



**Commission of the European Communities**

**ECLAIR Programme AGRE 0052**

**Starting date: January 1st, 1991**

**To Explore and Improve the  
Industrial Use of EC Wheats**

**TECHNICAL ANNEX**

**Updated Version: March 1st, 1993**

## **1. TITLE**

### **To Explore and Improve the Industrial Use of EC Wheats**

## **2. OBJECTIVES**

### **2.1 Goals and Objectives of the Project**

The main objectives of the project are to explore and improve the industrial use of EC wheats.

The rationale behind these objectives is manifold. For instance:

- Despite the fact that wheat is an essential crop for European agriculture and for the wheat-processing industry (milling, bread-making, biscuit-making and starch/gluten industries), EC wheats are not really adapted to this wide range of applications, especially to their future developments, because the various processes have not been clearly explained in terms of process requirements and wheat quality requirements.

- Whereas Europe is deficient in good quality strong wheat, the milling and baking industries require higher quality wheat because of modern developments in technology. In particular, the use of 'cold' methods in baking (refrigeration and deep-freezing of dough) makes it necessary to have available flours of higher protein content and greater and greater strength.

- On the other hand, the fact that current methods of breeding are predominantly focused on white-bread making and pasta production stands more and more in contrast to the current applications of wheat in wholemeal, biscuit manufacture, wheat/starch production, sweet leavened products and fermented products, and considering that quality is also related to flour extraction rate (the amount of white flour extractable from wheat), performance in flour blends and degree of sprout damage.

- The consistency of the quality of the greater part of existing wheat is insufficient because of too great a sensitivity to agronomic and climatic factors. In Southern Europe, the climate is often the factor limiting both yield and quality; in the coastal regions of Northern Europe, where the crop can be cultivated intensively, sprouting puts a severe strain on both yield and quality.

Based on these observations, the need to intensify research work aimed at exploring new outlets and developing new applications for wheat and at improving the quality of wheat was strongly emphasized during the conference organized by the CEC at San Angelo Lodigiano, Italy, in June 1987.

Therefore, the following specific objectives have been formulated:

- To stimulate breeding and development of novel wheat varieties that combine good agronomic character and excellent technological qualities which would satisfy simultaneously the farming and manufacturing industries and export markets;
- To maximize EC grown wheat quality by providing tools to minimize sprout damage and maximize milling quality;
- To further improve the economy of EC wheats by relating current processing requirements to wheat characteristics, thereby enabling traders, millers and breeders to select on these characters;
- To open new outlets for wheat by investigating and developing new applications of wheat and wheat products (flour, starch, gluten).

## 2.2 Abstract of the Project

The project is aimed at Exploring and Improving the Industrial Use of EC Wheats (*T. aestivum*) with the objective of filling the growing gap between process development and its understanding in terms of processing requirements and thus wheat quality requirements. A further objective is the stimulation of breeding and development of wheats capable of satisfying the present and future demands of European industry and the export market.

Improved use will result from better knowledge of the various applications of wheat (milling, white and wholemeal bread-making, starch/gluten industry, flour blends, fermented products and biscuit manufacture). Each main parameter of processing and its effect will be expressed in terms of functional properties of the wheat and related to specific wheat protein constituents and their interactions.

Combined functional/physico-chemical and biological advanced methodologies will be applied to quality determinants, which will result in a better understanding of their variability of composition, structure, and of their mechanism of action in the various industrial processes. As a consequence of the availability of genetic stocks and wheat samples produced in highly controlled environments of the various EC countries, the identification of improved breeding criteria (for sprouting resistance, milling quality, bread-making or biscuit-making quality, adaptation to starch/gluten separation) and the development of rapid tests for use in breeding programmes and trade will be obtained.

## 2.3 Project Methodology

As stated before this represents a completely new strategy not only innovative in this respect, but also in the advanced methodologies used to tackle the often complex problems. The general economic benefit from this is evident. Of the about 75 million tons of wheat grown annually in the EC, 15-16 million tons is surplus. Decreasing this surplus with minimizing the need to apply costly intervention regulations will save the EC millions ECU. In the following the objectives of the programme will be explained in more detail, using different research topics of the project.

## Processing Requirements and their Application to Wheat Selection and Quality Definition

As stated earlier most of today's research is focused on relations between protein content and composition and white bread-making quality. Modern wheat breeding for example exploits the relation between certain HMW glutenin subunits and bread-making quality. Recent data both from applied studies as from fundamental studies have indicated that knowledge of the interaction between flour components is lacking at this moment. The proposed project will focus on this approach using advanced biochemical and physico-chemical methodology (combination between subprogrammes A and B). Furthermore, the proposed project will try to fill the gap between wheat quality requirements and present wheat applications ('cold' methods in baking, wholemeal bread-making, starch/gluten separation, biscuit-making, sweet leavened products, and fermented products) by studying the suitability of wheats for these applications on an applied level. This will lead to a better understanding of different quality characters required, to rapid selection tools for use in breeding and trade and thereby to a better exploitation of EC grown wheats. Furthermore these studies will enable increased quality assurance, improved products and the development of new products and/or new processes. The economic benefits from this (eliminating the need for wheat imports, better use of EC wheats, improved quality through selection) are evident but not easy to quantify.

### Milling Quality

Milling quality is an aspect of wheat which has been necessarily left out of selection programmes until the last stages. Nevertheless, taking the amount of wheat produced annually in the EC, one percent increase in milling yield represents an advantage of 40 million ECU per year. The approach followed to tackle this problem is innovative through the use of image analysis techniques in combination with sensitive chemical assays. Strong cost reduction of image analysis equipment now enable the development of rapid test based on this equipment offering both a technical advantage as a economical advantage (decrease in labour costs, ability to select wheats on milling quality on intake or in early stages of breeding programmes).

### Starch/Gluten Separation

The application of wheat as a raw material for starch/gluten separation is relatively new. Using pilot scale equipment recently developed at the participating laboratories it is now possible to improve process economy by using enzyme methodology and by enabling the use of wholemeal flours in the new separation processes. Enzymes can be used to tackle the problem of variation in processing properties, allowing economical benefits in using locally grown cheaper wheats. The use of wholemeal enables a higher yield of starch/ton of wheat. The new processing enables reduced losses in terms of wastage and costs of waste water treatment.

## Sprout Damage

Prevention of sprout damage is an objective long yearned for in the EC. The average costs of sprout damage once in every five years (leading to 10 % loss in yield and reduction of the amount of bread-making quality by 50 %) is 50-60 million ECU per year. The approach envisaged in this project is entirely new in both concept as methodology. Instead of detecting levels of amylase work will focus on developing for example immunoassay-based fluorescence tests for factors related to dormancy. This will enable rapid detection at an early stage (technical advantage), prevention (economic advantage) and selection of sprouting resistance in breeding programmes.

*Several recent advances provide the potential to make a significant step forward in both more effective utilisation and in the development of better European wheat varieties for the future.*

1. The availability of isogenic, aneuploid and translocation stocks which enable to pinpoint the gene products that are important in functional performance.
2. The introduction of original approaches based on new concepts (*e.g.* intrinsic quality of wheat genotypes), or new protein fractions (*e.g.* friabilin, HMW-albumin, S-protein,...), that stand out clearly against the old classical Osborne's scheme.
3. The acknowledgement that quality is not determined (and cannot be predicted) solely by protein composition, but also by interaction of the proteins with various flour components: starch, pentosans, lipids.
4. The development of modern physical and spectroscopic methods that can observe the behaviour of individual components (*e.g.* proteins, lipids) in a complex mixture (in situ NMR spectroscopy, rheological measurements).
5. The demonstration of the potential of monoclonal antibodies to quantify specific components in a mixture and to probe their dynamics and distribution within various systems (dough development, seed dormancy).
6. The development of a range of physico-chemical techniques that determine interfacial and aggregation behaviour.

Apart from these purely scientific and technical aspects, *a particularly innovative element of this project is the establishment of a multidisciplinary programme (bringing together physical chemists, biochemists, immuno-chemists, rheologists and geneticists) and involving different industries (millers, bakers, biscuit manufacturers, starch/gluten manufacturers and breeders).* The large number of participants of this programme is without doubt the price one must pay in order to make progress on such a complex problem as satisfying, year after year, the industrial need for quality in wheat. The organisation of this programme as *three subprogrammes*, each one of which will benefit from the results of the other two, will greatly facilitate the scientific direction, and thus the chances of success, of the programme as a whole. It is also clear that the role of coordinator will be essential for making sure of good coordination between the participants.

*The likely principal results of this study would be the following:*

1. Better understanding of the physico-chemical basis of the industrial processing of wheat and flour (milling, white and wholemeal bread-making, starch/gluten industry, flour blends, fermented products and biscuit manufacture) which will allow each participant to apply his knowledge in his own industry.
2. Development of improved methods for the rapid and efficient analysis and characterization of lines in early stages of breeding programmes (quality indexes) and of wheat samples in trade.
3. Creation of a genetic base which breeders can utilise.
4. Better identification of quality determinants whose genes should be identified, cloned, sequenced and possibly transferred.
5. In the longer term and well beyond the limited framework of four years of work, the introduction of new varieties of wheat which bring together the desired agronomic and technological characteristics, particularly the stability of the expression of quality in various environmental conditions of development of the plant and with the minimum use of chemical treatments.

## 2.4 Summary of the Project

The research programme has been organized as three interdependent subprogrammes:

### **A - Industrial Processes**

### **B - Functional Components and their Interactions**

### **C - Biochemical-Genetics and Physiology**

All subprogrammes are to be spearheaded by combining the expertise of the laboratories that have taken a major part in the developments cited above, with the experience of industrial laboratories in controlling dough development and of private breeders.

**A - Subprogramme A** is aimed at *improving the industrial use of EC wheats*. This aim is approached along two broad lines of research.

1. Tools are developed in order *to maximize EC grown wheat milling quality*. Using image analysis and sensitive biochemical assays tests will be developed to predict milling quality.
2. A concerted effort will be made by laboratories from six EC member countries *to fill the gap between current wheat selection in breeding programmes and trade on one hand*

*and current applications of wheat on the other.* Applications of wheat in the wheat starch industry, in wholemeal bread-making, flour blends, fermented products (sour dough) and biscuit manufacture will be studied on an applied level (in connection with subprogramme B which studies processes on a fundamental level). This includes both the use of advanced biochemical and physico-chemical methodology as well as recently developed process technology. Studies are aimed at understanding processing requirements and their underlying physico-chemical/biochemical causes. This will lead to the identification of process customized *selection criteria*. This in turn will enable an improved use of EC wheats, improved guidelines and criteria for breeding and improved products and processing of wheat.

