To Explore and Improve the Industrial Use of EC Wheats

NEWSLETTER N° 1

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This first newsletter reports the activities of the ECLAIR programme 'To Explore and Improve the Industrial Quality of EC Wheats' up to June 1991, i.e. six months after the official starting date.

It comprises the reports of each subprogramme: A, B and C:

A - Industrial Processes, by Dr. R.J. Hamer
B - Functional Components and their Interactions, by Dr. J.J. Plijter
C - Biochemical-Genetics and Physiology, by Dr. N.E. Pogna.

In each section, a synthesis was made from individual progress reports prepared by each contributor, and was presented on either a task basis (A), or a partner basis (B and C).

In addition, this newsletter comprises a synthesis of the first answers to questionnaires prepared in each subprogramme: methods and equipments used for technological analyses (Annex I), list of the Southern Europe Network or North-Western Europe Network samples (subprogramme C) required by the industries or laboratories (Annex II), lists of 'available' samples (Annex III) and of 'wanted' samples (Annex IV) from subprogramme A laboratories.

**Administrative aspects:**

The work during this first period was greatly influenced by the delay in finalising the Contracts. To date all participants have started work and it can be said that the whole programme is progressing in a sound way. However, various local situations still exist due to different starting dates. Certain partners could advance funds and appoint people to work on the programme before the Contracts are signed. In particular, a warm acknowledgement must be given to TNO Organization, and to most participants in subprogramme C for having started work earlier. The latter held their first meeting as soon as October 1990 to prepare the sowings, so that wheat samples grown in highly controlled conditions can be made available in the 1991 harvest, avoiding thereby to other participants to lose one working year.

While many partners have already been able to report first results (see below the individual progress reports), some others could not start working before the official signing of the Contracts, so that their work is still in a preparatory phase, resulting in some slippages from the agreed schedule. How to manage with these slippages on some tasks, as far as work planning charts (interdependence of tasks) and date of production of the final report are concerned, will be discussed with the EC representative at the next meeting of the Scientific Management Committee in next September.

**Scientific aspects:**

Despite of the delay in contract negotiations that hampered some of the tasks, many promising results have already been reported. As an example:

- Development of a new image analysis method in milling studies,
- Measurement of work input requirements of wheat varieties (white bread and wholemeal bread) and flour
blends,
- Demonstration of the strong influence of low molecular weight wheat proteins in the formation of gelproteins in connection with rheological properties of the dough,
- Purification and characterization of the aggregative properties of gluten subfractions, especially LMW subunits of glutenin, and demonstration of their contribution to the French baking score,
- Development of a sequential extraction of wheat proteins based on Triton X114 phase partitioning and discovery of homologies between phospholipid transfer proteins,
- Examination of the role of 'friabilin' in a new context of homology with phospholipid binding proteins,
- Successful organisation of Southern Europe and North-Western Europe networks for producing samples required by Industrial and Laboratories,
- Production of lines and near-isogenic lines with different HMW, LMW, and gliadin compositions,
- Selection of dormant and non-dormant wheat types, and development of a bio-assay testing.

In addition, the scientific programme was more accurately described in a new version of the ‘Technical Annex’, completed by charts specifying Work Planning, Schedule, and Interdependence of Tasks.

Also, each subprogramme has organized fruitful meetings in which important resolutions were passed about coordination, collaboration with other subprogrammes, establishment of an inventory book on all technological, rheological and biochemical methods used, and on standardization of storage and handling of samples.

The problem of wheat samples is of capital importance and is worth thinking over:

As a matter of fact, the success of the whole programme largely depends on the choice of the wheat samples, the control of their growing conditions, their adequacy to the problems under study in each task, the use of common samples for some studies. It is essential, therefore, that each participant can be permanently informed of the availability of samples and can apply for specific cultivars or lines when necessary.

The technological evaluation of samples is a critical and essential point. It is critical because technological tests cost money, especially those performed at a pilot or an industrial scale. These cannot be performed undiscriminatingly on all available samples. It is an essential point because a major objective of our ECLAIIR programme is the stimulation of breeding and development of wheats capable of satisfying the present and future technologies of both Northern and Southern Europe. In contrast with most of the previous studies that considered the white flour breadmaking as their unique model, the study of the physicochemical bases of new technologies (biscuit-making, wholemeal breadmaking, separation starch/gluten through the use of enzymes, sour dough, etc.) is a particularly innovative element of the programme. The complete strategy of the programme will clearly appear in the fact that (i) biochemical tests (subprogramme B) will have to be calibrated not only on the basis of conventional breadmaking quality, but also with regard to new technologies investigated in subprogramme A. On the other hand (ii), it is crucial that, when new technologies are investigated, the samples (e.g. those produced in subprogramme C) be evaluated by both new and conventional tests.

A specific meeting of the participants to subprogramme C, including all industrial partners, scheduled on September 17 will be devoted to this problem of sample evaluation.

**Next progress report**

As indicated in the management section of the Technical Annex, the first annual report will have to be produced by the end of the first working year, i.e. December 31, 1991. All participants will be requested to
prepare their own contribution (to be sent to their subprogramme Manager) by November 15, 1991.

**General meeting of the programme**

A general meeting of the participants will be organized during the week of the 9th Wheat and Bread Congress in Paris (possibly on June 4, 1992). Such a meeting will be an unique opportunity to bring together representatives of all partners of the Contract, to present some important results already obtained, and to make a synthesis of the overall progress.
REVIEW OF ACTIVITIES

The period up to June 1991 was greatly influenced by the fact that contract negotiations continued. Also, the initial agreement to unofficially allow the work to start in September 1990 was revoked. As a consequence, the different activities of subprogramme A started at different times. In order to establish coordination and collaboration a first meeting of all participants was held in Wageningen, the Netherlands on March 11 and 12. This meeting was a great success for two reasons. First, all participants of the subprogramme were present at the meeting. Second, many resolutions were passed concerning coordinative and collaborative actions. The meeting was also attended by the programmes coordinator. At the meeting, an update was given of the different activities, including starting dates. The latter is summarized in the following table:

<table>
<thead>
<tr>
<th>project</th>
<th>participant</th>
<th>started at</th>
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<td>A.1.1 Milling quality</td>
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<td>1/91</td>
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<td>9/90</td>
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<td>1/91</td>
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<td>A.2.1 White bread</td>
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<td>A.2.5 Rheol/sweet bakery products</td>
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<td>A.2.6 Microorganisms</td>
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During the meeting in Wageningen resolutions were passed to establish a joint catalogue of wheat samples, to standardize storage and handling of samples and to document and standardize where possible the analytical and technological methods used. To ensure collaboration with subprogramme C it was decided to establish a so-called wanted samples list. This list describes the wheat samples needed in subprogramme A in the near future (e.g. the next year programme). Good progress has been made on these activities. To date the inventories of technological and rheological methods are ready. The Analytical methods 'book' is nearly ready as is the storage and handling protocol. A preliminary wanted samples list is send to the programme manager of subprogramme C and the programme coordinator. Finally, a next meeting was agreed in November 1991 which will be hosted by FMBRA at Chorleywood (UK).

