INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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INVESTIGATION OF WATER STRESS ON ANTIOXIDANT ENZYME ACTIVITIES IN GROUNDNUT VARIETIES (*Arachis hypogaea L*)

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ABSTRACT
Water, the most important component of life, is rapidly becoming a critically short commodity for humans and crop production. Limited water supply is one of the major abiotic factors that adversely affect agricultural crop production worldwide. Drought stress influences the normal physiology and growth of plants in many ways. In the present investigation drought induced changes pertaining to antioxidant enzyme activities were analyzed in two groundnut varieties (TAG-24 and TG-26). Plants were grown in pots under four regimes of water (100%, 80%, 60% and 40%). A 2×4 factorial experiment in complete randomized block design with 3 replications were conducted in Botanical garden of Fergusson College, Pune 411 004. Results showed that the activities of these enzymes were significantly increased with increase in drought stress level in leaves of both varieties of groundnut. Overall, the higher Peroxidase (POD), Catalase (CAT), Ascorbic acid oxidase **(**AAO) and Polyphenol oxidase (PPO) activities in leaves of *var.* TAG-24 than var.TG-26 may suggest the higher efficiency of tolerance under drought.

KEY WORDS: antioxidant enzymes, drought stress, groundnut.

INTRODUCTION
Groundnut (*Arachis hypogaea* L.) is popularly known as peanut. It is one of the world's most popular and universal crops, cultivated in more than 100 countries on six continents. China and India are the largest producers of groundnut. Groundnut is the largest source of edible oils in India and constitutes roughly about 50% of the total oilseeds production. Among the major groundnut growing states there has been consistent increase in area under cultivation in Andhra Pradesh, Tamilnadu, and Karnataka. However, in Gujarat and Maharashtra, the area under groundnut cultivation has shown continuous decreasing trend. The decrease in groundnut cultivation in Gujarat and Maharashtra is ascribed by bad weather conditions, drought, high temperature and salinity stresses[1,2]. Plants respond and adapt to environmental abiotic stresses. Drought is the most severe abiotic stress factor limiting plant growth and crop production. Among all abiotic stress factors, drought is the major environmental constraint to crop productivity worldwide[3]. Adequate water and nutrient supply are important factors affecting optimal plant growth and successful crop production. Water stress is one of the severe limitations of crop growth especially in arid and semiarid regions of the world as it has a vital role in plant growth and development at all growth stages[4]. According to[5] 45% of the world agricultural lands are subject to continuous or frequent drought. When plants are subjected to various abiotic stresses, some reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and singlet oxygen ($^1O_2$) are produced. Antioxidant enzymes such as superoxide dismutase, catalase and peroxidase, and low-molecular antioxidants such as ascorbic acid play a key role in scavenging those activated species [6]. Modulation of the activity of these enzymes may be an important factor in the tolerance of various plants to environmental stress. The relation between drought stress and enzymatic antioxidant systems has been studied in some plant species [7]. Decreases in plant growth under stress conditions are mainly associated with increases in ROS synthesis [8]. Plant responds to ROS by increasing the synthesis of antioxidant enzymes as a protection mechanism against cell damage. Antioxidant enzymes in plant cells play a major role in the preservation of membrane integrity, protection of DNA, and proteins degradation. Measurements of antioxidant enzyme concentrations, protein, and membrane integrity parameters provide an indication of the level of stress in plants. Therefore, in the present investigation drought induced changes pertaining to antioxidant enzyme activities were analyzed in two groundnut varieties (TAG-24 and TG-26) under pot experiment.

MATERIALS & METHODS
This research was carried out in Botanical garden at the Department of Botany, Fergusson College, Pune - 411004. Certified seeds of groundnut varieties TAG-24 and TG-26 were obtained from Mahatma Phule Agriculture College, Pune and tested for drought stress tolerance. A 2×4 factorial

experiment in complete randomized block design with 3 replications was conducted in earthen pots. Pots having a diameter of 35 cm and height of 30 cm. were used for pot experiments. Pots were filled with pot mixture containing garden soil and farmyard manure (3:1). The crop water requirements for four water regimes (100%, 80%, 60% and 40%) were premeditated on the basis of water holding capacity of soil. Seeds pretreated with fungicide were used for sowing. Uniform and undamaged 10 seeds were used per pot. On day 15 after emergence, seedlings were thinned to obtain 5 uniform seedlings per pot. After this, soil moisture status for each treatment was maintained until the plants were harvested. Normal cultural practices were followed during the growing season. Samples were collected from control and stressed plants. Fresh and physiologically active leaves (leaf no. 3 from top) of stressed and control plants were collected and cut into small pieces. Accurately 100 mg of leaf samples were homogenized in pre chilled mortar and pestle, in 3ml of 0.1M phosphate buffer (pH 7.0). The extract was centrifuged at 4^0C in cooling centrifuge at 15000×g for 10 minutes and the supernatant was used as source of enzymes. The catalase activity (CAT) was calculated by using Maxwell and Bateman method. The peroxidase (POD) activity was assayed by Vidyasekharan and Durairaj method. The ascorbic acid oxidase (AAO) was assayed by Sadasivam and Manickam method. The polyphenol oxidase (PPO) activity was assayed by Vidyasekharan and Durairaj method.

