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# Glycine Betaine and Shikimic Acid – Induced Modification in Growth Criteria, Water Relation and Productivity of Droughted *Sorghum bicolor* Plants

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With 2 Figures

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## Summary

IBRAHIM A. H. & ALDESUQUY H. S. 2003. Glycine betaine and shikimic acid – induced modification in growth criteria, water relation and productivity of droughted *Sorghum bicolor* plants. – Phyton (Horn, Austria) 43 (2): 351–363, with 2 figures. – English with German summary.

To evaluate the beneficial role of glycine betaine and shikimic acid and their combination on *Sorghum bicolor* plants under water deficit conditions, the plants were grown under greenhouse conditions and subjected to withholding water at days 40 from sowing. Analysis of growth, photosynthetic pigments, some solutes concentration, water relations and yield were carried out.

Water stress markedly reduced *S. bicolor* growth, chl a, chl b, carotenoids concentrations, RWC, transpiration rate and total leaf conductance at both lower and upper leaf side as well as yield components. On the other hand, total soluble sugars increased and proline accumulated in *S. bicolor* plants grown under drought conditions.

The applied chemicals mitigated the effect of water stress on *S. bicolor* growth and yield. The effect was more pronounced with glycine betaine + shikimic acid treatment. Used chemicals increased the formation of photosynthetic pigments as

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well as soluble sugars concentrations, and lowered proline concentration and transpiration rate of *S. bicolor* plants under the stress conditions as compared with untreated droughted plants.

The economic yield (grain yield) was found to be strongly positively correlated with shoot fresh and dry weights, leaf area, chlorophyll a concentration, transpiration rate, RWC and the biomass of developing grains. On the other hand, the economic yield was strongly negatively correlated with proline concentration of *S. bicolor* leaves.

## Zusammenfassung

IBRAHIM A. H. & ALDESUQUY H. S. 2003. Glycin Betain und Shikimisäure – Induzierte Modifikationen bei Wachstumskritierien, Wasserhaushalt und Produktivität von *Sorghum bicolor* unter Trockenstress. – Phyton (Horn, Austria) 43 (2): 351–363, with 2 figures. – Englisch mit deutscher Zusammenfassung.

Um die günstigen Eigenschaften von Glycin Betain und Shikimisäure sowie deren Kombination in *Sorghum bicolor* Pflanzen unter Wasserstressbedingungen abschätzen zu können, wurden Pflanzen im Glashaus angezogen und 40 Tage lang ab Aussaat einem Trockenstress unterworfen. Wachstumsanalysen, Analysen der Plastidenpigmente, einiger gelöster Stoffe, des Wasserhaushaltes und des Ertrages wurden durchgeführt. Der Wasserstress reduzierte deutlich das Wachstum, die Konzentration an Chlorophyll a und b sowie der Karotinoide, RWC, die Transpirationsrate und die Blattleitfähigkeit sowohl an der Ober- als auch an der Unterseite, darüber hinaus den Ertrag. Andererseits stiegen die Gesamtzucker an und das Prolin akkumulierte bei *S. bicolor* unter Trockenstress. Die zur Anwendung gekommenen Substanzen milderten den Effekt von Wasserstress am Wachstum und Ernte von *S. bicolor*. Die Reaktionen waren am besten ausgeprägt, wenn mit Glycin Betain und Shikimisäure gleichzeitig behandelt wurde. Die verwendeten Chemikalien bewirkten den Anstieg von photosynthetischen Pigmenten aber auch die Konzentration an löslichen Zuckern, verminderten jedoch die Prolinkonzentration und die Transpirationsrate von *S. bicolor* Pflanzen unter Stressbedingungen im Vergleich zu unbehandelten Pflanzen unter Trockenstress. Die Ernte (Kornertrag) war stark positiv mit dem Spross-, Frisch- und Trockengewicht, der Blattfläche, der Chlorophyll a-Konzentration, Transpirationsrate, dem RWC und der Biomasse der entwickelten Körner korreliert. Andererseits war der wirtschaftliche Ertrag stark negativ mit der Prolinkonzentration der *S. bicolor* Blätter korreliert.

## Introduction

Drought remains the single most important factor threatening the food security of people in the developing world. Therefore, improving crops drought resistance is an important objective (MCWILLIAM 1989). Reduction of crop yield by drought stress is associated with the multiple effects of water deficit such as decrease in leaf area development, net photosynthesis rate, and biomass accumulation (AGBOMA & al. 1997).

