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The Effect of Different Kinetin Application Methods on Some Chlorophyll Parameters of Two Crop Plants Grown Under Salinity Stress

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With 2 Figures

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Summary

SALAMA F. M. & AWADALLA A. A. 1987. The effect of different kinetin application methods on some chlorophyll parameters of two crop plants grown under salinity stress. – Phyton (Austria) 27 (2): 181–193, with 2 figures. – English with German summary.

Sand culture technique was employed to investigate the effect of soil salinity and kinetin on chlorophyll a and b contents, the a/b ratio and the chlorophyll stability to heat in *Sorghum bicolor* and *Gossypium barbadense* cultivars. Levels of osmotic potential of the soil solution between −3 and −13 bar were adjusted by irrigation with certain amounts of saline solutions containing NaCl and $CaCl_2$. Kinetin solutions (10 ppm) were applied by 3 methods: treatment of the seeds (presoaking), shoot treatment (spraying) and by irrigation. Kinetin can retain the chlorophyll content and even increases them in the two investigated plants when applied by presoaking method. The a : b ratio seems to be affected by salinity stress rather than by kinetin treatment. Kinetin enhances significantly the stability index of both chlorophyll types in particular when applied by presoaking method. The results proved that kinetin can alleviate salinity stress on chlorophyll parameters in both investigated plants when applied by presoaking method rather than by the other methods.

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Zusammenfassung

Salama F. M. & Awadalla A. A. 1987. Die Wirkung verschiedener Applikationsarten von Kinetin auf einige Chlorophyll-Parameter von zwei unter Salzstreß wachsenden Kulturpflanzen. – Phyton (Austria) 27 (1): 181–193, mit 2 Figuren. – Englisch mit deutscher Zusammenfassung.

An Sandkulturen von Cultivaren von *Gossypium barbadense* und *Sorghum bicolor* wurde der Einfluß von Salinität und Kinetin auf den Gehalt an Chlorophyll a und b sowie das a/b-Verhältnis und auf die Hitzestabilität der Chlorophylle untersucht. Das osmotische Potential der Bodenlösung wurde mit $CaCl_2$ + NaCl-Lösungen im Bereich von −0,3 – −13 bar eingestellt. Kinetin wurde durch Vorquellen der Samen, durch Besprühen der Sprosse sowie durch Bewässerung mit 10 ppm-Lösungen appliziert. Mit Kinetin kann der Chlorophyllgehalt erhalten bleiben, er ist aber bei beiden Versuchspflanzen nach Vorquellen in Kinetinlösungen erhöht. Das Verhältnis Chlorophyll a/b scheint durch Salzstreß stärker beeinflußt zu werden als durch Kinetin. Kinetin erhöht signifikant die Hitzestabilität der Chlorophylle, besonders nach Applikation durch Vorquellen. Die Ergebnisse legen nahe, daß Kinetin, durch Vorquellen der Samen geboten, die Streßwirkungen auf die untersuchten Chlorophyll-Parameter an beiden Versuchspflanzen stärker mindern kann als bei Applikation nach den beiden anderen Methoden. (Editor transl.)

Introduction

Salinity has been found to affect the pigment content of certain plant species by either increasing (Dostanova 1966) or decreasing its ratio (Carter & Myers 1963, Shimose 1973). Photosynthesis was found to be mostly reduced by salinity. This has been reported by a number of workers using various plant types and salinizing agents (Gale & al. 1967, Lapina & Popove 1970, Salama & Gasanov 1977 and Salama 1978).

Recently, some investigators used the plant growth regulators to alleviate the severe effect of salinity. Cytokinins, specially kinetin, are thought to be promising in that respect. The role of cytokinins as retardants of chlorophyll and protein degredation in senescing leaves is well documented (Anderson & Rowan 1968, Beevers 1968, Fletcher et al. 1968). Enhancement of chlorophyll synthesis by kinetin has also been demonstrated (Sugiura 1963, Banerji & Laloraya 1967). As pointed out by Pilet & Hofer (1966), kinetin may have two effects on chlorophyll: 1- increases the chlorophyll accumulation and 2- inhibits chlorophyll degredation.

The effects of kinetin on stimulation of chlorophyll formation may be due to an influence on the synthesis of protochlorophyllide (Stobart & al. 1972, Shlyk & Averina 1973). The role of kinetin in controlling chlorophyll degradation appears to be associated with its effect on chlorophyllase (Sabater & Rodriguez 1978).

