

PROTEINS DEPOSITION IN DEVELOPING DURUM WHEAT.
IMPLICATIONS IN TECHNOLOGICAL QUALITY.

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Durum wheat is widely considered as the best type of wheat for pasta products due to its excellent amber color and superior cooking quality. Differences in cooking quality are essentially attributed to the semolina protein content and protein quality (Feillet, 1979; 1983).

Gluten viscoelasticity - one of the most important parameter of intrinsic cooking quality of durum wheat cultivars - is associated to a particular group of proteins (gamma gliadins and LWG-glutenins) coded at locus G1B1, a multigenic family located on 1B chromosome (Autran and Berrier, 1984; Payne et al., 1984). Genotypes containing the type-45 allele (gliadin band 45 and LMW 2) have a strong gluten while those containing the type-42 allele (gliadin 42 and LMW 1) have a weak or poor gluten (Damidaux et al., 1980).

In recent works, it has been assumed that LMWG could play a primary role in determining gluten quality due to their strong aggregative properties, unlike gamma gliadins that could be genetic markers only. Autran et al. (1987) have presented evidences that the percentage of LMW in total proteins differentiates "type 45" and "type 42" genotypes: about 30% and 15% respectively. However, many aspects of the occurrence of the different wheat protein classes involved in durum wheat quality are still obscure

Although not many reports are available on proteins biosynthesis in durum wheat, it can be suggested that the way of synthesis and accumulation of the protein subunits might lead to a more or less aggregated state, which could directly influence the technological characteristics of semolina and pasta products.

Therefore, further investigations are needed on the temporal deposition and the aggregative behaviour of proteins during the grain development, in relationship to the nitrogen metabolism in the durum wheat plant. These may be of relevance to our understanding of the biochemical and physiological basis of variability in the expression of technological quality between (or within) the two main genetic classes: "type 42" and "type 45".

In the present paper, we are reporting the changes observed in several aspects of protein composition at various stages of grain development of five durum wheat cultivars, and we are discussing about the possible relationship of nitrogen metabolism to some physico-chemical basis of quality.

MATERIAL AND METHODS

Plant material

Plant material consisted in five durum wheat cultivars grown in Roma Experimental Station during 1984, 1985 and 1986 years, including four belonging to the genetic type "gamma-gliadin 45" and one to the genetic type "gamma-gliadin 42" (Fig. 1), the quality characteristics of which are shown on Table I.

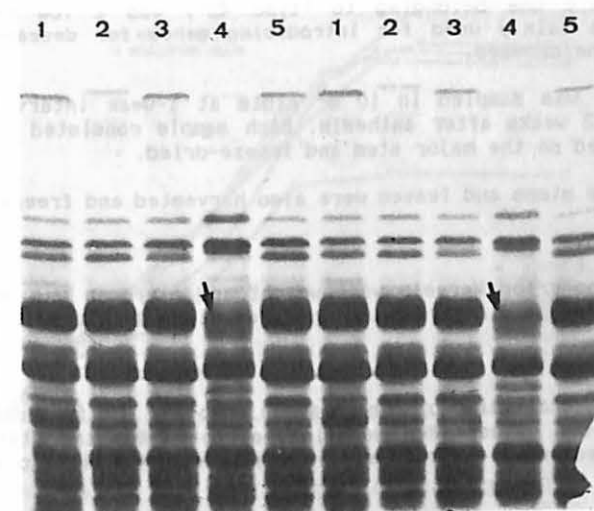


Fig. 1 : Electrophoretic patterns (SDS-PAGE) of the total reduced proteins of the 5 cultivars : (1) Trinakria; (2) Valfiora; (3) Valnova; (4) Lakota; (5) Tangarog. The arrow indicates the presence of glutenin LMW1 associated to genetic type "42" in cv. Lakota. The four other patterns contain glutenin LMW2 associated to type "45".

Cultivar	Genetic type	Grain weight	Protein content	Gluten elastic recovery	Pasta cooking quality
TRINAKRIA	45	44.3	16.1	1.81	+++
VALFIORA	45	43.5	14.0	1.85	+++
VALNOVA	45	43.0	15.4	1.84	++
LAKOTA	42	29.5	13.3	1.24	+
TANGAROG	45	49.0	13.7	1.68	++

Tab. 1 : Quality characteristics of the five cultivars.

- Trinakria and Valflora were high pasta quality cultivars with low agronomical yield

- Valnova was a medium pasta quality cultivar with very high agronomical yield

- Tangarog was a high-gluten type cultivar, which has been imported from Argentina for a long time and which may have a totally different behaviour when grown in Europe

- Lakota (the only one belonging to "type 42") was a low pasta quality cultivar which is mainly used for introducing genes for disease resistance (black rust) in the crosses.

