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Influence of nitrogen application on grain yield and end use quality in segregating generations of bread wheat (*Triticum aestivum L*)

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Variation in bread making quality of wheat (*Triticum aestivum L*) is a major consideration for suppliers and bakers. Grain protein concentration, protein quality, ash content and carbohydrates are major quality attributes of bread wheat. Breeders and their cereal chemist colleagues use predictors of these end use quality factors in the early generations to develop improved cultivars. Six populations of bread wheat derived from a previous diallel experiment were evaluated under three rates of nitrogen fertilizer for grain yield and end use quality. Increasing nitrogen (N) rates from low (40 kg fad^{-1}. N) to high (120 kg fad^{-1}. N) had an accelerating significant effect on grain yield and quality in F_2 and F_3 generations. Desirable quality types along with high grain yield were defined within the C3, C4 and C6 populations. The population C6 exhibited the highest flour protein (13.8%) and the lowest flour ash (0.42%) as well as good grain yield as compared with the check variety and other populations. In F_3 populations, 1000-kernel weight was an effective selection criterion for grain yield in pop. C3, C4 and C6 but it caused reduction in flour protein in pop. C1. Grain protein concentration showed an increasing trend in F_3 generation, confirming the positive relationship with grain yield. The expected response to selection of F_3 populations under high N level were 8.81% for grain yield, 8.0% for flour protein 5.74% for gluten and 4.98% for carbohydrates. The SDS-PAGE of grain storage proteins was performed in order to analyze molecular weight of gluten subunits (GS) and investigate genetic diversity among the selected populations. The population C6 exhibited the highest unique bands (5 from 7 bands) under high N level and followed by the population C4. The high N level generally increased total high-molecular weight-GS content in wheat grain, although different patterns of response to N rate were observed between populations.

Key words: Bread wheat, segregating populations, nitrogen and protein electrophoresis.

INTRODUCTION

Bread or common wheat (*Triticum aestivum L.*) is an important source of staple food and is widely cultivated worldwide because of its good nutritional and technical properties. Bread wheat improvement could be enhanced by the identification of germplasm with superior grain yield and end-use quality traits. In developing countries, wheat is the main source of energy in the diet where the bread represents the source of about of 70% of total energy obtained daily. Wheat is grown in a wide range of environments that affect overall performance, particularly grain yield and end-use quality. Wheat yield and end-use quality depend upon the environment, genotype, and their interaction (Bergman et al., 1998).

Early generation testing is a selection procedure based on initiating testing of genetically heterogeneous lines of families in an earlier generation than would normally be considered suitable for release (Anderson et al., 1991). When the concept is applied to development of homozygous cultivars in autogamous species, selection of homozygous lines from superior heterogeneous families permits the breeder to exploit the genotypic variance provided by inbreeding and to develop cultivars of suitable uniformity. Thus, the procedure has two phases, selection among heterogeneous families and selection of

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homozygous potential genotypes from superior families (Steven et al., 2002).

Many quality characteristics are important for the utilization of bread wheat, particularly flour extraction (milling yield), flour protein concentration, and some other chemical characteristics. These characteristics usually are influenced by cultivar and interactions of cultivar with environment (Souza et al., 2004).

Grain protein is of primary importance in determining the bread making quality of wheat. Variations in both protein content and composition significantly modify flour quality for bread-making. Gluten can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water-soluble constituents. Depending on the thoroughness of washing, the dry solid contains 75 – 85% protein and 5 – 10% lipids; most of the remainder is starch and non-starch carbohydrates (Wall, 1979; Preston et al., 1995). In practice, the term 'gluten' refers to the proteins, because they play a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesively, viscosity and elasticity on dough. Gluten, comprising roughly 78 to 85% of total wheat endosperm protein, is a very large complex composed mainly of polymeric (multiple polypeptide chains) and monomeric (single chain polypeptides) proteins known as glutenins and gliadins, respectively (Wieser et al., 2006). Glutenins confer elasticity to dough, where as gliadins are viscous and give extensibility to dough. Glutenin subunits are subdivided into two groups, low-molecular weight (LMW) glutenin subunits and high-molecular weight (HMW) glutenin subunits, and build polymeric proteins via inter-molecular disulfide bonds. All of these storage proteins are secretory proteins synthesized with a signal peptide that is cleaved when the protein is translocated into the lumen of the endoplasmic reticulum (Shewry et al. 1995).

