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Effect of Foliar Treatment of KCl on Chlorophyll, Total Sugars, Soluble Protein, *In Vivo* Nitrate Reductase Activity and Leaf Yield in Mulberry (*Morus alba* L. CV.S1)

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Foliar treatment with different concentrations of potassium chloride (KCl) to mulberry plants resulted in higher level of total chlorophyll, total sugars, soluble protein, *in vivo* nitrate reductase activity (NRA), net photosynthetic rate (NPR), pWUE and leaf yield. Optimal concentration was found to be 10.0 mM KCl with limited irrigation provided in the mulberry plantation planted in 90×90 cm spacing. The deleterious effect of soil moisture stress condition has been found to be overcome by KCl foliar spray twice at 15 days interval. Regression and correlation coefficients were analyzed, and a strong positive correlation was found between chlorophyll and total sugars, soluble protein and in vivo nitrate reductase activity, leaf dry weight and net photosynthetic rate and pWUE and net photosynthetic rate.

Key words: KCl, Mulberry, Chlorophyll, Total sugars, Soluble protein, Nitrate reductase activity, Photosynthesis

Introduction

In India, about 35 species of *Morus* are distributed from Jammu and Kashmir in the West to Manipur in the North East, sub-Himalayan belt and also in southern India. Some of these varieties are being cultivated under rainfed and irrigated condition for silkworm rearing. In irrigated condition farmers can harvest at least 5 crops but in rainfed area maximum 3 crops per year. KCl as foliar spray has been reported to influence the crop plants to overcome the water stress condition. In addition to this, sericulturists are interested for maximum production of foliage for rearing, so before leaf harvest a foliar spray can increase the essential metabolites required for silkworm and thus may help indirectly the cocoon crop production.

In the present investigation, the effect of KCl on four biochemical constituents *viz.*, chlorophyll, total sugars, soluble protein, *in vivo* nitrate reductase activity; two physiological parameters *viz.*, net photosynthetic rate (NPR) and physiological water use efficiency (pWUE) and total leaf yield/plant of *Morus alba* L. cv. S1 was studied.

Material and Methods

Conditions of treatment

The experiment was conducted in an existing plantation at Central Sericultural Research & Training Institute, Berhampore, West Bengal, India, planted in 90 x 90 cm spacing with limited irrigation to maintain a low soil moisture level of 9.27 – 11.28% as prevailed in rainfed area as compared to 27 to 30% soil moisture in irrigated garden. The soil containing 0.32% N, 0.52% P and 0.23% K at pH 6.8 and under natural conditions, with daily average maximum and minimum temperatures of 35°C and 25°C, respectively, 75% average relative humidity. Basal supplementation was provided as per zonal rainfed dose of N:P:K = 150:75:75 /ha/year just after pruning at the height of 15 cm from ground level.

Solutions with different KCl concentrations (0, 2.5, 5.0, 10.0 and 15.0 mM) were prepared in distilled water. Spray treatment consisted of an equal volume (100 cm^3. $plant^{-1}$) of solution from each concentration and was applied twice to the foliage of mulberry plants-first after 15 days of sprouting and the other on 30^{th} day after sprouting with

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the help of a micro sprayer. The design of the experiment was randomized block design (RBD) in three replications for each treatment.

Analyses

Total soluble protein was determined in fresh leaves as described by Lowry *et al.* (1951) using bovine serum albumin as standard. The *in vivo* nitrate reductase activity was assayed as per Hageman and Hucklesby (1971). Total chlorophyll contents were estimated from 80% (v/v) acetone extracts according to Arnon (1949). Total soluble sugar was measured spectrophotometrically using anthrone reagent (Morris, 1948). All the biochemical constituents were determined in fresh leaves from 30th day of spray treatment at fortnightly intervals and continued up to 60 days from the days of spray to synchronize with the silkworm rearing.

All the biochemical estimations were carried out in triplicate and repeated twice. The data were analyzed statistically using critical differences (CD) and correlation and regression coefficients.

Gas-exchange parameters

Net photosynthetic rate (NPR) and physiological water use efficiency (pWUE) were measured from the 5th expanding leaves using a portable photosynthetic system (LI-COR model 6200; Licor Instrument Inc, USA). The whole experiment was carried out between 11–12 hrs under natural condition with ambient temperature range of 28–30°C, relative humidity of 70–80% and photoperiod of 12 hrs.

