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Research Article

PHYTOCHEMICAL AND BIOCHEMICAL ANALYSIS OF GRAINOF *TRITICUM AESTIVUM L.* (6 VARIETIES) UNDER DROUGHT STRESS

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ABSTRACT

Wheat (*Triticum aestivum L.*) is an important crop which provides nutritional source and consumed all over the world. It is a main source of starch and glucose. Low availability of water affects the morphological status, physiological attributes and nutrient content of wheat grain as well. The present study explores the biochemical and phytochemical changes mediated by drought stress in sprouted wheat grains of 6 different varieties GW503, GW11, LOC-1, GW1255, GW173 and GW366. Presence of phytochemical components like tannins, terpenoids, cardiac glycosides and alkaloids was examined. All the varieties had higher glucose content in drought plants as compared to irrigated plants except LOC-1. Among all GW11 for starch content and GW1255 for glucose content proved to be the best variety to consume as well as to resist against drought stress.

INTRODUCTION

Wheat (*Triticum aestivum*) is a major crop and an important part of human diet, in developing countries in particular(1). Wheat is an essential source of carbohydrates(2). Internationally, it is the foremost source of vegetal protein in human food, containing a protein content of about 13%, which is comparatively higher than that of in other major cereals, but comparatively lower in context of protein quality for supplying essential amino acids(3). When eaten as the whole grain, wheat is a source of multiple nutrients and dietary fiber(2). Drought stress or less availability of water is viewed to be a major abiotic factor influencing plant growth, improvement and biochemical pathways like the synthesis of secondary metabolites and aromatic compounds(4)(5)(6)(7). Plant phytochemical compounds contentsuch astotal phenols, flavonoids and critical oil elements such as limonene, terpinolene, myrcene, quercetin and sabinene are mainly influenced by drought stress(2)(8)(9). In general, drought stress amongst cultivars brought on a clear limit in fresh weight, stem length, carotenoid and chlorophyll photosynthetic pigments, total soluble sugars, complete flavonoids and total phenolic compounds(10). In water scarcity, the life-cycle becomes shortened leading to reduced size of organs like leaves, tillers, spikes and affects physiological and biochemical tactics leading to reduced yields. During initial growth, drought reduces germination and emergence and reduces tiller number, plant height and leaf area, whilst at anthesis and throughout grain filling, drought decreases the share of fertile spikelets and wide variety of grains(11), per chance due to decreased pollination(12). During grain filling, water stress additionally alters the accumulation of starch and protein in the kernels, thereby reducing grain measurement and affecting grain yield and high-quality(13).

MATERIALS AND METHODS

In these experiments, 6 varieties of wheat GW503, GW11, LOC-1, GW1255, GW173, GW366 were taken and grains of these varieties were used which were sown during Rabi season and harvested in month of March. This investigation was performed at Biological and Life Sciences (Ahmedabad University). Seeds were procured from Gujarat state Seed Corporation Limited. Sprouted grains of both irrigated and drought conditioned plants were used.

Sample Preparation

Wheat grains were sprouted and extracted using distilled water and used as sample.

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Phytochemical analysis

1. ***Tannins:1*** to 2 drops of iron (III) chloride solution and 1 ml of distilled water were added in 0.5ml of grain extract prepared with distilled water. Color observation was observed then.
2. ***Terpenoids:1*** ml trichloromethaneand 1.5 ml of concentrated sulfuric acid were mixed in 1 ml grain extract.
3. ***Cardiac Glycosides:*** 1ml glacial acetic acidand one drop of iron (III) chloridesolution were mixed in 1ml extracted solution. This was then underneath with 1ml concentrated sulfuric acid.
4. ***Alkaloids:*** Few drops of Mayer's reagent were added to the grain extractand color change was observed. (14)

Biochemical Analysis

Glucose

Centrifuge tubes were taken and labeled for standard concentrations and samples. Different concentrations of glucose were prepared as standard. 3,5- dinitrosalicylic acidwas added to all the tubes and kept in water bath for 15 minutes and transferred to cold water bath till the temperature came down to the room temperature. Then, absorbance was measured in Eppendorf biospectrometer at 540nm.(15).