Each wheat was sampled in 10 m² plots at 1-week intervals generally commencing about 2 weeks after anthesis. Each sample consisted in the grains of 5 ears harvested on the major stem and freeze-dried.

In some cases stems and leaves were also harvested and freeze-dried.

Analytical methods:

Fresh and dried kernels were weight and nitrogen was determined by Kjeldahl method. The same analyses were made on stems and leaves.

Milling and pasta making:

Due to the small size of the immature samples, the freeze-dried grains were mill into flour rather than semolina, excepted for the standard mature sample that has been usually processed in a laboratory mill into semolina and then into pasta.

Gluten was also extracted and assessed for viscoelastic properties using a Viscoelastograph according to Damidaux and Feillet (1978).

Electrophoretic fractionations:

Ethanol-soluble proteins were fractionated in acidic PAGE, aluminium lactate buffer, pH 3.2, according to Bushuk and Zillman (1978)

Total reduced proteins were fractionated in SDS-PAGE, Tris-glycine buffer, pH 8.4, according to Payne and Corfield (1979).

Protein composition by differential solubility:

The major proteic fractions were determined by sequentially extracting 500 mg of flour in the following solvents:

- 1) NaCl 0.5 M (2 x 1 hour)
- 2) Ethanol 70 % (2 x 1 hour)
- 3) Propanol-1 50 % + 2 % Mercapto-ethanol (1 hour).
- 4) Acetic acid 0.1 % (1hour)

Protein content was determined by Kjeldahl method on pooled supernatants and on residue.

RESULTS AND DISCUSSION

The evolution in dry weight per grain during ripening is shown on Fig. 2

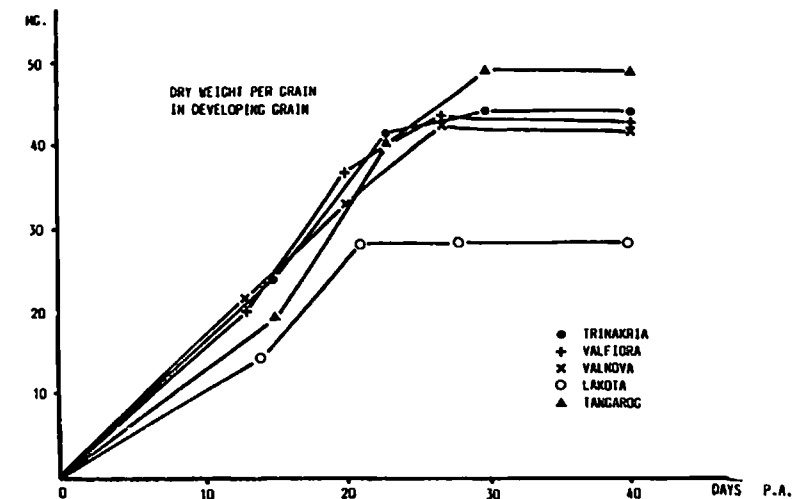


Fig. 2 : Dry weight (in mg.) per grain in developing grain.

It confirmed the rapid increase always observed during the first three or four weeks after flowering, followed, in the last phase, by a decline in the rate of increase of the dry weight that coincided with a rapid loss of water. It could be noticed that in cv. Lakota, the increase stopped earlier (20 days post anthesis) than in other cvs. such as Trinakria or Tangarok (30 days p.a.). Since the rate of the grain increase was similar for the different cultivars it seemed that a higher dry weight of the mature grain could be determined by a longer duration of the phase of rapid increase, although an alternative explanation could only involve the number of endosperm cells that are present at the end of the cell division phase.

The evolution of the protein content on a dry-weight basis is shown on Fig. 3. The protein content was relatively constant during the early stages when dry weight increases rapidly. Thereafter, the protein content increased steadily through to maturity with little difference between cultivars, excepted for cv. Lakota that caught up with the others at maturity only but after keeping lower values until the 34th day p.a., what confirmed the behaviour generally observed for "type 42" durum wheat cultivars (Grignac, personal communication).

However, when expressed as the amount of protein in 100 g of seed (like in Fig. 3), the protein content reflected not just the effects of genotype and/or environment upon the synthesis and accumulation of proteins, but also upon other substances among which starch is of a primary importance (the amount of starch strongly conditions the percentage value to be attributed to

a given amount of protein). For these reasons, several authors like Johnson et al. (1967) or Favret et al. (1970) suggested that protein content should be expressed as the amount in one seed, not as the percentage of seed weight.

