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Advances in improving wheat quality for different end-products in India

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ABSTRACT

India is the second largest wheat producer in the world and large number of end-use products of wheat such as chapati, bread, biscuit, cakes, pretzels, noodles and pasta products are consumed. During the last 10 years Indian wheat varieties and germplasm lines were characterized for gluten proteins, grain texture, starch properties, rheological tests and baking quality. Genotypes with superior quality traits have been identified and are being utilized in breeding programme. To select desirable traits in early segregating generations, microlevel tests requiring 1g or less than 1g whole meal flours were developed. Solvent retention capacities (SRC) of whole meals showed a highly significant negative correlation (r=-0.71, n=500) with cookie spread factor and positive correlation (r=0.91; n=300) with Farinograph Water Absorption. Alveographic studies exhibited that dough extensibility is the main limitation in many of the cultivars for bread and biscuit making quality. Electrophoretic profiles of 285 released varieties of wheat in India were developed using SDS-PAGE and Acid-PAGE of glutenins and gliadins, respectively. Relationship between specific combinations of HMW-GS and gluten strength has been established. Acid-PAGE technique exhibited large genetic diversity in Indian wheats and some gliadin patterns were observed in specific environments. PCR amplification of puroindoline genes indicated the prevalence of null mutation in pinA in released varieties in India leading to harder grains. New alleles of puroindoline B were identified having frame shift mutation. PCR amplification of waxy genes demonstrated the presence of Wx-4A null mutants in large number of wheat varieties in India. Germplasm lines including diploid progenitors have been identified with higher content of micronutrients and low phytic acid mutants have been developed. The information is being used to improve wheat for various end-use products.

Key Word: Wheat, Naphal, gluten, gliadin, biscuit quality

Wheat is the main source of world's food energy and nutrition. Large number of end-use products such as chapati, bread, biscuit and pasta products are made from wheat. There are quite large differences in grain composition and processing quality among wheat cultivars within a species. Hence, one cultivar may be suitable to prepare one food type but unsuitable to prepare the other one. Quality differences among wheat cultivars have gained even more importance in grain trading due to important global economic and social trends. Recently, many countries like India have adopted, or are in the process of adopting, free-market economies, impacting positively on the income of the population, particularly in urban areas. There is trend in India towards increased urbanization and increased demand for traditional and new convenient, processed wheat-based foods. For example, the demand for biscuit, cakes, pretzels, noodles, bread and pasta products is increasing @5% per annum in India. Millions of people are involved in milling, baking and pasta products industry with around 2.5 Billion US Dollars turnover per annum. To improve dough handling and baking quality of wheat, often chemical improvers are used. Most of these chemicals have toxic effects. Therefore, only alternative is to develop varieties suited to meet the requirements of these end-use products.

The development of product specific varieties depends on the knowledge of quality requirements of different end-use products and the genetic components controlling different quality traits. Soft wheat with low protein content and weak and extensible gluten are preferred for biscuit making. Hard wheat with high protein content (>13%) and strong and extensible gluten are preferred for bread making while hard wheat with medium strong gluten and high protein content for chapati making. Waxy wheats have been found associated with improved shelf life of breads. White salted Japanese noodles need partial waxy wheats with soft grain characteristics and medium protein content and gluten strength. Partial waxy wheats with comparatively lower amylose content and higher starch paste viscosity improve texture of white salted noodles. Yellow alkaline noodles need comparatively harder wheat with medium strong gluten and without waxy trait. Pasta made from durum wheat requires stronger gluten, high protein content and higher content of yellow pigments. In addition there are some nutritional quality traits important for human beings especially whose sole diets are cereal based. Micronutrient bioavailability, lysine deficiency and starch quality are major nutritional quality traits for improvement. The above facts clearly demonstrate that major grain components determining wheat quality are grain hardness, gluten strength, protein content, lysine and amylose content, beta carotene content and components related to water absorption. Research work conducted in India related to these aspects of wheat grain quality along with some relevant research done elsewhere is discussed below.