REVIEW OF PROJECTS

The milling quality project (A.1.1) is a collaborative study by FMBRA and TNO. In February Tony Evers of FMBRA visited Marcel Kelfkens of TNO to agree on a common approach and set up the collaborative experiments. Following this visit many wheat samples have been exchanged. At FMBRA milling studies were carried out and grains were analyzed. Recent work focused on the development of a new image analysis method allowing the measuring of larger quantities of grains. At TNO work focused on collecting
samples and milling and preparations for the analytical characterization. The work proceeds according to schedule.

The work on gluten-starch processing is carried out by TNO (A.1.2.1) and TUB (A.1.2.2). At both labs work is still in a preparatory phase. Some setbacks were encountered at TNO with the setting up of a small scale (10 gram) separation system. Problems have been solved but the project is a little behind schedule. At TUB it was decided not to build the pilot system as originally planned, but to redesign the lab scale system to allow a reliable characterization of the gluten produced from the wholemeal flour. Due to the administrative reasons stated earlier the project has only started in April.

Due to their start in September 1990 FMBRA was already able to present the first half year results on white bread (A.2.1) and wholemeal bread (A.2.2) at the Wageningen meeting. Interesting results were presented on the different work input requirements of wheat varieties. Blending studies show that stronger wheat varieties were approximately at the mean of the individual wheats. Work on wholemeal breadmaking performance demonstrated a lack of correlation with white breadmaking performance. Both projects have started quite well and are -for reasons stated before- ahead of the general schedule. Work at BSN (A.2.3) was also in a preparatory phase. Activities concerned the setting up of standardized rheological protocols together with FMBRA and Nuovo Crai. Also a laboratory test for semi-sweet biscuits was developed as well as the development of a pilot scale version. Work is somewhat behind the general schedule for administrative reasons.

The work on flour blends at TNO (A.2.4) is aimed to establish relations between biochemical and technological characters. With a first series of flour blends a good correlation was found with the amount of high molecular weight aggregates of glutenin and the amount of carbohydrates associated with the so-called 'gelprotein'. In reconstitution studies isolated LMW wheat proteins were demonstrated to influence the formation of gelproteins. This effect was paralleled by changes in rheological properties. This is an important result which may help explain the anomalous results in dough properties encountered upon blending of wheats. Work is proceeding very well on schedule.

The work at Nuovo CRAI focused on the evaluation of rheological protocols to assess dough properties on a small scale (A.2.5). Several protocols were discarded due to a lack of correlation with wheat protein content. Contacts have been established with BSN and FMBRA to assist in this. Work is approximately three months behind the average schedule.

At IATA work (A.2.6) has started with the selection of flours and microorganisms. This first phase of the project has already been completed. Again work has started later, but work is now well underway and progressing.

**MAIN CONCLUSIONS**

In general, the work of subprogramme A has met a delay of several months due to the delay in finalizing the contract. To date, all participants have started work. Some are still in a preparatory phase, others have already reported the first results. Some drawbacks have occurred: the setting up of the 10 gram gluten-starch system at TNO, the development of a small scale reliable rheological test at Nuovo CRAI. In both cases this does not require any changes in the initial objectives. In one case, the plan of work has been adjusted. At TUB it was decided not to construct the pilot separator, but to redesign the laboratory separator. This action is well justified and is aimed at improving the chance to reach the initial aim of the project. All in all work is now underway. The Wageningen meeting has demonstrated a great sense of collaboration, again demonstrated by the progress made in several joint activities.
INDIVIDUAL PROGRESS REPORTS


Partner 17 - TNO
Partner 14 - FMBRA

Progress report from 1-1-1991 to: 15-6-1991 (partner 17)
1-9-1990 to: 15-6-1991 (partner 14)

Key measures of achievement - Objectives:

First years milling assessment on 30 wheat varieties and development of image analysis system for estimates of endosperm content (objective). Milling results and image analysis procedure (deliverables).

Progress:

A wheat sample collection has been established including Dutch, French, and British wheat varieties with a wide variation in milling quality. Samples are obtained from the partners 05 and 14. The sample set is available for both partners in the task (14 and 17). Partners have made an inventory of methods for milling and kernel quality assessments carried out at both laboratories. The wheat samples are currently being milled and kernel assessments are being performed.

The possibility of using ferulic acid as a marker for bran has been investigated. It seems that due to differences in extractability the assessment of ferulic acid in very bran rich fractions is not possible. An arbitrary measure of bran friability can however be obtained by comparing flours at different extraction rates.

A presentation technique for the estimate of endosperm content in the kernel has been developed. With this technique it is possible to make macroscopic images of transsections of the kernel.

Earlier studies with image analysis applied to 1 grain at a time have failed to identify grain parameters which are consistently related to extraction rates of samples, possibly because the sixty or so grains measured were inadequate to represent samples. A different method is now being evaluated, in which 300 grains are measured in 10 sets of 30 grains simultaneously imaged on the Seescan Solitaire Prism 512 equipment. The test has been applied to wheats milled in earlier work and to a set of samples to be milled by both FMBRA and TNO Wageningen. Results on the first set are being processed but consideration of the second set will await completion of milling at both institutes.

Conclusions:

The project is proceeding according to schedule.

Task A.1.2.1 Improved separation of gluten and starch through the use of enzymes.

Partner 17: TNO

Progress report from 1-1-1991 to: 15-6-1991
**Key measures of achievement - Objectives**

Obtaining a better understanding of the mechanism of action of hemicellulases in starch-gluten separation and evaluate the use of enzymes as a processing aids. Objective: small scale separations.

**Progress**

A simplified model for gluten coagulation was developed. The system is operated at a 10 gram scale and corresponds well with a 5 kg pilot separating system. However, in the 10 gram system the action of some hemicellulase was evaluated, in contrast to the pilot scale system no clear improvement in separation characteristics was observed. Therefore the timing in the separation protocol was adapted. Now improvements are observed comparable to those of the 5 kg scale.

On a biochemical level, with the small scale samples no changes in carbohydrate content of the gluten could be observed. This could also be due to the smaller size of the samples, making it more difficult to analyze the small amounts of non starch polysaccharides associated with the gelprotein. On the other hand the amount and viscosity of gel protein (SDS-insoluble gluten proteins) were decreased. This was also observed in a model system of isolated gelproteins. This decrease may be due to contaminations of the hemicellulolytic enzymes with proteases. It is not clear yet if the proteases can also play a role in the separation of starch and gluten.

**Conclusions:**

The development of the small scale separation is ready, although adaptations to the protocol took more time than expected. Enzyme experiments are nearly ready. Work is a little behind schedule.

**Task A.1.2.2 Characterization of wheat gluten produced by new separation processes.**

Partner 13 - TUB Berlin.

Progress report from 1-4-1991 to 15-6-1991

**Key measures of achievement - Objectives:**

Investigation of the causes for the differences in the main rheological characteristics of gluten extracted from whole meal flour compared to gluten washed out of white flour (Objective). Pilot scale separation system (Deliverable). Completion of the deliverable December 1991.

