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## The Role of Shikimic Acid in Regulation of Growth, Transpiration, Pigmentation, Photosynthetic Activity and Productivity of *Vigna sinensis* Plants

By

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With 4 figures

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### Summary

ALDESUQUY H. S. & IBRAHIM A. H. A. 2000. The role of shikimic acid in regulation of growth, transpiration, pigmentation, photosynthetic activity and productivity of *Vigna sinensis* plants. – *Phyton* (Horn, Austria) 40 (2): 277-292, with 4 figures. – English with German summary.

The effect of shikimic acid on growth parameters, total leaf conductivity, transpiration, photosynthetic pigments,  $^{14}\text{C}$  assimilation and productivity of *Vigna sinensis* (*Fabaceae* – *Phaseoleae*) plants was studied. Shikimic acid application led to an increase in fresh and dry weights of cowpea plants and enhances leaf expansion as well as the root length and plant height.

Seed pretreatment with shikimic acid at various doses induces a marked increase in total leaf conductivity and transpiration rate at different stages of growth.

Shikimic acid at all concentrations was found to stimulate the production of Chl.a, Chl.b, Carotenoids and  $^{14}\text{C}$  fixation during leaf growth and development. Furthermore, shikimic acid at various doses applied improved yield and yield components of cowpea plants by increasing the number of pods/plant, length of pod, number of seeds/pod, seed biomass and 100-seed weight. The protein content of yielded seeds was increased in response to shikimic acid treatments. On the other hand, shikimic acid at all concentrations significantly decreased the polysaccharide content of developed cowpea seeds. In the majority of cases, the absolute values of

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total soluble sugars and sucrose in cowpea seeds appeared to be non-significantly affected in response to seed priming with shikimic acid.

### Zusammenfassung

ALDESUQUY H. S. & IBRAHIM A. H. A. 2000. Die Rolle der Shikimisäure bei der Wachstumsregulation, Transpiration, Pigmentation, Photosyntheseaktivität und Produktivität von *Vigna sinensis*-Pflanzen. – *Phyton* (Horn, Austria) 40 (2): 277–292, 4 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Wirkung der Shikimisäure auf Wachstumsparameter, Blattleitfähigkeit, Transpiration, Photosynthesepigmente,  $^{14}\text{C}$ -Assimilation sowie Produktivität wurde bei *Vigna sinensis* (*Fabaceae* – *Phaseoleae*) untersucht. Zugabe von Shikimisäure führte zu einem Anstieg in Frisch- und Trockengewicht bei *Vigna*-Pflanzen und vergrößerte sowohl die Blattfläche als auch die Wurzellänge und Pflanzengröße.

Vorbehandlung von Samen mit verschiedenen Mengen an Shikimisäure induzierte einen deutlichen Anstieg in der Blattleitfähigkeit und Transpirationsrate während verschiedener Wachstumsstadien.

Es zeigte sich, dass alle Konzentrationen an Shikimisäure die Produktion von Chl.a, Chl.b, Karotinoide und die  $^{14}\text{C}$ -Fixierung während Blattwachstum und -entwicklung stimulierte. Darüberhinaus verbesserten die verschiedenen Shikimisäuremengen den Gesamtertrag sowie den der Einzelkomponenten, indem die Zahl an Hülsen/Pflanze, die Länge der Hülsen, die Anzahl der Samen/Hülse, die Samenbiomasse und das 100-Korngewicht erhöht wurde. Der Proteingehalt der geernteten Samen stieg in Abhängigkeit von der Behandlung mit Shikimisäure an. Andererseits verminderten alle Konzentrationen von Shikimisäure signifikant den Polysaccharidgehalt in voll entwickelten *Vigna*-Samen. In der Mehrzahl der Fälle schienen die Absolutwerte der gesamtlöslichen Zucker, sowie Rohrzucker in den Samen von *Vigna* als Reaktion auf die Samenvorbehandlung mit Shikimisäure nicht signifikant beeinflusst worden zu sein.