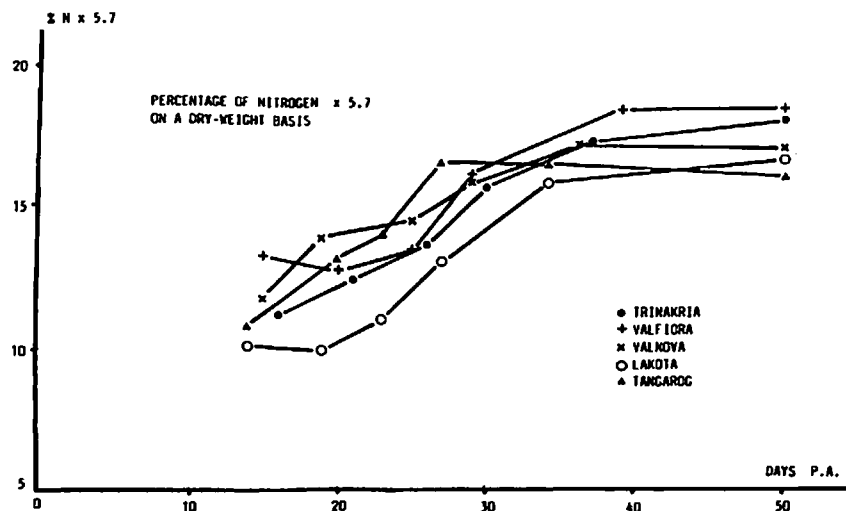


Fig. 3 : Percentage of nitrogen $\times 5.7$ on a dry weight basis in developing grain.

This evolution of the amount of protein (nitrogen $\times 5.7$) on a grain basis is shown on Fig 4. This time, a strong difference could be noted in the protein accumulation between cv. Lakota and all others.

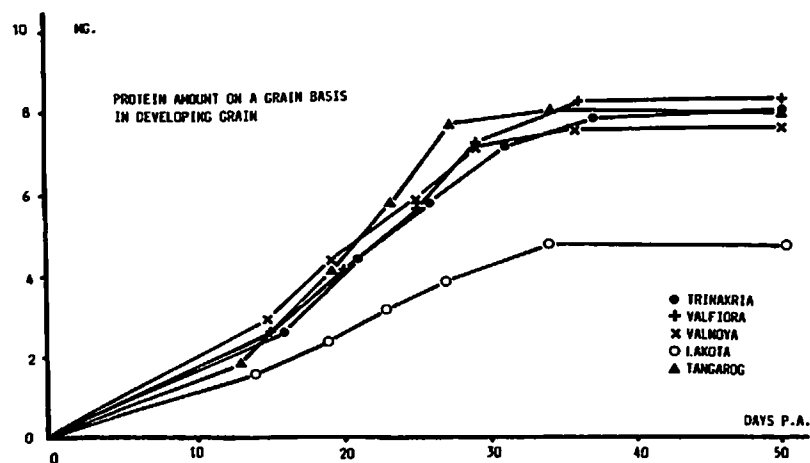


Fig. 4 : Protein amount (in mg.) on a grain basis in developing grain.

Generally speaking, differences in grain nitrogen evolution may be due: 1) to a different uptake in N or a different duration of the uptake phase or, 2) to a more or less efficient translocation of N from the vegetative parts to the grain (Johnson and Mattern, 1978) or, 3) to a distribution of the same amount of N to different amounts of dry matter (Nair and Abrol, 1979) or, 4) to more or less efficient enzymes in protein synthesis in the grain (Johnson et al., 1967).

It has been emphasized (Abrol et al., 1984) that the relative importance of these factors may vary in different genotypes and environments.

In the first analysis, unlike cvs Tangarog and Valflora which have a relatively low grain protein content and a high kernel weight, cv. Lakota marked off from others both by its small grain and a protein accumulation shifted to lower rates.

In order to go further into the understanding of these differences, fractionations of the proteins by electrophoresis and by differential solubility were undertaken, keeping in mind that much care must be exercised in interpreting comparative solubility data (for instance, freeze-drying can modify solubility or aggregative characteristics).

A characterization of ethanol-soluble protein extracts in PAGE (results not shown) indicated that gliadin bands appeared as soon as the 8th day after anthesis, which is in agreement of previous works on bread wheat and on durum wheat (Mecham et al., 1981 ; Tercé-Laforgue and Pernollet, 1982 ; Dell'Aquila et al., 1983). The patterns were nearly identical from the 12th day after anthesis to the end of grain development, with the exception of two omega gliadin bands (33-35) the ratio of which was progressively changing when maturity approached.