The Polyacrylamide-gel electrophoresis has been used to show that large size variation exists among LMW and HMW glutenin subunits, and it has been suggested that deletions and insertions within the repetitive region are responsible for these variations in length (Benmoussa et al., 2000). Allelic variation of high molecular weight (HMW) subunits of glutenin in 185 cultivars of bread wheat have been described by Xueli et al. (2005), where about 20 different major subunits were distinguished by SDS-PAGE. The high molecular weight (HMW) glutenin subunits from seven Pakistani wheat genotypes were also fractionated SDS PAGE, in order to characterize the plant material and test the variability within species (Khan et al., 2002).

Gliadins and glutenins are genetic markers allowing the expeditious and objective identification of a variety, determination of its genetic constitution, and determination of some important characteristics and traits. Genetic diversity is the basis for successful crop improvement and can be estimated by different methods such as morphological traits, end-use quality traits, and molecular markers (Fufa et al., 2005).

Improvement in grain protein concentration is a major objective in bread wheat making program world wide. Achieving this goal without a concurrent loss in grain yield has been difficult due to the well documented negative association between these two economically important traits (Costa and Kronstad, 1994; Dencic et al., 2000). Although reports of negative correlation between grain protein concentration and grain yield dominate in literature, some studies on winter wheat suggest that genetic improvement in grain yield and grain protein can occur simultaneously (Huebner et al., 1997; Mikhaylenko et al., 2000).

Nitrogen (N) is the most important environmental factor affecting protein content and composition, including contents of total protein and subunits. Fertilizer management is an important part of the overall management package target towards higher yield. Nitrogen fertilization strongly influences the quality of protein in wheat flour. In a detailed study of protein composition of 13 wheat cultivars grown under differential fertilization, Wieser and Seilmeier (1998) found that increased N fertilization decreased the proportion of hydrophobic proteins and increased the proportion of hydrophilic proteins. The effect of fertilization on protein content and composition varied significantly with wheat cultivars. Tolbert (2004) found that increasing nitrogen fertilizer increased protein content, flour and the arrival time of dough. Anne et al. (1995) concluded that a genetic increase in the amount of grain N could be accomplished by increasing N utilization efficiency through (i) increasing the N uptake efficiency of the crop or (ii) increasing N remobilization efficiency of the crop.

However, information about the impact of N on accumulations of HMW-GS in wheat is still limited. The objectives of this study were to (i) assess genetic variability of biomass yield, yield components and end use quality in early generations (ii) investigate the impact of nitrogen rate on the content of technological quality and grain yield (iii) confirm the results obtained through the electrophoretic separation of storage proteins

MATERIALS AND METHODS

Plant materials

The basic material consisted of six wheat crosses derived from previous diallel experiment by Bayoumi (2005). The crosses were chosen for this study based on their reputed differences in yield performance and 1000- kernel weight. The F_2 populations were planted in the 2005/2006 crop season at the Experimental farm of Faculty of Agriculture, Suez Canal Univ., Ismailia, Egypt, using seeds originated from the selected F_1 crosses (Table 1). In F_2 generation, each cross was represented by approximately 200 plants/replicate which grown in 12 rows. In 2006/2007 season, within each F_2 population selection was done based on 1000- kernel weight. A sample of the highest 20 families (a family is the progeny of an individual F_2 plant) were selected by taking one head from each selected plant to seed one F_3 head row. Each F_3 population consisted of 3 rows with individual row being 1.2m long and 0.3m apart.

Table 1. Wheat population identification by cross name, origin and pedigree developed to be selected for yield and quality.

Pop. No.	Cross Name	Origin	Pedigree
C 1	Nesser x Aclaimn	Mexico x Syria	(ICW85-1024-06AP-300AP-300L-IAP) x (ICW95-1273-QL-6OL-OAP)
C 2	Giza 168 x Korifla	Egypt x Portugal	(MRL/BUC//SERICM93046-8M-OY-OM-2Y-OB-OGZ) x(ICD84-0461-ABL-15AP-TR-AP-OTP)
C 3	Giza 168 x Aclaimn	Egypt x Syria	(MRL/BUC//SERI CM93046-8M-OY-OM-2Y-OB-OGZ) x (ICW95-1273-QL-6OL-OAP)
C 4	Gimmeza 7 x Korifla	Egypt x Portugal	Bb/7*2//YsoA/Kal*3/5/Skh8/4/Rrv/ww15/3/Bj'S//On*3/Bon CGm4024-1Gm-2Gm-oGm) x (ICD84-0461-ABL-15AP-TR-AP-OTP)
C 5	Korifla x Aclaimn	Portugal x Syria	(ICD84-0461-ABL-15AP-TR-AP-OTP) x (ICW95-1273-QL-6OL-OAP)
C 6	Gimmeza 7 x Aclaimn	Egypt x Syria	(Bb/7*2//YsoA/Kal*3/5/Skh8/4/Rrv/ww15/3/Bj'S//On*3/Bon CGm4024-1Gm-2Gm-oGm) x (ICW95-1273-QL-6OL-OAP)