B - The study of *the interactions and the development of dough* forms the objective of **Subprogramme B**, which has the following two main themes:

1. *Component interactions*: Proteins from glutenin and gliadin fractions which are linked to performance attributes will be prepared in sufficient quantities to study their water-binding by NMR), their aggregation with each other or with other components by NMR, by equilibrium sedimentation, ultracentrifugation, turbidimetry, SE-HPLC, etc.) and their hydrophobicity by RP-HPLC and TNS binding). These properties will be linked to performance tests in dough development and to associated indices of rheology. Study in lipids focus on the polar and protein-binding fractions using phosphorus NMR and fluorescence spectroscopy). The role of protein and lipid fractions in stabilizing the dough-gas bubble) interface will be determined by static and dynamic interfacial techniques. The minor protein components associated with starch granules will be also investigated to establish their role(s) in relation to functional properties of wheat, flour and isolated starch, to extend research on the role of starch granule protein Friabilin in controlling endosperm texture in wheat and to devise a predictive test of endosperm texture for use in plant breeding as a selection tool with single seeds (in connection with subprogramme C) and as a quality test at flour mill intake (subprogramme A).

2. *Dynamics of dough development*: The effect of heat and mechanical treatment on the distribution and mobility of protein components will be studied by NMR spectroscopy and linked with changes in dough rheology. Monoclonal antibodies will be used to label specific proteins and hemicelluloses to determine the dynamics of their distribution by immuno-gold labelling) within the developing dough particularly in relation to swelling and the formation of the biopolymer-gas interface. New oscillatory measurement techniques will be used to distinguish between two fundamental liquid and elastic contributions to the overall viscoelastic response.

C - **Subprogramme C** seeks to *analyse the biochemical, genetic and physiological bases of technological quality*. It is organized around the following topics:

1. Production of wheat samples in controlled conditions that are necessary to carry out studies of subprogrammes A and B. Evaluation of these wheats in various environments for yield potential and quality attributes.

2. Determination of the agronomic, physiological, genetic and biochemical factors affecting the technological quality and its stability of expression. They will include predictive values of biochemical tests.
3. Allelic composition, chromosomal location and genetic links of genes coding for the storage proteins subunits of HMW and LMW glutenins and gliadins, for certain albumins and S-proteins by analysing the lineage and chromosomal substitution lines between varieties. This study will be both qualitative (presence or absence of constituents) and quantitative contribution of LMW and HMW glutenin subunits to the total pool of wheat proteins).
4. Statistical analysis of a large collection of wheat cultivars in view to determine the relationships between allelic composition and baking quality. The protein fractions which appear to be correlated with qualitative characteristics (notably in view of trials carried out in industrial laboratories) will subsequently be purified so that their physico-chemical character can be determined within the framework of subprogramme B.
5. Development of rapid tests for dormancy and for initial stages of sprouting related to kernel constituents which will be used to produce wheat with a higher degree of sprouting resistance and early detection of sprouting damage in the field.



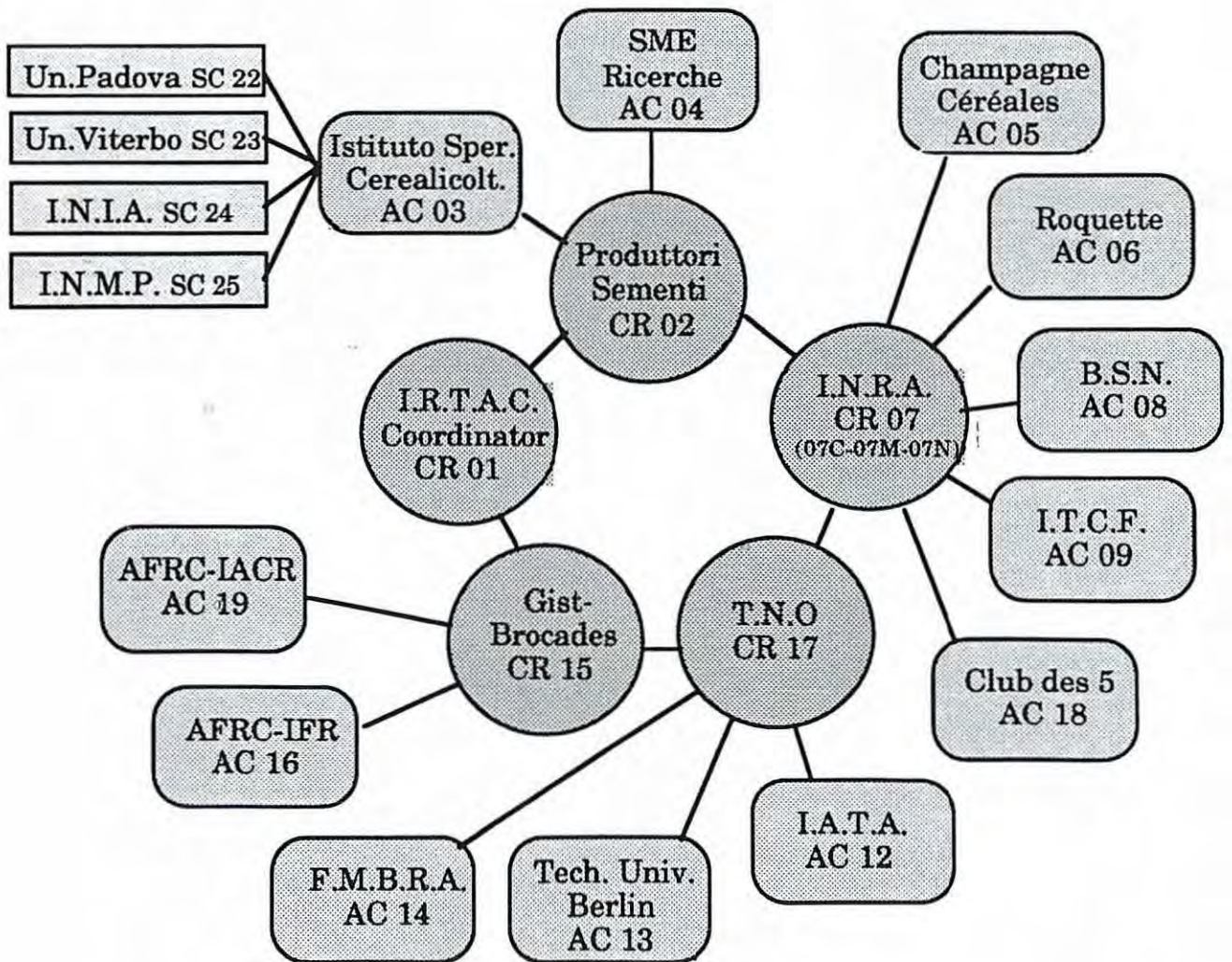
### **3. LIST OF PARTICIPANTS AND STRUCTURE OF THE PROJECT**

<u>Code</u>	<u>Name of Participants</u>	<u>Country</u>	<u>Role</u>
01	IRTAC, Paris	FR	Coordinator
02	Produttori Sementi, Bologna	IT	C
03	ISC, S. Angelo Lodigiano	IT	AC
04	SME Ricerche, Caserta	IT	AC
05	Champagne Céréales, Reims	FR	AC
06	Roquette Frères, Lestrem	FR	AC
07	INRA (Clermont-Ferrand + Montpellier + Nantes)	FR	C
08	BSN, Biscuit, Athis Mons	FR	AC
09	ITCF, Paris	FR	AC
12	IATA, Valencia	ES	AC
13	Technical University, Berlin	DE	AC
14	FMBRA, Chorleywood	GB	AC
15	Gist Brocades, Delft	NL	C
16	AFRC - ICR, Norwich	GB	AC
17	TNO, Zeist	NL	C
18	Club des 5, Paris	FR	AC
19	AFRC - IACR, Bristol	GB	AC
22	Università di Padova	IT	SC
23	Università di Viterbo	IT	SC
24	EERM, Jerez de la Frontera	ES	SC
25	ENMP, Elvas	PT	SC

[C = Contractor, AC = Associate Contractor, SC = Subcontractor]



## Organisational Structure



## **4. ROLE OF PARTICIPANTS**



Project Coordinator:           **01**

Organization:                   **Institut de Recherches Agro-alimentaires des  
Céréales**

Project Managers:            *Monique Richard and Jean-Claude Autran*

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Personnel background, responsibilities, outline of facilities

French representation to the International Association for Cereal Science and Technology, IRTAC, is a ltd association. It is supported by 18 professional associations involved in cereals production, breeding, storage and processing. Its main expertise is the identification and management of R+D programmes on cereals (production and transformation).

During the last seven years, IRTAC has managed several programmes in this field, such as:

- Standardization of wheat varieties identification by electrophoresis;
- Methods of evaluation of cereal fiber content and of gluten properties;
- Agronomic factors of mycotoxins development in maize;
- Assessment of the starch/gluten value of maize;
- Dough behaviour during freezing;
- Rheological properties of dough;
- Milling quality of bread wheats and durum wheats.

The administrative coordination of the ECLAIR programme is carried out by Mrs M. Richard.

The scientific coordination is carried out by Dr. J.-C. Autran, research director at the INRA (National Institute of Agricultural Research) Research Center, Montpellier, France. Dr. Autran received his PhD degree from Paris University in 1973 in the area of wheat histones and is also qualified as an engineer in agriculture and food industries. His principal research areas have included varietal identification of cereals by electrophoresis, chemistry of wheat proteins, biochemical basis of bread wheats and durum wheats quality, and development of biochemical tests for screening genotypes at the breeding stage. Dr. Autran is also teaching electrophoresis, cereals technology and biochemistry of plant proteins at the University of Montpellier.



### Bread-making quality

The problem of selecting for bread-making quality is tackled using NIRA in order to have an acceptable amount of protein in the grain. SDS-sedimentation test and electrophoretic analyses are carried out to select for good quality gluten. NIRA is also used to classify the selected lines according to their grain texture: hard or soft. Quality tests are performed starting from the F3 generation.

### Tissue culture

Regeneration from embryo calli has been achieved in a number of Italian genotypes. The first R2 population was screened in the fields in 1988.

Somaclonal variants differing from the original varieties in gliadin pattern and/or in grain texture were obtained and their bread-making quality is going to be assessed.

### Names and qualifications of the principal members of the team

- |               |  |
|---------------|--|
| E. Borasio    | - University degree in Agricultural Science.<br>- General Manager.   |
| E. DeAmbrogio | - Member of the National Academy of Agriculture.<br>- University degree in Agricultural Science.<br>- MSc in Plant Breeding In charge of the research division of Produttori Sementi Bologna from 1980.<br>- Member of the Italian Society of Agricultural Genetics, Eucarpia, International Association for Plant Tissue Cultures, NIAB (UK). |
| P. Jenabzadeh | - BSc in Biology.<br>- MSc in Biology-Plant Pathology. MSc in Plant Breeding.<br>- PhD in Cytology.  |
| S. Selleri    | - In charge of the Pathology Section of PSB.<br>- Senior Breeder. He was awarded the Todaro Prize (1986) by the National Academy of Agriculture for his contribution to wheat breeding.  |
| S. Ravaglia   | - University degree in Agricultural Sciences<br>- Junior breeder in charge of electrophoretic analyses.  |
| M. Paolini    | - University degree in Biological Sciences.<br>- In charge of the tissue culture laboratory.   |

### List of facilities, equipment and software to be used during the research programme

- Experimental fields located in farms of the province of Bologna and Ferrara
- Field equipment necessary for plot trials and screening nurseries: sowing machines, plot combine harvesters, seed cleaners, etc.;
- Greenhouses and growth cabinets (one greenhouse equipped with a cooling device to be used all the year round is available; a second greenhouse is going to be built).
- Laboratory equipment: Facilities for NIRA, SDS microsedimentation test, electrophoretic analysis of wheat storage proteins, hardware and software for recording, storing and analysing experimental data.