### Introduction

Shikimic acid is the known precursor of aromatic amino acids, L-phenylalanine, and L-tyrosine. These compounds are phenylpropane ( $\text{C}_6\text{-C}_3$ ) derivatives as are the building units of lignin, and not surprisingly, they were soon shown to be lignin precursors (HUMPHREY & al. 1999). Phenylalanine is an excellent precursor in all plants but tyrosine is only really effective in the grasses (STAFFORD 1974). Based on the observation of the direct incorporation of uniformly labeled C-13 glucose, and that glucose is converted via glycolysis and the shikimic acid pathway to tryptophan (YANG & CORDELL 1996). The shikimic pathway, a collection of seven enzymatic reactions whose end product is chorismate, has been studied for many years in a variety of microorganisms and plants. In microbial system, the end product of the pathway is used primarily for the synthesis of aromatic amino acids. In plants, chorismate is the precursor not only for the synthesis of aromatic amino acids ( i.e phenylalanine, tyrosine and trypto-

phan), but also for many secondary metabolites with diverse physiological roles (WEAVER & HERRMANN 1997).

Two derivatives of shikimic acid were isolated from croziers of the dwarf tree fern, *Dicksonia antarctica*, and their structures were elucidated as 4-o-caffeoylshikimic acid and 4-o-(p-coumaroyl) shikimic acid on the basis of mass spectrometric and NMR spectroscopic evidence (SAITO & al. 1997). The shikimic acid pathway participates in the biosynthesis of plant phenolics (LOGEMANN 1995), where the most abundant classes of phenolic compounds in plants are derived from phenylalanine via elimination of an ammonia molecule to form cinnamic acid (HAHLBROCK & SHEEL 1989). Simple phenolic compounds are widespread in vascular plants and appear to function in different capacities (SANDBERG & BENENBAUM 1989).

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 by-product. In some cases, phenolics treatment induces expression of the same genes and resistance against the same spectrum of pathogen as pathogen induced resistance (LAWTON & al. 1996). The resistance induced by chemical treatment can be very effective and may provide commercially useful broad spectrum-plant protection that is stable, long-lasting and environmental benign (GORLACH & al. 1996). Furthermore, NEERA & GARG 1989 found that application of caffeic acid (diphenol) at  $10^{-4}$  M and especially chlorogenic acid (polyphenol) to 40–50d old *Cicer arietinum* plants increased the number and weight of nodules/plant, N fixation, seed yield/plant, 100-seed weight and harvest index, compared with water-treated plant (control). Foliar application of 50,100 or 200 mg.m<sup>-2</sup> coumarin to *Vicia faba* increased plant height, number of branches, FW, DW, number of nodules, number of pods/plant and seeds/pod, and weight of seeds (GUPTA 1990).

In the light of the above limited reviews, it was thought of particular interest to study the effect of seed priming with shikimic acid on growth parameters, total leaf conductivity, photosynthetic pigments, <sup>14</sup>C assimilation and productivity of one important crop plant (i.e. *Vigna sinensis*).

Abbreviation: Chl a, chlorophyll a; Chl b, chlorophyll b; DW, dry weight; FW, fresh weight; IAA, indole acetic acid.

## Materials and Methods

### Plant material and growth conditions

A similar lot of *Vigna sinensis* (var. cream 7) seeds were surface sterilized with 0.001M HgCl<sub>2</sub> solution for three minutes and then washed thoroughly with distilled water. The sterilized seeds were divided into four sets. Seeds of the 1st set were soaked in distilled water to serve as control, the other three sets 2nd, 3rd, 4th were soaked in shikimic acid at 25,50,75 mg.m<sup>-3</sup> respectively for about three hours. The soaked seeds washed thoroughly with distilled water and then sowed on 19.6.1999, in earthenware pots (30cm in diameter) filled by 3kg loamy sandy soil (sand: clay 2/1v/v).

The pots were kept in greenhouse and the plants were subjected to natural day/night conditions (minimum/maximum temperature and relative humidity were: 29.2/33.2 and 35/45%, respectively at mid-day during the experimental period) and irrigated with normal tap water (free from magnesium or calcium carbonate). After two weeks only five uniform seedlings were left in each pot. One week from thinning, the plants received  $35\text{g N m}^{-2}$  as potassium nitrate and  $35\text{g P m}^{-2}$  as superphosphate.

Measurements started after the fully expansion of the 1st compound leaf (i.e 28 days from sowing) and finished with two measurements, one at flowering stage (i.e 45 days from sowing) and the other at fruiting (i.e 60 days from sowing). Data were obtained and the mean values per plant were computed for the following parameters: root length (the length of primary root in branching root system measured to the nearest cm.); plant height (cm.); total (above and below ground) fresh weight (g/plant); total dry weight (g/plant) and leaf area of the 1st compound leaf was measured by weighing the image of leaf (i.e that was of constant weight) and comparing that mass with the mass of a known area. At the harvest, ten plants from each treatment were taken and the mean per plant was calculated for the following parameters: pod length (cm.); number of pods/plant; number of seeds/pod; fresh and dry weight of seeds (g/seed) and 100- seed weight (g).