The check variety Sakha 94 was placed every 20 head rows among F_2 and F_3 populations.

The field experiments

The F_2 and F_3 populations of each cross along with check variety Sakha 94 were grown under three levels of nitrogen fertilizer i.e. 40, 80 and 120 kg fad^{-1}. in the form of ammonium nitrate (33.5%). Forty percent of total nitrogen was applied before sowing, while the remaining nitrogen was applied by topdressing in equal amounts at jointing and booting stages. Another 45 kg P_2O_5 fad^{-1} and 50 kg K_2O fad^{-1} were applied as basal fertilizer. Line source sprinkler irrigation system was used to impose irrigation. Irrigation was scheduled to fully meet water requirements of wheat by maintaining available soil moisture content in the top 60 cm of the root zone above 60%.

The observations of agronomic and quality characteristics were done as follows: a) Heading date: observation in days from the seedling emergency to the plant heading at the moment in which 50% of the plants reach heading. b) Spike length; average length in (cm), from the bottom rachis to the tip of the spike, excluding awns. c) 1000- kernel weight (g) was calculated as mean weight of three sets of 1000 grains per plot. d) Grain Yield: weight of grains /m^2, done only in the selected lines.

Quality analysis

The technological quality analyses were performed on the selected families in F_2 and F_3 generations. These parameters which were done at the Food Technology Department, Fac., of Agric., Suez Canal Univ., were;

Flour yield and extraction: Before milling, grain was tempered to 45% moisture content and held overnight after that milling was conducted according to method 44-16 (AACC, 2000). Break flour (the amount of flour obtained early in the milling process from the break roller) is a measure of how easily the grain can be milled to flour. The higher values desired and the straight grade flour (the total amount of flour obtained) were expressed as percent of total products.

Ash content: A low value of ash content is important in the assessment of quality. Ash content (on dry matter basis) was determined in duplicate on 5 g of sample by dry combustion for 16 h at 580°C (Method 08-01, AACC, 2000).

Protein and gluten contents: Generally, high protein and gluten content is associated with good flour. The protein content (N*5.7) was determined by Kjedhal analysis in duplicate (Method 46 -13, AACC, 2000). The result was adjusted on dry matter basis. Gluten content (%) was determined on 10 g of sample by washing with a 2% NaCl solution buffered at pH 6.8 (Method 30-10, AACC, 2000). The extracted wet gluten was dried in a heated plate, weighed and the result was adjusted on dry matter basis

Carbohydrate determination: The carbohydrates were spectrophotometerically determined in plant leaves by the phenol sulphoric acid method as described by Dubois et al. (1956).

Protein analysis electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the HMW and LMW glutenin subunits (Jackson et al., 1996). Gliadins were extracted with 70% (v/v) ethanol, and glutenin polymers reduced with 5 µg/µl DTT in 0.08 M Tris–HCl (pH 8.0) buffer and alkylated with iodoacetamide. Electrophoresis was performed on 12.5% acrylamide gels at 7.5 mA/gel constant current with a 10 kDa protein ladder (Gibco-BRL, 10–200kDa) as a molecular weight standard.

Statistical analysis

Yield and grain quality of the selected F_2 and F_3 populations were analyzed using a modification of a randomized blocks of split plot design with three replications. Main plots were designated for nitrogen fertilization (three levels 40, 80 and 120 kg fad^{-1}.N) while subplots were arranged for the six wheat populations. A computer program Genstat 8 Rel.PL16 was used for analyzing data. Analysis of variance of data from each cross in nitrogen treatments was performed for all the studied traits and the effects were considered random for populations and fixed for nitrogen treatment.

Narrow sense heritability (h^2) was estimated by parent offspring regression of selected families in the F_2 and F_3 generations according to Anderson et al. (1991). The expected response to family selection was calculated according to the method of Falconer and Mackay (1996).