Associate: **03**

Organization: **Istituto Sperimentale per la Cerealicoltura**  
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#### Involvement on a task by task basis

- Task C.1.1: Multilocal experiment and production of samples in controlled conditions: Southern Europe
- Task C.3: Experimentation on populations for breeding
- Task C.5: Genetic and technological aspects of HMW-glutenin subunits, HMW-albumins and S-proteins.
- Task C.6: Production and assessment of lines and near isogenic lines with different HMW glutenin subunit and gliadin compositions, and of null forms

#### Personnel background and responsibilities

Since 1932, the Experimental Institute for Cereal, Section of S. Angelo Lodigiano (EICR-SAL) has been working on genetic improvement of wheat. The first director was the internationally renowned Prof. N. Strampelli. After the second world war, Dr. R. Forlani carried out a pioneer work on interspecific hybridization. In the period 1953-1968, Dr. B. Rusmini released improved bread wheat varieties largely cultivated in the Po Valley and the first durum varieties suitable for the North Italy environments.

In 1972, the research activities were completely reorganized and new modern laboratories and equipments for experimental works were acquired. In 1980, a new building for offices and laboratories was built together with new facilities like greenhouses, growth chambers, granary, etc.

The activities carried out in the last 15 years include:

- 1) Release of new varieties. In 1980, the two varieties Salmone and Saliente were obtained. Salmone represents the first Italian variety with technological properties similar to those of hard wheats imported from North America. Saliente is a high yielding variety well adapted to the hilly areas of Italy and characterized by a good bread-making quality. With these varieties, the possibility of producing grain of good technological quality in the mountain districts was clearly demonstrated.

2) Agronomical and technological characterization of cultivated varieties. Since 1972, EICR-SAL coordinates a network of variety trials where the most popular cultivars and new varieties are grown in replicated plots at 40-60 locations in the most important wheat growing areas of Italy.

The agronomic results published in September before the next sowing season represent a valid instrument for the farmers in choosing varieties adapted to the different environmental conditions. The technological analyses carried out every year on grain samples derived from the network of variety trials allow a precise classification of the varieties according to their end-use value.

As a consequence of these studies and thanks to the publication of more than 30 papers on quality testing, a marketing contract of bread wheat based on technological value of the grain has been established in Italy.

3) Biochemical and genetic studies on storage proteins. The electrophoretic analysis of storage proteins from Italian and European varieties allowed a more rational choice of the parental varieties in the breeding programmes and an early screening of the segregating populations. The protein pattern polymorphism has been used for classification and identification of cultivars with practical application on wheat marketing.

4) Cultural initiatives. EICR-SAL plays an important cultural role at national level acting as reference point in the sectors of agronomy, genetic improvement, milling and processing of bread wheat.

At the international level, besides participating to the most important scientific meetings dealing with wheat, EICR-SAL has recently organized a meeting sponsored by the Commission of the European Communities entitled 'Hard Wheat *Triticum aestivum* L.: agronomic, technological, biochemical and genetic aspects' with the participation of 94 scientists from EEC Countries, Canada, USA, Yugoslavia and Hungary. The proceeding and recommendations of this meeting represent the economical and scientific justification of the present project.

5) Participation to coordinated activities. In 1979-84, EICR-SAL coordinated the national project entitled 'Improvement of bread wheat for bread-making quality and yield by means of genetic and agronomic interventions'. A second 5 years programme has ended in 1989 with the participation of 6 research institutes. EICR-SAL received a financial support from the National Research Council for physiological studies on 'grain filling'.

At the international level, EICR-SAL participates in the most important networks of variety or germplasm evaluation programmes organized by international agencies and is coordinator of a project entitled 'Genetic improvement of bread wheat for drought prone environments by means of physiological and biochemical indices' partially supported by EEC, Programme 'Science and Technology for Development', Subprogramme 'Tropical and Subtropical Agriculture' and carried out in cooperation with INRA Morocco.



On the basis of the information synthetically reported and on the list of the most recent publications, EICR-SAL believe it is qualified for carrying out the scientific activities envisaged in the different sectors of this project.

Names and qualifications of principal members of the team:

Norberto E. Pogna - Graduated in Biological Sciences  
 - Researcher at the EICR-SAL  
 Basilio Borghi - Graduated in Agricultural Sciences  
 - Specialized in Applied Genetics  
 - Head of EICR-SAL

List of facilities, samples, equipments and software to be used during the research programme

The Istituto Sperimentale per la Cerealicoltura (ISC) of S. Angelo Lodigiano has all the facilities, equipments, samples and software necessary for carrying out the activities envisaged in the research programme. In particular, ISC is located in a new building with laboratories, offices, granary, and a guest-house for visitors and students.

The following equipments and facilities are available.

- Biochemistry laboratories with electrophoretic apparatuses, HPLC densitometer, UV meter, centrifuges, deep freezers, etc.
- Rheology laboratory with alveograph, farinograph, mixograph, extensograph, baking equipments, laboratory mills, Falling Number apparatus, gluten extractor, etc.
- Growth chamber, vernalization room, greenhouses.
- Field equipments for plot sowing, harvesting, drying, cleaning and storage.
- Fields (30 ha) for agronomic trials.
- Hardware and software for recording storing and elaboration of any kind of experimental data.
- Wide collection of wheat cultivars, landraces and aneuploid lines.

Associate: **04**

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Involvement on a task by task basis

- |            |  |
|------------|--|
| Task A.2.5 | Applications of wheat products: Sweet bakery products and sour dough                           |
| Task C.1.1 | Multilocal experiment and production of samples in controlled conditions: Southern Europe      |
| Task C.1.2 | Multilocal experiment and production of samples in controlled conditions: North Western Europe |

Personal background and responsibilities:

Ing. Euro Giagnetich	Managing Director
Dr. Giancarlo Malgarini	Head of Food Technology Section
Dr. Giacomo Mazza	Head of Food Chemistry Section

List of facilities, equipments and software to be used during the research programme

The following equipment and facilities are available:

- Physical measurements laboratory equipped with:
  - Haake viscosimeter system RV20 with CV100 M5osc head for rotational and oscillatory measurements;
  - Instron Universal Testing Machine mod. 4301 fully equipped for extension, compression and squeezing measurements;
  - Chopin Alveograph;
  - Brabender Farinograph;
  - Mettler TA3000 Thermal Analysis System;
  - Thermal conductivity apparatus;
  - ERH apparatus.
- Chemical and Biochemical laboratory fully equipped with upgraded analytical instruments.
- Climatic testing cabinets, dough mixers, etc.
- Hardware and software for recording, storing and elaborating of any kind of experimental data.

Associate:                               **05**

Organization:                               **Champagne Céréales**

Project Manager:                           M. *François Plénier*

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Involvement on a task by task basis

- Task C.1.1     Multilocal experiment and production of samples in controlled conditions: Southern Europe
- Task C.1.2     Multilocal experiment and production of samples in controlled conditions: North Western Europe
- Task C.2.2     Genotype  $x$  environment interaction: Experimentation in controlled environment

Personnel background and responsibilities

François Plénier	- Directeur du Développement - Ingénieur ICAM - ICG
André Halipre	- Directeur Industriel Branche Meunerie - Ingénieur Agronome
Maryvonne Lemeur-Plunet	- Directrice Laboratoire GMR - Docteur ès-Sciences

Champagne Céréales is the Union of 9 Cooperatives of cereals.

Champagne Céréales is the mother firm of several industrial activities:

- milling
- malting
- maize
- dehydrated vegetables
- cereals specialities
- dietetics, etc.



Contractor: **07**

Organization: **INRA (Institut National de la Recherche Agronomique)**

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Laboratories involved in the programme

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Laboratory 07M: Laboratoire de Technologie des Céréales  
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Laboratory 07N: Laboratoire de Biochimie et de  
Technologie des Protéines  
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France's agricultural research Institute, the Institut National de la Recherche Agronomique, was founded in 1946. It is a public body which since 1984 has had the legal status of an 'Établissement Public à Caractère Scientifique et Technologique', or EPST - a status which confers on it a certain degree of autonomy. The Ministries responsible for INRA are the Ministry of Research and the Ministry of Agriculture and Fisheries. Research work at INRA concerns agricultural products at every stage of their production and processing. The researchers' aim is to ensure that technological advance is fully mastered in the short, medium and long term, and is made available to those involved at every stage in the sequence of events from seed to supper table: agricultural input manufacturers, farmers, food manufacturers, and of course consumers.

INRA's tasks are as follows:

- The physical and agronomic environment
- Crop production
- Livestock production
- Agricultural development
- The food industries
- Rural economics and sociology

Split up between 25 scientific research departments, and in geographical terms between 22 centres spread across the country, INRA comprises 8,200 people, 2,800 of whom are researchers and graduate agricultural scientists.

1) Laboratory 07C: **Station d'Amélioration des Plantes  
(Clermont-Ferrand)**

Organization: INRA (Institut National de la Recherche Agronomique)

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Involvement on a task by task basis

- |            |  |
|------------|--|
| Task C.1.1 | Multilocal experiment and production of samples in controlled conditions: Southern Europe      |
| Task C.1.2 | Multilocal experiment and production of samples in controlled conditions: North Western Europe |
| Task C.2.1 | Genotype $\times$ environment interaction: Ecophysiological approach                           |
| Task C.4   | Genetics of LMW glutenin subunits  |
| Task C.5   | Genetic and technological aspects of HMW-glutenin subunits, HMW-albumins and S-proteins.       |
| Task C.7   | Chromosomal location of storage protein genes  |
| Task C.8   | Biochemical markers  |

Personnel background and responsibilities

- |                 |  |
|-----------------|--|
| Michel Rousset  | - Ingénieur Agronome. Directeur de Recherches INRA.<br>- Wheat breeder: bread wheat breeding for quality improvement.  |
| Michel Bernard  | - Ingénieur Agronome. Directeur de Recherches INRA<br>- Wheat and Triticale cytogeneticist. Head of the wheat molecular mapping programme (RFLP).  |
| Gérard Branlard | - Ingénieur Agronome. Docteur-Ingénieur. Directeur de Recherches INRA.<br>- Geneticist and biochemist: biochemical and genetic diversity of gliadins and glutenins; technological quality of bread wheat, quality prediction for breeding. |

Mireille Dardevet	- Assistant-Ingénieur in the above-mentioned research. - Responsible for analytical technics: APAGE, SDS-PAGE, 2D electrophoresis and for genetic crosses.
Georges Gay	- Ingénieur d'Etudes INRA. - Cytogeneticist.
Pierre Bérard	- Ingénieur d'Etudes INRA. - Wheat breeder.
François Kaan	- Ingénieur Agronome. Directeur de Recherches INRA. - Wheat breeder.
Nathalie Robert	- Ingénieur des Techniques Agricoles. Docteur en Sciences Biologiques. Chargée de Recherches INRA.
Eugène Triboï	- Ingénieur Agronome. Docteur-Ingénieur. Directeur de Recherches INRA. - Agronomist and crop physiologist on bread wheat.

