

MORPHOLOGICAL, BIOCHEMICAL ANALYSIS AND RAPD MARKER BASED ASSESSMENT OF GENETIC DIVERSITY IN TRITICUM AESTIVUM L. AND TRITICUM DURUM DESF. IN ZONE V OF RAJASTHAN - A REVIEW

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ABSTRACT

*Wheat is an annual plant belonging to the family Poaceae (Gramineae). It is used by human being in the form of flour for making various different products. It has been used in various diseases such as anemia, liver disorders, digestion problems and cancer related diseases. The objective of this study is to assess the morphological characteristics of germplasm of bread and durum wheat (*Triticum aestivum* L. and *Triticum durum* Desf.) respectively. Whole study is carried out in Zone V of state Rajasthan, India. Ten varieties of wheat were planted under field condition and observe their morphological characters i.e. Days to heading, Days to maturity, Plant height, Ear head sq.m^{-1} , Spike length, Grain spike $^{-1}$, Weight of seed spike $^{-1}$, Texture, Thousand Kernel Weight (TKW), Grain yield and Hectoliter weight. Study reveals that this cereal plant being from used ancient times contains various primary metabolite i.e. protein, sugar, starch, lipids, phenol and secondary metabolites i.e. Flavonoids. Another objective of the present study is to assess the genetic diversity in wheat germplasm by using RAPD marker technique and to make available information about genetic similarity among the genotypes grown in various parts of Rajasthan state, which would be useful to wheat breeder or researcher.*

Keywords: *Flavonoids, Morphology, Metabolite, RAPD, Wheat*

I INTRODUCTION

In India, Wheat is the main staple food crop of the population after rice. The major wheat producing countries are China, India, USA, Russia, France, Canada, Australia, Pakistan, UK, Turkey, Iran and Italy. These countries contribute about 76% of the total world wheat production. Wheat offers a great wealth of material genetically studies due to its wide ecological distribution and enormous variation encountered for various morphological and physiological characters (Rangare *et al.*, 2013). Wheat is consumed as chapatti, bread, flour, suji, maida, biscuit, cookies, macroni, pasta etc. Genetic diversity of plants determines their potential for improved efficiency and hence

their use for breeding, which eventually may result in enhanced food production (Ormoli *et al.*, 2015). Most of the morphological characters in crop plants are quantitative in nature. Yield is one such character that results due to the action and interactions of various component characters. Direct selection for grain yield could be groundless; therefore, knowledge of the genetic variability in grain yield is helpful for making effective plant selection. By approaching the limits of biological productivity of wheat in the recent years has greatly increased the need of new initial material (Hailegiorgis, 2011; Graybosch and Peterson, 2010; Lanning *et al.*, 2010). Grain based foods like wheat which provide complex carbohydrates, is the best fuel for our bodies. Wheat is high in fiber, low in fat, full of vitamins (i.e. Thiamin, Riboflavin, Niacin) Folic acid and meager quantity of iron too. Wheat is used as so many other products that we use as straw particle board (wood) - used for kitchen cabinet, Hair conditioner, Milk replacer, Adhesives on postage stamps, water soluble ink, medical swabs, charcoal etc.

1.2 Taxonomic Position

Kingdom	: Plantae
Sub Kingdom	: Tracheobionta
Super Divison	: Spermatophyta
Division	: Mangoliophyta
Class	: Liliopsida
Sub Class	: Commelinidae
Order	: Cyperales
Family	: Poaceae (Gramineae)
Genus	: <i>Triticum</i>
Species	: <i>aestivum</i> and <i>durum</i>

English name- Wheat

Hindi name- Ganhua

Sanskrit name-Godhum



Fig. 1

The present study is designed to work out status of genetic variability among ten wheat genotypes at field experiment conducted during *Rabi*, 2015-16 and 2016-17. The observation were recorded on eleven different traits viz. Days to heading, Days to maturity, Plant height, Ear head sq.m^{-1} , Spike length, Grain spike $^{-1}$, Weight of seed spike $^{-1}$, Texture, Thousand Kernel Weight, Grain yield and Hectoliter weight.

II STUDY AREA and METHODOLOGY

The present investigation carried out in the field experimentation at farmer's field at village Dhakarkheri, Kaithoon Road of Kota District (Rajasthan), during the session of *rabi* 2015-16 and 2016-17. Experimental materials of studies comprised of 10 wheat varieties from Agriculture Research Station, Ummedganj, Kota viz. five genotypes of

Triticum aestivum L. (Raj 4037, Raj 4238, GW 322, GW 366, HI 1544) and five genotypes of *Triticum durum* desf. (Raj 6560, MPO 1215, HI 8498, HI 8737, HD 4728) sown in Randomized Block Design with three replications, 6 m X 1.20 cm gross Plot area, six rows were sown in 23 cm apart between row to row. A brief narration of the Pedigree and origin of these wheat genotypes is depicted in table 1.