GRAIN HARDNESS

There are two distinct wheat classes as soft and hard. Hard wheats tend to have more starch damage in flours suitable for bread making; while soft wheats have lower starch damage and have finer flour particle size suitable for biscuit quality. The Single Kernel Characterization System (SKCS) hardness index of 285 released varieties in India grown over

two years showed harder texture of grains of majority of varieties. Data demonstrated bimodial distribution of grain hardness index indicating one major gene for explaining variability in grain hardness (Fig.1). Grain hardness also showed very high heritability (H=0.91). Hardness Index (HI) varied from 40.2 to 101.3 with an average value of 82.0. Surprisingly only four varieties exhibited soft texture with HI around 40 and all other genotypes were hard with varying HI values. Since chapati has been the staple diet in India, the emphasis has been to develop wheat varieties suitable for this purpose. Harder grains and medium strong gluten

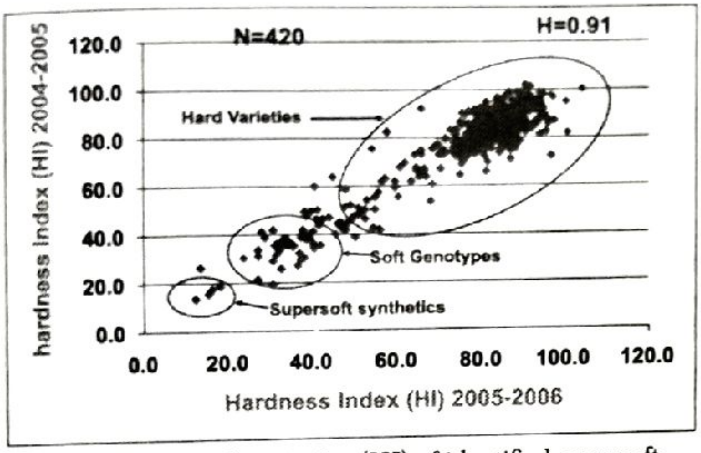


Fig. 1 Grain hardness index (HI) of identified supersoft synthetic hexaploids, soft germplasm lines and wheat varieties released in India grown over two year's period.

are preferred for good chapati making quality while softer grains and weak gluten for good cookie-making quality. The data clearly demonstrated the limited number of soft varieties in India for use in the development of cultivars for diverse products. This strongly supported the view that only within class hybridizations have been predominant in Indian wheat breeding programme with only few exceptions. Therefore, to widen gene pool diversity and to harness the new recombinants through interclass hybridizations, a large number of germplasm lines were evaluated for grain hardness. Seventy genotypes were identified with soft grain characteristics and better biscuit making quality. However, most of the identified genotypes were tall in nature and with lodging susceptibility. In addition, supersoft synthetic hexaploids were identified among a set of synthetic hexaploids supplied by CIMMYT (Ram *et al.* 2007). These sources are being used in the improvement of biscuit making quality so that the use of chemical improvers is minimized.

Molecular and biochemical studies demonstrated that puroindolines are the main determinants of grain texture. Biochemical studies during 1980's discovered friabilin, a 15kD starch bound protein, associated with grain softness (Greenwell & Schofield 1986). In this landmark paper they reported the presence of abundant 15kDa starch surface protein associated with soft wheat starch, small amount associated with hard wheat starch and none with durum starch. This report prompted research in several laboratories. Greenblatt *et al.* (1995) reported two classes of bound polar lipid (glyco & phospholipids) also follow the same pattern of occurrence as friabilin. However, the work of Jolly *et al.* (1996) demonstrated that friabilin was present in whole meal of hard and soft wheats. Although some hard wheat cultivars exhibited a reduced amount of friabilin, these data necessitated a re-evaluation of the then existing model which suggested that a quantitative difference in friabilin levels produced the difference between hard and soft wheats. Subsequent molecular studies exhibited that friabilin is composed of two components as puroindoline A (*pinA*) and puroindoline B (*pinB*) and either null mutation in *pinA* or glycine to serine to serine change in *pinB* caused harder texture (Gautier *et al.* 1994, Giroux & Morris 1997). Puroindoline are controlled by the gene on 5D chromosome where *Ha* locus is present.

A broad survey conducted in India by Sewa Ram *et al.* (2002, 2005) indicated prevalence of null mutation in pinA in varieties released in India. Recent studies by Lillemmo *et al.* (2006) also confirmed that *pinA* null mutations are predominant in wheat lines developed at CYMMIT and released in different parts of the world including India. In addition sequence analysis of both the puroindolines in two of the wheat varieties in India showed frame shift mutation in *pinB*. Recently large numbers of mutations in *Pina* and *Pinb* conferring hard endosperm have been identified. These mutations appear to have occurred independently from each other and some can be traced back to specific geographic areas. Whereas the *Pinb-D1b* allele prevails among the spring and winter wheats of North America, Europe, China and Australia (Cane *et al.* 2004, Chen *et al.* 2006, Lillemo and Morris 2000, Morris *et al.* 2001 and Xia *et al.* 2005), *Pinb-D1c* and *Pinb-D1d* are mostly found in Western Europe (Lillemo and Morris 2000). The three rare alleles *Pinb-D1e*, *Pinb-D1f*, *Pinb-D1g* are confined to North American cultivars (Morris *et al.* 2001) and *Pinb-D1p* has only been found in Chinese wheat (Chen *et al.* 2006, Ikeda *et al.* 2005 and Xia *et al.* 2005).

