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POTASSIUM NUTRITION OF COTTON (*GOSSYPIUM HIRSUTUM* L.) IN RELATION TO COTTON LEAF CURL VIRUS DISEASE IN ARIDISOLS

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Abstract

A greenhouse experiment was conducted using four cotton (*Gossypium hirsutum L.*) cultivars (CIM-448, CIM-1100, NIAB-Karishma, S-12), four rates of potassium (0, 62.5, 125.0, 250.0 kg K ha^{-1}) and two sources of potassium [sulphate of potash (K_2SO_4) and muriate of potash (KCl)] to determine response of potassium fertilizer in relation to infestation of cotton leaf curl virus disease (CLCuV), a whitefly (*Bemisia tabaci* Gennadius) transmitted geminivirus (genus *Begomovirus*) at the Central Cotton Research Institute, Multan, Pakistan. There were significant differences among the cultivars in incidence and intensity of CLCuV disease. The cultivars CIM-448 and CIM-1100 showed complete resistance to CLCuV, whereas cv. S-12 was highly susceptible and cv. Karishma moderately tolerant to the disease. There was 12 to 38% reduction in the disease incidence as a result of addition of 250 kg K ha^{-1}. The incidence and intensity of CLCuV disease were little affected due to the different sources of potassium fertilizer. The mild intensities of CLCuV disease in cv. NIAB-Karishma at day 30, 60 and 90 after planting were negatively correlated with increasing doses of potassium fertilizer. The relationship between intensity of CLCuV disease at day 90 after planting and potassium doses for cv. S-12 could be described by the regression equation (Y= 60.40-0.064x, r= -0.46**)

Introduction

Poor yields of cotton are often an outcome of biotic and abiotic stresses. Among these stresses, cotton leaf curl virus disease (CLCuV) has caused a widespread problem (Mahmood *et al*., 1995). This disease is caused by a whitefly (*Bemisia tabaci* Gennadius) transmitted geminiviruses (WTGs), known as cotton leaf curl virus (Hameed *et al*., 1994). The disease is characterized by upward or downward curling of leaves. Veins of the leaves become thickened, which is more pronounced on the underside. The disease results in stunted plant growth with loss in yield (Hameed *et al*., 1994). The plant's capacity to recuperate from damage will generally be increased through enhanced and well-balanced fertilizer usage, which in return reduces the susceptibility of cotton crop to CLCuV disease and increases seed cotton yield. A good supply of potassium may increase the plant's resistance due to its function in osmoregulation, in the energy status and in the synthesis of high molecular compounds (Beringer & Trolldenier, 1978; Marschner, 1995). Potassium (K) probably exerts its greatest effects on disease through specific metabolic functions that alter compatibility relationships of the host-parasite environment (Kafkafi *et al.*, 2001). The intricate relationships between K nutrition and metabolic functions and growth, as well as its interrelationships with various other nutrients within the plant and the soil provide ample opportunity for K to modify disease resistance or susceptibility.

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Cotton is known for being attacked by a multiplicity of pests and diseases. Various researachers (El-Gindy *et al.,* 1974) conducted pot experiments and reported that in susceptible varieties of cotton (*Gossypium barbadense* L.), significant reduction in wilt (*Fusarium oxysporum* f. *vasinfectum*) was noticeable at 500 ppm K when attacked by the fungus alone and at 1000 ppm K when the nematode (*Rotylenchus renifornis*) was also present. Chang & Tu, (1970) reported that attention should be paid to an adequate nitrogen: potassium (N: K) ratio since N and K play a major role in resistance to adverse pathogens; while N frequently reduces the resistance, K improves it. This was the decisive factor in obtaining high yields of Jute free from root rot. The incidence of blast disease in finger millet (*Eleusine coracana*) caused by *Pyricularia garisea* (Cke) Sacc. was reduced by 39% due to soil application of 30 kg K_2O ha^{-1} in combination with 1% KCl foliar spray at 45^{th} and 70^{th} day after transplanting (Murugesan *et al.*, 1995). Potassium effectively inhibited the cotton blight disease in southeastern Peoples Republic of China, and concurrently promoted root growth, delayed leaf drop and increased boll weight (Chang & Liang, 1978).