**Progress:**

Studies on a laboratory scale separation system have shown that gluten can be obtained from white flour as well as from wholemeal flour in a similar purity and yield, but that its main rheological characteristics depend on the kind of flour processed. The causes which affect the deviations of the gluten properties could not be investigated up till now, since the residence time of the starch and protein mass in the system is too long. This severely hampers the research programme as the change in gluten characteristics may partially depend on enzymatic activities, especially when wholemeal flour is processed under the present conditions. By the way, the effects of other process parameters can be cancelled. Therefore it is necessary to shorten the residence time of the mass in the system by technical means. This is the first objective in our research
A further disadvantage of any system having the same process design is connected to the fact, that several hours are required to achieve constant process conditions. This is mainly caused by the time consuming start-up procedure to reach a steady-state which is characterized by a constant concentration of solubles in the recirculated process water of around 6%. Due to this, the material demand of the system depends on its size. For that reason the scale of the system must be as small as possible. According to this precondition, the laboratory scale system meets the requirements to keep a constant mass flow far better than a pilot scale system as its material demand is much lower than that of the latter. This is also of great importance since the mass of the wheat lots which can be offered by the participants of Subprogramme C is limited. Therefore it was decided not to build the pilot system. Instead, the already existing laboratory system will be redesigned. The partial reconstruction of the laboratory system is mainly aimed to reduce the residence time of the mass in the system by a continuous concentration of the underflow of the sieving step using a decanter. This requires a machine of very small dimensions which has to be constructed as it can not be purchased. This is the main task of our present research work.

**Conclusions:**

The results of the investigations carried out show that we have to adapt the original approach by partially reconstructing the laboratory system. This is a necessary precondition to carry out the intended trials to investigate the influence of wheat quality and of the process parameters on the characteristics of the extracted gluten. Unfortunately, the delay caused by administrative problems in the signing of the EC-contract had the consequence that the official starting date had to be postponed. Therefore we are four months behind the planning schedule.

**Task A.2.1. The characteristics and processing requirements of wheat for specific end uses: white bread.**

Partner 14 - FMBRA

Progress report from 1-9-1990 to: 15-6-1991

**Key measures of achievement - Objectives:**

To assess the ability of strong gluten type varieties to carry varieties with weaker gluten characteristics, to determine work input requirements.

**Progress:**

Optimum work input requirements (Wh/kg dough) have been established for the Chorleywood Bread Process (CBP) using single wheat varieties from 1989 and 1990 harvests. Low work input requirement has been a consistent characteristic of poor breadmaking performance and high input with good performance.

Blends have been made to study the ability of strong gluten varieties to carry weak. Strong varieties successfully carried weak, work input and loaf properties were at approximately the mean of the blends.

Tests using traditional processes to investigate fermentation time requirements of varieties found in CBP to have clearly different work inputs are in progress.
Conclusions:

Work proceeds well as planned.

Task A.2.2. The characteristics and processing requirements of wheat for specific end uses: wholemeal bread.

Partner 14 - FMBRA.

Progress report from 1-9-1990 to: 15-6-1991

Key measures of achievement - Objectives:

To define factors that affect wholemeal bread-making quality of flours.

Progress:

Detailed analysis of results obtained when baking 14 UK and continental varieties in white, wholemeal and blended wholemeal (where the bran and offal components were interchanged with those of control UK variety) have shown that it was not possible to accurately predict the wholemeal baking performance from that of white when using a standard mixing regime (CBP at 40 kJ/kg work input).

There were indications that the drop in loaf volume caused by bran and offal components was smaller in the case of soft varieties, possibly due to the lower damaged starch level in such varieties.

This work has highlighted the need to establish the optimum work input requirement of the wholemeal flour and this aspect will be considered in the coming year.

Conclusions:

Work is well underway.

Task A.2.3. Evaluation of technological functionality of wheat flours and protein fractions in baked products.

Partner 08 - BSN Branche Biscuits.

Progress report from 1-4-1991 to: 15-6-1991

Progress:

We have participated in the preparation of common rules for the method book:

- We have analyzed the questionnaires proposed by partner 14 concerning the best methods for the rheological assessment of samples and the baking tests.

- We have proposed the test outline designed to be used for semi-sweet biscuits baking tests.
A laboratory scale baking test for semi-sweet biscuits of "Petit-Beurre" type has been developed. The pilot scale version of this test has also been finalised.

We propose to make the technological evaluation of the provided flours by using first the "Petit Beurre" baking test during the next six months. Tests on white bread will be made next year.

Conclusions:

Work just started.

**Task A.2.4. Processing properties of flour blends. Prediction and improvement.**

Partner 17 - TNO

Progress report from 1-1-1991 to: 15-6-1991

**Key measures of achievement - Objectives:**

Improvement of the insight in the relation between gluten composition and dough functional characteristics in order to predict and to improve the processing properties of EC flour blends and to compensate for year-to-year variations in flour quality.

**Progress:**

Wheat flour was milled from three varieties usually used for preparing flour blends. Wheat from two harvest years was used (1989 and 1990). The rheological and biochemical properties were determined on the flours and flour blends. With the results obtained with flour from the 1989 harvest a statistical model was developed in which the rheological properties were related to the biochemical properties. The amount of gelprotein (SDS-insoluble protein) and the amount of carbohydrates attached to the gelprotein could explain to a large extent the rheological properties of flour and flour blends. Furthermore, interaction effects could be observed, i.e. the rheological properties could not be predicted on basis of the properties of the separate base flours. With some flour blends, the rheological properties were similar as with the base flour which was present in the largest amount. Sensory evaluation showed a relation between the sensory characteristic "elastic" and the viscosity of dough as determined by a Weissenberg Rheogoniometer. The 1989 statistical model will be extended with the results from the 1990 harvest.

An effort was made to confirm the model by using fractionated wheat proteins in reconstitution studies. LMW wheat proteins were isolated and added to the base flours in concentrations up to 0.8 %. The three base flours were mixed for different times and the gelprotein content was determined directly after mixing and after 45 min of dough rest. With one flour the amount of gelprotein decreased fast. After resting for 45 min no increase in gelprotein could be observed. With the second flour the amount of gelprotein decreased only after longer mixing times. During resting the amount of gelprotein increased. With the third flour an intermediate behaviour was observed. LMW proteins are noted for their ability to enhance the breakdown of gel protein during mixing. It was shown in the project that these proteins become covalently incorporated into the glutenin matrix. The enhanced breakdown was found with two varieties but the stable variety was less sensitive for the effect of the LMW proteins. The stable variety contained the HMW glutenin-A subunits 5+10, which are noted for their positive effect on breadmaking quality. A second variety with this subunit composition was tested and behaved similar.
Conclusions:

A good statistical relation was found between rheological and biochemical characteristics of flour and flour blends. LMW proteins were found to be covalently linked with the backbone of gluten, the glutenins, which can explain their deleterious effect on dough structure. Good progress was made.

Task A.2.5. Rheological characterization of wheat samples and identification of specific processing requirements related to sweet bakery products and products with sour starters.

Partner 04 - SME Ricerche

Progress report from 1-4-1991 to 1-6-1991

Key measures of achievements - Objectives:

Rheological measurements in order to obtain a better understanding of the properties of wheat (Objective). Small scale tests (Deliverable). Completion March 1992.

