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## The Impact of Kinetin Application on Water Relations, Leaf Osmotic Potential and Soluble Carbon and Nitrogen Compound Contents in *Sorghum bicolor* Plants Growing at Varying Levels of Soil Acidity

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Abstract: Acidification of the root environment to pH 2, 3, 4, 5 and 6.5 (control) with sulfuric acid decreased transpiration rate, stomata! opening, soluble sugars, soluble proteins and total free amino acids content and increased leaf osmotic potential in Sorghum bicolor plants. Leaf area was markedly reduced at lower soil reaction (pH 3 and 2). However, leaf relative water content (RWC) was higher at pH 5 and pH 4 than at pH 6.5 and was lower at pH 3 and pH 2. Hydrolysable carbohydrates (KC.) were markedly reduced at pH 2. Application of kinetin enhanced transpiration rate, stomata! opening and improved leaf water content especially at higher soil acidity. Kinetin-treated plants had higer soluble sugars, hydrolysable carbohydrates, soluble proteins and total free amino acids content than in untreated plants. Leaf osmotic potential was lower (more negative value) in plants receiving kinetin solutions at all acidity levels (pH 6.5 receiving 5 mg dm<sup>-3</sup>) kinetin was an exception). Leaf growth was positively affected by the three kinetin concentrations at pH 2. The effects of soil acidity (A), kinetin (K) and their interaction (A×K) on the parameters tested were statistically significant. Applied kinetin had a dominant role (contributes to largest share in total treatment effect) in affecting plant water relations. The share of interaction (A × K) was dominant in affecting soluble sugars, hydrolysable carbohydrates, soluble proteins and total free amino acids (coefficient of determination ( $\eta^2$ ) = 0.47, 0.53, 0.80 and 0.81 respectively). The role of soil acidity was dominant for leaf area ( $\eta^2 = 0.62$ ). The results show that the application of kinetin can alleviate the deleterious effects of acidity on the parameters tested.

Key words: Acidity, kinetin, osmotic potential, Sorghum bicolor, transpirati

#### Introduction

Soils differ considerably in their degree of acidity or alkalinity and these differences are reflected in the vegetation that grows on them. Most crop plants grow well in soil that is neutral, mildly acid, or mildly basic. Soil that is strongly acid is not suited to crop culture, except for a few crops, which demand acid soils. Soil acidity is often associated with the presence of high concentrations of hydrogen (H<sup>+</sup>) and aluminium (A1<sup>3+</sup>) in exchangeable form (Medeiros *et al.*, 1994). Aluminum toxicity is a growth-limiting factor for plants grown on acid soil (Foy, 1992). In most humid areas (with high rainfall), acid soils are relatively common. The cause of this is the leaching of calcium and other exchangeable bases from the surface and their replacement by hydrogen ions (Arden and Kenneth, 1993).

Acid precipitation caused by the release of nitrogen and sulfur oxides into the upper atmosphere has becom a widespread environmental problem. Among its effects are accelerated nutrient leaching (Turner and Tingey, 1990) and causes changes in physiological processes dependent on membrane permeability (Sigal *et al.*, 1988). Effects of soil pH on membrane and ion transport properties in cereals (Wilkinson and Duncan, 1994) root elongation (Zaifnejad *et al.*, 1997) and shoot growth (Gadallah, 1994) has been studied.

The response of plants to stress involve complex of physicobiochemical changes including the balance between endogenous phytohormones. Acidification of the root environment is accompanied by changes in endogenous cytokinins (Cizkova, 1990). A large number of development and physiological processes (ex. cell division, branching of shoots, leaf senescence, chloroplast development, nutrient metabolism, membrane potential and ion transport) can be influenced by cytokinins or are correlated with changes in the endogenous cytokinin concentration (Schmulling *et al.*, 1997). Exogenous application of kinetin, one of the more widely studied cytokinins, can stimulate resistance against

unfavourable environmental factors and improved plant growth under stresses such as soil salinity, soil waterlogging and soil pollution (Gadallah, 1995a, b).