At least 16 viral and 2 microplasm-like (MLO) diseases have been reported in cotton (Watkins, 1981). The use of potassium fertilizer decreased the incidence of virus disease in 41% of the reported cases, and simultaneously K increased the yield of plants infested with viruses by 78% (Perrenoud, 1990). Results quoted by Potash and Phosphate Institute (Anon., 1993) showed that adequate K applications in cotton reduced the incidence rating for verticillium wilt, increased seed cotton yield and seed index. The application of potassium fertilizer in the form of KCl and K_2SO_4 has been reported to reduce the incidence of tobacco mosaic virus of *Nicotiana glutinosa* (Allington & Laird, 1954) and tomato mosaic virus (Chant & Gbaja, 1985). All factors, which improve nutrient availability in soil and uptake by plants, contribute to improve resistance / tolerance provided that fertilizer use and nutrient supply is balanced. Unfortunately, imbalanced fertilizer use is common in Pakistan's crop production system (Ahmad, 2000). The current NPK ratio of fertilizer use in Pakistan is 4:1:0.04, which in relation to crop nutrient removal indicates negative K balance and moreover soil K is being mined. Mining soil nutrients without appropriate replenishment leads to a loss of soil fertility, stagnating yields and higher disease incidence. Improved resistance of crops due to balanced nutrition requires less agrochemicals for plant protection.

Studies were therefore, undertaken to examine the incidence of CLCuV disease upto day 90 after planting and whether improved potassium nutrition could be effective in enhancing resistance to leaf curl virus disease in cotton crop.

Materials and Methods

A greenhouse experiment was conducted at Central Cotton Research Institute Multan, Pakistan. The soil was analyzed following standard methods (Ryan *et al.,* 2001). The soil was silt loam having alkaline reaction (8.3 pH). The soil contained 0.67% organic matter, 14.3 mg kg^{-1} available-P and 147 mg kg^{-1} exchangeable-K. The soil is moderately calcareous and medium in exchangeable-K, weakly structured and developed in an arid sub-tropical continental climate in the areas of sub-recent flood plains. The soil was alluvium of mixed mineralogy with smectites and mica being dominant clay minerals followed by kaolinites and chlorites with various degrees of weathering (Krauss *et al.,* 1996). The soil belongs to the Miani soil series being classified as Calcaric Cambisols and fine silty, mixed Hyperthermic Fluventic Haplocambids according to FAO (Anon., 1990; Hashmi *et al.,* 1993).

Thirty three kilogram of air-dried < 2 mm silt loam soil was added to each concrete pot (45.5 x 31.0 cm). The pots were internally lined with polyethylene sheet to avoid accretion of salts by the pot. A basal dose @ of 150 kg N and 22 kg P per $2x10^6$ kg ha^{-1} of soil was mixed with it at the time of planting seeds. The treatments consisted of (a) four cotton (*Gossypium hirsutum* L.) cultivars: S-12 (highly susceptible to CLCuV), NIAB-Karishma (moderately resistant to CLCuV), CIM-1100 and CIM-448 (both highly resistant to CLCuV); (b) four potassium fertilizer doses (0, 62.5,125.0, 250.0 kg K ha^{-1}) and (c) two potassium fertilizer sources [sulphate of potash (K_2SO_4) and muriate of potash (KCl)]. These cultivars were selected on the basis of tolerance rating to CLCuV disease and detection of geminivirus by Polymerase Chain Reaction (PCR) techniques (Mahmood *et al*., 1995). The design of the experiment was a completely randomized design with six replications. The average day temperature was 42 ± 3°C and the night temperature 29 ± 5°C. The day length was 14 hour and relative humidity ranged from 25.6 to 53.1%. There was no dearth of sunshine during the growth period. Twenty seeds of each cultivar were sown on May 28, 2000 at field capacity (35.2%) in each pot. Thinning was done 10 days after emergence, only 6 seedlings of uniform size were kept in each pot. Deionized water was used for irrigation purpose during the growth period.