Progress:

Preliminary rheological measurements were carried out with two types of flours, with different characteristics, using different flour/water ratios in order to find the best experiment conditions for testing. Viscosity measurements were carried out at constant temperature at different shear rates using a rotational viscometer. Measurements were made at 20 °C at shear rates of 0.1, 1, 5, 10, 50, 100, 400 sec\(^{-1}\). Slurries were exposed to shearing for a 10 min period so that a constant shear stress would have developed and hence a constant viscosity.

The experimental data were processed according to the power law and logarithmic shear stress vs logarithmic shear rate were obtained. The plotting of data shows a linear behaviour for each flour/water ratio and the linear regression is characterized by high coefficients. Then we calculate the coordination of structural units within a material.

The first results show that there is no significant change in the structural arrangements within differing concentrations of examined slurries as the coordination number remained approximately the same. One of the flours shows a slightly lower overall coordination number in comparison with the second sample of flour but the difference is not large enough to suggest a significant difference in molecular arrangements. Although there is a difference in the amount of protein within each flour, we can assume that the way in which these molecules are arranged is similar. The coordination number from our measurements can be approximated to "2" for both flours. This suggests that the arrangement of molecules within the slurries approximates to a monodimensional lamellar microstructure.

Conclusions:

Our future work will be the characterization of slurries with dynamic measurements to confirm structural data with the Bohlin theory, in order to establish small scale testing protocols. We consider a little delay of three months in the beginning of our research work, considering 1st April as our starting date.
**Task A.2.6. Interactions with microorganisms.**

**Partner 12 - IATA (Valencia, Spain).**

**Progress report from 1-4-1991 to 15-6-1991**

**Key measures of achievement - Objectives:**

Obtaining information about the effects of microorganisms on flour components and dough characteristics (Objective). Selection of microorganisms (Deliverable). Completion July 1991.

**Progress:**

A selection of wheat flour samples from a pool of twelve Spanish commercial flours located at different geographical areas, covering ranges of degree of extraction, proteolytic activity and flour quality has been formed on the basis of their physicochemical characteristics (moisture, protein, ash and fat contents) and rheological and fermentative properties (alveogram, farinogram, extensigram, maturogram, oven-rise record, and rheofermentogram). Also, pure cultures of lactic acid bacteria strains (homo- and heterofermentative) have been selected according to their acidification ability from the Cereal Laboratory collection. Commercial compressed yeast has been used for flour characterization purposes. Based on previous results, two strains of lactic acid bacteria - *Lactobacillus plantarum, Lactobacillus brevis* - have been selected for uses in two physical conditions - frozen and freeze-dried (on a sour dough matrix).

As physicochemical flour characteristics are concerned, the following ranges have been obtained: moisture: 12.75-15.31 %, protein: 10.85-14.56 % d.b., fat: 1.23-1.97 % d.b.; and ash 0.52-2.56 % d.b. Flour samples analyzed covered a wide range of rheological and fermentative values. Alveogram parameters of white flours varied from 100 to $252 \times 10^3$ ergs (W), 0.32 to 1.4 (P/L) and 11.26 to 36.65 (% of degradation). Main differences in rheological characteristics correspond to development time (farinogram: 1.5-13 min) and maximum resistance to extension (extensigram: 292-835 BU). Fermentative properties showed the widest value ranges for dough level (maturogram: 320-735 BU).

- As a general characteristic, the percent of degradation governed rheological and fermentative properties of Spanish commercial flours. This year, no flours without proteolytic activity could be found from our suppliers.

- Protein content was the main chemical parameter differentiating among with flours (75 % degree of extraction). The higher protein levels result in greater flour strength (W), water absorption, resistance to extension, energy and dough level.

- As expected an increasing degree of extraction leads to higher protein, fat and ash content. As a result, rheological and fermentative properties undergo deleterious effect as recorded in the extensogram, maturogram and impulsogram parameters. Higher water absorptions and degree of softening than for white flours, have been observed.

**Conclusions:**

On the basis of the above mentioned results, six flours will be selected after completion of some analytical determinations. Next period of phase 1, will deal with: Obtaining information about the effect of microorganisms on flour components and dough characteristics (Objective). Assessment of changes in functional properties of doughs and baking performance (Deliverable). Completion February 1992.
SUBPROGRAMME B: FUNCTIONAL COMPONENTS AND THEIR INTERACTIONS

Progress in subprogramme B up to June 1991 (Dr. J.J. Plijter)

REVIEW OF ACTIVITIES

The activities in this period were influenced by the fact that no contracts were signed, so that several partners involved in subprogramme B were actually not able to start research. As a consequence, the different activities in subprogramme B started at different times. Two meetings were held up till now; the first one on February 6 in Paris, where not everyone was present, was intended as a possibility for the people involved in subprogramme B to get acquainted with each other. The second scientific meeting was held in Montpellier, France, on July 11 and 12. This meeting was also a great success. Presentations were given by all participants involved in the programme.

REPORTS FROM COOPERATORS

Partner 07M - INRA Montpellier

Task B.1.1. Purification and characterization of gluten subfractions

Before selecting specific LMW subunits of glutenin for purification and study of functional properties, it was necessary to determine those that impart either high or poor breadmaking quality. Accordingly, a preliminary study of the composition in LMW (and HMW) subunits of glutenins was carried out in the frame of subprogramme C (see report of the task C.4 below): (i) to obtain a clear-cut separation between reduced glutenin polypeptides and gliadin monomers based on the Triton X114 extraction procedure, (ii) to enable a routine characterization of LMWG and HMWG by 1-D SDS-PAGE, (iii) to classify the most extensively grown French wheats according to their LMW pattern, and (iv) to determine statistical relations between the mean baking strength and the LMW type.

Partner 07N - INRA Nantes

Task B.1.1. Purification and characterization of gluten subfractions

Task B.1.2. Physicochemistry and functionality of wheat proteins

Gluten fractions composed of gliadins and of glutenin polymers with various sizes must be prepared and characterized without altering their functionalities in view of structural and rheological studies. The method of MacRitchie (1987), with some adjustments, was found to be usable for this purification. The characterization of the gluten proteins by SE-HPLC was improved by changing the elution buffer and introducing a mild sonication in the procedure.

Compositions of fractions extracted from gluten of wheat Aubaine by the procedures described above were analyzed. It was shown that fraction solubilities were related to their contents of glutenin polymers and
their sizes. Sizes of glutenin are related to their HMW-glutenin subunit contents.

Rheological analysis of gluten and gluten fractions of wheat Aubaine were performed. It was found that the viscoelastic behaviour of the fractions is very dependent on the length and the concentration of the gluten polymers.

