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RESEARCH ARTICLE

GROWTH AND BIOCHEMICAL MODIFICATIONS IN *ZEA MAYS* L. AS INDUCED BY TRIAZOLE UNDER DROUGHT STRESS

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ABSTRACT

In the present investigation, a pot culture experiment was conducted to estimate the ameliorating effect of triazoles such as triadimefon (TDM), tebuconazole (TBZ) and propiconazole (PCZ) on drought stressed maize. From 30 days after sowing (DAS), the plants were subjected to 4 days interval drought (DID) stress and drought with TDM at 15mg l^{-1}, TBZ at 10mg l^{-1} and PCZ 15 mg l^{-1} alone and one day interval irrigation was kept as control. The plants were separated into root, stem and leaf for estimating the growth and biochemical. The experiment was laid out in a completely randomized block design (CRBD) with seven replicates for each treatment. Individual and combined drought stress and triazole treatments decreased growth parameters like shoot length, total leaf area, whole plants fresh weight and whole plant dry weight but root length increased when compared to control. The biochemical compounds like sugar and sucrose contents increased, whereas starch content decreased under drought stressed plants.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the most important grown plants in the world. Commonly known as maize or corn belongs to the family Poaceae is the most widely grown grain crop all over the world. It is multipurpose crop, it is used as food for human beings, feed for animals, poultry and fodder for livestock, for producing alcohol and no alcohol drinks, built material, like a fuel, and like medical and ornamental plant (Bekric and Radosavljevic, 2008; Alahdadi *et al*., 2011; Khodarahmpour, 2011). Corn is a vital food crop and gives a big volume of raw materials for farm animal and many agro-related industries in the world (Bello *et al*., 2010; Randjelovic *et al*., 2011). Maize is a tropical plant but today it is grown in temperate, tropical and sub-tropical regions of the world (Ijaz *et al.*, 2015). The major maize production areas are located in temperate regions of the globe. In India, about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (for example starch and oil production) and 1% as seed (AICRP on Maize, 2007). Maize starch accounts for 80% of all starches. Till now, most studies about maize starch focused on the single modified starch, such as oxidized maize starch and cross linked maize starch. In general, oxidized starch has unique functional properties such as low viscosity at high solid concentration, clarity, film forming and binding properties, etc. (Kuakpetoon and Wang, 2006; Chang *et al*., 2008; Zhang *et al*., 2012). Drought stress is one of the major environmental stresses that seriously limits plant distribution, growth and yield worldwide (Zhao *et al*., 2011; Shi *et al*., 2012, 2013a, b; 2014). Drought is a meteorological term and is commonly defined as a period without significant rainfall. It is a complex physical–chemical process, in which many biological macro and micro molecules such as nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements involved (Moaveni, 2011). The drought is a factor frequently limiting growth, yield and N accumulation in plant production (Pandey *et al*., 2000, Deblonde and Ledent 2001, De Costa and Shanmugathasan 2002). Drought, cold and salinity are major forms of stress from abiotic sources that adversely affect plant growth and productivity (Nakashima *et al*., 2012) of which drought is considered as the most devastating. Generally, plants can tolerateor avoid drought stress through morphological, physiological and phenological mechanisms such as adjustment of biomass alloca-tion; modification of root length; adjustment of osmotic potential and enhancement of MDA, proline, soluble sugar, and antioxidative enzyme

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contents (Bartels and Sunkar, 2005; Xie *et al*., 2008; Li *et al*., 2013). The plant growth regulating properties of triazole compounds are the chemicals belong to a class of compounds known as ergosterol biosynthesis inhibitors and are used as fungicides as wells as plant growth regulators (Jaleel *et al*., 2007). The triazole compounds contain three nitrogen atoms and a pentagonal ring. Triazole makes changes in plant by preventing the enzyme activity of CytP450 (Zhu, 2004; Mohamadi and Rajaei, 2013; Amalan Rabert *et al*., 2013).

The drought stress amelioration by triazole compounds is of major research interest, because these compounds have innate potentiality for increasing antioxidant enzymes and molecules in oxidative stressed plants (Fletcher *et al*., 2000). Triazoles also increase tolerance of various plant species to biotic and abiotic stresses, including fungal pathogens, drought, air pollutants, low and high temperature stress, by reducing oxidative damage via elevation of antioxidants or reducing the activity of oxidative enzymes (Lin *et al*., 2006; Bahram, 2009; Pan *et al*., 2013). Among the triazoles, a class of antifungal agents that are used as pharmaceutical drugs, exert their fungicidal activity by inhibiting 14α-demethylase activity, which is involved in sterol biosynthesis (Ghannoum and Rice, 1999; Tamura *et al*., 2015).