RESULTS & DISCUSSION

Catalase(CAT): At higher stress level (40% soil moisture level), about 61.89% increase in CAT activity was observed in the leaf tissues of *var.* TAG-24, conversely only 50.31% increase in CAT activity was noted in *var.* TG-26. Thus the results suggest that CAT plays important role for drought tolerance in *var.* TAG-24, while it is not playing same role in *var.* TG-26 (Table.1).

Peroxidase (POD): The results on effect of increasing water deficit on activity of enzyme POD are depicted in table 2. The data show that leaf tissues of control plants of *var.* TAG-24 have nearly double POD activity (242.58 units g^{-1} FW) than *var.* TG-26 (124.56 units g^{-1} FW). From the results, it is clear that the POD activity of leaves linearly and significantly increased over the control under all levels of drought stress levels in both the varieties. This suggests that all levels of drought stress stimulated POD activity in both the varieties of groundnut. At higher stress level (40% soil moisture level), about 136.71% increase in POD activity was observed in the leaf tissues of *var.* TAG-24, conversely 93.87% increase in POD activity was noted in *var.* TG-26. Thus, the results indicate that by synthesizing more amount of POD, *var.* TAG-24 tolerates drought stress.

Ascorbic acid oxidase (AAO): The results on effect of increasing water stress on activity of enzyme AAO are illustrated in table 3. The data show that leaf tissues of control plants of *var.* TAG-24 have more AAO activity (22.45 units g^{-1} FW) than *var.* TG-26 (18.12 units g^{-1} FW). From the results, it is clear that the activity of enzyme ascorbic acid oxidase was stimulated at all concentrations of water stress in both the varieties, but stimulation was more in *var.* TAG-24 than *var.* TG-26. At higher stress level (40% soil moisture level), about 56.97% increase in AAO activity was observed in the leaf tissues of *var.* TAG-24, conversely 42.67% increase in AAO activity was noted in *var.* TG-26. Thus, the results indicate that by synthesizing more amount of AAO, *var.* TAG-24 tolerates drought stress. These results also indicate that *var.* TAG-24 has more powerful antioxidant system than *var.* TG-26.

TABLE 1: Effect of drought stress on Catalase activity in leaves of two varieties of groundnut

Soil Moisture Levels	*var.* TAG-24			*var.* TG-26		
	Mean	SM	PI/PD	Mean	SM	PI/PD
100% (Control)	533.25	0.00	00.00	425.26	0.00	00.00
80%	638.26	105.01	19.69	508.28	83.02	19.52
60%	745.28	212.03	39.76	572.58	147.32	34.64
40%	863.28	330.03	61.89	639.24	213.98	50.31
SEm±	3.32	--	--	10.01	--	--
CD (0.05)	27.02	--	--	84.03	--	--
CD (0.01)	50.38	--	--	156.70	--	--

SM: Shift in mean over control **PI:** Percent increase over control; **PD:** Percent decrease over control; **SEm:** Standard error of means; **CD:** Critical difference.

Polyphenol oxidase (PPO): The results on effect of increasing water stress on activity of enzyme PPO are given in table 4. The data show that leaf tissues of control plants of *var.* TAG-24 have more PPO activity (68.28 units g^{-1} FW) than *var.* TG-26 (55.86 units g^{-1} FW).

TABLE 2: Effect of drought stress on peroxidase activity in leaves of two varieties of groundnut

Soil Moisture Levels	*var.* TAG-24			*var.* TG-26		
	Mean	SM	PI/PD	Mean	SM	PI/PD
100% (Control)	242.58	0.00	000	124.56	0.00	000
80%	352.44	109.86	45.29	188.24	63.68	51.12
60%	462.58	220.00	90.69	228.62	104.06	83.54
40%	574.22	331.64	136.71	241.48	116.92	93.87
SEm±	2.50	--	--	4.48	--	--
CD (0.05)	20.23	--	--	38.66	--	--
CD (0.01)	37.73	--	--	72.09	--	--

SM: Shift in mean over control **PI:** Percent increase over control; **PD:** Percent decrease over control; **SEm:** Standard error of means; **CD:** Critical difference.

