

Original Research Article

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## Ameliorative Effect of Salicylic Acid on Yield, Biochemical and Antioxidant Parameters of Chickpea (*Cicer arietinum* L.) under Drought Stress

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### ABSTRACT

Drought stress is one of the major physiological stresses especially in arid and semiarid regions that severely affected crop production mainly leguminous crops. A pot experiment was carried out to assess growth, yield, biochemical and antioxidant contributing characters under semi-controlled environmental condition in the Department of Biological sciences, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, India. During November 2016 through March 2017 aiming to alleviate the drought stress effects on chickpea using salicylic acid (SA). Chickpea varieties C1021 and C1022 were used in the experiment. The experiment was conducted using Completely Randomized Design (CRD) in three replications with five levels of drought stress and Salicylic acid concentrations (T0: control, T1: 0.5 mM SA + 25, 50, 60 DAS of irrigation, T2: 1.0mM SA + 25, 65 DAS of irrigation, T3: 1.5mM SA+ 25, 50 DAS irrigation, T4: 2.0mM SA+25, 50 DAS of irrigation), even 0.3% potassium iodide at anthers formation. Different concentration of SA was applied as foliar spray and spraying was done on 30, 45 and 60 DAS. The data for chlorophyll content in leaf and relative water content (RWC), harvest index, antioxidant like proline, APX, SOD, H<sub>2</sub>O<sub>2</sub>, POD, MDA were observed. Yield and yield contributing characters were measured at maturity. Growth parameter was observed at different intervals of 30, 60, 90DAS. The results showed that drought decreased total dry weight, chlorophyll content, relative water content, plant height, number of branches, number of pods/ plant, pod length, 100-seed weight, number of seed/ pod and yield of chickpea. Foliar application of SA was highly significant (T3), then (T2) and then control (T0) of all two varieties best result observed in C1021 and C1022. At different doses under different drought stress conditions had the positive effects related to mitigation of drought stress effect but concentration at i.e., SA @ 1.5mM, respectively were found to alleviate the adverse effects significantly on the above parameters at drought condition.

#### Keywords

DAS, Salicylic acid, Ameliorates adverse effects, Chickpea, Flowering, Yield.

#### Article Info

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### Introduction

Chickpea (*Cicer arietinum* L.) is an important legume crop in the semi-arid tropics of the world. India is the world leader in chickpea production. In India, chickpea is cultivated on 8.68 mha with annual production of 8 mt and an average yield of 844 kgha-1.17 However,

it is under heavy stresses due to various abiotic factors that results in yield losses. The present study was performed to investigate the response of chickpea plants to exogenous application of SA and to examine the effect of SA on drought stress the activities of

antioxidant enzymes (POD), hydrogen peroxide and total proteins in this crop, since these are the most studied components implicated in plant defence against abiotic stresses.

Drought stress is a major abiotic stress limiting agricultural crop production worldwide. Plants respond to drought stress and acclimatize through various physiological and biochemical changes (Farooq *et al.*, 2009). Drought induces oxidative stress in plants, in which reactive oxygen species (ROS), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant metabolism (Hasegawa *et al.*, 2000). These mechanisms include β-carotenes, and enzymes including superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX) (Prochazkova *et al.*, 2001). Plants experience drought stress either when the water supply to roots is interrupted or when transpiration rate becomes very high. These two conditions often coincide under arid and semiarid climates. Salicylic acid (SA) is a naturally existing phenolic compound. Evidences put forward that externally applied SA increased plant's tolerance to several abiotic stresses, including drought (Azooz and Youssef, 2010). Exogenous SA reduced transpiration and increased nitrate reductase activity, flower longevity as well as the yield of some plants (Raskin, 1992), which overall suggest that SA may enhance the multiple types of stress tolerance in plants through which interactive effects on several functional molecules or other signaling molecules participating in more complex stress responses. Drought stress is a major concern affecting the agriculture production. Generally, pulses are very susceptible to drought stress. Chickpea (*Cicer arietinum* L.) is the third most important grain legume crop in the world experiences water stress during

pod set and seed filling stage (terminal drought) in India and the Mediterranean basin, leading to a substantial yield loss (Turner *et al.*, 2001). Drought stress during seed filling has been reported to be highly detrimental to yield in chickpea (Davis *et al.*, 1999). Comparatively meagre work is on record regarding role of SA on drought tolerance in chickpea. SA appears to have innate potentiality for increasing antioxidants and influencing antioxidant enzyme activity in plants subjected to oxidative stress (Hayat *et al.*, 2008). Therefore, the present investigation was conducted to evaluate the drought stress ameliorating ability of SA with special emphasis on growth and antioxidant system in drought stressed chickpea plants.

## **Materials and Methods**

A pot experiment was carried out during *rabi* season of 2016-2017 at Department of Biological Sciences, SHUATS, Allahabad (U.P.). The experiment was laid out in CRD design with five treatments with three replications for present investigation and out of 2 varieties of chickpea, 3 plants will be tagged randomly for the purpose of morphological, physiological/biochemical studies. Treatments will be denoted as T0, T1, T2, T3 and T4 replications as R1, R2 and R3 for variety C1021 and for variety C1022 treatment will be denoted as F0, F1, F2, F3, and F4 replication as R1, R2 and R3.

Spraying of potassium iodide @ 0.3g at anther formation in chickpea

## **Estimation of biochemical parameter**

### **Estimation of total chlorophyll content**

Chlorophyll was determined according to Wellborn (1983). One gram leaves sample was weighed and crushed with 80% acetone made the volume to 10 ml with 80% acetone,

centrifuged at 800 ppm for 5 minute. The supernatant was read under 663, 645 nanometre. The readings were fed in the following formula and results were determined under spectrophotometer.

