Wheat flour protein content and water absorption analysis in a doubled haploid population

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Abstract

A wheat_maize induced doubled haploid population that segregates at the Awned locus for awned and awnless phenotypes were studied at two field sites using a genetic linkage map. Interval QTL analysis indicated that significant QTLs for wheat flour water absorption and protein content were located on a linkage group associated with the morphological marker, awns. The QTL peak for flour water absorption was located at the Awned locus (B1, 5AL), whilst the QTL peak for protein content was located nearby, 10.1 cm away from the Awned locus. The locations of those QTL were confirmed by analysing data from two independent field trials conducted under different environment conditions. The QTL identified for water absorption controlled 12% and 11% of the observed variance at the two field trials, whilst for flour protein content the QTL explained 7% and 19% of the variance respectively. Variance component analysis indicated that the QTL for water absorption controlled approximately 14.8–25.0% and 13.6–23% of the genetic variance at the two sites studied (Roma and imbour) whilst the QTL for protein content explained between 12.8% and 30.4% of the genetic variance at Roma and 34.7–82.6% at Jimbour. Cross-site analysis with composite interval mapping approach resulted in significant LOD values of 6.12 and 9.94 for water absorption and protein content, respectively. The QTL for water absorption was independent from the hardness locus.

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1. Introduction

Flour protein content is not only an indicator of direct nutritional value, but is also an important influence on dough rheological properties (Payne et al., 1987; Wall, 1979). It is often related to bread-making quality (Halverson and Zeleny, 1988). Good bread flour has strong gluten that is indicated by high protein quantity (Campbell et al., 2001). In addition, wheat of high protein content usually commands a premium price because it is in demand for blending with low protein wheat for the production of bread flour (Halverson and Zeleny, 1988). Water absorption is the amount of water absorbed by the flour to produce dough of workable consistency. It is determined by the protein content of the flour, the amount of starch damaged during milling and the presence of non-starch carbohydrates (Finney et al., 1987; Simmonds, 1989). It is desirable that flours for bread-making possess a high water absorption capacity at normal working consistencies so that the yield of dough, and hence bread, will be relatively high.

There are two Awned genes in bread wheat, B1 on 5AL and B2 on 6BL (McIntosh et al., 1993). A connection between the awn phenotype and yield in wheat has been recognised for some time (Bayle and Suneson, 1940; Rosenquist, 1936). Most of the previous studies have been focused on the awn phenotype ignoring the conferring locus. In general, research has centred on effects of the awn phenotype on grain yield and photosynthesis (Olugbemi et al., 1976; Weyhrich et al., 1994), largely ignoring potential effects on quality attributes. Two previous reports have indicated that awn phenotype is related to grain protein content (Ibrahim and Abo Elenein, 1977; Sharma et al., 1983). However, the results were based on direct correlation analysis between the awn phenotype and protein content. It was not clear if the awn phenotype is the direct cause of high protein content. Here, we report a linkage between the awn phenotype and both flour water absorption and protein content in wheat, which was revealed by a QTL mapping approach.

2. Materials and methods

2.1. Genetic materials

A wheat_maize induced doubled haploid population (W21MMT70_Mendos) consisting of 92 lines was studied. The parents were selected using data from four quality assessment trials from 1991 and 1992 (P. Brennan, unpublished) grown at the Wellcamp test site (Queensland, Australia), which is one of the field stations for the Leslie Research Centre (formerly the Queensland Wheat Research Institute). W21MMT70, a wheat breeding line developed in Western Australia, expressed a relatively high dough extensibility value and awn phenotype that is controlled at the B1 Awned locus on the long arm of chromosome 5A. Mendos is an awnless Australian wheat cultivar that has a relatively low extensibility value. The cross W21MMT70_Mendos was made to primarily study dough extensibility.

2.2. Field trials

Field trials of this doubled haploid population were carried out in 1996 at two sites: the Queensland Department of Primary Industry Research Station, Roma, Queensland; and a commercial farm property at Jimbour, Queensland. Both sites are located within Queensland's wheat growing region. Irrigation was applied to the Roma trial, while the Jimbour trial was without any irrigation during the entire season. A Latinised row column design was used with two replications of each entry. Each replication was planted at a plot of 1.9m row_8m bay dimension with 742 seeds, equalling the commercial seeding rate of 10_6 plants/ha.