MATERIALS AND METHODS

The hybrid maize seeds variety NK 6240 were obtained from syngenta India private limited and used for this investigation. The experimental seeds were surface sterilized with 0.2% Mercuric chloride solution for five minutes with frequent shaking and thoroughly washed with tap water. In the preliminary study, under lab condition, 2,5,10, 15 and 20 mgL^{-1} of triazole compounds were tested and among the concentration tested, 15mg L^{-1} of Triadimefon (TDM), 10mg L^{-1} of Tebuconazole (TBZ) and 15 mg L^{-1} of Propiconazole (PCZ) treatments were prepared as optimum doses and used for further study. Plastic pots of 40 cm diameter and 45 cm height size were used for pot culture study. The pots were filled with 10 kg of soil mixture containing red soil; sand and farm yard manure in 1:1:1 ratio and the pots were arranged in Completely Randomized Block Design (CRBD). Totally 250 pots were used and one set containing 50 pots was kept as control and another set of 50 pots was used for drought stress inducement and the remaining three sets of 150 pots were used for drought stress with triazoles treatment. The treatments were given as soil drenching, 30 days after sowing (DAS). The plants were left for 30 DAS with alternative day irrigation. From 30^{th} to 60^{th} day, control plants were irrigated on every alternative day, drought treated and drought with triazole treated plants were irrigated at every 4 days interval. After drought treatment all the pots were irrigated on alternative day and it last up to harvest. Plants were uprooted randomly on 40^{th}, 50^{th} and 60^{th} DAS, washed with water and separated into root, stem and leaf for estimating growth and biochemical.

Measurement of growth (morphological) parameters

Root and shoot length

Root and shoot length were recorded on 40^{th}, 50^{th} and 60^{th} DAS. Below the point of root-stem transition to the tap root and the length of lateral roots were taken as total root length. The length between stem tip and point of root stem transition region was taken as stem length. The root length and the stem length were expressed in centimeters per plant.

Total leaf area

The total leaf area was measured using LICOR Photo Electric Area Meter (Model LI-3100, Lincoln, USA) and expressed in cm^2 per plant.

Whole plant fresh weight and dry weight

After washing the plants in the tap water, fresh weight of plant was determined by using an electronic balance (Model – XK3190-A7M) and the values were expressed in gram. After taking fresh weight, the plants were dried at 60 °C in hot air oven for 24 hours. After drying, the weight was measured and the values were expressed in gram.

Biochemical constituents

Starch

Starch content was estimated by following the method of Clegg (1956).

Extraction of starch and total sugar

In a pestle and mortar, five hundred milligrams of plant materials were homogenized with 10 ml of 80% ethanol and the homogenate was centrifuged at 800 g for 15 min and the supernatant was saved. The pellet was re-extracted with boiling 80% ethanol and the supernatants were pooled. Then the ethanol was evaporated and the extract was made upto 20 ml with distilled water. This extract was used immediately for the quantitative estimation of sugars. For starch extraction, the ethyl alcohol washed residue left behind after the soluble sugar extraction was taken. To the residue, 5 ml of distilled water was added and 6.5 ml of 52% perchloric acid (PCA) was also added and stirred well and heated at 80^{o} C in a water bath for 30 min. Then, 20 ml of distilled water was added and centrifuged for 15 min and the supernatant was saved in a 100 ml of volumetric flask. The residue was re-extracted and the supernatant was pooled and made upto 100 ml with distilled water. The extract was filtered through whatman No. 2 filter paper and the extract was used for the estimation of starch.

Estimation of starch

10 ml of cold anthrone reagent was added with 1 ml of perchloric acid (PCA) extract and it was diluted with 5 ml of deionised water. The test tube was heated for 10 min at 100^{o} C in a boiling water bath. The test tube was cooled rapidly and the absorbance was read at 630 nm in a spectrophotometer. Starch content was calculated by multiplying glucose equivalents with the conversion factor 0.9.