Table 2. Mean squares due to different sources of variation for evaluating the six wheat populations and check variety, for grain yield and quality.

Characters	S.O.V d.f	Rep. 2	Nitrogen(N) 2	Error 4	Populations 6	N x Pop. 12	Error 36
Heading date		12.73	146.4**	25.71	41183.4**	85.8**	15.6
Spike length		11.12	220.8**	13.86	910.75**	16.89*	8.58
1000-kernel weight		45.87	438.8**	52.53	2669.0**	124.1**	13.2
Grain yield		28.38	770.4**	45.47	9512.52**	413.7**	23.2
Flour yield		5.52	236.9**	12.96	1493.13**	41.14**	8.16
Flour protein		7.32	135.3**	10.58	819.77**	8.542**	1.5
Flour extraction		8.63	65.86**	11.05	1925.67**	28.11**	4.96
Flour ash		4.43	86.4**	5.31	1052.14**	18.28**	2.80
Gluten		2.13	34.64**	4.051	1218.96**	9.27**	1. 38
Carbohydrates		8.78	28.04**	6.01	2272.9**	63.16**	4.51

RESULTS AND DISCUSSIONS

General effects of populations and nitrogen treatments

The statistical analysis performed on the six populations and check variety Sakha 94, detected significant differences among populations, nitrogen levels and their interactions for all the studied traits (Table 2). The results indicated that there were sufficient variations among the selected populations. Nitrogen (N) supply had a significant impact on the studied traits, suggesting that varying N is important and therefore testing traits at various N levels would affect the performance of the populations.

The mean squares (MS) of populations (main breeding effect) were far greater than the MS for the interaction (more than 50 times greater than Population. × N. This may be interpreted as primary evidence that the interactt-ion, although statistically significant, may not represent major changes in population rankings for most attributes.

Effects of the breeding activity on grain yield and end use quality

Mean values of the selected F_2 populations for the studied traits are shown in Table 3. The heading date values indicated that the populations C1, C2 and C3 could be classified as having early maturity cycle while, C5 has a moderate maturity cycle. The late cycle of populations C4, and C6 is probably due to the fact that at least one parent of these populations is late maturity.

The data indicated that increasing nitrogen dose from low N (40 kg fad^{-1}. N) to high N (120 kg fad^{-1} N) had an accelerating significant effect on grain yield and quality. Among yield components, 1000-kernel weight increased throughout the increasing nitrogen dose from 33.8 to 36.7 (C1), 33.1to 37.6 (C2), 34.7 to 37.5 (C3), 34.2 to 40.5 (C4), 30.5 to 33.9 (C5) and 34.9 to 37.8 g (C6). The increase in kernels weight was associated with an increase in spike length and grain yield and exhibit a clear trend due to nitrogen treatments. The populations C3, C4 and C6 had a better performance for mean spike length, kernel weight and grain yield attained. Grain yield significantly varied among populations ranging from 591.3 to 840.6 g/m^2.

Protein content and gluten quality of wheat has long been recognized as the most important factors affecting bread-making properties (Huebner et al. 1997). The populations identified as C4, C5, and C6 showed the best performance in terms of protein and gluten content. The population C6 exhibited the highest flour protein (13.8 %) and the lowest flour ash (0.42 %) as well as good grain yield as compared with the check variety. Protein content was inversely proportional to ash content. This indicated strong relation with technological quality, according to the work done by Wall (1979) and Preston et al. (1995).

Nitrogen fertilizers enhanced protein content, where protein concentration had a major impact on the quality of end products. Huebner et al. (1997), Mikhaylenko et al. (2000) and Tolbert (2004) found that increasing nitrogen fertilizer increased protein content and flour yield.

The content of soluble carbohydrates is an important determinant of wheat quality (Shewry et al., 1995). The total carbohydrates concentration decreased remarkably with increasing nitrogen levels. Adequate amount of nitrogen in wheat plant tissue during spike initiation resulted in enough metabolites to be translocated to the developing spikes (Souza et al. 2004). The accumulation and remobilization of dry matter are complementary routes for increasing grain yield and when combined with improved N accumulation and redistribution, should result in family with high grain yield and protein concentration (Tolbert 2004). Although some populations had shown a good performance considering the technological quality parameters, it should be pointed out that the environmental conditions of cultivation, and grain handling could alter the values which express the quality of the lines (Anne et al. 1995).