The pots were kept in insect free cages. The plants were inoculated with CLCuV through whitefly transmission technique developed by Hashmi *et al*. (1993). The transmission, which involved introducing a large population of whitefly into a large muslin cage containing only CLCuV infected cotton plants and left them for 3-days. Then the potted healthy plants at the 2nd leaf stage were introduced and left for a period of 10-days. Later on, they were sprayed with insecticide Confidor 200 SL @ 625 ml ha^{-1}. Data on incidence and intensity of CLCuV disease were recorded during 90-day old crop. One hundred plants were taken at random from each treatment for recording the incidence and intensity of CLCuV disease at day 30, 60, and 90 after planting. The plants were graded by visual appearance of symptoms on leaf according to the system (Mahmood *et al.*, 1995). The grades were defined as No (no symptoms), MI= Mild (small scattered vein thickening), ME = Medium (large groups of vein thickening), S = Severe (severe curling and /or foliar regrowth, i.e., enations). Data on incidence and intensity of CLCuV disease (%) plants showing symptoms) were assessed on the basis of healthy and diseased plants showing differential infestation level, respectively.

The diagnostic leaves were collected at bloom stage (at day 60 after planting) consisting of fully expanded young leaves, usually 4th or 5th from the terminal. The plant material was dried in forced air oven at 70°C and potassium concentration was determined (Ryan *et al*., 2001). The seed cotton was hand picked in each plot measuring 64 m^2 and total yield calculated on an area basis. The data were subjected to statistical analyses (Montgomery, 1997).

Results and Discussion

The yield of seed cotton increased significantly with increasing levels of potassium fertilizer and cultivars differed significantly amongst themselves (Table 1). The addition of 250 kg K ha^{-1} caused a considerable improvement in seed cotton yield by 37% compared to zero K-rate treatment. The significant increase in seed cotton yield occurred on soil testing 147 mg kg^{-1} exchangeable-K. The relationship between seed cotton yield and levels of potassium fertilizer was highly significant and could be described by the regression equation ($Y= 1731.1+3.7645x-0.0053x^2$, $r= 0.53^{**}$) demonstrating the need to replenish potassium in the soil. Other researchers (Nemeth & Makhdum, 1981) reported that soils of Multan (Pakistan) have low K^+ selectivity and buffering capacity. This

indicates that the amount of K^+ ions released per unit time from soil reserves is not sufficient to meet the requirement of crops. Averaged across rates and sources of K-fertilizer, seed cotton yield of cv. CIM-448 was 58% greater than cv. S-12. Cultivars differed in their yield potential due to their genetic make-up and their differential tolerance level to CLCuV disease infestation under given environment. Plots fertilized with K_2SO_4 produced significantly higher yield compared to KCl. The positive influence of K_2SO_4 was due to its accompanying anion (SO_4^{2-}) in the sulphur deficient areas of Pakistan (Mengel, 1976). In the present study the narrowing of ratio between N: K (3:5) stimulated crop to show greater tolerance to CLCuV for increased seed cotton yield.

The susceptible cultivars (NIAB-Karishma, S-12) maintained lower concentration of K^+ in leaf tissues at flowering stage compared to cultivars (CIM-448 and CIM-1100) resistant to the disease (Table 2). Similar observations have been reported by Dastur & Bhatt (1964) that wilt-resistant flax took up and contained more K^+ than the susceptible variety. In grapes, increased resistance of K-fertilized plants to *Botrytis cinerea* was attributed to more rapid wound healing and the accumulation of fungitoxic compounds around wounds (Kiraly, 1976). Since viruses require a wound penetration, K fertilization decreased the number of susceptible sites on leaves of tobacco (*Nicotiana glutinosa*) by hastening wound healing as shown for tobacco mosaic virus (Allington & Laird, 1954). Other data suggest that addition of potassium fertilizer provided a well buffered supply of K to the cotton crop and that for achieving high yields of seed cotton, leaf-K concentration should be three percent.