Estimation of total sugar

Soluble sugars (reducing and non-reducing) were estimated by modified method of Nelson (1944).

Extraction

Non-reducing sugars were hydrolyzed to reducing sugar and total sugars were estimated.

Hydrolysis

1 ml of extract was evaporated to dryness in a boiling water bath. To the residue, 1 ml of distilled water and 1 ml of 6 N sulphuric acid were added. The mixture was hydrolyzed by incubating in a water bath at 50° C for 1 hour. The solution was neutralized with 1 N sodium hydroxide and made upto 10 ml with distilled water and used for the estimation of total sugars.

Estimation

A volume of 1 ml fresh copper reagent and 1 ml of extract [prepared by mixing copper tartrate solution and copper sulphate solution (25:1v/v)] were added. The mixture was heated in a Folin-Wu-tube with its mouth covered with a marble in a boiling water bath for 20 min, then cooled and 1 ml of arsenomolybdate reagent was added. The final volume was made upto 20 ml with distilled water. The resultant blue colour was read at 520 nm in a spectrophotometer against the appropriate blank. The sugar content was expressed in milligram per gram dry weight. The content of the sugar was calculated from the standard graph prepared with glucose.

Sucrose

Sucrose content was estimated by the method of Bernt and Bergmayer, 1970.

Estimation

For estimating sucrose, 1 ml of invertase (prepared by dissolving 250 units of yeast invertase in 500 ml of 0.2M sodium acetate buffer = pH 5.0) was added to 1ml of sugar extract and incubated at 37°C for 1h and, thereafter, the reaction was stopped by keeping the tubes in boiling water bath for 10 min. Under these conditions, sucrose was completely hydrolyzed. Glucose was determined by the glucose oxidase and peroxidase reaction (sigma) (Gascon and Lampen 1968) before and after invertase hydrolysis and the difference between these values was taken as the actual amount of sucrose in the sample.

Statistical Analysis

The pot culture was carried out in completely randomized design (CRBD). The data are expressed as mean ± SE for seven samples in each group.

RESULTS AND DISCUSSION

Effect of drought stress and drought with triazole combination on growth parameters

Water stress is characterized by reduction of water content, turgor, total water potential, wilting, closure of stomata and decrease cell enlargement and growth. Drought stress increased the root length in maize when compared to control (Fig.1). During stress condition, root growth can be greater because of increased partitioning of carbohydrates to roots (Prasad *et al*., 2008). Increased the root length in *Cannabis sativa* (Amaducci *et al.,* 2008) and in *Aspalathus linearis* (Lotter *et al*., 2014) under drought stress. Growth and development of the plant were dependent of the cell turgor, which practice a positive pressure that promotes through tissue extension mechanism (Kerbauy, 2004). Triazole treatments to the drought stressed plants increased the root length when compared to control. Triazoles increased the diameter and length of fibrous roots and enhanced the lateral root formation in tomato plants (Berova *et al.,* 2000; Mohamadi and Rajaei, 2013). Shoot length decreased in maize when compared to control under drought stress (Fig.2). The decreasing of water potential in meristem is a cause for lessening of the turgor pressure, that isn't sufficient for the cell growth. This is also one of the causes of decreasing protein synthesis and declining cell growth (Kochaki and Sarmadniya, 2004). Similar results were observed in avocado (Chartzoulakis *et al*., 2002) and *Aspalathus linearis* (Lotter *et al*., 2014). Drought stress with triazole treatments increased the shoot length when compared to drought stressed plants but it was lower than that of control. Triazole inhibits root formation depending on the plant species and concentration of chemical applied and at stimulatory concentration increase stem in the Harwood (Fletcher and Hofstra, 1988). Paclobutrazol suppressed shoot height in tomato (Pasian and Bennett, 2001).