2.3. Quality assessment

Water absorption was measured using the RACI Official Method for Physical Dough Testing (1995). All equipment was operated at 30 1C. Seven samples plus a control sample were tested each day based on a randomised sample order. Each flour sample (300 g) was tested twice and the mean value of two tests was used in further analyses. Protein content was determined using a LECO Nitrogen Determinator (FP-428, LECO Australia Pty Ltd.). Samples with extremely high or low values were subject to repeated testing (up to three times). The protein content was corrected for flour moisture content to 14% moisture basis using the equation: Protein content ¼ detected protein content_((100–14)/(100–flour moisture content)).

2.4. QTL analysis

A total of 432 markers (composed of a combination of amplified fragment length polymorphism (AFLP), rapid amplification of polymorphic DNA (RAPD), simple sequence repeat (SSR), glutenins and morphological markers) were used to construct a partial genetic linkage map for the DH population. Qgene Software (Nelson, 1995) was used to conduct interval mapping for flour water absorption and protein content based on individual field trial data with a 1 cm walking speed. Permutation tests (Churchill and Doerge, 1994) were conducted with MapManager/QT (Manly et al., 1997) to obtain empirical significance threshold values for the interval mapping. The permutation tests were conducted on a target linkage group containing the awn phenotype instead of the entire linkage map. To obtain highly significant threshold values, each permutation was performed 500 times at every 1 cM interval. The permutation tests gave three threshold values, viz: suggestive, significant and highly significant. By default, the suggestive, significant and highly significant LR thresholds corresponded to P-values of 0.1, 0.04 and 0.002, respectively. Cross-site analysis was conducted based on a mixed model approach (Zhu, 1999) using QTLMapper software (version 1.01b, Wang et al., 1999) with the background genetic variation controlled by other significant additive and epistatic QTLs (Po0.005, stepwise regression).

3. Results

3.1. Field trial data analysis

The segregation of the morphological marker Awned in the DH population fitted the 1:1 segregating pattern (Awned: Awnless ¼ 43:47, w_2 ¼ 0.17, P ¼ 0.68). The value of flour water absorption and protein content appeared to be normally distributed (Table 1 and Fig. 1). Comparing the mean values between the two trials, water absorption was significantly different between the two trials (F ¼ 6.93, $F_{0.01}$ ¼ 6.90, $F_{0.001}$ ¼ 11.21) while protein content was highly significantly different between the two field trials (F ¼ 65.85, $F_{0.001}$ ¼ 11.21). The values obtained for both traits were significantly higher at Jimbour than at Roma (Fig. 1).

Table 1
Descriptive statistics of trait data

Trial	Mean	Minimum	Maximum	Std. dev.	Skewness	Kurtosis	*P*
%H_2O							
R	59.8	54.7	65.3	2.11	0.046	−0.298	0.026
J	60.7	55.8	68.7	2.51	−0.507	0.075	0.003
%Protein							
R	12.1	9.1	15.7	1.32	0.369	−0.188	0.021
J	13.4	11.1	16.1	0.97	0.310	−0.015	0.511

R = Roma trial; J = Jimbour trial; Std. dev. = standard deviation; *P* = normality test results (Kolmogorov–Smirnov test), %H_2O = water absorption; % protein = protein content.

Variance components analysis (residual maximum likelihood, REML, Table 2) indicated that protein content were subject to large environmental influences (variance component of 37.6%) whilst water absorption was subject to medium levels of environmental influences (7.32%). Water absorption possessed relatively higher genetic variance component (47.9%) while protein content had lower genetic variance component (23.0%). Both traits had similar level of genetic by environment interactions (variance components of 11.6% and 7.7% for water absorption and protein content, respectively). The heritability of water absorption was 64.0% at Roma and 88.1% at Jimbour while that of protein content was 42.8% at Roma and 75.6% at Jimbour.