On the other hand, a characterization of total reduced storage proteins in SDS-PAGE (results not shown) indicated that bands in the gliadins and LMW-glutenins groups were present as soon as the 8th day after anthesis. HMW-glutenins began to appear at the 12th day only and were entirely present at the 14th day. Thereafter, the whole material increased in band intensity but with very little qualitative change through to maturity, confirming that within protein groups, the proteins are synchronously synthesized (Khan and Bushuk, 1976; Pernollet, 1985).

These experiments, however, gave information on the occurrence of storage polypeptides or subunits but not on aggregates or protein complexes. In order to get a better understanding of the kinetic of protein complexes formation, further studies have been carried out from sequential extraction.

Fig. 5 illustrates the evolution of the percentages of the salt-soluble, ethanol-soluble, isopropanol+ME-soluble, acetic acid-soluble and residue, at five times of grain development, in the case of cv. Trinakria.

Broadly speaking, salt-soluble fractions correspond to metabolic proteins (albumins and globulins) and also includes non protein nitrogen. On the other hand, ethanol-soluble (that we shall refer to as "gliadins", although we know that they contain a significant amount of partially aggregated LMW-glutenin-type material), isopropanol + mercaptoethanol-soluble glutenins and acetic acid-soluble glutenins correspond to other storage proteins whilst the residue could comprise both storage and structural (membranes) fractions.

As expected, it can be observed a marked decrease of the salt-soluble fractions, particularly in the first four weeks. A remarkable rise of the ethanol-soluble storage fraction was also noted until the 27th day p.a., after what this fraction reached a maximum and slightly declines, unlike the two next glutenin fractions (storage proteins too) that steadily increased through to maturity. Concerning the most insoluble glutenins (residue), their ratios were high at the beginning and at the end of development, but minimal around the 27th day p.a.

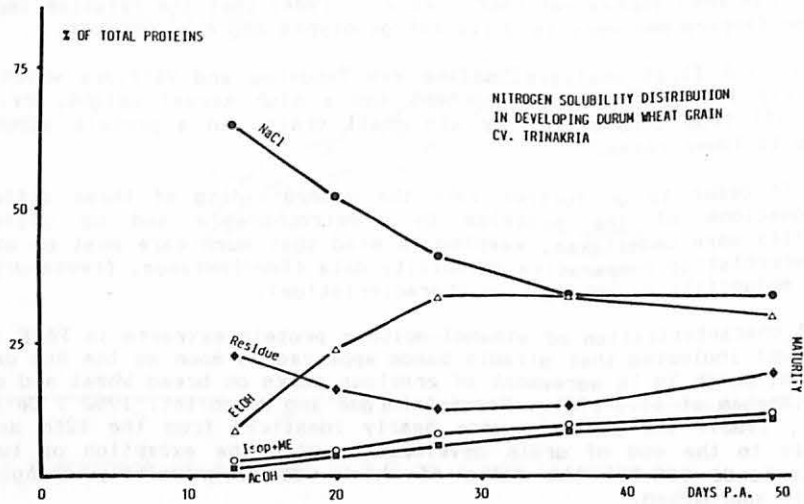


Fig. 5 : Nitrogen solubility distribution (% of total proteins) in developing durum wheat grain cv. Trinakria.

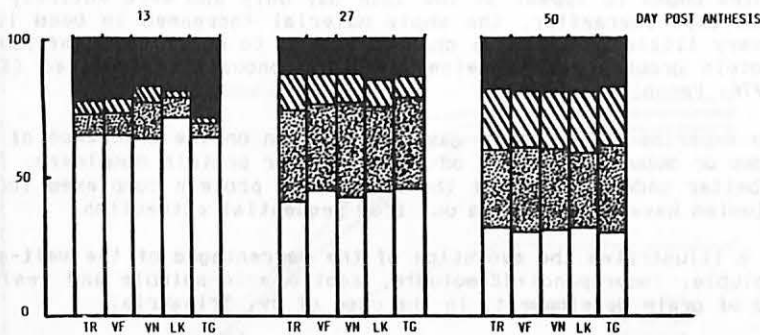


Fig. 6 : Proportions of total grain proteins as : salt-soluble (open), ethanol-soluble (stippled), isopropanol+ME- plus acetic acid-soluble (cross hatched) and residue (solid column) in developing grain.

