STUDIES ON BIOCHEMICAL QUALITY PARAMETERS OF WHEAT AS INFLUENCED BY LOCATION

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1. INTRODUCTION

Wheat the “versatile cereal food” is also described as the “stuff of life” or “king of cereals”. It continues to retain this coveted place with the roots ramifying in to the depth of human culture with evolutionary history parallel with the history of human civilization itself. Even today, it is the most important cereal crop in the world (Pena *et al.,* 2006) due to its feeding bowl to mankind. It is grown across a wide range of environments around the world and has the broadest adaptation. It is number one food grain consumed directly by human beings and the data suggest that more than 35 per cent of the world population depends on wheat (Borlaug, 1968 and Johnson *et al.,* 1978) as it supplies more nutrients than any other single crop (Ranum *et al.,* 1990). Besides fulfilling a nutritional role, a whole wheat diet has been shown to reduce the incidence of major human diseases such as diabetes, cardiovascular disease and cancer (Kritchevsky, 1999; Astrog *et al.,* 2002; McIntosh *et al.,* 2003; Willcox *et al.,* 2004 and Qu *et al.,* 2005).

Major countries growing this crop include China, India, USA, Russia, Canada and Mexico. In the world, wheat crop occupies 215.26 million ha with the production of 584.76 million tones and an average yield of 2797 kg per ha. In India, wheat is grown over an area of 26.80 million ha with production of 69.35 million tones with an average productivity of 2586 kg per ha. The states like Uttar Pradesh, Madhya Pradesh and Punjab are important from the point of both area and production (Anon., 2007).

Wheat cultivation in Karnataka is unique, wherein all three cultivated species *viz., Triticum aestivum*, *Triticum durum* and *Triticum dicoccum* are grown in typical hot tropical climate, characterized by the prevalence of high temperature during the crop growth. The area under wheat is about 2.23 lakh ha with the annual production of 1.25 lakh tones but the productivity is very low (564 kg/ha) as compared to national average.

The quality of wheat is largely dependent upon its chemical composition which is influenced by genetic and environmental factors and processing conditions. Consumers prefer wheat products with good palatability, good appearance and nutritive value.

Wheat is the principal source of energy, protein and dietary fiber for major portion of the world’s population (Abdel-Aal and Hucl, 2002). Wheat contains higher amount of protein than other cereals. Wheat protein quality mainly depends upon protein content and the balance of amino acid composition in the wheat grain (Li and Zhang, 2000 and Liu *et al.,* 2002).

Protein is an important quality parameter that decides the suitability of wheat for a particular type of product. Fractionation of wheat protein on the basis of solubility gives four types of components *viz.,* gliadin, glutenin, albumin and globulin. Gliadin (Monomeric) and glutenin (polymeric) together form a characteristic substance called ‘Gluten’ with water (MacRitchie, 1994 and Sapirstein, 1996). The unique feature of the wheat grain, *i.e.,* doughing property which is responsible for making wheat the most important source of protein in the human diet.

Glutenins confer elasticity to dough, whereas viscous gliadins give extensibility to dough (Payne *et al.,* 1984). More hydrophobic gliadins (g-gliadins) increase the volume of bread loaf, while gliadins from more hydrophilic part of the electrophoretic spectrum (□-gliadins) decrease the volume of a broad loaf (Van Lonkhuijsen *et al.,* 1992).

The amino acid composition of wheat protein is very unbalanced, especially for the total essential amino acid content, which is only 42 per cent of egg and milk protein (Zhai, 1991). The content of proteins and amino acids decrease due to the higher temperature generated during milling. Lysine, threonine and isoleucine are the main limiting amino acids in wheat. The lysine content of bread was reported to decrease with increase in temperature (Khadar and Dave, 1996).

Carbohydrates are the most abundant constituents of wheat kernel, forming about 83 per cent of the dry matter. Wheat contains starch, soluble sugar (2%) and cellulose (2-3 %). Starch is the major constituent of wheat endosperm. The main by-product of the oil extraction process is a defatted wheat germ meal, which has relatively high protein content (~30%) and high amount of essential amino acids. Wheat lipids have a high proportion of unsaturated and nutritionally important essential fatty acids. In whole wheat seed, linolenate, palmitate and oleate comprise approximately 96 per cent of the fatty acids. Common bread wheat grown under irrigated condition shows higher fat content as compared to those grown under dry conditions.

The minerals of wheat flour are not quantitatively large but may have considerable effect on the quality and behavior of the flour. The percentage of minerals present in flour usually gives an indication of grade of the flour (Ereifei and Shibli, 1993; Grover *et al.,* 1994 and Sangha *et al.,* 1998).

Phenolic compounds are major secondary metabolites (Klepacka and Fornal, 2006) and occur in either free or bound form in wheat grains (Krygier *et al.,* 1982; Sosulski *et al.,* 1982 and Abdel-Aal *et al.,* 2001). The phenolics in wheat grains are primarily derivatives of benzoic acid and cinnamic acid (Kim *et al.,* 2006).

Most of the scientific studies related to *T. dicoccum* wheat reveal that they are nutritionally superior over *T. aestivum* and *T. durum* wheat (Yenagi *et al.,* 1999) with respect to high protein and dietary fiber contents. The *T.dicoccum* based products are tastier, soft and have high satiety value. These products have low digestibility, low glycemic value and has been considered as a therapeutic food in the management of diabetes.

Wheat quality is partially determined by genetics, cultural environment and its interaction with the genotype (Busch *et al.,* 1969). The environmental effect is often larger than the genetic effect on wheat quality (Peterson *et al*., 1992). Such effects may include soil type, fertilizer level especially nitrogen (Paredez-Lopez *et al*., 1985 and Luo *et al*., 2000), distribution of rainfall level (Faridi and Finlay, 1989) and late season factors (Lookhart and Finney, 1984). In certain regions, elevated temperature during grain filling is possibly the most important environmental determinant of grain quality (Randall and Moss, 1990). High temperature during grain filling, especially greater than 35^0C, alters the protein biosynthetic pathways of grain, leading to protein compositional changes (Blumenthal *et al*., 1993).

Taking these facts into consideration, the present investigation was carried out to evaluate the biochemical parameters of *T. aestivum, T. durum* and T. *dicoccum* wheat varieties, which are classified in to different classes, exhibit different types of applications and differ in quantity and quality. The seeds were collected from two different locations *i.e*., Dharwad and Arabhavi. The *T. aestivum* varieties taken up for study were DWR-162, Raj-1006, GW-322, *T. durum* varieties were DWR-1006, MACS-2846 and NIDW-295 and *T. dicoccum* varieties were DDK-1009, DDK-1029 and NP-200 recommended for cultivation in peninsular zone with the following objectives:

1. To study the variation in biochemical quality parameters in different varieties of cultivated species of *T. aestivum, T. durum* and *T*. *dicoccum* wheat,

2. To study the variation in the micronutrient content of grains in three cultivated species of wheat and

3. To study the protein banding pattern of cultivated species of wheat grown under different environmental conditions.

2. REVIEW OF LITERATURE

Wheat is the world's most widely cultivated food plant which is used for the production of a wide range of food products. The quality evaluation of wheat grain varies with the genetic, agronomical and environmental factors on one hand and with the technology and processing on the other. Wheat is the staple food for majority of population in the world. The availability of nutrients from a particular food depends on its chemical composition. Available scientific information and pertinent literature on chemical composition of different wheat varieties are reviewed in this section.

2.1 Physicochemical characteristics of wheat and nutritional quality of wheat

Physicochemical characteristics of wheat is one of the important parameters which decides the quality of wheat in the field itself. It includes color, size, shape and weight of the grains. Apart from good physical characteristics of the grains, nutritional quality *i.e*., nutrient composition and availability of nutrients to the body are also very important.

2.1.1 Wheat varieties

Haridas Rao *et al.* (1976) studied the physicochemical characteristics of 24 Indian and three Canadian *T. durum* wheat varieties. The range values for crude protein (%), wet gluten (%) and pigments (ppm) were 10.60 to13.27, 28.20 to 34.3 and 2.72 to 4.56, respectively for Indian wheat, whereas for Canadian wheat the values were 13.26 to16.80, 40.60 to 52.60 and 4.87 to 6.47 ppm, respectively.

Bakshi and Bains (1984) studied the physicochemical characteristic of 27 *T. durum* and four bread wheat cultivars. The average protein content (%) of *T. durum* varieties was 11.80 which exceeded the bread wheat content by 1.30 per cent. The average pigment content of the *T. durum* flours was 5.30 ppm which far exceeded that of the bread wheats (3.60 ppm).

Henry (1985) analysed the concentrations of sugar soluble in 80 per cent ethanol in wheat, malt rye, rice, barley, oat and triticale. Wheat had 1.57 per cent soluble sugar. The highest concentration of sugar was found in wheat (10.30%) followed by rye (2.62%), triticle (2.37%), barley (1.93%) and oat (1.10%) and the lowest in rice (0.63%).

Physicochemical characteristics of 10 *T. durum* and two bread wheat cultivars were studied by Bakshi and Bains (1987). The mean ash (%), □-carotene (ppm), reducing and non-reducing sugars (%) of *T. durum* and bread wheat flours were 0.65 and 0.45; 3.90 and 1.80; 0.36 and 0.27 and 1.80 and 1.50, respectively.

Li and Posner (1989) determined the physicochemical characteristics, *viz.,* protein and ash content of each hard white winter (HWW) and hard red winter (HRW) wheats. The protein and ash content (%) of HWW wheat were 12.80 and 1.50, respectively and 13.00 and 1.39, respectively in hard red winter wheat.

Vatsala and Haridas Rao (1990) studied physicochemical characteristics of three *T. dicoccum* wheat varieties in comparison with three Bread and three *T. durum* wheat varieties. The mean total protein, ash and □-carotene contents of *T. dicoccum* wheats were 11.8 and 1.6 per cent and 2.72 ppm, respectively whereas *T. durum* wheats had 11.6 and 1.5 per cent and 3.16 ppm, respectively and Bread wheats 10.8 and 1.4 per cent and 2.44 ppm, respectively.

Hira *et al.* (1991) analyzed 19 wheat varieties for crude protein, available lysine, Ca, P, phytate, polyphenols, trace element and ionizable Fe. The mean values for crude protein and available lysine were 11.7 per cent and 2.43 g per 16 g N, respectively. Mean P, phytate, phenols, Ca, Fe, ionizable Fe, Mn, Cu and Zn were 430, 214, 274, 39, 8.2, 3.1, 4.6, 65, 4.25 mg per 100 g, respectively. Protein content was not found to be associated with phytate or phenol content.

Qualitative aspects of 14 new bread and four *T. durum* wheat cultivars were assessed by Nannor *et al.* (1995). Protein content was the highest in *T. durum* wheat. The □-

carotene content was higher in *T. durum* wheat. Bread wheat cultivars were reported to be superior over *T. durum* cultivars in water absorption capacity.

Piergiovanni *et al.* (1996) evaluated the chemical composition, □-carotene and gluten content and carried out SDS test in 50 accessions of *T. dicoccum* wheat varieties and compared with three *T. durum* wheat varieties. *T. dicoccum* had higher amount of mean protein (16.7%), fat (2.0%), dry gluten (10.9%) and SDS value (24 ml) than *T. durum* wheat varieties (14.1%, 1.53%, 7.5% and 32.33 ml, respectively). The mean □-carotene content was lower in *T. dicoccum* wheats (2.9 ppm) than *T. durum* wheat (4.66 ppm).

Reddy (1996) observed variation in total sugar content of *T. dicoccum* (1.85 %), *T. durum* (1.45 %) and *T. aestivum* (1.39 %) varieties and also reported that non-reducing sugar content of *T. durum* and *T. dicoccum* was higher than that of *T. aestivum* varieties.

Eleven *T. dicoccum*, one *T. durum* and one Bread wheat variety grown at eight locations were evaluated for physicochemical characteristic. Wheat grown in Ugar region had higher mean protein content (13.47%). Highest mean □-carotene content was found in Arabhavi (5.19 ppm) region (Anon., 1998).

Patil (1998) studied the physicochemical characteristics of three varieties of *T. dicoccum* wheat, two varieties of *T. durum* and one variety of *T. aestivum* wheat. The protein content (%) of *T. dicoccum* wheat vareied from 13.28 to 16.29 in *T. durum* 9.24 to 12.31 and in *T. aestivum* 15.29. Fat and ash content (%) of *T. dicoccum* ranged between 1.93-3.80 and 1.80-2.26, *T. durum* wheat 2.52-4.21 and 2.12-2.26, respectively and in *T. aestivum* wheat 2.15 and 2.46, respectively.

Reddy *et al.* (1998) studied the physicochemical characteristics of three varieties of *T. dicoccum*, two varieties of *T. durum* and two varieties of *T. aestivum. T. dicoccum* varieties were relatively rich in protein (12.5-13.7 %), total sugars (1.6 – 2.0 %) and non-reducing sugar content (1.1-1.8 %) and *T. durum* were rich in fat (1.6-1.7 %) and ash (1.8-1.9 %).

2.2 Important quality parameters of wheat

The quality of wheat is largely dependent upon its chemical composition which is influenced by genetic and environmental factors. Wheat is the principal source of energy, protein and dietary fiber in the diet. Protein content is an important quality factor that decides the suitability of wheat for a particular type of product. *i.e.* bread, spaghetti, pasta etc. Carbohydrates are the most abundant constituents of wheat kernel. Wheat flour contains a small amount of fat. The minerals of the wheat flour are not quantitatively large but may have considerable effect on the quality and behaviour of the flour.

2.2.1 Protein

Fathey and Michel (1987) studied accumulation of a high molecular weight protein during cold hardening of wheat. Soluble protein fractions from cold-tolerant winter wheats and cold-sensitive spring wheat were analysed. Single and two-dimensional polyacrylamide gel electrophoresis (PAGE) analysis revealed that cold hardening conditions include changes in the soluble protein patterns. The most important observation was the accumulation of a high molecular weight protein in the range of 200 KDa. The intensity of three protein bands (mol.wt 42, 47 and 48 KDa) increased while that of five others (mol. wt. 63, 67, 80, 89 and 93 KDa) decreased during hardening.

Singh *et al.* (1990a) studied total proteins from very strong wheat flour which were completely extracted without chemical reduction of disulfide bonds by applying mechanical shear with an ultrasonic probe in 2 per cent sodium dodecyl sulfate (SDS) solution at pH 6.9. Sonication was more efficient and hence required much less time to achieve complete extraction of proteins. The total unreduced flour proteins were fractionated in to three distinct peaks of decreasing size range, mainly glutenin, gliadin and albumin-globulin, respectively.

He and Hoseney (1992) observed the doughs containing more protein (11.5%) expanded at a faster rate than those containing less protein (7.0%).

Peltonen *et al.* (1994) reported that nitrogen fertilizer application improved bread making quality of wheat flour, mainly by increasing the quantity of low molecular weight

gliadins. The most positive effect of flour protein concentration and loaf volume was obtained with the application of granular, dicyandiamide – regulated, slow release nitrogen fertilizer.

Yves *et al.* (1998) observed that wheat storage proteins are responsible for the viscoelastic properties of dough. Their effect on dough rheology depends on the glutenin components and the proportion of gliadins, high and low molecular weight glutenin subunits.

James e*t al.* (2001) evaluated the multilocation yield potential, adaptation and end product quality of newly developed winter *T. durum* wheats. Seven winter *T. durum* lines, including 'Connie' and 'Stephens' were grown over six locations in 1999-2000 and planted at the same sites for evaluation in 2001. At four locations the *T. durum* lines had grain yields very comparable to Stephens. Grain protein content varied from 9.0-15.5 per cent, depending on the location and N rate. At three of six locations, average grain proteins (13 %) per cent were achieved with N treatment. At the other three locations, *T. durum* protein did not exceed 12.5 per cent, even at the highest N level.

Ganibelli *et al.* (2002) reported a new wheat endosperm protein subunit that was found in accessions belonging to different collections and was identified by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. In the reduced form, it had a protein fraction, which could be estimated by SDS and it had a protein fraction, which could be estimated by SDS-PAGE. Fractions collected after SE-HPLC and further characterized by SDS-PAGE confirmed that this protein participates in the glutenin polymeric structure.

Nutritional and structural properties of proteins extracted from defatted wheat germ were studied by Zhu *et al.* (2006). Defatted wheat germ contained relatively high amount of protein (34.9%) and well balanced amino acid profile. The albumin fraction exhibited 19 major polypeptide bands in the SDS-PAGE pattern and globulin fraction showed four distinct polypeptide group bands.

Singh *et al.* (2007) studied wheat germplasm with resistance to leaf and stripe rust, karnal bunt, loose smut, root aphids and high protein content. Protein content in commercially grown wheat varieties in India varies from 9 to 11 per cent. HD 2819 had 12.74 per cent grain protein content on an average and its mixography characters indicated better mixing time along with suitably high loaf volume. This genetic stock was also found to possess high *Glu-I* score that matched with a popular wheat variety C306 which possessed very high chapatti making characteristics. The observations with respect to disease and pest resistance and quality characters recorded at multi locations over the years (2001-2004) indicated that HD 2819 was a novel genetic stock. Since HD 2819 was having less than 100 cm plant height and performed favourably under rain fed and timely sown conditions, its novelty was enhanced.

Wang *et al.* (2007) studied the distribution of protein composition in bread wheat flour mill streams and relationship with bread making quality. The gliadin content of total flour protein was negatively and significantly correlated with loaf volume. These results indicated that the quantity and composition of protein among the mill streams was different which resulted in differences in bread making quality.

Muhammad *et al.* (2009a) studied the assessment of genetic diversity among Pakistani wheat advanced breeding lines using Radomly Amplified Polymorphic DNA (RAPD) and SDS-PAGE. Seed storage protein analysis produced 19 subunits ranging between 29-120 KDa. Similarity coefficients varied from 0.53-0.1 for the normal seeding date and from 0.47-1.0 for the rainfed group. High molecular weight subunits (particulars 120 KDa) showed greater polymorphism than the low molecular weight subunits.

Muhammad *et al.* (2009b) evaluated wheat by PAGE. The overall results showed low degree of heterogeneity and different varieties revealed differential protein banding pattern.

2.2.2 Gluten

Wheat flour proteins have long been known to be crucial in relation to bread making quality. Both the fractions of gluten *viz.,* glutenin and gliadin have been described here under to exhibit the elastic and extensible characteristics of the dough.

Quality attributes and grain protein composition in 60 advanced lines of cross breed wheats were correlated by Cressay *et al.* (1987). The presence of High Molecular Weight

Glutenin Subunits (HMW-GS) and specific gliadins measured the grain quality especially resistance of dough to extension.

Bushuk (1987) studied the relationship between HMW-GS and bread making quality of four Canadian grown wheats. The HMW-GS composition showed potential for bread making quality.

Singh *et al.* (1990b) selected total unreduced proteins from flours of 15 wheat cultivars of diverse origin and bread making quality were fractionated by SE-HPLC. Quantity of glutenin was highly positively correlated with loaf volume and dough resistance. The proportion of glutenin and gliadin fractions, particularly glutenin had a direct effect on functionality because very little flour was required.

Skepritt *et al.* (1990) studied LMW-GS which were compared with the other major wheat gluten polypeptides, HMW-GS and gliadins. Antibodies with specificities for similar groups of gliadins were bound to similar groups of glutenins. Some were bound to each of the major gliadins, LMW-GS and HMW-GS but not to other grain proteins. The solubility and immunochemical similarities and the known linkage between the genes for LMW-GS and certain gliadins indicated that LMW-GS may be responsible for many biochemical properties and quality effects usually attributed to gliadins.

Zhuge *et al.* (1992) reported that ground pearled wheat (GPW) were comparable with conventionally milled flour (CMF). The gluten from both GPW and flour contained 75.2-76.4 per cent protein. No significant differences were noted in the yield and purity of starch from GPW and CMF.

Morel (1994) studied Acid-PAGE of wheat glutenins, a new tool for separation of HMW-GS and LMW-GS. After sequential extraction of gliadin with 50 per cent isopropanol, glutenins were extracted with the same slowest containing dithiocrythritol and alkylated with 4-vinylpyridine. By two dimensional electrophoresis (Acid-PAGE x SDS-PAGE), HMW-GS were identified at the top of the Acid-PAGE pattern in contrast to LMW-GS that moved further.

Berot *et al.* (1996) studied the technological properties of the wheat gluten protein fractions. Gliadin rich fractions increased the extensibility of the dough and reduced its resistance to deformation. Glutenin rich fractions had opposite effect and increased dough resistance more than that of equally concentrated whole gluten.

Czukajowska *et al.* (1996) studied the effect of HMW-GS composition of seven soft white winter (SWW) and four club wheat cultivars on dough rheology. The SWW and club wheat grown under same conditions showed similar protein content (11.70 and 11.65%), but distinctly different values of total area of HMW-GS (0.90 to 1.90) for SWW and 0.70-1.30 for club wheat.

Dupuis *et al* (1996) studied characterization of acetic acid soluble and insoluble fractions of Glutenin of bread wheat. Interaction of Glutenin with gliadin appeared to be related to the solubility of Glutenin in acetic acid solution; the greater the interaction the higher was the solubility.

Sapirstein (1996) determined a new method for fractionation of monomeric (albumins, globulins and gliadins) and polymeric (native unreduced) Glutenin proteins of wheat flour. The results obtained from this study confirmed the importance of both the soluble and insoluble polymeric glutenin in determining flour strength. The protein isolation procedure was recommended for isolating pure glutenin from gliadin-glutenin mixtures.

Quantitative effects of total amounts of HMW-GS on bread making quality characteristics in 13 hard red spring wheat cultivars was determined by Huang and Khan (1997). The strong dough group cultivars had lowest solubolization of gluten proteins in SDS-buffer and the poor loaf group cultivars had highest. The SDS-soluble proteins of the strong dough group contained a smaller amount of HMW-GS than those of poor loaf group. The highest proportions of HMW-GS in total flour protein were found in the strong dough group, while the poor loaf group had the lowest percentage of HMW-GS in their total flour protein. It showed that total quantities of HMW-GS played an important role in dough mixing strength and bread-making performance of hard red spring wheat.

Peter *et al.* (2002) observed the structure and properties of gluten, an elastic protein from wheat grain. They observed that wheat gluten proteins corresponded with the major storage proteins that were deposited in the starchy endosperm cells of the developing grain. One group of gluten proteins, the high molecular mass subunits of glutenin was particularly important in conferring high levels of elasticity. These proteins were present in high molecular mass polymers that were stabilized by disulphide bonds and were considered to form the 'Elastic backbone' of gluten.

Moore *et al.* (2006) observed that one of the main problems associated with gluten free bread was obtaining a good structure. Transglutaminase (TGase), an enzyme that catalyzes acyl-transfer reactions through which proteins can be cross-linked. It could be a way to improve the structure of gluten-free breads. Results indicated that it was possible to form a protein network in gluten free bread with the addition of TGase. The efficiency of the enzyme was dependent on both the protein source and level of enzyme concentration.

Meredith *et al.* (2008) observed that the storage of wheat grains at elevated temperatures increased solubilisation of glutenin subunits. Analysis by SDS-PAGE and subsequent protein identification revealed that the most evident change protein that occurred during storage at 30^0C was an increase in the content of HMW-GS in the soluble fraction.

Influence of starch and gluten characteristics on Rheological properties of wheat flour gel at small and large deformation was observed by Tomoko *et al.* (2008). Gel mixed with the isolated gluten from waxy wheat lines appeared to have a weaker gel structure in dynamic viscoelasticity and compression tests. Starch properties of wheat were reported to be primarily responsible for rheological changes in wheat flour gel.

Hetty *et al.* (2009) observed that removing celiac-disease-related gluten proteins from bread wheat while retaining technological properties in a study with Chinese spring deletion lines. The deletion lines were technologically tested with respect to dough mixing properties and dough rheology. The result showed that removing □-gliadin locus from the short arm of chromosome 6 of the D-genome resulted in significant decrease in the presence of T-Cell stimulatory epitopes but also in a significant loss of technological properties. However, removing of ω-gliadin, □-gliadin and LMW-GS loci from the short arm of chromosome 1 of the D-genome removed T-cell stimulator epitopes from the proteome while maintaining technological properties.

2.2.3 Lipids and carbohydrates

Wheat flour contains a small amount of lipid. Triacylglycerol constitutes one of the major fractions of wheat flour lipid. Wheat lipid has a high proportion of unsaturated essential fatty acids.

Carbohydrates are the most abundant constituents of wheat. They include starch which is the major constituent of wheat endosperm and very small quantity of soluble sugars.

2.2.3.1 Lipids

The contribution of flour lipid fractions to soft wheat baking in sugar cookies was assessed by Kissell *et al.* (1971). Cookies from defatted flours were significantly smaller in diameter and poor in appearance and lighter than normal colour. Polar and non-polar lipid fractions alone were partially effective in improving defatted flour and were also required for restoration of quality. The addition of one half the normal level of free lipid to the extracted flour produced, cookies with larger diameter greater top grain and more intense cookie colour.

Chung *et al.* (1982) investigated the relation of polar lipid content with mixing requirement and loaf volume potential of hard red winter wheat flour. The non-polar lipid ratio and the amount of polar lipid and lipid-galactose of either wheat or flour were more highly correlated with loaf volume than with mixing time. Significant correlation of loaf volume and the lipid content, when both were correlated to a constant protein basis, indicating there by that polar lipids were related largely to protein quantity.

Yongfen *et al.* (2009) studied policosanol (PC) content and composition of wheat varieties as affected by environment. PC is a mixture of high molecular weight aliphatic primary saturated alcohols which possesses cholesterol lowering properties. Grain samples were collected from three varieties grown at three locations. Total PC content and its

composition in whole grain samples were determined using a gas chromatography system. Within each location a significant variety effect was observed. There was also a significant location-variety random effect on PC content.

2.2.3.2 Carbohydrates

Martin *et al.* (1991) observed that bread firming and starch recrystallization were not synonymous, although both occurred during bread storage. Factors such as baking time and presence of shortening influenced the water hydration capacity of the bread crumb. Bread firming resulted from cross – links (hydrogen bonds) between the continuous protein matrix and the discontinuous starch granules.

Chen *et al.* (1992) examined the carbohydrates of hard red spring wheat gluten and reported the levels of carbohydrate in three fractions. SDS-insoluble, SDS-soluble but 70 per cent ethanol-insoluble and SDS and 70 per cent ethanol-soluble were 11.63, 1.27 and 0.5 per cent (w/w), respectively. The major sugars in the SDS-insoluble fraction were glucose and xylose, whereas arabinose, galactose and mannose were in lesser amounts. SDS-soluble fraction contained glucose, galactose and mannose as the major sugars. The ethanol – insoluble fraction contained more sugars than the ethanol – soluble fractions for each neutral sugar component.

Chilkunda *et al.* (2001) studied caubohydrate composition of wheat, wheat bran, sorghum and bajra with good chapati/roti making quality. In wheat, Arabinoxylans were the major polysaccharides, other than starch and cellulose. The ratio of arabinose to xylose in whole wheat flour and wheat bran was nearly 1.25:1 but the hemicellulose A in wheat flour was mainly xylan type. Hemicellulose A had more arabinose than xylose but bajra had arabinose and xylose in equal amounts. Contents of dietary fiber was highest in wheat bran compare to other cereals.

Jhuma *et al.* (2003) studied four north Indian bread wheat cultivars *viz.,* C-360, HD-2009, WH-291 and WH-542 differing in flour and cooking quality. The study revealed a progressive decrease of total sugars reducing and non-reducing sugars in the developing grains and increase in starch content throughout the grain development period. The crude protein and soluble protein also increased during grain development.

Liu *et al.* (2007) studied *in vitro* digestibility and physicochemical properties of A and B type starch from soft and hard wheat flour. Wheat starches with different granular sizes not only had different degrees of enzymatic hydrolysis and thermal and pasting properties, but also had different molecular characteristics. Different amylose and protein content and branch chain length of amylopectin in A and B type wheat starch granules could also be the major factors besides granular size for different digestibility and other functional properties of starch.

2.3 Other parameters which influences the wheat quality

The minerals of wheat flour are not quantitatively large but may have considerable effect on the quality and behaviour of the flour and also on the enzyme activity and total phenol content also influence the wheat quality.

2.3.1 Enzyme activity

Chamberlain *et al.* (1981) reported that increasing □-amylase activity resulted in loss of crumb mechanical strength and increased the amount of starch degration products in the bread and thus there was an increase in crumb stickiness.

Matsuo *et al*. (1982) observed the effect of α-amylase activity of *T. durum* wheat on spaghetti quality. High amylolytic activity of semolina was found to result in high amylolytic activity in spaghetti and increased amount of residue in the cooking water and the level of reducing sugars in both semolina and spaghetti were instrumental in making the products slightly soft.

Ariyama and Khan (1990) studied the effect of storage on physicochemical and functional properties of both newly harvested hard red spring wheats and the same wheats sprouted in the laboratory for 12 and 14 h. Control samples generally showed an increase in milling extraction and flour protein content, decrease in proteolytic activity and improvement in baking quality. Sprouted samples showed an increase in milling extraction, flour protein

content and α-amylase activity and decrease in proteolytic activity. The heavily sprouted samples showed smaller changes than the lightly sprouted samples during storage.

The spaghetti making quality of commercial *T. durum* wheat samples with variable α-amylase activity was studied by Dexter *et al.* (1990). They reported that cooked spaghetti stickiness, firmness and resilience were not related to α-amylase activity of semolina. There was no evidence that high α-amylase activity was detrimental to spaghetti storage stability as measured by strand strength and spaghetti cooking quality.

Borrelli *et al.* (1999) investigated bright color of *T. durum* wheat products, resulting out of carotenoid pigment and their oxidative degradation by lipoxygenase. The β-carotene and α-tocopherol were found to inhibit activity of semolina lipoxygenase and improve pasta quality.

Devin *et al.* (2008) observed the effectiveness of dry heat, steam and microwave treatments in decreasing lipase activity, while retaining antioxidant activity to stabilize whole wheat flour against lipid degradation during storage. None of the treatments significantly decreased antioxidant activity. No significant differences in acceptance were found between the control and the samples either at baseline or after storage. They concluded that whole wheat flour may be stabilized against lipolysis by utilizing the treatment without decreasing antioxidant activity.

Effect of variation in amylase activity and puroindoline composition on bread quality in a hard spring wheat population was studied by Martin *et al.* (2008). Puroindoline (pon) A-D1b allele conferring grain hardness and Wx-B1b allele conferring lower amylase activity, causing a partial waxy phenotype, Pin B-D1b mutation had significantly softer kernels, higher break flour yield and higher loaf volume than pin A-D1b mutation. Wx-B1b had significantly lower kernel weight, lower amylase activity and higher flour swelling power than Wx-B1a.