Evidence of the eco-physiological role which plant growth regulators can play in adjusting the plants to salt stress conditions has been reported by several investigators. Plant response to salt stress such as chlorophyll content, its biosynthesis and its stability to heat when plants were treated with cytokinins is still not quite clear in the literature. The present study was designed to investigate the interactive effect of kinetin (applied by three different methods) and salinity stress on chlorophyll content (a and b), the a/b ratio and chlorophyll stability to heat (CSI) in salt stressed plants of cotton and millet. Of these three methods, one involved seed treatment (Presoaking) while the second method is plant treatment (Spraying) and finally the third application was through the (Irrigation) water.

Materials and Methods

Plants experimented with were each of cotton (*Gossypium barbadense.* cv. Dandara) and millet (*Sorghum bicolor* L. cv. Giza-3). Experimental plants were grown in plastic pots containing 1400 g air dry soil (sand/clay 2:1 v/v). The plants were twice watered with 100 ml portions of full strength Hoagland solution (HOAGLAND & ARNON 1950).

Five plants were allowed to grow in each pot, ψ_s levels were chosen at -3, -7, -10 and -13 bar, in addition to the control (-0.3 bar). For each potential level, three pots were assigned at random. Osmotic solution prepared according to the formula given by LAGERWERFF & EAGLE (1961) were used in irrigation to adjust ψ_s to the desired levels. A mixture of $CaCl_2$ and NaCl was used in the preparation of these solutions in which the sodium adsorption ratio was fixed at 12.5%. Solutions were added to the soil in such a way that the soil solution acquires the assigned potential at field capacity. Treatments of plants with saline solutions began when seedlings were 8 weeks old (except in presoaking experiment). On completing the treatment, the plants were watered with distilled water only. In this respect the moisture content of the soil was never allowed to fall beyond the field capacity. This was achieved by checking weights of pots twice daily. The plants were allowed to adjust to treatment for a period of two weeks before starting treatment with kinetin solution.

The kinetin concentration in the water solution used throughout the experiment was 10 ppm. Three different methods of kinetin application have been used in the present study, namely, presoaking, spraying and irrigation with the hormone solution. In presoaking method seeds of experimental plants were soaked in kinetin solution for 8 hours, then dried for 24 hours. The dried seeds were soaked again in the kinetin solution for another 8 hours and then air dried for 24 hours. The treated seeds were then sown in salinized pots containing 1400 g soil with the different levels of ψ_s as described. Another group of seeds were soaked in the same manner but in distilled water and were sown in other pots containing the same levels of ψ_s

in order to compare between the treated seeds with kinetin. In the spraying method, kinetin solution was applied by spraying the shoot system of the growing plants in each pot with 10 ml. of hormone solution. The control plants were sprayed with distilled water. Reapplication with kinetin was performed five days after the first spraying. The measurements were recorded 8 days after spraying with the second dose. In the irrigation method each pot was irrigated with 200 ml of kinetin solution over one week of intervals. The measurements were recorded 7 days after the last irrigation in which the pots were completed to field capacity by distilled water. The control plants were irrigated by distilled water throughout the entire expariment.

Chlorophyll a and b content was determined according to the method of TODD & BASLER (1965). Chlorophyll stability to heat was asessed according to the method of MURTY & MAJUMDER (1962), and the chlorophyll stability index (CSI) was modified as the percentage of chlorophyll content of the heated sample relative to its content in the fresh sample, as follows:

$$CSI = \frac{\text{content of chlorophyll in heated sample}}{\text{content of chlorophyll in fresh sample}} \times 100$$

The chlorophyll content was calculated by using the Mackinney equation given by VISHNIAC (1957). Chlorophyll a/b ratio was calculated in fresh samples only.

Proper statistical tests were used to elucidate the effects of single factors (ψ_s and kinetin) as well as the interaction ($\psi_s \times$ kinetin). The tests included analysis of varience (F values) and least significant difference test (LSD). In case of significant effect of any of the single factors or interaction, the relative contribution (share) of such factors in the total response to treatment combination are evaluated by means of the coefficient of determination (η^2).
In such case:

$$\eta^2 = \frac{\text{sum of squares due to the factor}}{\text{total sum of squares due to the treatment combinations}}$$

This coefficient is expressed as a fraction or a percentage. Such biometrical testes are applied according to the procedures of OSTLE (1963) and PLOXINSKI (1969).