Starch

Different concentrations ranging from 1-10% of starch solution were prepared. From each of the solution prepared, 500 µlvolume was transferred to the corresponding labeled tubes prepared for standard. All the tubes including sample tubes containing same volumes of samples were kept for incubation in water bath at 37ºC for 5 minutes. For blank 10% starch concentration was taken separately and incubated with other tubes.

Working solution for alpha amylase (25 µg/ml) was prepared from stock 4mg/ml, incubated at 37ºC for 5 minutes.

Both the solutions were removed from water bath and 500µl of alpha amylase was added to all the tubes and mixed gently. Again, all the tubes except blank were kept for incubation for 15minutes. Thereafter, 1ml 3,5- dinitrosalicylic acid was added to each tube, mixed thoroughlyand kept in boiling water bath for 2-3 minutes and allowed to cool down to theroom temperature. Volume of mixture in each tube was made up to 10ml by adding distilled water. Absorbancewas measuredin Eppendorf biospectrometer at 540nm(16).

RESULTS

Phytochemical analysis

Tannins: Extracted samples mixed withiron (III) chloride solution gave a negative result. Greencolor indicates presence of tannins and yellow color indicates absence. Here, yellow color was developed indicating negative result towards tannins as shown in table 1.1.

Terpenoids: Extracted samples were mixed with chloroformtrichloromethane and sulfuric acid to observe the presence or absence of terpenoids. All the varieties showed positive result towards terpenoids as it developed reddish brown color on addition of sulfuric acid. Therefore, terpenoids were present in all the wheat varieties as shown in table 1.1.

Cardiac Glycosides: Extracted sample were treated with glacial acetic acid containing a drop of iron (III) chloride and under laid with concentrated sulfuric acid. But, there was no development of violet colored ring which showed absence of cardiac glycosides in the samples as shown in table 1.1.

Alkaloids: Few drops of Mayer's reagent were added to the grain extract indicating yellow color for positive results and white color indicating negative results. Some varieties gave positive results while some had negative results. In irrigated conditions, GW503, LOC-1, GW173 and GW366 gave positive result while varieties GW11 and GW1255 had negative values. In drought conditions, varieties GW11, LOC-1, GW173 and GW366 showed positive results while GW503 and GW1255 had negative results as shown in table 1.2.

Table 1 Presence of Tannins, Terpenoids and Cardiac glycosides in GW503, GW11, LOC-1, GW1255, GW173 and GW366 under irrigated and drought conditions.

Variety	Constituents	Standard value	Observation	Irrigated Plants	Observation	Drought Plants
All	Tannins	Green color	Yellow	Negative	Yellow	Negative
All	Terpenoids	Reddish brown	Reddish brown	Positive	Reddish brown	Positive
All	Cardiac glycosides	Violet ring	No violet color	Negative	No violet color	Negative

Table 2 Presence of Alkaloids in GW503, GW11, LOC-1, GW1255, GW173 and GW366 under irrigated and drought conditions

Variety	Constituents	Standard value	Observation	Irrigated Plants	Observation	Drought Plants
GW503	Alkaloids	yellow	White	positive	yellow	negative
GW11	Alkaloids	yellow	Yellow	negative	white	positive
LOC-1	Alkaloids	yellow	White	positive	white	positive
GW1255	Alkaloids	yellow	yellow	negative	yellow	negative
GW173	Alkaloids	yellow	White	positive	white	positive
GW366	Alkaloids	yellow	White	positive	white	positive