The plant height (average of 5 plants/replication), leaf yield/plant (average of 5 plants/replication) and leaf dry weight were recorded once on 60th day as in general practice leaves are harvested during 60–70 days for rearing purposes. Regression and correlation coefficients were analyzes between leaves dry weight vs. net photosynthetic rate, pWUE vs. NPR and leaf yield vs. NRA.

Results and Discussion

The levels of total chlorophyll and total sugars in the leaves of control plants increased with time as observed during 60 days. The level of these constituents increased with all the concentrations of KCl applied throughout the study period. With 15.0 mM KCl, the levels of chlorophyll and total sugars were significantly less than with 10.0 mM KCl ($P<0.05$). The maximum levels of chlorophyll and sugars were observed in leaves treated with 10.0 mM KCl (Table 1). The regression analyses and correlation coefficient between chlorophyll and total sugars were analyzed by using all the data obtained from treated plants and the **r** value indicated a very high ($r=0.86$) positive correlation (Fig. 1). It confirms the findings of Ghosh and Srivastava (1993, 1995).

The *in vivo* NRA and soluble protein in control plants increased with age up to 60 days and the magnitude of increase was almost same. NRA as well as the soluble protein level was enhanced with all the concentrations of

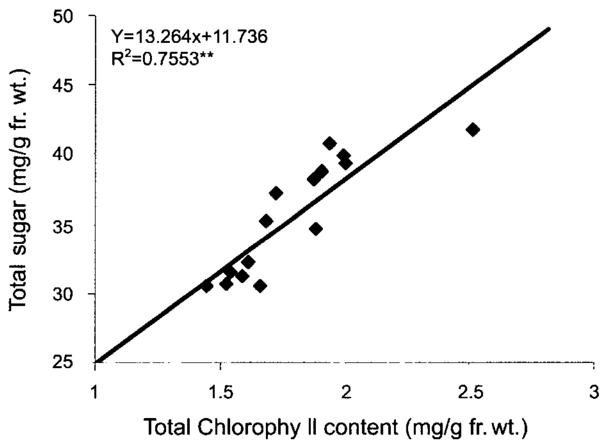

Fig. 1. Regression and correlation coefficients between chlorophyll and total sugars analyzed from all the data for the treated plants.

Table 1. Levels of total chlorophyll (mg/g fresh wt) and total sugars (% fresh wt) at different ages in the fresh leaves of control plants of *Morus alba* and of those sprayed with different concentrations of KCl

Conc. of KCl (mM)	Plant age (days after spray)					
	30		45		60	
	Chlorophyll	Sugars	Chlorophyll	Sugars	Chlorophyll	Sugars
0.0	1.448	30.58	1.523	30.66	1.589	31.32
2.5	1.558	31.59	1.612	32.30	1.686	35.17
5.0	1.729	37.28	1.910	38.72	2.003	39.34
10.0	1.876	38.15	1.994	39.86	2.514	41.77
15.0	1.658	30.58	1.882	34.71	1.937	40.80
CD at 5%	0.021	0.865	0.025	0.924	0.027	1.203

Table 2. *In-vivo* nitrate reductase activity (μmol NO_2^- $h^{-1}g^{-1}$ fresh wt) and soluble protein (SP) (mg/g fresh wt) in fresh leaves of control plants of *Morus alba* and of those sprayed with different concentrations of KCl

Conc. of KCl (mM)	Plant age (days after spray)					
	30		45		60	
	NRA	SP	NRA	SP	NRA	SP
0.0	4.83	20.45	5.03	20.89	5.25	21.23
2.5	5.36	21.82	6.12	22.67	6.89	24.21
5.0	6.62	22.47	7.33	24.28	7.95	25.61
10.0	6.94	22.69	8.55	24.89	8.76	26.59
15.0	5.37	22.10	6.25	23.28	6.93	25.17
CD at 5%	0.238	0.012	0.324	0.022	0.027	0.456

KCl but maximum increase was observed with 10.0 mM (Table 2). At 15.0 mM KCl, the levels of NRA and soluble protein were significantly less than at 10.0 mM KCl ($P < 0.05$). Thus the NRA and soluble protein were dose-dependent. The regression analyses and correlation coefficient between NRA and soluble protein were analyzed by using all the data obtained from treated plants and the **r** value indicated a very high ($r = 0.92$) positive correlation (Fig. 2). This observation is also in confirmation with Ghosh & Srivastava (1995).