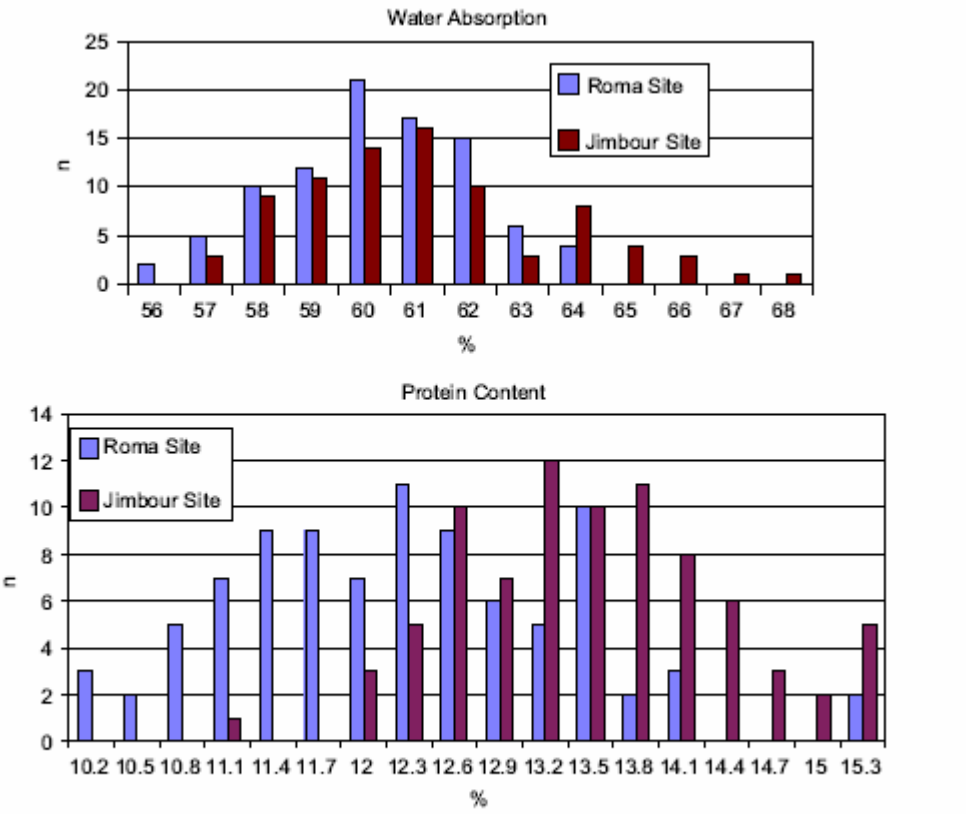

Fig. 1. Histograms of data distribution in the W21MMT70× Mendos DH population for the two traits studied.

Table 2
Variance component analysis for the two trials

Source	E	G	$G \times E$	Reps	e	$G/G \times E$
%H_2O						
Component	0.426	2.790	0.674	0	1.927	4.13*
	7.32%	47.9%	11.6%	0%	33.1%	4.49**
Std. error	0.632	0.569	0.284	0.017	0.214	
%Protein						
Component	0.8402	0.5138	0.1716	0.0001	0.7071	2.99*
	37.6%	23.0%	7.7%	0%	31.7%	3.25**
Std. error	1.1972	0.128	0.093	0.0061	0.0784	

E = environmental variance; G = genotype variance; $G \times E$ = genotype × environment variance; Reps = variance for replications in environments; e = pooled error. % H_2O = water absorption; % Protein = protein content; * = the actual $G/G \times E$ ratio of a relevant trait; ** = value using $G/G \times E$ ratio for grain yield as base line.

3.2. Linkage map construction and QTL mapping

By analysis of 432 markers among the DH population, 28 total linkage groups were constructed. The awn marker was located on a linkage group at 142 cm containing 8 AFLP markers (Fig. 2) at the Po0.01 level. The two flanking markers (paca-mctt-201, paac-mctt-188) had linkage distances of 16.7 and 10.1 cm (Kosambi function) to the Awned position, respectively.