#### Estimation of photosynthetic pigments

Chl.a, Chl.b and carotenoids were measured by spectrophotometric method as recommended by METZNER & al. 1965.

#### Measurements of total leaf conductance and transpiration rate

Total leaf conductance and transpiration rate of the 1st compound leaf of cowpea plants were measured using a Li- 1600M steady state Prometer. In order to get reliable data, the calibration of the apparatus should be carried out by adjusting the atmospheric pressure (PRES SET) and aperture area of the apparatus to 101.3 kPa and  $1\text{ cm}^2$  respectively.

#### Analysis of $^{14}\text{C}$ assimilation ( $^{14}\text{C}$ light fixation)

As described by GABER 1985 a definite fresh mass of 1st compound leaf discs was introduced into the fixation apparatus. An aqueous solution of  $^{14}\text{C}$ -sodium carbonate of known activity ( $3.7\text{MBQ cm}^{-3}$ ) was pipetted into the apparatus followed by 0.2 ml of  $\text{H}_2\text{SO}_4$  (10%). The evolved  $^{14}\text{CO}_2$  passed through the pores in the upper part of the inner container to the main apparatus where it could be photosynthesized by the green leaf discs and the radioactivity of the green leaf discs was measured using a Packard Scintillation Counter model 526. The count per minute (cpm) obtained were then calculated according to the efficiency of the apparatus used. The radioactivity measured is directly proportional to the amount of  $\text{CO}_2$  fixed in soluble organic compounds, which was calculated as cpm/mg fresh mass of leaf.

#### Extraction and determination of total soluble sugars, sucrose and polysaccharides

Sugars were extracted by the method of RIAZI & al. 1985. A known dry weight (0.03 g) was submerged in 10 ml 80% ethanol overnight with periodic shaking, then filtered through Whatman No. 1 filter paper, and the filtrate was made up to known volume with 80% ethanol. The total soluble sugars and sucrose content of developing seeds were determined by anthrone method as described by RIAZI & al. 1985 and recommended by BUYSSE & MERCKX 1993.

According to NAGUIB 1963 a known weight of the dried plant residue which remained after extraction of soluble sugars, was heated under reflux in 1.5 N  $H_2SO_4$  for 4 hours at 100 °C. The solution was neutralized, cleared with basic lead acetate (137g/L) and delead with  $Na_2HPO_4$  (M/3). The solution was made up to known volume. Polysaccharides content were estimated according to the procedure adopted by YOUNIS & al. 1969.

#### Estimation of protein

Protein content of cowpea seeds was extracted by reacting a known dry weight of seeds (~0.1g) with 5ml of 1N NaOH for 24 hrs. At the end of that time, the extract was filtered and raised to a known volume. Protein content was determined using the method of LOWRY & al. 1951.

#### Statistical analysis

The results were first subjected to the analysis of variance (Anova). When Anova showed a significant ( $P < 0.05$ ) effect, the least significant differences were used to compare treatments (SNEDECOR & COCHRAN 1976).

## Results and Discussion

### Changes in growth parameters, total leaf conductivity and transpiration rate

The available results for the effect of shikimic acid on growth parameters are shown in Fig. 1.a, 1.b, 1.c, 1.d and 1.e. In comparing with control plants, seed pretreatment with various concentrations of shikimic acid led to a marked increase ( $P < 0.05$ ) in root length, plant height, leaf area, fresh and dry weight of cowpea plants after 28, 45 and 60 days from planting. The previous pattern of results revealed that shikimic acid stimulates the growth parameters of cowpea plants.

The importance of the internal water balance in plant water relations is generally accepted because of the close relationship between this balance and turgidity, to the rates of physiological processes that control the quality and quantity of growth (ALDESUQUY 1988). Thus, the data presented in Fig. 2.a and 2.b showed that total leaf conductivity and transpiration rate in cowpea plants was 86.80 and 2.43 respectively after 28 days, followed by rapid increase up to 45 days, thereafter a massive decrease was noticed on the day 60. As compared to control plants, shikimic acid pretreatment at 25, 50, and 75  $mg \cdot m^{-3}$  induced the same pattern of changes in total leaf conductivity and transpiration rate, but the detected values of these parameters appeared generally to increase ( $P < 0.05$ ) drastically during the overall growth periods.