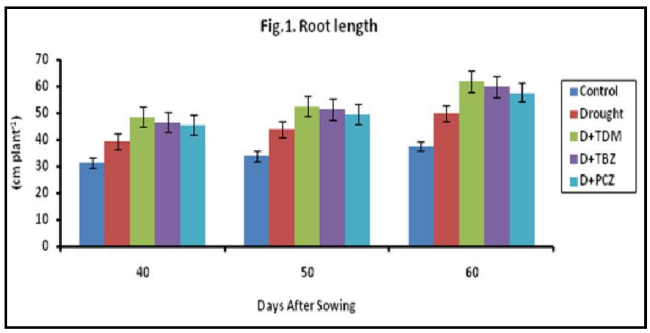

Figure.1. Effect of drought stress and drought with triazoles treatment on root length of maize

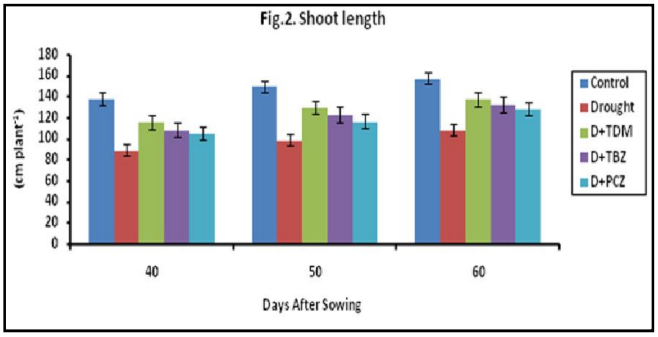

Figure.2. Effect of drought stress and drought with triazoles treatment on shoot length of maize

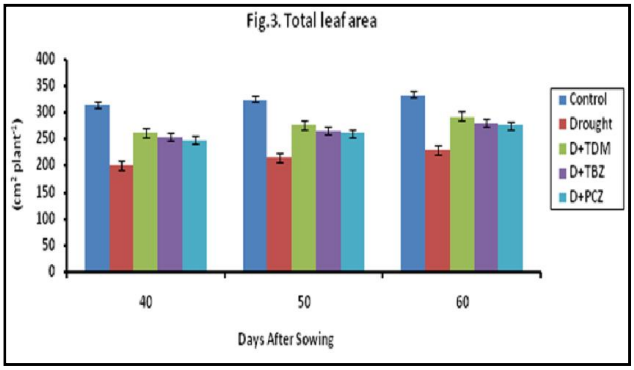

Figure.3. Effect of drought stress and drought with triazoles treatement on total leaf area of maize

Drought stress reduced the total leaf area when compared to control plants (Fig.3). Reduction in leaf area by water stress is an important cause of reduced crop yield through reduction in photosynthesis (Rucker *et al*., 1995 and Shao *et al*., 2008). Similar results were observed under drought stress in *Prunus persica* (Berman and Dejong, 1996) and wheat (Gong *et al*., 2003). Triazole treated plants showed increased total leaf area when compared to drought stress plants but it was lower than that of control. Paclobutrazol treatment also reduced the leaf area in *C. roseus* (Jaleel *et al*., 2006) and barley (Sunitha *et al*., 2004). Increased the triazole treatment the leaf area in rose plants (Jenks *et al.,* 2001) and in Ashwagandha (Sakthivel and Sridharan, 2015).