Results

I. Application of kinetin by sprayings

The results of the analysis of chlorophyll content, chlorophyll a/b ratio and chlorophyll stability index (CSI) for *Sorghum* plants either sprayed or

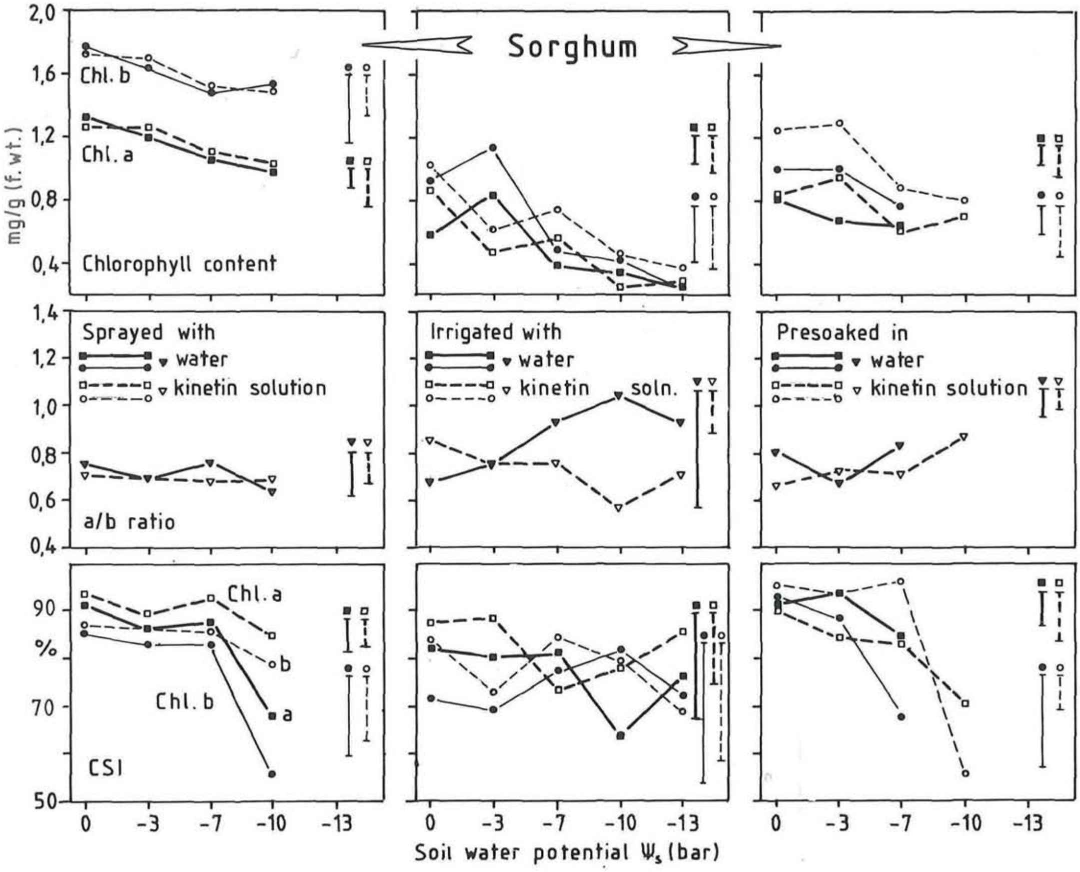

Fig. 1. Changes in chlorophyll a and b contents, a/b ratio and chlorophyll stability to heat in *Sorghum* plants in response to decreased soil osmotic potential and different kinetin application methods. (The vertical index bars represent the respective least significant differences at 5% level.)

not by kinetin solution are shown in Fig. 1. It is apparent that all of these parameters generally decreased with reducing ψ_s. The reduction in chlorophyll (a) content was significant at −3, −7 and −10 bar. No definite change was detected in the a/b ratio. The reduction in CSI due to salinity stress was only significant at −10 bar for both chlorophylls a and b. Spraying of *Sorghum* shoots with kinetin solution resulted in non significant effects on all the chlorophyll parameters tested except at − 10 bar when the CSI increased significantly. The analysis of the data of chlorophyll