Occurrently the increase in leaf area production caused by shikimic acid application could be resulted from the rapid rate of movement of nutrients and hormones transported through transpiration stream from root, which can accelerate the rate of leaf expansion in the developing leaves. Shikimic acid led to a marked increase in root length of cowpea

plants during the overall growth periods, therefore may increase the rate of water uptake from the soil and this effect may explain the significant increase in fresh weight of cowpea plants. Shikimic acid as being precursor of phenolic compounds has been shown to be of great importance in the regulation of growth and they are longer considered to be passive by-products (JAIN & SRIVASTAVA 1981). It was observed that the fall in shikimate dehydrogenase activity in cotyledons of *Capsicum annuum* coincides with a decrease in the concentrations of phenolics during primary leaf development (DIAZ & al. 1997). In connection with these results, it was reported that a low concentration of salicylic acid increases the growth of maize seedlings, while higher concentration inhibits it (JAIN & SRIVASTAVA 1981). Furthermore, ABO-HAMED & al. 1987 found that soil drench with salicylate led to an increase in fresh and dry weight of shoot and appeared at lower concentration  $800 \text{ mg.m}^{-3}$  to enhance plant height and leaf area of wheat plants.

The increase in transpiration rate of cowpea plants in response to shikimic acid application may result from the fact that shikimic acid increases the biosynthesis of phenolic compounds particularly coumarin (SAITO & al. 1997) which increases the number of both stomatal and epidermal cells per  $\text{mm}^2$  and therefore increases the rate of water vapor loss through stomata and finally resulted in an obvious increase in total leaf conductance in cowpea plants (GUPTA 1992). Moreover, GUPTA 1990 found that foliar application of 50, 100, or  $200 \text{ mg.m}^{-3}$  coumarin to *V. faba* increased plant height, number of branches, FW and DW. On the other hand, TAN & al. 1992 suggested that ferulic or P-coumaric acid increases the content of cell wall of rice seedlings by bound phenolic compounds which in turn decreases cell wall extensibility, resulting in inhibited cell growth.

Plant yield is a function of many factors among which the pigment content of the developing leaves is the most important (ALI 1999). Thus, the presented data in Fig. 3.a, 3.b, 3.c, 3.d and 3.e showed that, in control plants, Chl.a, Chl.b, Chl.a+b and carotenoids increased up to 45 days from planting, whereupon a noticeable decrease was manifested in these parameters after 60 days as the leaves undergone senescence. These results were in good agreement with those obtained by several workers in different plant species, i.e. in potato leaves (MORKRONOSOV & BAGAUTDINOVA 1974), in *Theobroma cacao* (BAKER & al. 1975). Grain priming with shikimic acid at different concentrations induced the same pattern of changes in photosynthetic pigments, as that occurred in control plants. On the other hand, shikimic acid enhances the production of photosynthetic pigments particularly Chl.a and Chl.b to much higher level if compared with those detected in control leaves. Moreover, the maximum production of pigments content in response to shikimic acid occurred after 45 days from planting where the treated plants produced high number of flowers. On the day 60, where leaves of control plants undergone senescence, shikimic acid may

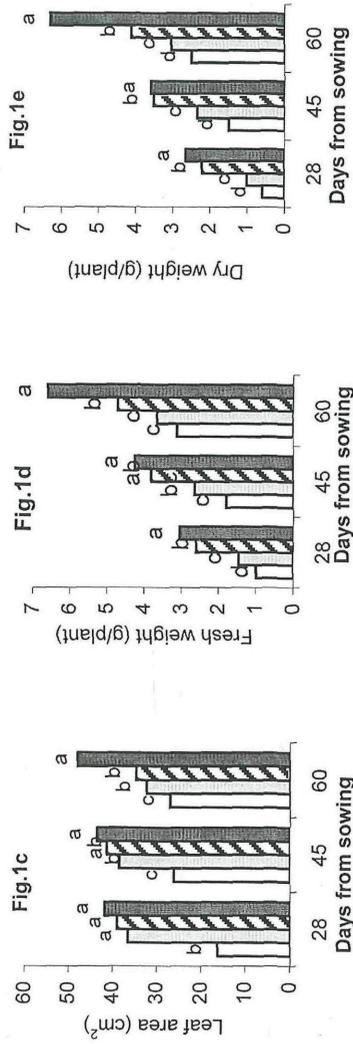
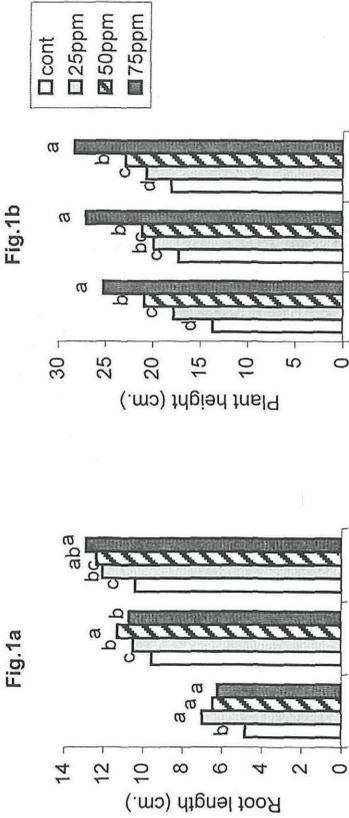


