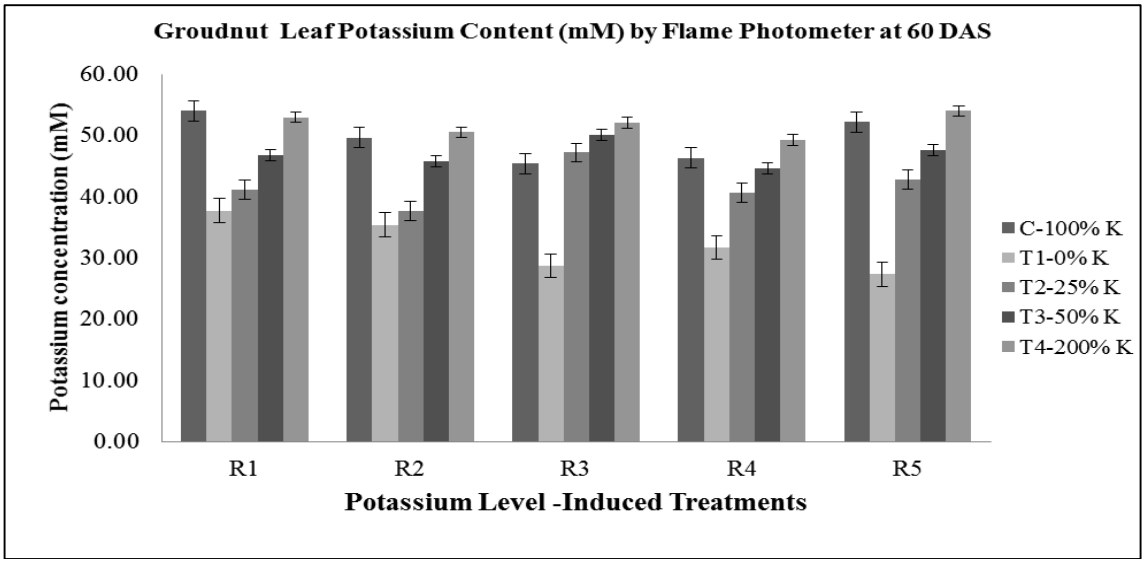

Fig 6: Potassium estimation from leaf samples at 60 DAS by flame photometry

Statistical Analysis: The experimental design of the induction of potassium deficiency was completely randomized, with five replicates for all treatments. The results were compared as mean ± SD of five replications. One-way analysis of variance (ANOVA) was used to compare the means. At $p<0.05$ the differences were considered significant and not significant at $p>0.05$. Statistical analyses were carried out using Tukey HSD calculator. (Tukey, 1949) [9].

Conclusions

In the present study, the groundnut plants were grown in marble sand as nutrient free medium followed by supplementation of nutrients via Hoagland Nutrient Solution (HNS) with a gradient or variable concentration of potassium mineral. The plants were induced with potassium level from deficient to double concentration as in normal HNS. The cultures were observed for different growth stages and morphological parameters for all the treatment such as emergence of cotyledons was observed at 5 DAS, at 7 DAS the cotyledons are flat and fully opens. First trifoliolate opening was noted at 10 DAS that ranged from 50% to 100% (2 to 4) of the seeds sown in the pots against the increasing gradient of potassium in the treatments. The beginning of first bloom as first reproductive stage ranged from 25-32 DAS for different treatments and K-deficit (T1) being most late. The day of first pegging was also observed as of first bloom, ranging from 31 to 41 DAS. Maximum yield at harvest stage was obtained in double K-level as of conventional HNS i.e. T4 treatments 64 pods/pot and K deficit being least with only 14 pods/pot.

Plant height (10.8-39.5 cm), leaf appearance, leaf chlorophyll content (0.3-1.8 mg/gm) and leaf morphology for potassium deficiency symptoms.

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