Glycine betaine (N,N,N-tri-methylglycine) is a quaternary ammonium compound, accumulated by many species of angiosperms, and is thought to contribute to salt and drought tolerance (GORHAM 1996, WOOD & al. 1996).

There is good evidence that it acts as a non-toxic cytoplasmic osmolyte and plays a central role in adaptation to stress (WYN JONES 1984). The osmoprotective effects of Glycine betaine are now generally interpreted in terms of compatibility with macromolecular structure and function (RHODES & HANSON 1993). In members of *Chenopodiaceae* and *Poaceae* families, it is derived from a two-step oxidation of choline, with two enzymes, choline mono-oxygenase (BROUQUISSE & al. 1989) and betaine aldehyde dehydrogenase (WEIGEL & al. 1986). Glycine betaine is metabolically inert and readily translocated from its site of synthesis in leaves to the other parts of plants; however it is readily degraded by soil microbes (AGBOMA & al. 1997).

There have been studies on the use of glycine betaine to alleviate the effects of salinity (HARINASUT & al. 1996) and drought (MÄKELÄ & al. 1996, AGBOMA & al. 1997) on plants and interest is increasing with better understanding of the physiological effects of salt and drought stress.

Shikimic acid is the known precursor of aromatic amino acids, L-pheynylalanin, and L-tyrosine. These compounds are phenylpropane (C6-C3) derivatives as are the building units of lignin, and not surprisingly, they were soon shown to be lignin precursors (HUMPHREY & al. 1999). Shikimic acid is a precursor of phenolic compounds. In this respect, JAIN & SRIVASTAVA 1981 found that phenolic compounds play an important role in regulation of plant growth and metabolism and they are longer considered to be passive bi-product. In some cases, phenolic treatment induces expression of the same genes and resistance against the same spectrum of pathogens as pathogen induced resistance (LAWTON & al. 1996).

Recently, ALDESUQUY & IBRAHIM 2000 found that pretreatment of seeds of Vigna sinensis with shikimic acid stimulated the production of chl.a, chl.b, carotenoids and 14C fixation during leaf growth and development. These findings were compatible with the observed increases in cowpea fresh and dry masses.

The objective of this study was to evaluate the beneficial effect of foliar application of glycine betaine and shikimic acid and their combination in increasing water stress tolerance through their effects on growth, photosynthetic pigments, some water relations and solutes concentration as well as yield components of *Sorghum* plants.

## Materials and Methods

### Plant Materials and Growth Conditions

Grains of *Sorghum* (*Sorghum bicolor* L. Moench var. Dorado) were used in this study. This variety is known to be more drought resistant than the other varieties which are common in Egypt (IBRAHIM 1999).

The grains were surface sterilized with 1 mol $m^{-3}$ $HgCl_2$ solution for three minutes and washed thoroughly with distilled water. The grains were soaked in distilled water for 3 hours and then allowed to germinate in Petri dishes for two days on filter paper moistened with water. The germinated grains were planted in plastic pots (twenty seeds per pot; 25 cm width (× 30 cm height) filled with 6 kg mixture of soil (clay and sand = 2:1, v/v). The pots were kept in a greenhouse, and the plants were subjected to natural day/night conditions (minimum/maximum air temperature and relative humidity were: 29.2/33.2°C and 63/68%, respectively at mid-day during the experimental period). Irrigation to field capacity was carried out when soil water content had fallen to 60% of its initial value. Twenty days after planting, the plants were thinned to five uniform seedlings per pot.

On the day 40 after planting the pots were allocated to eight groups. Each group contains twenty pots. These groups were arranged as the following treatments: control, water stress, glycine betaine control, glycine betaine + water stress, shikimic acid control, shikimic acid + water stress, glycine betaine + shikimic acid control, glycine betaine + shikimic acid + water stress.

For glycine betaine (75 mol $m^{-3}$) and shikimic (0.3 mol $m^{-3}$) treatments the plants were sprayed by these chemicals 48 h before starting the stress period and weekly during the stress period. Water deficit stress was applied by withholding water for 24 days; each droughted pot received 400 $cm^3$ of water at the mid of the stress period. At the end of the stress period, re-watering to the field capacity was carried out, and the plants were left to grow until grain maturation under normal irrigation.