Sorghum is an important food/feed crop throughout the world and is a cereal used by many people living in regions where soil are acidic (Zaifnejad *et al.*, 1997). Accordingly, the aim of the present work has been to study the changes in transpiration rate, stomata! opening, leaf relative water content, leaf area and leaf osmotic potential in *Sorghum bicolor* plants caused by acidification of the root environment and kinetin treatments (exogenously applied in different concentrations). In addition the contents of soluble carbon and nitrogen fractions which contributes to the osmoregulation and relates to stress tolerance *in* plants were determined.

#### Materials and Methods

Sorghum (Sorghum bicolor L. cv. Dorado) plants were grown under natural field conditions in plastic pots (2 dm<sup>-3</sup> volume) lined with double polyethylene bags containing 2400 g air-dry soil (sand/clay 2:1 v/v). The soil held 10.3 g water per 100 g oven dry weight at field capacity level. Soluble Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> content in the soil was 0.930, 0.080, 0.064, 0.375 and 0.057 mg  $g^{-1}$  oven dry soil respectively. After one week of growth, plants (five per pot) were twice watered with 200 cm<sup>3</sup> portions of full strength nutrient solution (Hoagland and Arnon, 1950). The soil was acidified using  $H_2SO_4$  to pH values of 2, 3, 4, 5 and 6.5 (control) in the soil saturation extract. Treatment of soil with acidic solution began when seedlings were 2 weeks old. The pots were irrigated daily by adding acidic solutions through perforated plastic tubes inserted into the soil to keep them at field capacity. This procedure aided in the uniformity distribution of the added solution. Three pots were arranged at random to each treatment combination at every acidity level. The plants were allowed to adjust to soil acidity for a period of 2 weeks before starting treatments with kinetin solutions.

Kinetin solutions (5, 10 and 15 mg dm<sup>-3</sup>) were applied three times at 5 day intervals by spraying the growing shoots in each pot with 15 cm<sup>3</sup> of kinetin solution. Control plants were sprayed with distilled  $H_2O$ . Plants were analysed a week after the last kinetin application.

Measurement of transpiration, stomatal aperture and leaf relative water content: Before starting transpiration measurement, the soil surface in the pots was covered with the projecting margins of the plastic bags lining the pots by wrapping them around the base of the stems. This procedure prevented water loss by direct evaporation from the soil and any measurable water loss, hence, represents loss by transpiration. Each pot was weighed at 3 hour interval, from 7 am to 7 pm Leaf area for each pot was measured by the disk method (Watson and Watson, 1933). Transpiration rate (mg H<sub>2</sub>O dm<sup>-2</sup> min<sup>-1</sup>) was calculated for the four daily periods I from 7 am to 10 am; II from 10 am to 1 pm; III from 1 pm to 4 pm and IV from 4 pm to 7 pm. Transpiration rate was obtained from the following formula:

Transpiration rate =  $\frac{\text{Water loss (mg)}}{\text{Leaf area (dm<sup>2</sup>) x time (min.)}}$ 

Length and width of the stomatal aperture on the upper and lower epidermis were determined using an ocular micrometer. The epidermis was removed from the leaf according to Rodriguez and Davies (1982). The epidermal strips were observed under light microscope in liquid paraffin to minimize post-experimental changes. In each treatment 40 stomata from four different strips were measured. Relative water content (RWC) of the fully-expanded leaf was measured by the method of Barrs and Weatherley (1962).

Wet and dry bulb temperature of air was measured by Aspiration Assmann Psychrometer. Actual and saturation vapour pressures (mm Hg) at the ambient temperatures were obtained from hygrometric tables and then vapour pressure deficit (VPD) was calculated.

Leaf osmotic potential and soluble carbon and nitrogen content: Leaf osmotic potential was determined by the cryoscopic method. The freezing point of the extract was determined by using a special ether evaporation device and a Beckman differential thermometer calibrated to 0.01°C. The osmotic potential was calculated as described by Abdel-Rahman and El-Sharkawi (1974).

Shoots (in triplicate) were harvested at noon, dried at  $70^{\circ}$ C in an aerated oven and ground to pass through 1 mm sieve. Soluble sugars and hydrolysable carbohydrates, total free amino acids and soluble proteins in shoots extract were determined using Bausch and Lomb spectrophotometer (Spectronic 2000) according to Buysse and Merckx (1993), Lee and Takahashi (1966) and Lowry *et al.* (1951), respectively.