Fig. 1. Effect of seed presoaking in shikimic acid on root length (Fig. 1a), plant height (Fig. 1b), leaf area (Fig. 1c), plant fresh weight (Fig. 1d) and plant dry weight (Fig. 1e) of cowpea plants at different stages of growth and development. Bars in grouping labeled with the same letter are not significant as indicated by LSD ( $P < 0.05$ ).

delay the senescence of cowpea leaves by retaining the chlorophylls. The stimulative effect exerted by shikimic acid on pigments biosynthesis might presumably due to the fact that shikimic acid increase the rate of transpiration and this will possibly increase the rate of translocation of minerals and cytokinin from root to the developing shoot. Thus, RICHMOND & LANG 1975 have shown that kinetin prevented chlorophyll loss in detached *Xanthium* leaves. Moreover, UHEDA & KURAIISHI 1978 found that kinetin increased both transpiration and chlorophyll synthesis. In connection with these results, KHURANA & MASHESHWARI 1980 found that in *Spirodela polyrrhiza* Sp<sub>20</sub> lower concentrations of salicylic acid ( $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$  and  $5 \times 10^{-5}$  M) stimulate the total chlorophyll synthesis, however the higher one  $10^{-4}$  M has a reversed effect.

#### Changes in <sup>14</sup>C assimilation

As can be seen from Fig. 4.a, 4.b, 4.c and 4.d, there is a gradual increase in soluble, insoluble and total photosynthates in control cowpea plants at all growth stages. The maximum accumulation of photosynthates occurred after 60 day from planting, whereas the setting seeds in developing pods begin its maturation.

Shikimic acid at 25, 50, 75 mg.m<sup>-3</sup> induced drastic increases in soluble, insoluble and total photosynthates. This pattern of changes appears similar to that occurred in control plants, but the absolute amount of photosynthates detected in treated plants were more higher if compared to control ones. The level of photosynthates appears to depend on the concentration of shikimic acid applied. Furthermore, shikimic acid treatments altered the ratios of soluble to insoluble photosynthates during the growth and development of cowpea plants. This promotive effect induced by shikimic acid may probably be due to its stimulative effect on leaf expansion and photosynthetic pigments as well as on the transpiration rate of cowpea plants during allover growth periods. On the other hand, shikimic acid may exert its effect on photosynthetic machinery at the mesophyll and chloroplast level by increasing plastid biogenesis through its action on increasing the biosynthesis of indole acetic acid (IAA) from tryptophan (YANG & CORDELL 1996). In connection to these results, there are many reports on IAA stimulation of chloroplast development as well as the whole photosynthetic machinery (WILD & al. 1981, SAKR 1985, KADIOGLU 1993, ALDESUQUY & GABER 1993).