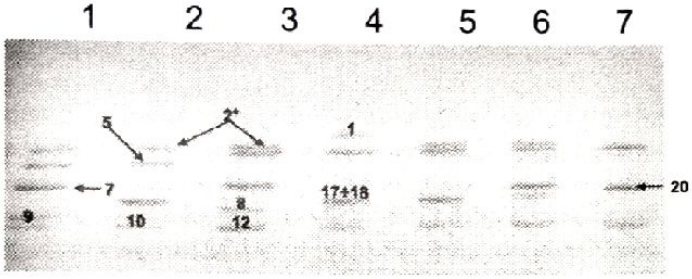
GLUTEN STRENGTH

Glutenins and gliadins constitute around 80% of the total seed proteins in wheat. These proteins impart the visco-elastic property to the dough which determines the end product quality. When flour is mixed with water, a visco-elastic complex called gluten is formed. Strong and extensible dough is good for bread making and weak and highly extensible for biscuit making quality. The strength and extensibility of the gluten are determined by the quality and quantity of glutenins and gliadins. Electrophoretic techniques such as SDS-PAGE and Acid PAGE have been employed to separate and characterize individual protein subunits of glutenins and gliadins respectively. Glutenins (acid soluble) are polymeric proteins whose monomeric units are divided into high (HMW) and low (LMW) molecular weight glutenin subunits and their molecular weight ranges between 67-130kDa and 35-45 kDa, respectively.

HMW GLUTENINS

In hexaploid wheat, six HMW glutenin genes are present, but only those coding subunits 1Bx, 1Dx are always expressed whereas the 1Ax and 1By are not always

expressed, and the 1Ay gene is not expressed (Gianibelli *et al.* 2000). Non-expression of 1Ay gene may be caused by nucleotide changes in the promoter region (Forde *et al.* 1985), by the presence of transposon like insertion in the coding region (Harberd *et al.* 1987) or presence of stop codon in down stream region (Bustos *et al.* 2000). Therefore, introduction of both Ax and Ay subunits from other sources as tetraploids or diploids wheats into hexaploid wheats can lead to enhanced genetic variability and improved technological properties (Lei *et al.* 2006). HMW profile of more than 280 wheat varieties released in India showed extensive variability (Ram 2003) (Fig.2). Nap Hal, an Indian land race of wheat, exhibits double null at *Glu-D1* locus where both subunit genes for 5+10 are absent. In addition, it possesses soft grain



1=PBW443, 2=GW273, 3=NW1014, 4=K9465, 5=K9644, 6=NW1012, 7=C306

Fig. 2 HMW glutenin profile of Indian wheat varieties

characteristics with wild form of puroindolines. This is the unique combination of both week gluten caused by *Glu-D1* double null and soft grain characteristics in nature (Ram *et al.* 2007).

The role of HMW-GS has been studied extensively in bread making (Payne *et al.* 1987; Kasarda *et al.* 1989; Shewry *et al.* 1992) and biscuit making (Souza *et al* 1994; Hou *et al.* 1996; Czuchajowska *et al.* 1996) quality. The role of individual HMW glutenin subunit in imparting dough strength has been identified and a *Glu-1* quality score for subunits has been developed (Payne and Lawrence 1983; Bushuk, 1998). Electrophoretic testing for HMW glutenin subunit composition is now used routinely in many breeding programs around the world. The correlation study in India showed significant positive effect (P=<0.001) of 2*, 5+10, 17+18 combinations of HMW-GS coded by *Glu-A1, Glu-B1* and *Glu-D1* loci respectively, on gluten strength. However, this combination of HMW-GS is present only in 17% of wheat varieties studied in India (Ram 2003). This shows that the strength in Indian wheat varieties can be enhanced by utilizing the above combinations of HMW-GS and hence the information can be valuable for breeding strategy to develop wheat varieties suitable for different end-use products as bread and biscuit. In addition, recently gene specific molecular markers for HMW glutenins have been identified which can discriminate between X and Y type glutenins. These markers can discriminate alleles which are not distinguishable by SDS-PAGE (Ahmad 2000; Gale 2005; Lei *et al.* 2006).