After thinning and before heading, the plants received 35 kg N $ha^{-1}$ as urea and 35 kg P $ha^{-1}$ as potassium dihydrogen phosphate as fertilizers.

Eight replicates for analyses of growth, photosynthetic pigments, total soluble sugars, proline and water relationships were carried out at the end of the stress period. The first fully expanded leaf from shoot apex was used for different analytical procedures. Ten samples per treatment were taken for yield analysis at the end of the growing season.

## Monitoring the Water Status of the Soil

Soil water content at the end of the stress period (SWC) was estimated by the destructive method as recommended by RITCHIE & al. 1990.

Estimation of leaf area:

Leaf area = length × maximum width × 0.74 (TURNER 1974)

Estimation of harvest index (HI):

Harvest index = Economic yield / Biological yield × 100 (BEADLE 1993)

Determination of photosynthetic pigments:

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method developed by LICHTENTHALER 1987.

Determination of total soluble sugars:

Total soluble sugars were extracted and determined by the anthrone method of RIAZI & al. 1985 as modified by IBRAHIM 1999.

Determination of proline:

The method adopted for estimation of proline was that described by BATES & al. 1973.

Estimation of relative water content (RWC):

Estimation of RWC was based on the methods described by RITCHIE & al. 1990 and PARDOSSI & al. 1992.

### Measurements of Total Leaf Conductance and Transpiration Rate

Total leaf conductance and transpiration rate of the 1st fully expanded leaf were measured using a Li-1600 M steady state porometer. The atmospheric pressure (PRES SET) and aperture area of the apparatus were adjusted to 101.3 kPa and 1 $cm^2$ respectively.

### Statistical Analysis

The main effect of factors (watering regime and both used chemicals ), and the interaction (watering regime × chemicals) were evaluated by general linear model (two way ANOVA) using SPSS program. Tests for significant differences between means at $P = 0.05$ were given by LSD test. The correlation coefficient between the economic yield and all evaluated criteria was also evaluated.

## Results

### Changes of Soil Moisture Content

Soil moisture content was markedly reduced at the end of the stress period, and it was found to be about 20.7 % and 4.8 % (% of oven soil dry weight) for control and droughted pots respectively.

### Growth Criteria

Results for growth criteria are shown in figure 1 (1A, 1B,1C and 1D). Water stress induced significant effect on shoot length, shoot fresh and dry weight, and leaf area. Water deficit reduced these criteria significantly. The used chemicals improved *Sorghum* growth and the main effect was significant for shoot fresh weight, shoot dry weight and leaf area. Furthermore, the interaction (watering regime × chemical) was significant for leaf area. Plants treated with Shikimic acid or shikimic + glycine betaine had a higher leaf area than those treated with glycine betaine only under the stress conditions.

### Photosynthetic Pigments

It is clear that water stress reduced chla, chl b and carotenoids concentration of *Sorghum* leaves significantly. The applied chemicals improved pigments concentration of *Sorghum* leaves. The interaction (watering regime × chemicals) was significant for chl b and carotenoids concentrations of *Sorghum*, whereas glycine betaine did not improve chl b or carotenoids of *Sorghum* plants grown under the stress conditions (Fig. 1E, F, G).

### Total Soluble Sugars and Proline

For total soluble sugars the main effects and the interaction (watering regime × chemicals) were significant. Water deficit appeared to increase total soluble sugars concentrations of *Sorghum* plants. Used chemicals improved total soluble sugars of *Sorghum* leaves. Furthermore, glycine betaine and shikimic acid treatments increased sugars concentration more than the other treatment under stress conditions (Fig. 2A).

For proline concentration it is clear that water regime and chemicals caused a significant effect. Proline was accumulated in *Sorghum* leaves in response to water deficit stress conditions, however *Sorghum* plants treated with chemicals had a lower proline concentration than non-treated plants (Fig. 2B).

## Water Relations

Water stress significantly reduced RWC of *Sorghum* plants. In this case the interaction (watering regime × chemicals) was significant. This reveals that the applied chemicals improved RWC of droughted plants only (Fig. 2C).

For transpiration rate the main effect of water regime and used chemicals and their interaction were significant. Water deficit markedly reduced transpiration rate on both leaf sides. Used chemicals appeared to decrease transpiration rate in *Sorghum* plants, and the effect was more pronounced with shikimic treatments under stress conditions (Fig. 2D, E).