F values and coefficients of determination (η^2) for significant effects of kinetin (applied by three different methods), salinity stress and their interaction on chlorophyll a and b contents, their stability to heat (CSI) and the a/b ratio in *Sorghum* and *Gossypium* plants

Application	Source of varience	Chlorophyll a F	Chlorophyll a η^2	Chlorophyll b F	Chlorophyll b η^2	a/b F	a/b η^2	CSI a F	CSI a η^2	CSI b F	CSI b η^2
		Sorghum									
Spraying	Kinetin	0.109	-	0.01	-	0.016	-	22.48**	0.01	4.15	-
	Salinity stress	163.77**	0,99	90.90**	0.99	81.05**	0.99	671.8**	0.98	110.7**	0.97
	Kinetin x stress	0.06	-	0.18	-	0.308	-	33.o**	0.01	1.89	-
Irrigating	Kinetin	0.029	-	0.24	-	2.660	-	1.69	-	0.37	-
	Salinity stress	23.81***	0.72	11.67**	0.80	0.150	-	2.35	-	0.84	-
	Kinetin x stress	9.07**	0.28	2.85	-	1.650	-	1.53	-	0.33	-
Pre-soaking	Kinetin	39.58**	0.08	39.67**	0.08	33.58**	0.03	51.95**	0.02	23.32**	0.03
	Salinity stress	100.76**	0.78	94.22**	0.82	152.36**	0.68	787.25**	0.84	16.79**	0.93
	Kinetin x stress	19.23**	0.14	9.08**	0.10	64.19**	0.29	77.50**	0.14	6.30**	0.04
		Gossypium									
Spraying	Kinetin	0.22	-	0.01	-	0.15	-	2.14	-	3.54	-
	Salinity stress	115.53**	0.97	1o.46**	0.97	0.70	-	2.60	-	9.36**	0.86
	Kinetin x stress	0.34	-	0.36	-	0.80	-	0.13	-	0.61	-
Irrigating	Kinetin	0.014	-	5.11*	0.10	5.23*	0.31	0.20	-	3.49	-
	Salinity stress	156.5**	0.92	9.95**	0.81	1.60	-	2.88	-	5.69**	0.56
	Kinetin x stress	52.20**	0.08	1.11	-	1.30	-	10.34**	0.78	3.52**	0.35
Pre-soaking	Kinetin	11.96**	0.05	7.89*	0.05	7.72*	0.06	50.72**	0.04	115.07**	0.08
	Salinity stress	8.07**	0.14	30.32**	0.83	28.63**	0.82	234.65**	0.70	257.50**	0.74
	Kinetin x stress	46.26**	0.81	4.43*	0.12	4.18*	0.12	89.64**	0.26	63.84**	0.18