The mechanism by which certain HMW-GS confer better bread making performance than others may be related to the differences in molecular size distribution of gluten polymers (Gupta & MacRitchie 1994). The polymer size primarily depends upon the number and position of cysteine residues and the size of the repetitive domain in the glutenins (Belton 1999). For example formation of larger or more branched polymers by 5Dx subunit has been attributed to the presence of additional cysteine residues. Gliadins and LMW-GS with one available cysteine residue function as chain terminators and therefore, the polymer is reduced by the introduction of these during polymer formation. Recently this information has been used in the development of transgenics for enhanced dough strength (Barrow *et al.* 1997, Rooke *et al.* 1999 and Alvarez *et al.* 2000, He *et al.* 2005).

LMW GLUTENINS

Though difficulty in separating single components arising from the complexity of the group has limited characterization of individual proteins, recent developments have given new insights into the structure and functionality of LMW glutenins (Cloutier *et al.* 2001; D'Ovidio and Masci 2004). LMW-GS constitute around 40% of gluten proteins and are separated into three groups called B-type, C-type and D-type by SDS-PAGE. LMW-GS are encoded at the *glu-3* loci on the short arm of chromosome-1 which are closely linked to *gli-1* loci (Singh and Shephard 1988) for gliadins. The C-type subunits are related to α and γ gliadins and D-type to gliadins and are considered as the mutated form of these gliadins respectively (Okita *et al.* 1985; Masci *et al.* 1991). Additionally LMW GSs contain a cysteine residue in the unrepetitive N-terminal domain which could be related to the ability of these polypeptides to form polymers. Based on the distribution of cysteine residues, the LMW-GS proteins can be classified into three types: i) those with one cysteine in n-terminal domain; ii) those with a cysteine residue in the repetitive domain; and iii) those with 8 cysteines in the C-terminal part of the protein. All the LMW glutenins were reported having first cysteine residue in the N-terminal part of the sequence until Masci *et al.* (1998) detected first cysteine residue in the repetitive domain in durum and bread wheat respectively and subsequently reported by Ram *et al.* 2006 in Indian wheat variety NP4. Zhang *et al.* 2004 developed markers specific to *Glu-A3* alleles. Long *et al.* 2005 identified nine groups of LMW-GS by deduced amino acid sequences of the highly conserved N-terminal domain of 69 known genes. They assigned each group of LMW-GS genes on a single chromosome arm and hence to a specific locus. These groups could be distinguished by LMW-GS group specific primer sets and hence could be utilized to select specific allelic variation in marker assisted breeding.

Various studies in the past indicated the importance of LMW-GS to dough properties and bread making quality (Gupta *et al.* 1994; Andrews & Skerritt 1996). In addition,

functional properties such as dough mixing, extensibility and resistance of product of individual LMW genes have been assayed using expression system in bacteria (Lee *et al.* 1999). The presence of specific LMW-GS encoded at the *Glu B-1* locus has been associated with pasta making quality in durum wheat (Pogna *et al.* 1991). Using a set of recombinant inbred lines, LMW-II marker related to gluten strength in durum wheat was identified in our laboratory (Fig.3). RILs were developed from a cross between HD4676 and NIDW15 with two fold difference in gluten strength. There was significant difference in gluten strength among RILs having LMW-I and LMW-II glutenin subunits. PCR analysis of *Glu-A3* locus using locus specific primers indicated the correspondence of LMW band and the PCR amplification pattern and strong association of LMW-II with gluten strength (Unpublished). Other reports also indicate significant effect of *Glu-B3* alleles

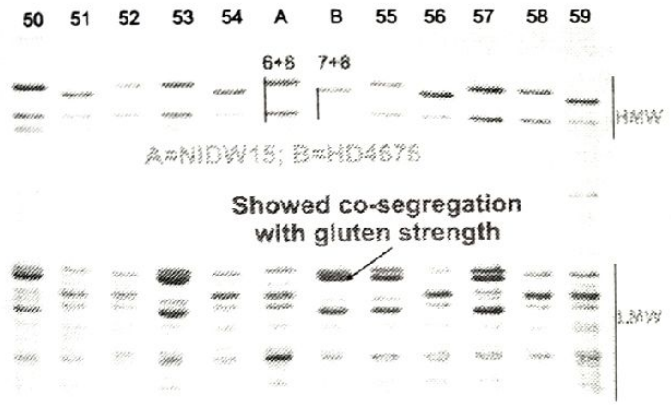


Fig. 3 LMW glutenin profile of parents and RILs indicating LMW II glutenin subunits associated with stronger gluten

on gluten strength (Luo *et al.* 2001; Vaccino *et al.* 2002). This demonstrated that HMW-GS are alone insufficient to account for differences in quality. LMW-GS must also be taken into consideration along with HMW-GS in identifying superior lines.