It was observed that the foliar spray of 10.0 mM KCl had a positive and most effective impact on the NPR, pWUE, plant height, leaf yield and leaf dry weight (Table 3). The regression analyses and correlation coefficient between leaf dry weight and photosynthesis (Fig. 3); pWUE vs. photosynthesis (Fig. 4) and leaf yield vs. NRA (Fig. 5) were analyzed by using all the data obtained from treated plants and the r values indicated a very high (leaf

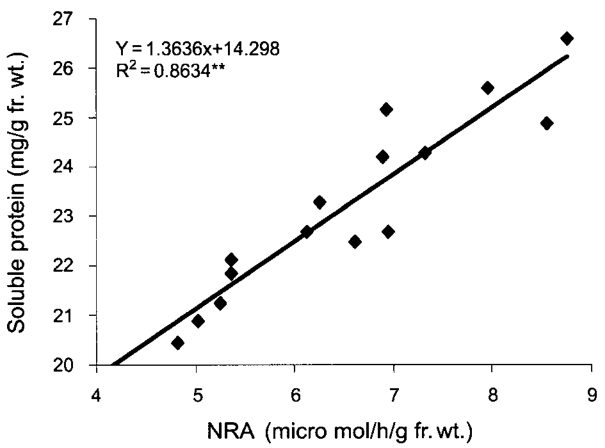

Fig. 2. Regression and correlation coefficients between nitrate reductase activity and soluble protein analyzed from all the data for the treated plants.

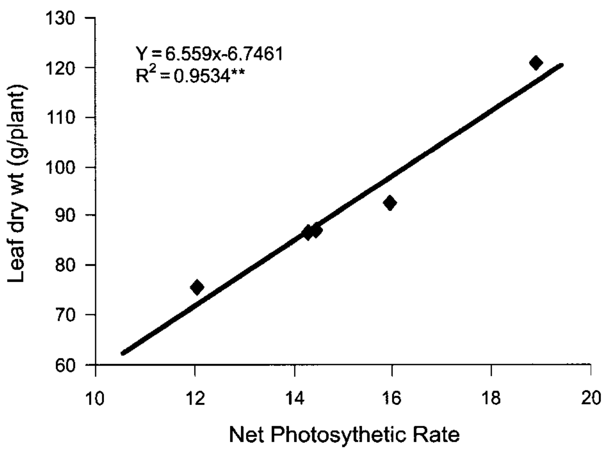

Fig. 3. Regression and correlation coefficients between leaf dry weight and net photosynthetic rate analyzed from all the data for the treated plants.

Table 3. Physiological and growth attributing parameters of control plants of *Morus alba* and of those sprayed with different concentrations of KCl on day 60

Conc. of KCl (mM)	Plant height (cm)	Leaf yield/plant (g)	Leaf dry wt/plant (g)	NPT (μmol/m²/sec)	pWUE
0.0	120	227	75.39	12.05	637.2
2.5	125	275	86.73	14.28	653.2
5.0	136	310	92.34	15.95	774.8
10.0	150	379	120.69	18.89	969.3
15.0	122	281	87.11	14.45	825.4
CD at 5%	5.24	8.78	10.19	1.89	156.6

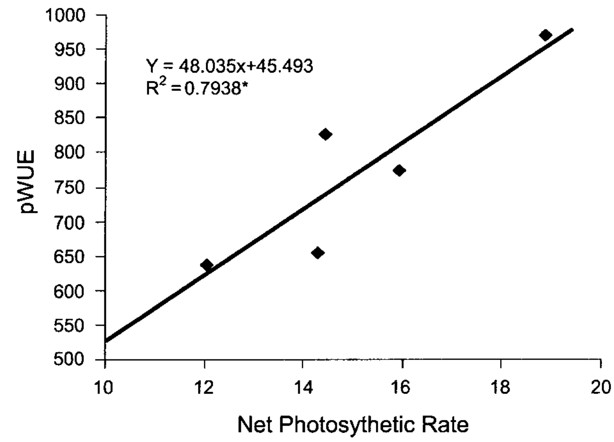

Fig. 4. Regression and correlation coefficients between pWUE and net photosynthetic rate analyzed from all the data for the treated plants.