Table 3. Estimated effect of nitrogen fertility on grain yield and end use quality for F_2 wheat populations and check variety (Sakha, 1994).

Trait		Population. no							
		C 1	C 2	C 3	C 4	C 5	C 6	Check	LSD
Heading date (days)	**L N**	82.3	84.7	81.9	91.6	90.6	92.1	88.2	1.03
	M N	84.6	85.7	83.2	97.3	91.3	94.3	92.4	1.24
	H N	87.9	87.8	86.8	99.2	92.4	100.5	98.5	1.33
	Mean	84.9	86.0	83.9	96.0	91.4	95.6	93.0	1.28
Spike length (cm)	**L N**	12.3	11.6	12.6	12.9	10.3	12.8	9.8	0.98
	M N	12.8	12.0	12.8	13.0	10.9	13.6	10.2	1.07
	H N	13.0	12.8	136	14.2	11.2	14.4	10.8	1.01
	Mean	12.7	12.1	13.0	13.3	10.8	13.6	10.2	0.85
1000-KW (g)	**L N**	33.8	33.1	34.7	34.2	30.5	34.9	30.1	0.87
	M N	34.1	34.5	35.8	35.4	31.2	35.8	32.8	1.11
	H N	36.7	37.6	37.5	40.5	33.9	37.8	34.6	1.34
	Mean	34.5	34.7	35.6	36.3	31.5	35.8	32.1	2.03
Grain yield (g) / m^2	**L N**	670	530	800	710	590	693	495	29.1
	M N	684	565	842	750	695	758	540	30.5
	H N	725	589	880	830	732	770	569	35.9
	Mean	693.0	591.3	840.6	763.3	672.3	740.3	534.6	40.8
Flour yield	**L N**	449.5	304.2	523.2	477.3	362.2	462.9	277.6	23.8
	M N	463.7	336.7	561.6	508.5	430.2	519.9	314.8	28.4
	H N	499.5	361.6	596.6	571.8	456.0	569.7	342.5	31.4
	Mean	470.5	333.4	559.8	519.0	415.5	507.1	311.1	30.9
Flour protein (%)	**L N**	12.3	11.9	10.5	12.7	12.9	13.4	9.8	0.88
	M N	12.6	12.2	11.3	13.6	13.4	13.8	10.4	0.64
	H N	13.0	12.4	11.7	14.0	13.7	14.2	10.9	0.29
	Mean	12.6	12.1	11.1	13.4	13.3	13.8	10.3	0.31
Flour extraction (%)	**L N**	67.1	57.4	65.4	67.3	61.4	66.8	56.1	2.43
	M N	67.8	59.6	66.7	67.8	61.9	68.6	58.3	2.84
	H N	68.9	61.4	67.8	68.9	62.3	70.1	60.2	3.02
	Mean	67.9	59.4	66.6	68.0	61.8	68.5	58.2	1.49
Flour ash (%)	**L N**	0.54	0.47	0.79	0.51	0.43	0.41	0.84	0.08
	M N	0.62	0.68	0.83	0.56	0.45	0.43	0.93	0.11
	H N	0.68	0.81	0.93	0.58	0.48	0.44	0.99	0.17
	Mean	0.61	0.65	0.85	0.55	0.45	0.42	0.92	0.15
Gluten (%)	**L N**	37.0	31.8	38.2	40.1	38.7	41.7	31.7	0.11
	M N	39.0	33.6	41.1	41.0	41.2	42.3	33.4	0.24
	H N	39.4	36.7	42.5	41.8	43.0	43.5	36.1	0.31
	Mean	38.4	34.0	40.6	40.9	40.9	42.5	33.7	0.78
Carbohydrates (%)	**L N**	27.1	30.4	28.5	26.5	28.6	25.1	34.7	2.15
	M N	24.9	28.4	26.3	26.1	27.3	23.4	32.8	1.98
	H N	25.4	26.3	23.8	25.0	25.4	22.6	31.5	1.77
	Mean	25.8	28.3	26.2	25.8	27.1	23.7	33.0	0.82

L N= low nitrogen (40 kg fad^{-1}); MN = moderate nitrogen (80 kg fad^{-1}); H N= high nitrogen (120 kg fad^{-1}).

Grain yield and quality response to selection in F_3 generation

Mean values for F_3 families under high N level were selected based on 1000- kernel weight (KW) for high yield and quality (Table 4). Significant differences were found among the F_3 families for all the studied traits. The grain yield and quality of the selected F_3 families was improved markedly through indirect selection for 1000-kernel weight except heading date and carbohydrates. The result indicated that 1000-KW was an effective selection criterion for grain yield in populations C3, C4

Table 4. Mean values for F_3 families selected for high yield and quality under high N level.