GLIADINS

Gliadins (alcohol soluble) represent around 50% of total gluten proteins in wheat. Gliadins are monomeric proteins and are divided into α, β, γ (30,000 daltons to 40,000 datons) and ω (44000 to 80000 daltons) according to their mobility in acid-PAGE. Most gliadin alleles are controlled in common wheat by six main loci on the chromosome on the first (*gli-1*) and sixth (*gli-2*) homoeological groups (Payne *et al.* 1986, Metakovsky 1991). There are also some minor loci as *Gli-3, Gli-5, Gli-6, Gli-D4* and *Gli-D5* which control few minor gliadin bands (Metakovsky 1997; Rodriguez & Carrillo 1996). *Gli-D4* controls γ type gliadins and is situated between centromere and the *Gli-D1* locus and *Gli-D5* controls ω -type gliadin and located between *Gli-D1* and telomere. Earlier studies demonstrated the association of γ gliadins (band 45) with pasta quality in durum wheat. However, subsequent studies indicated that low molecular weight glutenin subunits LMW2 were responsible for differences in quality (Payne *et al.* 1984; Pogna *et al.* 1988; Ruiz and Carrillo 1995). Recent developments in molecular techniques have given better insights into gene structure of gliadins. Zhang *et al.* 2003 identified SNPs and developed allele-specific PCR markers for γ-gliadins (tightly linked with specific LMW glutenins) associated with gluten strength in hexaploid wheats. Piston *et al.* 2006 identified four groups of γ-gliadin genes based on cDNA sequences and developed group specific PCR based primers to discriminate these groups using quantitative Real time PCR during grain development.

Because of their importance, the genetic polymorphism of the gliadins has been used to evaluate genetic diversity within several germplasms as Australia, Yugoslavia, Italy, France, Spain, Japan and India (Branlard *et al.* 2001, Metakovsky 1997; Ram *et al.* 2005). Extensive polymorphism (genetic diversity index (H) = 0.875) in gliadin pattern was observed in Indian cultivars (fig.4).

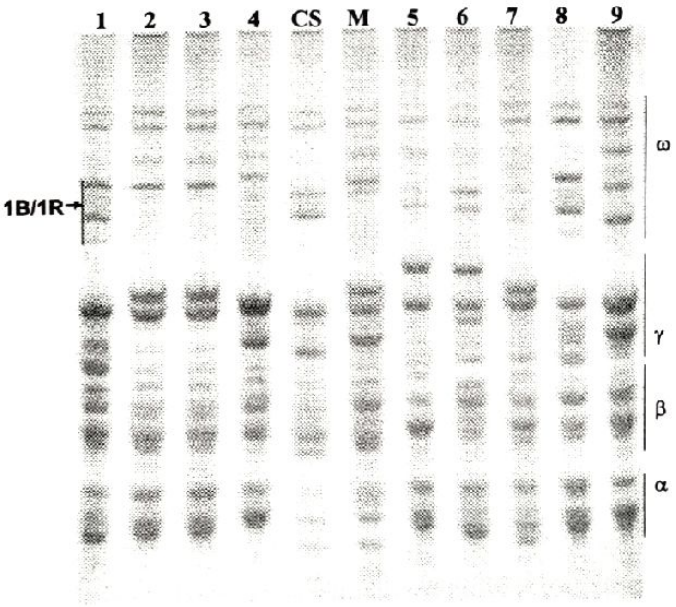


Fig. 4 Acid-PAGE Gliadin profile of Indian wheat varieties

A new system of classification of gliadins was developed based on the band pattern. A total of 147 band patterns were identified of which 45 different mobility bands were in the region of ω gliadins, 42 in the region of γ gliadins, 30 in the region of β and 29 in the region of α gliadins. Zone-wise and period-wise genetic diversity indices (H) of wheat cultivars are provided in the table below. The reduction in genetic diversity during 1990-onwards might be because of enhanced use of 1BL.1RS translocation. Some of the patterns were present predominantly in specific zones and may have role in adaptation to the conditions prevailing in these zones. There were loss of some gliadin bands and gain of new gliadins during successive years of release of cultivars.