Table 1. Effect of potassium fertilizer doses and sources on seed cotton yield [kg ha^{-1}] in four cotton cultivars.

Cultivar	KCl [kg K ha^{-1}]				K_2SO_4 [kg K ha^{-1}]			
	0	62.5	125	250	0	62.5	125	250
CIM-448	2204	2504	2601	2856	2204	2548	2680	3065
CIM-1100	1854	1940	2154	2332	1854	2167	2380	2470
Karishma	1548	1811	1818	2098	1548	1902	2003	2280
S-12	1340	1426	1775	1891	1340	1456	1799	2094

LSD ($p<0.05$)				
Cultivar	51.12**	Cv x S	69.92**
Dose	37.72**	D x S	57.06**
Source	34.96**	Cv x D x S	114.12**
Cv x D	75.43**		

** = Significant at the 0.01 level.

Table 2. Effect of potassium fertilizer rates and sources on K^+ concentration (%) in leaf tissues of four cotton cultivars.

Cultivar	KCl [kg K ha^{-1}]				K_2SO_4 [kg K ha^{-1}]			
	0	62.5	125	250	0	62.5	125	250
CIM-448	2.81	3.13	3.29	3.35	2.82	3.18	3.32	3.42
CIM-1100	2.73	2.99	3.18	3.28	2.77	3.12	3.19	3.33
Karishma	2.55	2.93	2.96	3.04	2.54	2.89	2.93	3.09
S-12	2.42	2.49	2.55	2.73	2.38	2.48	2.62	2.79

LSD ($p<0.05$)				
Cultivar	0.03**	Cv x S	0.03*
Dose	0.03**	D x S	0.03*
Source	0.02**	Cv x D x S	0.06*
Cv x D	0.05**		

ns = Not significant at the 0.05 level

** = Significant at the 0.05 and the 0.01 level, respectively

Table 3. Incidence of CLCuV disease (% plants with symptoms) as influenced by potassium nutrition.

Treatments		KCl				K_2SO_4			
Cultivar	**K Dose [kg ha^{-1}]**	**S**	**ME**	**MI**	**NO**	**S**	**ME**	**MI**	**NO**
At 30 DAP*									
Karishma	0	1	4	10	85	1	4	10	85
	62.5	0	4	9	87	0	4	9	87
	125	1	3	9	87	1	4	7	88
	250	0	5	9	86	1	3	8	88
S-12	0	1	5	12	82	1	5	12	82
	62.5	1	3	12	84	0	4	9	87
	125	1	5	10	84	1	5	8	86
	250	1	7	14	78	1	4	9	86
At 60 DAP									
Karishma	0	12	7	11	70	12	7	11	70
	62.5	18	7	11	64	14	10	11	65
	125	13	8	6	73	14	7	9	70
	250	10	12	5	73	8	10	8	74
S-12	0	21	11	15	53	21	11	15	53
	62.5	20	8	12	60	17	11	13	59
	125	17	12	7	64	20	9	15	56
	250	10	12	13	65	14	9	11	67
At 90 DAP									
Karishma	0	15	11	11	63	15	11	11	63
	62.5	16	11	13	60	13	11	13	63
	125	17	9	12	62	10	14	6	70
	250	12	14	14	60	13	13	11	63
S-12	0	59	21	20	0	59	21	20	0
	62.5	32	14	11	43	27	11	9	53
	125	29	15	12	44	26	12	10	52
	250	20	15	16	49	19	18	10	53

	30DAP			60DAP			90DAP		
LSD ($p< 0.05$)	Cultivar	Dose	Source	Cultivar	Dose	Source	Cultivar	Dose	Source
	3.77**	2.89 ns	1.36 ns	5.65**	4.57 ns	2.91 ns	7.28**	3.31 ns	3.25 ns

S = Severe (severe curling and /or enations), ns = Non-significant at the 0.05 level
ME = Medium (large groups of vein thickening) ** = Significant at the 0.01 level
MI = Minor (small scattered vein thickening),
NO = No symptoms of disease.
*DAP = Days after planting