2.3.2 Phenol content

Abdul *et al.* (2007) identified and isolated the low phytic acid wheat inbred lines/ mutants in Pakistan. The selected lines were grown at five locations which differed in soil types and environmental condition to determine G x E interaction on phytic acid contents. Cultivar Parvaz-94 gave consistently low and mutant NRL-0431 gave the highest concentration of phytic acid across the location. The effects of genotypes, environments (locations) and their interactions on phytic acid content were all highly significant, with the location having the largest effect. The highly significant interaction between genotype and environment suggests that the correct evaluation of wheat germplasm by phytic acid content should be conducted in multi-environments. Pham *et al.*(2008) observed total phenolic compounds and antioxidant capacity of wheat graded flours by polishing method. The total phenolic and flavonoid contents of free and bound phenolic extracts gradually increased in the order from the inner to the outer fractions. The wheat flours obtained from the outer parts of grain contained significantly higher amount of phenolics and exhibited significantly higher antioxidant capacity than did the whole grain. The inner wheat flour fractions milled from endosperm part had significantly higher amount of phenolics and exhibited significantly higher antioxidant capacity than did the white flour, which was milled by a conventional milling method.

Total phenolics and antioxidant potential in plasma and urine of human volunteers after consumption of wheat bran was studied by Ruth *et al.* (2008). when compared with ground rice, wheat bran gave significantly (P- 0.05) higher plasma total phenolics at 1h and significantly (P- 0.001) higher plasma antioxidant potential from 0.5 to 3 h. Comparisons with data from a range of other phenolic-rich food indicated that wheat bran phenolics were relatively well observed and may enhance antioxidant status.

2.3.3 Minerals

Peterson *et al.* (1983) studied flour and bran of 27 wheat varieties for protein and mineral content. The variation in protein content (g/100 g) in flour ranged between 10.2 and 17.1, whereas mineral (μg/g) content was as follows: Mn, 4.6 and 6.9; Ca, 178 and 244; Fe, 8.2 and 12.2 and Zn, 6.0 and 8.7. The mineral content among the genotypes was significant and stable and showed a positive correlation with protein content. The concentration of all the minerals was higher in bran than in flour.

Kumar and Kappor (1984) studied the Ca and trace mineral composition of different varieties of cereals. The content of Ca, Mn, Cu, Fe and Zn (mg/100 g) of flour of different varieties of wheat varied from 54.72 to 88.80; 1.80 to 2.96; 0.54 to 0.67; 6.48 to 8.89 and 2.30 to 2.80, respectively.

Another element of importance in nutrition is Mn. It has been recognised as an essential mineral nutrient for acquisition of photosynthetic competence in higher plants. However, it is not required in bacterial photosynthesis in which there is no photosystem II (PS II). Mn binds to the chloroplast at two kinds of sites and PS II contains 3 Mn atoms for 200 chlorophyll atoms. Some enzymes like phosphatases and carboxylases are required for Mn^{2+} synthesis (Gardner *et al*., 1988).

Copper is found in primary and secondary minerals but occurs primarily in organic complexes. Cu plays a role in photosynthesis as a part of chloroplast enzyme plastocyanin in the electron transport system. Cu is part of several oxidases such as ascorbic acid oxidases and polyphenol oxidases. Moreover, species and cultivars show difference in tolerance to copper (Gardner *et al*., 1988).

Eriefeh and Shib (1993) tested 10 *T. durum* wheat cultivars for their mineral composition. The cultivars varied signficiantly in Na, K, Ca, Zn, Mn, Cu, Fe, Mg and P contents. The mean mineral content of *T. durum* cultivars was as follows: Zn (2.5 mg/100 g) and Mg (85.1 mg/100 g). Mn (6.5 mg/100 g), Cu (3.8 mg/100 g), Fe (10.8 mg/100 g), Ca (66.5 mg/100 g), Na (16.1 mg/100 g), K (641.3 mg/100 g) and P (26.5 mg/100 g), respectively.

Zinc deficiency causes increased susceptibility to oxidative damage of membrane functions of some tissues. Although increased oxidative stress was small, a significant component of pathologies was observed in cardiovascular diseases (Singh *et al*., 1998). Abdallah and Samman (1993) observed that Zn may act as a biological antioxidant and further observed that high levels of Zn could also be pro-oxidant by eliciting a decline in the activity of erythrocyte Cu-Zn superoxide dismutase.

Sangha *et al.*(1998) analysed 10 wheat varieties for their mineral and phytate-P content and molar ratios. The total mineral content (mg/100 g) of wheat varied as follows: P from 21.3 to 40, Ca from 6.8 to 9.2, Fe from 2.2 to 4.4 and Zn from 50 to 78, respectively.

Anjum *et al*. (2002) observed phytate and mineral content in different milling fractions of some Pakistani spring wheat. The results indicated that the concentrations (ppm) of different milling fractions for total minerals *viz.,* Cu, Fe, Mn and Zn varied from 5.0 to 52.50, 26.0 to 147.50, 5.0 to 97 and 9.0 to 80.8, respectively.

Butt *et al.* (2004) developed minerals–enriched brown flour by utilizing wheat milling byproducts. Brown flour was developed by incorporating different proportions of wheat bran at 0, 5, 10, 15 and 20 per cent in to the residual flour. The highest iron content was observed in wheat bran (64.6 mg/kg), whereas iron content in different treatments of brown flour varied from 16.8 to 29.2 mg per kg and phytic acid content varied from 0.72 – 1.09 g per 100 g in different flour treatments.

Zinc is an essential element for human health and well-being. It has a structural and functional role in a large number of macromolecules and is required for over 300 enzymatic reactions. Zn ions participate in all the aspects of intermediary metabolism, transmission and regulation of the expression of genetic information, storage, synthesis action of peptide hormones and structural maintenance of chromatin and bio-membranes. Zn is thus needed for growth and development, protein and DNA synthesis, neuro-sensory functions, cell-mediated immunity, thyroid and bone metabolism (Meunier *et al*., 2005).

Hajra *et al.* (2006) studied the yield and micro-nutrient content of bread wheat under mineral fertilizer – Hal-tonic. The results indicated that Cu, Fe, Mn and Zn content of leaf, straw and grain of wheat increased with the application of varying levels of Hal-tonic and mineral fertilizers. The effect was more pronounced where 10 kg per ha Hal-tonic was applied with recommended dose of NP fertilizer.

Gujral *et al.* (2008) examined the effects of added minerals on pasting of partial waxy wheat flour, and starch and on noodle making properties. Mineral extracts (15.3% ash) isolated from wheat bran, when added to increase the ash content of wheat flour and lowered the pasting temperature of flour by 13.2 to 16.3 per cent. It did not however affect the pasting

properties of the isolated starch added ash which increased noodle thickness and lowered water retention of cooked noodle while it exhibited no significant effect on cooked noodle texture.

Zhao *et al.* (2008) observed variation in mineral micronutrient concentration in grains of wheat lines of diverse origin. Bread wheat, 150 lines representing diverse origin and 25 lines of *T. durum*, Spelt, Einkorn and Emmer wheat species, were analyzed for variation in micronutrient concentrations in grains. Spelt, Einkorn and Emmer wheat appeared to contain higher selenium concentration in grain than bread and *T. durum* wheats. Significant differences between bread wheat genotypes were found for grain Fe and Zn, but not Se concentration; the latter was influenced more by the soil supply. Grain Zn, but not Fe, concentration correlated negatively with grain yield, and there was a significant decreasing trend in grain Zn concentration with the date of variety release, suggesting that genetic improvement in yield resulted in dilution of Zn concentration in grain. Zn and Fe concentrations also correlated positively and significantly with grain protein content and P concentration, but the correlations with kernel size and weight or bran yield were weak. The results from the study were thought to be useful for developing micronutrient bio-fortification strategies.

Iskander and Davis (2009) observed 19 different bread wheat varieties at 13 different locations in Egypt. Sixteen elements were measured by Instrumental Neutron Activation Analysis (INAA) method. The overall average concentration of these elements in the investigated breads were as follows (μg/g): Br- 5.06, Ca-326, Cl-0.95, Co-0.044, Fe-54.0, K-2086, Mg-692, Mn-11.6, Na-1709, Rb-1.6, Sb-0.01, Se-0.28 and Zn-11.5.

The reviews collected reflect different wheat varieties, biochemical parameters and environments. The literature survey is exhaustive keeping in view the objectives of the present investigation.

3. MATERIAL AND METHODS

The present investigation was carried out during 2008-09 at Wheat Improvement Project Field, Wheat Laboratory, University of Agricultural Sciences, Dharwad. The details of the material used and techniques adopted during the course of this investigation are described in this chapter.

3.1 Selection of wheat samples

Seeds of three different species. *T. aestivum*, *T. durum* and *T. dicoccum* were collected from two different locations *i.e*., Dharwad and Arabhavi.

Three different wheat varieties that are popular and recently released from each of the three species were selected for the study, the details of which are presented in the Table 1. These selected varieties from three species were grown during *rabi* 2007-08 at MARS, Dharwad and ARS Arabhavi representing the irrigated and rain-fed ecology with distinct agro-climatic conditions of transitional and dry zones, respectively. The seed samples were collected at one lot, cleaned, stored in closed bins and used for entire study.

3.2 Quality parameters of wheat

Samples selected for the study were milled in a laboratory model Willey Mill (0.5 mm) and used for the assessment of the following biochemical quality parameters.

3.2.1 Carbohydrates

3.2.1.1 Estimation of total, reducing and non-reducing sugars

The soluble sugars were extracted from the dried wheat flour sample by alcohol extraction. Five hundred mg of sample was placed in a conical flask. Ethyl alcohol (80%) was added to it and boiled for 5 min on a hot water bath. The contents were cooled to room temperature and the supernatant was transferred to a volumetric flask. The extraction was carried out thrice and the final volume was made up to 10 ml. Total sugars were estimated in alcohol free extract using Nelson-Somogyi's method. After hydrolyzing 1 ml of the alcohol-free extract with 1N HCl at 50-60^{0}C, it was cooled and neutralised with 1N NaOH followed by 0.1N HCl using phenolphthalein as an indicator. The neutralized extract was made up to 5 ml with distilled water.

To one ml of neutralized extract, one ml of alkaline copper reagent was added. It was kept on the boiling water bath for 20 min. After cooling, one ml of arsenomolybdate reagent was added with immediate shaking and the final volume was made up to 15 ml. Absorbance was measured at 510 nm in a spectrophotometer using standard curve of D-glucose against a reagent blank.

Reducing sugars were estimated in the alcohol-free extract using Nelson-Somogyi's method (Sadasivam and Manikam, 1992).

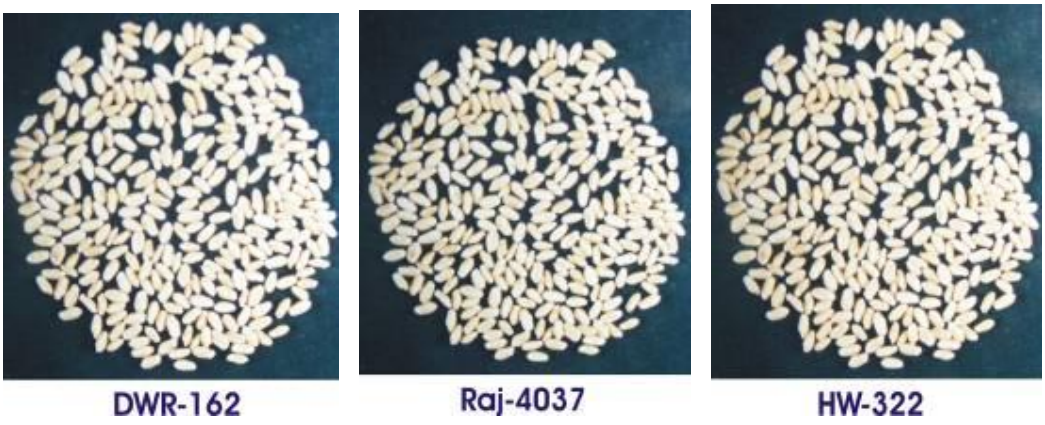
3.2.1.2 Total carbohydrates

Total carbohydrate content was estimated by anthrone method (Sadasivam and Manikam, 1992).

Table 1: Wheat varieties selected for the study

Varieties	Origin	Pedigree	Special features
T. aestivum			
DWR-162	Dharwad	Kavakaz/Buhol/ Kalyan sona/ Bob white	High grain yield, resistant to leaf stem and stripe rust diseases and heat tolerant (Irrigated condition)
Raj 4037	RAU, Durgapura	DL788-2 / RAJ-3717	High yielding resistance to all the three major rust and tough for threshing (Irrigated condition)
GW-322	GAU, Junagarh	PBW 173/GW 196	Uniform maturing, high yielding, high TGW, resistant to major races of rusts (Irrigated condition)
T. durum			
DWR-1006	Dharwad	DWL-5023/DON	High yielding, semi tall, tolerance to limited irrigation and multiple disease resistant and multiple disease resistant with diverse Sr. genes (Irrigated condition)
MACS-2846	ARI, Pune	CPAN 6079/ MACS 2340	High yielding, susceptible to leaf blight and uniform maturity (Irrigated condition)
NIDW-295	ARS, Niphad	BOOMER 33/ PLATA-8	High yielding superior over MACS-2846, high TGW and resistant to leaf blight (Irrigated condition)
T. dicoccum			
DDK-1009	Dharwad	NP 2004/ *NP-200 / ALTAR-84	Tolerant to leaf blight disease and high yielding (Irrigated condition)
DDK-1029	Dharwad	DDK 1012/HW-1093/ 276-15	Higher yield, resistant to brown and black rusts and spot blotch (Irrigated condition)
NP-200	IARI	Selection from Madhapalli local	Tall low yielding, high TGW, susceptible for lodging and most adopted (Irrigated Timely Sown)

T. aestivum



T. durum

T. dicoccum

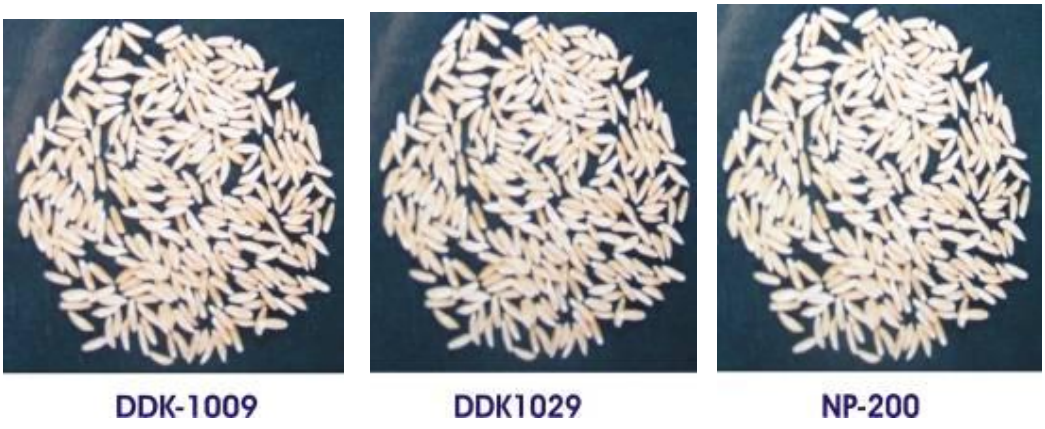
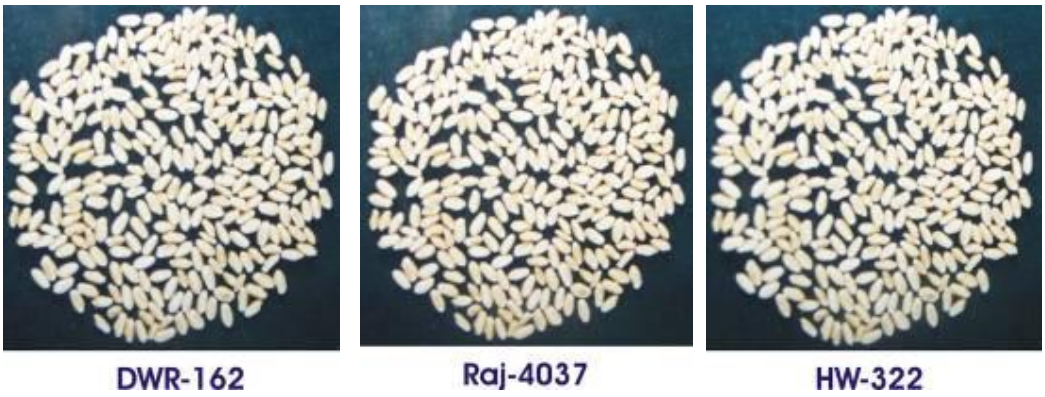


Plate 1. Physical appearance of wheat varieties grown at Dharwad location during rabi 2007-08

T. aestivum



T. durum

T. dicoccum

Plate 2. Physical appearance of wheat varieties grown at Arabhavi location during rabi 2007-08

Twenty five milligrams of dried homogenous wheat flour sample was placed in a boiling tube. Five ml of 2.5 N HCl was added to it and the mixture was kept in a boiling water bath for 3 h and cooled. When the tube attained room temperature, it was neutralized with sodium carbonate until the effervescences ceased. The volume was made up to 25 ml. It was then centrifuged for 10 min at 3000 rpm. The supernatant was decanted in to a 100 ml volumetric flask. The extraction was repeated thrice. The pooled supernatant was diluted with distilled water and made up to 100 ml. For one ml of extract in a tube kept on an ice bath, 4 ml of anthrone reagent was added and kept in a boiling water bath for 8 min and cooled. The green dark coloured complex developed was measured at 630 nm.

3.2.2 Available starch

Available starch content in the wheat flour sample was analyzed by hydrolyzing the wheat flour in perchloric acid by anthrone method (Sadasivam and Manikam, 1992).

Dried homogenous wheat flour sample (100 mg) was placed in a centrifuge tube, 5 ml of 80 per cent alcohol was added and placed on water bath at 80 to 80^{0}C for 5 to 10 min., cooled and centrifuged for 10 min at 3000 rpm. Alcoholic extraction was repeated thrice and supernatant was discarded. To the left over residue, 3 ml distilled water followed by 6.5 ml of 52 per cent perchloric acid was added. The contents were stirred for 5 min continuously and then occasionally for next 15 min. Little water was added and again centrifuged at 2000 rpm for 5 min. The supernatant was decanted in to a 100 ml volumetric flask. The extraction was repeated thrice, increasing the time from 15 to 25 min and finally to 30 min. To 1 ml of supernatant, 4 ml of anthrone reagent was added. It was kept in a boiling water bath for 8 min, cooled rapidly and the intensity of coloured complex was read at 630 nm, in a spectrophotometer using standard curve of D-glucose. The value thus obtained was multiplied by a factor 0.9 to get the contents of available starch.

3.2.3 Oil content

The oil was extracted from the dried wheat flour by Soxhlet method (Sadasivam and Manikam, 1992). Three grams of flour was placed in a thimble. A piece of cotton wool was placed on the top of the flour to evenly distribute the solvent as it drops on the sample directly during extraction. The thimble was placed in sample holder tube of the Soxhlet extraction apparatus. The oil was extracted using petroleum ether for 6 h without interruption by gental heating. The extracted solvent was evaporated on a water bath until no odour of ether was traced. It was cooled at room temperature. The dirt or moisture outside the flask was removed and flask was weighed. The amount of oil present in the wheat sample was calculated as follows:

$$\text{Oil content (\%)} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$

3.3 β-carotene and total free phenols

3.3.1 β-carotene content in wheat flour

β-carotene content of *T. dicoccum*, *T. durum* and *T. aestivum* wheat varieties was determined by the procedure of Mishra and Gupta (1995).

Wheat flour (8 g) was transferred to 150 ml glass stopperd Erlenmeyer flask and 40 ml of water saturated n-butanol was added to give a homogenous suspension. The contents were mixed for 5 min and allowed to stand overnight at room temperature in dark. Next day, the contents were mixed on a vortex mixer and filtered through Whatman No. 1 filter paper and made up to 50 ml. The absorbance of the clear filtrate was measured at 440 nm in a spectrophotometer. Water saturated n-butanol was used as a blank. The □-carotene content (ppm) of the sample was obtained by putting the absorption reading of unknown sample in the following equation:

β -carotene (ppm) = A x 23.5360 + 0.0105

where A = Absorbance at 440 nm

3.3.2 Total free phenols

Total free phenols from the wheat samples were extracted as reported by Adom *et al.* (2003). Five hundred mg of wheat flour in 10 ml of 80 per cent ethanol and kept on a shaker for 10 min at room temperature. Soluble components were separated from the bran by centrifugation at 2000 rpm for 10 min. This process was repeated twice and the final volume was made up to 10 ml with distilled water. Total free phenolic concentration was determined as described by Singleton *et al.* (1999).

To 1 ml of extract, 1 ml of Folin-Ciocalteu reagent (FCR) was added. After shaking well, 2 ml of sodium carbonate solution was added and the samples were placed in a boiling water bath for 1 min and cooled. The final volume was made up to 10 ml and the absorbance was measured at 650 nm in a spectrophotometer and the concentration of total free phenols was determined using standard catechol.

3.4 Proteins

3.4.1 Estimation of soluble proteins

The soluble proteins were extracted from wheat flour by placing 500 mg of flour in a beaker containing 10 ml of 0.1 M phosphate buffer, pH 6.8. The supernatant was transferred to a volumetric flask by using Whatman No 1 filter paper. From this, 2 ml aliquot was placed in another volumetric flask and final volume was made up to 10 ml with 0.1 M phosphate buffer, pH 6.8.

To 1 ml of extract, 5 ml of alkaline copper reagent was added, mixed well and allowed to react for 10 min. After 10 min, 0.5 ml of 1 N FCR was added, mixed well and kept in dark for 30 min. The absorbance of the coloured complex was measured at 660 nm. Total soluble protein content in the sample was calculated from a standard curve prepared using Bovine serum albumin (Lowry *et al*., 1951).

3.4.2 Available nitrogen

The available nitrogen in wheat flour was estimated by Micro-Kjeldhal method (Sadasivam and Manikam, 1992). Wheat sample was digested in con. H_2SO_4 with a pinch of digestation mixture comprising HgO, K_2SO_4 and $CuSO_4$. The digested sample was distilled with excess of 40 per cent sodium hydroxide and ammonia released was trapped in 2 per cent boric acid. Ammonium borate thus formed was titrated against 0.02N HCl and amount of nitrogen present in wheat sample was calculated as follows:

$$\% N = \frac{\text{Titre value} \times 0.02 \text{ N HCl} \times 0.014 \times 100}{\text{Weight of sample (mg)}}$$

3.4.3 Crude protein

The amount of crude protein present in the wheat sample was calculated by multiplying the N content by a factor 5.7.

Crude protein (g %) = % N x 5.7

3.5 Sodium dodecyl sulfate polyacrylamide gel Electrophorosis (SDS-PAGE)

The quality of *T.dicoccum*, *T.durum* and *T.aestivum* wheat varieties in relation to protein fractions was determined by SDS-PAGE as per the procedure of Damania *et al.* (1983).

3.5.1 Protein extraction

The wheat grains were ground to fine powder. Ten mg of powder was placed in 1.5 ml Eppendorf tube, 400 □l protein extraction buffer (Tris HCl buffer 0.05 M, pH 8.0, containing 0.02% SDS, 30.3% urea, 1% 2-mercaptoethanol) was added to each Eppendorf tube and kept over night at 40^0C. Soluble components were separated from the bran by centrifugation at 13000 rpm for 10 min. The supernatant was contained dissolved protein. The sample (27 □l) was loaded in a well of the electrophoretic gel.

3.5.2 Preparation of resolving gel (10% acrylamide gel)

The separating gel was prepared as follows:

Double distilled water	- 3.20 ml
Acrylamide (10 %)	- 2.64 ml
Tris-HCl buffer, pH 8.8	- 2.00 ml
SDS (10 %)	- 0.08 ml
Ammonium persulfate (10 %)	- 0.06 ml
TEMED	- 0.01 ml

3.5.3 Preparation of stacking gel (5%)

Double distilled water	- 2.70 ml
Acrylamide (5 %)	- 0.67 ml
Tris-HCl buffer, pH 6.8	- 0.50 ml
SDS (10 %)	- 0.04 ml
Ammonium persulfate (10 %)	- 0.03 ml
TEMED	- 0.004 ml

3.5.4 Tank buffer, pH 8.3

Tris	- 1.00 g
SDS	- 0.25 g
Glycine	- 3.60 g

The content were dissolved one by one in the distilled water and made up to 250 ml.

3.5.5 Gel preparation

Glass plates were cleaned with 70 per cent ethanol and fixed by using seal gasket and clips. Separating gel was poured in to the glass plate sandwich and layered with distilled water. After 30 min, distilled water was removed, stacking gel was added and comb was inserted in to the stacking gel.

3.5.6 Sample loading

The wells were cleaned with running buffer and the sample was loaded (27 □l) and protein molecular weight marker was loaded in the first well, using a micropipette.

3.5.7 Electrophoresis

Glass cabinet was fixed with electrophoresis apparatus and electrophoretic trays were filled with tank buffer, pH 8.3. The unit was connected to a power pack. Current was passed initially at 30 volts and then increased up to 60 volts after 2 h. The gel was run for 5-6 h till the tracking dye reached the bottom of the gel and power supply was disconnected.

3.5.8 Staining and destining

Gel was carefully detached from the glass plate and transferred to a tray containing staining solution (0.1% Coomassie Brilliant Blue) and shaken gently for 40 min.

Gel was de-stained in 7 per cent acetic acid until the background of gel became clear.

3.6 Enzyme assays

Enzyme assays were carried out colourimetrically for two enzymes, β-amylase and acid invertase in germinated wheat using dinitrosalicylic acid (DNSA) as a colouring reagent.

3.6.1 Sample extraction

Germinated and ungerminated wheat samples were extracted in chilled 0.1 M acetate buffer, pH 5.0.

3.6.2 Assay of β-amylase

The β-amylase activity in germinated and ungerminated seeds was estimated by DNSA method (Sadasivam and Manikam, 1992). The method involves:

i) Preparation of standard graph of maltose

ii) Enzyme assay and

iii) Estimation of soluble protein in the sample.

3.6.2.1 Preparation of maltose standard graph

One hundred mg of maltose was dissolved in distilled water and made up to 100 ml with distilled water in a volumetric flask. This solution contained 1 mg of glucose per ml which was used as a stock standard. In a series of labeled test tubes, suitable quantity of aliquots from standard (100-1000 μg) were pipetted out and made up to 1 ml with distilled water. A reagent blank with 1 ml distilled water was also maintained as a standard. DNSA reagent (0.5 ml) was added to all the test tubes, mixed well and kept in a boiling water bath for 5 min. Tubes were cooled and made up to 10 ml with distilled water. The per cent transmittance of standard and samples was recorded against a reagent blank which was adjusted to 100 per cent transmittance at 540 nm. The amount of reducing sugars present per gram of sample was calculated from the maltose standard graph.

DNSA reagent was prepared by dissolving 1g of 3, 5-dinitrosalicylic acid in a little amount of 2 N NaOH followed by 30 g of sodium potassium tartrate and made up to 100 ml with 2 N NaOH.

3.6.2.2 Enzyme assay

Wheat seeds, ungerminated and germinated for 24, 48 and 72 h were used for the assay of β-amylase. Seedling extracts were prepared in acetate buffer (0.1 M, pH 5.0) and used as an enzyme source. The enzyme activity was expressed as the amount of maltose released per min per mg of protein.

3.6.2.3 Soluble protein in the sample

Soluble protein was estimated by a colourimetric procedure of Lowry *et al.* (1951) as explained in 3.4.1.

3.6.3 Assay of invertase

Invertase is an enzyme which makes available reducing sugar like glucose and fructose for metabolic activities by hydrolyzing sucrose, a non-reducing sugar. The assay of acid invertase involves following steps:

i) Preparation of a standard graph of D-glucose,

ii) Enzyme assay and

iii) Estimation of soluble protein in the sample.

The enzyme assay was carried out by the method described by Sadasivam and Manikam (1992).

3.6.3.1 Preparation of standard graph of D-glucose

The standard graph of D-glucose was prepared as explained in 3.6.2.1 with the only change that D-glucose (10-100 μg) was used instead of maltose.

3.6.3.2 Enzyme assay

Enzyme assay was carried out as explained in 3.6.2.2, and the specific activity of acid invertase was expressed as amount of D-glucose released per min per mg of protein of the sample.

3.6.3.3 Soluble protein in the sample

Protein was estimated by a colorimetric procedure of Lowry *et al.* (1951) as explained in 3.6.2.3.

3.6.4 Residual effect

To identify residual activity of β-amylase and acid invertase in ungerminated dry seeds, seeds were powdered using mortar and pestle and crude enzymes were extracted using 0.1M acetate buffer, pH 5.0. Activities of β-amylase and acid-invertase were assayed by DNSA method and soluble protein estimation in the sample was carried out by Lowry *et al.* (1951) method as explained in 3.6.2.3.

3.7 Micronutrients

Micronutrients, Zn, Fe, Cu and Mn were extracted from wheat flour. Five grams of wheat flour was placed in a crucible. Charring and ashing was carried out according to the standard procedure of Anon. (2005). To the ash sample, 1 ml of distilled water and 4 ml of conc. HCl was added and kept in hot water bath till the contents got evapourated. Again 5 ml of conc. HCl was added and kept in hot water bath till the contents got evapourated. For the remaining residue, 1 ml of distilled water followed by 4 ml of conc.HCl was added and just warmed. The supernatant was filtered and collected in to a 100 ml volumetric flask. Final volume was made up to 100 ml with distilled water. The amount of micronutrients present in the sample (Zn, Fe, Cu and Mn) was determined by using atomic absorption spectrophotometer.

3.8 Statistical analysis

The data collected in triplicate for all the quality parameters were statistically analysed using Completely Randomised Design (Snedecor and Cochran, 1962).

4. EXPERIMENTAL RESULTS

The results of the present investigation to study biochemical quality parameters of *T. aestivum, T. durum* and *T. dicoccum* wheat varieties grown at All India Coordinated Wheat Improvement Project, Main Agricultural Research Station (MARS), Dharwad and at the Agricultural Research Station (ARS), Arabhavi during *rabi* season of 2007-2008 are presented below.

4.1 Carbohydrate profile

4.1.1 Total carbohydrate and Starch

The mean total carbohydrates content (Table 2) in grains was high in *T. aestivum* (72.30 %) and *T. durum* (71.07 %) varieties and was low (59.04 %) in *T. dicoccum* varieties tested. *T. aestivum* varieties differed significantly in their total carbohydrate content with change in location. For example, GW-322 recorded higher total carbohydrate (79.50 %) at Dharwad and 63.80 per cent at Arabhavi location. Similarly, Raj-4037 recorded 78.00 per cent at Arabhavi but only 70.60 per cent at Dharwad. *T. durum* varieties were consistent in their total carbohydrate content at both the locations, *viz.,* 70.57 per cent at Dharwad and 71.57 per cent at Arabhavi.