The changes in total leaf conductance were comparable to that of transpiration rate (Fig. 2F, J).

## Yield and Yield Components

The data presented in table 1 clearly showed that water stress reduced all evaluated yield components. Except for grain weight, used chemicals appeared to improve all *Sorghum* yield components. The interaction (watering regime × chemicals) was significant for shoot dry weight, panicle fresh weight, grain yield, 100 grains weight and harvest index. Glycine betaine + shikimic treatments appeared to mitigate the effect of water stress on *Sorghum* plants more than the other treatments.

The economic yield (grain yield) was found to be positively correlated ($r = 0.8 - 0.95$; $P < 0.01$) with shoot fresh and dry weights, leaf area, chlorophyll a concentration, transpiration rate, RWC and the biomass of developing grains. On the other hand, the economic yield was negatively correlated ($r = -0.94$; $P < 0.01$) with proline concentration of *Sorghum* leaves. Correlations with shoot length, total soluble sugars, panicle fresh weight and harvest index were not significant.

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Fig. 1. Interactive effects of glycine betaine and shikimic acid on growth criteria and pigments content of *Sorghum bicolor* plants grown under water deficit conditions. The vertical bars represent LSD values at $P = 0.05$.

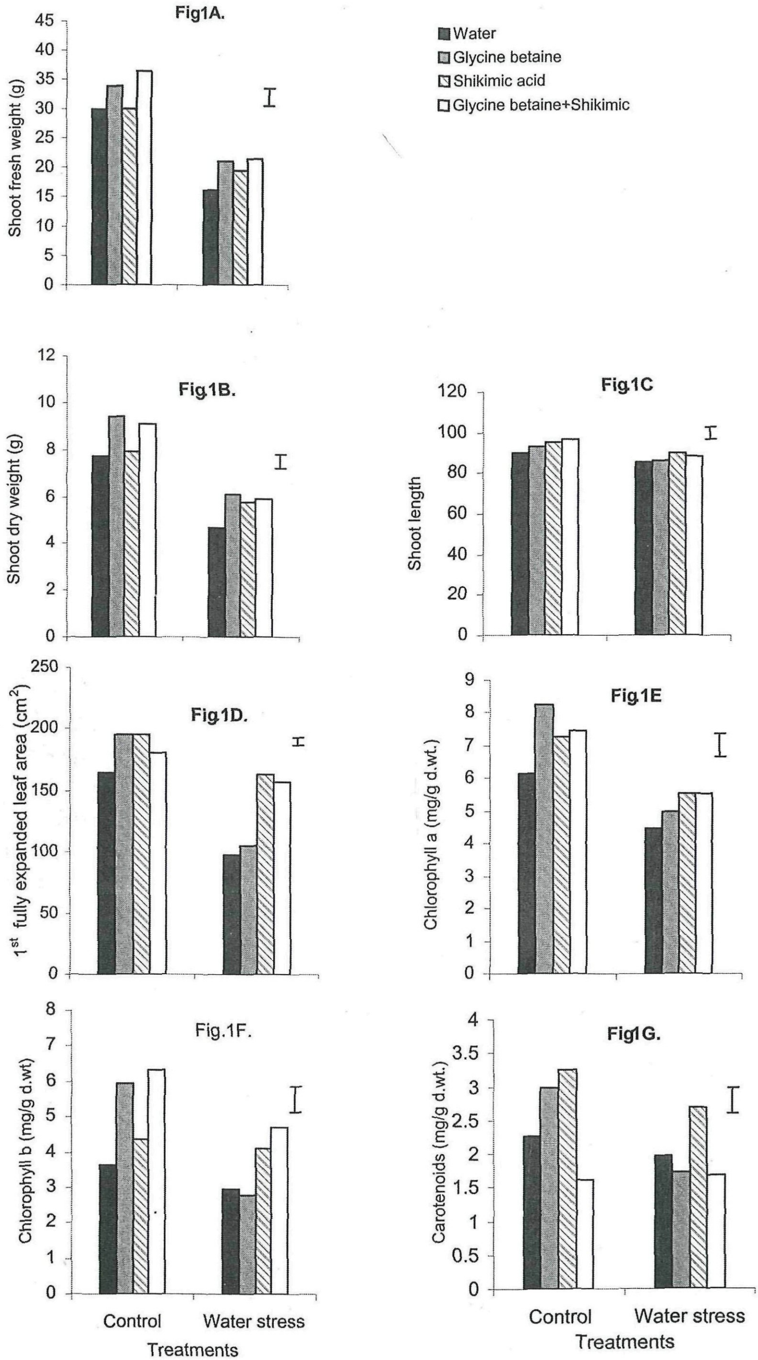
Fig1A.
Shoot fresh weight (g)
45
40
35
30
25
20
15
10
5
0
Water
Glycine betaine
Shikimic acid
Glycine betaine+Shikimic
Fig1B.
Shoot dry weight (g)
12
10
8
6
4
2
0
Fig1C
Shoot length
120
100
80
60
40
20
0
Fig1D.
1st fully expanded leaf area (cm2)
250
200
150
100
50
0
Fig1E
Chlorophyll a (mg/g d.wt.)
9
8
7
6
5
4
3
2
1
0
Fig.1F.
Chlorophyll b (mg/g d.wt)
8
7
6
5
4
3
2
1
0
Control
Water stress
Treatments
Fig1G.
Carotenoids (mg/g d.wt.)
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
Water stress
Treatments