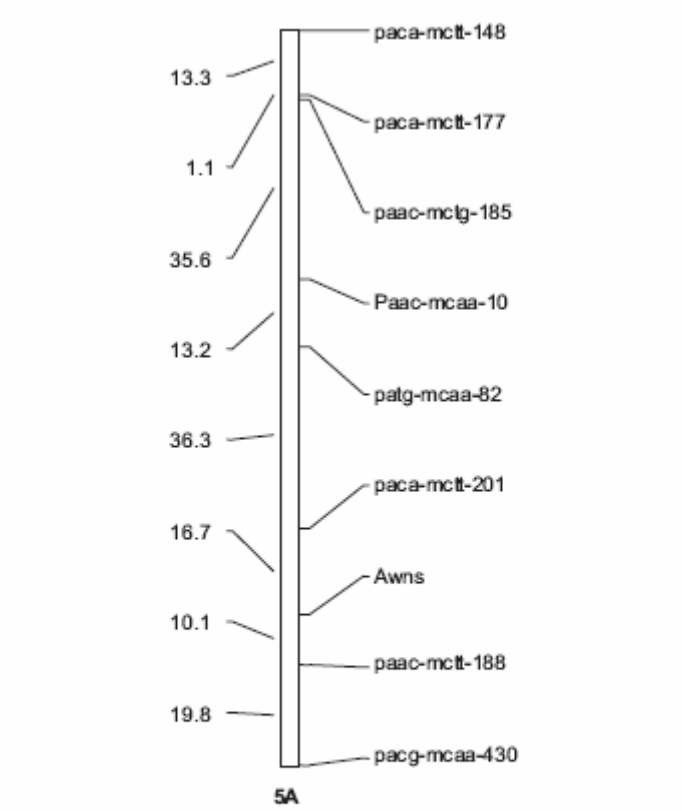

Fig. 2. Linkage groups located by Awned (the number at left side indicates linkage distance in cM between two linked markers. AFLP markers are named by their selective primer combination and fragment size, i.e. paca-mctt-148 means an AFLP fragment generated from primers Pst-ACA and Mse-CTT).

Single marker analysis indicated that the awn phenotype explained 12% of the phenotypic variance at Roma and 11% at Jimbour for water absorption. Interval analyses for water absorption using data from single environment suggested that a significant QTL was located on the target linkage group for both field trials, with the log likelihood ratio (LOD) peak located at the Awned position for both trials. The peak LOD value for the Roma trial was 2.88 and that for Jimbour was 2.29. Although these were not highly significant, based on permutation tests (highly significant LOD threshold ¼ 3.1 and 2.8 for Roma and Jimbour, respectively, corresponding to P ¼ 0.002), the two LOD peak points were well above the significant threshold value (Fig. 3, significant LOD threshold ¼ 1.5 and 1 for Roma and Jimbour, respectively, corresponding to P ¼ 0.04). Cross-site analysis (two environments, four replications, Table 3) indicated that this QTL controlled 5.27% of the total phenotypic variance with a LOD value of 6.12. No interaction was detected between this QTL and the two environments.

For protein content, single marker analysis indicated that the awn phenotype controlled 7% of the phenotypic variance at Roma (P ¼ 0.006) and 14% at Jimbour (P ¼ 5.85e-5). Interval mapping at each site revealed a QTL LOD peak located on the same region for both trials, which was located by AFLP marker paac-mctt-188 (a 188 bp product generated from AFLP primer pair Pst-AAC and Mse-CTT) and 10.1 cm away from the awn marker (Fig. 4). The peak LOD value for this QTL was 3.71 at the Jimbour site, which exceeded the highly significant threshold value (3.5, corresponding to P ¼ 0.002) and explained 19% of the phenotypic variance. At the Roma trial, this QTL had a peak LOD value of 1.66, which was significant (Po0.04) but not highly significant (Po0.002) according to permutation tests, and explained 7% of the observed variance for this trait. Cross-site analysis (Table 3) indicated that this QTL controlled 6.90% of the total phenotypic variance with a LOD value of 9.94. This QTL displayed significant interaction with environments (Q_E), which consisted of 1.7% of phenotypic variance (0.054P40.01 for both trials).

