

A Route-map to the Analysis of Drought Stress Tolerance in Wheat (*Triticum aestivum* L.) Genotypes

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Abstract:

The increasing drought stress in the world is a major threat to the increasing population of the world. Therefore, to overcome that highly important and burning issue of the world, we must carry out breeding programs throughout the world which will help us to release/evolve "Drought Tolerant Genotypes/varieties, especially for wheat crop". Because wheat is one of the most staple food crops of the world population. Therefore, this study has been prepared to release the proper and well-sequenced step-wise information up to the hands of all interested researchers/ scientists to show them a route-map to analyze the drought stress tolerance in wheat genotypes.

Key words: Drought, wheat genotypes, water potential, osmotic potential, Proline

Introduction to Drought

Drought is a one of the major world-wide constrains which covers about 43% of world land to different degrees. It is estimated that about 45% of 120 million hectares under wheat cultivation in developing countries, are lying under drought (Rajaram, 2001). In Pakistan almost 15 million hectares of cultivated land are drought affected (Mujtaba & Alam, 2002). Wheat is one of the major food crops Pakistan and it ranks first among all the cereal crops. In Pakistan, it is cultivated on about 8,900,700 million hectares with annual production of about 25,214000 kgs (Sidra- FAO, 2012). Abiotic stresses such as high temperature, drought and salinity considerably affects yield of the wheat crop. World food security has big threats of increasing food demand and decreasing required water availability. It is clear that present and future wheat food security will face water shortage. As water is most essential for wheat at its every stage from germination to maturation, so, drought stress is a major threat to crop production (Chaves *et al.*, 2003). In the light of these all facts, drought stress tolerance has got high importance to face the increasing demand of food and crop improvement. However, understanding and selection of the biochemical and physiological basis of water stress tolerance in plants is important for breeding the crop (Chaves *et al.*, 2003).

There is no doubt that drought is harmful for plant growth (Garg *et.al.*, 2004; Samarah *et.al.*, 2004). It is clear that under drought stress less water is available which results in decreased total nutrient uptake and frequently reduced the concentration of mineral nutrients in crop plants (Baligar *et al.*, 2001). Under drought stress conditions plant species and

genotypes within a species perform differently and mineral uptake is also varies accordingly (Garg, 2003). As drought reduces 30-50% crop yield, a research project can be prepared to evaluate/screen wheat genotypes for growing under drought affected areas of world. Wheat genotypes will be brought under study to evaluate the accumulation of some organic compounds (glycine-betaine, proline and sugars) considering imparting drought resistance. Also activities of various enzymes can be determined, which influence metabolic processes like nitrogen metabolism, photosynthesis, etc. In reference to stress tolerance, physiological processes are mandatory for a successful breeding program and this type of study will provide a selective criterion for wheat germ plasm / mutant in respective of stress condition. This study will provide information to plant breeders in developing new high yielding varieties for drought prone areas. The productivity of wheat could be enhanced by studying the nature of adaptation of wheat to drought and to find out possible physiological traits that possess tolerance under water stress conditions. This is essential for evolving the best adapted and high yielding wheat varieties for drought areas of arid regions of the world.

Materials and methods

Water Potential (Ψ_w)?

It is a quantitative description of the free energy states of water. The concepts of free energy and water potential are derived from the second law of thermodynamics. Water potential is a useful measurement to determine water-deficit stress in plants. Scientists use water potential measurements to determine drought tolerance in plants, the irrigation needs of different crops and how the water status of a plant affects the quality and yield of plants.

Water Potential in Plants

$$Y_w = \Psi_P + \Psi_S$$

Simplified Definition of Ψ_w :

$$Y_w = \Psi_P + \Psi_S$$

Where,

Ψ_P = pressure potential

- represents the pressure exerted by the cell wall inside the cell

Ψ_S = osmotic or solute potential

- represents the effect of dissolved solutes on water potential; addition of solutes will always lower the water potential

Water Potential of Plant Tissue has two components and is always negative

- Pressure Potential

+ Positive \longrightarrow Turgor (in cells with membranes)

- Osmotic or Solute Potential

- Negative

Water potential of pure water

- $\Psi_W = 0$ MPa

Pure H₂O, has no potential at all.