List of facilities, equipments and software to be used during the research programme

- Greenhouses.
- Growth cabinets.
- Microscopes.
- Molecular biology facilities: centrifuges, ultracentrifuge, DNA electrophoresis, deep freezer.
- Biochemistry equipment: 1D and 2D electrophoresis systems, power supplies, densitometer, image analyser for 2D gels (scanner, computer, software), spectrophotometers, computer for data processing and recording.
- Technological facilities: mills, Pelshenke apparatus, SDS sedimentation, Falling Number apparatus, Chopin Alveograph, nitrogen analyser (Technicon).



2) Laboratory 07M:                    **Laboratoire de Technologie des Céréales  
(Montpellier)**

Organization:                            INRA (Institut National de la Recherche Agronomique)

Project Manager:                        Dr. *Marie-Hélène Morel*

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INRA  
2 Place Viala  
34060 Montpellier Cedex 01

Telephone:                                (33) 67 61 24 33

Telefax:                                    (33) 67 52 20 94

Involvement on a task by task basis

- |            |   |
|------------|---|
| Task B.1.1 | Components interactions: Purification and characterization of gluten subfractions   |
| Task B.1.2 | Components interactions: Physico-chemistry and functionality of wheat proteins  |
| Task C.4   | Genetics of LMW glutenin subunits   |
| Task C.6   | Assessment of lines and near isogenic lines with different HMW glutenin subunit and gliadin compositions, and of null forms |

Personnel background and responsibilities

- |                        |  |
|------------------------|--|
| Jean-Claude Autran     | - Ingénieur ENSIA, Docteur ès-Sciences<br>- Directeur de Recherches INRA |
| Marie-Hélène Morel     | - Docteur en Biochimie<br>- Chargée de Recherches INRA                   |
| Marie-Françoise Samson | - Assistante-Ingénieur   |
| Joëlle Bonicel         | - Technicienne   |
| Valérie Mélas          | - Etudiante (Thèse de Sciences des Aliments)                             |

List of facilities, equipments and software to be used during the research programme

- Biochemistry equipment: various electrophoresis systems, 1D (A-PAGE, SDS-PAGE, IEF), 2D (IEF x SDS-PAGE), capillary electrophoresis, densitometer, image analyser for 1D gels (camera, computer, software), liquid chromatography, SE-HPLC, RP-HPLC, FPLC, spectrophotometer, centrifuges, ultracentrifuges, freeze-driers.
- Technological facilities: laboratory and pilot mills (durum wheat, bread wheat, maize), farinograph, alveograph, viscoelastograph, mixograph, bread-making, pasta making and biscuit making equipments.

3) Laboratory 07N: **Laboratoire de Biochimie et de Technologie des Protéines (Nantes)**

Organization: INRA (Institut National de la Recherche Agronomique)

Project Manager: *Dr. Yves Popineau*

Full address: Laboratoire de Biochimie et de Technologie des Protéines  
Centre de Recherches de Nantes  
INRA  
Rue de la Géraudière  
B.P. 527  
44026 Nantes Cedex 03

Telephone: (33) 40 67 50 00  
Telefax: (33) 40 67 50 05

Involvement on a task by task basis

- |            |   |
|------------|---|
| Task B.1.1 | Components interactions: Purification and characterization of gluten subfractions |
| Task B.1.2 | Components interactions: Physico-chemistry and functionality of wheat proteins    |
| Task B.1.5 | Components interactions: Lipid interactions                                       |

Personnel background and responsibilities

- |                  |  |
|------------------|--|
| Yves Popineau    | - Directeur de Recherches INRA<br>(Biochemistry of storage proteins)                       |
| Jacques Lefebvre | - Directeur de Recherches INRA<br>(Structure, conformation and rheology of macromolecules) |
| Didier Marion    | - Chargé de recherches INRA<br>(Biochemistry of lipids and lipid binding proteins)         |
| Martine Le Meste | - Ingénieur-chercheur (ENSBANA-Dijon)<br>(ESR studies of proteins)                         |
| William Loisel   | - Ingénieur de Recherches INRA<br>(Functional properties of wheat proteins)                |
| Colette Larré    | - Ingénieur de Recherches INRA<br>(Fractionation and purification of wheat proteins)       |

List of facilities and equipments to be used during the research programme

- Protein extraction.
- Analytical and preparative chromatography.
- Electrophoresis, SE- and RP-chromatography.

- Lipid binding determinations.
- Viscosimetry, viscoelastic measurements, tensiometer and Langmuir trough.
- Electron spin resonance.

Associate: **08**

Organization: **BSN/Branche Biscuits**

Project Manager: *Dr. Alette Verel*

Full address: Centre International de Recherche Jean Theves  
6 Rue Edouard Vaillant  
91200 Athis Mons  
FRANCE

Telephone: (33) 1 69 54 12 70  
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Involvement on a task by task basis

- Task A.2.3 Applications of wheat products: Evaluation in baked products
- Task C.1.1 Multilocal experiment and production of samples in controlled conditions: Southern Europe
- Task C.1.2 Multilocal experiment and production of samples in controlled conditions: North Western Europe

Personnel background and responsibilities

Alette Verel Doctor in Biochemistry. Head of Applied Research  
Department: twenty-two people (ten engineers and doctors,  
ten technicians, two administrative).

Involved directly in this area: two people (one engineer, one technician).

Other people are working on microbiology, biophysico-chemistry, nutrition. They can bring their help to the project if necessary.

List of facilities, equipments and software to be used during the research programme

- |                      |                       |                  |
|----------------------|-----------------------|------------------|
| Rheological analysis | - Farinograph         |                  |
|                      | - Alveograph          |                  |
|                      | - Extensograph        |                  |
|                      | - Dynamic rheometer   |                  |
|                      | - Viscosimeter        |                  |
| Baking tests         | - Laboratory mixers   | - Pilot mixers   |
| on laboratory scale  | - Laboratory sheeters | - Pilot sheeters |
| and on pilot scale   | - Laboratory ovens    | - Pilot ovens    |

- Proofers
- Dough moulder

Sensory evaluation - Expert panel

Physico-chemical analysis - Air oven

- Glutomatic for gluten quantification
- Kjeldahl apparatus
- Gas chromatography
- HPLC
- Laser granulometer
- Falling Number apparatus
- Texture analyser INSTRON
- Spectrophotometer

Data analysis

- Microcomputer
- Software Statgraphics: statistical graphic system
- Software Nemrod: new efficient methodology for research using optimal design.

Associate: **09**

Organization: **ITCF (Institut Technique des Céréales et Fourrages)**

Project Manager: *M. Michel Leuillet*

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Laboratoire Céréales  
16 Rue Nicolas Fortin  
75013 Paris  
FRANCE

Telephone: (33) 1 45 86 70 40  
Telefax: (33) 1 45 70 83 89

#### Involvement on a task by task basis

- |            |  |
|------------|--|
| Task C.1.1 | Multilocal experiment and production of samples in controlled conditions: Southern Europe      |
| Task C.1.2 | Multilocal experiment and production of samples in controlled conditions: North Western Europe |
| Task C.2.1 | Genotype $x$ environment interaction: Ecophysiological approach                                |
| Task C.2.2 | Genotype $x$ environment interaction: Experimentation in controlled environment                |

#### Personnel background and responsibilities

Michel Leuillet	- Head of Department 'Cereal Crops for Food and Industrial Uses'
Guy Martin	- Scientist (Cereal quality and crop production techniques)
Christine Bar	- Scientist (Wheat quality)
Marie-Hélène Bernicot	- Scientist (Wheat varieties)

#### List of facilities, equipments and software to be used during the research programme

Growth of wheat genotypes in a controlled environment	- Ordinary equipment for wheat production and harvest in experimental conditions - Sowing machine - Automatic meteorological station - Statistical programme for exploiting data.
Technological tests	- Ordinary equipment for rheological, chemical and baking tests

- Sample dividers
- Grinding apparatus
- Chopin Alveograph
- Brabender Farinograph and Viscograph
- NIR spectrometer
- Falling Number apparatus
- Glutomatic system-centrifuge-2015
- Electrophoresis equipment
- HPLC chromatography.

Associate: **12**

Organization: **IATA (Instituto de Agroquímica y Tecnología de Alimentos)**

Project Manager: *Dr. Carmen Benedito*

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SPAIN

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Telefax: (34) 6 39 3 0001

Involvement on a task by task basis

Task A.2.6 Applications of wheat products: Interactions with selected microorganisms and wheat flour components and their application to improve breadmaking processes

Personnel background, responsibilities, outline of facilities

Carmen Benedito	Dr. in Chemistry, Professor of Research
Concepción Collar	Dr. in Chemistry, Researcher
María Antonia Martínez-Anaya	Dr. in Chemistry, Researcher
Claudia Martínez	Food Engineer, Fellow
Ofelia Rouzaud	Chemical Engineer, Fellow
Two laboratory aids.	

The cereals laboratory of the Institute of Agrochemistry and Food Technology (IATA) has a many years experience in Cereal Research. From 1980 our subject has been devoted to the study of wheat bread dough fermentation. In this respect, several projects have been carried out, one of the most important is the study of the metabolic activities of micro-organisms involved in bread dough fermentation and in its relation with dough functionality and bread quality. As a result of this work, a great number of papers have already appeared on the subject. The research will benefit to the knowledge on gluten proteins at FMBRA and TNO. Research will be complemented by the work carried out in project A.2.4.

The members of the team will carry out the work described in A.2.6. This involves the analysis of biochemical changes in flour components and fermentation metabolites, the study of the mechanism of action of enzymes from flour and selected micro-organisms, both using biochemical, physico-chemical and functional methods of evaluation.



Associate: **13**

Organization: **TUB (Technische Universität Berlin)**

Project Manager: Prof. Dr. *Friedrich Meuser*

Full address: Technische Universität Berlin  
 Institut für Lebensmitteltechnologie  
 Getreidetechnologie  
 Seestraße, 11  
 1000 Berlin 65  
 GERMANY

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 Telefax: (49) 30 314 27 557

Involvement on a task by task basis

Task A.1.2 Gluten/starch separation: Characterization of wheat gluten produced by new separation processes

Personnel background, responsibilities, outline of facilities

Meuser, Friedrich	Prof. Dr.-Ing., Head of Department of Cereal Technology of the Institute of Food and Fermentation Technology
Pahne, Norbert	Dipl.-Ing., Scientist, coworker of Prof.Dr. Meuser
Rennau, Claudia	Chemical Assistant

Prof. Meuser has an outstanding reputation in cereal technology. Since 1976, Prof. Meuser and his team are working on wheat starch processing systems. There are special references about processing of waste water and starch/gluten separation.

The members of the team will carry out the work described in part A.1.2. This involves a reconstruction of an existing laboratory scale system for starch/gluten separation, processing of series of varieties and consequently analysis of process parameters and (by)products, detailed physico-chemical evaluation of gluten fractions obtained from a 'white flour' process and the 'wholemeal' process.

