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Biochemical tests in order to analyze durum wheat { *Triticum turgidum* L.(Thell.) conv. *durum* } single plant offsprings from a contrasted cross for quality

V. Gallia, J. C. Autran, M. F. Samson, J. David, C. Borriès, F. Kaan*

*Corresponding author: kaan@ensam.inra.fr
INRA UFR GAP and UTC, 2 Place Viala 34060 Montpellier cedex 1 France

Summary

The effect of temperature on the SDS volume test was evaluated. In the study of a contrasted cross between a genetic resource with outstanding SDS volume and a breeding line, SDS volume and the 4 heaviest SE HPLC fractions appeared heritable, protein content heritability was low in F4 offsprings. Protein content, HMW Glutenin Glu-B1 7+8 and SE HPLC F2 were found positively related with a higher SDS volume. SDS volume could be predicted through forward regression.
Keywords: SDS volume, SE HPLC fractions, glutenin electrophoresis, heritability.

Introduction

Minitests for the estimation of cooked pasta quality (firmness, elasticity, stickiness) should be a good way to appreciate the quality of durum wheat samples. However, these methods require cautious steps of semolina milling, pasta making, drying, cooking and rheological evaluation. The required weight of kernel is superior to 100 g; in contrast, breeding and genetic analyses are using single plants which yield only about 5 g of kernel. Biochemical predictive tests are simpler to realize and require smaller samples: half kernel for glutenin subunit Sodium Dodecyl Sulphate Poly-Acrylamid Gel Electrophoresis, 70 mg for protein content (Dumas method), 80 mg for Size Exclusion High Performance Liquid Chromatography of protein fractions, 6g for the classical SDS volume.

A new procedure of SDS volume test predictive of rheological value has been proposed by Peña *et al.* (1990) requiring only 1 g of kernel by sample. The first objective of this study was to assess this method in a range of laboratory temperature conditions.

In order to develop better durum cultivars for cooked pasta quality, genetic resources of *Triticum durum* (Kaan *et al.*, 1993) and *Triticum dicoccoides* (Joppa & Cantrell, 1990; Ciaffi *et al.*, 1991; Blanco *et al.*, 1995; Ciaffi *et al.*, 1995) were selected for high protein content or new glutenin subunits or high SDS volume. The second objective of this work was an attempt to study the segregation and relationships between biochemical predictors of cooked pasta quality factors in a set of recombinant F4 offsprings from a contrasted durum cross between a high quality genetic resource and a breeding line.

Material and methods

Plant material. A contrasted cross made in 1991 was investigated. The female parent was INRA 1599, a good quality breeding line of INRA program with the glutenin subunit genotype Glu-A1 HMW null, Glu-B1 HMW 7+8, Glu B3 LMW 2. The male parent was Iran 1, a genetic resource received from the Bari Germplasm Institute with the glutenin subunit genotype Glu-A1 HMW 1, Glu-B1 HMW 20, Glu B3 LMW 2. The SDS volume test of Iran 1 was outstanding. In 1994, 475 F4 seed offsprings were harvested harvested in Montpellier from individual random F3 plants belonging to 95 families from random F2 plants grown during the preceding season from a cross in 1992. These offsprings were analyzed for quality traits. The SDS volume test methodology study was performed on standard varieties for France: Cando, Ambral, Ardente, Arcour, Agathé, and lines of the preceding cross (102-3 and 33-5).
Glutenin subunit electrophoresis. Poly-Acrylamide Gel Electrophoresis was used in the presence of Sodium Dodecyl Sulphate (PAGE SDS), according to Laemmli (1970), modified by Payne & Corfield (1979) and Autran & Berrier (1984). The subunit nomenclature refers to Payne & Lawrence (1983).
Kernel protein content. Nitrogen content of about 70 mg of whole kernel flour from Cyclotec Tecator (0.5 mm sieve) was obtained after mineralization through flash combustion (Dumas AOAC 990.03) using the Fisons NA 2000 apparatus. It was then converted to protein content % of kernel (10% moisture) using a factor of 5.7.
Protein fractions through Size Exclusion High Performance Liquid Chromatography (SE HPLC). The method of Dachkevich & Autran (1989), in accordance with Bietz (1986), was used with 80 mg of whole flour on TosoHaas TSK G4000SW columns. The five main fractions were quantified. Fraction characteristics described by Dachkevich & Autran (1989) were confirmed by Bénétrix *et al.* (1994)
SDS Volume test. The method of Peña *et al.*(1990) was modified in our work as following: Eight ml of Coomassie blue R250 0.05% solution was placed in a 25 ml glass cylinder with stopper (0.2 ml graduation and 16 mm internal diameter). One g of whole meal was added. The cylinder was closed then manually shocked in order to tear off the flour from the cylinder wall. The contents was thoroughly mixed using Ika vibro-fix VF 10 vortex shaker at maximum speed during 5 sec. Then the cylinder was allowed to stand vertically for 2 min 45 sec, vortex for 3 sec, standing for 2 min., vortex for 3 sec, standing for 15 sec. Then 12 ml of a fresh working solution of lactic acid and SDS was added. The working solution was made from the mix up of the stock solution of SDS 2% w/v (480 ml) with the stock solution of lactic acid (10 ml). The stock solution of lactic acid was obtained after 2 days of gentle mixing of lactic acid and water (1:8 v/v).The cylinders were shaken with a Zeleny device for 1 min (40 cycles). The cylinders were then allowed to stand vertically for 14 min before the volume (ml) of the sediment was recorded.