### **Carotenoid content**

Carotenoid was determined according to Wellborn. (1983). 0.5 gm and homogenized in 10 ml of acetone (80% acetone), next to the centrifuged at 3000 rpm at 10 min. The absorbance was recorded at 470 nm.

### **Determination of total protein was estimated from seed by Lowry's method (Lowry *et al.*, 1951)**

#### **Reagents preparation**

##### **Reagent A**

Prepared by dissolving 2% Sodium carbonate in 0.1 N Sodium hydroxide.

##### **Reagent B**

Prepared by dissolving equal amount of 1% copper sulphate and 2.0% of potassium sodium tartarate.

##### **Reagent C**

Prepared by mixing 50 ml reagent A and 1ml reagent B and it was freshly prepared before use.

##### **Reagent D**

Folin – Ciocalteu reagent available commercially was used after 1:1, v/v dilution with distilled water.

### **Estimation of protein standard**

Estimation of protein was done by pipetting out 50 µl supernatant containing proteins into

test tubes in replicates of three and the total volume was made up to 1 ml. A tube with 1 ml distilled water served as a blank. 3 ml reagent C was added to each tube including the blank and after proper mixing the solution were allowed to stand for 30 min then 0.5 ml reagent D was added and after mixing, the tubes were left at room temperature in the dark for 60 min. Blue colour was developed in the solution. The absorbance was taken at 660 nm in UV- visible spectrophotometer.

### **Determination of proline**

Proline content was determined by the method adopted by Bates *et al.*, (1973). 0.5g of flesh leaf sample was homogenized in 10ml of 3% aqueous sulphosalicylic acid and the homogenate was filtered using what man's No.1 filter paper. Two ml filtrate was taken in a test tube and 2 ml of acid ninhydrin and 2ml of glacial acetic acid was added. This was allowed to react for 1 hour at 100<sup>0</sup>c in a boiling water bath. The reaction was terminated by placing the tube in an ice box. 4 ml of was added to the reaction mixture. The chromophore containing toluene was separated and absorbance was recorded at 520 nm wavelength using toluene as blank.

### **Determination of Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>)**

To estimate the hydrogen peroxide, content the method given by Velikovo *et al.*, (2000) was used in this present investigation. 500 mg of leaf tissue was homogenated with 5.0 ml of Trichloro Acetic acid (TCA) and cooled immediately in ice bath. Then it is centrifuged at 12000g for 15 min and the supernatant was collected. 0.5 ml of supernatant was transferred to test tube and 0.5 ml of 10mM phosphate buffer (pH 7.0) AND 1.0 ml of potassium iodide (1M) were added. After the addition, the solution was mixed vigorously and the absorbance was recorded at 390 nm. H<sub>2</sub>O<sub>2</sub> content was determined by using an

extinction coefficient (E) of  $0.28\mu\text{ m}^{-1}\text{cm}^{-1}$  and expressed as  $\text{n mol g}^{-1}\text{fw}$ .

### **Determination of peroxidase determination**

Phosphate buffer 100 mM.

Solution A: - contains 6.8 g of  $\text{KH}_2\text{PO}_4$  in 500 ml of distilled water.

Solution B: - contains 8.71g of  $\text{K}_2\text{HPO}_4$  in 500 ml of distilled Water.

Phosphate buffer was prepared by mixing 87 ml of solution A and 15 ml of solution B. And the pH was set to 6.1. It was prepared by dissolving 124 $\mu\text{l}$  of 30%  $\text{H}_2\text{O}_2$  (30 ml  $\text{H}_2\text{O}_2$  in 70 ml DW) in distilled water where the final volume was made up to 100ml. Guaicol (96mM) was prepared by dissolving 1075  $\mu\text{l}$  of guaicol in distilled water, where the final volume was made up to 100 ml.

The reaction mixture was prepared by adding 1.0 ml of phosphate buffer, 0.5 ml of guaicol, and 0.5 ml of  $\text{H}_2\text{O}_2$ , 0.1 ml of crude enzyme and 0.9 ml of distilled water. And the absorbance was measured at 470 nm.

### **Determination of Ascorbate peroxidase determination (APX)**

Ascorbate peroxidase is one of the most widely distributed antioxidant enzyme which reduce hydrogen peroxide to water using reduced ascorbate as the electron donor. It plays a more important role in scavenging ROS than other antioxidative enzyme since ascorbate, in addition to reacting with  $\text{H}_2\text{O}_2$  may react with superoxide, singlet oxygen and hydroxyl radical.

### **Reagents**

100 mM potassium phosphate buffer (pH 7.0), 15 mM L-ascorbate, 10 mM  $\text{H}_2\text{O}_2$

APX is assayed by the method of Nakano and Asada (1981)

1. Set up a reaction mixture (3.0 ml) by adding 2.7 ml of 100mM potassium phosphate buffer (pH 7.0). 0.1 ml L-ascorbate and 0.15 ml  $\text{H}_2\text{O}_2$ .

2. Initiate the reaction by adding 50  $\mu\text{l}$  of enzyme extract and record the decrease in absorbance at 290 nm spectrophotometrically for 2 min against blank which corresponds to the oxidation of ascorbic acid.

3. Calculation the enzyme activity using the molar extinction coefficient of  $2.8\text{ mM}^{-1}\text{ cm}^{-1}$  for ascorbate. One enzyme unit is expressed as the amount required to oxidize one n mole of ascorbate  $\text{min}^{-1}$

### **Determination of Superoxide Dismutase (SOD)**

Because of the instability of its substrate, all available assays of superoxide dismutase are indirect and depend upon its ability to scavenge  $\text{O}_2$  from reaction mixture and thus to inhibit the reaction caused by  $\text{O}_2$ . In one of the methods, superoxide dismutase was quantitated on the basis of its ability to inhibit photochemical reduction of nitro- blue tetrazolium (NBT).