Leaf Relative Water Contents (Turner (1986)).

2nd leaf is excised from each plant and fresh weight is recorded. After taking fresh weight, all the leaves are immersed in distilled water for 10 h then saturated weight of each leaf recorded. Samples are then oven dried at 70°C for 48 h and dry weight determined.

$$RWC (\%) = (Fwt - Dwt) / (Twt - Dwt) \times 100$$

Fwt = Fresh weight

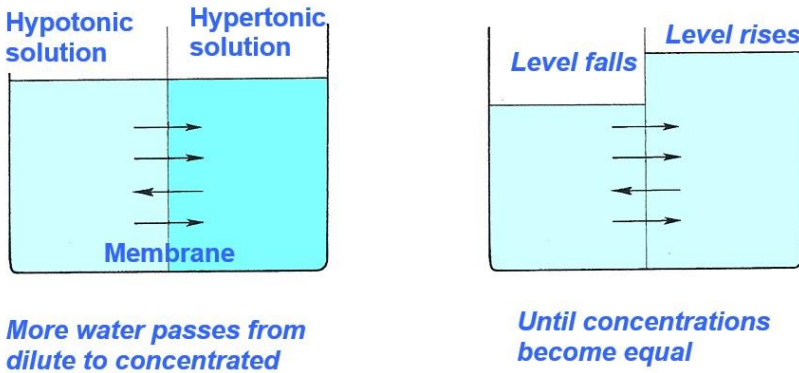
Dwt = Dry weight

Twt = Turgid weight

Osmotic potential

- **Osmosis** is the movement of water from a region of higher water concentration to a region of lower water concentration through a semi-permeable membrane.
- Hypotonic solution: More water, less solutes higher osmotic potential(less -ve values)
- Hypertonic solution: Less water, more solutes lower osmotic potential (More -ve values)

Osmotic potential: The potential of water molecule to move from a hypotonic solution to a hypertonic solution



Measurement of osmotic Potential

Osmolality is a measurement of the total no. of solutes in a liquid solution expressed in osmoles of solute per kg of solvent.

Organic analysis:

In the organic analysis, we normally determine following compounds

- Proline
- Betaine
- Sugar
- Enzymes

Proline determination (Bates *et al.* 1973)

Proline, which increases proportionately faster than other amino acids in plants under water stress, has been suggested as an evaluating parameter for irrigation scheduling and for selecting drought-resistant varieties. The necessity to analyze numerous samples from multiple replications of field grown materials prompted the development of a simple, rapid colorimetric determination of proline.

Reagent

1.25 gm Nin-hydrin in 30 ml G.A + 20 ml 6 M orthophosphoric acid

3 % sulfosalicylic acid

Fresh leaves samples (1- 0.5 gm) homogenized in 10 ml of 3 % sulfo-salicylic acid, filtered and filtrate is saved

2 ml filtrate

+ 2 ml Nin-hydrin solution

+ 2 ml Glacial Acetic acid

Heat at 100 °C for 1 hour.

Cool in ice bath

+ 4 ml toluene

vertices for 1 min:

Remove chromophore phase

Read at 520 nm



The proline concentration was determined from a standard curve and calculated on dry weight bases as follows:

Proline (μ moles / g dry Fresh wt) = O. D. x C. F x T.D.F / (mol.wt of proline)

Where,

O.D. =	optical density (520 nm)
Mol. Wt. of proline =	115.5
C.F. =	Curve Factor
T.D.F =	Total dilution factor

Betaine determination (Grieve *et al.*, 1983)

The periodide method for quaternary ammonium compounds (QAC) analysis was modified to permit rapid screening of numerous replicate plant samples.