Associate: 14

Organization: **Flour Milling and Baking Research Association (FMBRA)**

Project Managers: Dr. *Christopher J. Brock* (Tasks A.1.1, A.2.1 and A.2.2)  
Dr. *Philip Greenwell* (Task B.1.4)

Full address: The Flour Milling and Baking Research Association  
Chorleywood, Rickmansworth  
Hertfordshire WD3 5SH  
U.K.

Telephone: (44) 923 284111  
Telefax: (44) 923 284539

Involvement on a task by task basis

- Task A.1.1 Processing properties of wheat: Milling quality
- Task A.2.1 Applications of wheat products: The characteristics and processing requirements of wheat for specific end-uses: White bread
- Task A.2.2 Applications of wheat products: The characteristics and processing requirements of wheat for specific end-uses: Wholemeal bread
- Task B.1.4 The role of minor protein components associated with starch granules

Personnel background, responsibilities, outline of facilities

Dr. C.J. Brock, MA, PhD, Head of Cereal Science Group. Dr. Brock has more than fifteen years experience in protein biochemistry. Recently he has been Head of Biochemistry Section at RHM Research and Engineering Ltd., and Head of Cereal Science group at FMBRA where he has created and led multidisciplinary research projects in cereal science bringing together biochemistry, physical sciences, microscopy and genetics to explain processing performance and product quality.

**Task A.1.1**

Mr. A.D. Evers, MSc, CBiol, MIBiol, Head of Physical Sciences and Microscopy Section. Mr. Evers has extensive research experience in the area of cereal grain microstructure in relation to grain development and milling and baking performance. He has in recent years developed an interest in the application of image analysis in this area, and facilities have been established for such work. Preliminary experiments have given

promising indications of the usefulness of this technique for milling quality assessment.

#### **Task A.2.1/2**

Mr. T.H. Collins, Nat. Bakery Dip., CGIA, Head of Bread Section. Mr. Collins has many years experience of applied research in bread-making. He has made many significant contributions to the development of the UK bread-making industry, his most notable contribution being the very significant role he played in the development of the Chorleywood Bread Process, by which about 75% of UK bread is baked. His recent work, in collaboration with Dr. Hook (see below) includes research on the effect of milling procedure on wholemeal bread-making performance including the response to added vital wheat gluten. His input is essential for the research on optimisation of the wholemeal baking procedures and assessments of different wheats.

Dr. P. Pritchard, BSc, PhD, Head of Biochemistry Section. Dr. Pritchard has many years experience of strategic basic research related to the processing of bread. He has carried out much research in the past on the effect of  $\alpha$ -amylases during bread-making, but in recent years his interests have focused on the effect of dough processing on the gluten proteins of flour. His role in the project will be in the analysis of structural changes in dough proteins as a function of work input by biochemical techniques and determining the biochemical basis for differences between wheats.

Miss K. Little, C & G, B/Tec. Dip., Technologist in the Bread Section. Miss Little has worked at the FMBRA for five years. Her recent project work has included the study of the mixing requirements and baking quality of single wheat varieties of UK and continental European origin.

Mr. G. Oliver, BSc, Senior Scientist. Mr. Oliver has several years experience of research in measuring the thermal and rheological properties of flours and bakery goods. His recent work has involved developing fundamental rheological methods for predicting the baking performance of wheat varieties. In the project he will be studying the rheology of glutenin proteins and the effect of work input on dough rheology.

Miss B.E. Sang, HNC, C & G, Technologist in Bread Section. Miss Sang has five years experience of research in bread-making. Work has included lengthy investigations into the use of wholemeal flour and its effect on baking quality. More recent studies have involved the use of single wheat varieties with investigation centering on their tolerance when using the bulk fermentation process.

#### **Task B.1.4**

Dr. P. Greenwell, BA, DPhil, Senior Research Associate in the Biochemistry Section has over twenty years' research experience in the area of protein biochemistry. For the last ten years he has worked on the biochemistry of the proteins in wheat flour. In recent years he has pioneered research on the proteins of the wheat starch granule in relation to their possible functional role.

Miss B.M. Bell, BSc, has long experience of lipid-related topics within the bread-making industry, especially those concerning fat-improvement in the Chorleywood

Bread Process and recently the challenging analysis of grain surface constituents. Her involvement in the Project is necessary as lipid advisor and analyst, as lipids have been shown to be associated with starch granule proteins. Techniques will be devised to determine total lipid present and analyse the minute amounts of lipids involved.

Dr. S.S. Sahi, BSc, PhD, MRSC, CChem, Senior Scientist has considerable experience in the field of surface chemistry gained during his work for a PhD. and as a postdoctoral fellow where he studied food surfactants and protein/lipid materials. At FMBRA he has led the work on gas cell stabilisation in bread doughs and success of his work has promoted the use of these techniques to tackle other areas of research being carried out at the RA.

Mr. D. Smith, HNC, Technician.

**Contractor:**                   **15**

**Organization:**               **Gist Brocades N.V.**

**Project Manager:**           ***Ir. Johan J. Plijter***

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   **2600 MA Delft**  
   **THE NETHERLANDS**

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**Telefax:**                     **(31) 15 793 911**

**Involvement on a task by task basis**

- Task B.1.2    Components interactions: Physico-chemistry and functionality of wheat proteins
- Task B.1.3    Component interactions: Gluten hydration and interactions with other components
- Task B.1.5    Component interactions: Lipid interactions

**Personnel background and responsibilities**

Dr. J.D.R. Hille, Biochemist.  
Dr. J.J. Plijter, Biophysicist.  
Technical assistant

Associate: **16**

Organization: **AFRC-IFR (Institute of Food Research)**

Project Manager: *Dr. Ian J. Colquhoun*

Full address: AFRC Institute of Food Research  
Norwich Laboratory  
Norwich Research Park  
Colney  
Norwich NR4 7UA  
UK

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#### Involvement on a task by task basis

- Task B.1.3 Component interactions: Gluten hydration and interactions with other components
- Task B.2.1 Dynamics of dough development: Development of microscopic techniques for examining bread doughs
- Task B.2.2 Dynamics of dough development: Polyclonal and monoclonal antibodies to wheat pentosans
- Task B.2.3 Dynamics of dough development: Role of pentosans in the structure of dough and baked products
- Task B.2.4 Dynamics of dough development: Interactions between proteins, pentosans and lipids in doughs and baked products
- Task B.2.5 Dynamics of dough development: Effects of heat and mechanical work

#### Personnel background and responsibilities

Dr. P.S. Belton	BSc, PhD, Head of Norwich Laboratory.
Dr. M.R.A. Morgan	BSc, PhD, Head of Department of Food Molecular Biochemistry.
Dr. I.J. Colquhoun	MA, PhD, Head of Molecular Spectroscopy Group.
Dr. E.N.C. Mills	BSc, PhD, Biorecognition and Immunotechnology Group.
A. Grant	BSc, Molecular Spectroscopy Group.
S. Holden	Biorecognition and Immunotechnology Group.
Dr. M.L. Parker	BSc, PhD, Head of Electron Microscopy Group.

List of facilities, equipments and software to be used during the research programme

Spectroscopy	<ul style="list-style-type: none"><li>- 3 super conducting magnet multinuclear NMR systems.</li><li>- 3 Fourier transform infrared spectrometers.</li><li>- 1 Fourier transform Raman spectrometer.</li></ul>
Immunoassay	<ul style="list-style-type: none"><li>- Full facilities for producing monoclonal and polyclonal antibodies and methods for labelling these.</li></ul>
Microscopy	<ul style="list-style-type: none"><li>- 2 transmission electron microscopes.</li><li>- 1 scanning electron microscope.</li><li>- Light microscopes.</li></ul>
Software	<ul style="list-style-type: none"><li>- Programmes for analysis of relaxation time data and simulation of spectra and relaxation times.</li><li>- Data transfer software.</li></ul>

**Contractor:** 17

**Organization:** **TNO Biochemistry and Chemistry Institute**

**Project Managers:** Dr. *Robert J. Hamer* (Tasks A.1.1 and C.10)  
Ir. *Peeter L. Weegels* (Tasks A.1.2.1 and A.2.4)

**Full address:** TNO-CIVO  
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3700 AJ Zeist  
THE NETHERLANDS

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Involvement on a task by task basis

Task A.1.1 Processing properties of wheat: Milling quality

Task A.1.2.1 Gluten/starch separation: Use of enzymes

Task A.2.4 Applications of wheat products: Flour blends

Task C.10 Sprouting resistance

Personnel background, responsibilities, outline of facilities

Dr. R.J. Hamer,	- Biochemist, Head Biochemistry Physical Chemistry Department
Ir. M. Kelfkens,	- Scientist, Head of Cereals Section
Ing. A.M. van de Pijpekamp	- Technical assistant
Ing. W.J. Lichtendonk,	- Chemical assistant
T. Gröneveld,	- Chemical assistant
Ir. R. Orsel	- Physical chemist
Ir. P.L. Weegels,	- Biochemist
J.P. Marseille	- Technician
P. Bosveld	- Technician
Dr. H.P.M. van Laarhoven	- Biologist

The department of biochemistry and physical chemistry, derived from the former TNO Cereals, Flour and Bread Institute has since its foundation in 1946 gained an outstanding reputation in the field of Cereal Chemistry and Technology.

Dr. Hamer and his team have a broad experience in the field of chemical cereal analysis including proteins and non-starch polysaccharides.



Ir. Kelfkens has conducted research on the relation between kernel morphology and milling quality and developed models to evaluate the milling process. The milling department of the Institute has milling equipment at his disposal ranging from micro scale to pilot scale. TNO will carry out milling tests and will evaluate the milling quality of varieties. TNO will also assess the morphological characteristics of the wheats investigated and analyse the distribution of kernel constituents in milling fractions using biochemical techniques. Research will be complemented with work on milling quality carried out by FMBRA making use of image analysis. TNO will combine the results of both institutes as a basis for a milling assay.

Ir Kelfkens has also a broad experience in evaluating quality aspects of wheat from an agricultural and physiological point of view. The Institute has a broad experience (more than 25 years) on the assessment of sprout damages in various ways and has insights in its complexity.

Members of the team will carry out the work described in C.10 on sprouting resistance. This involves immunoassays for wheat germ agglutinin, abscisic acid, setting up a system to rapidly analyse amylase isoenzyme patterns. Analysis of large series of wheat samples, either from breeding stocks, controlled sprouting experiments or variety trials, in close co-operation and with assistance of breeding companies. Antibody fluorescent probe conjugates will be prepared and tested.

Associate: **18**

Organization: **Club des 5**

Project Manager: *M. Hervé Haslé*

Full address: GIE de Recherches Génétiques Céréales  
83 Avenue de la Grande Armée  
75782 Paris Cedex 16  
FRANCE

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Telefax: (33) 1 40 67 98 67

#### Involvement on a task by task basis

- Task C.1.1: Multilocal experiment and production of samples in controlled conditions: Southern Europe
- Task C.1.2: Multilocal experiment and production of samples in controlled conditions: North Western Europe
- Task C.2.1: Genotype  $\times$  environment interaction: Ecophysiological approach of the genotypic expression
- Task C.2.2: Genotype  $\times$  environment interaction: Experimentation in controlled environment

#### Personnel background, responsibilities, outline of facilities

Status: GIE (Groupement d'Intérêt Economique) Recherches Génétiques Céréales: 'Club des 5', created in April 1983.