### **Reagents**

Methionine 300g of methionine (mol.wt.150) in 100 ml of double distilled water

Riboflavin solution (75 $\mu\text{M}$ )

Dissolve 28.5 mg riboflavin (mol. wt. 376)

Nitro -blue tetrazolium solution (1.125mM)

Dissolve 46 mg of NBT (mol. wt. 817) in 50 ml of double distilled water

EDTA disodium solution (1.5mM)

Dissolve 56 mg of EDTA – disodium salt (mol. wt. 372) in 100 ml of double distilled water.

Phosphate buffer (50 Mm; pH 7.0)

Dissolve 0.265 g of  $\text{KH}_2\text{PO}_4$  (mol. wt. 174) in 50 ml of distilled water. Adjust the Ph to 7.0, if there is need to do so, and make up the volume to 100ml with distilled water.

Phosphate buffer (50Mm: pH 7.8).

Dissolve 0.058 g of  $\text{KH}_2\text{PO}_4$  (mol.wt.136) and 0.8 of  $\text{K}_2\text{HPO}_4$  (mol.wt.174) in 50 ml of distilled water. Adjust the pH to 7.0, if there is need to do so, and make up the volume to 100 ml with distilled water.

### Results and Discussion

The various morphological, yield, biochemical and antioxidant characters studied and portrayed in different figures have been described in this chapter.

### Growth parameters

Among all the two variety C1021 and C1022 the plant height (cm) at 30DAS, 60DAS, 90DAS and No. of primary branches was

maximum in C1022 in T3 (salicylic acid concentration 1.5mM) followed by C1021 in T3 (salicylic acid concentration 1.5mM). In case of the relative water content it was found maximum in variety C1021 in T3 (salicylic acid concentration 1.5mM) followed by variety C1022 in T3 (salicylic acid concentration 1.5mM).

### Yield parameters

Among all the two variety C1021 and C1022 in all treatment the number of pods/plant, seed weight per pod, seed yield per plant, 100-seed weight and harvest index was greater in variety C1022- 47.83 pods in T3 (salicylic acid concentration 1.5mM) followed by C1021- 46.33 in T3 (salicylic acid concentration 1.5mM).

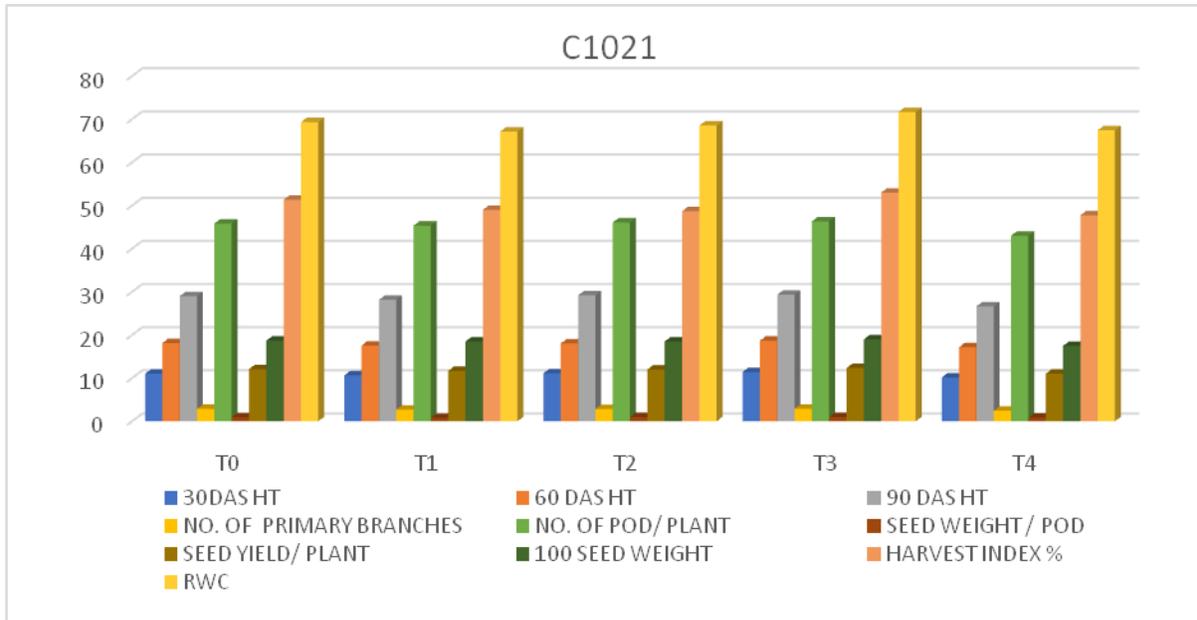
### Bio chemical parameters

Among all the two variety C1021 and C1022 at 60 DAS the maximum chlorophyll a was observed in C1021 in T3 (salicylic acid concentration 1.5mM) followed by C1022 in T3 (salicylic acid concentration 1.5mM).

