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Effects Of Triazole Compounds On Pigment And Proline Content Of *Solanum Tuberosum* Under Drought Conditions

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Abstract: The effects of triazole treatments on the non-enzymatic antioxidant and proline metabolizing enzymes of *Solanum tuberosum* (Potato) was researched in the study. The triazole compounds namely Triadimefon (TDM) 15mg l^{-1}, Tebuconazole (TBZ) 10mg l^{-1} and Propiconazole (PCZ) 15 mg l^{-1} was given to plants by soil drenching 30, 40 and 50 days after sowing (DAS). From 30 DAS the plants were subjected to 4 days interval drought (DID) stress and drought with triazole compounds and one day interval irrigation was kept in control. The plants were uprooted on 40, 50 and 60 DAS separated into root, stem and leaf for estimating the pigment and proline contents. Individual and combined drought stress and triazole treatments increased photosynthetic pigments like chlorophyll a, b and total and biochemical content like amino acid and proline when compared to control.

Keywords: Triazole, Potato, Triadimefon, Tebuconazole, Propiconazole, Drought.

I. INTRODUCTION

Water stress is an environmental factor limiting plant growth and is a major concern in climate change. Desertification processes in arid areas are the result of the degradation of vegetation cover since plant growth is seriously limited in arid sites (Evelin et al., 2009). Drought stress is one of the main environmental factors limiting plant growth and yield and it is the most prevalent cause of crop yield loss due to an increase in temperature and decrease in water availability respectively, that departs from the optimal condition for plant life (Larcher, 2003). Drought and temperature extremes can cause extensive economic loss to agriculture (Peng et al., 2004; NCDC, 2011), an effect that is likely to increase as global climate change progresses (Keane et al., 2009). The Drought reduces plant growth by affecting several physiological and biochemical processes, such as photo- synthesis, respiration, nutrient transport and hormone balance, leading to the reduction of fresh and dry biomass (Farooq et al., 2012). Comprehensive biochemical and physiological studies in plants adapted to severe water deficit conditions are needed in order to get a better understanding of drought resistance mechanisms and acclimation processes. The present study was undertaken to thoroughly evaluate the effect of drought stress and drought with triazole treatment in potato on non-enzymatic antioxidant and proline metabolizing enzymes.

The impact of water stress and drought on agricultural development is complex and exhibits variability across different geographical areas, crop types, and agricultural methods. Regions that mainly depend on rainfed agriculture and lack adequate irrigation infrastructure are especially susceptible to the impacts of water stress and drought. The inadequacy of water availability can lead to crop failures, shortfalls in food supply, and economic setbacks, hence intensifying concerns regarding food security (Talaat et al., 2015).]. Water stress is characterized by an insufficient quantity of water for agricultural purposes, whereas drought is defined as a prolonged period of very low precipitation. Both of these conditions have the potential to lead to a decrease in water availability, which can have a detrimental impact on the growth and development of plants. The occurrence of water stress and drought commonly results in dehydration and restricted nutrient absorption, hence causing a reduction in crop productivity (Hussain, and AL-Taey, 2020). Water stress is characterized by an insufficient quantity of water for agricultural purposes, whereas drought is defined as a prolonged period of very low precipitation. Both of these conditions have the potential to lead to a decrease in water availability, which can have a detrimental impact on the growth and development of plants. The occurrence of water stress and drought commonly results in dehydration and restricted nutrient absorption, hence causing a reduction in crop productivity (AL-Taey et al., 2022). The impact of water stress and drought on agricultural development is complex and exhibits variability across different geographical areas, crop types, and agricultural techniques. Regions that mainly depend on rainfed agriculture and lack adequate irrigation infrastructure are especially susceptible to the impacts of water stress and drought. Inadequate water availability can lead to crop failures, shortfalls in food supply, and economic losses, hence increasing challenges related to food security [Olesen and Bindi, 2002].