When expressed in percentage of the total proteins, similar evolutions (decrease in salt-soluble fractions, increase in gliadins, decrease and then increase of residual fractions), with little varietal differences, were observed (Fig. 6).

More interesting differences, however, were noticed when the protein fractions were expressed as milligrams per grain.

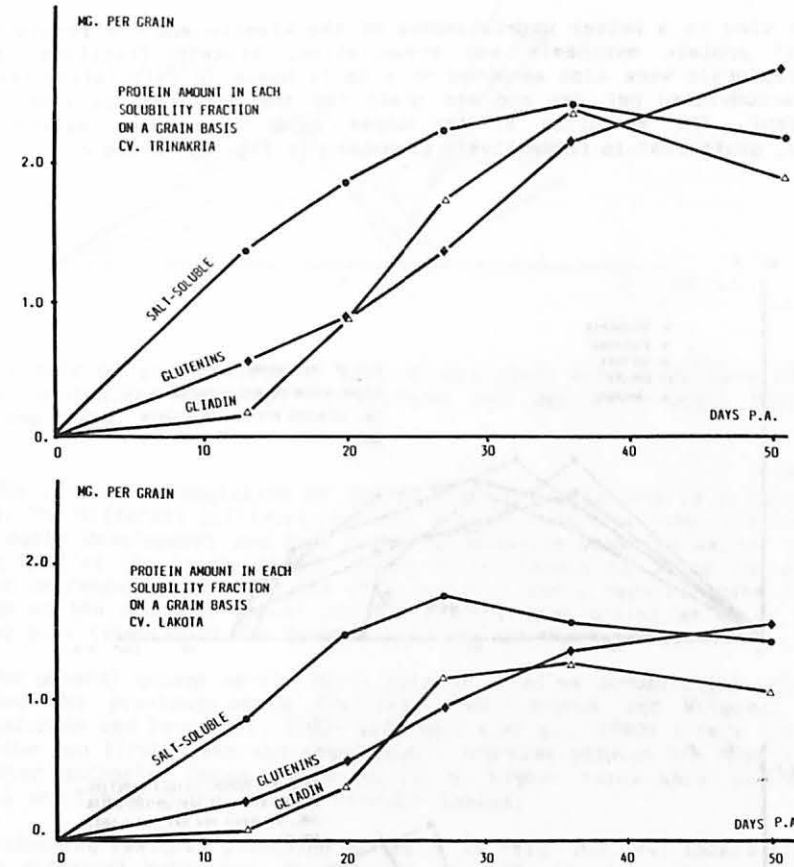


Fig. 7 : Protein amount (mg. nitrogen x 5.7) in each solubility fraction on a grain basis in developing grain : cv. Trinakria (7a) and cv. Lakota (7b).

In cv. Trinakria (Fig. 7a), for example, salt-soluble fractions were accumulated until the 36th day p.a., while in cv. Lakota (Fig. 7b) the maximum was already reached on the 27th day and followed by a marked decrease. Also, in cv. Trinakria, ethanol-soluble proteins were accumulated at a high rate until the 36th day and then markedly decline, while, in cv. Lakota, both

gluten proteins (gliadins and glutenins) were accumulated at a lower rate and during a shorter time

It is particularly important to notice that the loss of gliadins during the two last weeks before maturity coincides with an increase of the glutenins (essentially residual glutenins), as previously observed by Dexter and Matsuo (1977). These complementary phenomena were particularly obvious in cv. Trinakria but much attenuated in cv. Lakota.

In view to a better understanding of the kinetic and the physiological basis of protein synthesis and accumulation, protein fractions in the developing grain were also examined on a daily basis in calculating the mean amount accumulated per day and per grain for the different periods of the development. The evolution of the three major fractions (salt-soluble, gliadins, glutenins) is respectively presented in Fig. 8a, b and c.