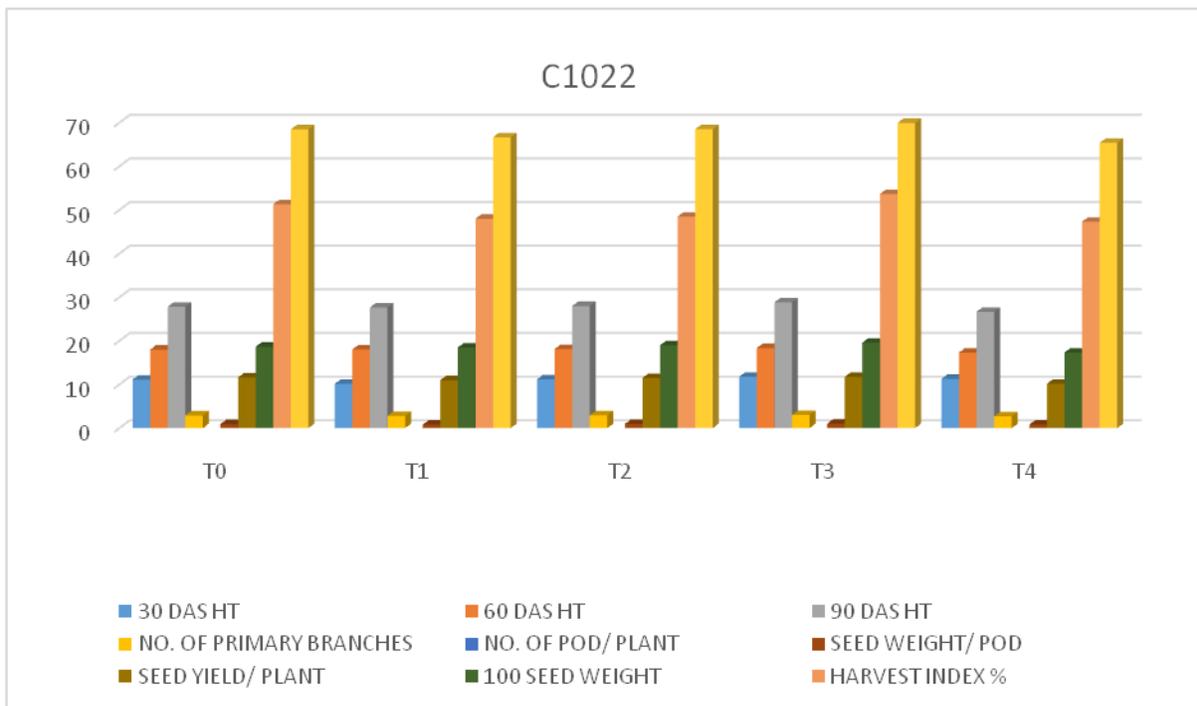
**Table.1** Treatment details

Treatment	Chemical	Concentration	Irrigation days	Spray	Spraying DAS
T0	Control	-----	Well irrigated	-----	-----
T1	Salicylic acid	0.5mM	25,50,60 DAS	foliar	30,45 and 60
T2	Salicylic acid	1.0mM	25,65 DAS	foliar	30,45 and 60
T3	Salicylic acid	1.5mM	25,50 DAS	foliar	30,45 and 60
T4	Salicylic acid	2.0mM	25,50 DAS	foliar	30,45 and 60

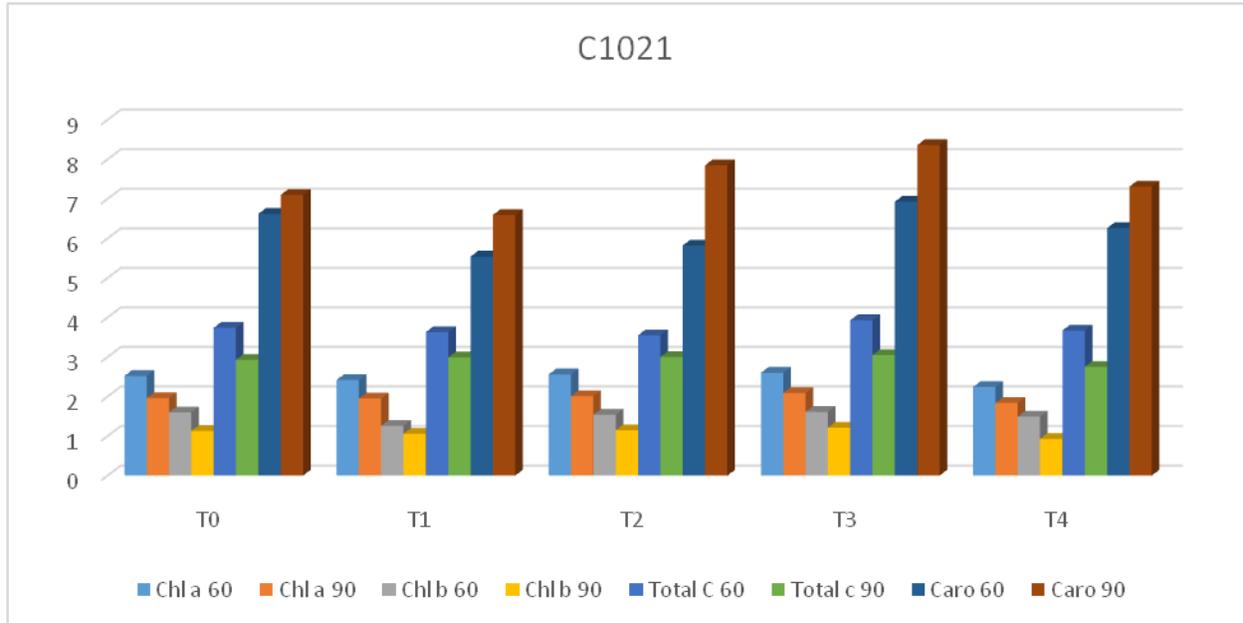
**Fig.1** Effect of drought stress and salicylic acid on plant height (30,60,90DAS), No. of primary branches, No. of pod /plant, seed weight/plant, seed weight/pod, 100 seed weight, Harvest index %, RWC in variety C1022