Among the varieties tested, Raj-4037, a *T. aestivum* variety recorded significantly higher total carbohydrate content of 74.30 per cent and the rest of the *T. aestivum* and *T. durum* varieties were on par (70.60 – 71.60 %) with each other. However, *T. dicoccum* varieties recorded significantly low total carbohydrate content ranging between 58.10 and 61.10 per cent. However, *T. dicoccum* varieties recorded significantly higher total carbohydrate (61.87 %) at Arabhavi as compared to Dharwad (57.00 %) location, whereas *T. aestivum* varieties recorded significantly higher total carbohydrate (74.17 %) at Dharwad location as compared to Arabhavi (70.47 %) location.

The total starch content (Table 2) in wheat grains was high in *T. aestivum* (68.44 %) and *T. durum* (63.37 %) but low in *T. dicoccum* (54.37 %). *T. aestivum* and *T. dicoccum* varieties differed significantly in their total starch content with the change in location. For example, GW-322 recorded higher starch content of 73.39 per cent at Arabhavi but low content of 67.23 per cent at Dharwad location. Similarly, *T. dicoccum* variety NP-200 recorded higher starch content of 62.60 per cent at Arabhavi but low at Dharwad (50.03 %) location. *T. durum* varieties were consistent in their starch content at both the locations (Dharwad, 63.40 % and Arabhavi, 63.33 %).

Among the varieties tested, *T. aestivum* variety GW-322 recorded significantly higher starch content of 70.31 per cent and the rest of the *T. aestivum* varieties were on par with each other (67.26-67.74 %). *T. dicoccum* varieties recorded significantly low starch content ranging between 49.10 and 57.60 per cent. However, *T. dicoccum* varieties recorded significantly higher starch content of 57.53 per cent at Arabhavi as compared to Dharwad with 51.30 per cent. *T. aestivum* varieties however, recorded significantly higher starch content at Arabhavi (69.48 %) as compared to Dharwad (67.48 %).

4.1.2 Reducing, non-reducing and total sugars

The reducing, non-reducing and total sugar (Table 3) in wheat grains was high in *T. durum* (1.11 %) varieties and low in *T. aestivum* (0.88 %) varieties tested. *T. aestivum, T. durum* and *T. dicoccum* varieties differed significantly in their non-reducing and total sugar contents with the change in location. For example, DWR-162 recorded higher non-reducing sugar (1.22 %) at Dharwad but was 0.88 per cent at Arabhavi. Similarly, GW-322 recorded 1.41 per cent of non-reducing sugar at Arabhavi but only 0.95 per cent at Dharwad. *T. durum* varieties DWR-1006 and NIDW-295 recorded higher non-reducing sugar content (1.76 and 1.49 %, respectively) at Dharwad but was low at Arabhavi (1.32 and 1.14 %). *T. dicoccum* varieties DDK-1009, DDK-1029 and NP-200 recorded higher non-reducing sugar (1.80, 1.59 and 1.79 %, respectively) at Arabhavi but lesser amount of non-reducing sugar (0.92, 0.91 and 1.09 %, respectively) at Dharwad.

Table 2. Total carbohydrate and Starch content in *T. aestivum*, *T. durum* and *T.dicoccum* wheat varieties, location-wise

Varieties	Total Carbohydrate (%)			Starch (%)		
	Dharwad	**Arabhavi**	**Mean**	**Dharwad**	**Arabhavi**	**Mean**
T. aestivum						
DWR-162	72.40	69.60	71.00	68.30	67.12	67.74
Raj-4037	70.60	78.00	74.30	66.90	67.62	67.26
GW-322	79.50	63.80	71.60	67.23	73.39	70.31
Mean	**74.17**	**70.47**	**72.30**	**67.48**	**69.48**	**68.44**
T. durum						
DWR-1006	70.60	72.50	71.55	61.43	60.38	60.91
MACS-2846	71.70	70.30	71.00	63.67	62.62	63.15
NIDW-295	69.40	71.90	70.60	65.09	67.00	66.05
Mean	**70.57**	**71.57**	**71.07**	**63.40**	**63.33**	**63.37**
T. dicoccum						
DDK-1009	58.40	59.60	59.00	55.30	60.00	57.60
DDK-1029	55.30	61.00	58.10	48.30	50.00	49.10
NP-200	57.30	65.00	61.10	50.03	62.60	56.45
Mean	**57.00**	**61.87**	**59.04**	**51.30**	**57.53**	**54.37**
	Treatment	**Location**	**Interaction**	**Treatment**	**Location**	**Interaction**
CD at 5%	**2.00**	**0.50**	**1.00**	**2.10**	**1.70**	**2.03**

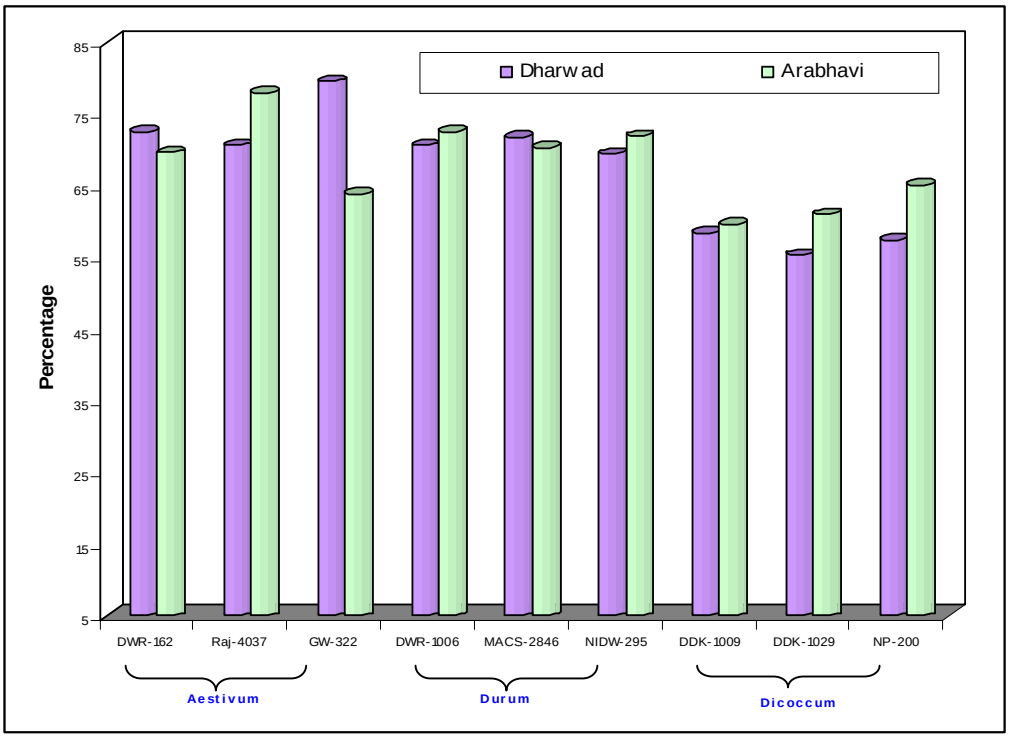


Fig. 1a. Total carbohydrate content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

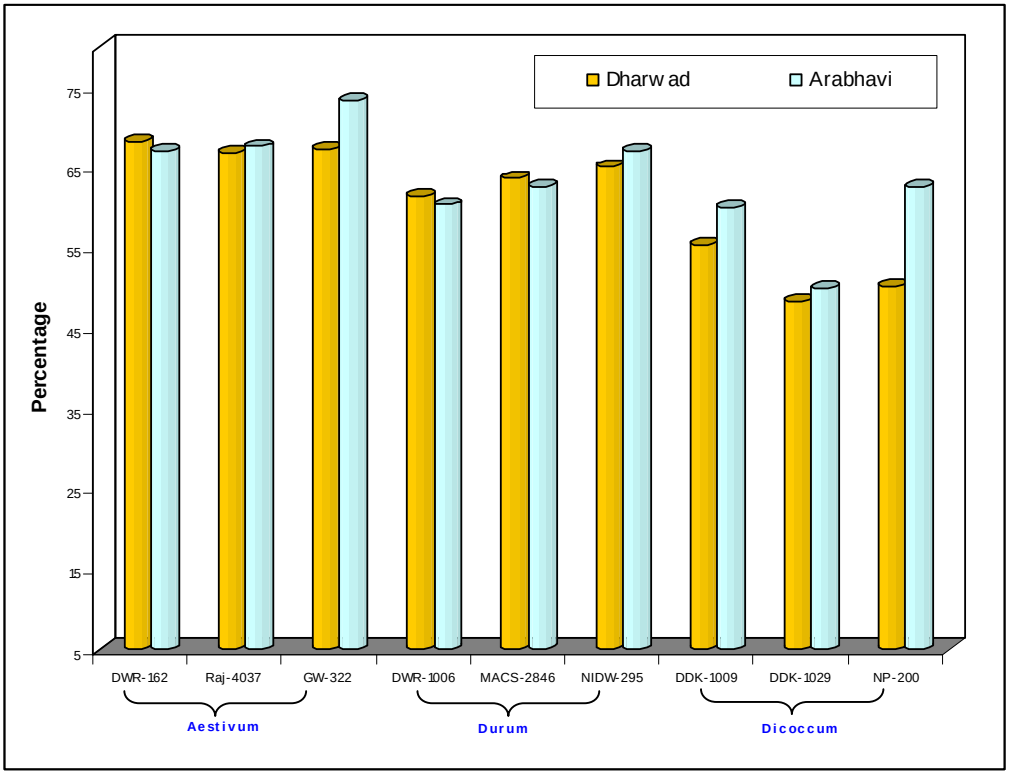


Fig. 1b. Starch content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

Table 3. Reducing, Non-reducing and Total sugar content of *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	Dharwad			Arabhavi			
	Reducing sugar (%)	Non-reducing sugar (%)	Total sugars (%)	Reducing sugar (%)	Non-reducing sugar (%)	Total sugars (%)	Mean
T. aestivum							
DWR-162	0.14	1.22	1.42	0.08	0.88	1.01	0.79
Raj-4037	0.13	1.17	1.36	0.11	1.33	1.51	0.94
GW-322	0.17	0.95	1.12	0.12	1.41	1.61	0.90
Mean	**0.15**	**1.11**	**1.30**	**0.10**	**1.21**	**1.38**	**0.88**
T. durum							
DWR-1006	0.16	1.76	2.01	0.10	1.32	1.49	1.14
MACS-2846	0.24	1.60	1.92	0.19	1.41	1.67	1.17
NIDW-295	0.22	1.49	1.79	0.13	1.14	1.33	1.02
Mean	**0.21**	**1.62**	**1.91**	**0.14**	**1.29**	**1.50**	**1.11**
T. dicoccum							
DDK-1009	0.22	0.92	1.19	0.11	1.80	2.01	1.04
DDK-1029	0.21	0.91	1.17	0.18	1.59	1.85	0.99
NP-200	0.20	1.09	1.35	0.24	1.79	2.13	1.13
Mean	**0.21**	**0.97**	**1.24**	**0.18**	**1.73**	**2.00**	**1.05**
	Treatment			**Location**		**Interaction**	
CD at 5%	**0.11**			**0.07**		**0.05**	

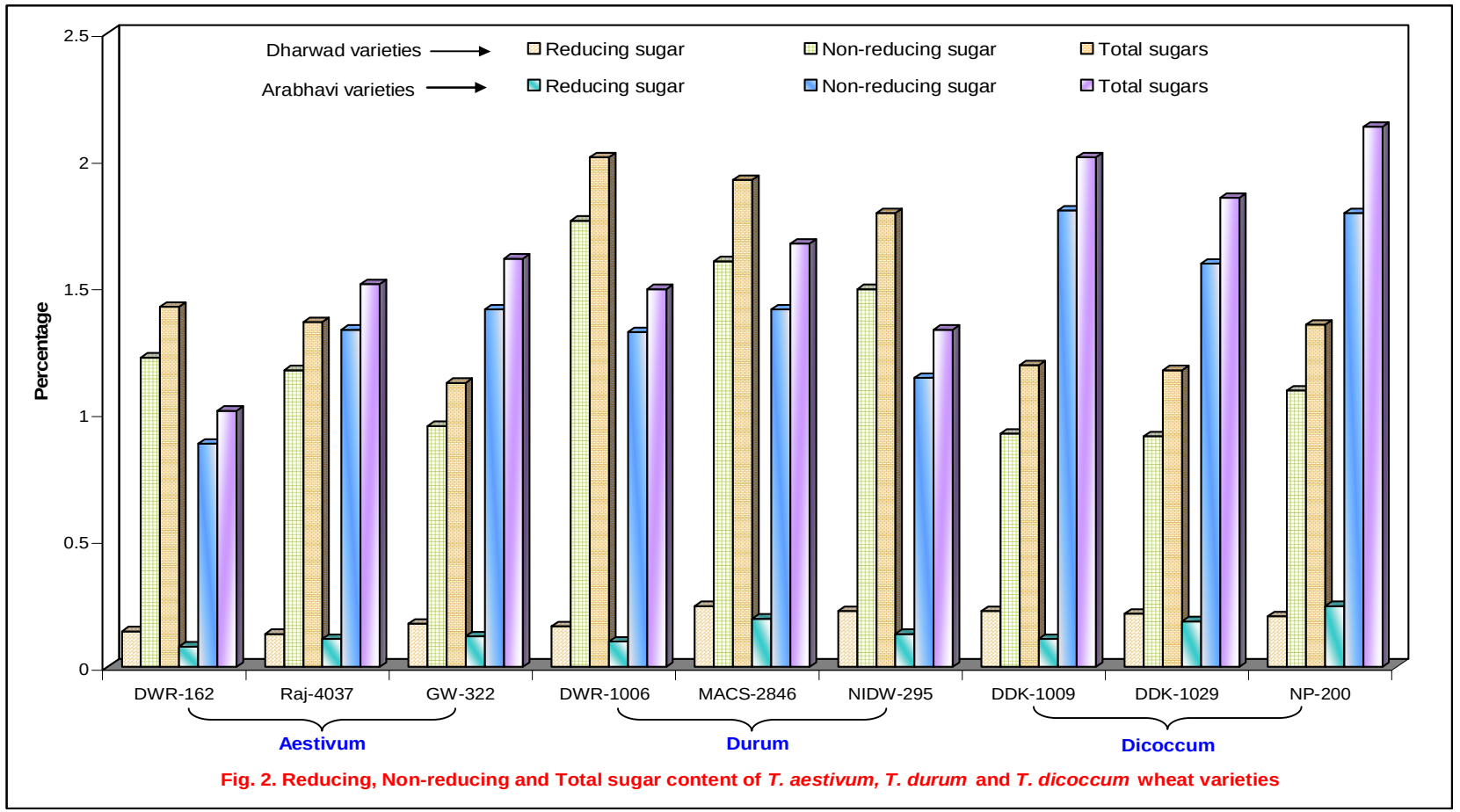


Fig. 2. Reducing, Non-reducing and Total sugar content of T. aestivum, T. durum and T. dicoccum wheat varieties

T. aestivum, T. durum and *T. dicoccum* varieties differed significantly in their total sugar content with the change in location. For example, DWR-162 recorded higher non-reducing sugar (1.42 %) at Dharwad but was 1.01 per cent at Arabhavi location. Similarly, GW-322 recorded 1.61 per cent of non-reducing sugar at Arabhavi but was only 1.12 per cent at Dharwad. *T. durum* varieties, DWR-1006 and NIDW-295 recorded higher non-reducing sugar content (2.01 and 1.79 %, respectively) at Dharwad location but was low at Arabhavi (1.49 and 1.33 %, respectively). *T. dicoccum* varieties, DDK-1009, DDK-1029 and NP-200 recorded higher non-reducing sugar (2.01, 1.85 and 2.13 %, respectively) at Arabhavi but contained lesser amount of non-reducing sugar (1.19, 1.17 and 1.35 %, respectively) at Dharwad.

Among the varieties tested, *T. durum* variety MACS-2846 recorded significantly higher sugar content (1.17 %), whereas rest of the *T. durum* varieties were on par (1.02-1.14 %) with each other. *T. aestivum* varieties, however, recorded significantly low sugar (0.79-0.94 %) content. The term sugar here includes reducing, non- reducing and total sugars and the values are mean of the values for both the locations.

4.2 Protein profile

4.2.1 Nitrogen and Crude protein

Nitrogen content (Table 4) in wheat grains was high in *T. dicoccum* (3.01 %) and low in *T. durum* (2.01 %) varieties tested. *T. aestivum* varieties differed significantly in their nitrogen content with the change in location. GW-322 variety of *T. aestivum* recorded higher nitrogen (2.50 %) at Arabhavi but was low at Dharwad (1.66 %).location. *T. durum* varieties at Dharwad (1.80 %) and Arabhavi (2.23 %) were consistent in their nitrogen content. Similarly, *T. dicoccum* varieties were consistent in their nitrogen content at both Dharwad (3.14 %) and Arabhavi (2.87 %) locations.

Among the varieties tested, *T. dicoccum* variety DDK-1009 recorded significantly higher nitrogen content (3.13 %), whereas remaining *T. dicoccum* varieties recorded significant nitrogen content but were on par with each other (2.90-3.00 %). *T. aestivum* varieties recorded significantly higher nitrogen content of 2.44 per cent at Arabhavi as compared to 1.97 per cent at Dharwad.

The crude protein content (Table 4) was high in *T. dicoccum* (17.16 %) and low in *T. durum* (11.65 %) varieties tested. *T. aestivum, T. durum* and *T. dicoccum* varieties significantly differed in their crude protein content with the change in location. For example, *T. aestivum* variety GW-322 recorded higher crude protein content of 14.25 per cent at Arabhavi and 9.46 per cent at Dharwad. *T. durum* variety MACS-2846 recorded higher crude protein content of 13.26 per cent at Arabhavi but low at Dharwad (9.50 %) location. However, NP-200 variety of *T. dicoccum* recorded significantly higher crude protein content of 18.24 per cent at Dharwad but only 15.96 per cent at Arabhavi location.

Among the varieties tested, *T. dicoccum* variety DDK-1009 had significantly higher crude protein content of 17.84 per cent and the rest of the varieties were on par (16.32-17.10 %) with each other. *T. durum* varieties recorded low amount of protein content (11.18-12.39 %). *T. aestivum* and *T. durum* varieties recorded significantly higher crude protein content at Arabhavi (13.94 and 12.75 %) as compared to Dharwad (11.24 and 10.28 %) location, whereas *T. dicoccum* varieties had significantly higher level (17.91 %) at Dharwad but recorded low value (16.39 %) at Arabhavi.

4.2.2 Soluble protein and wet gluten

Soluble protein content (Table 5) was high in grains of *T. dicoccum* (1.47 %) varieties but was low in *T. durum* (1.29 %) varieties tested. *T. durum* varieties differed significantly in their soluble protein content with the change in location. Variety DWR-1006 recorded higher soluble protein content (1.46 %) at Dharwad but low (1.21 %) at Arabhavi. *T. dicoccum* varieties were consistent in their soluble protein content both at Dharwad (1.48%) and Arabhavi (1.46%) locations.

Among *T. dicoccum* varieties tested, DDK-1029 recorded significantly higher soluble protein content (1.67%) and the rest of the varieties were on par (1.30%-1.46%) with each other. *T. durum* varieties recorded significantly low soluble protein (1.21-1.34 %) content.

Table 4. Nitrogen and crude protein content in *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	Nitrogen (%)			Crude protein (%)		
	Dharwad	Arabhavi	Mean	Dharwad	Arabhavi	Mean
T. aestivum						
DWR-162	2.29	2.21	2.25	13.05	12.60	12.83
Raj-4037	1.97	2.63	2.30	11.21	14.99	13.10
GW-322	1.66	2.50	2.08	9.46	14.25	11.86
Mean	**1.97**	**2.44**	**2.21**	**11.24**	**13.94**	**12.60**
T. durum						
DWR-1006	1.78	2.14	1.96	10.16	12.20	11.18
MACS-2846	1.66	2.32	1.99	9.50	13.26	11.38
NIDW-295	1.96	2.24	2.10	11.20	12.80	12.39
Mean	**1.80**	**2.23**	**2.01**	**10.28**	**12.75**	**11.65**
T. dicoccum						
DDK-1009	3.16	3.10	3.13	18.01	17.67	17.84
DDK-1029	3.07	2.73	2.90	17.49	15.56	16.32
NP-200	3.20	2.80	3.00	18.24	15.96	17.10
Mean	**3.14**	**2.87**	**3.01**	**17.91**	**16.39**	**17.16**
	Treatment	**Location**	**Interaction**	**Treatment**	**Location**	**Interaction**
CD at 5%	**0.42**	**0.30**	**0.20**	**2.00**	**1.10**	**1.01**

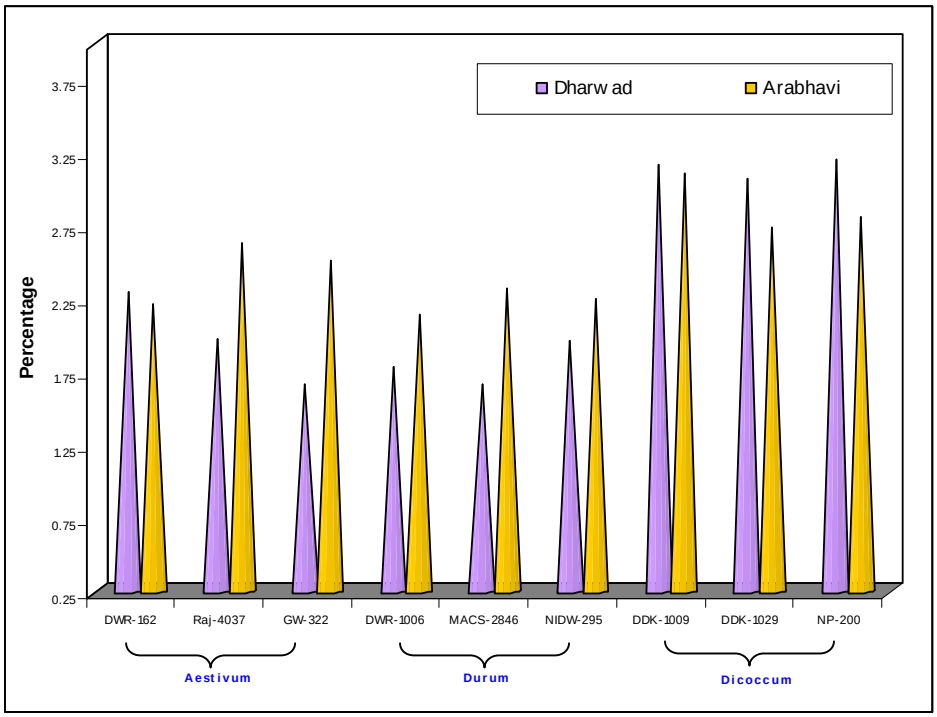


Fig. 3a. Nitrogen content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

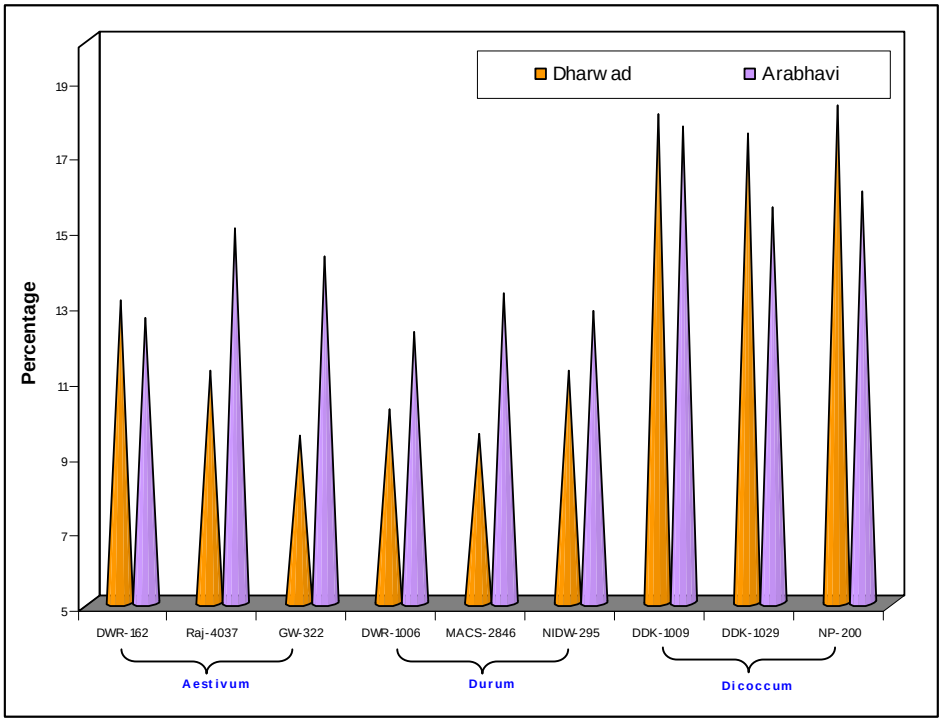


Fig. 3b. Crude protein content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

Table 5. Soluble proteins and wet gluten content in *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	Soluble Protein (%)			Wet Gluten (%)		
	Dharwad	**Arabhavi**	**Mean**	**Dharwad**	**Arabhavi**	**Mean**
T. aestivum						
DWR-162	1.38	1.43	1.40	31.80	33.50	32.60
Raj-4037	1.33	1.43	1.38	25.10	30.80	27.90
GW-322	1.31	1.43	1.37	26.50	24.50	25.00
Mean	**1.34**	**1.43**	**1.38**	**27.80**	**29.60**	**28.50**
T. durum						
DWR-1006	1.46	1.21	1.34	43.70	50.70	47.20
MACS-2846	1.10	1.32	1.21	30.30	41.00	35.60
NIDW-295	1.22	1.45	1.33	30.90	37.50	34.20
Mean	**1.26**	**1.32**	**1.29**	**34.96**	**43.06**	**39.00**
T. dicoccum						
DDK-1009	1.38	1.22	1.30	34.40	26.60	30.50
DDK-1029	1.61	1.73	1.67	42.50	37.80	40.10
NP-200	1.44	1.42	1.46	34.90	37.90	36.40
Mean	**1.48**	**1.46**	**1.47**	**37.26**	**34.10**	**35.67**
	Treatment	**Location**	**Interaction**	**Treatment**	**Location**	**Interaction**
CD at 5%	**0.048**	**0.034**	**0.02**	**0.30**	**0.10**	**0.14**

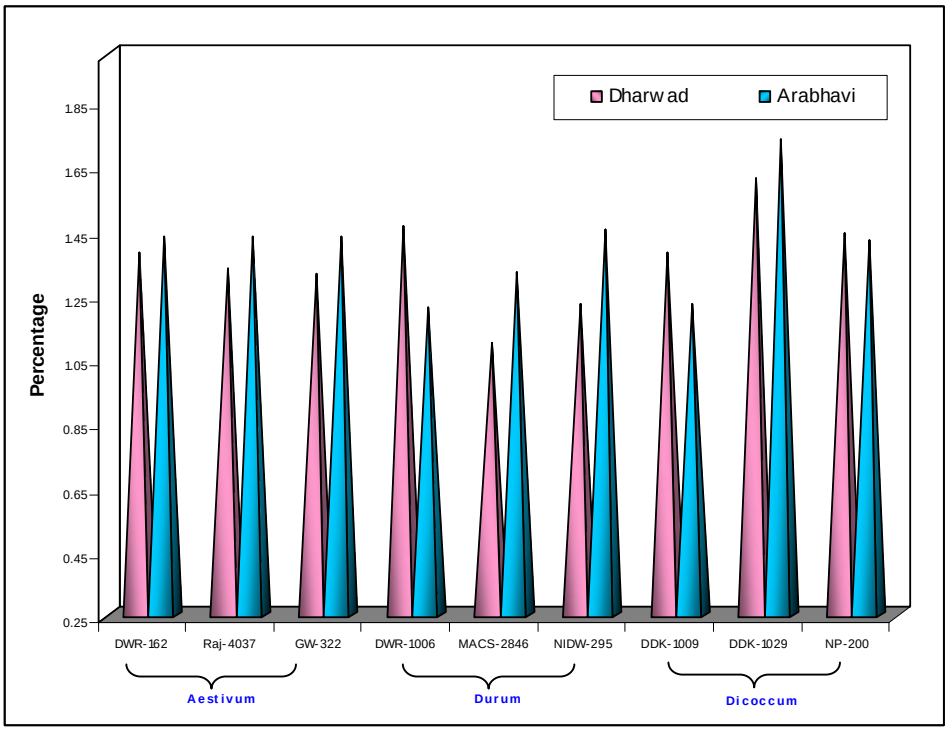


Fig. 4a. Soluble protein content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

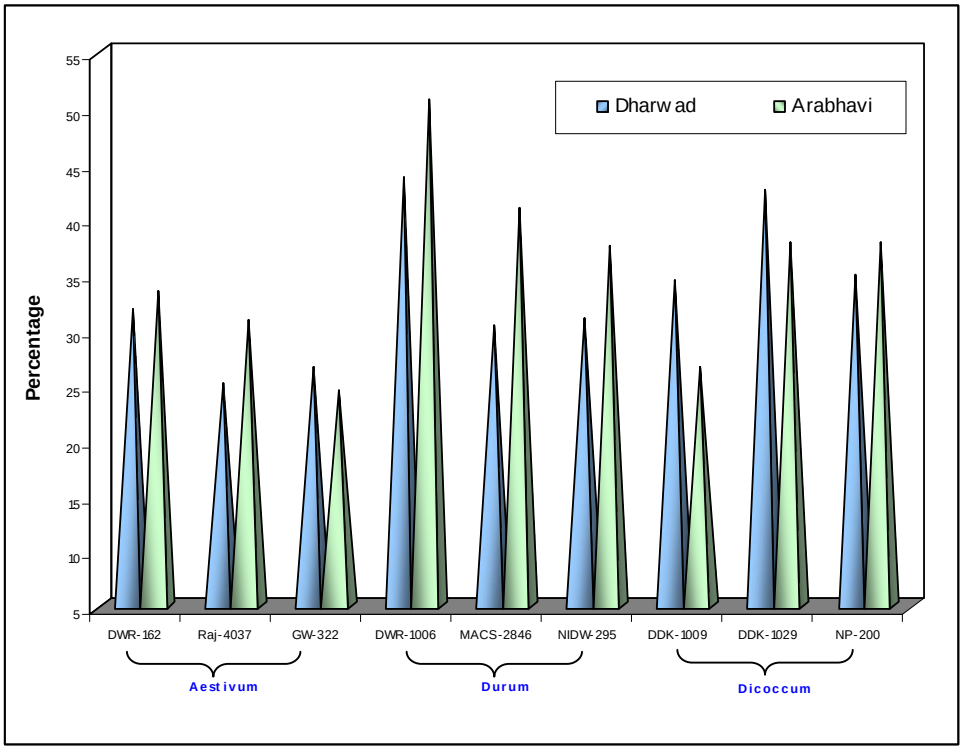


Fig. 4b. Wet gluten content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

However, *T. aestivum* and *T. durum* varieties recorded significantly higher soluble protein content (1.43 and 1.32 %, respectively) at Arabhavi as compared to Dharwad (1.34 and 1.26 %, respectively).