Biochemical Analysis

Glucose: In irrigated plants, variety GW503, GW11, LOC-1 GW1255, GW173 and GW366 had glucose concentrations of 169.37mg/ml, 84.37 mg/ml, 349.37 mg/ml, 301.25 mg/ml, 142.5 mg/ml and 156.87 mg/ml while drought plants had glucose concentrations of 193.75 mg/ml, 453.75 mg/ml, 224.37 mg/ml, 586.87 mg/ml, 226.87 mg/ml and 216.25 mg/ml in GW503, GW11, LOC-1 GW1255, GW173 and GW366 respectively. Among all, GW1255 (586.87 mg/ml) had the highest glucose content. In irrigated plant LOC-1 (349.37 mg/ml) had the highest glucose content and GW1255 (586.87 mg/ml) had highest glucose content in drought plants. Moderate glucose content was present in GW503 (169.37 mg/ml), GW1255 (301.25 mg/ml), GW173 (142.5 mg/ml), GW366 (156.87 mg/ml) in irrigated plant and GW11 (453.75 mg/ml), LOC-1 (224.37 mg/ml), GW173 (226.87 mg/ml), GW366 (216.25 mg/ml) in drought plant. Lowest glucose content was present in GW11 (84.37 mg/ml) in irrigated and GW503 (193.75 mg/ml) in drought plant.

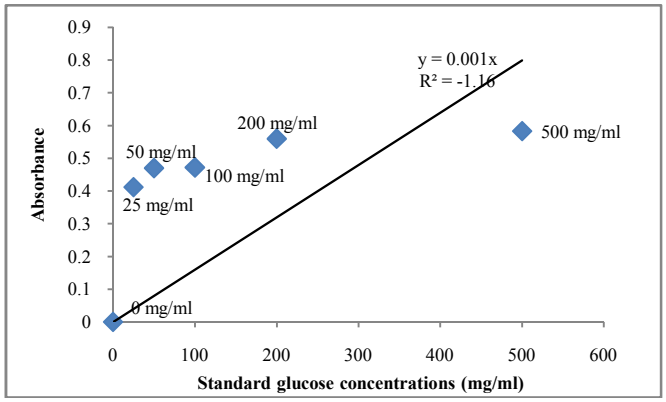

Fig 1 Standard curve for glucose concentrations (mg/ml)

Table 3 Concentration of glucose (mg/ml) in all varieties calculated based on standard curve

Variety	Irrigated (mg/ml)	Drought (mg/ml)
GW503	169.375	193.75
GW11	84.375	453.75
LOC-1	349.375	224.375
GW1255	301.25	586.875
GW173	142.5	226.875
GW366	156.875	216.25

Starch: Varieties GW503, GW11, LOC-1 GW1255, GW173 and GW366 in irrigated plants had starch concentrations of 17.46 mg/ml, 6.27 mg/ml, 9.32 mg/ml, 30.68 mg/ml, 8.98 mg/ml and 12.88 mg/ml and drought plants had starch concentrations of 12.71 mg/ml, 17.97 mg/ml, 8.81 mg/ml, 24.07 mg/ml, 8.3 mg/ml and 7.12 mg/ml respectively. GW1255 had highest starch content in irrigated plant (30.68 mg/ml) as well as in drought plant (24.07 mg/ml). Lowest starch content was observed in GW11 (6.27 mg/ml) in irrigated plants and GW366 (7.12 mg/ml) in drought plants. Among all, highest starch content was observed in GW1255 (30.68 mg/ml) under irrigated condition.

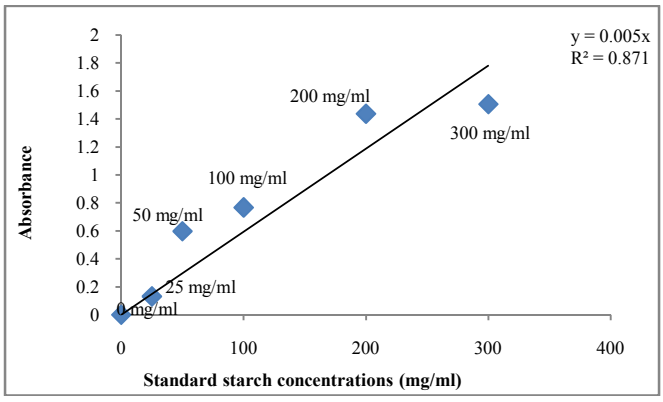

Fig 2 Standard curve for starch concentrations (mg/ml)