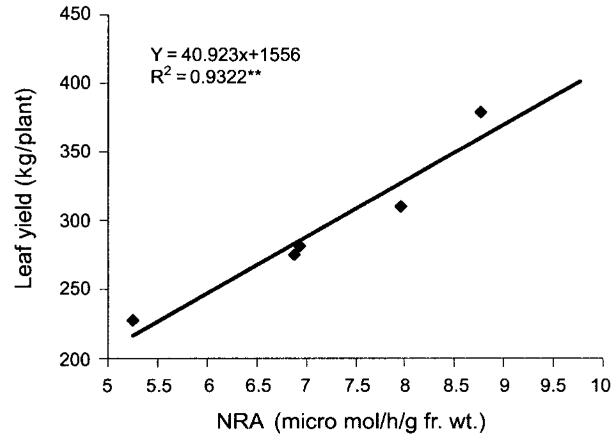

Fig. 5. Regression and correlation coefficients between leaf yield and nitrate reductase activity analyzed from all the data for the treated plants.

dry weight and photosynthesis r = 0.97; pWUE vs. NPR r = 0.89 and leaf yield vs. NRA r = 0.96) positive correlation.

The importance of various anions and cations for general cell physiology is well described (Steward and Sutcliffe, 1959; Steward, 1963; Evans and Sorger, 1966; Epstein, 1972; Rains, 1976). Foliar fertilization has frequently been proven to be a satisfactory method of supplying nutrients to mulberry and oak (Das *et al.*, 2002; Dixon *et al.*, 1981).

The results indicated that the response of mulberry plants to foliar treatment of KCl was concentration-dependent, being optimal at 10.0 mM, where higher levels of chlorophyll, total sugars, soluble protein, NRA, NPR, pWUE, plant height, leaf yield and leaf dry weight were maintained for 60 days. In addition to these, potassium helps the mulberry plants to overcome the soil moisture stress as the plants grown with limited irrigation facility having soil moisture level of 9.272 – 11.28% in comparison to 30% in irrigated garden.

Potassium is a macronutrient although it does not form any organic complex in the plant. It is present in abundance in the vacuoles of the plant and one of its roles is in osmotic and ionic regulation. K^+ ions are the essential component of the K-shuttle, a mechanism that regulates and enhances uptake of nitrate ions, which are the basis for amino acid synthesis and all other organic nitrogen compounds in the leaves. Therefore, increasing the K-shuttle capacity by foliar fertilization may lead to enhanced levels of organic nitrogen compounds and NRA in the leaves (Lips *et al.*, 1970). K^+ can also bind through ionic bonds to protein and other compounds with negative functional groups. In the phosphorylation mechanism both in mitochondria and in chloroplasts the proton transport mechanism is involved (Boyer *et al.*, 1977) which is associated with K^+ uptake. Potassium functions as a cofactor or activator for many enzymes of carbohydrate and protein metabolism more effectively at an optimal concentration which varies for different plants.

The stimulation of nitrate reductase activity by potassium salts is generally in agreement with the response reported earlier by different workers in a number of plants (Beevers and Hageman, 1969; Hewitt, 1975; Hewitt *et al.*, 1976). K^+ ions have been shown to affect nitrate reductase and nitrite reductase in various ways (Hewitt and Nicholas, 1964; Dilworth, 1974; Hewitt, 1975). The improved levels of other parameters (chlorophyll, total sugars, soluble protein, leaf yield *etc.*) might result from a general improvement in the metabolic activity of the cells.

The high level of soluble protein was found only in those treatments of KCl where the NRA was also higher, and vice versa. Thus, *in vivo* NRA can be taken as an index of higher soluble protein level in mulberry leaves. Johnson *et al.* (1976) suggested that NR is a parameter ultimately determines yield or leaf biomass which is in support of the observation recorded in this investigation. Moreover, the coefficients of correlation between chlorophyll vs. total sugars, soluble protein vs. NRA, leaf yield vs. NRA, leaf yield vs. NPR analyzed for all the data were positive.

Therefore, spray with the optimal dose (10 mM) of potassium chloride which increased NRA was useful in the sense that the treatment increased protein level in the leaves and that protein enriched leaves are beneficial for silkworm larval growth. In addition, foliar spray of potassium not only helps the plants to overcome the soil moisture stress condition but also increases the leaf yield by about 52% as compare to control. This recommendation would definitely help the farmers of rainfed area of mulberry cultivation where poor productivity of foliage is the set back for the industry.

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