Wet gluten content (Table 5) in wheat grains was high in *T. durum* varieties (39.00 %) and low in *T. aestivum* (28.50 %) varieties tested. *T. durum* varieties differed significantly in their wet gluten content with the change in location. For example, MACS-2846 recorded higher value at Arabhavi (41.00 %) but low (30.30 %) at Dharwad. Similarly DWR-1006 recorded higher wet gluten content at Arabhavi (50.70 %) but low at Dharwad (43.70 %).

Among the *T. durum* varieties tested, variety DWR-1006 recorded significantly higher wet gluten content (47.20 %) whereas, the other two *T. durum* varieties were on par (34.20-35.60 %) with each other. *T. aestivum* varieties recorded significantly low wet gluten (25.00-32.60 %) content. However, *T. aestivum* and *T. durum* varieties tested recorded significantly higher wet gluten (29.60 and 43.06 %, respectively) at Arabhavi as compared to Dharwad (27.80 and 34.96 %, respectively), whereas *T. dicoccum* varieties tested recorded significantly higher wet gluten content (37.26 %) at Dharwad as compared to Arabhavi (34.10 %).

4.3 Antioxidant and protective elements

β-carotene content (Table 6) in wheat grains was high in *T. durum* varieties (4.32 mg/g) while it was low (2.35 mg/g) in *T. aestivum* varieties. *T. durum* varieties differed significantly in their β-carotene content with the change in location. *T. durum* variety, MACS-2846 recorded higher β-carotene content (4.36 mg/g) at Dharwad and lower (3.25 mg/g) at Arabhavi. *T. aestivum* and *T. dicoccum* varieties were consistent in their β-carotene content at both the locations, Dharwad (2.44 and 2.25 mg/g, respectively) and Arabhavi (2.27 and 2.55 mg/g, respectively).

Among the *T. durum* varieties, variety DWR-1006 recorded significantly higher β-carotene (5.09 mg/g) content, whereas the other two varieties of *T. durum* were on par (3.80-4.07 mg/g) with each other. *T. aestivum* varieties recorded significantly low β-carotene (1.93-2.99 mg/g) content. However, *T. aestivum* and *T. durum* varieties recorded significantly higher β-carotene content at Dharwad (2.44 and 4.62 mg/g, respectively) as compared to Arabhavi (2.27 and 4.02 mg/g, respectively). Similarly, *T. dicoccum* varieties recorded significantly higher β-carotene content at Arabhavi (2.55 mg/g) as compared to Dharwad (2.25 mg/g).

The total phenol content (Table 6) in wheat grains was high in *T. dicoccum* varieties (0.84 mg/g) and low in *T. durum* (0.75 mg/g) varieties. *T. aestivum* and *T. durum* varieties differed significantly in their total phenol content with the change in location. For example, DWR-162 of *T. aestivum* had higher amount of total phenol at Dharwad (1.03 mg/g) and low at Arabhavi (0.76 mg/g). Similarly, *T. durum* variety NIDW-295 recorded higher total phenol content (0.90 mg/g) at Dharwad as compared to Arabhavi (0.67 mg/g). *T. dicoccum* varieties were consistent in their total phenol content at Dharwad (0.86 mg/g) and Arabhavi (0.83 mg/g).

Among the varieties tested, *T. dicoccum* variety DDK-1029 recorded significantly higher total phenol (0.97 mg/g) content, whereas rest of the varieties were on par (0.74-0.83 mg/g) with each other. *T. durum* varieties recorded significantly low total phenol (0.66-0.81 mg/g) content. *T. aestivum* and *T. durum* varieties recorded higher values at Dharwad (0.79 and 0.78 mg/g, respectively) as compared to Arabhavi (0.72 and 0.71 mg/g, respectively).

4.4 Oil content

The oil content (Table 7) in wheat grains was high in *T. dicoccum* (2.20 %) and low in *T. aestivum* and *T. durum* (1.53 and 1.25 %, respectively) varieties. *T. dicoccum* varieties recorded significantly differed in their oil content with the change in location. *T. dicoccum* varieties, DDK-1029 and NP-200 recorded significantly higher oil content at Arabhavi (3.30 and 2.70 %, respectively) and low oil content at Dharwad (2.20 and 1.50 %, respectively).

Table 6. β-carotene and total phenol content in *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	β carotene (mg / g)			Total phenols (mg/g)		
	Dharwad	**Arabhavi**	**Mean**	**Dharwad**	**Arabhavi**	**Mean**
T. aestivum						
DWR-162	3.19	2.79	2.99	1.03	0.76	0.89
Raj-4037	2.30	1.57	1.93	0.69	0.70	0.70
GW-322	1.84	2.46	2.15	0.67	0.72	0.70
Mean	**2.44**	**2.27**	**2.35**	**0.79**	**0.72**	**0.76**
T. durum						
DWR-1006	5.50	4.68	5.09	0.80	0.82	0.81
MACS-2846	4.36	3.25	3.80	0.66	0.66	0.66
NIDW-295	4.00	4.15	4.07	0.90	0.67	0.79
Mean	**4.62**	**4.02**	**4.32**	**0.78**	**0.71**	**0.75**
T. dicoccum						
DDK-1009	2.61	3.10	2.85	0.83	0.82	0.83
DDK-1029	2.05	2.43	2.24	0.91	1.04	0.97
NP-200	2.10	2.14	2.12	0.84	0.65	0.74
Mean	**2.25**	**2.55**	**2.40**	**0.86**	**0.83**	**0.84**
	Treatment	**Location**	**Interaction**	**Treatment**	**Location**	**Interaction**
CD at 5%	**0.50**	**0.13**	**0.23**	**0.15**	**0.03**	**0.04**

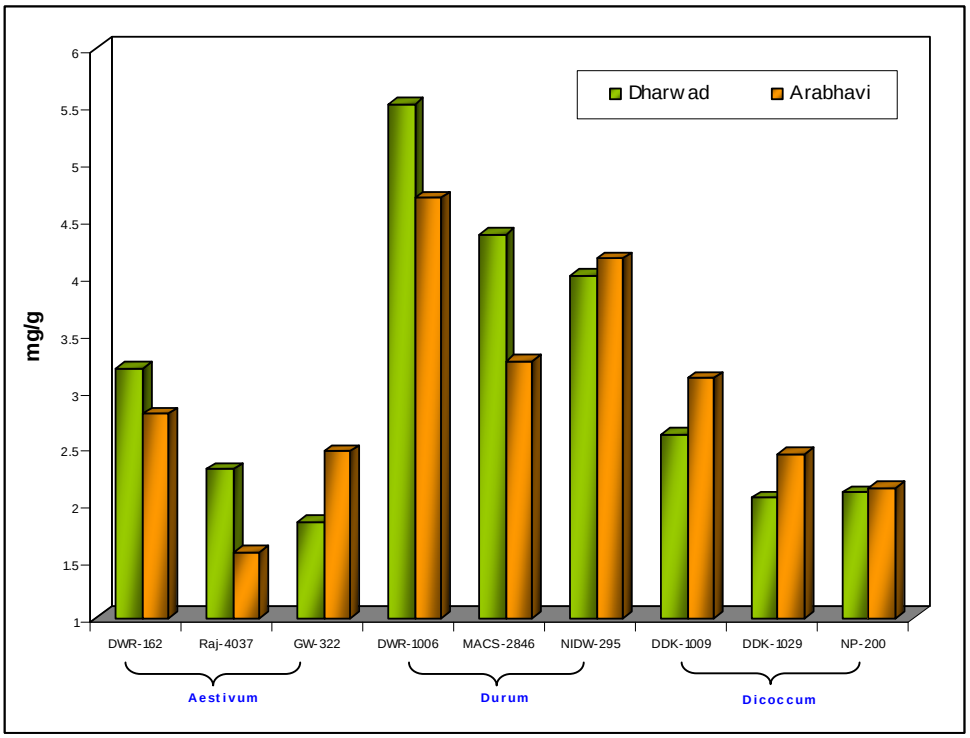


Fig. 5a. β-carotene content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

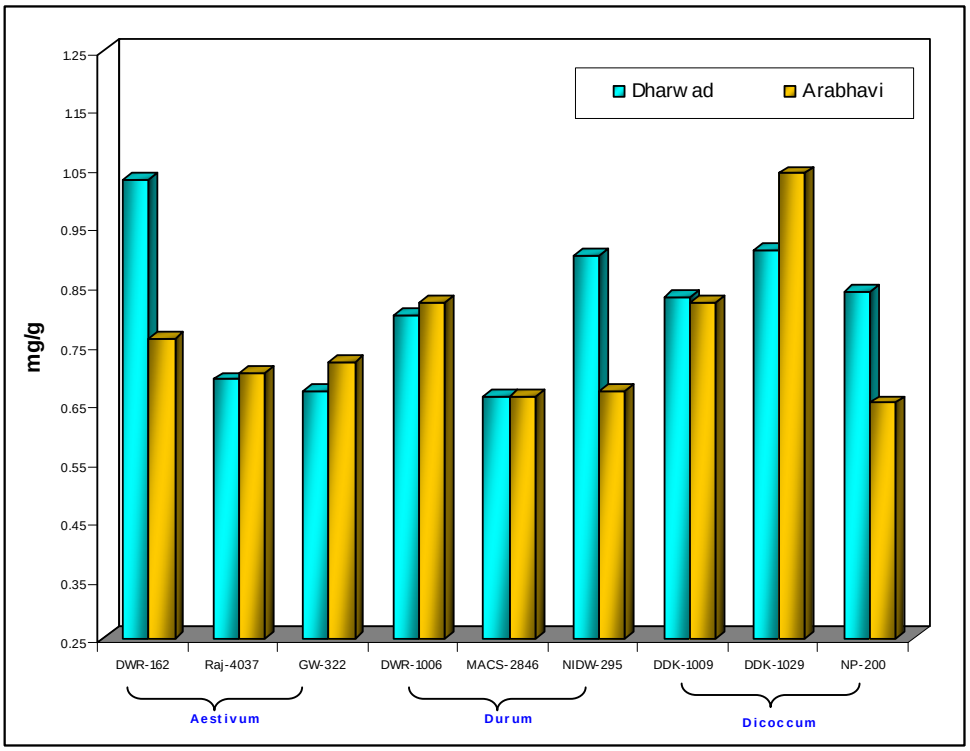


Fig. 5b. Total phenol content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

Among the *T. dicoccum* varieties tested, variety DDK-1029 recorded significantly higher (2.70 %) oil content and rest of the varieties were on par (1.80-2.10 %) with each other. *T. durum* varieties recorded low oil (1.35-1.40 %) content. Location wise, *T. aestivum, T. durum* and *T. dicoccum* varieties recorded significantly higher oil content at Arabhavi (1.93, 1.53 and 2.73 %, respectively) than at Dharwad (1.13, 0.96 and 1.70 %, respectively).

4.5 Micronutrient content

The micronutrient content of copper (Cu) in wheat grains was higher in *T. durum* (1.52 mg/100g) and low in *T. aestivum* (1.03 mg/100g) varieties tested (Table 8). *T. dicoccum* variety, DDK-1029 differed significantly in total Cu content. At Dharwad it was high (1.83 mg/100g) as compared to Arabhavi (1.02 mg/100g). However, *T. durum* variety, NIDW-295 recorded significantly higher Cu content. It was however higher at Dharwad (2.17 mg/100g) as against low value at Arabhavi (1.01 mg/100g).

Among *T. durum* varieties tested, variety DWR-1006 recorded significantly higher Cu content (1.77 mg/100g) and the rest of the *T. durum* varieties were on par (1.22-1.59 mg/100) with each other. *T. aestivum* varieties tested recorded significantly low Cu (0.90-1.11 mg/100g) content. *T. aestivum* (1.28 mg/100g), *T. durum* (1.90 mg/100g) and *T. dicoccum* (1.56 mg/100g) varieties recorded significantly higher Cu content at Dharwad as compared to Arabhavi (0.79, 1.14 and 0.93 mg/100g, respectively).

The zinc (Zn) content (Table 8) in wheat grains was high in *T. durum* (2.23 mg/100g) and low in *T. dicoccum* (1.43 mg/100g) varieties tested. *T. durum* and *T. dicoccum* varieties differed significantly in their Zn content with the change in location. For example, variety NIDW-295 recorded significantly higher Zn content (2.47 mg/100g) at Dharwad but low Zn content (1.73 mg/100g) at Arabhavi, whereas DDK-1029 variety, *T. dicoccum* recorded higher Zn content (2.03 mg/100g) at Dharwad but low Zn content at Arabhavi (1.00 mg/100g). *T. aestivum* varieties were consistent in their Zn content both at Dharwad (1.64 mg/100g) and Arabhavi (1.37 mg/100g).

Among the *T. durum* varieties tested, DWR-1006 recorded significantly higher Zn content (2.58 mg/100g) and the remaining *T. durum* varieties were on par (2.03-2.10 mg/100g) with each other. *T. dicoccum* varieties recorded significantly low Zn content (1.35-1.52 mg/100g). However, *T. durum* (2.43 mg/100g) and *T. dicoccum* (1.71 mg/100g) varieties recorded higher Zn content at Dharwad as compared to Arabhavi (2.04 and 1.15 mg/100g, respectively).

Iron (Fe) content (Table 9) in wheat grains was high in *T. durum* varieties tested (7.96 mg/100g) and low in *T. aestivum* (6.97 mg/100g) varieties tested. *T. durum* varieties differed significantly in their Fe content with the change in location. Variety NIDW-295 recorded higher Fe content at Dharwad (8.30 mg/100g) but low at Arabhavi (7.50 mg/100g). Similarly, DWR-1006 also recorded higher Fe content (8.77 mg/100g) at Dharwad, but was significantly low at Arabhavi (8.03 mg/100g). Although *T. aestivum* varieties contained low Fe content, variety DWR-162 had high amount of Fe (8.17 mg/100g) at Dharwad but was low at Arabhavi (7.50 mg/100g). However, *T. dicoccum* varieties were consistent in their Fe content at both Dharwad (7.24 mg/100g) and Arabhavi (7.30 mg/100g).

Among the *T. durum* varieties tested, the variety DWR-1006 recorded higher Fe content (8.40 mg/100g) and the rest of the *T. durum* varieties were on par (7.60-7.90 mg/100g) with each other. *T. aestivum* varieties contained low amount of Fe content (6.15-7.83 mg/100g). However, *T. aestivum* and *T. durum* varieties recorded significantly higher Fe content at Dharwad (7.62 and 8.33 mg/100g, respectively) as compared to Arabhavi (6.33 and 7.60 mg/100g, respectively).

The manganese (Mn) content (Table 9) in wheat grains was high in *T. durum* varieties (3.36 mg/100g) and low in *T. aestivum* (2.56 mg/100g) and *T. dicoccum* (2.60 mg/100g) varieties tested. *T. aestivum* varieties differed significantly in their Mn content with the change in location. For example, GW-322 recorded higher Mn content at Dharwad (3.5 mg/100g) over Arabhavi (2.10 mg/100g).

Table 7. Oil content in *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	Oil content (%)		
	Dharwad	**Arabhavi**	**Mean**
T. aestivum			
DWR-162	1.30	2.10	1.70
Raj-4037	1.00	1.70	1.35
GW-322	1.10	2.00	1.55
Mean	**1.13**	**1.93**	**1.53**
T. durum			
DWR-1006	1.00	1.70	1.35
MACS-2846	1.20	1.60	1.40
NIDW-295	0.70	1.30	1.00
Mean	**0.96**	**1.53**	**1.25**
T. dicoccum			
DDK-1009	1.40	2.20	1.80
DDK-1029	2.20	3.30	2.70
NP-200	1.50	2.70	2.10
Mean	**1.70**	**2.73**	**2.20**
	Treatment	**Location**	**Interaction**
CD at 5%	**0.04**	**0.03**	**0.02**

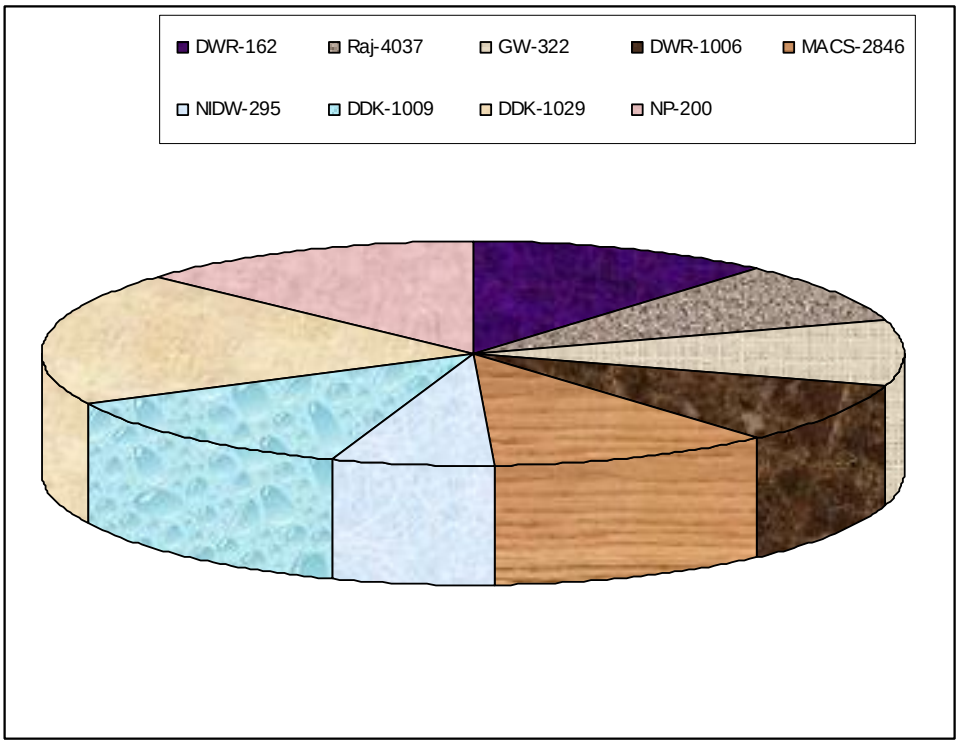


Fig. 6a. Oil content of T. aestivum, T. durum and T. dicoccum wheat varieties at Dharwad location

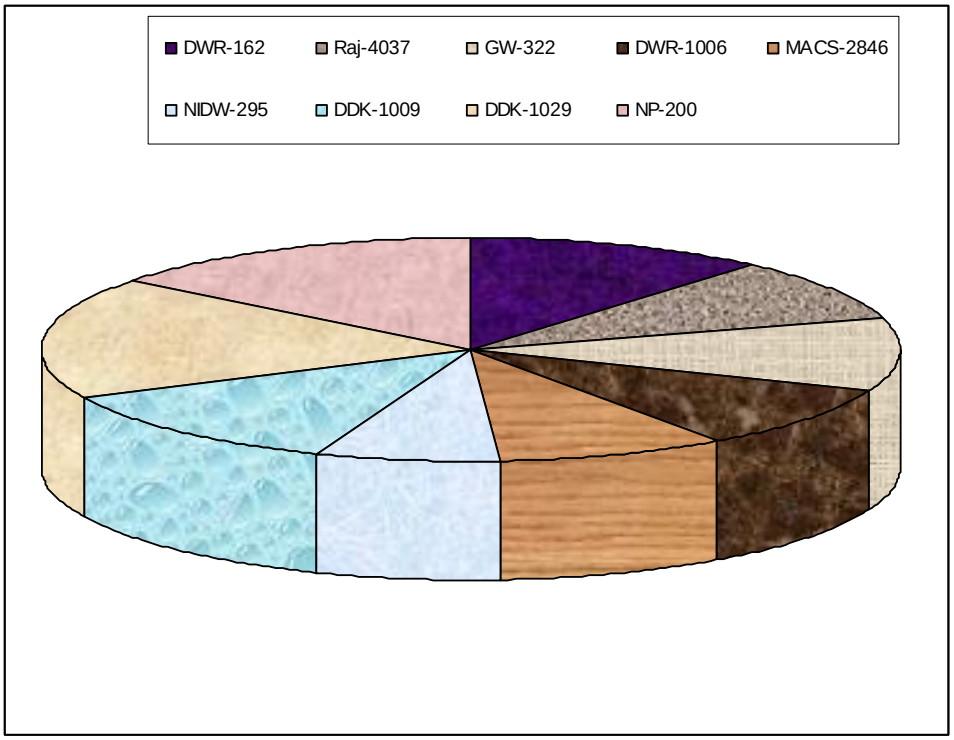


Fig. 6b. Oil content of T. aestivum, T. durum and T. dicoccum wheat varieties at Arabhavi location

Table 8. Copper and Zinc content in *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	Cu (mg / 100g)			Zn (mg / 100g)		
	Dharwad	**Arabhavi**	**Mean**	**Dharwad**	**Arabhavi**	**Mean**
T. aestivum						
DWR-162	1.27	0.96	1.11	2.07	1.83	1.95
Raj-4037	1.25	0.55	0.90	1.23	0.99	1.11
GW-322	1.33	0.88	1.10	1.63	1.30	1.47
Mean	**1.28**	**0.79**	**1.03**	**1.64**	**1.37**	**1.51**
T. durum						
DWR-1006	2.10	1.43	1.77	2.73	2.43	2.58
MACS-2846	1.43	1.00	1.22	2.10	1.97	2.03
NIDW-295	2.17	1.01	1.59	2.47	1.73	2.10
Mean	**1.90**	**1.14**	**1.52**	**2.43**	**2.04**	**2.23**
T. dicoccum						
DDK-1009	1.37	0.89	1.13	1.60	1.27	1.43
DDK-1029	1.83	1.02	1.43	2.03	1.00	1.52
NP-200	1.50	0.89	1.19	1.50	1.20	1.35
Mean	**1.56**	**0.93**	**1.25**	**1.71**	**1.15**	**1.43**
	Treatment	**Location**	**Interaction**	**Treatment**	**Location**	**Interaction**
CD at 5%	**0.50**	**0.36**	**0.24**	**0.50**	**0.36**	**0.24**

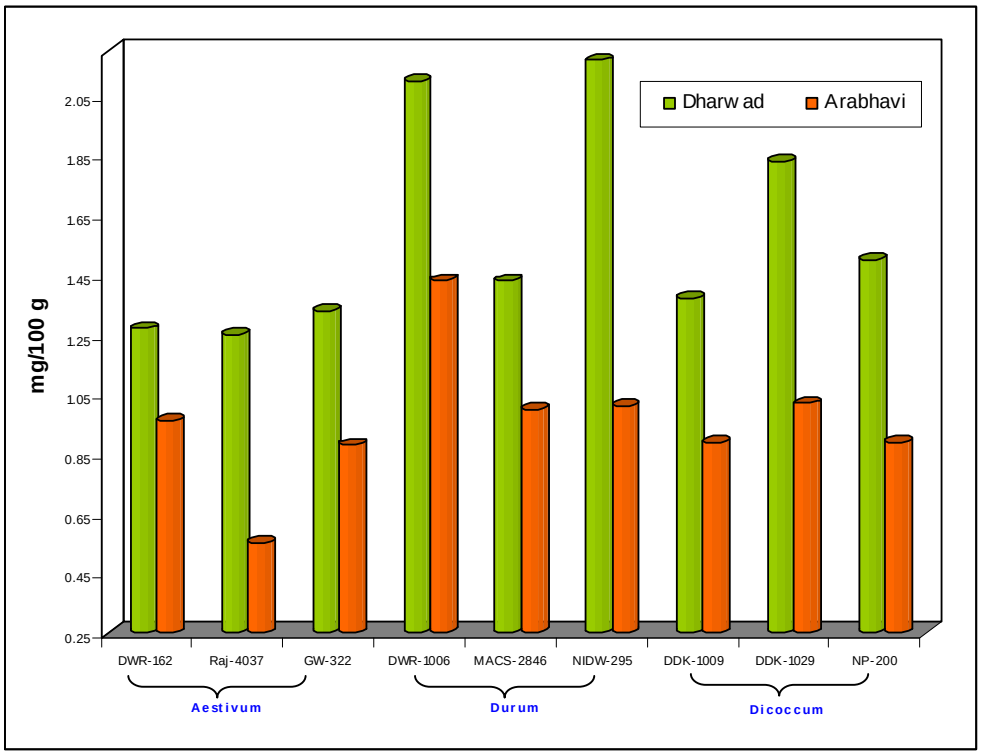


Fig. 7a. Copper content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

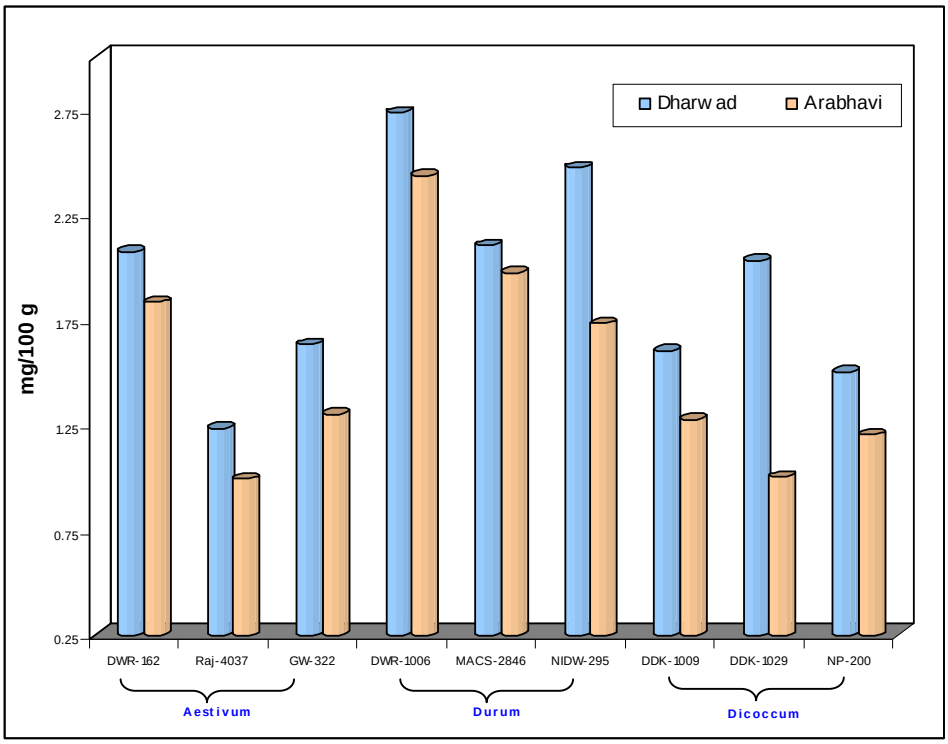


Fig. 7b. Zinc content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

Among the *T. durum* varieties, the variety DWR-1006 recorded significantly higher Mn content (3.63 mg/100g) and the rest of the varieties were on par (2.98-3.48 mg/100g) with each other. *T. aestivum* varieties (1.97-2.93 mg/100g) and *T. dicoccum* (1.85-3.27 mg/100g) varieties contained low Mn content. *T. aestivum, T. durum* and *T. dicoccum* varieties recorded higher Mn content (2.94, 3.67 and 2.94 mg/100g, respectively) at Dharwad as compared to Arabhavi (2.19, 3.05 and 2.26 mg/100g, respectively).

4.6 Enzyme activities

Data on the activities of hydrolytic enzymes, β-amylase and acid invertase are depicted in Tables 10 and 11. The units of specific activities are expressed as µg of maltose and glucose produced per min per mg of protein, respectively in the ungerminated and germinated wheat grains up to 72 h.

At Dharwad, the β-amylase activity was maximum in ungerminated seeds. Maximum activity was observed in *T. aestivum* variety Raj-4037 (2.00) which differed significantly from *T. durum* (1.33-1.65) and *T. dicoccum* varieties (1.03-1.21). *T. aestivum* varieties, Raj-4037 (2.00) and GW-322 (1.67) were on par with each other, whereas DWR-162 (1.77) differed significantly from GW-322. *T. durum* wheat varieties, DWR-1006 (1.33) and MACS-2846 (1.45) were on par with each other, whereas NIDW-295 (1.65) differed significantly from DWR-1006. However, *T. dicoccum* varieties, DDK-1009 (1.21), DDK-1029 (1.03) and NP-200 (1.13) were on par with each other.

After 24 h of germination, maximum β-amylase activity was observed in *T. aestivum* variety, Raj-4037 (2.25) which differed significantly from *T. durum* which was ranging between1.67 and 1.97 and that of *T. dicoccum* varieties between 1.25 and 1.51. *T. aestivum* varieties, DWR-162 (1.99), Raj-4037 (2.25) and GW-322 (2.23) were on par with each other. *T. durum* wheat varieties, DWR-1006 (1.67) and MACS-2846 (1.82) were also on par with each other, whereas NIDW-295 (1.97) differed significantly from DWR-1006. *T. dicoccum* varieties, DDK-1009 (1.51), DDK-1029 (1.25) and NP-200 (1.43) were on par with each other.

After 48 h of germination, β-amylase activity was found to increase in all the varieties. Raj-4037 showed maximum β-amylase activity of 2.97, which differed significantly from *T. durum* (2.15-2.51) and *T. dicoccum* varieties (1.68-1.98). *T. aestivum* varieties, DWR-162 (2.53) and GW-322 (2.67) were on par with each other, whereas Raj-4037 (2.97) differed significantly from DWR-162. *T. durum* wheat varieties, DWR-1006 (2.15) and MACS-2846 (2.33) were on par with each other, whereas NIDW-295 (2.51) differed significantly from them. *T. dicoccum* varieties, DDK-1009 (1.98) and NP-200 (1.77) were on par with each other, whereas DDK-1029 with 1.68 units of activity differed significantly from DDK-1009.