## Discussion

The evaluated growth criteria of *Sorghum* plants (shoot fresh and dry weight weights, shoot length and leaf area) were reduced at the end of the water stress period. These disorders caused by drought stress are mainly due to loss of turgor which affects the rate of cell expansion and ultimate cell size, consequently it decreases growth rate, stem elongation, leaf expansion and stomatal aperture (HALE & ORCUTT 1987). The reduction of dry matter production under water stress conditions was reported to be mainly due to the reduction of leaf area (TERBEA & al. 1995) and the reduction of net photosynthesis and the higher increases in the rate of photorespiration (PERRY & al. 1983).

The available results indicate that chl a, chl b and carotenoids concentrations were reduced in *Sorghum* leaves at the end of the stress period. Furthermore, carotenoids concentration appeared to be less affected than chlorophyll. These results are in good conformity with those obtained by EL-KHEIR & al. 1994. The enhancement of chlorophyll degradation in leaves of stressed plants can probably be due to the disturbance in hormonal balance. Such disturbance may be manifested by diminished kinetin biosynthesis and increased abscisic acid. The former is known to inhibit chlorophyllase activity, whereas the latter is known to accelerate it (DRĄŻKIEWICZ 1994).

The applied chemicals appeared to improve the growth vigour by increasing shoot dry weight, leaf area and chlorophyll concentration of *Sorghum* plants. The protective role of glycine betaine on *Sorghum* growth can be related to its role in osmotic adjustment where it acts as a non-toxic cytoplasmic osmolyte (WYN JONES 1984, AGBOMA & al. 1997). Regarding the effect of shikimic acid ALDESUQUY & IBRAHIM 2000 found that Shikimic acid led to a marked increase in root length of cowpea plants during the overall growth periods, therefore the rate of water uptake from the soil may increase and this effect may explain the significant increase in fresh weight of stressed *Sorghum* plants. Furthermore, ALDESUQUY & IBRAHIM 2000 suggested that the increase in leaf area production caused by shikimic acid application could result from the rapid rate of movement of nutrients and hormones transported in the transpiration stream from the root, which can accelerate the rate of leaf expansion in developing *Sorghum* leaves. The stimulative effect of shikimic acid on pigments content of *Sorghum* leaves is in accordance with the findings of MENGHINI & al. 1992 who observed that the addition of shikimic acid to the culture media of *Agastache foeniculum* increased contents of chlorophyll a and b, lutein, zeaxanthin and β-carotene.

Water stress increased total soluble sugars in *Sorghum* plants. Application of chemicals induced a further increase in soluble sugars concentration under water deficit conditions. This increase in soluble sugars

# Erratum

IBRAHIM A. H. & ALDESUQUY H. S.: Glycine betaine and shikimic acid – induced modification in growth criteria, water relation and productivity of droughted *Sorghum bicolour* plants. – Phyton (Horn, Austria) 43(2): 351–363.

Table 1 is missing in the above article. Please insert it between p. 358 and 359.