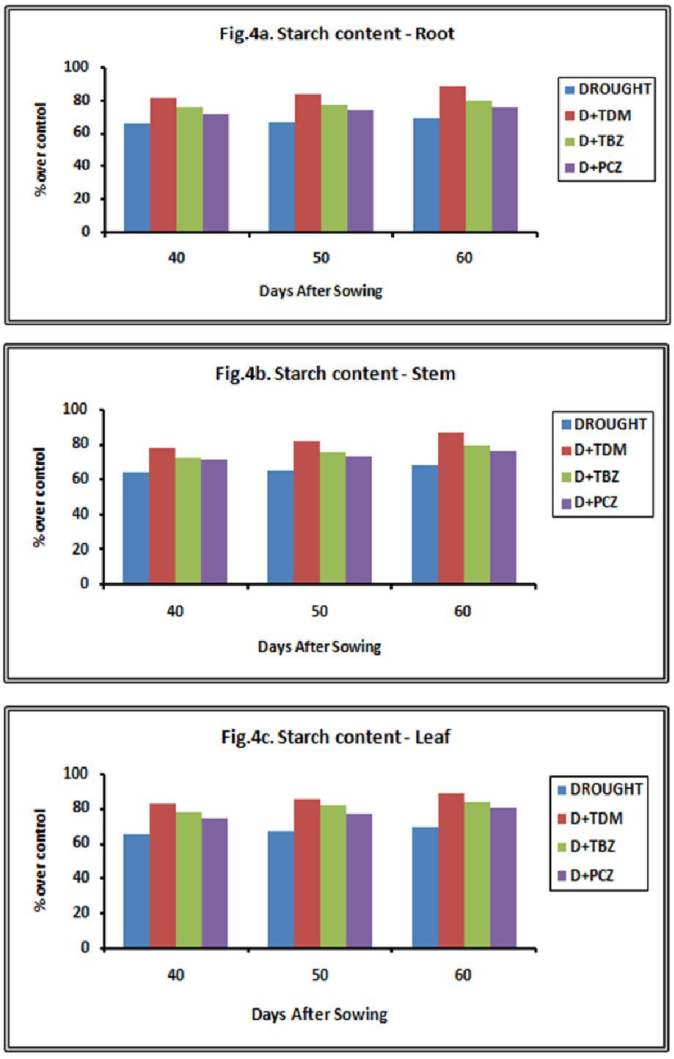

Figure.4. Effect of drought stress and drought with triazoles treatment on starch content of maize

Drought stress caused decreased the whole plant fresh and dry weight accumulation in maize when compared to control (Table 1). The reduction of the fresh and dry weight was observed in the varieties of *Triticum aestivum* and it was more pronounced in the sensitive varieties (Abdalla and El-Khoshiban, 2007). Under water deficit stress the biomass production was decreased in *Populus cathayana* and drought severely affected all growth parameters (Yin *et al*., 2005). Decreased total dry weight may be due to the considerable decrease in plant growth, photosynthesis and canopy structure as indicated by leaf senescence during drought stress in *Ricinus communies* (Schurr *et al*., 2000). Triazole treatments to the drought stressed plants significantly increased the whole plant fresh and dry weight when compared to drought stress. Triadimefon treatment showed increased plant fresh weight in *Lycopersicon esculentum* (Mohamadi and Rajaei, 2013). The triazole treatments increased the dry weight in *Plectranthus aromaticus* and *Plectranthus vettiveroides* (Meena Rajalekshmi *et al*., 2009).

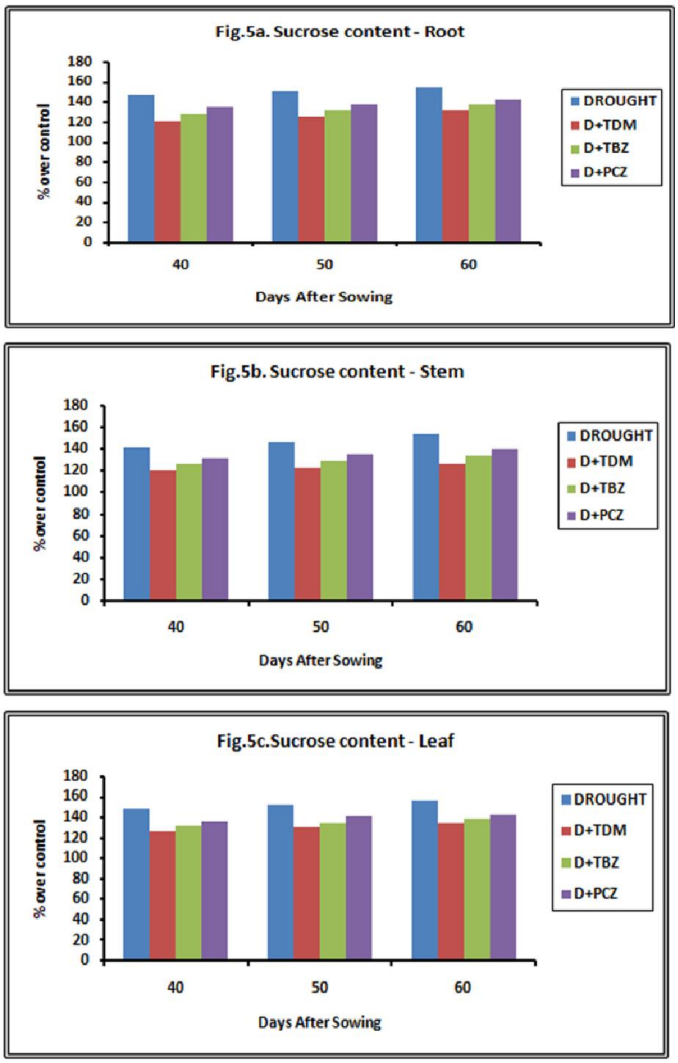

Figure.5. Effect of drought stress and drought with triazoles treatment on sucrose content of maize