More over, the occurrence of water stress and drought has the potential to adversely impact the overall stability of agricultural systems. Farmers encounter several obstacles when it comes to the cultivation of crops, preservation of soil fertility, and control of pests and diseases. These a fore mentioned causes exacerbate the decline in agricultural output and have the potential to result in enduring implications for both food security and rural livelihoods (Lei et al., 2006). In order to mitigate the effects of water stress and drought, farmers and policymakers implement a range of methods. The implementation of efficient irrigation systems, the adoption of drought-resistant crop varieties, the practice of conservation agriculture, and the promotion of sustainable water management techniques are some of the strategies employed to optimize water utilization, enhance crop resilience, and ensure longer agricultural sustainability (Rajasekar and Manivannan, 2015; AL-Taey et al., 2022). Farmers and politicians adopt a variety of methods in order to lessen the impact of water stress and drought on their operations. These may include the installation of effective irrigation systems, the selection of crop types that are resistant to drought, the practice of conservation agriculture, and the promotion of environmentally responsible methods of water management. These strategies aim to maximize the efficiency with which water is used, improve crop resilience, and assure the long-term viability of agriculture (Schlenker and Roberts, 2009).

Triazoles are a group of growth inhibitor chemical compounds that widely used as fungicides. The compounds contain three nitrogen atoms and a pentagonal ring. It can be noted to many types of triazole such as uniconazol, paclobutrazol, triamidefon, tebuconazole, propiconazole and hexaconazole (Zhu et al., 2004). Triadimefon, tebuconazole and propiconazole is a triazole compound having fungicidal as well as plant growth regulating properties. Plant growth regulators (GA3, PBZ and 6-BA) play important roles in plant growth, development, yield and qualities formation (Zheng et al., 2011). Triazoles, a class of antifungal agents that are widely used as fungicides or pharmaceutical drugs, exert their fungicidal activity by inhibiting 14α-demethylase activity, which is involved in sterol biosynthesis (Ghannoum and Rice, 1999). Triazoles protect plants against various stresses including drought, low and high temperatures, UV light and air pollution. They have been referred to as plant ‘‘multi-protectants’’ because of their ability to induce tolerance in plants to environmental and chemical stresses (Gupta et al., 2004). The use of plant growth regulators, as GA3, PBZ, 6-BA or their compounds, is becoming popular to ensure efficient production. Remarkable accomplishments of plant growth regulators such as manipulating plant growth and crop yield have been actualized in recent years (Zvi and Eduardo, 2011).

Drought strongly inhibits key physiological and biochemical processes, leading to poor plant performance and tuber yield loss. The magnitude of this loss, however, mostly depends on the duration and severity of drought episodes as well as plant growth stage and cultivar (Hill et al., 2021).Drought during the early growth stage is considered to be the most harmful as it substantially reduces total leaf area, reduces photosynthetic rates, and assimilates partitioning to tubers leading to poor tuber initiation, bulking, and tuber yield (Obidiegwu et al., 2015). Drought during tuberization leads to fewer stolon per stem, reflected by lower tuber number and yield (Eiasu et al., 2007). If potato plants experience drought during the tuber bulking stage, they will produce fewer and smaller-sized tubers. Never the less, it has been suggested that the initiation of stolon and the formation of tuber are the most critical stages of drought stress (Aliche et al., 2020). The reduction in tuber yield under drought is suggested to be mainly associated with the inhibition of photosynthesis (Plich et al., 2020).

Potatoes (*Solanum tuberosum*) occupy a crucial position in the global food supply chain. Potatoes, being extensively consumed and grown, serve as a substantial dietary source of nutrients and caloric intake for a considerable worldwide population. Potatoes are a very adaptable food item that is abundant in carbs, essential vitamins, and vital minerals. Potatoes has the capacity to thrive in a wide range of climatic circumstances and exhibit adaptability to numerous soil compositions, therefore rendering them viable for cultivation in places characterized by limited agricultural resources. Potatoes, due to their considerable yield potential and capacity to flourish in adverse conditions, play a crucial role as a significant crop for food security. They make a substantial contribution to nourishing populations and maintaining a reliable global food supply (AL-Taey et al., 2019). The scarcity of water has had a significant influence on potato farming. The insufficiency of water resources has resulted in reduced crop yields and compromised the overall quality of potato production. The limited availability of water has hindered the growth and development of potato plants, leading to stunted growth and smaller tuber sizes. Additionally, the lack of water has increased the susceptibility of potato crops to various diseases and pests, further exacerbating the negative effects on yield. Water scarcity and drought exert a substantial influence on global agricultural progress and crop productivity. The presence of these environmental elements can present significant obstacles for agricultural practitioners, hence impacting their capacity to generate an adequate supply of crops that meet the desired standards of quality. In order to comprehensively comprehend the impacts of water stress and drought on agriculture, it is imperative to take into account a multitude of factors (Smith and Steduto, 2010).