Table 1 Zone-wise and period-wise genetic diversity indices (H) of wheat cultivars based on α, β, γ and ω gliadin patterns (Sewa Ram *et al.* 2005)

Zone / Period	Number of cultivars	Genetic diversity index (H)				
		H(α)	H (β)	H (γ)	H (ω)	Mean
Zone						
NWPZ	54	0.889	0.893	0.913	0.919	0.904
NEPZ	37	0.890	0.784	0.916	0.922	0.878
CZ	25	0.854	0.790	0.911	0.900	0.864
PZ	19	0.867	0.797	0.820	0.891	0.844
NHZ	22	0.825	0.855	0.880	0.785	0.836
Period						
1961-70	13	0.840	0.876	0.899	0.899	0.879
1971-80	27	0.933	0.848	0.936	0.944	0.915
1981-90	53	0.872	0.824	0.928	0.914	0.885
1991- onwards	59	0.925	0.847	0.876	0.822	0.868

STARCH QUALITY

The key features of starch deposition in endosperm that control functionality are starch content, grain hardness, granule size, distribution and shape, presence of endogenous lipids in the granule, amylopection structure and the ratio of amylose to amylopectin. Small starch granules have been reported to increase the extensibility of the dough whereas large granules increase the resistance to extension (Larson *et al.* 1997). The starch serves as the source of carbon during yeast fermentation in bread making, involves setting of bread loaf and plays key role in retrogradation during storage. Higher damaged starch in wheat flour is detrimental to the biscuit making quality. Reports indicate that genetic variations for starch traits amongst different wheat genotypes are responsible for variation in end-product quality (Morris 1998). Large differences occur in starch pasting and rheology with the small reduction in amylose content resulting from waxy allele at the three- homoeological loci of GBSS located at chromosome 7A (*wx-A1*), 4A (*wx-B1*) and 7D (*wx-D1*) and affect eating quality of Japanese noodles (Yamamori *et al.* 1994; Zeng *et al.* 1997; Zhao *et al.* 1998). The study of wheat lines having null allele at GBSS locus indicated that starch viscosity as well as swelling volume increased without significant change in the relative amylose content (Zhao *et al.* 1998).

Starch with high pasting viscosity in wheat flour is beneficial in the production of noodles with a smooth surface and soft texture. As the swelling ability of starch granules is largely responsible for the degree of paste viscosity observed when a starch suspension is heated, it is suggested to use flour swelling power as a simple test to assess the inter-cultivar differences in starch properties (Crosbie *et al.* 1992). High swelling degree is positively related to the softness of Japanese white salted noodles and negatively correlated with the firmness of yellow alkaline noodles. In our study, large variations were observed in flour swelling power of Indian wheats. The whole meal (WM) flour FSP varied from 7.18 to11.47 with the average value of 9.29. Flour FSP varied from 8.20 to 12.95 with the average value of 10.59. On an average there was higher flour FSP than WM FSP because of dilution effect of bran and other particles on starch in the whole meal flour. There was highly significant positive correlation between flour FSP and whole meal FSP ($R2=0.479$; $p<0.001$). Strong significant positive correlations were observed between peak viscosity in RVA and FSP ($R2=0.374$; $p<0.001$). Since FSP needs only 40 mg of the flour and easy to do as well as large number of samples can be done in a day, it is very useful in breeding for improving noodle making quality. Earlier reports also indicated positive correlation between FSP and noodle quality (Crosbie *et al.* 1992; Fu *et al.* 1998). This has additional advantage of predicting noodle starch quality for wheat products using simple whole meal flour test. This is because whole meal can be obtained with very small amount and several hundred whole meal samples can be ground in a day. The data demonstrated the utility of whole meal tests in predicting wheat quality based on starch swelling.

SOLVENT RETENTION CAPACITY (SRC) TESTS

Biscuit quality of wheat flour depends primarily upon the chemical constituents in the flour responsible for water holding and the quality and quantity of the gluten proteins determining dough strength. Generally soft wheats with weak gluten and low protein content are preferred for biscuit making. Based on the above quality characteristics required for cookie-making solvent retention capacity tests were developed and adopted by AACC as method 56-11 (2000). Solvent retention capacity is the weight of solvent held by flour after centrifugation and expressed as the percentage of flour weight (14% mb). In this test four different solutions as lactic acid, sucrose, water and sodium carbonate are utilized

for providing information on chemical and physical aspects of wheat samples. A combined pattern is considered useful for predicting commercial baking performance. Generally lactic acid SRC is associated with glutenin characteristics, sodium carbonate SRC with starch damage, sucrose SRC with pentosan content and gliadin characteristics and water SRC with all of those four constituents. In our study SRC profile explained 70 % variability in biscuit spread factor (Ram and Singh 2003). Since baking tests are time consuming, labour intensive, expensive and require large quantity of sample, small-scale tests developed (using 1 gram flour) can be used to predict the biscuit making quality of early generation breeding material.