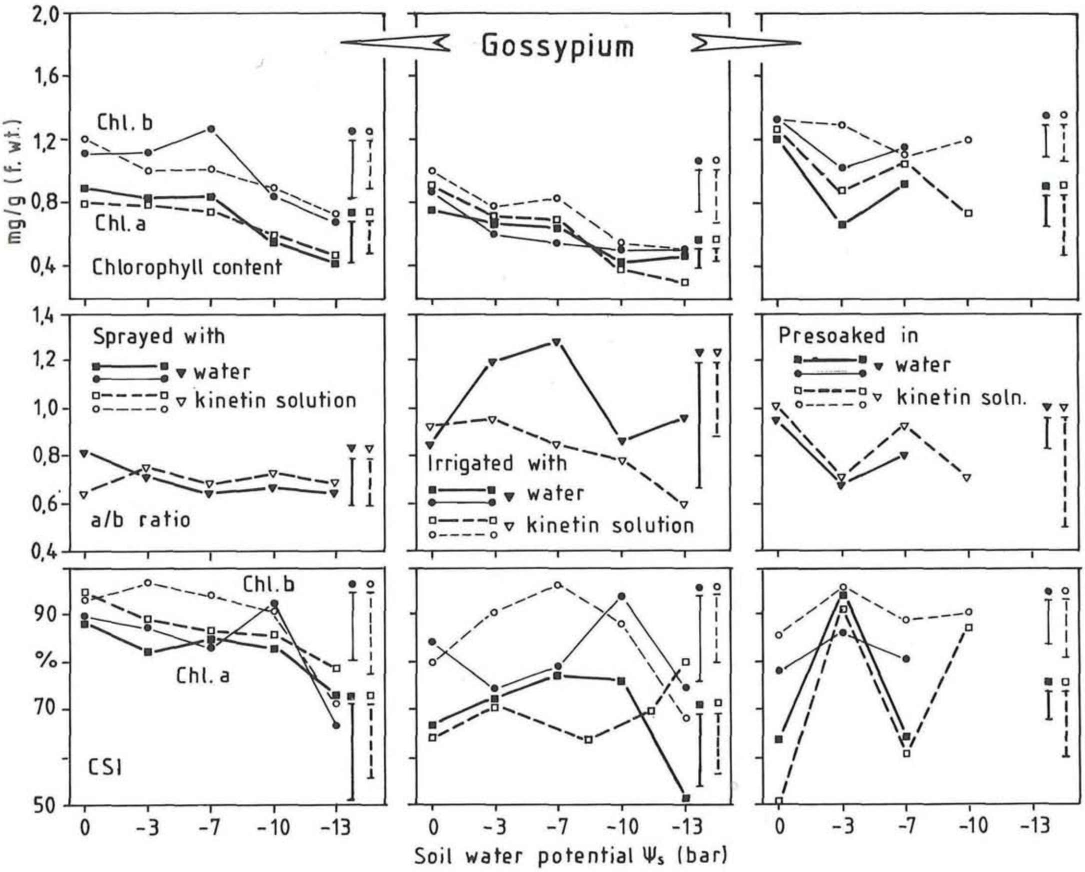

Fig. 2. Changes in chlorophyll a and b contents, a/b ratio and chlorophyll stability to heat in *Gossypium* plants in response to decreased soil osmotic potential and different kinetin application methods. (Index bars see legend to Fig. 1.)

content, a/b ratio and CSI in *Sorghum* plants sprayed or not by kinetin solution are shown in Table 1. Only salinity stress showed a significant F value in all chlorophyll data but neither kinetin nor its interaction with salinity had significant F values except CSI for chlorophyll a where all treatments had highly significant values. In *Gossypium* (Fig. 2), the content of both chlorophyll a and b was less than that in *Sorghum* plants but it revealed the same trend with respect to salinity stress. The chlorophyll a content decreased significantly at −10 bar as well as at −13 bar. The reduction in chlorophyll b content was significant at −13 bar. Spraying of

Gossypium shoots with kinetin solution did not affect the chlorophyll content, its stability to heat or the a/b ratio as shown in Fig. 2. The analysis of the data of chlorophyll content, a/b ratio and CSI of the *Gossypium* plants sprayed or not with kinetin solution reveald the same trend as that of *Sorghum* plants with one exception that, F value was highly significant for CSI of chlorophyll b for salinity stress only.

II. Application of kinetin by irrigation

Data of the analysis of chlorophyll a and b contents, a/b ratio and CSI for *Sorghum* plants either irrigated or not with kinetin solution are shown in Fig. 1. Generally, the chlorophyll b content was greater than the chlorophyll (a) content. The contents of the two types of chlorophyll increased with increasing soil salinity to −3 bar. This increase was significant in case of chlorophyll a only. The chlorophyll contents of both chlorophyll a and b decreased significantly with reducing ψ_s to −7, −10 and −13 bar as compared with non stressed plants. Irrigation with kinetin solution increased the chlorophyll a content significantly and b content non-significantly in non-stressed *Sorghum* plants. The chlorophyll a and b contents were significantly decreased at −3 bar but was rather increased non–significantly at −7 bar. It is also clear from the figure that the chlorophyll contents in the *Sorghum* plants irrigated with kinetin solution increased in most levels specially chlorophyll b.