After 72 h of germination, a decrease in β-amylase activity was observed in all the treatments, whereas *T. aestivum* variety Raj-4037 (2.57) showed maximum β-amylase activity, which differed significantly from *T. durum* (2.00-2.27) and *T. dicoccum* varieties (1.43-1.73). *T. aestivum* varieties, Raj-4037 (2.57) and GW-322 (2.37) were on par with each other, whereas DWR-162 (2.03) differed significantly from them. *T. durum* wheat varieties, DWR-1006 (2.01), MACS-2846 (2.00) and NIDW-295 (2.27) were on par with each other. *T. dicoccum* varieties, DDK-1029 (1.43) and NP-200 (1.57) were on par with each other, whereas DDK-1009 (1.73) differed significantly from DDK-1029.

At Arabhavi, β-amylase activity was studied in ungerminated seeds. Maximum activity was observed in *T. aestivum* variety, Raj-4037 (2.13), which differed significantly from *T. durum* (1.45-2.00) and *T. dicoccum* (1.07-1.41) varieties. *T. aestivum* varieties, Raj-4037 (2.13) and GW-322 (2.03) were on par with each other, whereas DWR-162 (1.70) differed significantly from Raj-4037. *T. durum* wheat varieties, MACS-2846 (1.68) and NIDW-295 (2.00) were on par with each other, whereas DWR-1006 (1.45) differed significantly from NIDW-295. *T. dicoccum* varieties, DDK-1009 (1.33) and NP-200 (1.41) were on par with each other, whereas DDK-1029 (1.07) differed significantly from NP-200.

Table 9. Iron and Manganese content in *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	Fe (mg / 100g)			Mn (mg / 100g)		
	Dharwad	**Arabhavi**	**Mean**	**Dharwad**	**Arabhavi**	**Mean**
T. aestivum						
DWR-162	8.17	7.50	7.83	3.07	2.80	2.93
Raj-4037	7.00	5.30	6.15	2.27	1.67	1.97
GW-322	7.70	6.20	6.95	3.50	2.10	2.80
Mean	**7.62**	**6.33**	**6.97**	**2.94**	**2.19**	**2.56**
T. durum						
DWR-1006	8.77	8.03	8.40	4.00	3.27	3.63
MACS-2846	7.93	7.27	7.60	3.13	2.83	2.98
NIDW-295	8.30	7.50	7.90	3.90	3.07	3.48
Mean	**8.33**	**7.60**	**7.96**	**3.67**	**3.05**	**3.36**
T. dicoccum						
DDK-1009	7.63	6.97	7.30	2.13	1.57	1.85
DDK-1029	7.27	7.77	7.52	3.07	2.33	2.70
NP-200	6.83	7.17	7.00	3.63	2.90	3.27
Mean	**7.24**	**7.30**	**7.27**	**2.94**	**2.26**	**2.60**
	Treatment	**Location**	**Interaction**	**Treatment**	**Location**	**Interaction**
CD at 5%	**0.63**	**0.45**	**0.29**	**0.63**	**0.45**	**0.29**

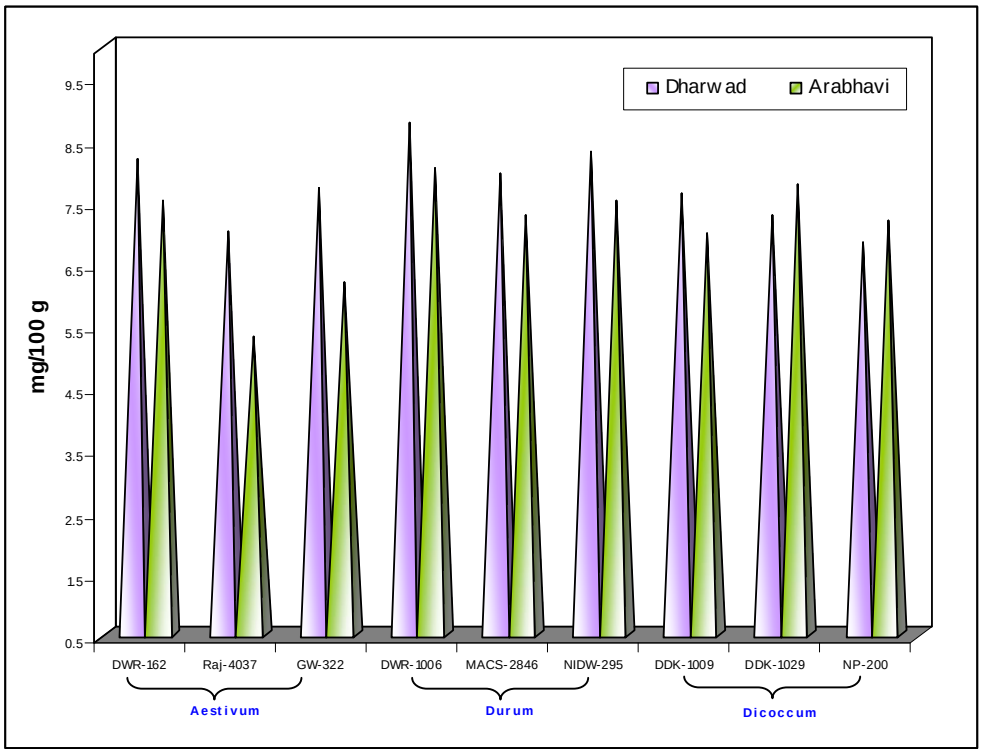


Fig. 8a. Iron content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

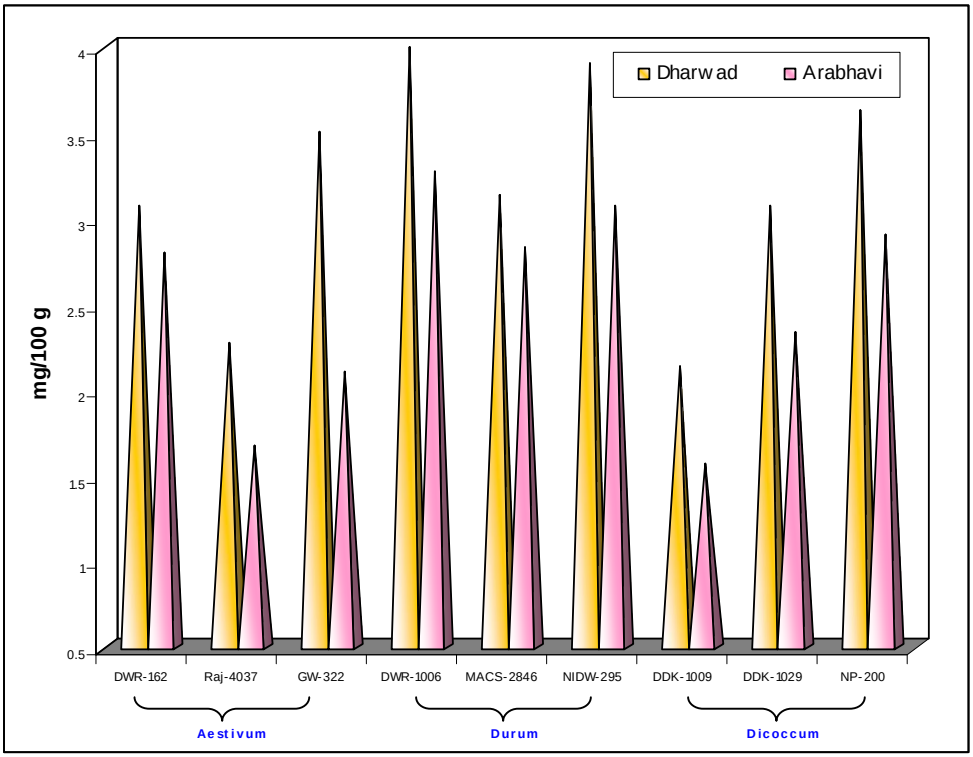


Fig. 8b. Manganese content of T. aestivum, T. durum and T. dicoccum wheat varieties location-wise

Table 10. Specific activity of β-amylase in *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	Specific activity of β-amylase (µg of maltose produced/min/mg of protein)								
	Dharwad				Arabhavi				
	Ungerminated seeds	24 h germination	48 h germination	72h germination	Ungerminated seeds	24 h germination	48 h germination	72h germination	Mean
T. aestivum									
DWR-162	1.77	1.99	2.53	2.03	1.70	2.12	2.89	2.65	2.21
Raj-4037	2.00	2.25	2.97	2.57	2.13	2.38	3.16	2.88	2.54
GW-322	1.67	2.23	2.67	2.37	2.03	2.26	3.03	2.79	2.38
Mean	**1.81**	**2.16**	**2.72**	**2.32**	**1.95**	**2.25**	**3.03**	**2.77**	**2.38**
T. durum									
DWR-1006	1.33	1.67	2.15	2.01	1.45	2.02	2.53	2.41	1.94
MACS-2846	1.45	1.82	2.33	2.00	1.68	2.10	2.69	2.43	1.88
NIDW-295	1.65	1.97	2.51	2.27	2.00	2.20	2.53	2.32	1.97
Man	**1.48**	**1.82**	**2.33**	**2.09**	**1.71**	**2.11**	**2.58**	**2.39**	**1.93**
T. dicoccum									
DDK-1009	1.21	1.51	1.98	1.73	1.33	1.76	2.48	2.25	1.63
DDK-1029	1.03	1.25	1.68	1.43	1.07	1.29	1.84	1.59	1.26
NP-200	1.13	1.43	1.77	1.57	1.41	1.79	2.33	2.14	1.55
Mean	**1.12**	**1.40**	**1.81**	**1.58**	**1.27**	**1.61**	**2.22**	**1.99**	**1.48**
	Treatment			**Location**			**Interaction**		
CD at 5%	**0.25**			**0.12**			**0.20**		

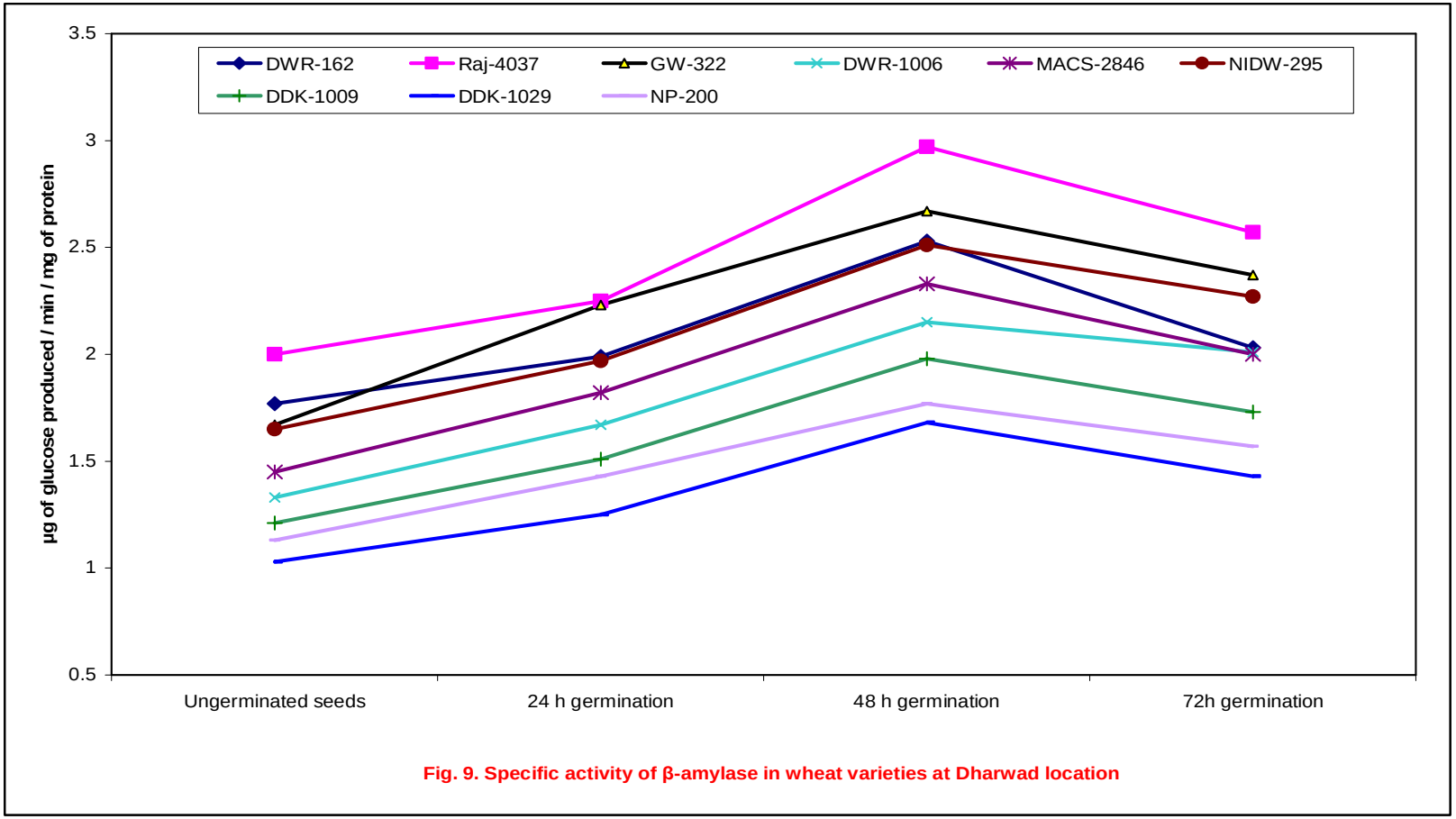


Fig. 9. Specific activity of β-amylase in wheat varieties at Dharwad location

Fig. 9. Specific activity of β-amylase in wheat varieties at Dharwad location

After 24 h of germination, maximum β-amylase activity was observed in Raj-4037 (2.38) which differed significantly from *T. durum* (2.02-2.20) and *T. dicoccum* varieties (1.29-1.79). *T. aestivum* varieties, DWR-162 (2.12), Raj-4037 (2.38) and GW-322 (2.26) were on par with each other. *T. durum* wheat varieties, DWR-1006 (2.02), MACS-2846 (2.10) and NIDW-295 (2.20) were on par with each other. *T. dicoccum* varieties, DDK-1009 (1.76) and NP-200 (1.79) were on par with each other, whereas DDK-1029 (1.29) differed significantly from them.

After 48 h of germination, increased β-amylase activity was observed in all the varieties. Raj-4037 showed maximum β-amylase activity (3.16), which differed significantly from *T. durum* (2.53-2.69) and *T. dicoccum* (1.84-2.48) varieties. *T. aestivum* varieties, DWR-162 (2.89), Raj-4037 (3.16) and GW-322 (3.03) were on par with each other. *T. durum* wheat varieties, DWR-1006 (2.53), MACS-2846 (2.69) and NIDW-295 (2.53) were on par with each other. *T. dicoccum* varieties, DDK-1009 (2.48) and NP-200 (2.33) were on par with each other, whereas DDK-1029 (1.84) differed significantly from them.

After 72 h of germination, decreased β-amylase activity was observed in all the varieties. *T. aestivum* variety Raj-4037 (2.88) differed significantly from *T. durum* (2.32-2.43) and *T. dicoccum* (1.59-2.25) varieties. *T. aestivum* varieties, DWR-162 (2.65), Raj-4037 (2.88) and GW-322 (2.79) were on par with each other. *T. durum* wheat varieties, DWR-1006 (2.41), MACS-2846 (2.43) and NIDW-295 (2.32) were on par with each other. *T. dicoccum* varieties, DDK-1009 (2.25) and NP-200 (2.14) were on par with each other, whereas DDK-1029 (1.59) differed significantly from them.

When location-wise β-amylase activity was studied in ungerminated seeds, *T. aestivum* variety GW-322 differed significantly at both the locations (Dharwad 1.67 and Arabhavi 2.03, respectively). *T. durum* varieties, MACS-2846 (1.68) and NIDW-295 (2.00) at Arabhavi did not differ significantly amongst themselves but differed significantly among themselves at Dharwad (1.45 and 1.65, respectively). *T. dicoccum* variety, NP-200 differed significantly at both the locations (Dharwad 1.13 and Arabhavi 1.41, respectively).

After 24 h germination, *T. aestivum* varieties, DWR-162, Raj-4037 and GW-322 did not differ significantly at both the locations (Dharwad 1.99, 2.25 and 2.23 and Arabhavi 2.12, 2.38 and 2.26, respectively). *T. durum* wheat varieties, DWR-1006 (2.02), MACS-2846 (2.10) and NIDW-295 (2.20) at Arabhavi did not differ significantly amongst themselves but differed significantly at Dharwad (1.67, 1.82 and 1.97, respectively). At Dharwad, *T. dicoccum* varieties DDK-1009 (1.33) and NP-200 (1.41) did not differ significantly amongst themselves but differed significantly at Arabhavi (1.76 and 1.79, respectively).

After 48 h germination, *T. aestivum* varieties, DWR-162 (2.89) and GW-322 (3.03) at Arabhavi did not differ significantly amongst themselves but differed significantly at Dharwad (2.53 and 2.67, respectively). *T. durum* wheat varieties, DWR-1006 (2.53), MACS-2846 (2.69) and NIDW-295 (2.53) at Arabhavi did not differ significantly but differed significantly among themselves at Dharwad (2.15 and 2.33, respectively). At Dharwad, *T. dicoccum* varieties, DDK-1009 (1.98) and NP-200 (1.77) did not differ significantly but differed significantly among themselves at Arabhavi (2.48 and 2.33, respectively).

After 72 h germination, *T. aestivum* varieties, DWR-162 (2.03) and GW-322 (2.37) at Dharwad did not differ significantly amongst themselves but differed significantly at Arabhavi (2.65 and 2.79). *T. durum* wheat varieties, DWR-1006 (2.41) and MACS-2846 (2.43) at Arabhavi did not differ significantly amongst themselves but differed significantly at Dharwad (2.01 and 2.00, respectively). At Dharwad, *T. dicoccum* varieties, DDK-1029 (1.43) and NP-200 (1.77) did not differ significantly amongst themselves but differed significantly among themselves at Arabhavi (2.25 and 2.14, respectively).

As far as mean β-amylase activity was concerned, irrespective of location, *T. aestivum* (2.21-2.54) varieties significantly differed from *T. durum* (1.88-1.97) and *T. dicoccum* (1.26-1.63) varieties.

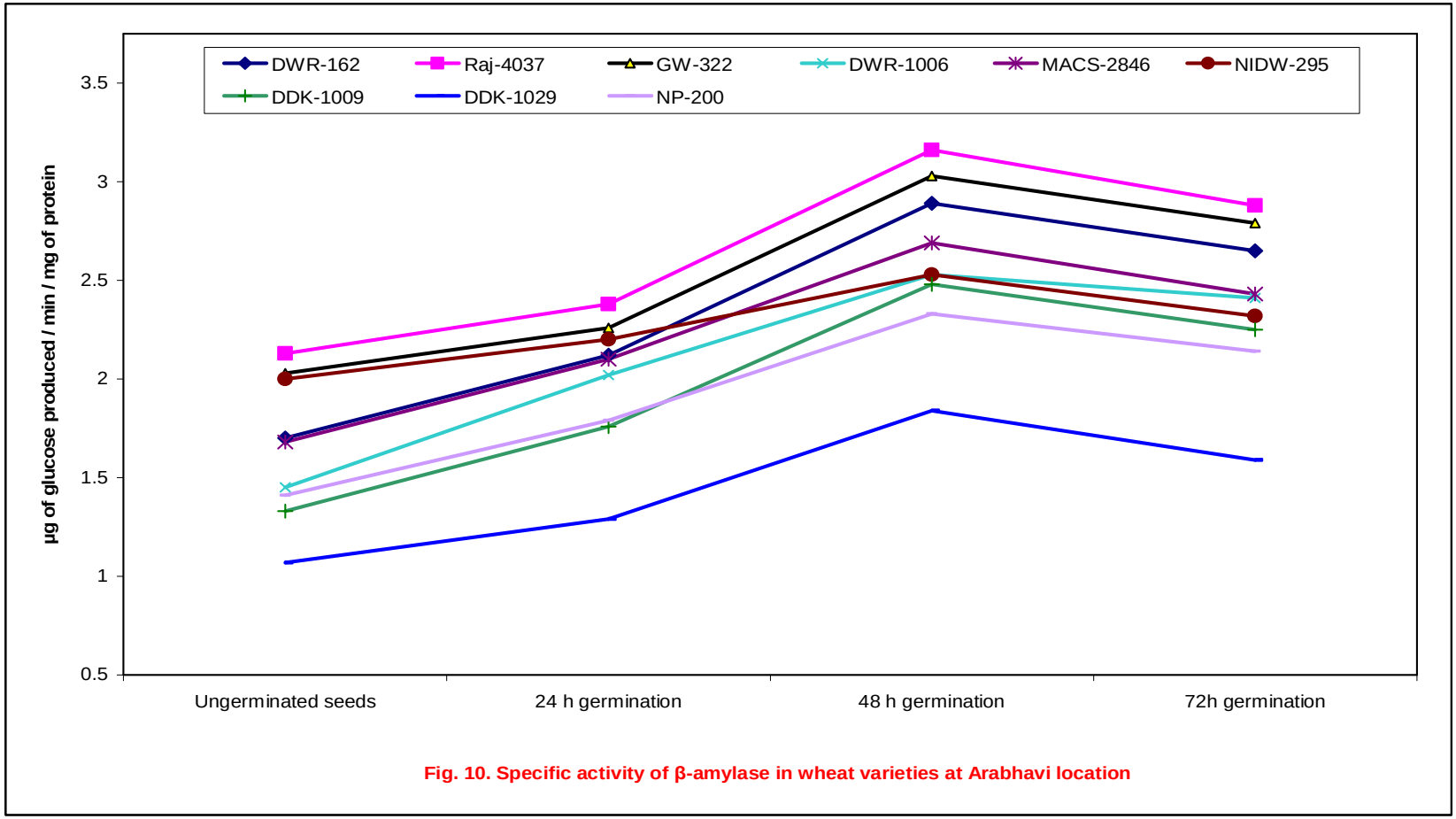


Fig. 10. Specific activity of β-amylase in wheat varieties at Arabhavi location

Fig. 10. Specific activity of β-amylase in wheat varieties at Arabhavi location

Table 11. Specific activity of Acid Invertase activity in *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties, location-wise

Varieties	Specific activity of acid Invertase (µg of glucose produced/min/mg of protein)								
	Dharwad				Arabhavi				
	Ungerminated seeds	24 h germination	48 h germination	72h germination	Ungerminated seeds	24 h germination	48 h germination	72h germination	Mean
T. aestivum									
DWR-162	1.33	1.83	2.12	2.00	1.45	1.95	2.26	2.13	1.88
Raj-4037	1.31	1.52	1.98	1.73	1.53	2.10	2.58	2.33	1.88
GW-322	1.23	1.47	1.87	1.56	1.62	2.21	2.67	2.46	1.88
Mean	**1.29**	**1.61**	**1.99**	**1.76**	**1.53**	**2.09**	**2.50**	**2.31**	**1.88**
T. durum									
DWR-1006	1.12	1.16	1.47	1.23	1.35	1.56	2.12	1.95	1.49
MACS-2846	1.18	1.21	1.59	1.43	1.32	1.53	2.13	1.88	1.53
NIDW-295	1.22	1.35	1.67	1.46	1.42	1.84	2.22	2.10	1.66
Mean	**1.17**	**1.24**	**1.58**	**1.37**	**1.36**	**1.64**	**2.16**	**1.98**	**1.56**
T. dicoccum									
DDK-1009	0.99	1.00	1.13	1.10	1.12	1.26	1.58	1.33	1.18
DDK-1029	1.01	1.09	1.25	1.11	1.13	1.34	1.74	1.53	1.28
NP-200	1.10	1.13	1.38	1.25	1.15	1.29	1.67	1.43	1.16
Mean	**1.03**	**1.07**	**1.25**	**1.15**	**1.13**	**1.30**	**1.66**	**1.43**	**1.21**
	Treatment			**Location**			**Interaction**		
CD at 5%	**0.20**			**0.11**			**0.12**		

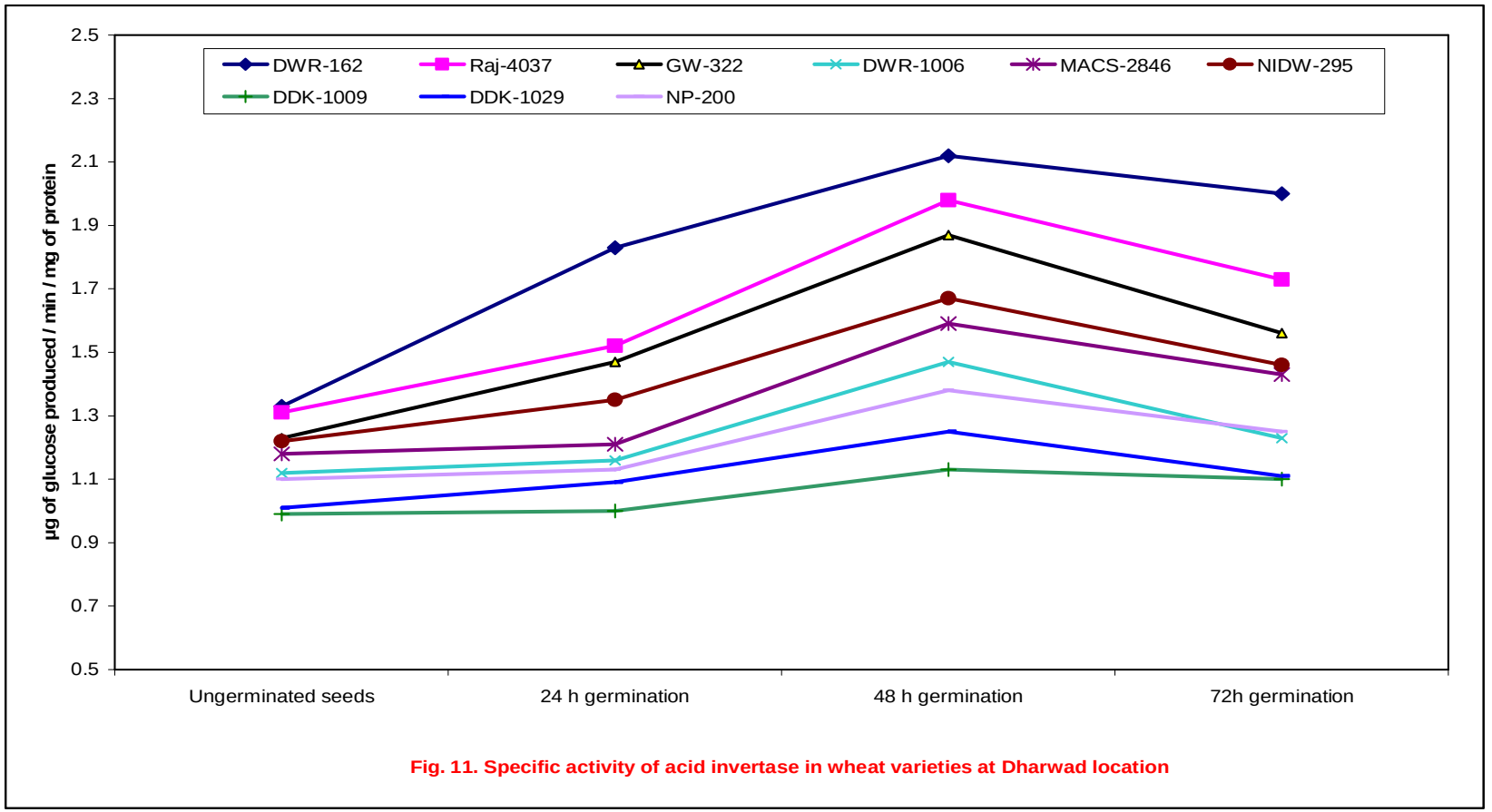


Fig. 11. Specific activity of acid invertase in wheat varieties at Dharwad location

Fig. 11. Specific activity of acid invertase in wheat varieties at Dharwad location

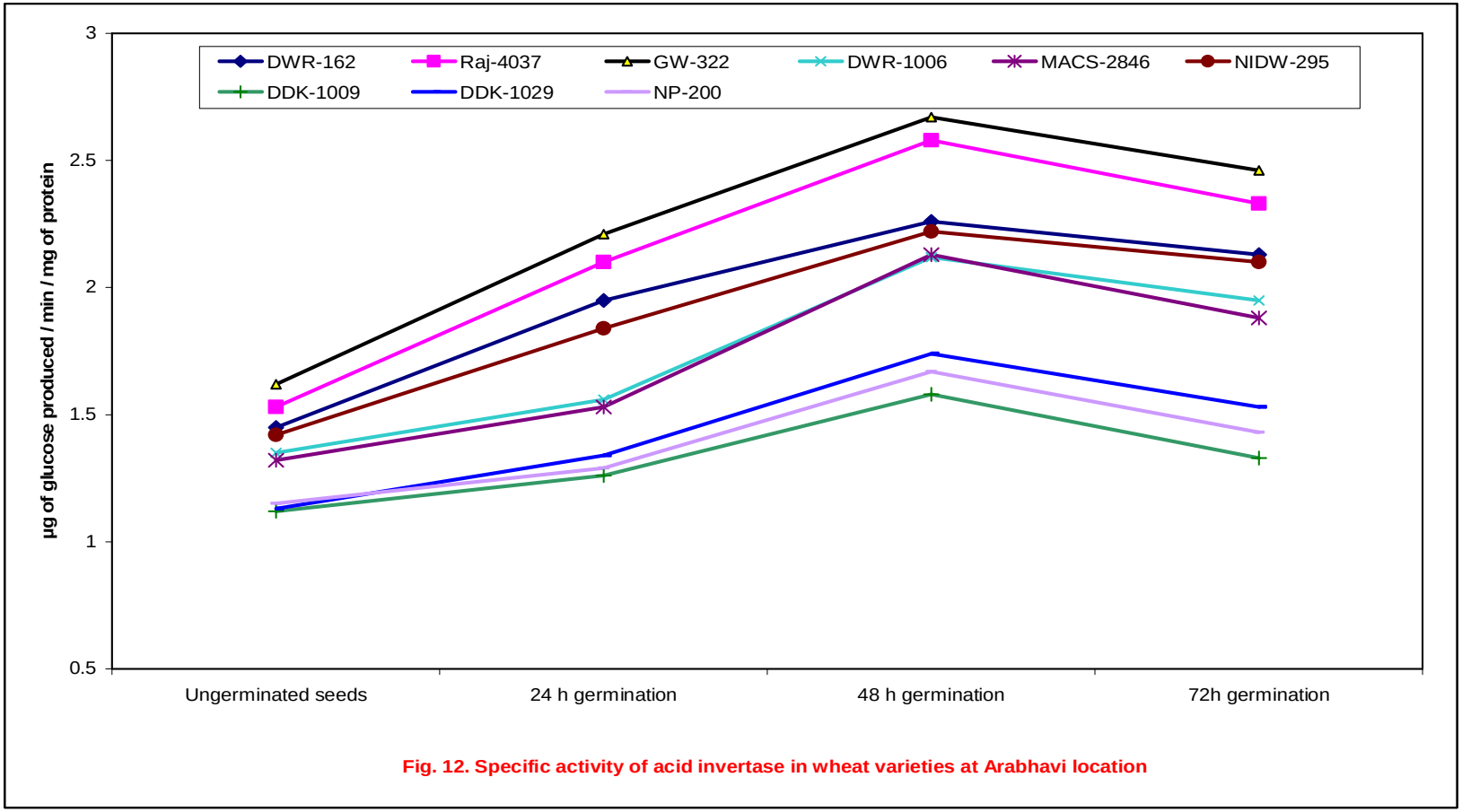


Fig. 12. Specific activity of acid invertase in wheat varieties at Arabhavi location

Fig. 12. Specific activity of acid invertase in wheat varieties at Arabhavi location

At Dharwad, when acid invertase activity was studied in ungerminated seeds, maximum activity was observed in *T. aestivum* variety DWR-162 (1.33) which differed significantly from *T. durum* (1.12-1.22) and *T. dicoccum* varieties (0.99-1.10). *T. aestivum* varieties, DWR-162 (1.33), Raj-4037 (1.31) and GW-322 (1.23) were on par with each other. *T. durum* wheat varieties, DWR-1006 (1.12), MACS-2846 (1.18) and NIDW-295 (1.22) were also on par with each other. Similarly, *T. dicoccum* varieties, DDK-1009 (0.99), DDK-1029 (1.01) and NP-200 (1.10) were on par with each other.