The cultivars CIM-448 and CIM-1100 showed complete resistance to CLCuV disease and are, therefore, omitted from Table 3. This agrees with the results reported by Shah *et al*., (1999) who did not detect virus particles in cvs. CIM-448 and CIM-1100 by TAS-ELISA at 100 days after grafting and also no band of expected size was seen in PCR amplified product. They concluded that cultivars CIM-1100 and CIM-448 were highly resistant/immune to CLCuV infection. The incidence of CLCuV disease infestation was, however, significant in cvs. NIAB-Karishma and S-12, but without significant effect due to potassium fertilizer doses and types (Table 3). Similarly, the

intensity of the disease graded as medium and severe at day 30, 60 and 90 after planting was not markedly affected by potassium nutrition. However, intensity of disease graded as mild was significantly influenced by various treatments (Table 4). The application of potassium fertilizer resulted in reduction of spread of disease at its mild infestation level. The incidence percentage of CLCuV disease increased with an advancement of crop growth (Table 3). There were no interaction that included cultivars, potassium fertilizer doses and sources and were non-significant for increasing tolerance to CLCuV disease during the growth period.

Table 4. Intensity of CLCuV disease (%) as influenced by potassium nutrition.

Treatments		KCl			K_2SO_4		
Cultivar	**K Dose [kg ha^{-1}]**	**MI**	**ME**	**S**	**MI**	**ME**	**S**
At 30 DAP							
Karishma	0	81	17	2	73	23	4
	62.5	79	21	0	75	25	0
	125	77	20	3	67	23	10
	250	71	29	0	70	24	6
S-12	0	77	23	0	77	21	2
	62.5	74	20	6	71	26	3
	125	70	38	2	68	30	2
	250	65	33	2	68	32	0
At 60 DAP							
Karishma	0	29	20	51	35	20	45
	62.5	31	20	49	32	25	43
	125	29	31	40	28	25	47
	250	25	32	43	24	35	41
S-12	0	36	21	43	33	20	47
	62.5	32	20	48	29	25	46
	125	26	31	43	27	24	49
	250	23	28	49	22	28	50
At 90 DAP							
Karishma	0	30	27	43	36	25	39
	62.5	31	28	41	35	31	34
	125	33	25	42	23	44	33
	250	33	34	33	21	49	30
S-12	0	23	20	57	18	20	62
	62.5	22	24	54	18	24	58
	125	25	25	50	18	26	56
	250	21	29	50	18	32	50

LSD ($p< 0.05$)

Variable	30 DAP			60 DAP			90 DAP		
	MI	**ME**	**S**	**MI**	**ME**	**S**	**MI**	**ME**	**S**
Cultivar	0.29**	0.52**	0.64**	0.13**	0.35**	0.47**	6.81**	7.22**	7.25**
Dose	0.33**	0.31 ns	0.46 ns	0.25**	0.33 ns	0.37 ns	4.57**	3.73 ns	4.60 ns
Source	0.27 ns	0.23 ns	0.40 ns	0.21 ns	0.24 ns	0.22 ns	2.66 ns	2.86 ns	3.85 ns

ns = Not significant at the 0.05 level

** = Significant at the 0.01 level

Table 5. Relationship between intensity of CLCuV disease and potassium fertilizer doses in two cotton cultivars (*n*= 32 for each cultivar).

Dependent variable		Regression equation	Correlation co-efficient (*r*)
Intensity of disease	**Days after planting**		
cv. Karishma			
Mild	30	76.60 – 0.0217	-0.16 ^{ns}
	60	36.43 – 0.043 x	-0.55 ^{ns}
	90	32.25 + 0.083 x	-0.08 ^{ns}
Medium	30	23.55 + 0.010 x	0.13 ^{ns}
	60	22.23 + 0.035 x	0.47**
	90	29.80 + 0.017 x	0.18 ^{ns}
Severe	30	3.63 – 0.166 x	0.056 ^{ns}
	60	49.15 – 0.025 x	-0.30 ^{ns}
	90	38.60 – 0.013 x	-0.12 ^{ns}
cv. S-12			
Mild	30	73.23 – 0.0224 x	-0.18 ^{ns}
	60	34.33 – 0.071 x	0.35**
	90	19.78 + 0.022 x	0.41*
Medium	30	25.90 + 0.036 x	0.39*
	60	22.98 + 0.015 x	0.19 ^{ns}
	90	20.43 + 0.041 x	0.55**
Severe	30	1.38 + 0.04 x	-0.08 ^{ns}
	60	45.33 + 0.018 x	0.30 ^{ns}
	90	60.40 – 0.063 x	-0.46**