#### Changes in yield components

It is clear from Table 1 that the application of shikimic acid at various concentrations greatly affected the different yield components of cowpea plants. Thus seed priming with shikimic at all doses increased significantly ( $P < 0.05$ ) number of pods/plant, pod length, seeds number/pod, seed fresh and dry weight as well as 100- seed weight. The magnitude of increase

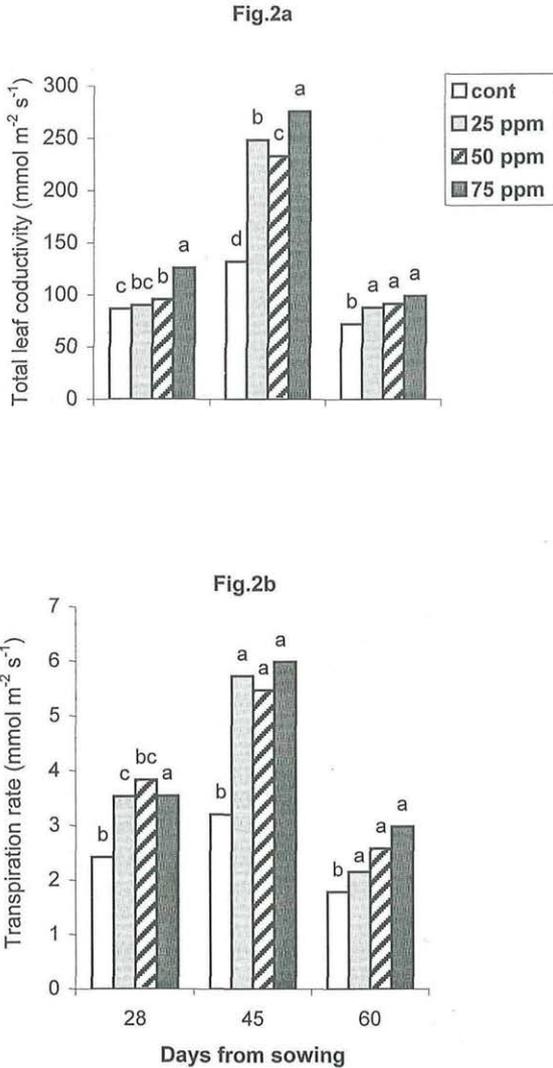


Fig. 2. Effect of seed presoaking in shikimic acid on total leaf conductivity (Fig. 2a) and transpiration rate (Fig. 2b) of cowpea plants at different stages of growth and development. Bars in grouping labeled with the same letter are not significant as indicated by LSD ( $P < 0.05$ ).

appears to depend mainly on the concentration used, whereas the concentration increases there is simultaneous increase in the above parameters. The improving in yield capacity of cowpea plants in response to

shikimic acid application might be mediated through increased longevity of leaves by retaining chlorophylls which perhaps contributed to seed filling by enhancing the duration of photosynthates supply to developing seeds. In connection to these results GURBAKSH & SHARMA 1982 found that salicylic acid induced a profound increase in yield of groundnuts (dry pod yield and 100- seed weight). Application of caffeic or chlorogenic acid at  $10^{-4}$  M to *C. arietinum* increased seed yield, 100- seed weight and harvest index compared with water treated plants (control), but the application of vanillic acid and salicylic acid decreased the values of the above indices (NEERA & GARG 1989). Moreover, GUPTA 1990 found that foliar application of coumarin at 50,100,500  $\text{mg.m}^{-3}$  to *V. faba* plants increased number of pods/plants and seeds/pod and weight of seeds particularly at the lower doses while at 50  $\text{mg.m}^{-3}$  decreased pods number/plant, pod length, seed number/pod and seed fresh and dry weight.

#### Changes in some biochemical aspects

Seed priming with shikimic acid at 25  $\text{mg.m}^{-3}$  induced massive ( $P < 0.05$ ) or slight increases respectively in total soluble sugars and sucrose in developing seeds of cowpea plants (Table 1). Shikimic acid as a precursor of many phenolic compounds may be mediated its effect via phytohormones (ALDESUQUY & al. 1998). Many phenolic compounds are known to provide protection to auxins against oxidation (SCHNEIDER & WHITMAN 1974). The increased levels of auxins may result in an increase in invertase enzyme as it has been demonstrated by GLASZIOU & al. 1966. This fact could explain the observed increase in total soluble sugars content in developing seeds. Sucrose might be the main pool of sugars and it is the transportable form which should be exported to the developing seeds and part of which will be hydrolysed by the induced invertase (ALDESUQUY & al. 1998). From another point of view, shikimic acid at all concentrations applied stimulate the degradation of polysaccharide and consequently decreased it significantly ( $P < 0.05$ ) within the developing seeds as it is clear in table 1. Finally, the effect of shikimic acid upon the polysaccharide level may explain the observed increase in total soluble sugars and sucrose in cowpea seed particularly at 25  $\text{mg.m}^{-2}$  shikimic acid.