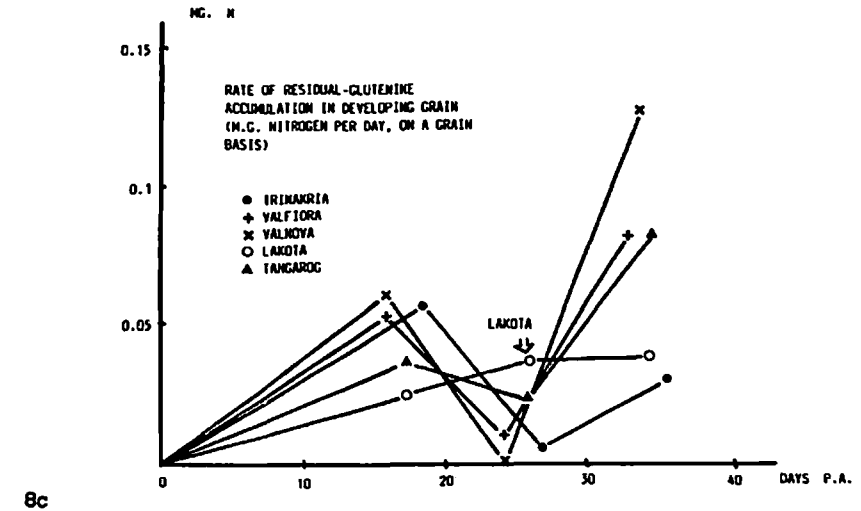
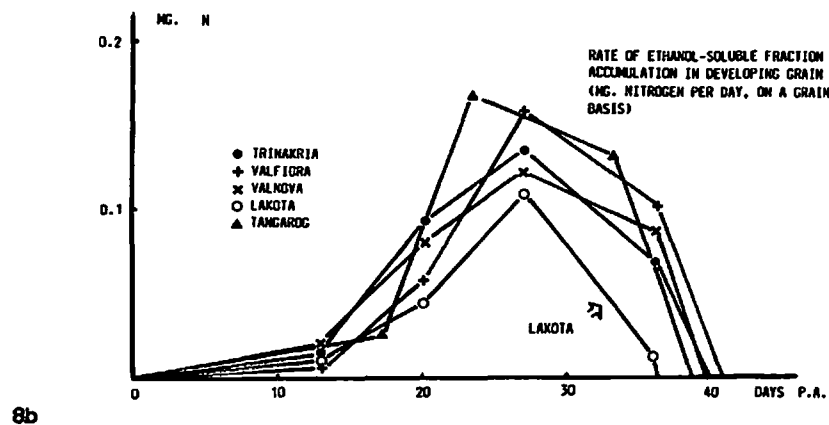
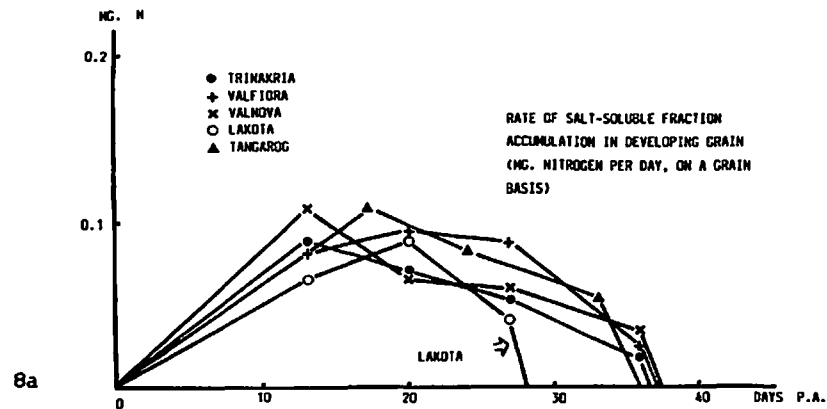


Fig. 8 : Rate of accumulation of salt-soluble (8a), ethanol-soluble (8b) and residual glutamine (8c) (mg. of nitrogen per day on a grain basis) in developing grain.

The rate of accumulation of the salt-soluble fractions is presented on Fig. 8a. The different cultivars showed a similar evolution (the rate was high in the early development and they cease accumulating when the kernel reached about a half of its final weight), excepted cv. Lakota for which there was a dramatic decrease as early as the 20th day p.a. and a negative rate (i.e. a decrease of the absolute amount of this fraction per grain) as early as the 28th day p.a. (instead of the 36-38th days for all the other cultivars).

The general scheme of the daily rate of gliadins accumulation (Fig. 8b) confirmed the previous works (Fellet, 1965; Bushuk and Wrigley, 1971; Tercé-Laforgue and Pernollet, 1982; Dell'Aquila et al., 1983) : very low level during the two first weeks and considerable increase between the days 17th to 27th after anthesis. Among the 5 cultivars, higher rates were noticed for Valfiora and Tangarog and a lower rate for Lakota.

Concerning residual glutenins accumulation (Fig. 8c), cv. Lakota also had a quite different behaviour. As a matter of fact, the four other cultivars, showed three clearly separated phases during the development:

1) A rapid synthesis (from anthesis to the 16th day p.a.) that coincides with the accumulation of the salt-soluble nitrogen and that could correspond to the formation of the structural fractions (membrane proteins ?) of the glutenin-type proteins.