TABLE 1 List of wheat genotypes with pedigree used in present investigation

S. No.	Name of Varieties	Centre of Origin	Year of Release	Pedigree	Description of variety
1	Raj 4037	SKNAU, Durgapura (Rajasthan)	2003	DL788-2/RAJ3717	IR, TS
2	Raj 4238	SKNAU, Durgapura (Rajasthan)	2016	HW2021/RAJ3765	IR, LS
3	GW 322	JAU, Junagarh (Gujarat)	2002	GW173/GW196	IR, TS
4	GW 366	JAU, Junagarh (Gujarat)	2006	DL802-3/GW232	IR, TS
5	HI 1544	IARI, Indore (M.P.)	2008	HINDI62/BOBWHITE/CPAN2099	IR, TS
6	Raj 6560	SKNAU, Durgapura (Rajasthan)	2005	TOPDY6	IR, TS
7	MPO 1215	JNKVV, Powarkhera (M.P.)	2009	GW1113/GW1114//HI8381	IR, TS
8	HI 8498	IARI, Indore (M.P.)	1999	RAJ6070/RAJ911	IR, TS
9	HI 8737	IARI, Indore (M.P.)	2014	HI8177/HI8158//HI8498	IR, TS
10	HD 4728	IARI, New Delhi	2016	ALTAR84/STINT//SILVER_45/3/SOM AT_3.1/4/GREEN_14//YAV_10/AUK	IR, TS

*IR- Irrigated, TS-Timely sown, LS-Late sown

The observations will be recorded on five randomly selected competitive plants in each entry of each replication for all the characters except for days to 75% flowering and days to maturity, which will recorded on plot basis. Analysis of variance suggested by Panse and Sukhatme (1967).

III MORPHOLOGICAL METHODS

Five competitive plants will be randomly selected from each replication in genotypes for recording observation. The selected plants will be tagged and data on individual plant will be recorded for the following characters:

3.1 Days to Heading: It is calculated as days taken from sowing to emergence of 75% of ears (spikes) in a plot.

Observation on off-type plants should not be considered.

3.2 Days to Maturity: Total days taken from sowing to maturity when all plants in a plot show natural senescence and the grains become hard and fit for harvesting.

3.3 Plant Height: Measured at the time of maturity in centimeters from the ground level upto the terminal spikelet.

Care should be taken to record the measurement from the most commonly representative plants in the plot.

3.4 Ear head sq. m⁻¹: Total ear's in one sq. m area.

- 3.5 Spike length (cm): Length of spikes of five representative spike heads from base of spike to top of spike awns from each plot were measured and averaged to record mean length of spike in c.m.
- 3.6 Grain Spike⁻¹: Five representative spike heads selected from sample rows of each plot will threshed, winnowed and their grain number counted and averaged to record mean number of grain per spike.
- 3.7 Weight of seed spike⁻¹: Representative ear head selected from sample rows of each plot will threshed, winnowed and their grain weighed in grams and recorded as weight of seed per spike.
- 3.8 Texture: Hardness of matured seed (Soft, Semi Hard and Hard).
- 3.9 Thousand kernel weight: A small seed sample will taken from the produce of each plot harvested and 1000 seeds were counted, weighed in grams and recorded as test weight or TKW.
- 3.10 Grain Yield: The sun dried bundles from each plot will thresh, winnowed and the grain so obtained will weigh with physical balance to record seed yield per plot.
- 3.11 Hectoliter weight: Hectoliter weight usually determines the plumpness of the grain. Flour yield increase and flour ash decrease with the increase in hectoliter weight. Kernal size and shape are the most important features which influences the overall hectoliter weight of the grain.

IV BIOCHEMICAL ANALYSIS

4.1 Primary Metabolite

Biochemical study of the plant is a necessary prerequisite in order to evaluate their importance in the overall metabolism of the plant.

- 4.1.1 Protein Content (%): Protein is a fundamental quality test of wheat since it forms the basis for payment to farmers and is related to its end-product processing potential. Protein level is an estimation based on the amount of nitrogen present in the grain. It is important parameter for making different products of bread wheat. The protein requirement are >12.0%, 10.0-12.0 and <10.0% for making good quality bread, Chapatti and biscuit respectively. Protein content will be estimated using the spectrophotometer (Lowry *et al*, 1951).
- 4.1.2 Total soluble Sugar & Starch: The estimation of total soluble sugar & starch test will be done by phenol-sulfuric method (Dubois *et al*, 1951).
- 4.1.3 Lipids: Lipids will estimated by the method suggested by (Jayaraman J., 1980).
- 4.1.4 Total phenols: Total Phenol content in each sample will evaluate the method suggested by Bray & Thorpe, 1951.