It is clear from this investigation that seed priming with shikimic acid improves the growth parameters of cowpea plants by increasing the turgidity, stimulating leaves expansion, enhancing the production of photosynthetic pigments as well as the massive increase in photosynthetic activity. Furthermore, shikimic acid increases the yield capacity of cowpea plants by inducing a massive increase in the pod length, numbers of pod/plant, numbers of seeds/pod and seed biomass as well as increases the protein, total soluble sugars, sucrose contents and decreasing the polysaccharides level. In future, this study will be extended to include further investigations on the effect of shikimic acid on some different metabolic

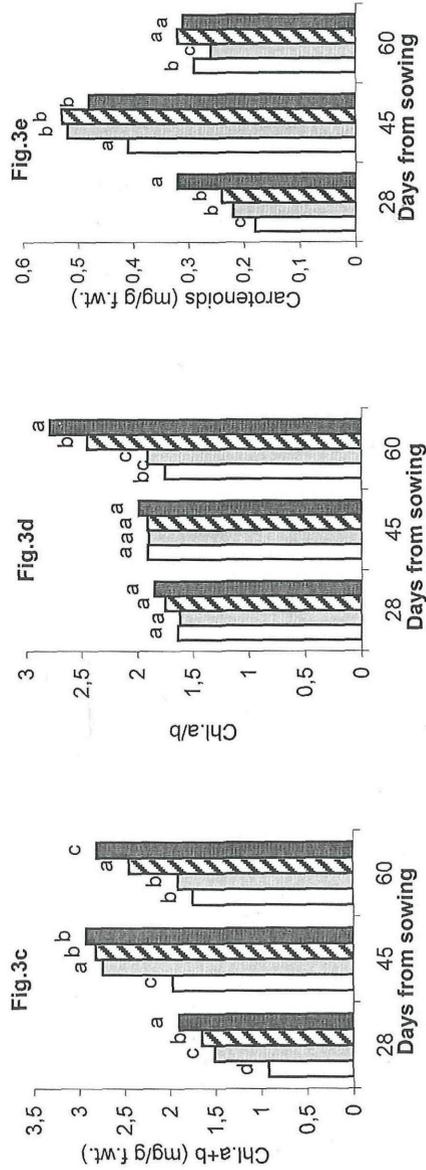
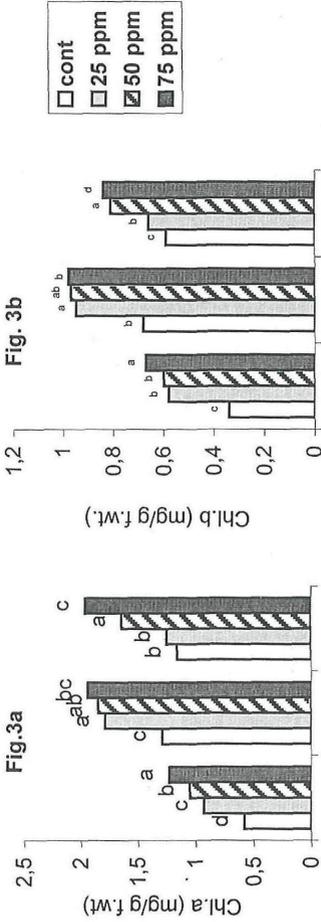


Fig. 3. Effect of seed presoaking in shikimic acid on chlorophyll a (Fig. 3a), chlorophyll b (Fig. 3b), chlorophyll a+b (Fig. 3c), chl.a/b (Fig. 3d) and carotenoids (Fig. 3e) of cowpea plants at different stages of growth and development. Bars in grouping labeled with the same letter are not significant as indicated by LSD ( $P < 0.05$ ).

pathways, different enzymes, endogenous hormonal levels, ultrastructure of chloroplast of C<sub>3</sub> & C<sub>4</sub> plants as well as the resistance against the microbial infections through lignification of cell wall in susceptible plants.