Statistical tests applied were: least significant differences (LSD) and analysis of variance (Ostle, 1963). The relative effect of each single factor and interaction in contributing to the total response was evaluated by the coefficient of determination  $(n^2)$ . In such case:

Sum of squares due to the factor

 $\eta^2 = \frac{1}{\text{Total sum of squares due to the treatment combination}}$ 

#### Results

Transpiration rate: Diurnal fluctuation in transpiration rate generally corresponded to diurnal fluctuations in air

temperature and vapour pressure deficit (VPD) (Fig. 1). Maximum transpiration rate was attained at about 2.30 pm. Higher soil acidity and treatment of plants with kinetin caused some changes in the diurnal pattern of transpiration at particular pH levels. For example, with K<sub>2</sub> and K3, plants reached peaks of transpiration before noon. In the absence of kinetin, transpiration rates were reduced with increased soil acidity. Decrease in transpiration due to soil acidity was significant at midday but, not in the early morning or late afternoon. However, the control (pH 6.51 rate during the day times was lower than that of some stressed plants which received kinetin (Fig. 1). Kinetin treatments significantly increased transpiration rates in both control and stressed plants from 7 am to 1 pm. However, in the late afternoon (4-7 pm), transpiration rates were lower at most pH levels with all kinetin concentrations as compared to untreated plants.

Table 1: Mean daily transpiration, stomatal pore area, leaf relative water content and leaf area in *Sorghum bicolor* at different pH levels and kinetin (K) concentrations

	рΗ	Kinetin (mg dm $^{-3}$ )						
		0	5	10	15			
Transpiration rate	6.5	6.96	6.71	8.24	8.50			
(mg H <sub>2</sub> O)dm <sup>2</sup> min <sup>-1</sup> )	5	4.73	5.80	6.65	7.44			
	4	5.32	5.94	6.55	7.02			
	3	5.58	7.21	7.00	5.95			
	2	4.34	7.00	8.03	7.07			
Stomatal pore area	6.5	41.38	54.08	61.29	54.69			
(μm²)	5	27.00	30.58	32.53	42.21			
	4	30.32	28.42	30.02	37.60			
	3	25.56	24.11	33.77	29.59			
	2	10.27	19.23	24.34	23.30			
Relative	6.5	80.86	84.22	83.54	83.22			
water content	5	84.24	87.40	86.79	88.54			
(%)	4	83.91	87.37	84.46	85.36			
	3	76.16	87.90	86.81	89.61			
	2	76.65	86.84	83.97	83.77			
Leaf area	6.5	5.37	5.27	4.53	4.98			
(dM <sup>2</sup> )	5	4.74	4.99	5.18	4.58			
	4	4.48	4.56	4.64	4.98			
	3	3.72	4.25	4.43	430			
	2	3.49	4.38	4.78	4.56			

In the absence of kinetin, lowering the pH resulted in reduction in the average daily transpiration rate compared to pH 6.5 (control) (Table 1). Transpiration rates of kinetin-treated plants were generally higher than those of non kinetin-treated plants. Statistically significant effect for kinetin (K.), soil acidity (A) and their interaction (A × K) on transpiration rate was shown by analysis of variance (F values Table 2). Based on calculated coefficient of determination (n<sup>2</sup>), K had a greater effect than A on changes in transpiration rate is dominant ( $\eta^2$ =0.51). A×k interaction and A were equally subdominant ( $\eta^2$ -0.25 and 0.24 respectively).

**Stomatal pore area:** Generally, stomata of unstressed plants (pH 6.5) opened in the early part of the day and maximum stomatal pore area occurred at 1 pm (Fig. 2). Then, stomatal aperture decreased progressively to a minimum at 7 pm. Plants at pH 4 without added kinetin showed initial decreases in stomatal pore area in the early part of the day (7-10 am) and increasing progressively toward a maximum at 4 pm. Plants at pH 5 and 4 with added kinetin (K<sub>2</sub>) showed a progressive decrease in stomatal opening from early in the morning (7 am) toward a minimum at evening (7 pm). The same trend was observed at pH 3 with the highest kinetin concentration.