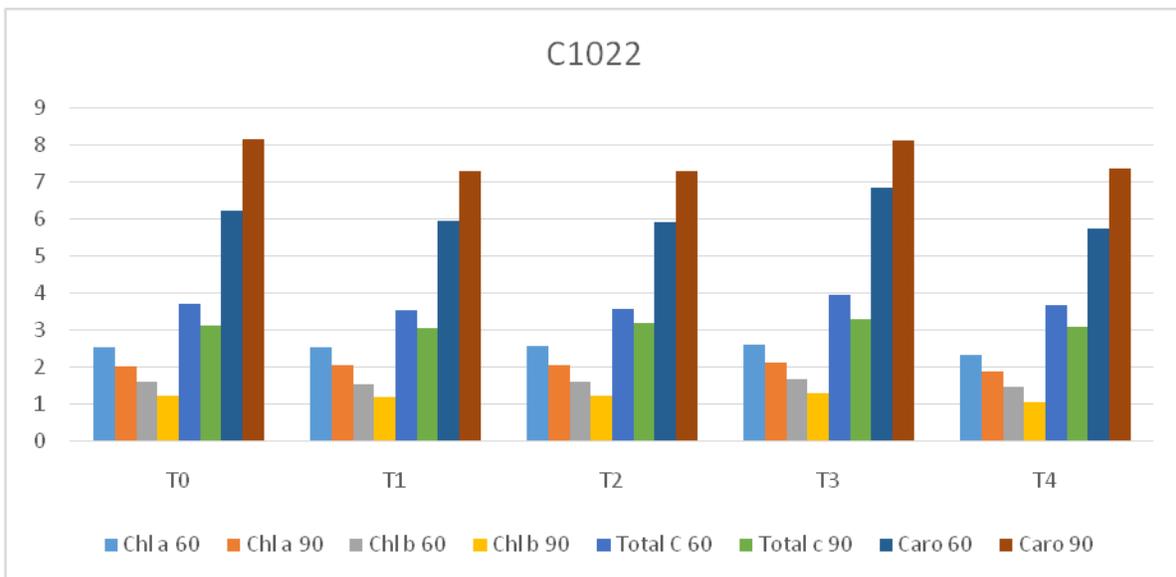
**Fig.2** Effect of drought stress and salicylic acid on plant height (30,60,90DAS), No. of primary branches, No. of pod /plant, seed weight/plant, seed weight/pod, 100 seed weight, Harvest index %, RWC in variety C1021



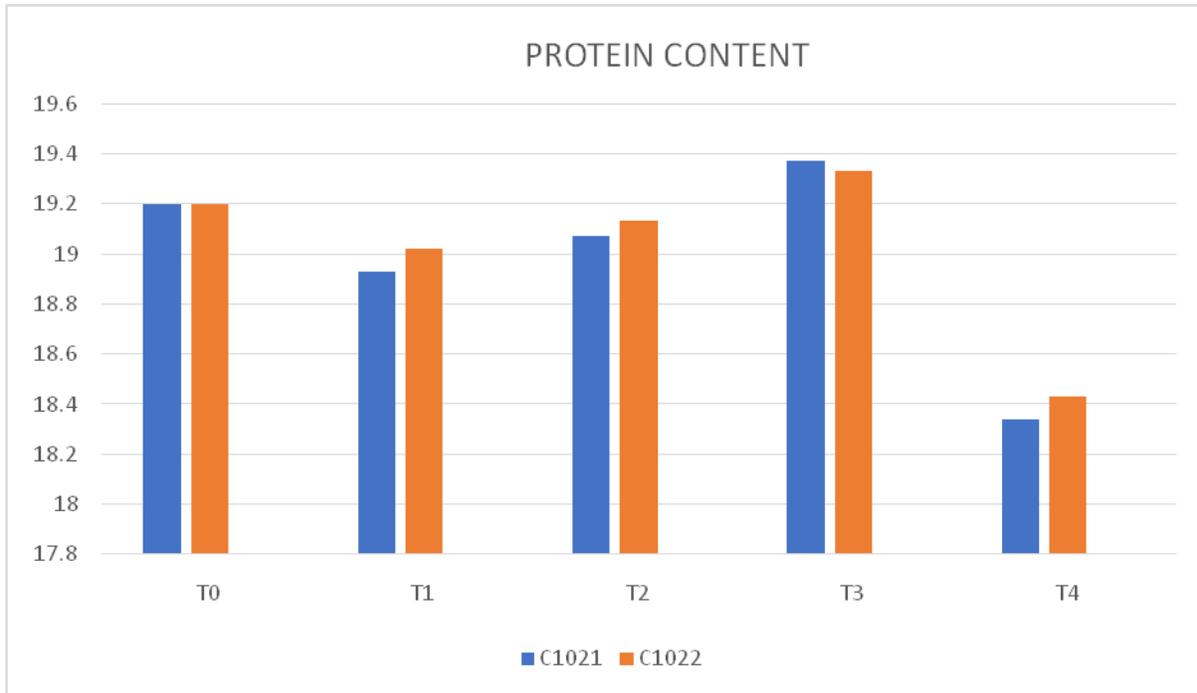
**Fig.3** Effect of drought stress and salicylic acid on Chlorophyll a (60,90DAS), Chlorophyll b (60,90DAS), Total Chlorophyll (60,90DAS), Carotenoids (60,90DAS) in C1021