II. MATERIAL AND METHODS

The potato seeds were used this investigation. 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 experimental potato 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 mg L^{-1} of triazole compounds were tested and among the concentration tested, 15mg L^{-1} of Triadimefon (TDM), 10mg L^{-1} of Tebuconzole (TBZ) and 15 mg L^{-1} of Propiconazole (PCZ) treatments were prepared as optimum doses and used for further study. The treatments were given as soil drenching, 30 days after planting (DAP). The plants were left for 30 DAS with alternative day irrigation. From 30th to 60th 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 40th, 50th and 60th DAS, washed with water and separated into root, stem and leaf for estimating pigment, amino acids and proline contents.

2.1 CHLOROPHYLL CONTENT

Chlorophyll and carotenoid contents were extracted from the leaves and estimated according to the method of Arnon (1949).

2.2 Extraction

Five hundred milligram of fresh leaf material was ground with 10 ml of 80 per cent acetone at 4˚C in a pestle and mortar and centrifuged at 2,500 g for 10 min at 4˚C. The residue was re-extracted with 80 per cent acetone until the green colour disappeared in the residue and the extracts were pooled and transferred to graduated tube and made up to 20 ml with 80 per cent acetone and assayed immediately.

2.3 Estimation

Three milliliters of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm in a Spectrophotometer (U-2001–Hitachi) against 80 per cent acetone as a blank. Chlorophyll content was calculated using the formula of Arnon (1949).

Total chlorophyll (mg/ml) = 0.0202) X (A.645) + (0.00802) X (A.663)

Chlorophyll ‘a’ (mg/ml) = (0.0127) X (A.663) – 0.00269) X (A. 645)

Chlorophyll ‘b’ (mg/ml) = (0.0229) X (A.645) – (0.00468) X (A. 663)

2.4 Estimation of Total Free Amino acid content

Total free amino acids were extracted and estimated by following the method of Moore and Stein (1948).

2.5 Extraction

Five hundred milligram of fresh plant material was homogenized in a mortar and pestle with 10 ml of 80% boiled ethanol. The extract was centrifuged at 800 g for 15 minutes and the supernatant was made upto 10 ml with 80% ethanol and used for the estimation.

2.6 Estimation

In 25 ml test tube, one milliliter of ethanol extract was taken and neutralized with 0.1 N NaOH using methyl red indicator. To which, 1 ml of ninhydrin reagent was added. The content was boiled in a boiling water bath for 20 minutes, and then 5ml of diluting solution was added, cooled and made up to 25 ml with distilled water. The absorbance was read at 570 nm in a Spectrophotometer (U-2001–Hitachi) against an appropriate blank. The standard graph was prepared by using Leucine as standard and the amino acid content was calculated using the standard graph and the results were expressed in milligram per gram dry weight.

Reagent

Ninhydrin Reagent

Solution I: 80 mg of stannous chloride in 50 ml citrate buffer at pH 5.0.

Solution II: 2 gram of Ninhydrin in 50 ml methyl cellosolve, both solutions were mixed freshly.

Diluting Reagent

Distilled water and n-propanol mixed in equal volume (1:1 v/v).

2.7 Determination of proline content

Proline was extracted and estimated by following the method of Bates et al. (1973).