Concerning a/b ratio, it seems that salinity stress did not affect this ratio since all differences were non-significant. The a/b ratio exhibited another trend in case of *Sorghum* plants irrigated with kinetin solution for it increased in the non-stressed plants and then decreased non-significantly at −3 and −7 and highly significant at −10 and −13 bar. Treatment of *Sorghum* plants with kinetin solution increased the CSI of chlorophyll a which was significant at −3 bar as compared with the corresponding salinized levels and not irrigated with kinetin solution. All other differences were non-significant. The analysis of the data of chlorophyll in *Sorghum* plants irrigated or not with kinetin solution is shown in Table 1. Obviously, the application of kinetin by irrigation method does not affect chlorophyll in this plant. The F values were only significant for salinity stress in chlorophyll a and b content and the interaction between kinetin and salinity in case of chlorophyll a only. In *Gossypium* (Fig. 2), the chlorophyll a and b contents decreased significantly with reduced ψ_s. Irrigation with kinetin solution resulted in significant increase in chlorophyll a content in non-stressed plants and another significant decrease in the same chlorophyll at −13 bar as compared with the corresponding salinized levels. Chlorophyll b content increased non-significantly at all levels tested. The chlorophyll a/b ratio also non-significantly increased with reduced ψ_s in *Gossypium* plants until −10 bar. Treatment with kinetin solution by irrigation resulted in decreasing a/b ratio and this decrease was significant at −7 and −13 bar as compared with the corresponding salinized levels. CSI for chlorophyll a and b contents did not differ significantly with respect to the control, with reducing ψ_s. With respect to the *Gossypium* plants irrigated with kinetin solution, the CSI for chlorophyll a increased significantly at −13 bar, while for chlorophyll b the increase was at −3 and −7 bar as compared with the corresponding salinized levels. The analysis of the data of chlorophyll a and b contents, the ratio a/b and CSI for a and b chlorophylls in *Gossypium* plants are shown in Table 1. Kinetin effect has significant F value in case of chlorophyll b content and a/b ratio. Salinity stress has significant F value in case of chlorophyll a and b contents and CSI for chlorophyll b only. The interaction between kinetin and salinity showed a significant F value with respect to chlorophyll a content and CSI for both a and b chlorophylls. It is also observed that in CSI for the two types of chlorophyll, the interaction has a high relative contribution in the total response to treatment combinations (η^2 = 78% in chl. a and η^2 = 35% in chl. b).

III. Application of kinetin by presoaking

Figure 1 shows the chlorophyll data (content, a/b ratio and CSI) in *Sorghum* plants either their seeds were previously presoaked or not in kinetin solution. It is clear from the figure that both chlorophyll a and b contents significantly decreased with reducing ψ_s. Treatment with kinetin solution by presoaking method tend to increase both chlorophyll a and b contents as compared with corresponding salinized levels. The ratio a/b in untreated plants with kinetin decreased significantly with reduced ψ_s. Presoaking in kinetin solution resulted in significant reduction in a/b ratio in non-stressed plants. CSI for both chlorophyll a and b decreased with reducing ψ_s. Treatment with kinetin solution decreased the stability of chlorophyll a to heat. In contrast, the presoaking in kinetin solution tend to increase CSI of chlorophyll b, the increase was only significant at −7 bar. The analyses of the data of chlorophyll in *Sorghum* plants either treated or not with kinetin solution by the presoaking method are shown in Table 1. It is clear from the table that all factors (kinetin, salinity stress and their interaction) significantly affected the chlorophyll content, a/b radio and CSI. The relative contribution of salinity stress in the total response to treatment combinations was the greatest followed by the interaction and finally the kinetin as monitored by (η^2, Table 1). Apparently the application of kinetin by the presoaking method is the only case in which all treatments affected significantly all chlorophyll parameters. The chlorophyll a and b contents, the ratio a/b and CSI for both a and b chlorophylls in *Gossypium* plants either their seeds presoaked in kinetin solution or in distilled water are shown in Fig. 2. Both chlorophyll a and b contents significantly decreased in *Gossypium* plants presoaked in distilled water, with increasing soil salinity as compared with the control plants. Presoaking in kinetin solution increased the chlorophyll a and b contents as compared with the corresponding salinized levels. The ratio a/b for both treated and untreated plants have had the same trend, but the kinetin treatments increased the values of the a/b ratio at all salinity levels. The CSI of chlorophyll a and b significantly increased at − 3 bar in plants presoaked in distilled water. Presooking in kinetin solution seemed to decrease the CSI of chlorophyll a, while increased CSI of chlorophyll b, but, however, non-significantly. The analysis of the data of chlorophyll in *Gossypium* plants is shown in Table 1. The F values for all factors tested were significant with respect to all chlorophyll parameters. The relative contribution of the interaction between kinetin and salinity has a very high value in the total response to treatment combination as indicated by η^2 values.