Reagent

A Potassium tri-iodide (KI₃) (7.5 gm Iodine + 10.0 gm KI) dissolved in 100 ml of 1 N HCL)

B 1-2, dichloro- ethane (must be stored at - 10 0C before use)

C 2.0 N HCL

Sample preparation

1.0 gm ground dried material or 1.0 gm chopped fresh material

+ 50 ml toluene water mixture (0.5 % toluene)

Shaken mechanically for 24 hr at 25 C

Filtered and volumed to 100 ml

1 ml filtrate

+ 2 ml 2N HCL

+ 0.5 ml KI₃ solution

Shaking in ice bath for 90 min.

+ 2 ml ice cooled water + 2 ml 1-2, dichloro ethane

Air passing or vertexing

Two layers formed

Lower yellow colour is taken (colour stable for 30 min)

Read at 365 nm

Total sugar (Riazi. A. Matruda.K. and Arslam. A. 1985)

Measurement methods currently employed in the determination of total sugar contents in wheat crop.

0.1 ml ethanol extract

+ 3 ml freshly prepared anthrone.

Heated at 97°C for 10 min

Cool in ice bath

Read at 630 nm

Formula = $(OD \times CF \times T.Dil / Mol.wt = \mu mole/gm)$

Enzymatic analysis:

Nitrate Reductase Activity (Bordon, 1984)

Enzymatic analysis is a useful tool to determine concentrations of some components. The enzyme is used as an analytical reagent to catalyze a specific reaction of the compound to be determined. The substrates, products, and rate of the reaction can be equated to the concentration of the compound. Products and coenzymes are typically measured.



Reagent

A 0.2 M Phosphate buffer (pH 7.5)
containing 0.02 M KNO₃

B 1 % Sulphanilamide in 3 N HCl

C 0.02 N (1- Naphtyl) – ethylene diaminedihydrochlorid
1 gm chopped leaves segments

+ 10 ml 0.2 M Phosphate buffer (pH 7.5) containing 0.02 M KNO₃

Incubate in dark at 300 C for 1 hr

1 ml of above

+ 0.5 ml 1 % Sulphanilamide in 3 N HCl

+ 0.5 ml 0.02 N (1- Naphthyl) – ethylene diaminedihydrochloride

Incubate at room temp for 20 min

Read at 542 nm

Photosynthetic parameters

Pigment contents (chlorophyll contents)

By Extraction method (classical method or Destructive Method)

CHLOROPHYLL DETERMINATION (Lichten Thaler, H.K. 1987.)

Take 1.0 gm fresh chopped leaves



Add 10ml 80% acetone



Freeze for 24 hours in Refrigerator



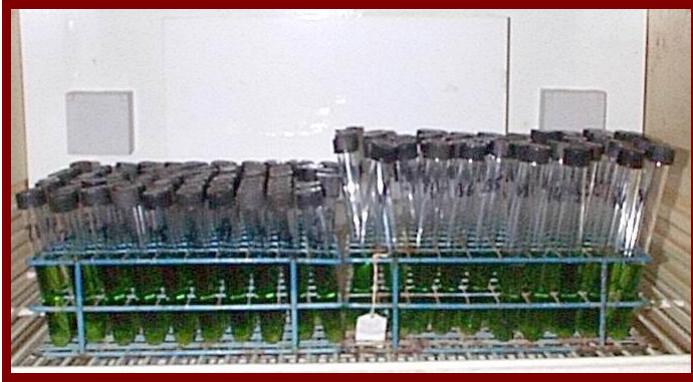
Read at 645 and 663 n.m.

Formula:-

Chlorophyll a: $[12.70 (\text{O.D } 663) - 2.69(\text{O.D. } 645)] \times V/w \times 1000$

Chlorophyll b: $[22.90 (\text{O.D } 645) - 4.68(\text{O.D. } 663)] \times V/w \times 1000$

Total Chlorophyll: $[20.20 (\text{O.D } 645) + 8.02(\text{O.D. } 663)] \times V/w \times 1000$



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