Y = Intensity of CLCuV disease at days after planting; X = Potassium fertilizer doses
ns = Non-significant at the 0.05 level *, ** = Significant at the 0.01 level and 0.05 level

The CLCuV disease was not significantly affected due to different sources of potassium fertilizer. In earlier studies, Mengel (1976) and Huber & Arny (1985) who reported that intricate relationship of K nutrition to metabolic function and growth, as well as its interrelationship with the various nutrients within the plant and soil, provide ample opportunity for K^+ to modify disease resistance or susceptibility. As mobile regulator of enzyme activity, K^+ is involved in essentially all cellular functions that influence disease severity. Therefore, K probably exerts its greatest effect on disease through specific metabolic functions that alter relationships of the host-parasite environment.

The intensity of CLCuV disease during the growth period was negatively related to potassium fertilizer doses only in cv. S-12, but not always significant (Table 5, Fig.1). The intensity of disease was reduced by 12 to 38% by the application of 62.5 to 250 kg K ha^{-1}. The decreasing trend in disease virulence indicated the plant's capacity to withstand an infection towards CLCuV disease, through well-balanced fertilizer usage.

The linear equations were the best fit to depict the relationships between potassium concentration in leaf tissues during bloom stage (at day 60 after planting) and corresponding intensity of CLCuV disease (Fig. 2). Cultivar NIAB-Karishma showed higher tolerance to disease compared to cv. S-12 at the same of level of potassium concentration in leaf tissues. These results corroborates with those of (Mengel & Kirkby, 1978) that potassium promotes the development of thicker outer walls in epidermal cells, thus preventing disease attack. Furthermore, plant metabolism is very much influenced by potassium, therefore, plant's defense against diseases may be favoured by changes in metabolism associated with high plant potassium content.

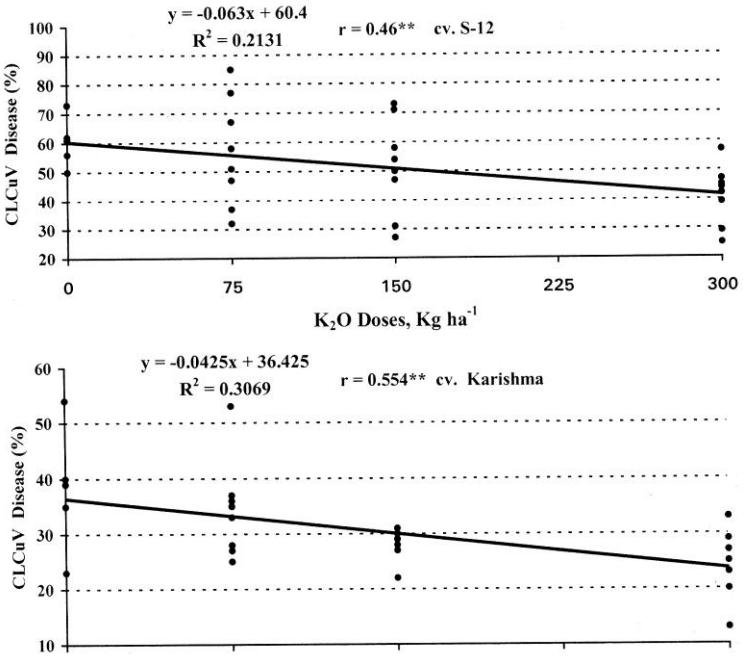

Fig. 1. Relationships between potassium fertilizer doses and intensity of CLCuV disease.