Effect of drought stress and drought with triazole combination on biochemical constituents

Drought stress decreased the starch content in all the treatments of maize when compared to control (Fig.4). Starch forms the major component of sorghum grain, therefore grain yield reduction is mainly caused by the reduction of starch accumulation (Duffus 1992; Emes *et al.*, 2003). Drought stress during flowing stage reduced the starch synthesis enzyme activities, starch accumulation in grains, and the differences between starch components were also demonstrated under drought stress (Bing *et al*., 2014). Drought stress with triazole treatments increased the starch content when compared to drought stress.

Table 1. Effect of drought stress and drought with triazoles treatment on whole plant fresh and dry weight of maize

Das	Control	Drought	D + tdm	D + tbz	D+pcz
Whole plant fresh weight (expressed in grams plant^{-1})					
40	214.68±0.757	132.10±1.342	171.16±1.417	162.30±1.556	156.23±0.962
50	235.22±1.203	151.55±1.545	189.96±1.398	184.30±1.254	177.03±1.047
60	247.06±1.587	166.50±1.644	209.50±1.644	189.47±1.806	189.47±1.806
Whole plant dry weight (expressed in grams plant^{-1})					
40	72.31±0.905	45.16±1.423	57.86±0.875	55.63±0.883	53.38±1.050
50	77.14±0.914	50.63±1.645	64.06±1.344	60.86±1.137	59.41±1.199
60	82.83±1.614	55.29±1.207	71.21±0.995	69.39±1.350	65.54±1.129

Values are mean ± SE of seven replicates

Table 2. Effect of drought stress and drought with triazole treatments on total soluble sugar content of maize

(Expressed in mg/g dry weight)

Das	Control	Drought	D + tdm	D + tbz	D+pcz
Root					
40	0.756±0.010	1.104±0.011	0.941±0.009	0.973±0.005	1.000±0.011
50	0.967±0.006	1.456±0.007	1.214±0.019	1.289±0.010	1.334±0.006
60	1.179±0.008	1.810±0.010	1.540±0.010	1.613±0.011	1.654±0.009
Stem					
40	0.520±0.010	0.750±0.010	0.631±0.007	0.660±0.005	0.699±0.008
50	0.810±0.009	1.180±0.012	1.011±0.008	1.056±0.006	1.107±0.010
60	1.066±0.006	1.651±0.008	1.391±0.011	1.444±0.010	1.513±0.008
Leaf					
40	1.129±0.005	1.674±0.006	1.450±0.008	1.511±0.008	1.554±0.009
50	1.350±0.008	2.093±0.009	1.796±0.010	1.856±0.010	1.914±0.009
60	1.516±0.009	2.406±0.010	2.054±0.010	2.134±0.006	2.211±0.008

Values are mean ± SE of seven replicates

Triadimefon treatment, uniconazole and ectaconazole increased the total non-structural carbohydrates in potato and *Poa pratensis* (Kane and Smiley, 1983; Kapur *et al.,* 1993). Total sugar content increased in all parts of plants under drought stress when compared to control (Table 2). Increase in sugar content has been observed in *chickpea* (Mafakheri *et al*., 2011), *Dactylis glomerata* and *Poa bulbosa* (Volaire *et al*., 2001) under drought stress. Water stress significantly increased total sugar content in sorghum (Yadav *et al*., 2005). Triazole treatment to the drought stressed plants increased the sugar content when compared to control but it was lower than that of drought stress. Triazole treatment increased the sugar content in rye grass (Hampton and Habbeith Waite, 1985) and *Vigna aconitifolia* callus (Gehlot *et al.,* 1989). Drought stress increased the sucrose content in all the parts of maize plants when compared to control (Fig.5). The sucrose concentration was found higher in soybean under drought stress (Fulia Liu *et al*., 2004). Triazole treatment with drought stressed plants decreased the sucrose content when compared to drought stress but it was higher than that of control. The sucrose content increased with triazole treatment in leaves and tubers of tapioca. The requirement for higher level of sucrose for successful tuber induction in invitro systems suggests that sucrose may play a vital role in tuber induction process (Fernie and Willmitzer, 2001).

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