Table 1. Interactive effects of glycine and shikimic acid on yield and yield components of sorghum plants grown under water deficit conditions.

| Treatments | Yield components | | | | | | |
|---|---|---|---|---|---|---|---|
| | Shoot fresh weight (g) | Shoot dry weight (g) | Panicle fresh weight (g) | Grain yield (g/plant) | 100 grains fresh weight (g) | 100 grains dry weight (g) | Harvest Index |
| Control | 37.56 | 21.11 | 10.82 | 8.09 | 4.16 | 3.47 | 44.00 |
| Water stress | 23.40 | 10.27 | 4.42 | 3.51 | 2.51 | 2.33 | 31.00 |
| Glycine betaine (control) | 38.91 | 17.53 | 9.72 | 8.79 | 4.06 | 3.50 | 59.00 |
| Glycine betaine + Water stress | 24.19 | 11.92 | 6.57 | 4.50 | 2.34 | 2.17 | 38.00 |
| Shikimic ( Control) | 51.44 | 23.64 | 10.81 | 8.50 | 3.83 | 3.26 | 35.00 |
| Shikimic + water stress | 27.12 | 13.65 | 6.86 | 3.74 | 2.41 | 2.23 | 36.00 |
| GB + Sh (Control) | 33.39 | 19.01 | 10.88 | 9.15 | 3.61 | 3.20 | 48.00 |
| GB + Sh + Water stress | 32.38 | 18.07 | 9.55 | 6.55 | 2.90 | 2.53 | 37.00 |
| LSD at $P = 0.05$ | 9.10 | 3.10 | 2.1 | 1.8 | 0.30 | 0.20 | 0.08 |

Abbreviations: GB, glycine betaine ; Sh, shikimic acid.

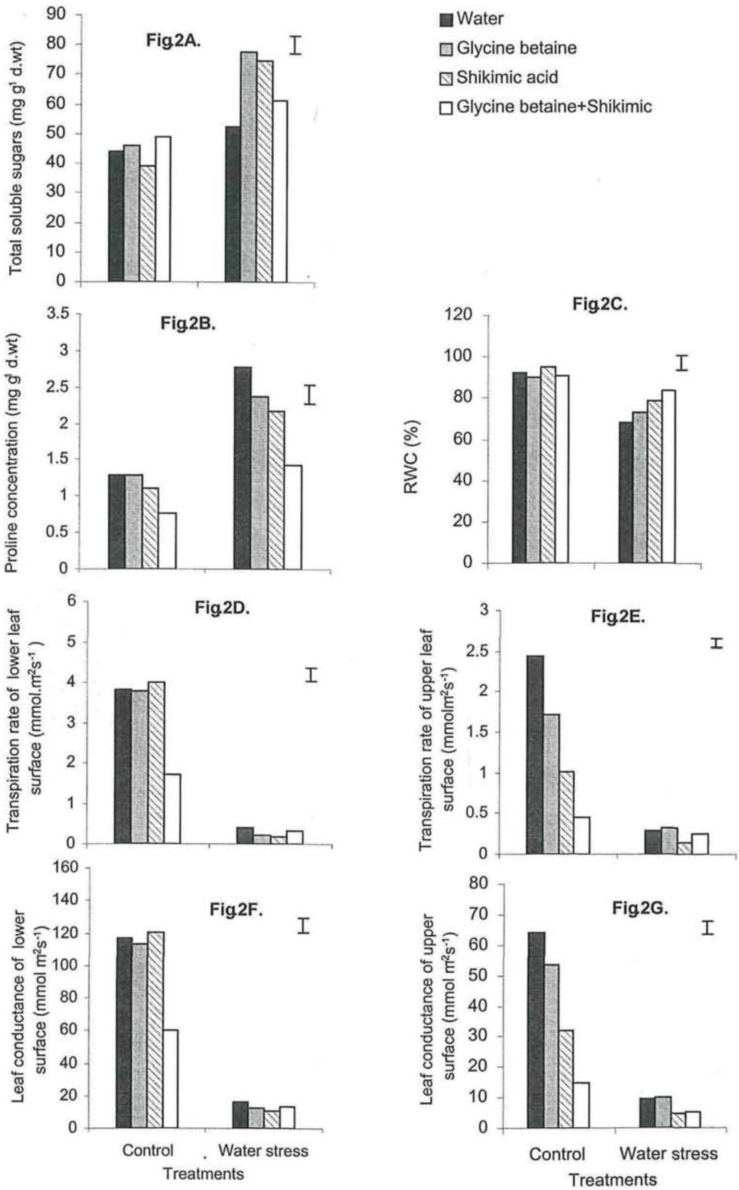


Fig. 2. Interactive effects of glycine betaine and shikimic acid on total soluble sugars, proline and water relation of *Sorghum bicolor* plants grown under water deficit conditions. The vertical bars represent LSD values at P = 0.05.