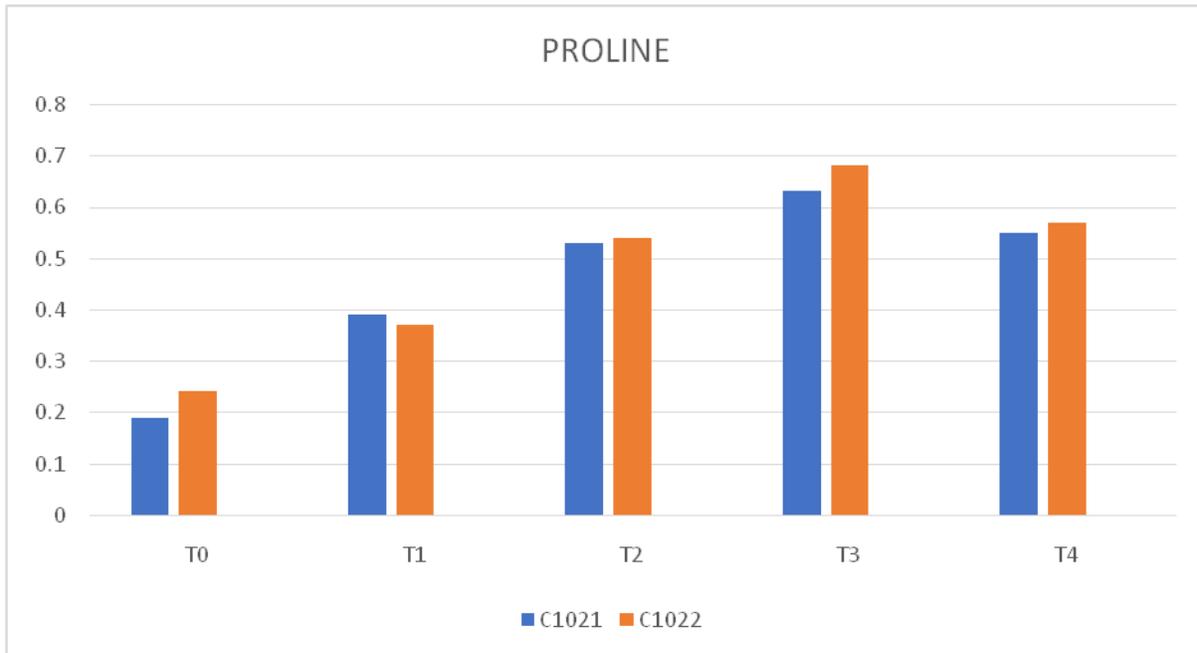
**Fig.4** Effect of drought stress and salicylic acid on Chlorophyll a (60,90DAS), Chlorophyll b (60,90DAS), Total Chlorophyll (60,90DAS), Carotenoids (60,90DAS) in C1022



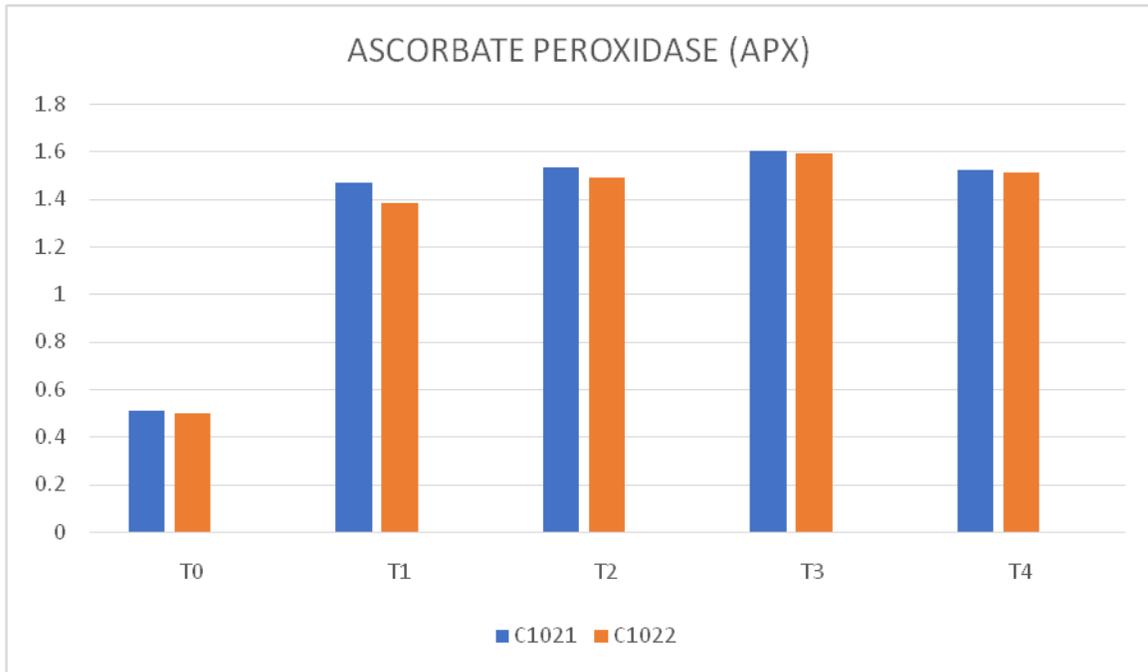
**Fig.5** Effect of drought stress and salicylic acid on protein content in chickpea varieties, antioxidant