President: Pierre Benoist, Plant breeder.

Members: The most important French plant breeders in wheat: about 60 % of the today wheat varieties in the French seed production.

1. - C.C. Benoist (wheat, barley, sunflower), 78700 Orgerus.
  - 2 wheat breeding stations: Orgerus (78) and Levet (18).
  - 35 breeders and technicians
  - Quality and diseases laboratories.
  - Main own wheat varieties today: Récital, Festival, Prinqual, Talent, Tango, Génial, Fortal. Durum wheat Ambral.

2. - Fl. Desprez (wheat, barley, oat, rye, beats, forage), 59242 Templeneuve
  - 3 wheat breeding stations: Cappelle (59), Jouy (28), Lectoure (32).
  - 50 breeders and technicians.
  - Quality and diseases and vitro methods laboratories.
  - Main own wheat varieties today: Soissons, Viking, Austerlitz, Aboukir, Rivoli.
  
3. - Rustica Seed Co. (wheat, barley, sunflower, maize, soybean), 31700 Blagnac.
  - Associated with GAE (wheat, barley), 91720 Maise.
  - 3 wheat breeding stations: Mondonville (31), Maise (91), Orchies (59).
  - 60 breeders and technicians.
  - Quality and diseases and vitro methods laboratories.
  - Main own wheat varieties today: Gala, Foison, Vizir, Ecrin, Festin (Rustica), Créneau (GAE), durum wheats Capdur, Primadur, Mondur (GAE)
  
4. - Serasem (wheat, barley, oat, rapeseed, pea).
  - of UNCAC/Ringot Group.
  - 3 wheat breeding stations: Premesques (59), La Brosse-Montceaux (77), Montbartier (82).
  - 40 breeders and technicians.
  - Quality and diseases and vitro methods laboratories.
  - Main own wheat varieties today: Baroudeur, Fandango, Artaban, Promentin, Centurion, Garant.
  
5. - Verneuil Seed Co. (wheat, barley, oat, forage, maize, sunflower), 77390 Verneuil-l'Étang.
  - 2 wheat breeding stations: Verneuil (77), Castelnaudary (11).
  - 40 breeders and technicians.
  - Quality and diseases and vitro methods laboratories.
  - Main own wheat varieties today: Thésée, Scipion, Darius, Caton, Pistou, But, Fief.

Associate: **19**

Organization: **AFRC-IACR (Institut of Arable Crops Research)**

Project Manager: Prof. *Peter S. Shewry*

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Long Ashton Research Station  
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UK

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Involvement on a task by task basis

Task B.1.1 Component interactions: Purification and characterization of gluten subfractions

Personnel background and responsibilities

Peter R. Shewry BSc, PhD, DSc, Head of Biochemistry Department

Arthur S. Tatham BSc, PhD, Senior Scientific Officer

List of facilities, equipments and software to be used during the research programme

- Glasshouse, controlled environment and field facilities for production of wheat under controlled conditions.
- Full range of equipment for protein separation and purification including electrophoresis, isoelectric focusing (analytical and preparative), HPLC, FPLC and conventional column chromatography.
- Range of modern centrifuges: new Beckman scanning attachment to be purchased for Sedimentation Equilibrium studies.
- Laser gel scanner with data handling.
- Mainframe (VAX 750) and personal computer (IBM opus) facilities.
- Facilities for amino acid analysis, N-terminal amino acid sequencing and peptide synthesis are available at the University of Bristol Molecular Recognition Centre.

Subcontractor:                   **22**

Organization:                   **Università di Padova**

Project Manager:                Prof. *Angelo D.B. Peruffo*

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 Università di Padova  
 Via Gradenigo 6  
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 ITALY

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 Telefax:                         (39) 49 807 0517

Involvement on a task by task basis

Task B.1.1      Component interactions: Purification and characterization of  
 gluten subfractions

Personnel background and responsibilities

Angelo D.B. Peruffo	- University degree in Biochemistry - Full Professor
Andrea Curioni	- Member of American Association of Cereal Chemists - University degree in Agricultural Sciences - Researcher.
Giovanna Pressi	- University degree in Biological Sciences - Associate Researcher.

List of facilities, equipments and software to be used during the research programme

- Electrophoretic apparatuses (Rotofor, ELFE, Transblot System)
- Equipments for chromatography, amino acid analyses, Uvimeter, densitometer, freeze-drier, centrifuges, etc.
- HPLC
- Hardware and software for recording and elaboration of data.

**Subcontractor:**                   **23**

**Organization:**                   **Università di Viterbo**

**Project Manager:**               **Prof. *Domenico Lafiandra***

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**Involvement on a task by task basis**

Task B.1.1    Component interactions: Purification and characterization of  
 gluten subfractions

Task C.4       Genetics of LMW glutenin subunits

**Personnel background and responsibilities**

Domenico Lafiandra	- Doctor degree in Chemistry, University of Bari. - Associate Professor Dept. of Agrobiology and Agrochemistry, University of Tuscia, Viterbo.
M. Ciaffi	- University degree in Agricultural Science, University of Perugia.
C. Tomassini	- University degree in Agricultural Science, University of Perugia.
Stefania Masci	- Doctoral Degree in Biological Science - Researcher

Subcontractor:                   **24**

Organization:                   **INIA**

Project Manager:                *Dr. Jorge de Juan Aracil*

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Telefax:                         (34) 56 18 23 52

Involvement on a task by task basis

Task C.1.1    Multilocal experiment and production of samples in controlled conditions: Southern Europe

Personnel background and responsibilities

Dr. Jorge de Juan Aracil   Head of Cereals Department

**Subcontractor:**                   **25**

**Organization:**                   **ENMP**

**Project Manager:**               **Dr. *Francisco Bagulho***

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**Involvement on a task by task basis**

- |            |  |
|------------|--|
| Task C.1.1 | Multilocal experiment and production of samples in controlled conditions: Southern Europe  |
| Task C.6   | Production and assessment of lines and near isogenic lines with different HMW glutenin subunit and gliadin compositions, and of null forms |

**Personnel background and responsibilities**

Francisco Bagulho - Cereal Breeder, Station Director  
Coordinator of Cereals National Programme in  
INIA (Portugal); qualified as 'Investigador Principal'  
He is the main responsible to the introduction  
of 25 new varieties in the National Catalogue.

**List of facilities, equipments and software to be used during the research programme**

- Fields and all the equipment to sow the material
- Electrophoresis equipments
- Software facilities (MSTAT)
- Chopin Alveograph



## **5. MANAGEMENT OF THE PROJECT**

## 5.1 - Organisation

The research programme has been organised as three interdependent subprogrammes, each of which is under the direction of a scientific programme manager:

1. Industrial Processes: (*Dr. R.J. Hamer*, TNO Biochemistry and Chemistry Institute, Zeist, The Netherlands).
2. Functional Components and their Interactions: (*Dr. J.J. Plijter*, Gist Brocades, Delft, The Netherlands).
3. Biochemical-Genetics and Physiology: (*Dr. N.E. Pogna*, Istituto Sperimentale per la Cerealicoltura, Milano, Italy)

*Dr. J.C. Autran* (INRA-IRTAC, Montpellier, France) will be the scientific coordinator. *Ms M. Richard* (IRTAC, Paris, France) will be in charge of the administrative aspects.

In addition, a **Scientific Management Committee** comprising Drs. J.C. Autran (INRA-IRTAC, Montpellier); R.J. Hamer (TNO, Zeist): subprogramme A; J.J. Plijter (Gist Brocades, Delft): subprogramme B; N.E. Pogna (Istituto Sperimentale per la Cerealicoltura, Milano): subprogramme C, has been set up. This Committee will be in charge of the cohesion of the whole Programme.

## 5.2 - Decision-making Processes

After the 10th month from the commencement date of the project will undergo the first assessment, and be followed by three further assessments every twelve months thereafter.

For each assessment, the progress of the project will be evaluated and compared to the expected timetable herein (see charts) and the overall objectives of the project. The completion of tasks, deliverables, etc. will be measured/announced as well as many unforeseen problems and delays. As a result of each assessment, the technical annex may be modified accordingly and if considered necessary to achieve the objectives of the project. Amendments may consist of changes in timing, tasks and roles.

## 5.3 - Reports

- All documents prepared in the frame of this contract shall be in English;
- They shall be prepared in a sufficient number of copies to be distributed to laboratories participating in the work and to the Commission;
- All documents shall indicate the contract number on the 1st page;
- Documents including newsletters, progress and final reports shall be typed on plain white paper with no logos or any other commercial indication;

- To limit retyping when preparing the final version of the various documents, all reports, newsletters, documents, etc., will be made using WORD or WORDPERFECT and be submitted in hard copy and diskette format.
- Progress reports and final reports should not exceed 100 pages.
- It is essential that all documents (newsletters, progress reports, final report) be prepared sufficiently in advance (please strictly follow the indicated schedule) to allow their examination by all participants before the various meetings and workshops are held.
- All publications resulting from the research will be submitted to the relevant subprogramme manager for approval, prior to publication. The Scientific Management Committee may also be consulted.
- All publications will clearly state that *"this research is in part supported by the Commission of the European Communities under the ECLAIR programme"*. The Commission will receive copies of all publications, reports, newsletters etc.

### **5.3.1 - Newsletters**

Every year, half-way between annual reports, a newsletter will be compiled by the Scientific Coordinator and distributed to all the participants. The Subprogramme Managers will seek the individual contributions to the newsletter from the respective subprogramme participants in not more than one page per participant. The newsletters will contain:

- progress of the work to date;
- main results obtained;
- any delays or deviations that occur which would require modifying the following period of reporting.

### **5.3.2 - Progress Reports**

Every year, an annual progress report will be compiled by the Scientific Coordinator and distributed to all the participants. The Subprogramme Managers will seek the individual contributions to the report from the respective subprogramme participants in not more than two pages per participant, including 1-2 tables or figures. The annual progress report will contain:

- a full description of the work carried out during the reporting period;
- the results obtained in relation to each task and subtask described in the charts;
- a critical evaluation of the progress (in relation to the objectives and deliverables);
- a short discussion of remaining problems and suggested modifications if required.

The progress reports will be produced for month 10, 22, 34, from the commencement date.

### **5.3.3 - Final Report**

A final report will be compiled from the annual reports and contain a detailed summary (maximum 10 pages) of the full report including the objectives and the main conclusions of the work.

The draft will be submitted within 2 months of the actual completion of the work.

## 5.4 - Collaborations between Subprogrammes

The organisation of the research programme as three interdependent subprogrammes was imagined to make easier the preparation of the project and its management. However, the three different approaches (industrial processes, physico-chemistry and biochemical-genetics) have to work in close connection. **A good coordination between subprogrammes** (cf. Scientific Management Committee § 5.2) and **a collaboration between research groups is an essential condition to ensure the success of the programme as a whole and to fill the gap between process development and its understanding in terms of wheat quality requirements and development of suitable breeding tests.** Any scientific problem identified in one subprogramme should benefit from the expertise of the other two, and cross actions should be developed every time it is possible.