Task B.1.5 Lipid interactions

Lipid binding proteins could be purified with the aid of Triton X114 phase partitioning, followed by size-exclusion, ion-exchange and reversed-phase high performance chromatographies. A wheat phospholipid transfer protein was purified from wheat flour using ammonium sulfate precipitation, size-exclusion and ion-exchange chromatographies. A consequence of the extraction procedure with Triton X114 is the development of a new sequential fractionation of wheat proteins. In this way, pure albumin-globulin, gliadin and glutenin fractions can be purified. The proteins partitioning in the Triton X114 rich phase are two χ-gliadins and a series of low molecular weight cystein rich proteins. N-terminal amino acid analysis showed that, excepted for the purothionins, all proteins are unknown. They have a pl above 8. The phospholipid transfer protein is a monomeric protein of 90 residues, MW = 9607, a pl of 10 and has 8 disulfide bridges. It shows strong homologies with the other known proteins of this family isolated from e.g. barley, rice, maize.

Partner 14 - FMBRA

Task B.1.4. The role of minor protein components associated with starch granules

Work since April 1990 has been devoted entirely to the starch granule surface protein "friabilin" (experimental approaches (a) and (b), as planned). A monoclonal antibody has been produced to assay friabilin content of whole endosperm, for comparison with isolated starches. T. durum and T. aestivum had zero and high contents as expected, but hard T. aestivum had a high content in contrast to the low level on its starch. The immunoassay cannot therefore predict endosperm texture as hoped. This important objective will be pursued for a further year (with deferral of experimental approaches (c) and (d) on starch granule properties). N-terminal aminoacid sequence of friabilin shows homology with the phospholipid binding proteins being studied under task B.1.5, therefore their role in endosperm texture will be examined in this context.

Partner 15 - Gist Brocades

Task B.1.2. Physicochemistry and functionality of wheat proteins

Task B.1.3. Gluten hydration and interactions of gluten proteins with other components

Task B.1.5. Lipid interactions

Work has been done on the interfacial behaviour of dough during mixing. Dough was prepared from a commercial flour, with no other additive than salt, sugar, yeast and water. Samples were taken at different mixing times. The interfacial behaviour was studied with the aid of an overflowing cylinder. During mixing the components which determine the surface behaviour changed from a high molecular character to a low molecular weight character.
Partner 16 - AFRC-IFR Norwich

Task B.1.3. Gluten hydration and interactions of gluten proteins with other components

Task B.2. Dynamics of dough development

Work on the project has not yet commenced because of the delay in signing the contract.

The necessary methodology in NMR spectroscopy has been developed over the last year in an independently funded project on a related theme. Because of this and because for the NMR work the staff was in place, no serious slippage is anticipated. Deuterium NMR relaxation measurements have been used to study the effects of hydration (by D$_2$O) and heating on gluten proteins. Since the deuterium can exchange with some labile protons on the protein and the relaxation time of the deuterium nucleus will differ greatly, depending on its surrounding. Therefore, the water relaxation can be used to monitor changes in the state of protein (denaturation, aggregation, etc.). The relaxation behaviour was studied in a good and bad baking quality gluten. Unfortunately, the relaxation behaviour was mainly determined by the gelation of residual starch present in the glutens, so no differences could be detected. NMR relaxation experiments on purified proteins (HMW subunits) and the availability of well characterized glutens under the ECLAIR programme will probably provide the solution to this problem.

The work done on the monoclonal antibodies has not been started, but it is anticipated that it can begin on September 1st.

Partner 19 - AFRC-IACR Bristol

B.1.1. Purification and characterization of gluten subfractions

No progress since it was not able to recruit a scientist to work on the project until the contracts were signed. Therefore, there is a slippage on the agreed programme of work of at least six months.

Partner 22 - Università di Padova

B.1.1. Purification and characterization of gluten subfractions

The aim of the research at the University of Padova is to purify the LMW glutenins. A purification technique tested is based on adsorption chromatography on controlled pore glass. In this way, it was possible to purify native gluten free of monomeric proteins, which is essential since in reduced conditions the LMW-glutenin subunits have a similar molecular mass as those of other wheat proteins (mainly gliadins). The native gluten prepared this way (one of the two peaks present), is constituted by at least two classes of aggregates, but it is completely free of monomeric proteins. In the reduced state, the two aggregates disappear and we can observe the HMW- and LMW-molecular weight glutenin subunits peculiar to the wheat cultivars used.

Partner 23 - Università di Viterbo

B.1.1. Purification and characterization of gluten subfractions
Different lines of research are explored at this moment:

- Studies on the variation for protein components important for bread wheat technological properties in wild and cultivated material.
- Purification and biochemical characterization of some components of the gliadin and glutenin fractions.
- Isolation and characterization of null lines for certain gliadin and glutenin components.
- Introduction, in cultivated wheats, of the new detected allelic variants for HMW and LMW glutenin subunits and evaluation of their effect on quality.
FOREWORD

This section summarizes the research activities carried out by participants in the "Subprogramme C" of the ECLAIR Programme "To Explore and Improve the Industrial Use of EC Wheats" from October 8, 1990 to June 15, 1991. It comprises: (i) reports from Cooperators; (ii) reports on the two sessions of the Subprogramme C Working Group, and (iii) Synthesis of the answers to the Subprogramme C questionnaire.

A warm acknowledgement for advice and ideas is given to all participants in Subprogramme C. Their creative advice and encouragement at critical moments are greatly appreciated. At I.S.C. S.Angelo Lodigiano, Carmen Cominotti Borghi efficiently and carefully made sure that correspondence and documents were done promptly and well.

Norberto E. Pogna

REPORTS FROM COOPERATORS

Partner 02 - Produttori Sementi, Bologna, Italy

Participants: E. Borasio and E. De Ambrogio

Task C.1.1. - Multilocal experiments: Southern Europe Network (SEN)

Due to the late sowing, the emergence was irregular and the density of some of the plots was lower than optimal. Nitrogen fertilizer was supplied the 21st of January and the 21st of March (40 and 92 Units/ha respectively); on the 13th of May, 42 Units/ha of nitrogen were supplied to half of the plots. In spite of the difficult start, it looks as it will be possible to get useful information from the trial and to supply seed samples to other cooperators.

Task C.9. - Somaclonal variation for factors affecting breadmaking quality

In October 1990 we sowed the lines from R3 to R5 deriving from our work on tissue culture. At the moment we are particularly interested in some of the R5 lines deriving from plants that we studied in cooperation with Prof. Lafiandra (University of Viterbo, Subcontractor 23). These R5 lines belong to the cultivars Salmone, Oderzo and Gemini. The Salmone variants were studied in more detail thanks to the availability of easily detectable markers such as "Red glume colour" and "Hairy glume" that are linked to some gliadin loci. We found lines missing one of the mentioned markers along with the linked $\chi$- or $\xi$-gliadins. As to the Oderzo variants, it seems that some of them are lacking $\alpha$-gliadins. We have now to verify whether the lack of gliadin fractions is stably inherited and, if it is so, we will make available seed samples of these lines to cooperators interested to study the impact on quality of the missing gliadins.
Gemini, a hard wheat cultivar, we found a variant producing grain having a soft texture; if this is confirmed, also seed from this line will be made available to cooperators.

Task C.3. - Experimentation on populations for breeding

Because of the uncertainties about the starting date of the programme, we did not start the experimentation on populations for breeding, that we have in cooperation with I.S.C. S.Angelo Lodigiano (Associated Contractor 03); the populations will be sown in our location next October.