After 24 h of germination, maximum acid invertase activity was observed in *T. aestivum* variety DWR-162 (1.83) which differed significantly from *T. durum* (1.16-1.35) and *T. dicoccum* varieties (1.00-1.13). *T. aestivum* varieties, DWR-162 (1.83) and Raj-4037 (1.52) were on par with each other, whereas GW-322 (1.47) differed significantly from DWR-162. *T. durum* wheat varieties, DWR-1006 (1.16), MACS-2846 (1.21) and NIDW-295 (1.35) were also on par with each other. Similarly, *T. dicoccum* varieties, DDK-1009 (1.00), DDK-1029 (1.09) and NP-200 (1.13) were on par with each other.

After 48 h of germination, increased acid invertase activity was found in all the varieties. DWR-162 showed maximum acid invertase activity (2.12), which differed significantly from *T. durum* (1.47-1.67) and *T. dicoccum* varieties (1.13-1.38). *T. aestivum* varieties, DWR-162 (2.12), Raj-4037 (1.98) and GW-322 (1.87) were on par with each other. *T. durum* wheat varieties, DWR-1006 (1.47), MACS-2846 (1.59) and NIDW-295 (1.67) were also on par with each other. *T. dicoccum* varieties, DDK-1009 (1.13) and DDK-1029 (1.25) were on par with each other, whereas NP-200 (1.38) differed significantly from DDK-1009.

After 72 h of germination, *T. aestivum* variety DWR-162 (2.00) showed maximum acid invertase activity, which differed significantly from *T. durum* (1.23-1.46) and *T. dicoccum* (1.10-1.25) varieties. *T. aestivum* varieties, Raj-4037 (1.73) and GW-322 (1.56) were on par with each other, whereas DWR-162 (2.00) differed significantly from GW-322 (1.56). *T. durum* wheat varieties, DWR-1006 (1.23), MACS-2846 (1.43) and NIDW-295 (1.46) were on par with each other. *T. dicoccum* varieties, DDK-1009 (1.10), DDK-1029 (1.11) and NP-200 (1.25) were also on par with each other.

In ungerminated seeds, the activity of acid invertase was maximum in *T. aestivum* variety GW-322 (1.62) which differed significantly from *T. durum* (1.32-1.42) and *T. dicoccum* (1.12-1.15) varieties. *T. aestivum* varieties, DWR-162 (1.45), Raj-4037 (1.53) and GW-322 (1.62) were on par with each other. *T. durum* wheat varieties, DWR-1006 (1.35), MACS-2846 (1.32) and NIDW-295 (1.42) were also on par with each other. Similarly, *T. dicoccum* varieties, DDK-1009 (1.12), DDK-1029 (1.13) and NP-200 (1.15) were on par with each other.

After 24 h of germination, maximum acid invertase activity was found in GW-322 (2.21) which differed significantly from *T. durum* (1.53-1.84) and *T. dicoccum* (1.26-1.34) varieties. *T. aestivum* varieties, DWR-162 (1.95) and Raj-4037 (2.10) were on par with each other, whereas GW-322 (2.21) differed significantly from DWR-162. *T. durum* wheat varieties, DWR-1006 (1.56) and MACS-2846 (1.53) were on par with each other, whereas NIDW-295 (1.84) differed significantly from them. *T. dicoccum* varieties, DDK-1009 (1.26), DDK-1029 (1.34) and NP-200 (1.29) were on par with each other.

After 48 h of germination, increased acid invertase activity was observed in all the wheat varieties. *T. aestivum* variety GW-322 showed maximum activity (2.67) which differed significantly from *T. durum* (2.12-2.22) and *T. dicoccum* (1.58-1.74) varieties. *T. aestivum* varieties, Raj-4037 (2.58) and GW-322 (2.67) were on par with each other, whereas DWR-162 (2.26) differed significantly from them. *T. durum* wheat varieties, DWR-1006 (2.12), MACS-2846 (2.13) and NIDW-295 (2.22) were on par with each other. *T. dicoccum* varieties, DDK-1009 (1.58), DDK-1029 (1.74) and NP-200 (1.67) were also on par with each other.

After 72 h of germination, decline in the invertase activity was found in all the varieties. *T. aestivum* variety GW-322 showed maximum activity (2.46) which differed significantly from *T. durum* (1.88-2.10) and *T. dicoccum* (1.33-1.53) varieties. *T. aestivum* varieties, Raj-4037 (2.33) and GW-322 (2.46) were on par with each other, whereas DWR-162 (2.13) differed significantly from GW-322. *T. durum* wheat varieties, DWR-1006 (1.95), MACS-2846 (1.88) and NIDW-295 (2.10) were also on par with each other.

Similarly *T. dicoccum* varieties, DDK-1009 (1.33), DDK-1029 (1.53) and NP-200 (1.43) were on par with each other.

Location wise, acid invertase activity in ungerminated seeds revealed that *T. aestivum* varieties Raj-4037 (1.53) and GW-322 (1.62) did not differ significantly among themselves at Arabhavi but differed significantly at Dharwad (1.31 and 1.23, respectively). *T. durum* varieties, DWR-1006 (1.12), MACS-2846 (1.18) and NIDW-295 (1.22) did not differ significantly amongst themselves at Dharwad but differed significantly among themselves at Arabhavi (1.35, 1.32 and 1.42, respectively). *T. dicoccum* varieties, DDK-1009, DDK-1029 and NP-200 did not differ significantly at both the locations (Dharwad 0.99, 1.01 and 1.10 and Arabhavi 1.12, 1.13 and 1.15, respectively).

After 24 h germination, *T. aestivum* varieties, Raj-4037 (2.10) and GW-322 (2.21) at Arabhavi differed significantly amongst themselves at Arabhavi and Dharwad (1.52 and 1.47, respectively). *T. durum* varieties, DWR-1006 (1.16) and MACS-2846 (1.21) did not differ significantly amongst themselves at Dharwad but differed significantly from themselves at Arabhavi (1.56 and 1.53, respectively). *T. dicoccum* varieties, DDK-1009 (0.99), DDK-1029 (1.01) and NP-200 (1.10) at Dharwad did not differ significantly amongst themselves but differed significantly amongst themselves at Arabhavi (1.26, 1.34 and 1.29, respectively).

After 48 h germination, *T. aestivum* varieties, DWR-162 (2.12) and Raj-4037 (1.98) did not differ significantly amongst themselves at Dharwad but differed significantly at Arabhavi (2.26 and 2.58, respectively). *T. durum* wheat varieties, DWR-1006 (1.47), MACS-2846 (1.59) and NIDW-295 (1.67) did not differ significantly amongst themselves at Dharwad but differed significantly at Arabhavi (2.12, 2.13 and 2.22, respectively). *T. dicoccum* varieties, DDK-1009 (1.13) and DDK-1029 (1.25) did not differ significantly amongst themselves at Dharwad but differed significantly at Arabhavi (1.58 and 1.74, respectively).

After 72 h germination, *T. aestivum* variety Raj-4037 differed significantly at both the locations (Dharwad 1.73 and Arabhavi 2.33, respectively). *T. durum* varieties, DWR-1006 (1.23), MACS-2846 (1.43) and NIDW-295 (1.46) did not differ significantly amongst themselves at Dharwad but differed at Arabhavi (1.95, 1.88 and 2.10, respectively). *T. dicoccum* varieties, DDK-1009 (1.33), DDK-1029 (1.53) and NP-200 (1.43) did not differ significantly amongst themselves at Arabhavi but differed significantly at Dharwad (1.10, 1.11 and 1.25, respectively).

As far as mean acid invertase activity was concerned, irrespective of the location, *T. aestivum* varieties (1.88) significantly differed from *T. durum* (1.49-1.66) and *T. dicoccum* varieties (1.16-1.28).

4.7 Protein banding pattern

Location-wise evaluation of protein banding patterns of *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties are presented in Figs.13 and 14. The protein bands were identified by SDS-PAGE using a protein marker. The bands in 10-70 KDa range were considered as Low Molecular Weight Glutenin Subunits (LMW-GS) and the bands in the range of 71-100 KDa were considered as High Molecular Weight Glutenin Subunits (HMW-GS) according to Bietz and Wall (1972).

The results of the protein banding patterns of different varieties raised at Dharwad and Arabhavi, when compared with standard molecular weight markers, reveal that the total number of *T. aestivum* varieties at Dharwad and Arabhavi depicted 19 and 20 bands between 10-100KDa, respectively.

Variety DWR-162 exhibited total seven bands in the range 10-100KDa both at Dharwad and Arabhavi. It did not exhibit any band of 10KDa at Dharwad, whereas at Arabhavi, one band was observed at 10 KDa. Both the locations exhibited one band each in the range of 10-20 and 20-30 KDa. In the 30-40KDa range at Dharwad, DWR-162 did not show any band, whereas one band was observed at Arabhavi. At Dharwad, the same variety in the range of 40-50KDa exhibited one band, whereas at Arabhavi it did not exhibit any band in that range. Both the locations exhibited one band each in the range of 60-70 KDa and 70-85 KDa. In between 85-100 KDa, DWR-162 at Dharwad contained one band near 85 KDa, whereas at Arabhavi it exhibited one band very close to 100 KDa.

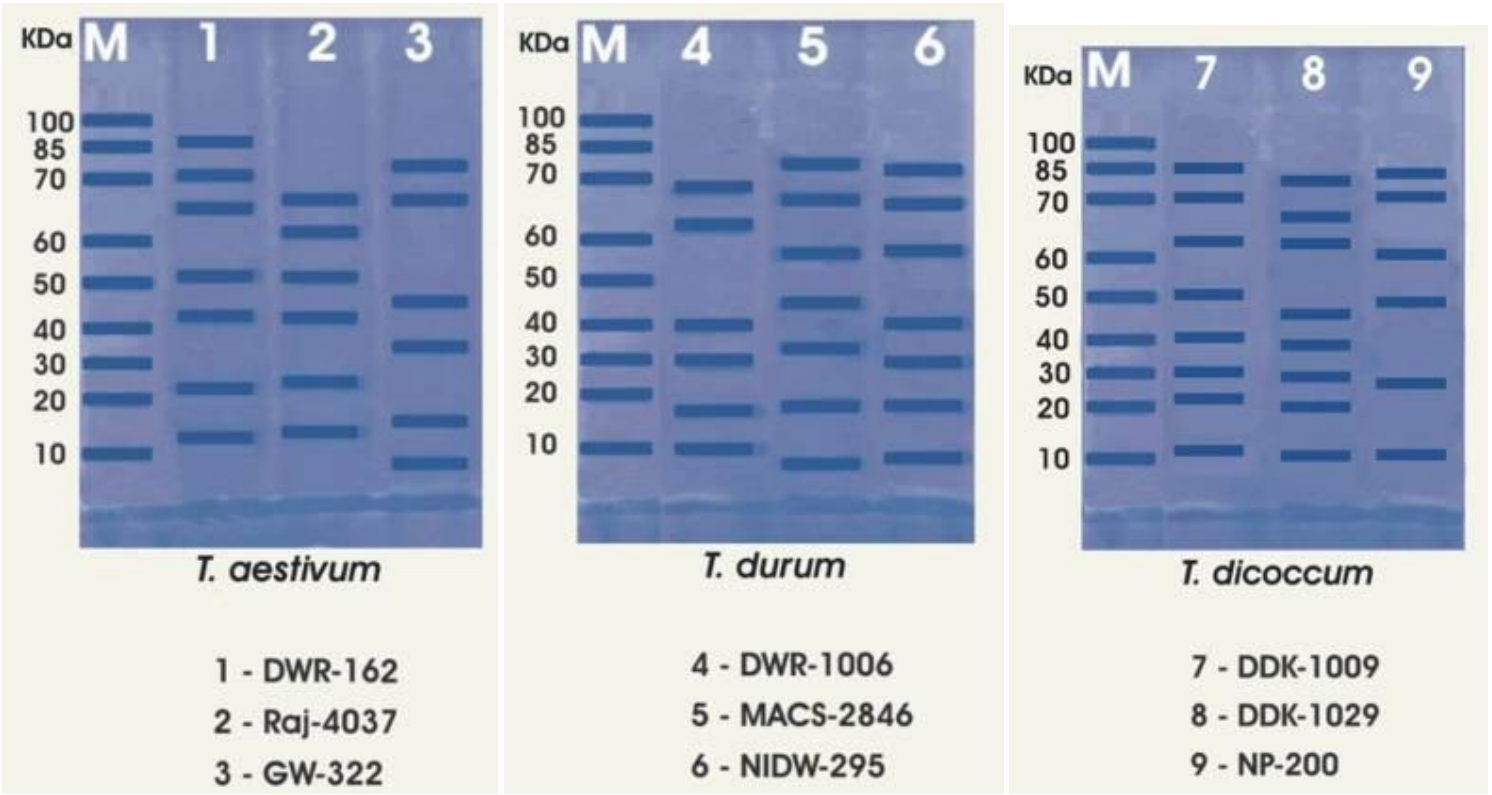


Fig.13. Protein banding pattern of T. aestivum, T. durum and T. dicoccum at Dharwad location

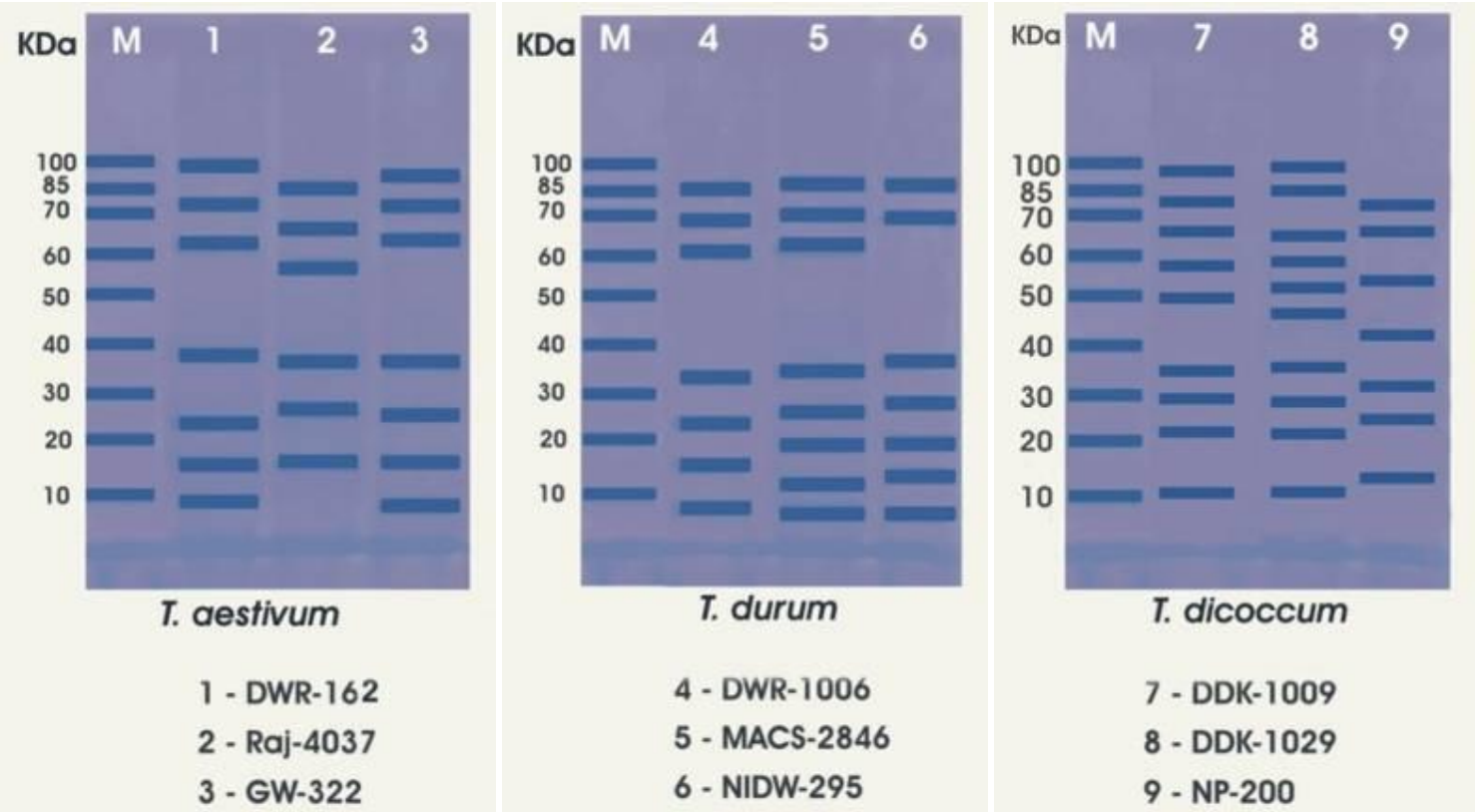


Fig. 14. Protein banding pattern of T. aestivum, T. durum and T. dicoccum at Arabhavi location

Another *T. aestivum* variety, Raj-4037 exhibited six bands at both the locations in the range of 10-100KDa. Both the locations showed one band each in the range of 10-20 and 20-30 KDa. In 30-40 KDa range at Dharwad it did not exhibit any band, whereas at Arabhavi it contained one band in that range. At Dharwad one band was found in the range of 40-50 KDa, whereas at Arabhavi no bands were observed in that range. In 50-60 KDa range, one band each was found at Dharwad and at Arabhavi. At Dharwad, two bands were observed in the range of 60-70 KDa, whereas at Arabhavi it contained only one band in that range. In the range of 70-85 KDa both at Arabhavi and Dharwad, no band was observed. At both the locations, Raj-4037 did not exhibit any band at 100 KDa range.

The *T. aestivum* variety, GW-322 exhibited six bands at Dharwad and seven at Arabhavi in the range of 10-100 KDa. At both the locations, it contained one band each below the range of 10 KDa and 10-20 KDa. In 20-30 KDa range, it did not exhibit any band at Dharwad, whereas at Arabhavi it exhibited one band in that range. In the range of 30-40 KDa at both the locations, one band each was observed. At Dharwad one band was observed in 40-50 KDa range, whereas at Arabhavi no band was observed in that range. Both the locations exhibited one band each in the range of 60-70 and 70-85 KDa. At Arabhavi, variety GW-322 exhibited one band in the range of 85-100 KDa, whereas no band was located in that range at Dharwad.

The total number of bands in *T. durum* varieties at Dharwad and Arabhavi were 20 and 22 between 10-100 KDa, respectively. Variety DWR-1006 exhibited a total of six bands at Dharwad, whereas at Arabhavi it exhibited seven bands in the range of 10-100 KDa. At Arabhavi, DWR-1006 exhibited one band below the range of 10 KDa, whereas at Dharwad only one band was located in that range. In 10-20 KDa range, it exhibited one band each at Dharwad and at Arabhavi. Only one band was located in 20-30 range at Arabhavi, whereas one band was located at 30 KDa at Dharwad. No bands were exhibited in the range of 40-50 and 50-60 KDa at both the locations, whereas just above 40 KDa one band was located at Dharwad and one band was seen at 60 KDa at Arabhavi. In 70-85 KDa range, no bands were observed at Dharwad and at Arabhavi, whereas one band was observed just above 85 KDa at Arabhavi.

Second *T. durum* variety MACS-2846 exhibited seven bands at Dharwad and eight bands at Arabhavi in the range of 10-100KDa. At both the locations, it exhibited one band each below the range of 10 KDa. In 10-20 KDa range, one band was found at Dharwad, whereas at Arabhavi two bands were observed. At Dharwad, no band was found in the range 20-30 KDa, whereas at Arabhavi one band was observed in that range. At both the locations, it exhibited one band each in the range of 30-40 KDa. At Dharwad one band each was found in the range of 40-50 and 50-60 KDa, whereas at Arabhavi no band was observed in that range. In 60-70 KDa range, one band was found at Dharwad, whereas at Arabhavi two bands were observed in that range. At Dharwad MACS-2846 contained one band in 70-85 KDa range, whereas at Arabhavi it exhibited one band between 85-100 KDa.

The *T. durum* variety NIDW-295 exhibited a total of seven bands in the range of 10-100 KDa, both at Dharwad and Arabhavi. At both the locations, it exhibited one band each below the range of 10 KDa. At Dharwad, one band was observed in 10-20 KDa range, whereas at Arabhavi two bands were observed. At both the locations, it exhibited one band each in the range of 20-30 and 30-40 KDa. No band was observed in 40-50 KDa range at both the locations. In 50-60 KDa one band was observed at Dharwad, whereas at Arabhavi no band was observed. At both the locations, NIDW-295 exhibited one band each in the range of 60-70 and 70-85 KDa. None of the *T. durum* varieties exhibited 100 KDa band at Dharwad and Arabhavi.

The total number of bands in *T. dicoccum* varieties at Dharwad and Arabhavi were 22 and 26, respectively, in the range of 10-100 KDa. Variety DDK-1009 exhibited two bands in 10-20 KDa range at both the locations. At Arabhavi, it exhibited two bands in the range of 20-30, one each in 30-40 and 40-50 KDa range. One band each was observed in 50-60 KDa range at Dharwad and Arabhavi. In 60-70 KDa range DDK-1009 exhibited one band each at Dharwad and Arabhavi. In the same variety, one band was observed in 70-85 KDa range at Arabhavi, whereas two bands were observed at Dharwad in 85-100 KDa range.

Another variety of *T. dicoccum*, DDK-1029 exhibited eight bands at Dharwad and 10 at Arabhavi in the range of 10-100 KDa. At both the locations, it exhibited one band each in

10-30 KDa range. At Dharwad, DDK-1029 exhibited one band at 40 KDa, whereas at Arabhavi one band was observed in 30-40 KDa range. At both the locations, it exhibited one band each in 40-50 KDa range. In 50-60 KDa range at Dharwad, no bands were observed, whereas at Arabhavi two bands were observed in that range. At Dharwad two bands were observed in the range of 60-70 KDa, whereas at Arabhavi one band was observed in that range. At both the locations it exhibited one band each in the range of 70-85 KDa. At Arabhavi and Dharwad, variety DDK-1029 exhibited one band each in the range of 85-100 KDa.

The *T. dicoccum* variety NP-200 exhibited six bands at Dharwad and seven bands at Arabhavi in the range of 10-100 KDa. At Dharwad, one band of 10 KDa was observed, whereas at Arabhavi no band was found. In the range of 10-20 KDa, no band was observed at Dharwad, whereas at Arabhavi one band was observed. At both the locations, it exhibited one band each in the range of 20-30 KDa. At Arabhavi, NP-200 exhibited one band in 30-40 KDa range, whereas at Dharwad no bands were found. At both the locations, it exhibited one band each in the range of 40-50, 50-60, 60-70 and 70-85 KDa. No band was found in the 100 KDa range at both the locations.

The overall results provide conclusive evidence that there was effect of environmental variation in different biochemical parameters in the different genotypes studied in the present investigation.

5. DISCUSSION

The biochemical quality parameters of different *T. aestivum*, *T. durum* and *T. dicoccum* verities of wheat, grown in the Peninsular Zone by the All India Coordinated Wheat Improvement Project, University of Agricultural Sciences, Dharwad were studied in the grains collected from the crop grown at transitional (Dharwad) and dry agroclimatic (Arabhavi) zones of Karnataka during *rabi* 2007-2008. The biochemical quality parameters of *T. aestivum*, *T. durum* and *T. dicoccum* wheat varieties were examined. The results obtained during the course of this investigation are discussed hereunder.

5.1 Biochemical quality parameters of wheat

Quality, the most important criteria for any seed is largely dependent upon its chemical composition. It is, in turn, influenced by genetic and environmental factors. Food stuffs contain proteins, carbohydrates, fats, vitamins, minerals and other accessory food factors in varying proportions. All these nutrients play specific roles in human nutrition.

Nature of carbohydrates and their distribution in grains reflects the grain quality. Sweetness of the product is related to simple sugars, whereas nature of starch depicts the cooking quality. Carbohydrate profile of wheat varieties *viz.,* total sugars, reducing and non-reducing sugars, starch and total carbohydrate components are assessed to know its nutritional and functional qualities.

A glance at the total carbohydrate and starch content (Table 2) reveals that *T. aestivum* varieties contained higher amount of carbohydrate followed by *T. durum* and *T. dicoccum* varieties. *T. aestivum* variety GW-322 contained higher percentage of carbohydrate at Dharwad as compared to Arabhavi. As far as carbohydrate content is concerned, DWR-162 performed better at Dharwad than Arabhavi, whereas Raj-4037 was an exceptional case because it performed very well at Arabhavi as compared to Dharwad. *T. durum* variety NIDW-295 performed better at Arabhavi than at Dharwad, whereas DWR-1006 and MACS-2846 performed better at both the locations. *T. dicoccum* varieties, DDK-1029 and NP-200, contained good amount of carbohydrate at Arabhavi as compared to Dharwad, whereas DDK-1009 performed better at both the locations. Overall, the data of the present investigation reveal that *T. durum* and *T. dicoccum* varieties performed better at Arabhavi than at Dharwad. It may be concluded that *T. aestivum* varieties are better suited for Dharwad location as compared to Arabhavi location with respect to carbohydrate content.

It may be appropriate to mention here that starch content (Table 2) of *T. aestivum* variety GW-322 showed 6.2 per cent increase at Arabhavi as compared to its own performance at Dharwad, whereas DWR-162 and Raj-4037 performed equally well at both the locations. *T. durum* variety NIDW-295 performed better at Arabhavi than at Dharwad, whereas DDK-1006 and MACS-2846 performed equally well at both the locations. *T. dicoccum* varieties, DDK-1009 and NP-200, contained very good amount of starch at Arabhavi as compared to Dharwad, whereas DDK-1029 performed equally well at both the locations. Moreover, significant interaction effect was observed among *T. aestivum*, *T. durum* and *T. dicoccum* varieties.

Jhuma *et al.* (2003) studied four north Indian bread wheat cultivars *viz.,* C-360, HD-2009, WH-291 and WH-542 differing in flour and cooking quality. The study revealed a progressive decrease in total sugars, reducing and non-reducing sugars in the developing grains and increase in starch content throughout the grain development period. The results of the present investigation thus receive support from the finding of Jhuma and his coworkers.

The status of reducing, non-reducing and total sugars is depicted in Table 3. The *T. aestivum* varieties, DWR-162, Raj-4037 and GW-322 contained relatively lower amount of reducing sugars as compared to *T. durum* and *T. dicoccum* varieties. *T. durum* varieties, DWR-1006, MACS-2846 and NIDW-295 contained good amount of reducing sugars at both the locations. *T. dicoccum* varieties, DDK-1009, DDK-1029 and NP-200 contained the highest amount of reducing sugars than *T. aestivum* and *T. durum* and there was no significant difference location-wise since they performed better at both the locations.

T. durum varieties were found to contain higher amount of non-reducing sugars as compared to *T. aestivum* and *T. dicoccum* varieties. It may be noted that *T. aestivum* variety DWR-162 contained higher amount of total sugars at Dharwad as compared to its own performance at Arabhavi, whereas Raj-4037 and GW-322 performed equally well at both the locations.

These observations receive support from the studies of Bakshi and Bains (1987) who observed higher amount of non-reducing sugar content in 10 *T. durum* wheat varieties (1.50-2.20 %) than in two bread wheat varieties and higher amount of reducing sugars in *T. durum* varieties than in Bread varieties. Observation of this investigation reveal that higher amount of non-reducing sugar was observed in *T. durum* (1.49-1.78 %) varieties, whereas, the amount of reducing sugar was higher in *T. dicoccum* (0.20-0.22 %) than *T. durum* and *T. aestivum* varieties.

Reddy (1996) observed variation in total sugar content of *T. dicoccum* (1.85 %), *T. durum* (1.45 %) and *T. aestivum* (1.39 %) varieties and also reported that non-reducing sugar content of *T. durum* and *T. dicoccum* was higher than that of *T. aestivum* varieties. It is evident from data that *T. durum* and *T. dicoccum* contained higher amount of non-reducing sugar than *T. aestivum* at Arabhavi, whereas at Dharwad *T. durum* and *T. aestivum* varieties contained higher amount of non-reducing sugar than *T. dicoccum* varieties. The requirement of soluble sugars in wheat grain for the germination purpose is well known. In location-wise study like this these sugars assume greater importance.

The increase in nitrogen availability (Table 4) observed in this investigation may be attributed to improved soil properties which might have led to enhanced nitrogen fixation and mineralization of organic nitrogen. Minimized nitrogen loss might have helped in increasing protein content. It can be seen from the data that lowest nitrogen content was observed in *T. durum* varieties at Dharwad (1.66-1.96). All the *T. aestivum* varieties showed higher nitrogen content at Arabhavi than Dharwad. Raj-4037 was placed first at Arabhavi as compared to its performance at Dharwad followed by GW-322, whereas DWR-162 performed equally well at Arabhavi and Dharwad location, suggesting that it is suitable for both the locations. *T. dicoccum* varieties at Dharwad contained good amount of nitrogen and DDK-1009 performed equally well at both the locations. *T. dicoccum* varieties seem to be better suited for Dharwad as compared to Arabhavi location as far as nitrogen availability is concerned in the present investigation, although less variation was found at Arabhavi.