Gadallah and Sayed: Acidi	ty, kinetin,	osmotic	potential, S	Sorghum	bicolor,	transpiration
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Table 2: F and n<sup>2</sup> values for the effect of acidity (AI, kinetin (K) and their interaction (A × K) on transpiration rate, stomata' opening, leaf relative water content (RWC) and leaf area

Source	Transpiration	Transpiration rate		Stomata' opening		RWC		Leaf area	
of Varience									
	F	η²	F	η²	F	η²	F	η²	
A	16.91**	0.24	49.00**	0.19	74.58**	0.12	18.83**	0.62	
К	47.32**	0.51	137.37**	0.53	642.30**	0.80	3.5	0.10	
A×K	5.77**	0.25	72.09* *	0.28	14.25* *	0.08	2.92	0.28	





Fig. 1: Diurnal fluctuation in transpiration rate in Sorghum bicolor plants growing at different acidity levels (pH) and Kinetin (K) concentrations. Corresponding effective climatic varieties are indicated above. The vertical index bars represent the respective LSD at 5% level

Except for  $K_0$  plants at pH 4 from 4 to 7 pm and pH 3 with  $K_2$  at 7 am, stomatal pore area was wider for plants at pH 6.5 at any time of the day.

In the absence of kinetin ( $K_0$ ), stomata! opening decreased significantly with increasing acidity throughout the day (Fig. 2). Spraying of kinetin enhanced stomatal opening especially in the early part of the day. No definite pattern was observed in stomata! opening response under different kinetin concentrations in the afternoon.

Average daily stomata! aperture decreased with increasing soil acidity (Table 1). Application of kinetin induced an increase in average daily stomatal opening at all soil acidities, except at pH 4 and 3 with K<sub>1</sub> and at pH 4 with K<sub>2</sub>. The role of kinetin, soil acidity and their interaction on stomatal opening was shown by analysis of variance to be statistically significant. The relative role of kinetin (Table 2) is dominant ( $\eta^2 = 0.53$ ) over acidity in affecting stomata! opening and that of A x K interaction is subsidiary.

**Relative water content:** Diurnal fluctuation in leaf relative water content (RWC) was observed at all soil pH values and kinetin concentrations (Fig. 3). In both presence or absence of kinetin, plants at the lowest

reaction showed a progressive decrease of RWC in the early morning toward a minimum at 1 pm. However, they tended to regain turgidity early in the afternoon. RWC fluctuated during the late part of the day (4-7 pm) and ended up at a lower (e.g. pH 4) or higher (e.g. pH 6.5) i levels at sunset (7 pm) relative to its value at sunrise (7 am). Control (pH 6.5) plants at K<sub>0</sub> and K<sub>1</sub> showed increasing RWC early in the morning (until 10 a.m). The same trend was observed with K<sub>1</sub> (pH 4), K2 (pH 4 and 3) and K3 (pH 5).

In the absence of kinetin, RWC of plants grown in acid soil (pH 5 to pH 3) was significantly higher than that at pH 6.5 in the morning (Fig. 3). The reverse was noticed in the afternoon. Soil acidity yielded non significant decrease in RWC at midday. Kinetin-treated plants generally had higher RWC than untreated analogues for most of the day time.

Mean daily leaf RWC (Table 1) was higher at pH 5 and 4 than control but the opposite was true at pH 3 and 2. Kinetin enhanced leaf RWC especially at pH 3 and 2. Soil acidity, kinetin and soil acidity x kinetin interaction (Table 2) had highly significant effect on leaf relative water content. The share of K in affecting leaf RWC was predominant ( $\eta^2 = 0.80$ ) but that of A and A×K was subsidiary (Table 2).

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Fig. 2: Diurnal pattern in leaf relative water content of *Sorghum bicolor* plants growing at different acidity levels (pH) and Kinetin (K) concentrations. The vertical index bars represent the respective LSD at 5% level

Table 3:	Contents	of	soluble	surgars	(S.5). hydi	rolysable
	carbohydra	tes ۱	NI.C.), sc	luble prot	eins (S.P.) a	and tota
	free amino	aci	ds (T.A.A	.) conten	ts and leaf	osmotic
	potential (I	MPa)	in <i>Sorgl</i>	hum bicol	or plants at	different
	pH levels a	nd ki	netin (K)	concentrat	ions	