2) A rapid decrease (17th-24th day) which could be explained by the fact that the developing grain had satisfied its requirements for cytoplasmic and structural proteins, most of the additional nitrogen being then channelled into the synthesis of the gliadin-type proteins.

3) Another rapid increase (25th-35th day), that could mean a synthesis of new storage proteins, or more probably (since this phase coincides with the decrease of the ethanol-soluble nitrogen) an internal rehandling, within the storage proteins. A part of the ethanol-soluble proteins could undergo some change in solubility during the drying phases of the development, perhaps giving rise to more highly aggregated fractions and making them belonging to a glutenin-type complex.

Conversely, cv. Lakota showed a lower and a steady rate of accumulation of the most insoluble glutenins until the 26th day p.a. and almost no increase thereafter, either as if there was no or little occurrence of new highly aggregated fractions, or as if there was no or little conversion process of the ethanol-soluble fractions into the residual one.

Complementary informations on the nitrogen metabolism in relationship to proteins deposition in the grain were obtained from the study of the nitrogen levels in the vegetative parts of the wheat plant.

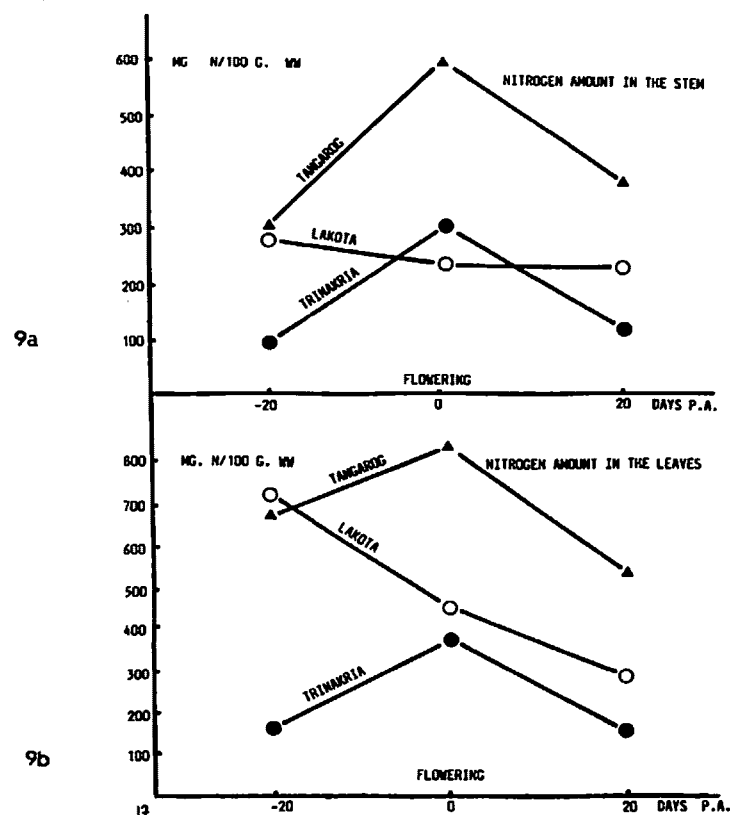


Fig. 9 : Nitrogen amount (mg. in 100g., wet basis) in the stem (9a) and in the leaves (9b) in the developing plant.

In the leaves of cvs. such as Trinakria or Tangarog (Fig. 9a), the nitrogen level showed an increase in the pre-flowering phase until a maximum and then declined during the grain development. A similar kinetic was observed in the stem of these cvs. (Fig. 9b).

Conversely, in the case of cv. Lakota, no maximum amount of leave or stem nitrogen was evidenced: the nitrogen level, relatively high at the beginning, steadily decreased, what could be interpreted as both a poor nitrogen uptake in the leaves and a weak ability to nitrogen translocation from the vegetative parts to the grain.

This result was consistent with the observation of an early decline of the metabolic (salt-soluble) nitrogen in the cv. Lakota. Hence, all these results go to prove an early decrease of the metabolic activity in this cv. and an even early death of the plant, from which it would result a less efficient accumulation process of the storage fractions during the last periods of grain development.

GENERAL DISCUSSION

In this work on durum wheat grain development, we have confirmed that salt-soluble proteins were made earlier than the others and, owing to an expression of the results in nitrogen synthesized per day on a grain basis, we could be able to show that they decreased to about half at maturity, indicating some turnover in this fraction. On the other hand, gliadins, as may be expected from their storage function, were present in significant amounts only after two weeks after anthesis and were deposited in considerable amounts during the next two weeks. In the last phase, a decrease of ethanol-fractions expressed on a grain basis occurred concomitantly to an increase of the most insoluble glutenin fractions, which is of particular importance since the aggregation state of the proteins (presence of long linear polymers) is considered to influence directly the viscoelastic properties of gluten. This would agree with results reported by Dexter and Matsuo () who found an improvement of pasta cooking quality when maturation proceeds.