Trait	Population. no								
	C 1	C 2	C 3	C 4	C 5	C 6	Mean	Check	LSD
Heading date (days)	88.5	90.0	87.1	100.2	99.0	102.5	94.5	89.5	0.49
Spike length (cm)	13.2	13.0	13.8	14.7	11.6	14.8	13.5	10.6	0.23
1000-KW (g)	37.0	37.9	37.6	41.2	35.2	39.6	38.1	34.7	0.86
Grain yield (g) / m^2	730	595	897	843	762	810	773	570	36.7
Flour yield	496	364	599	577	471	554	510	350	40.3
Flour protein (%)	10.2	12.8	12.3	14.6	14.0	14.5	13.06	10.9	0.65
Flour extraction (%)	68.0	61.2	67.0	68.5	62.0	69.2	65.9	60.7	1.64
Flour ash (%)	0.94	0.63	0.42	0.51	0.84	0.41	0.62	0.98	0.15
Gluten (%)	39.6	37.1	42.8	42.3	43.1	43.8	41.4	36.3	1.13
Carbohydrates (%)	25.9	26.8	24.6	26.1	21.3	20.4	24.1	32.1	1.26

Table 5. Heritabilities of the studied traits determined by regression of F_3 on F_2.

Trait	Population. no						
	C 1	C 2	C 3	C 4	C 5	C 6	Mean h^2
Heading date (days)	0.71	0.66	0.65	0.79	0.68	0.69	0.69
Spike length (cm)	0.46	0.47	0.49	0.53	0.48	0.57	0.50
1000-KW (g)	0.49	0.43	0.40	0.62	0.69	0.61	0.54
Grain yield (g) / m^2	0.29	0.17	0.38	0.41	0.37	0.44	0.34
Flour yield	0.34	0.41	0.45	0.52	0.48	0.59	0.46
Flour protein (%)	0.49	0.51	0.64	0.63	0.56	0.76	0.60
Flour extraction (%)	0.30	0.41	0.43	0.52	0.46	0.60	0.45
Flour ash (%)	0.21	0.18	0.23	0.24	0.19	0.27	0.22
Gluten (%)	0.52	0.58	0.62	0.64	0.61	0.65	0.60
Carbohydrates (%)	0.61	0.63	0.65	0.72	0.60	0.74	0.65

and C6 but it caused reduction in flour protein in population. C1 when compared with the mean of all populations and check variety exhibited the lowest trend for the most traits Grain protein concentration showed an increasing trend in F_3 generation, confirming the positive relationship with grain yield. Increased protein storage may be obtained by improving the efficiency of N utilization (Bergman et al. 1998). This could be achieved through either higher N uptake capacity of root system or through greater metabolization of nitrogenous compounds from the vegetative organs to the grain (Dencic et al., 2000).

Comparing the selected F_3 families with the check variety Sakha 94, it was able to show that mean grain yield increased (773 g/m^2 versus 570 g/m^2), flour yield (510 g/m^2 versus 350 g/m^2), flour protein (13.06% versus 10.9%), flour extraction (65.9% versus 60.7%) and gluten (41.4% versus 36.3%). Selection for high grain yield and protein is expected to delay the maturity and induce a reduction in carbohydrates.

Heritability and expected response to selection

Heritability (h^2) of a trait is important in determining its response to selection. Heritability estimates were computed by applying parent- progeny regression to F_2 and F_3 data (Table 5). Mean narrow sense heritability estimates ranged from 0.22 to 0.69. The wide range in the heritability values suggest that where selection is practiced for these traits, reasonable levels of genetic progress would be expected. The mean heritability estimates were 0.50, 0.54 and 0.34 for spike length, 1000-kernel weight and grain yield, respectively. The mean heritabilities for grain quality were relatively high for Protein content, gluten and carbohydrates. The Literature reports mixed results on the effectiveness of early generation selection. Weegels et al. (1996) found that early generation selection resulted in low values for yield in wheat while Preston et al. (1995) and Steven et al. (2002) found that F_2 performance was good indicator of F_3 performance for yield and quality.