2.8 Extraction

Five hundred milligram of fresh plant material was homogenized in a mortar and pestle with 10 ml of 3% aqueous sulfosalicyclic acid. Then the homogenate was filtered through Whatmann No.1 filter paper. The residue was re-extracted and pooled and the filtrate was made up to 20 ml with aqueous sulfosalicylic acid and this extract was used for the estimation of proline.

2.9 Estimation

To 2 ml of proline extract, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added. The mixture was incubated for an hour at 100 °C in a boiling water bath. Then the test tubes were transferred to an ice bath to terminate the reaction. Then 4 ml of toluene was added and mixed vigorously using a test tube stirrer for 20 seconds and the toluene containing the chromophore was separated from the aqueous phase with the help of a separating funnel and the absorbance was measured at 520 nm in a spectrophotometer using a reagent blank. The proline content was determined from a standard curve with proline and the results were expressed in milligram per gram dry weight.

Acid-Ninhydrin Reagent

To 1.25 gm of ninhydrin, 30 ml warm glacial acetic acid, 20 ml of 6 M phosphoric acid were added with agitation.

3.Statistical analysis

Statistical analysis was performed using the one way analysis of variance (ANOVA) followed by the Duncan's Multiple Range Test (DMRT). The values are mean ± SE for seven samples in each group. p values ≤ 0.05 were considered as significant.

4. Results and Discussion

4.1Total chlorophyll content

Drought stress decreased the total chlorophyll content to a larger extent when compared with control potato plants on 40, 50 and 60 DAS (Table 1). A reduction in chlorophyll content was reported in drought stressed soybean plants (Zhang et al., 2006), and cotton (Massacci et al., 2008). However, stomatal and non-stomatal limitation was generally accepted to be the main determinant of photosynthesis under drought stress (Farooq et al., 2009). Under condition of water stress, photosynthetic electron transport through PS II is inhibited (Zlatev et al., 2010). Triazole treatment to the drought stressed sunflower plants increased the total chlorophyll content when compared to drought stress. Paclobutrazol treatment to the drought stress increased the pigments in olive (Thakur et al., 1998), jack pine, white spruce and black spruce (Marshall et al., 1991). Triazole treated leaves were dark green due to high chlorophyll a and b in Zea mays (Khalil et al., 1990), potato (Tekalign et al., 2005) and barley seedlings (Sunitha et al., 2004).

Table 1. Effect of drought stress and drought with triazoles treatment on Chlorophyll content of potato Expressed in mg/ gm fresh weight)

DAS	CONTROL	DROUGHT	D + HEX	D + TBZ	D+PCZ
Chlorophyll 'a'					
40	0.304±0.032	0.167±0.046	0.203±0.037	0.192±0.039	0.189±0.044
50	0.381±0.044	0.235±0.048	0.267±0.052	0.266±0.049	0.255±0.038
60	0.463±0.059	0.336±0.043	0.366±0.039	0.358±0.048	0.345±0.041
Chlorophyll 'b'					
40	0.159±0.014	0.108±0.016	0.122±0.016	0.119±0.015	0.108±0.013
50	0.221±0.019	0.146±0.021	0.161±0.017	0.158±0.020	0.161±0.021
60	0.315±0.017	0.227±0.018	0.251±0.015	0.248±0.017	0.246±0.019
Total Chlorophyll					
40	0.503±0.054	0.302±0.098	0.355±0.093	0.344±0.098	0.337±0.091
50	0.602±0.089	0.391±0.107	0.442±0.088	0.434±0101	0.425±0.095
60	0.787±0.092	0.554±0.095	0.606±0.084	0.593±0.094	0.593±0.096

Values are mean ± SE of seven replicates

4.2 AMINO ACID CONTENT

Drought stress increased the free amino acid content when compared to control in potato plants on 40, 50 and 60 DAS (Table 2). The amino acid content increased under drought condition in sunflower (Manivannan et al., 2007) and in *Catharanthus roseus* (Jaleel et al., 2007). The amino acid content increased under drought condition in and Marsh grasses (Maricle et al., 2008). Accumulated amino acid may be occurring in response to the change in osmotic adjustment of their cellular contents (Shao et al., 2007). The amino acid content increased to higher level immediately after treatment, later it declined as day progresses. The amino acid content has been shown to increase under drought condition in coconut (Kasturi Bai and Rajagopal, 2000). Amino acid accumulation plays a very important role in drought tolerance, probably through osmotic adjustment in different plant species, such as Radix astragali (Tan et al., 2006). Triazole treatment to the drought stressed potato plants lowered the amino acid content when compared to drought stress but it was higher than that of control. Similar results were observed in *Abelmoschus esculentus* (Amalan et al., 2013).