Partner 03 - Istituto Sperimentale Cerealicolture (I.S.C.), S.Angelo Lodigiano, Italy

Participants: B. Borghi, R. Redaelli, A. Biancardi, T. Lafranchis and N.E. Pogna

Task C.1.1. - Multilocal Experiments: Southern Europe Network (SEN)

The SEN trials were sown in S.Angelo Lodigiano (North Italy), Tolentino (Central Italy) and Foggia (South Italy). At S.Angelo Lodigiano emergence took place late in December. Previous crop was maize. Nitrogen was applied twice during winter for a total of 90 kg/ha. Late nitrogen application on three replications was given as an urea foliar application at heading (25 kg/ha of nitrogen).

Weather conditions were unfavourable during autumn and winter, with heavy rains in October, early frost in December and -15 °C in February without snow cover. Late frost (-1 °C) occurred in April 20th.

In spite of this unfavourable conditions, the crop appeared in good vegetative conditions during spring. Plant height and biomass appeared reduced. Diseases were not severe with a slight mildew attack. No chemical control against foliar diseases was applied.

Most of the foreign varieties appear unsuitable for our condition because of lateness in heading or high stature.

Harvesting is expected for the first days of July.

Task C.3. - Experimental populations for breeding

We have grown the SINT population mentioned in the programme for a total of 100 S3 lines on 1.5 m² plots and two replications. By visual selection we expect to reduce the number of lines to be harvested and evaluated for breadmaking quality to about 40.

Task C.5. - Genetic and technological aspects of HMW glutenin subunits, HMW-alboumins and S-proteins

- Novel HMW glutenin subunits encoded by the *Glu-B1* locus were found in two new Italian durum wheat cultivars (Parsifal and Fenice) and in two bread wheat cultivars grown in North Africa (Acsad 67 and Algerien G). Five new *Glu-A1* alleles were observed in *Triticum monococcum* lines grown in Central Italy. The cvs Parsival and Fenice were crossed with reference cultivars and the resulting progeny will be used for genetic and technological studies.

- Water-soluble proteins from 50 Italian bread wheat cultivars and aneuploid lines of Chinese Spring were fractionated by PAGE at acidic pH (A-PAGE) and SDS-PAGE. Genetic variation was found for both
HMW (about 60 kDa) and LMW (14-14 kDa) albumin components. Some HMW albumins were found to be coded by genes assigned to chromosome 4AL (redesignated as 4BL), whereas some LMW albumin components were found to be coded by chromosome 3Ds. F2 segregating progenies from six crosses between bread wheat parents having contrasting HMW albumin patterns are currently being investigated for allelism and genetical linkage by SDS-PAGE.

Task C.6. - Production of lines and near-isogenic lines with different HMW glutenin subunit, and gliadin compositions, and of null forms

- Fifteen near-isogenic lines of the bread wheat cultivar Alpe were grown in replicated plots. These lines have contrasting HMW glutenin subunits (subunits 7+8 vs 7+9, 2+12 vs 5+10) and gliadins (χ-40 vs χ-43.5, 1D- encoded χ-gliadins present vs absent). Rheological tests will be carried out on these lines in order to study the effects of the different alleles as well as of the allelic interactions on gluten quality.
- Null forms lacking both Gli-A1 and Gli-D1 encoded gliadins and LMW glutenin subunits were grown in replicated plots. More than 20 lines will be analysed using rheological tests. Moreover, null forms lacking Gli-A1, Gli-B1, Gli-D1, Gli-A2 and Gli-D2 encoded gliadins have been isolated within the bread wheat line S29. These forms are currently being crossed in order to produce multiple Nulls.
- The Canadian cv Neepawa was crossed with the Italian cv Costantino. These parents have contrasting allelic variants for gliadins and LMW glutenin subunits coded by the Gli-1 and Glu-3 loci respectively. At each generation, seeds from 6 spikes segregating at both Gli-B1 and Glu-B3 loci were grown in head-rows. F6 head-rows have currently being grown in the field. Moreover, genetical analyses of segregating progeny allowed us to isolate one mutant line lacking chromosome 1B encoded gliadins and LMW glutenin subunits.

Partner 05 - Champagne Céréales, Reims, France

Participants: S. Varieras, F. Plénier, M. Lemeur-Plunet

Tasks C.1. - Multilocal experiment: Southern Europe Network (SEN) and North Western Europe Network (NWEN)

Tasks C.2.2. - Genotype x environment interaction

Wheat seed samples from SEN and NWEN along with samples from G x E interaction study will be evaluated for their technological characteristics. Moreover, we supplied Dr. Kelfkens (TNO, Wageningen) with 11 seed samples (15 kg each) of wheat cultivars Armide, Aubaine, Baroudeur, Camp Remy, Festival, Futur, Génial, Récital, Soissons, Thésée, Ventura.

Partner 06 - Roquette Fréres S.A., Lestrem, France

Participants: M. Flèche, M. Huchette, J.J. Caboche and M. Dumont

Partner 08 - BSN/Branche Biscuits, Athis Mons, France

Participant: A. Verel
Task C.1. - Multilocal experiment: Southern Europe Network (SEN) and North Western Europe Network (NWEN)

Task C.2.2. - Genotype x environment interaction

The seed samples from SEN, NWEN and G x E interaction study will be characterized by rheological tests. Our research activity will be organized during the "Subprogramme C" meeting scheduled for September 17, 1991.

**Partner 07C - INRA - Station d'Amélioration des Plantes, Clermont-Ferrand, France, and INRA - Station d'Amélioration des Plantes, Montpellier, France**

**Partner 09 - Institut Technique des Céréales et Fourrages, ITCF, Paris, France**

**Partner 18 - Club Des 5, Paris, France**

Tasks C.1.1. and C.1.2. - Multilocal experiment: Southern Europe Network (SEN) and North Western Europe Network (NWEN)

Participants: 07C: M. Rousset and N. Robert (Clermont), F. Kaan (Montpellier)  
09: M.H. Bernicot  
18: H. Haslé

**Keys measures of achievement - Objectives**

Two experimental networks have been organized to produce samples grown in different environments. The samples will be used to study the effect of genotype x environment interaction on quality and to provide raw material to partners of subprogrammes A and B.

**Progress**

Southern Europe Network (SEN)  
24 high quality varieties have been sown in  
- 2 locations (partner 07C and 07M)  
- 2 locations (partner 18)  
Treatments: two nitrogen manure conditions, two repeats for each condition.

North Western Europe Network (NWEN)  
12 varieties have been sown in  
- 4 locations (partner 07)  
- 3 locations (partner 18)  
- 2 locations (partner 09)  
Treatments: cultivation with or without fungicide, two repeats for each condition

There is no problem of growing, except in Clermont-Ferrand where, for both networks, damages due to spring frost were very severe. It is probable that the trials can not be exploited in this location.
Financial status

1.5 man months (1 scientist, 1 Ingeneer)

Task C.2.: Genotype x Environment interaction: Ecophysiological approach

Participant 07C: N. Robert

Keys measures of achievement - Objectives

Analysis of the grain filling period i.e. accumulation of dry matter and storage proteins, is a first approach for studying the stability of quality. It has been undertaken in Clermont-Ferrand to relate variations of accumulation kinetics, amounts and ratios of storage proteins to stability of technological quality. Variation of storage proteins (mainly glutenins and gliadins) in relation to variation of quality will be also analysed on samples from both experimental networks.