Indirect selection for increased yield of F3 populations was done by selection for 1000-KW among the highest populations in protein content. The grain yield of the F_3 selected families was improved markedly through indirect selection for 1000-KW especially under high N level. The results in Table 6 showed that the expected response of

Table 6. The expected response of selection (%) of F_3 families under high and low N levels.

Trait	Population. no							
	N	C 1	C 2	C 3	C 4	C 5	C 6	Mean
Heading date (days)	**L N**	-7.3	-6.9	-7.5	-0.63	0.13	1.86	-3.39
	H N	-3.4	-4.2	-5.1	-0.41	1.23	2.87	-1.5
Spike length (cm)	**L N**	1.33	0.94	8.7	9.6	0.62	10.1	5.21
	H N	2.48	1.54	10.3	11.9	2.68	14.5	7.23
1000-KW (g)	**L N**	1.98	1.87	7.8	10.5	1.9	9.9	5.65
	H N	3.74	3.69	10.5	13.9	4.6	11.2	7.95
Grain yield (g) / m^2	**L N**	5.6	3.4	6.9	8.4	3.5	8.8	6.1
	H N	8.3	6.2	9.3	10.8	5.4	12.9	8.81
Flour yield	**L N**	7.2	4.9	9.1	11.6	5.2	11.1	8.18
	H N	9.8	6.3	11.2	12.8	7.8	14.6	10.41
Flour protein (%)	**L N**	-0.8	9.9	6.9	4.5	5.5	5.9	5.31
	H N	-0.2	10.9	8.8	8.5	9.8	10.2	8.0
Flour extraction (%)	**L N**	1.4	5.8	3.8	10.7	6.1	11.2	6.5
	H N	2.3	5.9	4.1	10.9	7.2	11.8	7.0
Flour ash (%)	**L N**	-0.03	-0.07	-0.06	-0.12	-0.04	-0.13	-0.07
	H N	-0.8	-0.08	-0.1	-0.23	-0.09	-0.19	-0.24
Gluten (%)	**L N**	1.56	3.36	3.90	5.25	2.25	5.46	3.63
	H N	2.81	4.53	5.94	7.84	3.84	7.9	5.47
Carbohydrates (%)	**L N**	2.13	4.47	5.74	6.69	3.6	8.65	5.21
	H N	1.9	3.21	6.82	5.42	5.8	6.74	4.98

Table 7. Distribution of polymorphism bands among wheat populations under high N level.

Polymorphism bands	Population. no					
	C1	C2	C3	C4	C5	C6
Monomorphic bands	5	5	5	5	5	5
Polymorphic (without unique)	5	5	9	11	6	7
Unique bands	0	0	1	1	0	5
Polymorphic + Unique	5	5	10	12	6	12
Total bands	10	10	15	17	11	17
Polymorphism %	83.33					

selection of F_3 populations for high N level were -1.5% for heading date, 7.23% for spike length, 7.95% for 1000-kernel weight and 8.81 % for grain yield.

The gain in flour yield, flour protein, gluten and carbohydrates represented approximately, 10.41, 8.0, 5.47 and 4.98% of the base population (F_2), respectively. Costa and Kronstad (1994), Preston et al. (1995) and Dencic et al. (2000) have been able to demonstrate isolated examples of success in increasing yield and protein by selecting for yield components in early generations. However, most breeders have found that the yield component selection technique is ineffective in increasing yield and protein. It is worth to mention that, the efficiency of selection for high yielding and quality among F_3 families was enhanced by growing plants under high N level. This may be due to the fact that F_3 families grown in high N environment should have had a greater opportunity to express their genetic potential (Bergman, 1998).

Effect of nitrogen application on the protein patterns using SDS-PAGE

SDS-PAGE is now the most usual procedure for separating and quantifying gluten subunits, although other methods have been developed (Benmoussa et al., 2000). The electrophorogram showing proteins banding pattern of different wheat populations are given in Figure 1. Detection of proteins whose levels are altered by nitrogen treatment was done by comparing pattern from low, moderate and high nitrogen treated plants. Protein bands detected to different molecular weight were 30 bands and ranged from 14 kDa to 116 kDa**.** Most of these bands ex-