However, a very important varietal difference was evidenced in the kinetic of accumulation of the different protein fractions, including metabolic fractions, between cv. Lakota - the only one belonging to "type 42" - and the others which belong to "type 45", so that it could be wondered if a peculiar nitrogen metabolism could not be involved in the origine of the different physico-chemical characteristics of cv. Lakota proteins, resulting in a poorer cooking quality potential.

As recently reviewed by Porceddu et al. (1983) and Simmonds and O'Brien (1981), it is largely accepted that once fertilization has occurred, both the newly synthesized amino acids and those derived from the hydrolysis of proteins from different parts of the plant migrate towards the developing seeds in which the genes specifying the synthesis of storage proteins are transcribed and translated in addition to those governing the synthesis of metabolic proteins.

Little is known, however, about the changes that wheat storage proteins undergo after their synthesis. It is considered that monomeric units of proteins are synthesized on the rough endoplasmic reticulum, pass into the lumen and then migrate to their site of deposition in the protein bodies (Miflin et al., 1983). But the condensation level that affects the proteins according to the stage of grain development is controversial: John and

Carnegie (1971) assumed that inter-protein disulfide bonding occurred during the drying out of the grain only, while Miflin et al. (1983) found very high molecular weight aggregates in the protein bodies with a molecular weight distribution similar as in the gluten obtained from the mature seed. On the other hand, Pernollet (1985) emphasized the physical forces occurring during the growth of starch granules and during the grain drying, that might originate for protein bodies membrane disruption and for building up new associations between proteins or between proteins and other constituents.

Whatever it may be, when maturation proceeds, we think that the composition of such aggregates is likely to evolve from predominantly structural complexes in the first days of grain development to predominantly storage complexes. From solubility studies that clearly showed a decrease in ethanol-soluble material coming with an increase of the residual fraction during the two last weeks before maturity, we would agree with a flexible conversion process turning the initially low size fractions into highly aggregated complexes. Of particular importance, therefore, would be those factors (climate, temperature, humidity) that affect the kinetic of grain drying. At this stage taking place immediately before maturity, this could largely influence the aggregation process and would have a great importance in determining the phenotypic quality within a given genotype (i.e. from a given pool of monomeric proteins or subunits).

Moreover, a such process is likely to have a varietal basis. If environmental factors only were involved, a cultivar like Lakota, grown in the same conditions than the others and the grains of which dry out and cease their synthetic activity earlier, would have a higher (or at least equal) ratio of protein insolubilization or aggregation, which is not the case. Considering that a conversion process would occur at the end of the grain development, it is likely to be influenced by the protein biosynthesis and turn over at preceding stages, especially in some cultivars having a very peculiar nitrogen metabolism like cv. Lakota, type 42.

When several durum wheat genotypes were compared, the differences in intrinsic quality had been first attributed to the presence of different alleles (such as allele 42 or allele 45), assuming that each allele might code for proteins having different functional properties (Autran, 1981) or, more recently, for different amounts of aggregative (LMW-glutenins) proteins (Autran et al., 1987). From our present results, an alternative explanation of the lower gluten quality of type 42 cultivars would involve the nitrogen metabolism (nitrogen translocation) before flowering and during the first part of grain development of these types of cultivars. A mechanism associated to an earlier decrease in the synthesis and that remains to be explained (absence of certain active enzymes? deficiency in some metabolic system?) could impair the ratio of final conversion of the polypeptides into large aggregates and would be a causative factor of a poor intrinsic quality (without necessarily altering the protein content of the grain: Lakota has a protein content similar to Tangarok or Valflora).

In the future, a larger number of cultivars should be investigated in view to a better understanding of the physico-chemical basis of wheats quality. It seems essential to focus on an accurate estimation of the aggregation level of proteins (eventually in association to other components like lipids) which is likely to be a key of their functional properties in gluten or in pasta. Electrophoretic techniques that involve a disruption of the protein complexes are certainly not suitable, while SE-HPLC techniques are much recommended for this purpose. Dynamical approaches like the study of protein aggregates, at different physiological states and in connection to a study of the ribosomal and total RNA levels should give basic insights in the understanding of wheats quality and allow its improvement through genetics and breeding.

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