Table 2. Effect of drought stress and drought with triazoles treatment on amino acid content of potato (Expressed in mg g-1 dry weight)

DAS	CONTROL	DROUGHT	D + HEX	D + TBZ	D+PCZ
Root					
40	4.833±0.47	7.289±0.42	6.342±0.40	6.386±0.38	6.332±0.45
50	6.282±0.48	9.589±0.43	8.467±0.45	8.425±0.39	8.330±0.46
60	10.316±0.39	13.122±0.45	12.475±0.47	12.314±0.45	12.304±0.43
Stem					
40	3.456±0.39	5.473±0.37	5.088±0.44	4.933±0.37	4.833±0.42
50	6.088±035	7.577±0.38	6.456±0.29	6.330±0.40	6.266±0.39
60	9.190±0.43	11.253±0.40	10.481±0.38	10.327±0.43	10.231±0.42
Leaf					
40	5.723±0.40	8.685±0.46	7.881±0.38	7.654±0.47	7.594±0.38
50	7.878±0.48	10.933±0.40	10.033±0.46	9.842±0.40	9.771±0.43
60	11.431±0.43	14.161±0.47	11.558±0.44	11.449±0.42	11.336±0.46

Values are mean ± SE of seven replicates

4.3 PROLINE CONTENT

Drought stress caused a higher accumulation of proline content in all parts of the potato plants when compared to control in 40, 50 and 60 DAS (Table 3). Increased proline accumulation was reported in (Vendruscolo, et al., 2007). Proline accumulation in plants might be a scavenger and acting as an osmolyte. The reduced proline oxidase may be the reason for increasing proline accumulation. Proline accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress (Jaleel et al., 2007). Proline as an osmo protectant compound, plays a major role in osmoregulation and osmotolerance (Demir, 2000). However, its definite role in exerting stress resistance continues to be a debate (Demiral and Turkan, 2006). Triazole treatment with drought stress caused an enhancement in proline content when compared to control but it was lower than that of drought stressed potato plants. Triazoles resulted in increased proline content in *Eruca sativa* seedlings (Mathur and Bohra, 1992). Triazole induced a transient raise in abscisic acid content (Fletcher et al., 2000) and sunflower (Amalan et al., 2013).

Table 3. Effect of drought stress and drought with triazoles treatment on Proline content of potato (Expressed in mg g-1 dry weight)

DAS	CONTROL	DROUGHT	D + HEX	D + TBZ	D+PCZ
Root					
40	0.623±0.057	0.946±0.074	0.783±0.058	0.794±0.058	0.798±0.063
50	0.906±0.083	1.457±0.088	1.142±0.064	1.185±0.072	1.198±0.077
60	1.347±0.063	2.401±0.072	1.703±0.057	1.728±0.083	1.755±0.081
Stem					
40	0.653±0.045	0.854±0.071	0.694±0.055	0.713±0.048	0.724±0.074
50	0.891±0.063	1.485±0.048	1.142±0.073	1.175±0.051	1.186±0.068
60	1.025±0.055	1.798±0.066	1.315±0.079	1.336±0.063	1.343±0.046
Leaf					
40	1.357±0.089	2.105±0.098	1.725±0.099	1.763±0.084	1.796±0.097
50	1.803±0.086	3.051±0.089	2.356±0.122	2.402±0.105	2.427±0.1
60	2.086±0.082	3.775±0.086	2.684±0.086	2.708±0.122	2.722±0.097

Values are mean ± SE of seven replicates

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