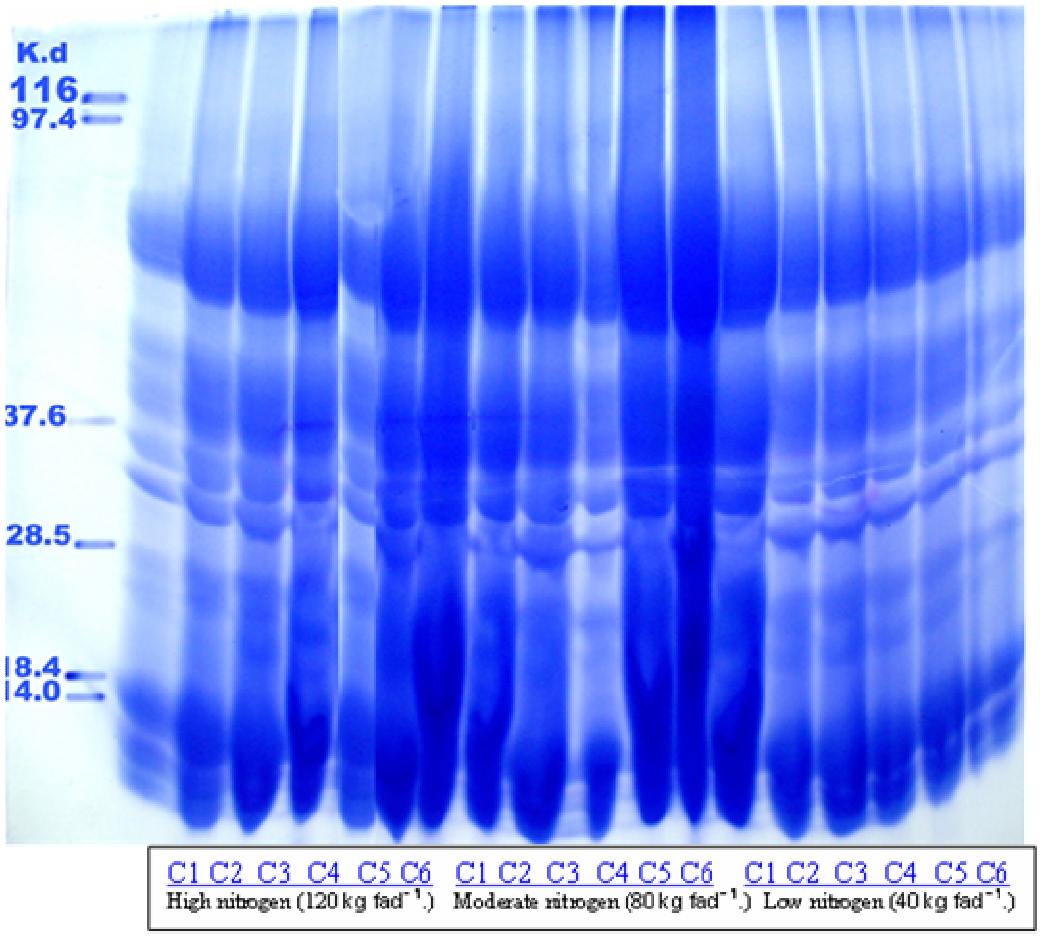

Figure 1. SDS-PAGE of the various wheat populations under three levels of nitrogen fertilizer

hibit a specific trend to high and moderate nitrogen treatments.

N generally increased total HMW-GS content in wheat grain, although different patterns of response to N rate were observed between populations. In general, total HMW-GS content increased with increasing N rate from 40 to 120 kg fad^{-1}. Luo et al. (2006) concluded that the amount of HMW-GS and LMW-GS was genetically determined and their amounts are increased by (late) N application. N application was also reported to increase HMW-GS content by 56–101% (Wieser and Seilmeier, 1998).

The analysis software for the gel together with image was used to interpret the selected populations (Table 7). The variance analysis showed that about 83.3 % of polymorphism was accounted for by the gluten subunit composition, reflecting more diversity among the selected populations. The population C6 exhibited the highest unique bands (5 from 7 bands) under high N level and followed by the population C4. Moreover, the darkest bands usually have to be set at high N level while the lightest bands noticed at low N level .This results con-firmed the major effect of N on grain quality which obtain-ed from field measurements.

Conclusions

Both agronomic characteristics of high grain yield along with information on technological quality performance are important in the development of advanced breeding lines which will potentially produce new cultivars. The data presented here shows that certain superior populations can combine the desired characteristics of the wheat breeding priorities, helping the farmer and at the same time the miller, the baker and the final consumer to have a better product. Nitrogen fertility is one of the key factors affecting wheat yield and quality. Less starch is accumulated in the grain, and higher final grain protein concentration, along with high grain yield result as a response to high N application in wheat.

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