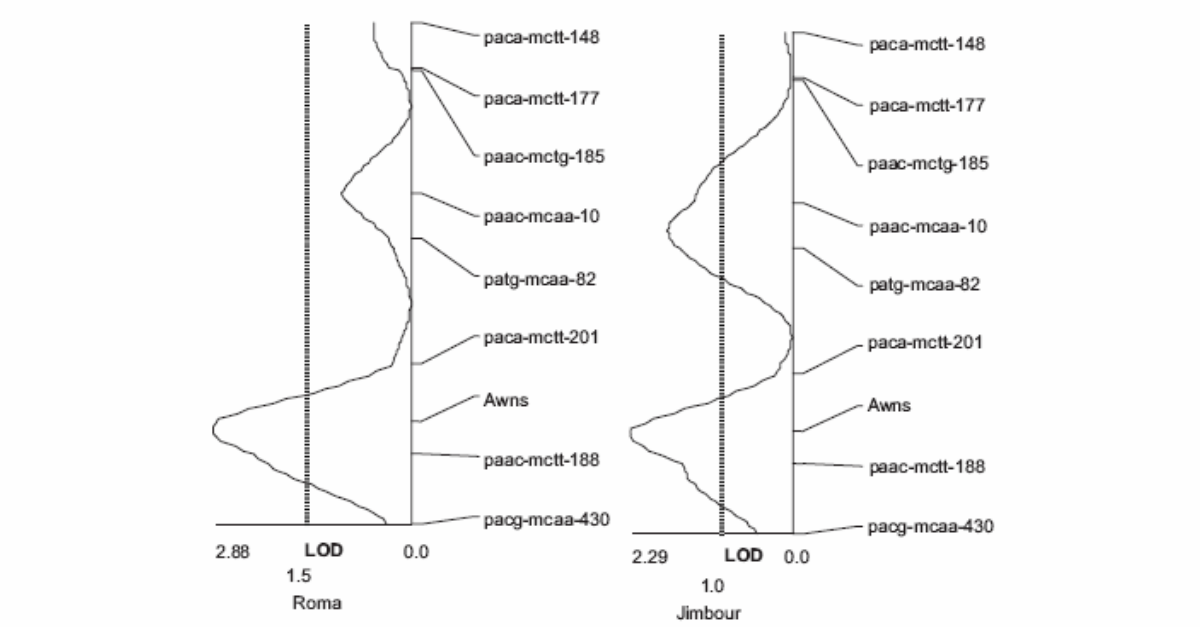

Fig. 3. Interval mapping results for flour water absorption (the empirical threshold values based on permutation tests are marked, corresponding to $P = 0.04$).

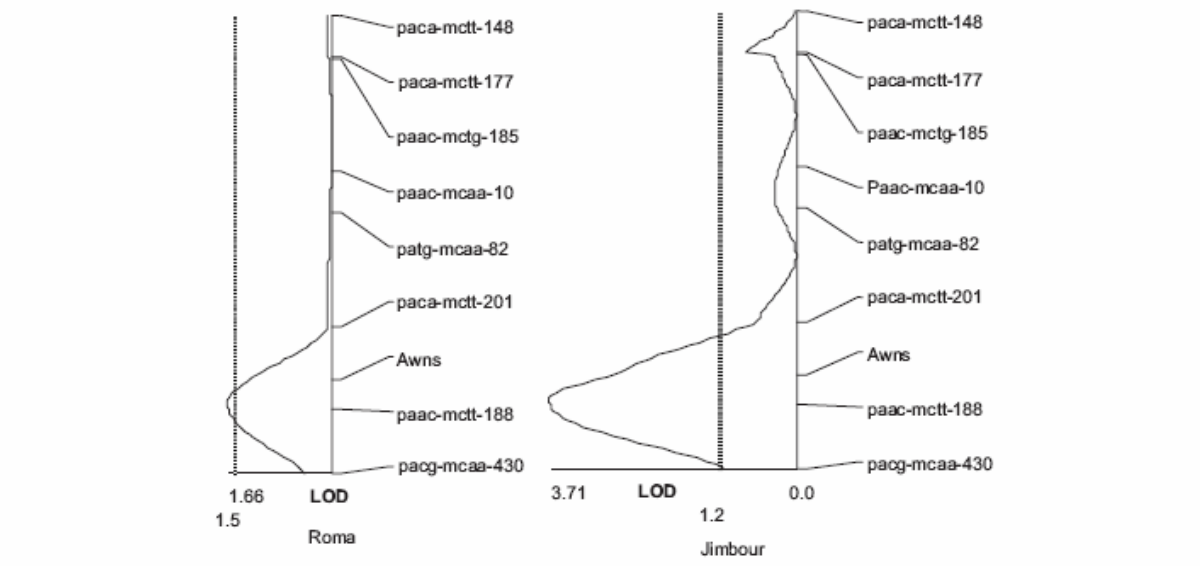

Fig. 4. Interval mapping results for protein content (the empirical threshold values based on permutation tests are marked, corresponding to $P = 0.04$).