TABLE 3: Effect of drought stress on Ascorbic acid oxidase activity in leaves of two varieties of groundnut

Soil Moisture Levels	*var.* TAG-24			*var.* TG-26		
	Mean	SM	PI/PD	Mean	SM	PI/PD
100% (Control)	22.45	0.00	000	18.42	0.00	000
80%	24.28	1.83	8.15	20.14	1.72	9.34
60%	29.82	7.37	32.83	22.48	4.06	22.04
40%	35.24	12.79	56.97	26.28	7.86	42.67
SEm±	0.92	--	--	0.94	--	--
CD (0.05)	7.35	--	--	7.60	--	--
CD (0.01)	13.71	--	--	14.18	--	--

SM: Shift in mean over control **PI:** Percent increase over control; **PD:** Percent decrease over control; **SEm:** Standard error of means; **CD:** Critical difference.

TABLE 4: Effect of drought stress on Polyphenol oxidase activity in leaves of two varieties of groundnut

Soil Moisture Levels	*var.* TAG-24			*var.* TG-26		
	Mean	SM	PI/PD	Mean	SM	PI/PD
100% (Control)	68.28	0.00	000	55.86	0.00	000
80%	102.86	34.58	50.64	72.22	16.36	29.29
60%	129.68	61.40	89.92	94.28	38.42	68.78
40%	143.56	75.28	110.25	108.24	52.38	93.77
SEm±	1.77	--	--	2.29	--	--
CD (0.05)	16.18	--	--	18.86	--	--
CD (0.01)	30.18	--	--	35.16	--	--

SM: Shift in mean over control **PI:** Percent increase over control; **PD:** Percent decrease over control; **SEm:** Standard error of means; **CD:** Critical difference.

From the data, it is evident that in *var.* TAG-24 and *var.* TG-26, the activity of PPO increased linearly with increase in all levels of drought stress over the control. It is evident from the results that the activity of enzyme PPO was stimulated under water stress conditions in both the varieties, but stimulation was more in *var.* TAG-24 than *var.* TG-26. At higher stress level (40% soil moisture level), about 110.25% increase in PPO activity was observed in the leaf tissues of *var.* TAG-24, conversely 93.77% increase in PPO activity was noted in *var.* TG-26. Thus, the results indicate that by synthesizing more amount of PPO, *var.* TAG-24 tolerates drought stress. This reflects that *var.* TAG-24 has more powerful antioxidant system than *var.* TG-26. Active oxygen can accumulate in plants under water stress. There is a defensive system in plants, that is to say, plants have an internal protective enzyme-catalyzed cleanup system, which is fine and elaborate enough to avoid injuries of active oxygen, thus guaranteeing normal cellular function [9]. When the plants suffer from water stress, the whole defensive system needs to be activated in order to resist the active oxygen injuries. Single antioxidant enzymes or antioxidant cannot resist these injuries.

It was found that the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of many plants were affected by drought [10]. Numerous studies have shown that the degree of injury caused by drought is negatively correlated to the increase of activities of SOD, POD and CAT. Increased SOD, POD and CAT activities in response to water stress have also been reported by [11] and [12]. But some results are different from this; for example [9] found increase in SOD and POD activities and decrease in CAT activity under water stress[13]. Reported that the POD activity was increased with increase water stress for all the rice varieties [14]. Observed significant increase in CAT and POD activity in the leaves of *Vicia faba* cultivars under drought stress and concluded that CAT and POD play an essential role in scavenging the H_2O_2 toxicity. According to[15] under different water stress treatments, the activities of

SOD, CAT and POD in leaves and roots first increased drastically and then declined during the period of maize development [16]. Showed that severe stress increased POD, CAT and PPO activities in leaves and roots, of cultivars of sesame[17]. Stated that the activity of POD and PPO significantly increased with increasing salinity levels in groundnut. Our results showed that drought caused changes in antioxidant enzymes activity in leaves of both varieties of groundnut. Overall, the higher POD, CAT, AAO and PPO activities in leaves of *var.* TAG-24 may suggest the higher efficiency of tolerance under drought. These results are similar in part to results obtained by Ghorbanli [18].Reported that in salt-tolerant tomato SOD and CAT were effective antioxidant enzymes. Also, [19] reported that in salt tolerant *Setaria italica*, POD activity was found to be higher to protect plants against the stress. These observations are similar in part to results obtained by[11]. In present study, data pertaining to CAT and POD clearly show that under drought, CAT and POD activity increased significantly in the leaves of both the varieties of groundnut. However, the level of CAT and POD activity was found to higher in the tolerant *var.* TAG-24 than the sensitive *var.* TG-26 under control as well as under drought conditions, suggesting better capacity of *var.* TAG-24 to decompose H_2O_2 under drought stress. These results are in a same line of [20] they observed higher constitutive level of CAT and POD activity in drought tolerant *Phaseolus acutifolius*. Lin and Kao concluded that SOD, GR and POD play a protective role in scavenging reactive oxygen species (ROS). Increasing CAT and POD activities may have contribution in drought stress tolerance in *var.* TAG-24.

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