Results and discussion

SDS volume method. The micromethod described was tested in comparison with the classical method (Quick & Donnelly, 1980) for the checks on 3 time series. We found the same variety classification using Duncan multiple range test (table 1). However, the recent micromethod was less precise within a time series, due to possible sampling problems. Moreover, we found an important variation between series with a coefficient of variation of about 7% for each genotype (table 1).

Table 1. *Average SDS volumes of the checks compared between 2 methods.*

	Micromethod (ml)								Classical Method (ml)	
	Exp. 1		Exp. 2		Exp. 3		Average			
	Mean	CV %	Mean	CV %	Mean	CV %	Mean	CV %	Mean	CV %
Agathé	6.4 a	1.1	7.4 a	1.0	7.6 a	2.4	7.1 a	8.3	34.5 a	2.0
Ardente	6.4 a	0.0	7.1 a	2.0	7.2 a	3.9	6.9 a	5.4	33.0 a	0.0
Arcour	6.3 a	6.7	7.1 a	0.0	7.3 a	3.8	6.9 a	7.7	33.0 a	0.0
Ambral	4.1 b	1.7	4.6 b	0.9	4.9 b	2.2	4.5 b	8.8	24.5 b	2.9
Cando	3.4 c	4.1	3.6 c	1.9	3.8 c	1.1	3.6 c	5.0	23.0 c	0.0
Mean	5.3		6.0		6.2		5.8		29.6	

Means of the same column with different letters were significantly different ($P<0.05$ Duncan test).

We inferred that environmental conditions were implied in this variation, particularly temperature as emphasized by Dick & Quick (1983) and Mc Donald (1985). Additional evaluations were made at different temperature using air conditioning from 18°C to 25°C. The regressions of SDS volume data on temperature were statistically ($P<0.05$) significant for 5 checks (>5 observations) (table 2).

Table 2. *Regression of SDS volume on temperature for different genotypes.*

Genotype	Number of Observations	Slope of Regression	Intercept	SDS (22°C)
Cando	20	-0.14	7.0	3.88
Ambral	6	-0.13	8.1	5.16
Ardente	6	-0.10	9.2	6.92
Arcour	6	-0.08	8.7	6.96
Agathé	20	-0.23	12.6	7.45
102-3	3	-0.28	16.1	9.99
33-5	3	-0.37	20.4	12.17