James *et al*. (2001) evaluated multi-location yield potential, adaptation and end product quality of winter *T. durum* wheats. At three out of six locations, 13.0 per cent protein was recorded by *T. durum* wheat varieties, at other three locations, *T. durum* protein did not exceed 12.5 per cent even after the application of highest nitrogen level. They opined that improper nitrogen uptake or other environmental factors might have affected the protein levels in the *T. durum* varieties studied at three locations.

In this context, observations of Peltonen *et al*. (1994) are worth mentioning here who reported that nitrogen fertilizer application improved bread making quality of wheat flour, mainly by increasing the quantity of low-molecular weight gliadin. The most positive effect of flour protein concentration and loaf volume was obtained by them with the application of granular, dicyandiamide-regulated, slow release N-fertilizer.

Protein is an important quality parameter that decides the suitability of wheat for a particular type of product. The unique feature of the wheat grain is dough-forming property making it responsible for the most important source of protein in the human diet.

The significant interaction effect observed in *T. aestivum*, *T. durum* and *T. dicoccum* varieties irrespective of locations in relation to crude protein (Table 4) indicates the importance of conducting multi-location trials for growing wheat. Perusal of data reveal that at Arabhavi, 3.78 and 4.79 per cent increase in crude protein was registered by *T. aestivum* varieties Raj-4037 and GW-322 over Dharwad. When *T. aestivum* varieties were ranked with respect to their locations, GW-322 was placed first at Arabhavi followed by Raj-4037 as compared to its performance at Dharwad, whereas DWR-162 performed equally well at Dharwad and Arabhavi suggesting thereby that DWR-162 may be suitable for both the locations. Similarly, all the *T. durum* varieties also performed better at Arabhavi than Dharwad location. It may be noted here that *T. dicoccum* varieties at Dharwad contained good amount of crude protein than Arabhavi. Overall, the data of the present investigation reveal that two

varieties of *T. aestivum* (DWR-162 and Raj-4037) and all the *T. durum* varieties studied performed better at Arabhavi than at Dharwad and *T. dicoccum* varieties were found to be better suited for Dharwad as compared to Arabhavi location as far as protein is concerned.

It may be noted here that Vatsala and Haridas Rao (1990) observed variation in the mean protein content of *T. aestivum*, *T. durum* and *T. dicoccum* varieties (10.8-11.8 %). Their findings received from support Patil (1998) who studied three *T. dicoccum* and two *T. durum* varieties. The mean protein content in his study varied from 12.23-16.29 per cent in *T. dicoccum* and 9.24-12.31 per cent in *T. durum* varieties and oil content from 1.9-3.8 per cent in *T. dicoccum* and 2.5-4.2 per cent *T. durum* varieties. These observations support the results of the present investigation in which there was variation in mean protein content (11.65-17.15 %) and oil content (1.2-2.2 %) of *T. aestivum*, *T. durum* and *T. dicoccum* varieties.

The results of James *et al*. (2001) also lend support to the observations of this investigation on protein content. They made multi-location evaluation of the yield potential, adaptation and end-product quality of newly developed winter *T. durum* wheats. The grain proteins were found to vary from 9.0-15.5 per cent depending on the location and rate of nitrogen application. At three out of six locations, 13.0 per cent protein was recorded by *T. durum* wheat varieties. At other three locations, *T. durum* protein did not exceed 12.5 per cent even at the application of highest level of nitrogen. They attributed these observations to the location variation where environmental factors might have affected the quality of wheat.

Singh *et al*. (2007) reported protein content in commercially grown wheat varieties in India which ranged between 9.0 and 11.0 per cent. Among them, *T. durum* variety HD 2819 had 12.74 per cent and better mixing time along with high loaf volume. The observations with respect to disease resistance, pest resistance and quality characters recorded at multi-locations indicated that HD 2819 was a novel genetic stock. The study further emphasized the necessity and importance of multi-location trials.

It appears that Arabhavi location is better for increasing protein in wheat over Dharwad location. Many factors like soil type and its pH and EC, soil microflora, salinity of water used for wheat cultivation, temperature, rain fall etc affect the yield and quality of the wheat. Randall and Moss (1990) observed that elevated temperature during grain filling is possibly the most important environmental determinant of grain quality. High temperature during grain filling, especially greater than 35^0 C alter has been reported to the protein biosynthetic pathways of grain, leading to protein compositional changes (Blumenthal *et al*., 1993).

Perusal of data from Table 5 reveal that at Arabhavi, Aestivum verities GW-322 and Raj-4037 and *T. durum* varieties MACS-2846 and NIDW-295 had better soluble protein content at Arabhavi, whereas DWR-1006 performed better at Dharwad over Arabhavi. *T. dicoccum* variety DDK-1009 had higher soluble protein content at Dharwad than Arabhavi, however DDK-1029 performed better at Arabhavi as compared to Dharwad. It is interesting to note at the same time that NP-200 performed equally well at both the locations. Significant interaction effect was observed among *T. aestivum*, *T. durum* and *T. dicoccum* varieties irrespective of locations.

The observations of Karimzadeh *et al*. (2003) regarding changes in the callus soluble protein of winter and spring wheat cultivars following cold treatment are worthy of mention here. Significant cold-induced increase in protein quantity was observed by them during the low temperature treatment, irrespective of the cultivars as compared with the controls. Both the winter and spring wheat cultivars showed differences in their callus soluble protein content during the experimental period in response to temperature alterations.

It has been reported that the unique feature of the wheat grain is its dough making property which makes wheat as the most important source of protein in all the cereals. Gliadin and Glutenin together form a characteristic substance called ‘Gluten’ with water (MacRitchie, 1994 and Fu and Sapirstein., 1996). Glutenin confers elasticity to dough, whereas viscous gliadins provide extensibility to dough (Payne *et al*., 1984). It has been reported that more hydrophobic gliadins increase the volume of bread loaf, while gliadins from more hydrophilic part of the electrophoretic spectrum decrease the volume of bread loaf (Van Lonkhuijsen *et al*., 1992).

It may be noted from Table 5 that wet gluten content of *T. aestivum* varieties DWR-162, Raj-4037 and GW-322 was on higher side at Arabhavi over Dharwad. *T. durum* varieties DDK-1009 and DDK-1029 contained higher amount of wet gluten at Dharwad than Arabhavi, whereas NP-200 had higher gluten content at Arabhavi as compared to its content at Dharwad. It implies that depending upon the requirement of a particular type of wheat with specific gluten content, breeders may attempt to develop such lines.

The results of Haridas Rao *et al*. (1976) lend support to the observations of this investigation on gluten content. They studied 24 Indian and three Canadian *T. durum* wheat varieties. The range of values for Indian wheat gluten were 28.20-34.30 per cent and the Canadian values ranged between 40.60-52.60 per cent.

It has been well documented that quantity of glutenin in wheat flours is positively correlated with loaf volume and dough resistance and proportion of glutenin and gliadin fractions, particularly glutenin has a direct effect on the functionality because of little flour requirement (Singh *et al*., 1990b).

The data from Table 7 reveal that percentage of oil was found to be very less in wheat. The content of oil in wheat varieties at Arabhavi was higher as compared to their oil content at Dharwad. Significant interaction effect observed in *T. aestivum*, *T. durum* and *T. dicoccum* varieties irrespective of locations is indicative of the various factors involved in imparting the varieties those properties which allow them to perform batter at one location over another.

With the increase in awareness of cholesterol content of different food stuffs and its relationship with atherosclerosis, Policosanol (PC) of wheat assumes great importance since it has been reported to possess cholesterol-lowering property. Youngfen *et al*. (2009) studied PC content and composition of wheat varieties affected by the environment. PC is a mixture of high-molecular-weight aliphatic primary saturated alcohols which possess cholesterol-lowering property. They collected grain samples from three varieties grown at three locations and found a significant variety effect within each location. There was also a significant location-variety random effect on PC content. Although PC content was not studied in the present investigation, it may be worthwhile to study this parameter in future keeping in view its cholesterol-lowering property. Breeders may explore the possibility of introducing those genes responsible for increasing PC content of different wheat genotypes.

An attempt was made in this investigation to study the changes in starch and sucrose content of wheat varieties through related hydrolytic enzyme activities in the ungerminated and germinated seeds up to 72 h (Tables 10 and 11). The β-amylase is an enzyme which hydrolyzes starch, the reserve carbohydrate in plants, whereas enzyme invertase hydrolyses sucrose in to D-glucose and D-fructose units. Invertase cleaves the O-C (fructose) glycosidic bond, whereas the amylase cleaves the O-C (glucose) bond. Both the enzyme activities were studied in the crude extract of ungerminated and germinated wheat.

It may be noted that highest activity of β-amylase was recorded in Raj-4037 at Arabhavi (48 h) as compared to its activity at Dharwad and minimum activity was recorded in *T. dicoccum* variety DDK-1029 at Dharwad (Table 10). Location-wise Raj-4037 performed better at Arabhavi than Dharwad which may be mainly due to higher germination percentage. Maximum germination was observed in *T. aestivum* varieties than *T. durum* and *T. dicoccum* varieties. In the ungerminated seeds, the specific activity of β-amylase was studied.

In case of acid invertase, the maximum activity was observed in GW-322 at Arabhavi, as compared to its performance with other varieties (Table 11) and minimum activity was observed in *T. dicoccum* variety DDK-1009, whereas location wise DDK-1009 scored better at Arabhavi than Dharwad. In the ungerminated seeds, the specific activity of acid invertase was highest in GW-322 at Arabhavi. It is well documented that lower the activities of amylase and invertase, higher will be the keeping quality due to less deterioration of seeds during storage. These enzymatic parameters go hand in hand with seed moisture content. It may be noted that if moisture content is brought down, the hydrolytic activities of these enzymes come down considerably providing stability to the stored seeds against insect attack and deterioration.

It may be worthwhile to note that Matsuo *et al*. (1982) observed the effect of α-amylase activity in *T. durum* wheat on spaghetti quality. High amylolytic activity of semolina was found to result in high amylolytic activity in spaghetti and increased amount of residue in

the cooking water and the level of reducing sugars in both semolina and spaghetti were instrumental in making the products slightly soft.

Amani (2008) studied the effect of cold-shock on wheat enzymes. The changes in sucrose synthase and acid-invertase were determined by micro-plate reader. Both the enzymes exhibited parallel increase after 2, 4 and 6 h of transferring the seedlings to cold conditions and they were found to be involved in the plant response to chilling stress. This observation indicates that wheat enzymes are prone to different stresses.

Wheat contains higher amount of protein than other cereals. Wheat protein quality mainly depends upon HMW-GS and LMW-GS contents and the balance of amino acid composition in the wheat grain (Li and Zhang, 2000 and Liu *et al*., 2002).

It may be noted that among *T. aestivum* varieties, Dharwad DWR-162 was found to have seven bands in the electrophorogram (Figs.13 and 14). It was conspicuous by not showing a band at 50 and 60 KDa at Arabhavi and Dharwad, respectively. In case of Raj-4037, a 50 KDa band was found to be missing at Arabhavi, whereas it was prominent at Dharwad.

Most striking observation of the present investigation is total lack of 50 KDa protein band in all the *T. aestivum* and *T. durum* varieties studied at Arabhavi as compared to Dharwad. It is noteworthy that GW-322 did not express at 50 KDa at both the locations. It may be seen from Figs. 13 and 14 that DWR-162 had a band in the 100 KDa range, at Arabhavi, whereas it was missing at Dharwad in that region. Appearance of 70 or near 70 KDa band in all the *T. aestivum*, *T. durum* and *T. dicoccum* varieties studied at Arabhavi and Dharwad, except in *T. aestivum* variety Raj-4037 at Dharwad also needs further exploration. Appearance of protein bands below 10 KDa in *T. durum* and *T. aestivum* varieties at both the locations is also a striking feature. It may be noted that none of the *T. dicoccum* varieties at both the locations exhibited any bands below 10 KDa.

The studies of Muhammad *et al*. (2009a) who evaluated wheat by SDS-PAGE, observed low degree of heterogeneity which lend support to the observations of this investigation on the varieties revealing differential protein banding pattern. They expressed that the SDS-PAGE analysis of wheat endosperm protein was found to be useful for evaluation of genetic variability.

Muhammad *et al*. (2009b) studied genetic diversity among wheat grown in Pakistan using RAPD and SDS-PAGE. Seed storage protein analysis produced 19 subunits ranging between 29-120 KDa. According to them HMW-GS showed greater polymorphism than LMW-GS.

In the present investigation, a 10-100 KDa protein marker was used and RAPD was not employed. It is, therefore, difficult to comment on polymorphism in HMW-GS and LMW-GS based on the observations of this investigation in different wheat varieties.

It has been well documented that cold hardening condition improves soluble protein patterns leading to the accumulation of high molecular weight protein in the range of 200 KDa (Fathey and Michel, 1987). During the present investigation, the temperature variation at Dharwad during *rabi* season was between 26-31^{0}C (Appendix II) as compared to the temperature variation of 31-32 ^{0}C observed at Arabhavi (Appendix III). The protein marker used in this study was 10-100 KDa. Hence effect of cold hardening in the range of 200 KDa could not be ascertained. Moreover, temperature variation was not indicative of severe cold during *rabi* 2007-08.

Wheat quality is partially determined by genetics, cultural environment and its interaction with genotype (Busch *et al.,* 1969). The environmental effect is often larger than the genetic effect on wheat quality (Peterson *et al*., 1992). Such effects may include soil type, fertilizer level especially nitrogen (Paredez-Lopez *et al*., 1985 and Luo *et al*., 2000), distribution of rainfall level (Faridi and Finlay, 1989) and late season factors (Lookhart and Finney, 1984). In certain regions, elevated temperature during grain filling is possibly the most important environmental determinant of grain quality (Randall and Moss, 1990). High temperature during grain filling, especially greater than 35^{0}C, alters the protein biosynthetic pathways of grain, leading to protein compositional changes (Blumenthal *et al*., 1993). Four hypotheses were proposed by Blumental *et al*., (1996) that account for changes in dough strength that are caused by heat stress: (1) Changes in the ratio of glutenin to gliadin, (2)

alteration in the formation of disulphide bonds between glutenin peptides, thus leading to a reduction of the size of the glutenin polymers, (3) direct effects of heat-shock proteins on dough strength, and (4) changes that heat-shock proteins and chaperons impose on the folding and polymerization of polypeptides during polymer formation.

Fathey and Michel (1987) observed that the intensity of three protein bands of 42, 47 and 48 KDa increased, while that of five other bands *viz*., 63, 67, 80, 89 and 93 KDa decreased due to hardening. In the present investigation, the temperature variation in Dharwad was from 11.8-31^{0}C during *rabi*, whereas at Arabhavi it was from 11.37-32.92^{0}C (Appendix III). As compared to the severe cold atmosphere found in western countries and hilly region of India, this variation may not be considered as deleterious for the growth of the wheat varieties. It appears from the weather data during the *rabi* season of 2007-2008 that the average rainfall received was 64.8 mm at Dharwad and 35 mm at Arabhavi (Appendices II and III). It is also noteworthy that during the period of growth of wheat varieties in this investigation, the relative humidity varied from 66-96 at Arabhavi as compared to 23-93 at Dharwad. All these factors along with soil nutrient status and water quality might have found expression in various biochemical quality parameters studied in the present investigation leading to better performance of *T. dicoccum* varieties at Dharwad than Arabhavi.

Cressay *et al*. (1987) opined, based on their study of quality attributes and grain composition in 60 advanced lines of cross breed wheats, the presence of HMW-GS and specific gliadins decide the grain quality especially resistance of dough to extension. Similarly, Peltonen *et al.* (1994) reported that nitrogen fertilizer application improved bread making quality of wheat flour mainly by increasing the quantity of LMW-GS.

Wheat finds its utility in different food products mainly chapati, bread, pasta, spaghetti etc, wherein different qualities of wheat play important roles. It is, therefore, desirable for a breeder to decide the suitability of a particular wheat in different forms and attempt to modify the genetic make up of the wheat protein.

5.2 Protective elements/antioxidants

β-carotene or yellow pigment is an organic compound, classified as a terpenoid. It is a strongly-coloured pigment abundant in plants and fruits. It is a precursor of Vitamin A and it is responsible for colour of the grain.

In the present investigation, *T. aestivum* varieties had better β-carotene content (Table 6) at Dharwad than Arabhavi. *T. durum* varieties also performed better at Dharwad over Arabhavi, whereas NIDW-295 performed equally well at both the locations. *T. dicoccum* varieties performed better at both the locations, indicating thereby that they were suitable for both the locations. When all the varieties were ranked with respect to β-carotene content, *T. durum* varieties ranked first followed by *T. dicoccum* and *T. aestivum* and there was significant interaction effect observed among them irrespective of locations. It may be noted that the suitability of *T. durum* pasta product depends upon its yellow colour and β-carotene content is generally high in *T. durum* wheat varieties (Nannor *et al*., 1995).

The Dharwad progress report (North Karnataka) published by the Directorate of Wheat Research, Karnal (Anon., 1998) reports that 11 *T. dicoccum*, one *T. durum* and one Bread wheat varieties were grown at eight locations. Wheat grown in Ugar region had higher mean protein when compared with other regions, whereas Arabhavi region had higher mean β-carotene content (5.19 ppm) when compared with other locations indicating thereby location-wise variation in □-carotene content and utility of such an evaluation.

Plant phenolics are secondary metabolites that encompass several classes of structurally diverse natural products, biogenetically arising from the shikimate-phenylpropanoid-flavonoid pathways. Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions (Vincenzo *et al*., 2006).

Total phenolic contents observed in this investigation in wheat flour (Table 6) reveal that *T. aestivum* variety DWR-162 at Dharwad had higher phenolic content than at Arabhavi. Raj-4037 and GW-322 performed equally well at both the locations. *T. durum* variety NIDW-295 performed well at Dharwad as compared to its performance at Arabhavi, whereas DWR-1006 and MACS-2846 were found suitable for their protective elements at both the locations,

since they performed equally well at both the locations. *T. dicoccum* variety NP-200 at Dharwad also contained higher amount of phenols, whereas DDK-1009 was suitable for both the locations. DDK-1029 performed better among all the varieties at Arabhavi as compared to Dharwad. Moreover, there was significant interaction effect observed among *T. aestivum*, *T. durum* and *T. dicoccum* varieties irrespective of locations. When varieties were ranked with respect to the presence of phenols, *T. dicoccum* was placed first following by *T. aestivum* and *T. durum* varieties.

Although phytic acid content was not studied in the present investigation, it may be worthwhile to study this parameter as it has been reported to act as a protective element. Abdul *et al*. (2007) identified and isolated the low phytic acid wheat inbred mutants in Pakistan. The selected mutants were grown at five locations which differed in soil types and environmental conditions to determine G x E interaction on phytic acid content. The effect of genotypes, locations and their interactions on phytic acid content were all highly significant.

Considering antioxidant and protective elemental factors, this investigation reveals that between Dharwad and Arabhavi locations, the performance of the varieties at Dharwad was better as compared to their performance at Arabhavi.

5.3 Trace elements

In the present investigation, trace elements (Cu. Mn, Zn and Fe) were studied in different varieties of wheat grains obtained from two locations, Dharwad and Arabhavi. It has been reported in the scientific literature that these micronutrients are essential for various biological functions (Gardner *et al*., 1988; Singh *et al*., 1998; Abdallah and Simman, 1993 and Meunier *et al*., 2005).

Minerals of wheat flour are not quantitatively large but may have considerable effect on the quality and behavior of the flour. The percentage of minerals present in flour usually gives an indication of grade of the flour (Ereifeh and Shib., 1993; Grover *et al*., 1994 and Sangha *et al*., 1998).

It may be recalled that the highest Cu content was observed in *T. durum* varieties as compared to *T. aestivum* and *T. dicoccum* varieties (Table 8). *T. aestivum* variety Raj-4037 performed better at Dharwad as compared to its own performance at Arabhavi, whereas DWR-162 and GW-322 performed equally well at both the locations. *T. durum* variety NIDW-295 contained good amount of Cu as compared to at Arabhavi, whereas *T. dicoccum* varieties DDK-1029 and NP-200 performed better at Dharwad than at Arabhavi, whereas DDK-1009 was favorable for both the locations, since it performed equally well at both the locations, confirming location-wise mixed response by the varieties.

Copper is found in primary and secondary minerals but occurs primarily in organic complexes. Cu plays a role in photosynthesis as a part of chloroplast enzyme plastocyanin in the electron transport system. Cu is part of several oxidases such as ascorbic acid oxidases and polyphenol oxidases. Moreover, species and cultivars show difference in tolerance to copper (Gardner *et al*., 1988). The soil Cu content at both the locations (Appendix IV and V), does not point out towards any variation in the wheat quality due to Cu content.

Zinc is an essential element for human health and well-being. It has a structural and functional role in a large number of macromolecules and is required for over 300 enzymic reactions, especially dehydrogenases. Zn ions participate in all the aspects of intermediary metabolism, transmission and regulation of the expression of genetic information, storage, synthesis of peptide hormones and structural maintenance of chromatin and biomembranes. Zn is also needed for growth and development, protein and DNA synthesis, neuro-sensory functions, cell-mediated immunity, thyroid and bone metabolism (Meunier *et al*., 2005). It may be noted that despite low level of Zn in the soils of Arabhavi, the wheat grain Zn content was quite high at Dharwad.

Perusal of data on Zn (Table 8) contained highest Zn in *T. durum* varieties as compared to *T. aestivum* and *T. dicoccum*. It may be noted that *T. aestivum* varieties DWR-162, Raj-4037 and GW-322 performed equally well at both the locations, indicating thereby that they are favourable for both the locations with respect to environmental conditions, soil type etc. Moreover, significant interaction effect was observed among *T. aestivum*, *T. durum* and *T. dicoccum* varieties irrespective of locations.

Zhao *et al*. (2008) observed variation in mineral micronutrient concentration in grain of wheat of diverse origin. Significant differences between bread wheat genotypes were found for grain Fe and Zn. Grain Zn, but not Fe, concentration correlated negatively with the grain yield and there was a significant decreasing trend in grain Zn concentration with the data of variety release, suggesting that genetic improvement in yield had resulted in a dilution of Zn concentration in the grain. Both grain Zn and Fe concentrations were also found to be correlated positively and significantly with grain protein content and P concentration. However, the correlation with kernel size, kernel weight or bran yield were weak. It is necessary that genetic improvement in yield has to be monitored carefully since Zn concentration in the grain gets diluted, leading to disturbances in the oxidative metabolism of grain.

Another element of importance in nutrition is Mn. It has been recognized as an essential mineral nutrient for acquisition of photosynthetic competence in higher plants. However, it is not required in bacterial photosynthesis in which there is no photosystem II (PS II). Mn binds to the chloroplast at two kinds of sites and PS II contains three Mn atoms for 200 chlorophyll atoms.

Mn exists in soil as a divalent ion (Mn^{2+}) in the soil solution; uptake of Mn^{2+} is active, which competes with other cations like Fe^{2+}. It is an activator of several enzymes specially those involved in fatty acid and nucleotide synthesis and is essential for respiration and photosynthesis. It also activates synthesis of an auxin, IAA through IAA oxidase which results in maintaining less IAA concentration in tissues, since at higher concentration its activity is inhibitory.

The role of Fe in oxidative metabolism and in the electron transport chain needs no special mention. Table 9 reveals that Fe and Mn content of wheat varieties were highest in *T. durum* varieties as compared to *T. aestivum* and *T. dicoccum*. *T. aestivum* varieties Raj-4037 and GW-322 performed better at Dharwad as compared to their performance at Arabhavi. *T. durum* varieties DWR-1006 and NIDW-295 contained good amount of Fe at Dharwad than at Arabhavi, whereas MACS-2846 had good amount of Fe at both the locations. *T. dicoccum* varieties DDK-1009, DDK-1029 and NP-200 performed well at both the locations. Significant interaction effect was also observed in *T. aestivum*, *T. durum* and *T. dicoccum* varieties irrespective of locations with respect to Fe content of wheat varieties.

Looking in to the role of Mn in plant kingdom, Mn has shown promise at both the locations (Table 9). *T. aestivum* variety, GW-322 at Dharwad contained good amount of Mn than at Arabahvi, whereas DWR-162 and Raj-4037 performed well at both the locations. *T. durum* varieties DWR-1006 and NIDW-295 performed better at Dharwad than at Arabhavi. However, *T. dicoccum* varieties DDK01029 and NP-200 were found to contain better Mn at Dharwad as compared to Arabhavi. Significant interaction effect was observed in *T. aestivum*, *T. durum* and *T. dicoccum* varieties irrespective of locations as was seen in case of Fe.

It has been reported that Fe, Mn, Zn and Cu fertilization causes significant increase in grain yield, straw yield, 1000 grain weight, number of seeds per spiklet and grain protein content (Ziaeian amd Malakouti, 2001). In the present investigation, the micronutrient status of soil as influenced by organic and inorganic sources of nutrients was studied (Tables 8 and 9). It may be noted here that except Mn and Zn contents, which were on higher side at Arabhavi than Dharwad, other micronutrients were within the normal range in different wheat verities at both the locations. Further, EC content varied from 0.11-0.15 dS per m at Dharwad and 0.17-0.27 dS per m at Arabhavi (Appendices IV and V).

All these factors along with different soil nutrients, EC, pH and water quality might have found expression in various biochemical quality parameters studied in the present investigation (Appendices IV and V) leading to a better performance of *T. dicoccum* wheat varieties at Dharwad than Arabhavi.

The ICAR project of multi-location trials on different crops has made a great impact in India. Wheat being a staple food for majority of population in India, focus on multi-location trials of wheat has brought out so much of data that the dream of self sufficiency in wheat production is within our reach now. The factors which affect the wheat production are many and it is difficult to study them in one attempt. This investigation was an attempt to evaluate the biochemical parameters of popular varieties of wheat in the Peninsular zone and it may

not be an exaggeration to say that the objectives of this investigation could be achieved to a great extent.

Future line of work

1. There is a need to study the amino acid composition and its sequencing in different varieties of wheat since differential pattern of protein banding has been observed in the present study.
2. There is a need to study the isoleucine, methionine and phenylalanine fractions in wheat which are closely related to celiac disease toxicity.
3. There is a need to study the role of glutenin and gliadins in celiac disease toxicity since higher concentration of these proteins pose a threat to the person who is affected by the disease.
4. Very little work has been done on low molecular weight glutenin subunits (LMW-GS) *i.e.,* gliadins and their types □, □, □ and □ with respect to disease resistance and insect attack. It may be undertaken in future studies on wheat.
5. There is a need to work on polymerase chain reaction (PCR) to know the genetic diversity and RAPD to know the polymorphism among the different wheat varieties.
6. Total lack of 50 KDa protein subunits in all the *T. aestivum* and *T. durum* varieties at Arabhavi as compared to its appearance in them at Dharwad needs further exploration.
7. Appearance of bands below 10 KDa in *T. durum* and *T. aestivum* varieties at both the locations and their absence in *T. dicoccum* varieties at both the locations may be related to their location-wise differential performance. It may be studied in depth in future, since appearance and disappearance of bands is also dependent on temperature variation.
8. Studies on heat shock proteins and their relevance in wheat needs special attention since they impose changes in folding of polypeptide chains.
9. There is a need to study the policosanol content of wheat which possesses cholesterol lowering properties, in view of a large amount of wheat consumed by Indian population providing natural protection against development of atherosclerosis.
10. There is a need to study and analyse environmental factors like temperature, soil type, EC and pH of soil, rainfall, relative humidity, micronutrient content of soil and pH and EC of water used for irrigating wheat under multi-location trials since these factors affect the wheat quality.

6. SUMMARY

An investigation was carried out to study the effect of two different locations namely, Dharwad and Arabhavi on the quality parameters of different *T.aestivum*, *T.durum* and *T.dicoccum* varieties of wheat grown during *rabi* season in the Peninsular Zone by the All India Coordinated Wheat Improvement Project, University of Agricultural Sciences, Dharwad during 2007-2008. Efforts were made to find out differences in the biochemical quality parameters of different varieties of wheat. The salient features of the findings are outlined below:

1. Higher total carbohydrate content was registered by *T. aestivum* varieties at Dharwad, whereas *T. durum* varieties registered higher amount at Arabhavi. Starch content of *T. aestivum* varieties was comparable at both the locations.

2. The percentage of sugars inclusive of reducing, non-reducing and total sugars of *T. dicoccum* varieties was on higher side at Arabhavi, whereas it was high in *T. durum* varieties at Dharwad.

3. *T.dicoccum* varieties exhibited higher nitrogen content at both the locations as compared to *T. durum* and *T. aestivum* varieties.

4. Oil and crude protein content of *T. dicoccum* varieties registered higher values at Dharwad than at Arabhavi.

5. *T. durum* varieties registered increased β-carotene content at Dharwad as compared to Arabhavi. Soluble proteins were higher in *T. dicoccum* varieties at both the locations.

6. Wet gluten content of *T.durum* varieties exhibited higher values at Arabhavi as compared to Dharwad, whereas *T. dicoccum* varieties exhibited higher amount of total phenols at both the locations.

7. Micronutrient content showed an increase in *T. durum* varieties at both the locations.

8. Specific activities of hydrolytic enzymes, β-amylase and acid invertase exhibited increasing trend in their activities up to 72 h of germination as compared to ungerminated seeds. Both the hydrolytic enzymes exhibited increasing trend up to 48 h of germination and thereafter decline. The hydrolytic enzymes exhibited higher activities in the germinated seeds of *T. aestivum* varieties at both the locations as compared to *T.dicoccum and T. durum* varieties.

9. Some varieties exhibited noteworthy biochemical parameters at Dharwad like nitrogen, crude protein, soluble protein, β-carotene, total phenol and micronutrients, whereas some varieties exhibited higher values at Arabhavi location like total carbohydrate, starch, wet gluten content and specific activities of β-amylase and acid-invertase.

10. For making available good amount of protein, antioxidants and micronutrients from wheat, Dharwad environmental conditions seem to be more favourable to grow wheat.

11. Looking in to genotype and environmental interaction, *T. dicoccum* and *T.aestivum* genotypes seem to be more suitable for Dharwad environmental conditions and *T. durum* genotype for Arabhavi environment.

12. Future line of work has been suggested.

REFERENCES

Abdallah, S. M. and Samman, S., 1993, The effect of increasing dietary Zn on the activity of superoxide dismutase and Zn concentration in erythrocytes of healthy female subjects. Eur. J. Clin. Nutr., 47: 327-332.

Abdel-Aal, E. S. M. and Hucl, P., 2002, Amino acid composition and invitro protein digestibility of selected ancient wheats and their end products. J. Food compos. Anal., 15: 737-747.

Abdel-Aal, E. S. M., Hucl, P., Sosulski, F. W., Graf, R., Gillott, C. and Pietzak, L., 2001, Screening spring wheat for midge resistance in relation to ferulic acid content. J. Agric. Food Chem., 49: 3559-3566.