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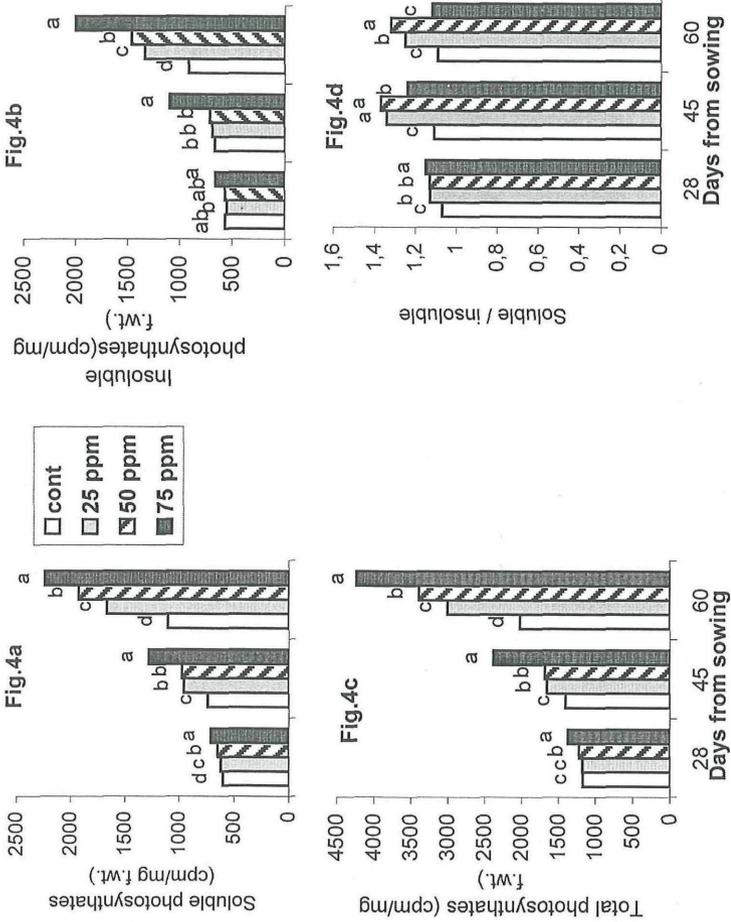


Fig. 4. Effect of seed presoaking in shikimic acid on soluble photosynthates (Fig. 4a), insoluble photosynthates (Fig. 4b), total photosynthates (Fig. 4c) and soluble/insoluble (Fig. 4d) of cowpea plants at different stages of growth and development. Bars in grouping labeled with the same letter are not significant as indicated by LSD ( $P < 0.05$ ).

Table 1.  
Effect of seed presoaking in shikimic acid on yield components and some biochemical aspects of yielded seeds of cowpea plants. Values of treatments with the same letter in each row are not significantly different at  $P < 0.05$ .

Presoaking treatments (mg g <sup>-3</sup> )	Number of pods/plant	Pod length (cm.)	Number of seeds/pod	100-seed weight (g)	Seed biomass		Protein (mg/g d.wt)	Total soluble sugars	Carbohydrates (mg/g d.wt.)	
					Seed FW*	Seed DW**			Suc.	Polys.
Control	1.66 <sup>b</sup>	8.06 <sup>c</sup>	3.00 <sup>b</sup>	45.9 <sup>b</sup>	0.5 <sup>c</sup>	0.4 <sup>b</sup>	394.69 <sup>c</sup>	39.97 <sup>b</sup>	22.26 <sup>a</sup>	77.31 <sup>a</sup>
25	2.66 <sup>b</sup>	9.50 <sup>bc</sup>	5.00 <sup>b</sup>	52.9 <sup>ab</sup>	0.6 <sup>b</sup>	0.5 <sup>a</sup>	408.03 <sup>c</sup>	47.33 <sup>a</sup>	27.54 <sup>a</sup>	43.81 <sup>b</sup>
Shikimic acid	50	11.46 <sup>ab</sup>	6.66 <sup>ab</sup>	56.8 <sup>a</sup>	0.6 <sup>ab</sup>	0.5 <sup>a</sup>	452.70 <sup>a</sup>	40.47 <sup>b</sup>	24.48 <sup>a</sup>	18.01 <sup>c</sup>
75	5.66 <sup>a</sup>	13.52 <sup>a</sup>	10.06 <sup>a</sup>	61.2 <sup>a</sup>	0.6 <sup>ab</sup>	0.5 <sup>a</sup>	427.94 <sup>b</sup>	42.89 <sup>b</sup>	23.66 <sup>a</sup>	11.06 <sup>c</sup>
LSD at 5%	1.99	2.15	4.61	8.66	0.06	0.05	16.29	3.53	5.95	12.13

FW\* = Fresh weight.

DW\*\* = Dry weight.

Suc. = Sucrose

Polys. = Polysaccharides

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