4.2 Secondary Metabolite

Generally, phenolic compounds are categorized as phenolic acids, flavonoids, stilbenes, lignans, coumarines and tannins (Slavin JL 2000) However, the most abundant phenolic compounds found in cereals are phenolic acids and flavonoids (Zilic S *et al.*, 2011). The flavonoid pigments are usually yellow in color and are widely distributed in nature. They are difficult to separate and purify by crystallization due to their similar solubility relationships and their tendency to form mixed crystals. (Gage *et al.*, 1948). Hence flavonoids are polyphenolic secondary plant

metabolites which impart color and flavor to the plant foods. Many of these compounds play a protective role in the plants and are biochemically active. Flavonoids have been regarded both for nutritive purpose as vitamins and also as anticarcinogenesis. (Rusznayak S *et al.*, 1936)

4.2.1 Flavonoids

According to observations and experiences man has learned that some crops/plants have capacity to protect humans from various disease including rapid as well as chronic ones. Herbal medicine is widely practiced throughout the world from long time as these medicines are safe and environmental friendly. According to the WHO fact sheet, 2008, “In some of the Asian and African countries, “near about 80% of the world's population depends upon traditional system of health care”. Genus *Triticum* has been traditionally known to have several medicinal properties to treat various disorders. It has been used as a general purpose health tonic for several years. It is used to treat a number of conditions including the thalassmia, hypolipidemic, hypoglycemic, ulcers, cleansing, fissures, injuries, cuts and wounds. Young leaves of cereal plant were used in ancient times. In folk medicine, practitioners used wheatgrass for the treatment of cystitis, arthritis, rheumatic pain, chronic skin disorders and constipation. It also used in many of the ailments such as anemia, liver disorders and cancer related diseases. They are mostly rich in antioxidant properties. Whole grain of wheat, primarily their aleurone layer, germ and bran are abundant sources of phytochemicals including carotenoids, vitamin E, Phenolic compound. Lignans, β -glucans, sterols and starch etc. He & Giusti (2010) revealed that flavonoids acted as antioxidants, possessed anti-inflammatory, anti-carcinogenic activity and diabetes alleviation properties and could be associated with cardiovascular disease prevention and obesity control. However, so far very little work has been done on the activity of these plant extracts. The study showed a high level of flavonoids, keeping these in view, the plant extracts rich in flavonoids can be used in treating the diseases such as cancer, stomach ailments, anemia and other blood related diseases that result mainly due to the free radicals generated in the body. Further, we plan to study the component flavonoids present in the sample using the gas chromatography-mass spectrometry (GC-MS).

V. RAPD MARKER TECHNIQUE

The RAPD is the fast technique, easy to perform and comparatively cheap. RAPD is a PCR based technology. Random Amplified Polymorphic DNA (RAPD) marker estimation will be done by (Williams *et. al.* 1990). Molecular marker are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification in the earlier stages of plant development. Among the different DNA marker types RAPD, RFLP, SCAR, AFLP etc., Random Amplified Polymorphic DNA (RAPD) marker have frequently been used for genetic analysis (Langridge *et al.*, 2001) due to its simplicity, efficiency and non requirement of sequence information. RAPDs have been widely used for identification of genotypes in crop plants, for investigating the genetic variability within species and to show

relationship among populations (Freitas *et al.*, 2000) Genetic diversity can be accessed from pedigree analysis, morphological traits or using molecular markers (Pejic *et al.*, 1998)

In 1990, William *et al.* has indicated that Random Amplified Polymorphic DNA (RAPD) technique has not limited to single copy sequence but also amplify fragments from repetitive region of genome, has been effectively used for the evaluation of genetic diversity in diploid, tetraploid and hexaploid wheat. The technique has better resolving power than the morphological characteristics. Molecular marker are useful tools for estimating genetic diversity as these are not influenced by environment, are abundant and do not require previous pedigree information (Bohn *et al.*, 1999) but there are some advantages and limitations of this powerful techniques.

5.1 Advantages

- It has many advantages over other techniques. It can be used with uncharacterized genomes and can be applied to cases in which only small quantities of DNA are available.
- Absolutely no knowledge of the target genome is required. It can be used on any DNA sample.
- Even if the same primer is used with different samples, they will produce different results and different bands patterns that may allow for recognition of the various strains.
- RAPD is an inexpensive technique; it will be used to study genetic polymorphism between closely related species and also used to select variants of microbial isolates.

5.2 Limitations

- The primer targeting is random, it is absolutely essential to have a large genome template.
- The primer sequence has to be right to produce the right results.
- The quality of DNA used in the technique, concentration of PCR reagents, their purity and the conditions in which the reaction is carried out will all affect the results.
- RAPD requires knowledge and careful operation for good results and for these results to be reproducible.
- Mutation of target DNA can give wrong profile.

VI. CONCLUSION

The present research provided information on genotype characteristics studied and its grouping. The ample availability of information on this plant facilitates the study on it since ages various parts of this plant are being used for medicinal use. In present years, traditional as well as pharmacological uses of plants products received much attention because it is believed that they are safe for human use. So, these are necessary approaches in search of new treatment of diseases. Flavonoids also exhibited a high level of antioxidant properties. So, we plan to study the component flavonoids present in the sample using the gas chromatography-mass spectrometry (GC-MS). This work will surely help to increase the bioactivity of wheat by specificity.

The genetic diversity study of wheat material obtained from diverse regions of country by RAPD marker techniques will support breeders to enhance the genetic variation of breeding accessions and utilizing the studied wheat resources further effectively.

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