Abdul, J. K., Akhtar, A., Farooq-I-Azam. and Aurang zeb., 2007, Identification and isolation of low phytic acid wheat (*Triticum aestivum* L.) inbred lines / mutants. Pak. J. Bot., 39 (6): 2051-2058.

Adom, K. K., Sorrells, E. and Liu, R. H., 2003, Phytochemical profiles and antioxidant activity of wheat varieties. J. Agric. Food Chem., 53: 2297-2306.

Amani, A. L., 2008, Activity of sucrose synthase and acid invertase in wheat seedlings during a cold-shock using microplate reader assays. Aust. J. Basic Appl. Sci., 2(1): 53-56.

Anjum, F. M., Tenu, W. and Johson, T., 2002, Phytate and mineral content in different milling fractions of some Pakistani spring wheat. Cereal Chem., 79(3): 203-207.

Anonymous, 1998, Resource Management Programme Vol. IV DWR Progress Report, Ed. D. S. Chauhan., R. K.Sharma., A. S. Kharub and S. Nagarajn, AICWIP Directorate of Wheat Research, Karnal, Haryana, India.

Anonymous, 2005, Official Methods of Analysis of AOAC International, 18th Edition. Gaithersburg, Maryland, USA.

Anonymous, 2007, Agriculture centre for monitoring economy. Year Book., 27: 111-118.

Ariyama, T. and Khan, K., 1990, Effect of laboratory sprouting and storage on Physico-chemical and bread making properties of hard red spring wheat. Cereal Chem., 67(1): 53-58.

Astrog, P., Boutron-Ruoult, C., Andrieux, C., Blachier, F., Blottiere, H., Bonithonkopp, C., Clavel-Chapelon, F., Corpet, D., Duee, P. H., Gerber, Eflah, K., Enanteau, J. and Siess, M. H., 2002, Dietary fibers and colorectal cancer. Experimental studies, epidemiology, mechanisms. Gastroenterol. Clin. Biol., 26: 893-912.

Bakshi, A. K. and Bains, G. S., 1984, Study of the physico-chemical, rheological characteristic of durum and bread wheat cultivars. Bull. Grain Technol.., 22(1): 3-12.

Bakshi, A. K. and Bains, G. S., 1987, Study of the physico-chemical, rheological, baking and noodle quality of improved durum and bread wheat cultivars. J. Food Sci. Tech., 24(5): 217-221.

Berot, S., Chiron, H., Nicolas, M., Gautiens., Godon, B. and Popineau, Y., 1996, Pilot scale preparation of wheat gluten protein fraction II technological properties of the fractions. J. Food Sci. Tech., 31(1):77-83.

Bietz, J. A. and Wall, J. S., 1972, Wheat gluten subunits: Molecular weights determined by SDS-PAGE. Cereal Chem., 49(1):61-68.

Blumenthal, C., Barlow, E. and Wrigley, C. W., 1993, Growth environment and wheat quality: The effect of heat stress on dough properties and gluten preoperties. J. Cereal Sci., 18: 3-21.

Blumenthal, C., Stone, P. J., Gras, P. W. and Appels, R., 1996, Heat-shock protein 70 and dough quality changes resulting from heat stress during grain filling in wheat. Cereal Chem., 73(3):603-609.

Borlaug, N. C., 1968, Wheat breeding and its impact on world food. *In : Proceedings of Third International Wheat genet. Symposia*. Canberra, Australia, pp. 1-36.

Borrelli, G. M., Jroccoli, A., Difonzo, N. and Fares, C., 1999, Durum wheat lipoxygenase activity and other quality parameters that affect pasta colour. Cereal Chem., 76(1):57-59.

Busch, R. H., Shuey, W. C. and Frohberg, R. C., 1969, Response of Aestivum to environments in relation to quality characteristics. Crop Sci., 9: 813-817.

Bushuk, W., 1987, Glutenin of marquis wheat as a reference for estimating molecular weight of glutenin subunits by SDS-PAGE. Cereal Chem., 64(5): 324-327.

Butt, M. S., Ihsanullah Qamar., Anjum, F. M., Abdul Aziz. and Arif Randhawa., 2004, Development of mineral-enriched brown flour by utilizing wheat milling by products. Nutr. Food Sci., 34(4): 161-165.

Chamberlain, N., Collins, T. H. and Medermott, E. E., 1981, Alpha amylase and bread properties. J. Food Tech., 16: 127-152.

Chen, J., Khan, K., Shelton, D. R. and Appolonia, L. D., 1992, Isolation and fractionation of carbohydrate-containing Proteins from wheat Gluten. Cereal Chem., 69(5): 703-705.

Chilkunda, D., Nandini. and Paramahansa, V. S., 2001, Carbohydrate composition of wheat, wheat bran, sorghum and bajra with good chapatti/roti (Indian flat bread) making quality. Food chem., 73(2): 197-203.

Chung, O. K., Pomeranz, Y., Jacobs, R. M. and Howard, B. G., 1982, Relation of polar lipid content to mixing requirement and loaf volume. J. Food Sci., 45: 1168-1180.

Cressay, P. J., Campbell, W. P., Wrigley, C. W. and Griffin, W. B., 1987, Statistical correlation between quality attributes and grain protein composition of sixty advanced lines of cross bread wheat. Cereal Chem., 64(5): 324-327.

Czukajowska, Z., Po-ying, L. N. and Smoliski, S., 1996, Role in dough rheology of high molecular weight glutenin subunits of soft white winter and club wheats. Cereal Chem., 73(3): 338-345.

Damania, A. B., Porceddu, E. and Jackson, M. T., 1983, A rapid method for the evaluation of variation in germplasm collection of cereals using polyacrylamide gel electrophoresis. Euphytica., 32: 877-883.

Devin, E., Pete, S. B. and Bayputt, K., 2008, Effectiveness of dry heat, steam and microwave treatment in decreasing lipase activity, while retaining antioxidant activity to stabilize whole wheat flour against lipid degradation during storage. Cereal Chem., 85(1): 77-83.

Dexter, J. E., Matsuo. R. R. and Kruger, J. E., 1990, The spaghetti making quality of commercial durum wheat samples with variable α-amylase activity. Cereal Chem., 67(1): 79-81.

Dupuis, B., Bushuk, W. and Sapirstein, H. D., 1996, Characterization of Acetic Acid Soluble and Insoluble fraction of Glutenin of Bread Wheat. Cereal Chem., 73(1): 103-108.

Ereifei, K. I. and Shibli, R., 1993, Rheological and baking quality of flour from a landrace and durum wheat cultivars grown in Jordan. Cereal Chem., 70 (1):253-255.

Eriefeh, J. and Shib. T., 1993, Ten durum wheat cultivars for mineral composition. Cereal Chem., 70 (1): 307-311.

Faridi, H. and Finlay, J. W., 1989, Improved wheat for baking. Food Sci. Nutr., 28: 175-209.

Fathey, S. and Michel, P., 1987, Accumulation of a high molecular weight protein during cold hardening of wheat. Plant Cell Physiol., 28(7): 1173-1179.

Fu, B. X. and Sapirstein, H. D., 1996, Procedure for isolating monomeric proteins and polymeric glutenin of wheat flour. Cereal Chem., 73(1): 143-152.

Ganibelli, M. C., Masci., Larroque, O. R., Lafiandra, D. and MacRitchie, F., 2002, Biochemical characterization of a Novel Polymeric Protein Subunit from Bread Wheat (*T. aestivum*). J. Cereal Sci., 35(3):265-276.

Gardner, F. P., Pearce, R. B. and Mitchell, R. L., 1988, Mineral nutrition, In: Physiology of Crop Plants, Scientific Publishers, Jodhpur, Rajastan, India, pp. 98-131.

Grover, K., Sadana, B. K. and Hira, C. K., 1994, Availability of trace mineral from cereal preparations. Indian Food Packer, 48: 39-44.

Gujral, B., Pete, B. and Law. D., 2008, Effects of added minerals on pasting of partial waxy wheat flour and starch on noodle making properties, Cereal Chem., 85(3): 311-315.

Hajra, Khan., Zia-ul-Hassan. and Ali Akbas Maitto., 2006, Yield and micronutrient contents of bread wheat (*T.aestvum*) under a multinutrient fertilizer-hal-tonic. Int. J. Agri. Biol., 8(3): 366-370.

Haridas Rao, P., Rahim, A., Prabhavathi, C. and Shurpalekar, S. R., 1976, Physico-chemical, rheological and milling characteristics of India durum wheats. J. Sci. Food Agri., 13(6): 317-322.

He, H. and Hoseney, R. C., 1992, Effect of the quality of wheat flour protein on bread loaf volume. Cereal Chem., 69(4):557-601.

Henry, P. J., 1985, A comparison of the non-starch carbohydrates in cereal grains. J. Sci. Food Agric., 36: 1243-1253.

Hetty, C. Vanden Broeck., Teun W. J. M., Van Herpen., Cees Schuit., Elma M. J. S., Liesbeth Dekking., Dirk Bosch., Rob, J. H., Marinus, J. M. S., Ludoviscus, J. W. J., Gilessen. and Ingrid, M. Vandermeer., 2009, Removing of Celiac-disease-related gluten proteins from bred wheat while retaining technological properties: A studies with Chinese spring deletion lines. Bio. Med. Central Plant Biol., 9: 41-45.

Hira, C. K., Kocchar, A., Sadana, B. K. and Sharma, K. K., 1991, Protein, lusin, mineral and phenol contents of some Indian wheat (*T.aestivum*) varities. J. Food Sci. Tech., 28(2): 112-117.

Huang, D. Y. and Khan, K., 1997, Qualitative determination of high molecular weight glutenin subunits of hard bread spring wheat by SDS-PAGE qualitative effects of total amount on bread making quality characteristic. Cereal Chem., 74(6): 613-615.

Iskander, F. Y. and Davis, K. R., 2009, Mineral and trace element contents in bread. J. Cereal Sci., 49 (2): 270-275.

James, P., Warren, E. and Kronstad E. C., 2001, Multi-location evaluation of the yield potential, adaptation, and end-product quality of newly developed winter durum wheats. *Oregon Wheat Commission Report*., pp. 8-10.

Jhuma, M., Shashi, M. and Muhammad, Y., 2003, Carbohydrate and protein content of the developing grains of wheat cultivars. J. Food Sci. Tech., 40(2): 219-221.

Johnson, V. A., Briggle, L. W., Axtell, J. D., Bouman, L. P., Leng, E. R. and Johnson, T. R., 1978, Grain Crops, In: Protein Resources and Tech., (Milner, M. *et al*., Eds) *AVI Publishing Co., Westport CT*, pp. 239-255.

Karimzadeh, I. J., Osborne, B. G. and Cornish, G. B., 2003, Changes on the callus soluble protein of winter and spring wheat cultivars following cold treatment. J. Cereal Sci., 34: 125-133.

Khadar, V. and Dave, R., 1996, Baking temperature and time on the quality and nutritive value of germinated wheat bread. Indian. J. Nutr. Diet., 23: 106-114.

Kim, K. H., Tsao, R., Yang, R. and Cui, S. W., 2006, Phenolics acids profiles and antioxidant activities of wheat bran extracts and the effect of hydrolytic conditions. Food Chem., 95: 466-473.

Kissell, T., Pomeranz, Y. and Yamazaki, W. T., 1971, Effect of flour lipids on cookie quality. Cereal Chem., 48(6): 665-667.

Klepacka, J. and Fornal, L., 2006, Ferulic acid and its position among the phenolic compounds of wheat. Crit. Rev. Food Sci. Nutr., 46: 639-647.

Kritchevsky, D., 1999, Protective role of wheat bran fiber: Preclinical data, Am. J. Med., 106: 285-315.

Krygier, K., Sosulski, F. and Hogge, L., 1982, Free, esterified and insoluble-bound phenolic acids. 1. Extraction and purification procedure. J. Agri. Food Chem., 30: 330-334.

Kumar, B. and Kappor, F., 1984, Ca and trace mineral composition of different varities of cereals. Cereal Chem., 61(3): 403-405.

Li, W. H. and Zhang, D. H., 2000, The balance analysis of the amino acid content in seed filling period of wheat. Seed, 2: 21-23.

Li, Y. Z. and Posner, E. S., 1989, Determination of wheat milling potential and its influence on flour quality determination rate. Cereal Chem., 66(5): 365-369.

Liu, Q., Gu, Z., Donner, E., Tetlow, I. and Emes, M., 2007, Investigation of digestibility in vitro and physico chemical properties of A- and B- type starch from soft and hard wheat flour. Cereal Chem., 84(1): 15-21.

Liu, Y. P., Que, S. Y., Li, X. P., Lan, S. Q., Lie, Y. H. and Li, J. P., 2002, Protein content and amino acid composition and qualities of different blue and purple grain wheat. Acta. Agric. Boreal. Sci., 17: 103-107.

Lookhart, G. L. and Finney, K. F., 1984, Polyacrylamide electrophoresis of wheat gliadins: The effect of environmental and germination. Cereal Chem., 61(2): 143-147.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., 1951, Protein measurement with Folin-phenol reagent. J. Biol.Chem., 193: 265-267.

Luo, C., Branlard, G. and Griffin, W. B., 2000, The effect of nitrogen and sulphur fertilisation and their interaction with genotype on wheat glutenins and quality parameters. J. Cereal Sci., 31: 185-194.

MacRitchie, F., 1994, Role of polymeric proteins in flour functionality in wheat kernel proteins: molecular and functional aspects. Bitervo, Italy, *Universita Degli Studi Della* Tuscia. pp 145-150.

Martin, C., Bains, G. and Collins, E., 1991, Bread firming and starch recrystallization are not synonymous. Cereal Chem., 68(3): 403-408.

Martin, J. M., Sherman, J. D., Lanning, S. P., Talvert, L. F. and Giroux, M. J., 2008, Effect of variation in amylase content and puroindolin composition in bread quality in a hard spring wheat population. Cereal Chem., 85(2): 258-266.

Matsuo, R. R., Dexter, J. E. and MacGregor, A. W., 1982, Effect of sprout damage on durum wheat and spaghetti quality. Cereal Chem., 59(6): 468-472.

McIntosh, G. H., Noakes, M., Royle, P. J. and Foster, P. R., 2003, Whole grain rye and wheat foods and markers of bowel health in over weight middle-aged men. Am. J. Clin. Nutr., 77: 967-974.

Meredith. E., Marina C. and Emanuele M., 2008, Storage of wheat grains at elevated temperatures increase solubalisation of glutenin subunits. Cereal Chem., 85(2): 183-187.

Meunier, N., Connor, J. M. O., Maiani, G., Cashman, K. D., Secker, D. L., Ferry, M., Roussel, A. M. and Coudray, C., 2005, Importance of Zn in the Elderly: The zenith study. Euro. J. Clin. Nutr., 59: 515-519.

Mishra, D. K. and Gupta, P. K., 1995, Protocol for evaluation of wheat quality. *Technical Bulletin No.3*, Directorate of Wheat Research, Karnal, India. pp 3-7.

Moore, M. M., Heinbockel, M., Docker, P., Ulmer, H. M. and Arendt, E. S., 2006, Network formation in gluten free bread with application of transglutaminase. Cereal Chem., 83(1): 28-36.

Morel, M. H., 1994, Acid-Polyacrylamide Gel Electophoresis of Wheat Glutenins: A new tool for the separation of High and Low molecular weight subunits. Cereal Chem., 71(3): 511-514.

Muhammad, F. A., Muhammad, I., Muhammad, S. M., Malik, A. R.and Muhammad, M., 2009a, Assessment of genetic diversity among Pakistani wheat (*T. aestivum*) advanced breeding lines using RAPD and SDS-PAGE. Electronic. J. Biotechnol., 13(3): 13-15.

Muhammad, N., Masood, B., Faquir, M. A., Kamran, S and Rashid, M., 2003, Effect of moisture on the shelf life of wheat flour. Intr. J. Agri. Biol., 5(4): 458-459.

Muhammad, S., Mohsin, J., Haji Akbar, Israr, K. and Rehmana K., 2009b, Evaluation of wheat by PAGE. African. J. Biotech., 9(2): 243-247.

Nannor, B. S., Chougole, B. A., Kotecha, P. M. and Pharande, A. L., 1995, Studies on some qualitative aspects of new wheat cultivars. J. Maharashtra agric. Univ., 20(2): 180-182.

Paredez-Lopez, O., Corravioubas, M. and Barquin, J., 1985, Influence of nitrogen fertilization on the physicochemical and functional properties of bread wheats. Cereal Chem., 62(1):114-118.

Patil. R. B., 1998, Dicoccum wheat semolina and product quality of different grades in comparision with durum and bread wheat. *MHSc. Thesis*, Univ. Agric. Sci., Dharwad.

Payne, P. I., Holt, L. M., Jackson, E. A. and Law, C. N., 1984, Wheat storage proteins: Their genetics and their potential for manipulation for plant breeding. Phil. Trans. R.Soc.London *B*., 304: 359-371.

Peltonen, F., Parmar, A. K. and Catignani, G. L., 1994, Nitrogen fertilizer application improved bread making quality of wheat flour. Cereal Chem., 71(2): 237-243.

Pena, E., Bernardo, A., Soler, C. and Jouve, N., 2006, Do tyrosine cross links contribute to the formation of the gluten network in common wheat dough. Cereal Chem., 83(1):69-75.

Peter, R. S., Nigel, G. H., Pete, S. B. and Arthur, S. T., 2002, The structure and properties of gluten: an elastic protein from wheat grain. Phil. Trans. R. Soc. Biol. Sci., 357(1418): 133-142.

Peterson, C. J., Graybosch, R. A. and Grombacher, A., 1992, Genotype and environment effects on quality characteristics of hard red winter wheat. Crop Sci., 32: 98-103.

Peterson, J., Collins, F. and Masci, T., 1983, Flour and bran of 27 wheat varities of protein and mineral content. Cereal Chem., 60 (1): 117-121.

Pham V. H., Tomoko, M., Kazutaka, I. and Naofumi, M., 2008, Total phenolic compounds and antioxidant capacity of wheat graded flours by polishing method. Inter Food Res., 42(1): 185-190.

Piergiovanni, A. R., Laghetti, G. and Perrino, P., 1996, Characteristic of meal from hulled wheats (*T. dicoccum* Schrank and *T. spelta* L); An evolution of selected accessions. Cereal Chem., 73(6): 623-625.

Qu, H. Y., Madl, R. L., Takemoto, D. J., Baybutt, R. C. and Wang, W. Q., 2005, Ligans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. J. Nutr., 135:598-602.

Raghuvanshi, R. S., Shukla, P. and Sharma, S., 1993, Proximate composition and gluten content of wheat varities (*T.aestivum*). Bull. Grain Technol.., 31(2): 117-119.

Randall, P. J. and Moss, H. J., 1990, Some effects of temperature regime during grain filling on wheat quality. Austr. J. Agric. Res. 41: 603-617.

Ranum, P. M., Barrett, F. F., Zoewe, R. J. and Kulp, K., 1990, Nutrient levels in internationally milled wheat flours. Cereal Chem., 67(2):114-117.

Reddy, M. M., Yunagi, N. B., Meera, Rao, Srinivasan, C. N. and Hanchinal, R.R., 1998, Grain and gluten quality of some cultivars of wheat species and their suitability for preparation of traditional south Indian seed products. J. Food Sci. Tech., 35(5): 441-444.

Reddy, S. B., 1996, Study of the physico-chemical properties and variation in sugar content of some wheat cultivars. Cereal Chem., 73(6): 651-653.

Ruth, S., Hucl, P. and Graf, R., 2008, Total phenolics and antioxidant potential in plasma and urine of humans after consumption of wheat bran. J. Nutr., 87: 107-113.

Sadasivam, S. and Manikam, A., 1992, In: Biochemical Methods for Agricultural Sciences, Wiley Eastern Limited, New Delhi and TNAU, Coimbatore, India, pp. 5-11, 22, 34, 122-123.

Sangha, J. K., Parmar, A. K. and Sachdeva, R., 1998, Mineral content of wheat cultivars with reference to their molar ratios. J. Food Sci and Tech., 35: 151-153.

Sapirstein, H. D., 1996, Procedure for Isolating monomeric proteins and polymeric glutenin of wheat flour. Cereal Chem., 73(1): 178-183.

Singh, B., Tomar S. M. S., Vinod, R. and Anil K., 2007, HD2819 (IC546938; INGR0704) a wheat *(Triticum durum)* germplasm with resistance to leaf and stripe Rust, karnal Bunt, loose smut, root aphids and high protein content. J. Plant Genet. Resou., 20 (3):249-271.

Singh, M. N., Daftary, C. and Kulp, K., 1990a, Total proteins from strong wheat flour were extracted without chemical reduction of disulfide bonds by applying mechanical shear. Cereal Chem., 67 (2): 105-108.

Singh, N. K., Donovan, R. and MacRitchie., 1990b, Use of sonication and SE-HPLC in the study of wheat flour proteins II. Relative quantity of glutenin as a measure of Bread making quality. Cereal Chem., 67(3): 207-211.

Singh, R. B., Naiz, M., Rastogi, S., Bajaj, S., Gaoli, Z. and Shoumin, Z., 1998, Current Zn intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations of north India. J. Am. Nutr., 17: 564-570.

Singleton, V. L., Orthofer, R. and Lamuela, R. M., 1999, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299: 152-178.

Skepritt, S., Kocchar, A. and Hamer, R. J., 1990, LMW-GS compared with the major wheat polypeptides, HMW-GS and gliadins. Cereal Chem., 67(1): 11-13.

Snedecor, G. W. and Cochran, W. G., 1962, Statistical methods. Iowa State University Press: Ames, Iowa.

Sosulski, F., Krygier, K. and Hogge, L., 1982, Free, esterified and insoluble bound phenolic acids, three compositions of phenolic acid and potato flours. J. Agric. Food. Chem., 30: 337-340.

Tomoko, A., Sawaya, W. N. and Bayonmi, M. J., 2008, Influence of starch and gluten characteristics on rheological properties of wheat flour gel at small and large deformation. Cereal Chem., 85(2): 114-117.

Van Lonkhuijsen, H. J., Hamer, R. J. and Schreuder, C., 1992, Influence of specific gliadins on the bread making quality of wheat. Cereal Chem., 69(1): 112-116.

Vatsala, C. N. and Haridas Rao, P., 1990, Physico-chemical and rhelogical characteristics of Indian *T. dicoccum* wheat in comparison with *T.aestivum* and *T.durum*. Indian Miller., 11(2): 3-8.

Vincenzo, L., Veronica, M. T. and Angela, C., 2006, Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochem. Adv. Res., 81:23-67.

Wang, Y. G., Khan. K., Hareland, G. and Nygard, G., 2007, Distribution of protein composition in bread wheat flour mil streams and relationship to bread making quality. Cereal Chem., 84(4): 271-275.

Willcox, J. K., Ash, S. L. and Catignani, G. L., 2004, Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr., 44: 275-295.

Yenagi, N. B., Hanchinal, R. R. and Suma, C., 1999, Nutritional quality of emmer wheat semolina and its use in planning therapeutic diets. In : Nutritional Society of

India, 32nd Annual Meeting, Nov 25-26, Abstract, Avinashilingam University, Coimbatore, pp.85.

Yongfen, C., Nurhan, T. D., Jeff Edwards., Brett, C. and Carla, G., 2009, Policosanol content and composition of wheat varieties as affected by environment. J. Food Sci. Agri., 89 (2):310-314.

Yves N., Jean P. M., Sandra D. P. and Yves P., 1998, Analysis of Wheat storage proteins by Exhaustive Sequential Extraction Followed by RP-HPLC Nitrogen determination., J. Sci. Food Agric., 77: 96-102.

Zhai, F. L., 1991, Physiology and heredity of quality improvement for plant protein, In: Crop Quality Breeding, (F. L. Zhai, F. L., ed.), China Agricultural Press: Beijing. pp. 44-45.

Zhao, F. J., Su, Y. H., Dunham, S. J., Rakszegi, M., Bedo, Z., McGrath, S. P. and Shewry, P. R., 2008, Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. J. Cereal Sci., 49(2): 290-295.

Zhu, K. X., Zhou, H. M. and Quian, H. F., 2006, Proteins extracted from defatted wheat germ: Nutritional and structural properties. Cereal Chem., 83(3): 301-303.

Zhuge, Q., Persaud, J. N., Posener, E. S. and Deyoe, C. W., 1992, Prediction of physical dough properties from glutenin subunit composition in bread wheats: correlation studies. Cereal Chem., 69(4):615-617.

Ziaeian, A. H. and Malakouti, M. J., 2001, Effect of Fe, Mn, Zn and Cu fertilization on the yield and grain quality of wheat in the calcareous soils of Iran In: Plant Nutrition- Food Security and Sustainability of agro-ecosystems., Eds: Horst, W. J *et al*., Netherlands, pp. 840-841.

APPENDIX

Appendix I: Preparation of resolving gel (10% Acrylamide gel)

The separating gel was prepared as follows:

Double distilled water	- 3.2 ml
Acrylamide	- 2.64 ml
Tris-HCl buffer pH 8.8	- 2.00 ml
SDS (10 %)	- 0.08 ml
Ammonium persulfate (10 %)	- 0.06 ml
TEMED	- 0.01 ml

Preparation of stacking gel (5%)

Double distilled water	- 2.7 ml
Acrylamide	- 0.67 ml
Tris-HCl buffer pH 6.8	- 0.50 ml
SDS (10 %)	- 0.04 ml
Ammonium persulfate (10 %)	- 0.03 ml
TEMED	- 0.004 ml

Tank buffer, pH 8.3

Tris	- 1.0 g
SDS	- 0.25 g
Glycine	- 3.6 g

The contents were dissolved one by one in the distilled water, pH was adjusted to 8.3 and made up to 250 ml.

Appendix II: Whether data at Dharwad during *rabi* 2007-08

Year : 2007-08
Zone : Peninsular Zone
State: Karnataka

Latitude : $15^0 26'$ N
Longitude : $75^0 07'$ E
Altitude : 678 m

Months	Temperature (^{0}C)		Rainfall (mm)	Rainy days	Relative humidity	
	Max.	Min.			Morning	Evening
September, 07	27.40	20.37	43.6	2	92.00	74.25
October, 07	29.76	19.40	16.12	1	78.20	59.00
November, 07	29.42	14.40	48.20	0	68.75	36.00
December, 07	28.74	14.67	0.00	0	82.85	49.50
January, 08	29.76	12.94	0.00	0	66.20	26.80
February, 08	31.50	16.82	0.00	0	62.25	31.50

Source : All India Co-ordinated Wheat Improvement Project (MARS, Dharwad)

Appendix III: Whether data at Arabhavi during *rabi* 2007-08

Year : 2007-08
Zone : Peninsular Zone
State: Karnataka

Latitude : $15^0 26'$ N
Longitude : $75^0 07'$ E
Altitude : 678 m

Months	Temperature (^{0}C)		Rainfall (mm)	Rainy days	Relative humidity	
	Max.	Min.			Morning	Evening
September, 07	32.92	22.27	62.70	7	90.97	80.90
October, 07	32.45	19.35	34.70	2	93.09	69.70
November, 07	32.72	14.52	4.60	1	96.41	65.30
December, 07	31.69	14.83	0.00	0	85.87	66.06
January, 08	32.01	11.27	0.00	0	90.61	67.14
February, 08	33.10	15.51	0.00	0	91.58	62.93

Source : All India Co-ordinated Wheat Improvement Project (ARS, Dharwad)

Appendix IV : Major nutrient and micro nutrient status of soil as influenced by organic and inorganic sources of nutrients at MARS, Dharwad during *rabi* 2007-08

Treatments	pH	EC	N	P_2O_5	K_2O	Cu	Zn	Fe	Mn
		dS/m	kg/ha			mg/Kg			
RDF	6.09	0.11	5.40	19.30	304.80	3.78	0.70	20.93	47.74
RDF + FYM	6.27	0.13	7.50	31.10	472.80	4.15	0.81	28.33	54.82
100% organic	6.63	0.15	8.70	26.80	379.20	4.16	0.77	23.21	52.16
75% organic	6.42	0.12	6.90	24.60	390.00	4.09	0.70	23.87	50.45
50% organic + 50% inorganic	6.44	0.12	7.20	25.70	394.80	3.75	0.72	22.31	47.45

Appendix V : Major nutrient and micro nutrient status of soil as influenced by organic and inorganic sources of nutrients at ARS, Arabhavi during *rabi* 2007-08

Treatments	pH	EC	N	P_2O_5	K_2O	Cu	Zn	Fe	Mn
		dS/m	kg/ha			mg/kg			
RDF	8.26	0.25	223.30	16.65	307.30	3.81	1.05	23.84	42.29
RDF + FYM	8.24	0.27	232.90	19.90	276.70	4.60	1.17	28.05	46.06
100% organic	8.22	0.24	235.50	18.48	349.40	4.22	1.09	25.22	40.26
75% organic	8.30	0.17	219.00	13.50	331.00	4.61	0.88	27.00	44.25
50% organic + 50% inorganic	8.49	0.23	247.90	16.18	293.40	3.86	0.89	24.92	44.47

STUDIES ON BIOCHEMICAL QUALITY PARAMETERS OF WHEAT AS INFLUENCED BY LOCATION

SHABANA NADAF 2010 Dr. P. W. BASARKAR
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ABSTRACT

Three different wheat varieties that are popular and recently released from each of the three species *T. aestivum*, *T. durum* and *T. dicoccum* grown during *rabi* 2007-2008 at MARS, Dharwad and ARS, Arabhavi were studied for biochemical parameters. Total carbohydrate content of *T. aestivum* varieties registered higher value at Dharwad, whereas *T. durum* varieties registered higher value at Arabhavi. Starch content of *T. aestivum* was comparable at both the locations. *T. dicoccum* varieties had higher nitrogen, crude protein and oil content at Dharwad location. *T. durum* varieties registered increase in β-carotene content at Dharwad over Arabhavi. Soluble proteins were higher in *T. dicoccum* varieties at both the locations. Wet gluten content of *T. durum* varieties exhibited higher values at Arabhavi, whereas *T. dicoccum* contained higher phenol content at both the locations. Micronutrient content of *T. durum* varieties was high at Dharwad. DWR-1006 had good micronutrient content. Specific activities of hydrolytic enzymes, β-amylase and acid invertase exhibited increasing trends in their activities up to 72 h of germination as compared to ungerminated seeds. The hydrolytic enzymes registered higher activities in germinated *T. aestivum* varieties at both the locations. Fractionation of HMW-GS and LMW-GS of protein carried out by SDS PAGE revealed different patterns. *T. dicoccum* and *T. aestivum* genotypes were more suitable for Dharwad environmental conditions, whereas *T. durum* varieties were suitable for Arabhavi.