	pН	Kinetin (	Kinetin (mg dm <sup></sup> )					
		0	5	10	15			
S.S.	6.5	33.09	25.30	23.28	17.64			
(mg $g^{-1}$ d.wt)	5	20.04	25.46	24.35	23.23			
	4	18.59	28.77	29.23	30.61			
	3	15.76	34.82	35.40	28.94			
	2	11.18	26.45	21.77	24.95			
H.C.	6.5	1.64	7.65	9.20	7.90			
(mg g <sup>-1</sup> d.wt)	5	2.12	3.85	3.37	5.36			
	4	1.64	2.48	5.28	8.09			
	3	1.29	1.65	3.91	4.87			
	2	0.91	4.74	12.22	3.57			
S.P.	6.5	68.13	72.44	77.46	77.00			
(mg $g^{-1}$ d.wt)	5	43.45	51.71	48.17	31.58			
	4	39.65	45.70	48.35	49.24			
	3	34.00	48.57	55.24	62.96			
	2	30.61	61.17	59.59	55.66			
T.A.A.	6.5	30.68	4.48	12.23	10.15			
(mg $g^{-1}$ d.wt)	5	4.27	7.30	10.77	13.79			
	4	3.20	5.94	18.05	6.75			
	3	1.13	12.92	7.82	16.49			
	2	5.04	22.44	14.03	15.89			
MPa	6.5	-1.39	-0.97	-1.75	-1.43			
	5	-0.64	-1.63	-1.71	-1.29			
	4	-0.66	-1.39	-1.50	-2.74			
	3	-0.64	-1.32	-1.27	-1.36			
	2	-0.51	-1.09	-1.44	-1.62			

Leaf area: Acidification of root environment to pH 3 and 2 caused about 29 and 35% reduction in leaf area as

compared to the unacidified control (pH 6.5) (Table 1). Kinetin application enhanced leaf growth especially at pH 3 and pH 2. The role of soil acidity (A) in affecting leaf area was dominant ( $\eta^2 = 0.62$ ) but the role of pH x K interaction was subdominant ( $\eta^2 = 0.281$  and the share of kinetin was the minor ones (Table 2).

**Soluble carbon and nitrogen components:** In the absence of kinetin, soluble sugar content decreased progressively with increasing soil acidity (Table 3). This is not the case, however, with hydrolysable carbohydrates, where its content was negligible affected by soil acidity with respect to control. Kinetin application reduced soluble sugars accumulation at pH 6.5 and increased the content in the acid range from pH 4 to pH 2 compared to  $K_o$  plants. Kinetin-treated plants, either acid-stressed or not, had higher hydrolysable carbohydrates content than untreated analogues.

Increasing soil acidity reduced progressively soluble proteins and total free amino acids contents. Spraying with kinetin solutions enhanced soluble proteins accumulation in both unstressed and stressed plants compared to untreated plants except at pH 5 sprayed with 15 mg dm<sup>-3</sup> kinetin where the plants had lower soluble proteins than untreated plants. Unstressed plants (pH 6.5) receiving kinetin solutions accumulated less free amino acids than in kinetin untreated analogues but the revers was true in acid-stressed plants. Each of kinetin, soil pH and shier interaction (Table 4) had highly significant effect on shoot soluble sugars, hydrolysable carbohydrates, soluble proteins and total free amino acids as indicated by F value. The relative role of  $(A \times K)$  interaction (Table 4) in affecting soluble sugars, hydrolysable carbohydrates, soluble proteins and total free amino acids was dominant ( $n^2 = 0.47$ , 0.53, 0.80 and 0.81 respectively).

Gadallah and Sayed: Acidity	, kinetin, osmotic	potential, Sorghum	bicolor, transpiration
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Table 4: F and n<sup>2</sup> values for the effect of acidity (A), kinetin (K) and their interaction (A × K) on contents of soluble sugars (5.5.) hydrolysalfm\* carbohydrates (N.C.), soluble proteins (S.P.) and total free amino acids (T.A.A.) contents

Source	S.S		H.C		S.P		A.A		0.P		
of Varience											
	F	η²	F	η²	F	η²	F	η²			
A	12.03**	0.24	12.87**	0.19	34.58**	0.10	93.85**	0.14	15.22**	0.10	
К	19.23"	0.29	24.29"	0.28	47.01**	0.10	43.67"	0.05	92.30**	0.44	
A×K	7.69"	0.47	11.66**	0.53	96.61**	0.80	181.51**	0.81	23.50**	0.46	
* 0' ' ' '	E 0/ ()	**0		o/ (* )							

\*Significant at 5% confidence level. \*\*Significant at 1% confidence level.