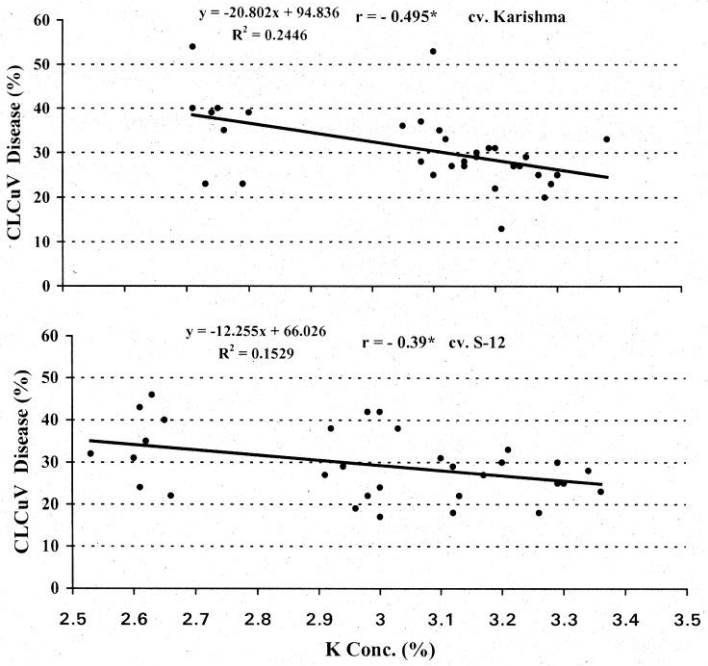

Fig. 2. Relationships between K concentration in leaf tissues at blooming and intensity of CLCuV disease.

Potassium affects metabolism and in K deficiency, soluble compopunds of low molecular weight accumulate, especially soluble nitrogen compounds and sugars because of increased activity of decomposing enzymes and reduced phosphorylation. This is frequently accompanied by better parasite development probably because of such compounds constitute a particularly suitable diet for them. Adequate potassium nutrition increases the content of phenols which can also play a beneficial role in plant resistance. Potassium affects plant morphology, hardening the tissues with resulting improvement in resistance to disease penetration. Stomata are open for longer than necessary in potassium deficiency increasing the chances of disease penetration. Fuchs & Grossmann (1972). Jeffers *et al*., (1982) reported that added potassium nearly always decreased moldy soybean seed caused by the pod and stem blight organism (*Diaporthe sojae*) and that the K^+ concentration in leaves closely paralleled the K treatments and the soil K status. Various researachers (Munster, 1964; Alagianagalingam *et al*., 1977; Timm *et al*., 1986) imply a separates role for K^+ relative to disease severity. It is generally important that a balanced fertility programme be maintained. An imbalance of any essential nutrient has major ramification that are manifest throughout the physiological processes of a plant. Mariani (1952) found that application of KCl to a soil which had received no potassium fertilizer for more than 10-years reduced blast attack of panicl sterility and markedly increased the weight of panicles. Wanscher (1952) also reported 46% decrease in infestation of leafroll of potatio by addition of K_2SO_4 in the soil. Later on, Munster (1964) found 41 to 58% reduction in the application of K_2SO_4 in the soil. While reviewing data on potassium and plant health, Perrenoud (1990) reported that KCl had a beneficial effects in 66% of the cases and K_2SO_4 in 51% of the cases.

The role of K in a plant depends on the availability of Mg^{2+} and Ca^{2+}, which are, in turn, influenced by pH. Thus, K^+ availability is enhanced by Ca^{2+} in neutral but not in acid soils (Huber, 1978). Therse interactions could drastically influence the observed effects of K on disease (Huber & Arny, 1985). As a mobile regulator of enzyme activity, K is involved in essentially all cellular functions that influence disease severity. Resistance of some plants has been correlated with higher levels of K than obtained in tissues of susceptible cultivars. A wilt-resistant flax takes up and contains more K^+ than a susceptible variety (Dastur & Bhatt, 1964; Huber & Arny, 1985).

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