Functional properties of flour including water absorption are measured using Mixograph and Farinograph that are used to differentiate wheat flours of good and poor baking quality. The determination of optimum water needed to obtain a certain dough consistency is essential to give dough with optimal handling characteristics suitable for rheological testing as well as product quality. Flours of lower water absorption are preferred for biscuit making and higher water absorption for chapati and bread making. However, Farinograph and Mixograph tests are time consuming, labour intensive and expensive; small-scale tests are required to predict the end-use quality of grain from early generation breeding material where limited quantity of grain available is available. SRC profile as well as Mixograph and Farinograph profiles of flours were developed using 192 wheat genotypes including 160 released varieties in India. Based on multiple regression analysis an equation was developed to predict FWA which explained very large variability (R=0.93, N=300) in actual FWA (Ram *et al.* 2005) (Fig.5). Since large number of genotypes were used in this investigation, it has obvious implications in breeding programme for the improvement of wheat quality.

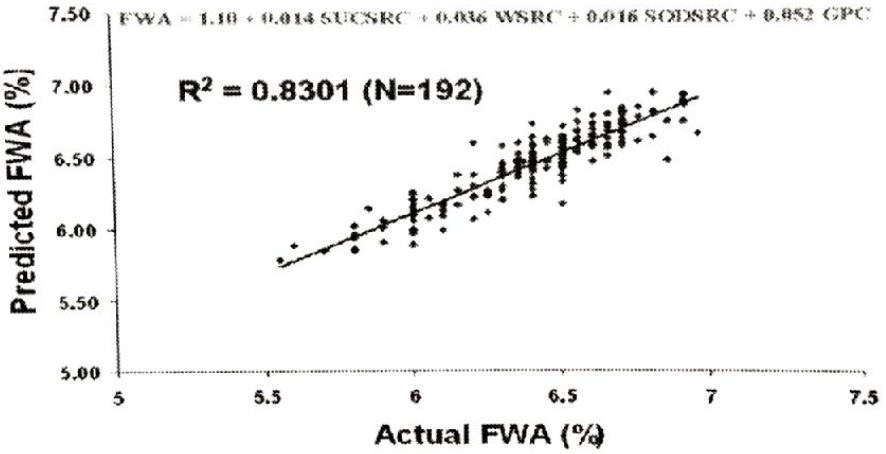


Fig. 5 Relationship between actual Farinograph water absorption and predicted Farinograph water absorption

Gluten has the important property of swelling in various non reducing solvents (dilute acetic acid, lactic acid and SDS) and the swelling volume appears to be directly related to quantity and quality of glutenin present Therefore, small-scale tests used to predict bread making quality are based on glutenin swelling capacity or directly on insoluble glutenin content. Sedimentation tests utilizing 6g material is laborious, time consuming and requires

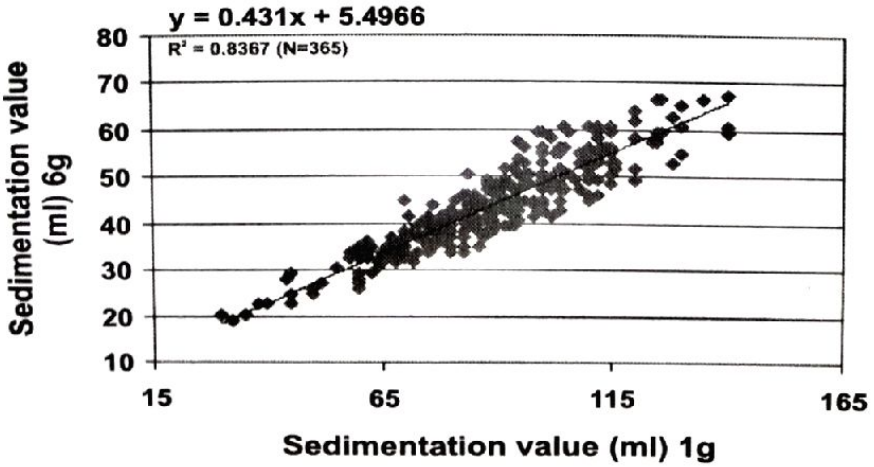


Fig. 6 Relationship between 1g and 6g sedimentation tests

more amount of chemicals. One g test was developed for measuring sedimentation volumes to expedite the breeding programme. In this investigation 365 diverse wheat genotypes were used to see correlation between the values of 1g and 6g tests and their utility in breeding programme (Fig. 6). Very high positive correlation (R2=0.84) was observed between these tests. Since 1g test utilizes lesser amount of whole meal flour and also large number of samples (200 samples in a day) can be analyzed, it is very useful in predicting dough strength in early segregating generation.