Fig. 3: Diurnal fluctuation in stomata! pore area of *Sorghum bicolor plants* growing at different acidity levels (pH) and Kinetin (K) concentrations. The vertical index bars represent the respective LSD at 5% level

**Osmotic potential of the leaf:** In the absence of kinetin (Table 3), lowering the soil pH caused progressive increase in leaf osmotic potential (less negative values), which reached about -0.5 MPa at pH 2 i.e. only 37% of control plants. Kinetin at all three concentrations decreased leaf osmotic potential (more negative values) over the entire pH range (from 6.5 to 2) except in unstressed plants treated with 5 mg dm<sup>-3</sup> kinetin where their osmotic potential was higher than kinetin untreated plants. Both kinetin and soil acidity as well as thier interaction significantly affected leaf osmotic potential. The share of kinetin is nearly equally dominant as  $A \times K$  interaction ( $\eta^2 = 0.44$  and 0.46, respectively) in affecting leaf osmotic potential, but the role of acidity is a minor one ( $\eta^2 = 0.10$ ).

#### Discussion

Increasing the acidity of the root environment (Fig. 1) caused a reduction in transpiration rate compared to the control (pH 6.5). The decrease in transpiration rate with increasing soil acidity could be induced by inactivation of the endogenous cytokinins in the leaves (Cizkova, 1990) which has been reported to be responsible for stimulation of transpiration (Henson and Wareing, 1976). Reduction in leaf area (Table 1) and stomata! opening (Fig. 2) could be an alternative explanation for the low transpiration in acid stressed plants. The reduction in transpiration may directly help to avoid acidity stress by minimizing water loss. Kinetin treatment induced higher transpiration rates than in untreated plants. Such an effect throught to be to promotion of stomatal opening by kinetin (Table 1). Plants exposed to the highest acidity level (pH 2) showed a higher response to kinetin than the control (pH 6.5) may be *due* to the combined effects of (1) a reduction in the activity of endogenous cytokinins by acidity in stressed plants (Cizkova, 1990) and (2) exogenously applied kinetin acting as a substitute for the inactivated endogenous cytokinins.

The data of Fig. 2, indicates a progressive decrease in stomatal pore area with increasing soil acidity. The decrease could be induced by changes in internal plant water balance and/or increasing abscisic acid content of plants (Cizkova, 1990). The increase in abscisic acid is suggested to cause stomata( closure (Henson and Turner, 1990). Acidity-induced stomatal closure therefore greatly reduces transpiration (Fig. 1), thereby improving water status or arresting its decline (decreasing about 4% only between pH 6.5 and pH 2). Kinetin treatment generally increased stomatal pore area in unstressed and stressed sorghum plants. The increase is attributed to the combined effects of (1) reduction of CO<sub>2</sub> concentration inside leaves and greater rate of photosynthesis in guard cells than in mesophyll (Hans, 1967), thus favoring stomatal opening, 2) kinetin causes stomata to open osmopassively and turgorpassively (Pallas and Box, 1970). Promotion of stomatal opening by kinetin is important for enhancement gaseous exchange since the guard and subsidiary cells control over 95% of the gaseous exchange between leaves and the atmosphere (Willmer and Fricker, 1996).

The data on changes in relative water content (Fig. 3) show that plants at higher acidity levels (pH 3 and 2) maintained lower water content than the control (pH 6.5). The

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explanation given is that sulfuric acid forms sulfates in soils. The plasma membrane of the majority of cells is impermeable to sulfates which must be transported actively by carriers or permeases (Anderson, 1972). Thus the cell wall acidified considerably by hydrogen ions and a relatively high anion concentration appear outside the cell. It is well known that acidification of cell walls affects the calcium balance resulting in decrease of cell wall stability (Percy and Baker, 1988). Combination of acidity with a high external osmotic potential result in decrease of the water content. In addition soil acidity increased solubility of aluminum (Al<sup>3+</sup>), manganese (Mn<sup>2</sup>) and iron (Fe<sup>3+</sup>) to the extent that they become toxic (Arden and Kenneth, 1993). These cations affect trans-membrane and trans-root potentials and also Hion efflux and root growth (Foy, 1992), Kinetin sprayed plants had higher relative water content than unsprayed plants (K<sub>0</sub>). This could be due to effects of kinetin on membrane stability and permeability to water and solutes; (Gadallah, 1995a). The maintenance of relatively high leaf RWC in kinetin treated plants may also be achieved by osmotic adjustment which involves the accumulation of soluble sugars and free amino acids (Table 3) in acidstressed plants received kinetin.