The slope of the regression was negative and related to the SDS adjusted at 22°C with the preceding regression procedure. If we estimate:

$$SDS = p_g * T + c_g \quad (1)$$

where SDS was the volume data, T the temperature p_g the slope of the regression for the genotype g and c_g the intercept, we can estimate a second regression between slope of (1) regression and SDS (table 2):

$$p_g = -0.0317 * SDS_{g22} + 0.0477 \quad (2)$$

statistically significant ($p<0.05$), where SDS_{g22} was the genotypic value for SDS at 22°C. From (1) and (2) we computed directly the value from SDS data:

$$SDS_{g22} = \frac{SDS - 0.0477*T + 1.0494}{1.694 - 0.0317*T}$$

After correction, the genotype variance was maintained, the replication variance divided by three, and the genotype by replication interaction became not significant.

Glutenin subunits. 468 kernels from different F4 offsprings were analyzed. The comparison with expected segregation indicated a small deficit of heterozygous possibly due to confusion between heterozygous and homozygous for Glu-A1 locus. Glu-A1 null observations were in light excess. The segregations were however essentially Mendelian.

Heritability estimates. Protein content, SDS_{g22}, SE HPLC fractions, were found quantitatively distributed. Individual variations for protein content (range 8.09 to 17.63) and SDS_{g22} (range 4.24 to 13.23) were important in the cross studied. Heritability estimates were approximated using between F3 families and residual variance estimates: Mean squares MS_F and MS_R were estimated from the model $Y_{ij} = \mu + F_i + R_{ij}$

MS_F were the between family mean squares, and MS_R the residual mean squares.

Estimation of between families variance: $\sigma^2_F = \sigma^2_B = \frac{MS_F - MS_R}{n}$ n was the average number of individuals in a family.

Estimation of residual variance = within family variance + environmental variance =

$$\sigma^2_W + \sigma^2_E = MS_R$$

In accordance with classical results (Gallais, 1990) the heritability of F3 individual families should be:

$$h^2_I = \frac{\sigma^2_B}{\sigma^2_B + \sigma^2_W + \sigma^2_E} = \frac{\sigma^2_B}{\sigma^2_B + \sigma^2_R}$$

and the heritability of F2 offsprings: $h^2_F = \frac{\sigma^2_B}{\sigma^2_B + \frac{\sigma^2_R}{n}}$

SDS_{g22} and the 4 heaviest SE HPLC fractions appear heritable and as frequently observed protein content heritability was low (table 3).

Table 3. *Estimation of variances and heritabilities for quantitative traits*

Trait	n	$MS_R = \sigma^2_R$	MS_F	$\sigma^2_F = \sigma^2_B$	h^2_I	h^2_F
SDS_{g22}	3.92	1.45	9.49	2.05	0.58	0.85
Protein content	3.67	3.97	6.76	0.76	0.17	0.41
SE HPLC F1	4.33	0.72	8.86	1.88	0.72	0.92
SE HPLC F2	4.33	0.49	5.00	1.04	0.68	0.90
SE HPLC F3	4.33	1.26	13.70	2.87	0.69	0.91
SE HPLC F4	4.33	2.13	32.82	7.09	0.76	0.94
SE HPLC F5	4.33	1.85	3.05	0.28	0.13	0.39

Relations between characters

Phenotypic correlations between quantitative traits.

Protein content, SDS_{g22}, and the 5 SE HPLC yet described F1, F2, F3, F4, F5 fractions were correlated on a sample of 45 to 52 offsprings from 12 families (table 4).

Table 4. *Correlation coefficients between SDS_{g22}, protein%, and HPLC fractions*

	Protein	F1	F2	F3	F4	F5
SDS_{g22}	+0.44**	+0.12	+0.39**	+0.21	-0.25	-0.26
Protein		-0.46**	-0.39**	+0.39**	+0.47**	-0.69**
F1			+0.49**	+0.04	-0.76**	+0.01
F2				-0.02	-0.76**	+0.19
F3					-0.36**	-0.72**
F4						-0.06