Table 2 Concentration of starch (mg/ml) in all varieties calculated based on standard curve

Variety	Irrigated (mg/ml)	Drought (mg/ml)
GW503	17.45763	12.71186
GW11	6.271186	17.9661
LOC-1	9.322034	8.813559
GW1255	30.67797	24.0678
GW173	8.983051	8.305085
GW366	12.88136	7.118644

DISCUSSION

Phytochemicals are chemical compounds that are synthesized by plants via primary or secondary metabolism in order to help them to survive under stress conditions caused by either biotic or abiotic factors. Effects of phytochemicals on health are still under research and hence they are not considered as "essential nutrients"(14). Tannins which are also known as tannic acid, prevent from converting the absorbed nutrients to new body substances due to which tannin rich foods are considered to be of low nutritional value. Hence, absence of tannin in wheat increases its nutritional value(17). Terpenoids (isoprenoids) exhibit the biggest and most diversecategory of chemicals among the various compounds created by plants. Plants use metabolites of terpenoids for a various basic functions involved in growth and development but generally utilize the most of terpenoids for more compact chemical interactions and for protection against abiotic and biotic stress(18). Terpenoids in irrigated as well as in drought plants showed positive response. Terpenoids were present in all the drought conditioned grains, so most of the grains were healthily developed, even though plants were in drought condition, presence of terpenoids made the grains resistant and they were equally nutritious as compared to irrigated grains. Cardiac glycosides are a category of organic compounds that increase the output force of the heart and increase its contractions' rate by functioning on the cellular sodium-potassium AT Pase pump(19). Hence, they are toxic to animals if consumed in high concentration. Thus, study presents that wheat is safe to consume as cardiac glycosides are absent in wheat. Plants produce alkaloids as final products of nitrogen metabolism which play the role of growth regulators as their structures resemble to those of regulators as well as protective agents against attack by predators(20). GW503 irrigated were positive and drought was negative. GW11 irrigated was negative and drought was positive. LOC-1, GW173 and GW366 both irrigated and drought was positive. In GW1255 both were negative. Depending on the variety, presence of alkaloid changes. In wheat endosperm, starch is the representative storage compound that occupies 65-75% of the total dry weight of a grain. It is synthesized within the amyloplast of reproductive structure cells since four days after anthesis (DAA)(21)(22). Soil drought has been incontestable to influence starch synthesis by control the activities of starch synthesizing enzymes(23). During this study, it was absolutely ascertained that, compared with the irrigated plants, enzyme activities in wheat grains were considerably increased, however considerably decreased by the drought plants. All irrigated plants had higher starch content than drought plants but GW11 had higher starch content than irrigated plant. It can be said the GW11 increased starch accumulation and proved to be a good variety as compared to other drought plants. For glucose, trehalose is a non-reducing sugar containing two glucose units linked with an α,α-1,1-glycosidic linkage. This glucose is present in a wide variety of organisms and may serve as an energy source as well as carbon source and also as a protective molecule against abiotic stresses(24). Like sucrose, trehalose can play a role of a carbohydrate storage molecule as well as a transport sugar.(25) When exposed to stress, it can also stabilize proteins and plant membranes by replacing hydrogen bonding through polar residues and thus prevents protein denaturation and membrane fusion(26). Drought plants had

considerably higher glucose content than irrigated plants except LOC-1. This shows that this variety has poor response towards drought stress due to deprived functioning or poor accumulation of trehalose.

CONCLUSION

In alkaloids, some of the varieties had negative responses and some of them had positive responses. Tannins, terpenoids and cardiac glycosides had negative responses in all the varieties. So, results for phytochemical analysis are same for all the varieties. GW1255 was proved to be a good variety as it had highest glucose content in drought conditions and for starch content, GW11 proved to be a good variety. So, it can be said that GW11 and GW1255 varieties proved to be tolerant towards drought conditions. LOC-1 had poor response towards drought stress so, LOC-1 was unable to overcome drought stress.

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