A number of actions will be encouraged to improve further collaborations between subprogrammes:

### 5.4.1 Meetings

The organisation of meetings will be responsibility of the Subprogramme Managers and the Scientific Management Committee. All the participants in the project will be informed of all meetings, regardless of the subject and at least two months before the meeting date. Each meeting should be held at a different venue and promote the widest possible interaction between the participants. The minutes of all meetings will be published in the newsletter.

a) All the three **subprogrammes** will organise two meetings per year in alternating the meeting places. Each subprogramme manager will organise these meetings, that may include visits to different laboratories, etc., as deemed necessary and useful. These meetings should be primarily aimed at stimulating co-operation, exchanging information, preparing reports, comparing methodologies, etc.

b) **Technical meetings** or **cross groups meetings** will be organised on topics whose progress is essential and for which collaboration between subprogrammes must be intensified. For instance:

- HMW subunits of glutenin
- LMW subunits of glutenin
- Pentosanes/pentosanases
- Rheology

The technical meetings will also serve as pre-evaluation meetings for the Subprogramme Manager in order to prepare him for the following Scientific Management Committee meeting. Therefore, any modifications to the technical annex will be discussed and agreed on by the participants.

### c) Plenary Meetings of the Programme

At least two plenary meetings of the programme will be organised. The first one in the course of the second year and the second one near the end of the programme to help in

clearing the major results obtained and drawing the main conclusions in view to the final report.

#### **5.4.2 Availability and Evaluation of Samples**

The problem of wheat samples is of capital importance. 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 sets of samples for some studies.

It is essential 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 point because technological tests cost money, especially those performed at a pilot or industrial scale. These cannot be performed indiscriminately on all available samples. It is also an essential point because a major objective of this ECLAIR 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 physico-chemical bases of new technologies (biscuit-making, wholemeal breadmaking, gluten/starch separation, 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.

#### **5.4.3 Books of Methods**

Two books of methods will be completed. A book of quality related methods will be set up by participants to subprogramme A and a book of biochemical procedures will be set up by participants to subprogramme B. Both books will be distributed to each partner. To make updating easier, it is recommended to have these books transferred into a word processor form.

#### **5.4.4 Profile Sheets of Participants**

To make clear which are the aims in the different partners of the programme, and to make the collaborations easier, a book of profile sheets of participants will be prepared. Based on the updated content of the technical annex, this set of record cards will contain the address, phone, fax, languages spoken and picture of each participant, with a short description of the field of expertise (key words), so that everybody can easily know and contact the relevant person for any problem and can detect where the other subprogrammes are the most supportive.

## Subprogramme A: INDUSTRIAL PROCESSES

Subprogramme A consists of two parts: A1 and A2. Part A1 is aimed at **relating processing characteristics to wheat characteristics**. Part A2 is aimed at developing **new applications of wheat**.

### A.1 - PROCESSING PROPERTIES OF WHEAT

A quality factor of underestimated economic significance is **milling quality** (A.1.1). Project A.1.1 tackles the problems both from a breeders as for a growers and trade points of view. The breeder needs new and improved criteria for selection, the trader needs reliable and rapid tools to evaluate quality of wheat lots. Subtasks A.1.2.1 and A.1.2.2 deal with an important new outlet of wheat, its **processing by the wheat starch industry**. This has been demonstrated to imply requirements for the quality of wheat other than for bread-making. Nevertheless the use of wheat in this respect is hampered by large variations in processing quality and losses in wastage and waste water treatment. Project A.1.2.1 will investigate new possibilities for correcting variations in processing quality, project A.1.2.2 will investigate the possibility of using whole wheat flour as a starting material for wheat starch production.

#### Task A.1.1: MILLING QUALITY (Partners 14, 17)

##### Starting point

Although a considerable effort is and has been devoted to the improvement of wheat in terms of bread-making quality, another quality criterion, milling quality, has had minor attention. Milling quality has been shown to vary greatly between different wheat varieties (up to 10 % variation in yield). Also seasonal effects are present. Milling quality can be defined as the ease with which kernels can be milled into flour and the yield of pure white flour that can be extracted from the kernels. The factors determining milling quality are complex. At present attempts at prediction are made by reference to several factors. These include visual appearance (discolouring), specific weight and grain hardness. Their use is however severely restricted and unsuitable for selection of new varieties. The first estimation of milling quality is usually made when selection is almost finished, that is when varieties enter government trials. Until now little research has been aimed at discovering determinants of milling quality. This is mainly due to the limited choice of kernel material and methodological limitations to tackle this complex topic. Recently in Partners 14 and 17 preliminary studies have been carried out to discover kernel factors related to milling quality. In this study advanced techniques have been used to characterize kernel morphology (image analysis) and to characterize milling in terms of an empirical model containing bran friability and endosperm-bran interaction factors. This has led to new and improved

ways of understanding and predicting milling quality. Since these studies are based on a limited number of samples and relied upon milling data from laboratory millings, these results need to be corroborated experimentally and validated using results from commercial mills.

### Objectives

The present project is aimed at:

- providing a wider and more commercially relevant experimental basis for the prediction of Milling quality using image analysis; and further at:
- identifying the relative importance and nature of factors determining milling quality (genetic or environmental);
- producing a method for predicting milling extraction rate.

### Experimental approach

- a. Establish the factors of milling in terms of bran friability and other milling factors.

Wheat varieties will be selected from all relevant commercial sources in the EC as well as from collections of wheat breeding companies. Selection criteria are grain hardness (as measured by their content of friabilin) and kernel shape (as measured by image analysis). 20-30 wheat varieties will be collected during two further harvesting years in order to study year-to-year variation in milling quality and to distinguish between genetic and/or environmental effects (Partners 14 and 17). Well characterized wheat lots will be milled into flour under highly standardized conditions by a Buhler experimental mill (Partner 17 with commercial flour mill). Wheat will be evaluated by current quality tests such as specific weight, protein content, Falling Number, etc. (Partner 14, 17). The different flour fractions will be biochemically characterized in terms of markers for the different anatomical components of the kernel (Partner 17). This will lead to a detailed description of the distribution of each anatomical component (*e.g.* bran, endosperm, aleurone layer) in the different milling fractions. The methods applied should be very accurate. Potential markers are phytic acid, ferulic acid and lignin, to be determined with HPLC or colorimetric methods.

- b. Estimation of the contribution of kernel features to milling quality by means of image analysis.

Good progress has been made within the programme, towards establishing the grain size threshold below which endosperm content is significantly reduced. The threshold is lower than was previously largely believed to be the case. The sensitivity of image analysis techniques demonstrated even on low magnification images will be exploited in identification within samples of sub-standard grains and non-wheat inclusions. Relationships detected milling behaviour of wheats from different parts of Europe and their morphological characteristics will be further examined.

More detailed assessments of anatomical characteristics will continue so that these can be related to the modelling results described in (a) above.

- c. Statistical evaluation.

At this stage, results obtained in phase 1 and 2 will be analysed statistically in order to reveal possible correlations between kernel morphological characteristics, biochemical composition, milling factors and milling yield (Partner 17). This will lead to a predictive model for milling quality.

d. Development of a predictive test for milling quality.

Exploratory work will begin to evaluate how the 'research' image analysis system can be developed to provide a suitable system for commercial use by millers and grain traders (Partner 14). This will require robustness, ease of operation and reliability coupled with low capital costs. The needs for breeders will also be considered. Their requirements include the use of small sample sizes, the need for non-destructive analysis and possible evaluation of pre-ripe material. Some preliminary tasks will be performed to enable a reduced operator time. When developed this method will be tested by flour millers and breeding companies in the EC (flour millers and breeding companies throughout the EC are associated with this programme through labs 14 and 17).

Possible benefit to European Industry and Agriculture

The ability to select wheat on the basis of a simple test will enable milling quality to be used as a selection criteria in breeding programmes. Further, this test will enable the selection of wheat at intake. This will offer savings of verification and experimentation and ensure purchase of stocks from which extraction of the highest value products can be maximised.

Manpower

Partner 14	18 man-months
Partner 17	21 man-months

Deliverables

- Completion milling studies, providing figures for statistical analysis, evaluations at end of year 1 and 2;
- Development of markers to estimate milling factors. Evaluation of possibilities. Completion at year 1;
- Development of image analysis, evaluation of technical possibilities at end of year 1. Completion of image analysis system in year 2;
- Statistical evaluation of the results of phase A and B, leading to predictive model, evaluation at 2nd quarter of year 3;
- Development of predictive test, evaluation and completion at end of year 4.

Interdependence with other tasks

See 'Diagram of Interdependence'

Duration



48 months from month 1 to month 48

## **6. DETAILED DESCRIPTION OF TASKS**

## Task A.1.2 - GLUTEN/STARCH SEPARATION (Partners 17, 13)

The wheat starch industry is currently the fastest growing wheat processing industry in the EC. The processing of wheat into wheat starch and gluten signifies a growing alternative outlet for EC grown wheat. This attractive new application is however not without practical problems. At present, the processing of wheat stands for increased wastage, increased costs for waste water treatment and problems with raw material selection due to strong variations in processing properties between different wheat varieties and harvesting years. These problems put the attractiveness of wheat as a raw material for the starch industry at risk. It is therefore urgent to study the process of starch/gluten separation in detail, to control this process (1.2.1) and to enable the successful implementation of new processes (1.2.2). Project 1.2.1 is carried out by Partner 17, project 1.2.2 by Partner 13. Both laboratories will collaborate by using the same wheat lots and by exchanging experimental results.

### Subtask A.1.2.1 - Improved Separation of Gluten and Starch through the Use of Enzymes (Partner 17)

#### Starting point

The rate of gluten coagulation (RGC) is one of the most important aspects governing starch/gluten separation. Recent studies at Partner 17 have identified a role of wheat hemicellulose in determining the RGC: modification of hemicellulose by adding hemicellulases strongly affected the RGC. At this moment it is not clear by what mechanism hemicelluloses or - in general - non starch polysaccharides operate and to what extent enzymes can be used as processing aids in starch/gluten separation.

#### Objectives

This project is aimed at:

- obtaining a better understanding of the role of non starch polysaccharides in starch/gluten separation
- obtaining a better understanding of the mechanism of action of hemicellulases in starch/gluten separation;
- evaluating the use of enzymes as processing aids.

#### Experimental approach

a. Study of hemicellulolytic enzymes in a simplified model.

Selected commercially available and purified hemicellulolytic enzymes will be characterized biochemically and tested in a simplified system for gluten protein coagulation. Tests will be performed at different addition levels with or without added purified wheat pentosans / hemicellulose. Gluten coagulation will be estimated by

turbidity measurements and changes in glutenin solubility in SDS. Enzyme action will be evaluated from HPLC/GLC carbohydrate analyses. Also viscosity measurements will be performed.

b. Validation of model experiments.

Separations will be carried out on pilot scale to test the results obtained with the model system. Again, separations will be accompanied by biochemical analyses. The results of (a) and (b) will lead to a theoretical model describing the role of pentosans / hemicellulose in gluten coagulation.

c. Correcting differences in processing properties. Model studies.