NUTRITIONAL QUALITY STUDIES AND FUTURE PERSPECTIVES

The large part of the population in the country faces problem of micronutrient deficiency (especially Fe and Zn). This problem is more predominant in infants and pregnant women who require comparatively larger quantity of micronutrients. Genetically increasing the levels of micronutrients and their bioavailability is especially relevant for poor grain consuming families living in isolated rural areas for which vegetable sources are often in short supply. Reports indicate that the bioavailability of micronutrients is reduced by the presence of anti-nutritional factors such as phytic acid in the grain. Phytic acid inhibits the release of Fe and Zn in the intestine and thus their absorption is reduced. The inhibitory effect of phytic acid can be reduced by decreasing phytic acid content and enhancing phytase activity in the grain. Phytase releases phosphate groups from phytic acid and consequently its binding to micronutrients is decreased. Therefore, identifying genotypes having low phytic acid content and high phytase activity along with higher Fe and Zn content is necessary before we proceed to improve nutritional quality of wheat. Low phytic acid mutants have been developed and identified in the back ground of Indian wheat varieties being cultivated in large part of the country. Large variations have been reported in micronutrient content in hexaploids as well as their diploid progenitors in India (Chhuneja *et al.* 2006). Work done at DWR; Karnal indicates large variation in micronutrient content in grains of different varieties.

In addition to micronutrient deficiency, protein malnutrition is a major problem in areas where cereal based diet is predominant. Wheat is deficient in essential amino-acid like lysine and needs improvement. In recent years, major progress has been made in understanding the metabolic pathways of essential amino acids and sulfur metabolites in plants, as well in the identification of regulatory and limiting steps in these metabolic pathways that could be overcome by metabolic engineering. As limited variability exists in wheat for lysine content, therefore, transgenic approach has potential for enhancing lysine content (Forsyth *et al.* 2005). Manipulation of starch composition by changing amylose to amylopectin ratio in wheat endosperm has significant potential to improve human health through its resistant starch content (Regina *et al.* 2006). Resistant starch can improve large-bowel function by inducing synthesis of short chain fatty acids. Carotenoids are other natural compounds that reduce the oxidative damage to biological membrane by scavenging peroxi-radicals such as those involved in certain human diseases and in the ageing process. Natural antioxidants in human foods may have to maintain the quality of products by inhibiting free radicals and oxidative processes. Beta-carotenes have also been implicated in reducing the risk of cancer in human beings. Vitamin A deficiency also increases susceptibility to malaria and diarrhoeal disease, and reduces the bioavailability of micronutrients, including iron, important to the health of all family members (WHO 2002). Therefore, an improvement in the endogenous quantity of beta-carotenes will enhance the nutritional value of pasta products. This can be acheved by using existing variability in wheat and by transgenic approach. Two candidate genes phytoene synthase and beta cyclase associated with levels of beta-carotene have been characterized in *Arabidopsis* and other plants. Recently "Golden rice" has been developed using metabolic engineering approach by transforming rice using two genes of beta-carotene biosynthetic pathway taken from bacterial and plant sources (Ye *et al.* 2000 and Paine *et al.* 2005). This increased beta-carotene from zero ppm to 37 ppm indicating the potential of improving beta-carotene content in wheats using transgenic approach.

CONCLUSION

In conclusion, germplasm lines having superior quality traits have been identified and are being used in breeding programme. Nap Hal, an Indian land race of wheat, showed the presence of both soft grain characteristics and weak gluten suitable for biscuit making quality. High and low molecular weight glutenins and gliadins have been separated electrophoretically from 285 released varieties of wheat in India using SDS-PAGE and Acid-PAGE techniques. Relationship between specific combinations of HMW-GS and gluten strength has been established. Acid-PAGE technique exhibited large genetic diversity in Indian wheats and some gliadin patterns were observed in specific environments. PCR amplification of puroindoline genes indicated the prevalence of null mutation in pinA in released varieties in India and had harder grains. Two exceptional cultivars exhibited frame shift mutation in pinB causing harder grain texture. Solvent retention capacities (SRC) showed a significant negative correlation with both biscuit diameter and spread factor. SRC profile along with protein content can be used in predicting Farinograph water absorption (FWA) as it explained 88% variability in FWA. Rheological studied (alveograph tests) exhibited that dough extensibility is the main limitation in many of the cultivars for bread and biscuit making quality. PCR amplification of waxy genes demonstrated the presence of Wx-4A null

mutants in large numbers of wheat varieties in India. Germplasm lines including diploid progenitors have been identified with higher content of micronutrients and low phytic acid mutants have been developed. The information is being used to improve wheat for various end-use products.

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