Discussion

The effect of increased salinity stress and different methods of kinetin application on chlorophyll content in the experimental plants proved that kinetin treatment can retain the pigment content in each of *Sorghum* and *Gossypium* plants and that the former is the most responding to both factors when treated by presoaking method. Decrease in chlorophyll content in the experimental plants under increased salinity agrees with the findings of MAZEN (1981) on the same plants; CARTER & MAYERS (1963) working with sugar beet; SHIMOSE (1973) with rice and HEIKAL (1975) with sunflower, but in variance, with the findings of MARGOLINA (1950) DOSTANOVA (1966) working on sugar beet. It is clear from the results of this study that kinetin can retain the chlorophyll content (a and b) and even increase them in the two experimental plants when applied by presoaking method. This is supported by the results of kinetin application by presoaking method where all factors affected significantly the chlorophyll contents in both investigated plants. It was rather clear that the η^2 value for the interactive effect was relatively high. These results support those of VARSHNEY & BAIJAL (1979) who found that kinetin treatment retained the total chlorophyll in salt stressed grasses and VARSHNEY (1980) who found that kinetin retained chlorophyll content in two varieties of Guar *(Cyamopsis tetragonoloba)* grown under salinity stress. Chlorophyll stability to heat was noticed to decrease (in general) in the two experimental plants under decreased osmotic potential. In contrast to what was found by MAZEN (1981) on the same species. Kinetin and its interaction with salinity stress, its effect on chlorophyll stability to heat can be obviously noticed in both *Sorghum* and *Gossypium* plants (specially chlorophyll b) when treated by the presoaking method. Table 1 indicates that the effect of the investigated factors (kinetin, salinity stress and their interaction) on chlorophylls a and b stability to heat in the two experimental plants, has a highly significant F values with respect to presoaking method. η^2 indicated that, the relative contribution of either kinetin or its interaction with salinity stress in the total response to treatment combinations, had considerable values when kinetin was applied by such method.

Accordingly, it could be concluded that kinetin is positively effective on chlorophyll stability to heat when applied by presoaking method. The high stability of pigments to heat in experimental plants may be attributed to the fact that these plants are tropical plants and they should consequently be heat enduring. Interpretation of curves of ratio "Chl.a/Ch.b" and those of chlorophyll a and b stability to heat may help in bearing an idea about the mode by which reduction in pigments contents takes place (whether it is a heat destruction or an inhibition of chlorophyll synthesis).

On the basis of the above discussion concerning all parameters of experimental plants under salinity stress and treated with kinetin solution by three different methods, it can be concluded that, the presoaking method is the best method for kinetin application. The results of this study proved that, kinetin can alleviate salinity stress in *Sorghum* and *Gossypium* plants when applied by presoaking method rather than the other two methods.

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Recensio

MANSFELD R. 1986. Verzeichnis landwirtschaftlicher und gärtnerischer Kulturpflanzen (ohne Zierpflanzen). Herausgegeben von Jürgen SCHULTZE-MOTEL. Unter Mitarbeit von E. H. BENEDIX †, R. FRITSCH, I. GREBENSCIKOV, K. HAMMER, P. HANELT, J. KRUSE, H. I. MAASS, H. OHLE, K. PISTRICK, A. RIETH, J. SCHULTZE-MOTEL, C. TITTEL. Zweite, neubearbeitete und wesentlich erweiterte Auflage. – Gr. 8°, Band 1: Seiten XVI + 578, 79 Abb., Band 2: Seiten VI + 579–1126, 74 Abb., Band 3: Seiten IV + 1127–1696, 88 Abb., Band 4: Seiten IV + 1697–1998; Kunststoffband. – Springer Verlag Berlin, Heidelberg, New York, Tokyo. – DM 440,–; ISBN 3-540-15966-5.

Dieses Werk folgt dem „Vorläufigen Verzeichnis landwirtschaftlich oder gärtnerisch kultivierter Pflanzenarten“ von Rudolf MANSFELD aus dem Jahre 1959. Hier von Neuauflage zu sprechen ist, wenn man die beiden Werke vergleicht (früher 659 Seiten, jetzt 1998 Seiten, früher ca. 1430 Arten behandelt, jetzt ca. 4800, früher keine, jetzt 241 Abb., früher keine, jetzt zahlreiche Literaturhinweise), schlicht eine Untertreibung. Das Konzept ist gleich geblieben, davon abgesehen liegt aber praktisch ein neues Werk vor. Der Begriff Kulturpflanze wird hier weit gefaßt; neben den eindeutig domestizierten Pflanzen sind darüber hinaus all diejenigen Arten aufgenommen, für