Leaf area was negatively affected by soil acidity. About 35% decreased in leaf area was observed at higher acidity level (pH 2) as compared to the unacidified control (pH 6.5). Reduction in leaf growth is in accordance with Zaifnejad *et al.* (1997) who found that plants grown on acid soil with high exchangeable Al<sup>3+</sup> often show decreased growth and water deficit symptoms. This could be due to the deficiency of calcium and magnesium ions needed by the plant for normal metabolism (Arden and Kenneth, 1993). The sensitivity of leaf expansion in sorghum plants to foliar water status could be an alternative explanation for leaf growth reduction especially at high acidity levels. The reduction in leaf area may improve foliar water status and help to restriction of water loss, thus enabling the sorghum plants to survive severe soil acidity.

Stressed plants in the acid range from pH 5 to pH 2 accumulated lower soluble sugars and hydrolysable carbohydrates than in the control in accordance with the findings of Zaib-un-Nisa and Rafiq (1983). Such reduction may be due to the inhibition of various photosynthetic processes including chlorophyll biosynthesis (Gadallah, 1994), activities of photosynthetic enzymes (Zaib-un-Nisa and Rafig, 1983) and stomata! effectiveness, (Fig. 2). Acid stressed plants treated with kinetin had higher soluble sugars and hydrolysable carbohydrates than kinetinuntreated plants. Such enhancement may be an outcome through increasing chlorophyll content (Gadallah, 1994, 1995a, b), photosynthetic activity and/or increased starch degradation by kinetin (Berridge and Ralph, 1971). The increase soluble sugars may be an adaptive response which involves adjustment of osmotic potential that facilitates the maintainance of favourable water balance (Gnanasiri et al., 1992).

Soluble proteins and total free amino acids contents were reduced under acidic conditions, The reduction may be due to inhibition of their de novo synthesis and/or increased degradation in acid-stressed plants. Soluble proteins and total free amino acids were higher in kinetin-treated plants. This is mainly due to enhancement of kinetin to proteins and amino acids synthesis (Gadallah, 1995a). Accumulation of soluble proteins improves water retention properties especially under severely acidic conditions.

Leaf osmotic potential increased progressively (less negative values) with increasing soil acidity. This was due to reduction in the accumulation of osmotically active

metabolites such as soluble sugars and free amino acids (Table 3) and mineral ions (Turner and Tingey, 1990) which contribute to osmoregulation where soil acidity cause insolublity of phosphorus, potassium and other minerals and deficiency of calcium and magnesium (Arden and Kenneth, 1993) and alter the absorption of ions at the root epidermal plasma membrane (Medeiros et al., 1994). Application of kinetin caused decrease in negative osmotic water potential of leaf through enhancement of soluble sugars and free amino acids accumulation (Table 3) and decreased ion leakage through effects kinetin on the stability of leaf membranes (Gadallah, 1995a). The increase in soluble sugars, soluble proteins, total free amino acids content and osmotic adjustment (lowering the leaf osmotic water potential) by kinetin suggests that marked improvement in growth of plants grown on acidic soil may be obtained by kinetin application in order to increase the adjustment of plant water relations to such conditions. Despite to soil acidity and kinetin treatment combinations the results indicate significant interactions between soil acidity and kinetin on most parameters tested. The role of  $\mathsf{A} \times \mathsf{K}$ interaction was dominant for soluble sugars, hydrolysable carbohydrates, soluble proteins total free amino acids and leaf osmotic potential. This may signify that soluble carbon and nitrogen components as well as leaf osmotic potential in sorghum plants were slightly affected by soil acidity and kinetin as single factors but largely affected by their interactions.

In summary, the deleterious effect of stress provoked by acidification of root environment on plant water relations, leaf area and osmotic potential and some metabolites was ameliorated by spraying the growing shoots with kinetin solutions.

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