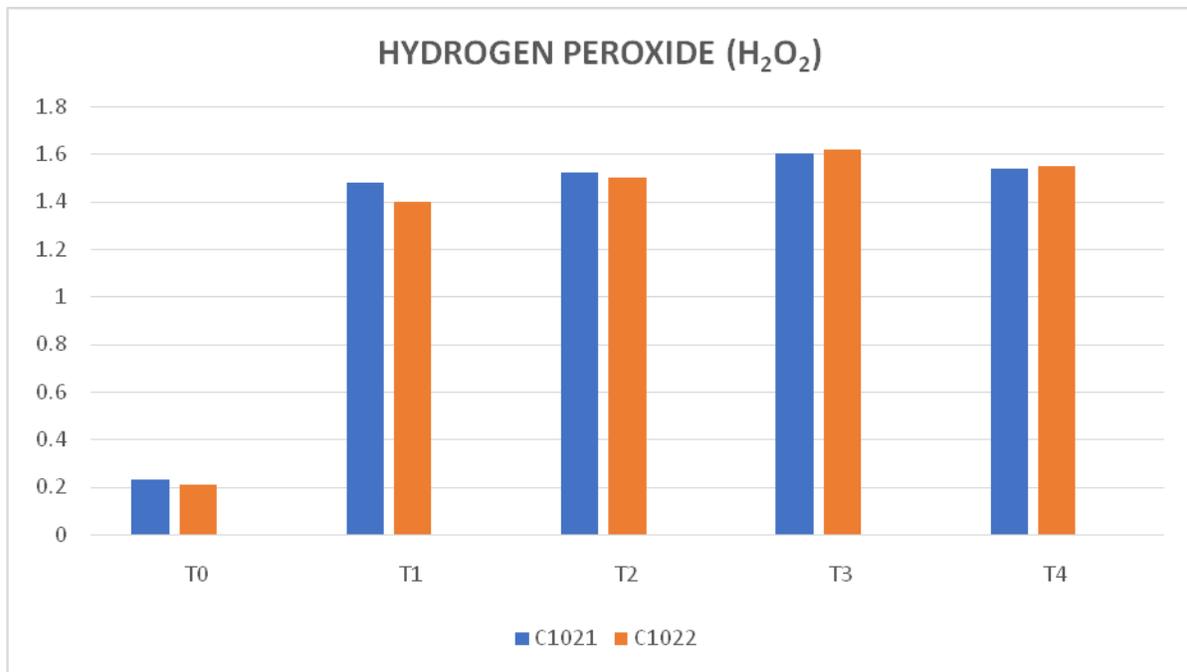
**Fig.6** Effect of drought stress and salicylic acid on proline in chickpea varieties



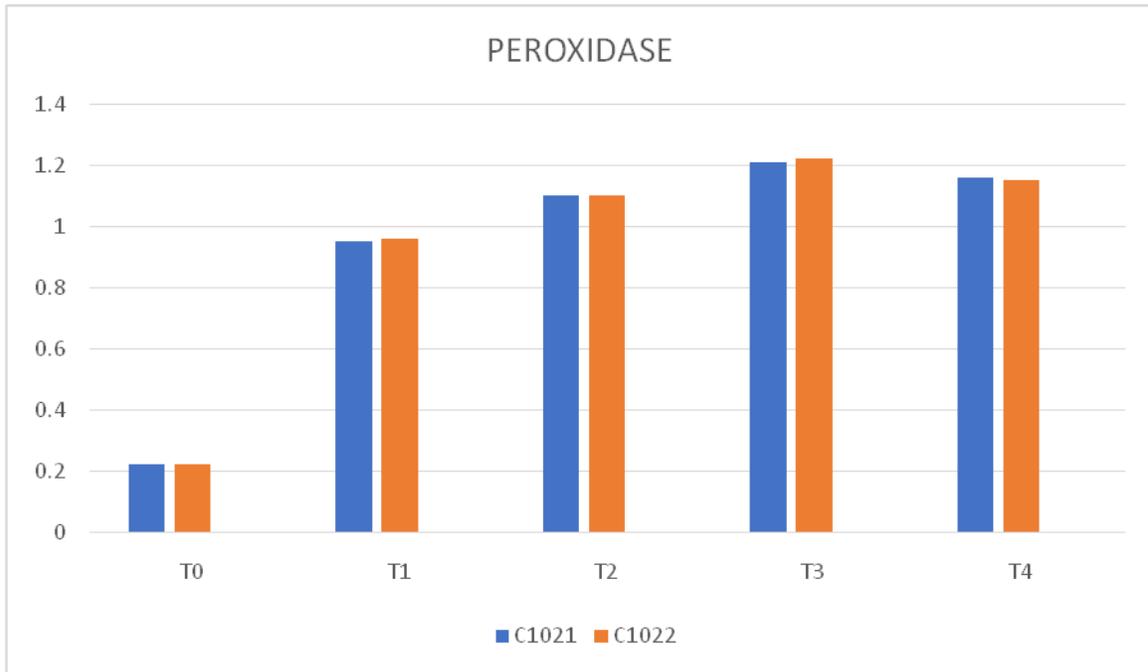
**Fig.7** Effect of drought stress and salicylic acid on ascorbate peroxidase in chickpea varieties