Progress

Four varieties (Talent, Capitole, Camp Remy and Apollo) grown under two nitrogen fertilization conditions have been cutting up from flowering time. Dry matter accumulation in stem, leaves and spike, and storage proteins accumulation in the grain will be followed till maturity.

Financial status

1 man month (1 scientist, 1 technical assistant)

Task C.2.2. - Genotype x Environment interaction: Controlled environment

This experiment has not been undertaken yet.

Task C.4. - Genetic of LMW glutenin subunits

Participant 07C: G. Branlard

The LMW D zone proteins analysed by a 2 steps acidic-PAGE/SDS-PAGE technique were investigated on bread wheat cultivars. Twelve mobility bands were observed in 193 different varieties. Using nullitetrasomic and chromosome substitution lines the chromosomal locations of these bands were attributed to chromosome 1AS, 1BS and 1DS. Their allelism is currently under investigation.

Task C.7. - Chromosomal location of storage protein genes, chromosome interaction on protein synthesis and development of new germplasm

Participant 07C: A. Bouguennec, M. Bernard, M. Rousset

Keys measures of achievement - Objectives
Thirty-six intervarietal substitution lines (with duplications) have been obtained by INRA in the recipient variety Courtout. Six varieties, with various breadmaking qualities, have been used as donors for the substituted chromosomes (group 1 and 6 chromosomes). This material will be used to study the genetic information of these chromosomes and their effects, mainly on quality.

Progress

All the substitution lines and their parents have been already analysed biochemically and in field for agromorphological traits and quality characteristics. This work has allowed us to eliminate some suspicious lines and notice some lines which seem to differ from Courtot.

For 1990-91, two trials have been sown with 3 replications in two locations. In addition, these lines are being analysed by C-banding and RFLP methods.

Financial status

6 man months: 1 Ph.D. student, 1 master student

Task C.8. - Biochemical markers for screening early generations

Participant 07C: G. Branlard

The quality score based on the relationship between individual LMW subunits of glutenin and gluten quality will be established after completion of analysis on the allelic variation of the LMW D zone proteins. Currently, a theoretical study on the efficiency of electrophoretic separation of biochemical markers in breeding has been developed. The effects of (1) methods of sampling, (2) number of implicated loci, (3) frequency of the alleles to be screened and (4) generation of the grain analysed, on the efficiency of the electrophoresis of biochemical markers in breeding were particularly studied.

Partner 07M - INRA - Laboratoire de Technologie des Céréales, Montpellier, France

Participants: M.H. Morel, M.F. Samson, J-C. Autran

Task C.4. - Genetics of LMW Glutenin Subunits

Objectives: Determining the LMWG and HMWG subunits composition among a collection of hexaploid wheats licensed in France.

Progress: To obtain a clear-cut separation between reduced glutenin polypeptides and gliadin monomers and enable a routine characterization of LMWG and HMWG by 1-D SDS-PAGE, albumins, globulins and gliadins were selectively extracted. We used Triton X114 extraction procedure similar to that used in membrane protein studies, followed by a more conventional extraction with 70% ethanol. The last pellet containing glutenins was then extracted with an SDS-β-mercaptoethanol solvent. After SDS-PAGE, the HMWG subunit composition was determined according to the nomenclature of Payne and Lawrence. The components with relative mobilities between 750 and 850 were assumed to consist of LMWG type B subunits and the different patterns where clustered taking into account homologous patterns.

Among the 70 cultivars analysed, 50 were clustered in 4 groups. The remaining 20 cultivars gave fuzzy patterns or were present in less than 3 genotypes and were discarded from the subsequent analysis. Some
characteristics of each of the four groups are presented in Table 1.

Multiple variance analysis between the W value of the Chopin Alveograph (a parameter highly related to French baking test) and the glutenin composition is given in Table 2. A mean square value highly significant for LMWG group was obtained. Other varieties will be analysed to confirm this result.

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<th>Table 1.</th>
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<td>Number of cultivars</td>
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<td>Molecular weight</td>
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<td>Mean W (a)</td>
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<td>s.d.</td>
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<th>Frequency (%) of HMWG subunits in four groups of cultivars.</th>
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<tbody>
<tr>
<td>HMWG subunits</td>
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<td>Chromosome 1A</td>
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<td>1</td>
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<tr>
<td>2*</td>
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<td>null</td>
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<tr>
<td>Chromosome 1B</td>
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<td>6-8</td>
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<td>7</td>
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<td>7-8</td>
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<td>7-9</td>
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<td>Chromosome 1D</td>
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<td>5-10</td>
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<td>2-12</td>
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a : W value given by Chopin alveograph
s.d.: standard deviation

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<thead>
<tr>
<th>Table 2</th>
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<tr>
<td>Origine of variation</td>
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<tr>
<td>Main effects</td>
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<tr>
<td>LMW groups</td>
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<tr>
<td>Glu-A1</td>
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<tr>
<td>Glu-B1</td>
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<td>Glu-D1</td>
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<tr>
<td>Residual</td>
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Partner 17 - TNO Nutrition and Food Research, Wageningen, The Netherlands

Participant: M. Kelfkens

Task C.10. - Sprouting resistance

During the first half year, progress has been made on the following items:

a) Development of germplasm with genetic variation.

A breeding program has been developed in cooperation with four wheat breeders, consisting of crosses between material varying in dormancy as well as \(-\)-amylase-content in the absence of sprout damage. Material from the parents will be available in the 1991 harvest to characterize this material accurately. Progenies are multiplied by the breeders and will be available by the end of the program.

b) Selection of dormant and non-dormant types.

Varieties with a dormant or non-dormant character have been selected using a germination test. The character of the varieties has been confirmed by ABA-sensitivity testing, which showed that dormant varieties are more sensitive to ABA-inhibition. These results confirm that the germination test reveals a biochemically based form of dormancy.

c) Bio-assay development.

A bio-assay has been developed to test the inhibitory effects of ABA or wheat extracts on germinating embryos. Results show that the test is able to discriminate between sensitive and non-sensitive varieties. Also, inhibitory effects of extracts can be measured with this bio-assay.

d) Isolation of markers.

Work has started on the isolation of the markers for dormancy in wheat bran. First results of bio-assay testing show that inhibitory action is present in water-soluble, heat-stable and ethanol-soluble extracts. This indicates that markers for dormancy are present in the extracts.

Results so far, show that no modifications in the program are necessary. The isolation of the markers can be continued according to plan.

Partner 24 - Estacion Experimental Rancho de la Merced, Jerez de la Frontera, Spain

Participant: J. de Juan Aracil Lopez

Task C.1.1. - Multilocal experiment: Southern Europe Network (SEN)

SEN trials were sown in the following three places: (1) Rancho de la Merced, Jerez de la Frontera, Cadiz; (2) Cortijo Tomejil, Carmona, Sevilla and (3) Cortijo Castro, Camino de Purchil, Granada.