4. Discussion

4.1. QTL analysis

In this study the genetic effects (explained variance) detected were lower for cross-site analysis than for single trial analysis. This may be because the single trial analysis did not use other significant QTLs to control genetic background, while the cross-site analysis used other additive and epistatic markers as background markers in the analysis, so that part of the genetic effects were absorbed. Nevertheless, cross-site analysis revealed higher significance (higher LOD value) than single trial analysis at both trials. The cross-site analysis provided further information for evaluating the general performance of the detected QTLs more accurately. It is worth noting that even though two widely different environments were used and several different mapping approaches were tested, the positions of the QTLs remained consistent. Many other loci were found to be linked with water absorption and protein content; however, they were only significant at one field trial. These loci were not significant against cross-site analysis.

Table 3
Cross-site analysis results for the two detected QTLs

Trait	LOD	P (a)	H^2 (a) (%)	H^2 (ae) (%)
%H_2O	6.12	<10e−4	5.27	0
%Protein	9.94	<10e−4	6.90	1.7

%H_2O = water absorption; % PROTEIN = protein content; H^2 (a) = total variance explained by the additive QTL, H^2 (ae) = total amount of the variance explained by QTL by environment interaction ($Q \times E$).

4.2. Water absorption

The analysis over all 28 linkage groups (interval mapping using data from single field trial) revealed that only one QTL for water absorption was significant across the two field trials (Po0.04), and the peak position was located on the Awned position (chromosome 5A). The QTL controlled 12% and 11% of the observed variance at Roma and Jimbour, respectively. Considering the 64.0% heritability at Roma and 88.1% at Jimbour for this trait, the awned phenotype consisted of approximately 18.75% and 12.5% of the genetic variance for water absorption at Roma and Jimbour, respectively. It is worth noting that no G_E interaction was detected with this QTL using the two very different sets of environment conditions. It is known that starch damage (related with grain hardness) and protein content are often associated with flour water absorption (Finney et al., 1987; Simmonds, 1989). Since the grain hardness is controlled by the main locus, Ha, that is located on the short arm of chromosome 5D (Symes, 1965), it is clear that the QTL at the Awned locus (5AL), identified in this study, is independent of the main hardness locus. In addition, the water absorption QTL is 10.1 cm away from the QTL associated with protein content identified in this study. As this was confirmed by two independent field trials and with various mapping approaches, it indicates that in this population water absorption is genetically linked to, but not directly, influenced by protein content.

The B1 awned locus has been co-located with growth factors in wheat including the Rht12 dwarf gene (McIntosh et al., 1993; Rebetzke and Richards, 2000). It is not clear whether the increased water absorption linked with the B1 locus is due to the physiological function of the awn, such as increased photosynthesis, or to tightly linked genes of unknown function. Nevertheless, the association between awned phenotype and flour water absorption has not been reported previously. Since the awn phenotype is perhaps the most notable phenotype in wheat and the QTL identified is a major genetic component for water absorption in this study and it is independent from grain hardness, it is expected that the current results may be valuable in spring wheat breeding programs where the awn trait is segregating at B1 locus.

4.3. Protein content

Due to the close linkage to the protein QTL detected in this study, the awned phenotype may be very useful in breeding varieties with high protein content in spring wheats when the B1 locus is segregating. Various QTL loci have been identified previously for wheat protein content. Those including loci on chromosome 2DL (Prasad et al., 1999), 5DL (Law et al., 1978), 4B, 5A, 6A, 6B, 7B (durum wheat, Blanco et al., 1996), and on 2B, 1A, 5AL, 7AL (Campbell et al., 2001). It is unclear that if there are any relationships between the main QTL for protein content identified in this study and the QTLs previously identified on 5A in Durum wheat (Blanco et al., 1996) and on 5AL in bread wheat (Campbell et al., 2001). Whilst most wheat storage proteins are encoded by group 1 chromosomes (Payne et al., 1982; Pogna et al., 1990; Singh and Shepherd, 1988), the majority of the identified loci for protein content are not located on group 1 chromosomes. The relationship between those protein content loci and the protein encoding genes is yet to be determined.

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