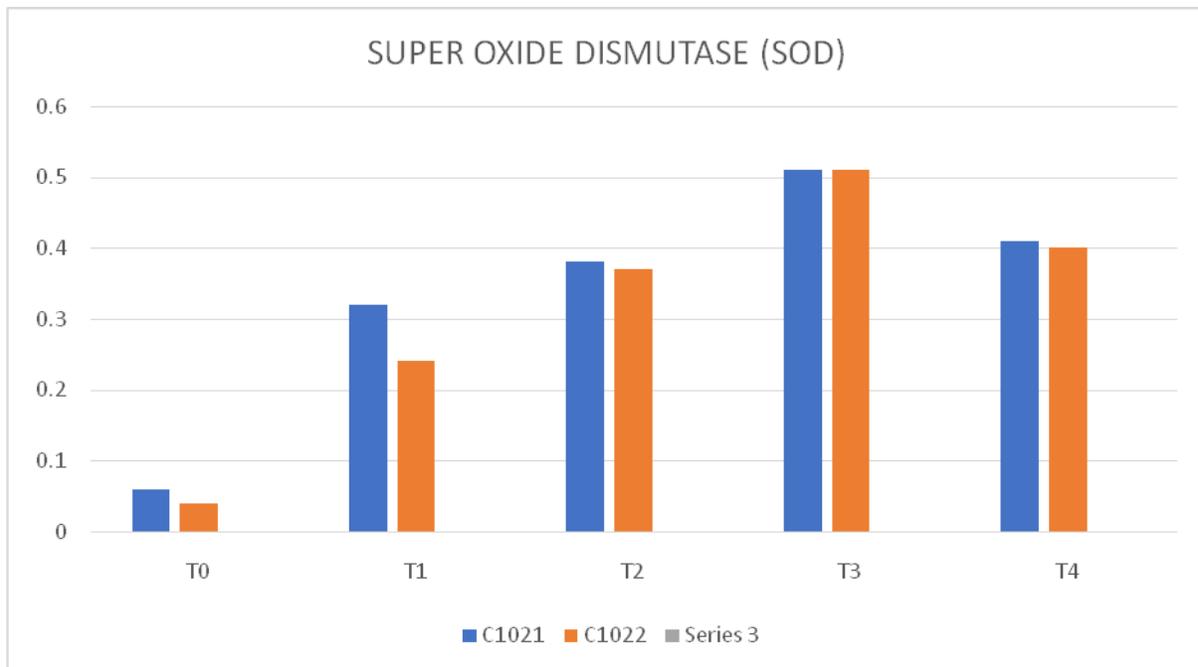
**Fig.8** Effect of drought stress and salicylic acid on hydrogen peroxide in chickpea varieties



**Fig.9** Effect of drought stress and salicylic acid on peroxidase in chickpea varieties



**Fig.10** Effect of drought stress and salicylic acid on super oxide dismutase in chickpea varieties



In case of 90 DAS the maximum chlorophyll a was observed in variety C1022 in T3 (salicylic acid concentration 1.5mM) followed

by variety C1021 in T3 (salicylic acid concentration 1.5mM). In 60 DAS the maximum chlorophyll b was observed in

variety C1022 in T3 (salicylic acid concentration 1.5mM) and followed by in T3 (salicylic acid concentration 1.5mM). In case of 90DAS was maximum in C1022 in T3 (salicylic acid concentration 1.5mM) followed by C1021 in T3 (salicylic acid concentration 1.5mM). At 6DAS the maximum total chlorophyll 60 DAS was observed in both the variety C1021 and C1022 of treatment T3 (salicylic acid concentration 1.5mM) followed by the variety C1021 in T0 (control). In case of 90 DAS the maximum total chlorophyll was observed in variety C1022 in T3 (salicylic acid concentration 1.5mM).

Maximum carotenoid at 60DAS was observed in variety C1021 in T3 (salicylic acid concentration 1.5mM) followed by C1022 in T3 (salicylic acid concentration 1.5mM). In case 90DAS was observed in variety C1021 in T3 (salicylic acid concentration 1.5mM) followed by C1022 T3 (salicylic acid concentration 1.5mM).

In protein content it was observed in C1021 in T3 (salicylic acid concentration 1.5mM) followed by C1022 in T3 (salicylic acid concentration 1.5mM). In case of proline maximum was found in C1022 in T3 (salicylic acid concentration 1.5mM) followed by C1021 in T3 (salicylic acid concentration 1.5mM).

In case of APX it was observed in C1021 in T3 (salicylic acid concentration 1.5mM) followed by C1022 in T3 (salicylic acid concentration 1.5mM).

In case of H<sub>2</sub>O<sub>2</sub> was observed in C1022 in T3 (salicylic acid concentration 1.5mM) followed by C1021 in T3 (salicylic acid concentration 1.5mM) and maximum peroxidase was observed in variety C1022- in T3 (salicylic acid concentration 1.5mM) followed by C1021 in T3 (salicylic acid concentration 1.5mM). In case of SOD it was observed

maximum in the both variety C1021- 0.51 in T3 (salicylic acid concentration 1.5mM) and C1021- 0.51 in T3 (salicylic acid concentration 1.5mM).

### **Protein content**

The effect of different concentration of Salicylic acid applied on chickpea crop was studied by protein content in varieties C1021 and C1022.

### **Proline**

The effect of different concentration of Salicylic acid applied on plants was studied on Chickpea plant proline in varieties C1021 and C1022.

### **Ascorbate peroxidase (APX)**

The effect of different concentration of Salicylic acid applied on plants was studied on Chickpea plant ascorbate peroxidase (APX) in varieties C1021 and C1022.

### **Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)**

The effect of different concentration of Salicylic acid applied on plants was studied on Chickpea plants, Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in varieties C1021 and C1022.

### **Peroxidase**

The effect of different concentration of Salicylic acid applied on plants was studied on Chickpea plants, Peroxidase in variety C1021 and C1022.

### **Super Oxide Dismutase (SOD)**

The effect of different concentration of Salicylic acid applied on plants was studied on Chickpea plants, Super Oxide Dismutase (SOD) in variety C1021 and C1022.

In conclusion, based on present study, it was concluded that drought stress was deleterious to chickpea growth and yield. The result shows that C1021 T3 (salicylic acid concentration 1.5mM) showed superior adoptive traits and yield (12.17g/seed /plant). Among two varieties (C1021 and C1022) and the treatment T3 (salicylic acid concentration 1.5mM) were found to show higher ameliorating effect with yield (11.74g/seed/plant in C1022) than other treatments under drought stress and the T2 (salicylic acid concentration 1.0mM) also showed good result and T4 (salicylic acid concentration 2.0mM) treatment. The exogenous application of SA (salicylic acid) improved the flowering, yield, biochemical and antioxidant parameters by decreasing the harmful effect of drought stress.

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