The first two places are under dry conditions, whereas the third can be irrigated. The previous crop were: Chickpeas (Rancho de la Merced); Sunflower (Cortijo Tomejil) and Onion (Cortijo Castro).
Seeding was done with Hege seedmaster in Rancho de la Merced, and with Wintersteiger seedmaster in the other places. Weather was very good in Jerez with an unusual cloudy spring which can affect quality. Moreover, a very dry wind blew the last ten days in May, fastening the maturity. In Carmona, the weather was very dry in December and January; however, tillering has made the crop better than it was expected.

In Granada three irrigations saved the crop. In Herez and Carmona a hard attack of leaf rust and a weaker attack of Septoria tritici occurred, whereas in Granada the highest injury was produced by powdery mildew (Erisiphe graminis). Insect attack was not very important, whereas birds were a problem mainly in Granada.

In June 12, cultivars were ready to be harvested in Jerez and Carmona. In Granada they were at state 23-24 of the Romig's scale.

**Partner 25 - Estacao Nacional De Melhoramento de Plantas, Elvas, Portugal**

Participants: F. Baguhlo and B. Macas

**Task C.1.1. - Multilocal experiment: Southern Europe Network (SEN)**

In the frame of our participation on SEN we have planted two trials; one in Elvas and the other in Beja. The sowing data was adequate for spring varieties but not for alternative types. Moreover, we received some seed samples late. Spring was very dry especially in April and May, and heavy water stress occurred in Beja. For this reason in the next season we should separate the varieties according to their growth cycle and make two trials, one for alternative types and another for spring genotypes. In the conditions of dry spring the alternative genotypes are expected to produce very poor quality grain. This will occur this season in Beja. By the end of June we will harvest. We are planning to determine, in our laboratory, protein content and the SDS-Sedimentation volumes. However, some problems can occur with funds to buy reagents if we do not receive money from this project soon.

**REPORTS ON THE TWO SESSIONS OF THE SUBPROGRAMME C (SP-C) WORKING GROUP**

The first session of the SP-C working group was held in Montpellier, France, in October 2, 1990. At this session the working group discussed the following items and took the following decisions:

(i). It discussed at length the objectives of the "Multilocal experiment" (Tasks C.1.1. and C.1.2.) and of "Genotype x Environment interaction" (Tasks C.2.1. and C.2.2.).

(ii). It agreed to start the multilocal experiments and the research activity referring to Task C.2.1. (Ecophysiological Approach to the Genotype Expression) during the sowing season 1990. This decision was taken to provide biochemists and physicists with the raw material for execution of the researches envisaged in Subprogrammes A and B.

Accordingly, 24 bread wheat cultivars (10 from Italy, 6 from France, 5 from Portugal and 3 from Spain) were sown in 14 locations in France, Italy, Portugal and Spain. These cultivars are important high quality wheat cultivars whose yield potentials and expression of quality attributes will be evaluated in different environments of the Southern Europe Network (SEN). Moreover, 12 wheat cultivars were sown in 9 locations of the North-Western Europe Network (NWEN) and four wheat cultivars were grown under
two N levels for studying the genotypic expression of breadmaking quality following an ecophysiological approach.

(iii). The report on the first session was distributed to all participants in Subprogramme C, as well as to the scientific managers of Subprogrammes A and B.

The **second session** of the SP-C working group was held in Paris in February 20, 1991. The report on this session was distributed to all participants in Subprogramme C and to the scientific managers of Subprogrammes A and B. The working group discussed the following items and took the following decision:

(i). It agreed to develop a guideline for collecting agronomical, physiological and technological data for SEN and NWEN cultivars. The guideline was prepared following suggestions and comments to a draft made by I.S.C. (S.Angelo Lodigiano). It contains instructions for recording field performance data along with technological data.

(ii). It also agreed to carry out few preliminary technological analyses (protein content, grain moisture, SDS-Sedimentation test and grain hardness) on the wheat samples from SEN and NWEN. The results of the above mentioned analyses are scheduled to be available by the end of September 1991.

(iii). It decided to prepare a questionnaire and distribute it to the potential users of the wheat samples from SEN and NWEN. The questionnaire was intended to obtain information on type and quantity of raw material required by cooperators for biochemical analyses and industrial uses (see point iv of this document).

(iv). It finally decided to hold the third session in April 1991. However, this session was cancelled because of budget restrictions and deferred to September 17, 1991.

**REPORT ON "SUBPROGRAMME C QUESTIONNAIRE"

As previously noted, the SP-C working group decided to prepared a questionnaire inviting all colleagues to give information about type and amount of raw material from SEN and NWEN they would require for research purposes. Other objectives were (i) to give an inventory of the seed samples and of the methods for the technological analyses carried out by SEN and NWEN cooperators and (ii) to obtain opinions and help from participants in Subprogramme A and B in optimizing future work.

The following main conclusions were drawn from the questionnaire.

(i). **Methods, techniques and equipments in the examination of SEN and NWEN cultivars:** to follow the "Instructions for SEN" in recording agronomical and technological data and to send data and notes from SEN to I.S.C. S.Angelo Lodigiano, for statistical treatment of data. The preliminary technological analyses of SEN and NWEN cultivars should include moisture content, protein content, SDS-sedimentation volume and grain hardness. The results are scheduled to be distributed to all collaborators by the end of September 1991. Since different methods and equipments will be used for quality measurements, 5 to 8 wheat samples (50-60 g each) from each SEN collaborator will be sent within August 18, to INRA-Clermont Ferrand for reference analyses.

(ii). **Collaboration with Industries:** to define during the session scheduled for September 17, 1991, the rheological and breadmaking analyses to be performed by the Industries (AC 04, AC 05, AC 06 and AC
08) on samples from SEN and NWEN.

(iii). **Methods and Equipments used for technological analysis**: to set up collaborations in order to establish standard methods for technological evaluation of wheat samples. Annex I reports the methods and instruments used by the laboratories involved in Subprogramme C. The exchange of reference wheat samples (high quality, medium quality and poor quality wheat cultivars) should be encouraged in order to check the different methods and adapt them when necessary. Agreement must be reached for standardization of the analytical techniques in collaboration with colleagues of Subprogramme A.

(iv). **Wheat samples required by Industrial and Laboratories**: Annex II reports the list of the SEN or NWEN samples required by Industries and Laboratories. Other specific requests were: (i) small number of samples, selected on the basis of widely different milling and baking qualities and/or genetic background (from FMBRA) and (ii) multiplication of some Australian lines (HMW subunit null forms) for rheological studies (from INRA, Nantes). It is quite obvious that some requests can not be fulfilled this year taking into account the large amount of grain required. Therefore it is important to intensify collaborations with Industries and Laboratories in order to accommodate specific wishes from Subprogrammes A and B in 1991-92.

A questionnaire prepared by TNO has been distributed to make a list of requested samples. This should help us organize the next sowing. Finally, your attention is drawn to three critical terms: (1) **September 17, 1991**: meeting of participants in Subprogramme C, (2) **September 30**: distribution of agronomical and technological data of SEN and NWEN and (3) **October 30**: deadline for receipt of requests of SEN and NWEN samples (1991 harvest) from Industries and Laboratories.