**: significant P<0.01; *: significant P<0.05

Glutenin related heavy fractions F1 and F2 were positively significantly correlated (+0.49). Both were negatively significantly correlated with smaller weight gliadin related F4 fraction (-0.76). Protein content was significantly related to all quantitative characters: positively with SDS_{g22} (+0.44), negatively with heavy fractions F1 (-0.46) and F2 (-0.39), positively with light fractions F3 (+0.39) and F4 (+0.47), negatively with F5 fraction. These results essentially confirmed Dachkevitch & Autran (1989) observations. SDS_{g22}, apart from its classical positive relationship with protein (+0.44) was only significantly positively correlated to F2 fraction associated with LMW glutenins (+ 0.39).This could be new, in contrast with Ciaffi *et al.* (1995) and Liu *et al.* (1994) who found a strong negative relation between quality predictors and SE HPLC fractions related to gliadins.

Relations between HMW glutenin alleles and quantitative traits.

No significant relation was found between kernel protein content and Glu-A1 or Glu-B1 genotypes. Some significant relations were found between HMW glutenin alleles and respectively SDS_{g22} and SE HPLC fraction 3 (table 5).

Table 5 *Relationship between glutenin alleles and traits in homozygous F4 offsprings*

Homozygous HMW Subunit	Number of F4 offsprings	SDS_{g22}	Number of F4 offsprings	SE HPLC F3
Glu-A1 null	185	9.11	29	19.62 a
Glu-A1 1	142	9.37	23	18.43 b
F probability		>0.05		0.04
Standard error		1.89		1.90
Glu-B1 20	137	8.42	20	18.36 a
Glu-B1 7+8	162	9.81	32	19.56 b
F probability		0.0001		0.02
Standard error		1.75		1.88

The absence of statistically significant relation between rare subunit Glu-A1 1 and SDS_{g22} was in contrast with Ciaffi *et al.*(1991), Blanco *et al.* (1995), Kaan *et al.* (1993) who found a positive relationship between subunit 1 from genetic resources (*T. durum*, *T. dicoccoides*) and SDS ratings. The positive relationship between Glu- B1 HMW glutenin 7+8 subunit and SDS_{g22} was moderate compared with total

variation of this cross for SDS_{g22}. It confirms however the results of Pogna *et al.* (1990) in other crosses with the same allelic segregation at Glu-B1 locus.

Prediction of SDS_{g22} using quality tests.

Forward regression method using SAS software was realized on the individual offsprings analyzed for the protein, glutenin subunit and SE HPLC. The main variable was the kernel protein content, explaining 19.3% of total SDS_{g22} variance. Among the 2 variable models, protein content and SE HPLC F2 were the best explaining combination. The third and fourth variables were the glutenin subunit 7+8 or 20 and subunit 1 or null. Until now SE HPLC was not significant but its contribution enhanced F test. We obtained the final model:

$$SDS_{g22}=-11.0+0.59*Prot\%+\begin{cases}-1.46 \text{ if Null}\\ 0.00 \text{ if 1}\end{cases}+\begin{cases}1.59 \text{ if (7+8)}\\ 0.00 \text{ if 20}\end{cases}+0.38*F1+0.38*F2$$

This explains 71.4 % of variation (coefficient of determination R^2), 36.4 % with protein %, 12.1 % with Glu-A1 allele, 11.1 % with Glu-B1 allele, 6.9 % with F1 SE HPLC fraction, 5.0 % with F2 SE HPLC fraction. If the family variable is added, Glu-A1 and Glu-B1 factors disappear because they were included in the families and F2 fraction is no more significant. The model is now explaining 84.8 % of variation (R^2) but the family factor is typically of a random type.

In conclusion, we assume that an new source of variation for high SDS volume test was found in the Iran 1 accession. The relation with glutenin subunits and HPLC fractions was not very close but in accordance with other results about the positive effects of HMW fractions analogous to Glu-A1 1 and Glu-B1 7+8 fractions. The modest contribution of HPLC fractions F1 and F2 to the prediction of high SDS offsprings partially confirmed the results of Liu *et al.*(1994), Autran *et al.*(1988) and Ciaffi *et al.*(1995).

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