would be advantageous for osmotic adjustment (PREMACHANDRA & al. 1995) and may increase the internal resistance to water flow, thereby minimizing water loss (SHIELDS 1958). ALDESUQUY & IBRAHIM 2000 suggested that, shikimic acid stimulates the degradation of polysaccharide in cowpea plants and this may explain the observed increase in total soluble sugars in stressed *Sorghum* leaves.

Proline accumulated in *Sorghum* leaves in response to water stress. Several possible roles have been attributed to supraoptimal levels of proline: osmoregulation under drought and salinity conditions (WYN JONES & STOREY 1978, stabilization of proteins (SCHOBERT & TSCHESCHE 1978), conservation of nitrogen and energy for the post-stress period (BARNETT & NAYLOR 1966) and detoxification of free radicals by forming long lived adducts with them (FLOYD & ZS-NAGY 1984).

Interestingly, the application of chemicals reduced proline concentration in *Sorghum* plants under stress conditions as compared with non-chemical treated plants. This is compatible with the finding of LARTHER & al. 1996 in rape leaf discs. They observed that proline accumulation was lower when the stress media contained glycine betaine. They suggested that Glycine betaine might exert some effect at the membrane level since it was found that triethylammonium chloride mimicked the Glycine betaine inhibitory role on the osmoinduced proline response.

Our findings indicate that water deficit greatly reduced RWC, transpiration rate and total leaf conductance on both leaf sides. Upper leaf side appeared to be more affected than the lower leaf side. These results are expected and consistent with that of many investigators (RITCHIE & al. 1990, CHETTI & al. 1996, WOOD & GOLDSBROUGH 1997).

The provided chemicals in many cases decreased transpiration rate and total leaf conductance of stressed plants. In this connection AGBOMA & al. 1997 found that exogenous application of glycine betaine to soybean reduced transpiration rate, under all conditions, to 85% of untreated plants. Maintenance of high water content could have delayed the destruction of chlorophylls and other leaf compounds until plants were exposed to greater level of stress (GUMMULURU & al. 1989). Reduction of transpiration rate during development of water stress is mainly due to stomatal closure and may be an adaptive criterion which helps the plants to achieve suitable water balance under drought conditions (ARNON & GUPTA 1995). Furthermore, the decrease in transpiration rate of *Sorghum* plants in response to shikimic acid may result from the effect of shikimic acid on the biosynthesis of phenolic compounds that may decrease the number of both stomatal and epidermal cells per $cm^2$ and therefore decreases the rate of water vapor loss through stomata and finally resulted in an obvious decrease in total leaf conductance. In this connection, ARTECA 1996 reported that shikimic acid play an important role in the biosynthesis of salicylic acid via cinnamic acid.

Yield is a result of the integration of metabolic reactions in the plants, consequently any factor that influences this metabolic activity at any period of plant growth can affect the yield (IBRAHIM 1999). Our data show that all yield and yield components were reduced by drought treatments. The strong negative correlation between grain yield and proline concentration indicate that proline concentration may act as a symptom of stress rather than indication of tolerance and this is compatible with reports by IBARRA-CABALLERO & al. 1988 and IBRAHIM 1999.

The applied chemicals appeared to mitigate the effect of water stress on *Sorghum* yield and the effect was more pronounced with (glycine betaine + shikimic acid) treatment. This improvement would result from the beneficial effect of the provided chemicals on growth and metabolism of *Sorghum* plants under water deficit conditions.

It is clear from this investigation that the effect of water stress on *Sorghum* had a negative effect on growth and productivity. On the other hand, the exogenous application of glycine betaine and shikimic acid improves the growth parameters of *Sorghum* plants by increasing the turgidity, stimulating leaf expansion and enhancing the production of photosynthetic pigments. Therefore, the applied chemicals increase the yield capacity of *Sorghum* plants.

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