Processing properties can vary strongly from one wheat variety to another or from one harvesting year to another. Varieties representing the wheat currently used within the EC for starch/gluten processing will be milled into flour, characterized and tested in the model system. Using model experiments, enzymes and dosages will be selected to standardize the RGC.

d. Correcting differences in processing properties. Pilot experiments.

The validity of the experiments outlined under (c) will be checked on a pilot scale. This allows also determination of quality and yields of gluten and starch and amounts of wastage produced.

Possible benefit to European Industry and Agriculture

The EC has always been confronted with strong variations in the quality of wheat. This limits their application in more than one way. If it proves possible to correct for these variations this strongly improves the economy of EC wheats by enabling the use of locally grown wheats and minimizing wastage.

Manpower

Partner 17                      16 man-months

Deliverables

- Results of small scale separations, first theory on role of hemicellulose / pentosans: corrections model I;
- Results of pilot scale separations, confirmation / correction of model I, resulting in correction model II;
- Validation of model II using commercial flours, ending in final correction model enabling and demonstrating an improved separation of gluten and starch.

Interdependence with other tasks

See 'Diagram of Interdependence'

Duration

48 months from month 1 to month 48

<b>Subtask A.1.2.2 - Characterization of Wheat Gluten Produced by New Separation Processes (Partner 13)</b>
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### Starting point

As already mentioned, one effect of the EC agricultural policy is that wheat is now preferred to corn as raw material for starch extraction. This has led to a considerable expansion of wheat starch and gluten production which has, in turn, necessitated the expansion of wheat starch factories and the building of new ones. A result is the requirement for advanced processing technologies capable of economically processing the raw material, hitherto only wheat flour.

The primary disadvantage of all current wheat starch processes are the high costs involved in the dry substance recovery as well as the excessively high process water consumption. Neither the techniques employed for effluent disposal nor for the recovery of the dissolved and suspended dry substance are anywhere near cost effective. Therefore, the most meaningful approach to overcome the existing problems in wheat starch production is a restructuring of the process. This should be possible on the basis of the technical progress made over the past decade in gluten agglomeration by high-pressure homogenisation and the separation of starch and gluten using centrifuges. This may even open ways to directly process wholemeal flours.

For this purpose, such a process has been recently developed on a laboratory scale. The process is designed to constantly process 1 kg wholemeal or white flour per hour. All the necessary trials to evaluate the advantages of the process can now be carried out. The experiments are intended to demonstrate that the concentration of the process water circuit can be significantly increased while maintaining purity, yield and characteristics of the end-products. According to the results gained thus far, one side-effect of this process is, that the gluten extracted from wholemeal flour differs in its main rheological characteristics from gluten washed out of white flour. Up till now, neither the cause is known which affects the deviation of these characteristics, nor whether it can be influenced by the parameters. Therefore it is extremely important to investigate the influence of wheat quality characteristics and the process parameters on the characteristics of the separated gluten. As long as no further knowledge is available on the development of the gluten characteristics the implementation of this newly developed process in industry is at risk.

This is mainly due to the fact, that wheat can only compete with corn as a raw material if its extractable constituents enable a comparable profitability to those extracted from corn. With regard to profitability, it is interesting to note that the competitive capacity of wheat starch factories as opposed to corn starch factories is largely guaranteed by the demand of the baking industry for vital gluten. Concerning dough mixing and bread-making, the gluten must therefore have specific quality characteristics which are related to its water binding capacity, rehydration rate, and elasticity.

The change in gluten characteristics may at least partly depend on enzymatic activities, mainly from proteases, which in wholemeal flour are much higher than in white flour.

## Objectives

This project is aimed at:

- investigating the causes for these differences; and subsequently:
- understanding the effect of process parameters on gluten viscoelastic properties.

There is an urgent need to perform this research work as at this moment large technological changes are taking place within the EC wheat starch industry.

## Experimental approach

### a. Production of gluten on laboratory scale.

An existing laboratory scale system will be reconstructed in order to improve the reproducibility of the gluten recovery and to shorten the residence time of the mass in the system by technical means. 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. The equipment will be designed to process about 2 kg flour per hour.

### b. Separation of different wheat varieties, analysis.

Different wheat lots representing both wheat commonly used in the European wheat starch industry as well as lower quality EC wheats will be analysed for their contents of protein (water soluble/insoluble), starch and non-starch polysaccharides. These will be processed using the laboratory separation system. The resulting gluten fractions and samples of process waters will be freeze-dried for further relevant physical and chemical characterization. In this connection determination of proteases in the freeze-dried fractions is of paramount importance. Their action can be best evaluated from changes in the soluble proteins of the process water.

### c. Physico-chemical evaluation.

Gluten fractions obtained with both types of processes (*i.e.* process with white flour and process with wholemeal) will be compared in detail using physico-chemical analyses. Selected fractions will undergo more extensive biochemical characterization (protein composition, protein amino acid composition, content of starch and non-starch polysaccharides). The results of a and b will possibly lead to the discovery of a systematic relationships between wheat processing properties and the composition of raw materials as well as the physico-chemical properties of the resulting gluten.

## Possible benefit to European Industry and Agriculture

The introduction of a new process for wheat starch production would considerably improve the economic situation of the EC wheat starch industry. The existing waste water problem, one of the main disadvantages of using wheat instead of corn or maize as a raw material could be solved.

As mentioned in 1.4.1, samples of wheat (flour) will be exchanged and results will be compared.

Manpower:

Partner 13                      36 man-months

Deliverables

- Laboratory scale separation system; initial evaluation;
- Laboratory scale separations, comparisons with conventional separations (with Partner 17), collection of fractionated flours;
- Optimized process, compositional characteristics, qualities and physico-chemical characteristics of gluten fractions obtained from white and wholemeal flours;
- Evaluation of possible differences between gluten produced from white or wholemeal flours. Insight in the nature of these differences and possibilities to eliminate quality differences;

Interdependence with other tasks

See 'Diagram of Interdependence'

Duration

36 months from month 1 to month 36



## A.2 - APPLICATIONS OF WHEAT PRODUCTS

Most of the current research and breeding is devoted to one sole purpose: the production of wheat (flour) for white bread-making. Changes in bread consumption from white bread to whole meal bread, development of a wide range of bread and baked products have made a reorientation in this respect necessary. Processing properties of whole meals and selection criteria for wheat differ widely from those of white flour. Definition of processing requirements of wheats and translation of these processing requirements into characteristics of wheat (constituents) is a new and better approach to evaluate and improve the application of wheat in Europe. Processing requirements can differ greatly due to the wide variation in manufacturing processes and formulations. In sections 2.1 to 2.6 industrial and industry research groups from France, Germany, Great-Britain, Holland and Italy will perform a joint effort. Sections 2.1 and 2.2 and 2.3 deal with processing requirements of wheats for wholemeal and white bread-making. Section 2.4 is focused on the typical aspects of flour blending and supports work in Section 2.1 with regard to the suitability of very strong flours to 'carry' weaker types. Although blending of flours is common practice, this forms a completely new area of research, but nevertheless, a very important one in view of the inferior and unpredictable processing properties of 100 % EC blends (with or without gluten) as compared to EC flours with USA/Canadian flours. Section 2.3 is concerned with the rheological characterization and subsequent technological evaluation of white bread and whole wheat bread formulas. Here flours and mixtures of flour with isolated wheat fractions originating from other parts of the programme (*i.e.* subprogramme B and subprogramme A, section 2.4) will be characterized using advanced and improved rheological techniques in part 2.5. In this part also the knowledge on specific differences in processing requirements will be further extended to sweet leavened products (high sugar, high lipids) and products with sour starters. Fermented products from wheat also form a growing market having its own requirements. Improved understanding of the interaction of starter cultures with wheat flour components and improved applications of wheat flour in fermented products are the aims of Section 2.6.

**Task A.2.1/2 - THE CHARACTERISTICS AND PROCESSING REQUIREMENTS OF WHEAT FOR SPECIFIC END USES: WHITE BREAD AND WHOLEMEAL BREAD (Partner 14)**

In response to results obtained in the first year of the project, Task A.2.2 wholemeal bread was amalgamated with task A.2.1 white bread with the agreement of the subprogramme A manager with effect from April 1991. A common set of wheat samples has been used in subsequent work and results have been communicated in single reports covering both tasks.

**A.2.1 - White bread**

Starting point

In different parts of Europe grists become dominated by single wheat varieties. Farmers choose wheat varieties on the basis of agronomic performance characteristics as well as quality for a particular end use. If crops are going to be dominated by single varieties it may not necessarily be the most desirable quality for the particular intended end use. As a result of the already well developed knowledge of the role of certain gluten proteins in determining bread-making potential new, allegedly 'very strong', varieties are beginning to emerge from breeding programmes (*e.g.* the variety Fresco). Apart from the fact that these varieties have not been primarily selected on other very important criteria such as milling quality, it is very well possible that these varieties are not compatible with contemporary baking equipment and bread-making processes. As has been experienced before with the very strong variety Glenlea this may render new varieties completely unsuitable. In fact, it has already been established that Fresco gives dough with excessively high work input requirements during mixing. On the other hand the exceptional characteristics of these new wheat varieties merit investigation of the underlying physico-chemical reasons. In this respect the project is closely associated with the projects on binary mixtures (Section 2.4: 'Processing properties of wheat flour blends').

Objectives

The present project has the following aims:

- To assess the ability of these new 'strong' gluten type varieties to carry varieties with weaker gluten characteristics, and to determine the extent to which the work input requirements of such blends are compatible with contemporary baking equipment and bread-making processes;
- To determine the underlying physico-chemical reasons for these differences in gluten strength and bread-making quality and thus provide feedback that will focus to plant breeding programmes aimed at producing wheats with superior bread-making quality;
- To develop small scale tests suitable for use in plant breeding or in grain trading that are capable of differentiating between wheats with different gluten properties.

### Experimental approach

a. A new machine for measurement of work input during bread dough mixing has been designed and built at Partner 14 which will provide more precise data on work input levels than has been possible hitherto. The machine is being used to determine the work input requirements of a selected range of single bread wheat varieties for producing bread of optimum quality. The varieties include the new allegedly very strong gluten types as well as conventional types. The investigation is being repeated on wheat from more than one harvesting year and from different growing regions. The potential of the new stronger gluten varieties to carry established wheats with weak gluten properties in flours milled with binary blends of such wheats and the effect on work input requirement will be determined. This work will be carried out in conjunction with project 2.4 carried out at Partner 17.

b. Doughs and isolated glutens will be analysed by advanced rheological techniques using a Bohlin parallel plate Rheometer in both oscillatory and 'creep' modes. This should define in physical terms the rheological parameters associated with good bread-making quality. Similar experiments will be performed on doughs and glutens from the binary blends of strong and weak gluten wheats.

c. Biochemical analysis to determine the molecular basis for variation in gluten strength will include measurements of SDS insoluble 'gel protein' amount and its dynamics during mixing in the presence and absence of oxidizing improvers used in bread-making. Size exclusion HPLC will be used to determine the relationship of gluten molecular size distribution in flour and its dynamics during mixing to variation in gluten strength. The ratios of glutenin to gliadin proteins in the gluten proteins will be determined using selective extraction techniques, and new techniques for quantifying different classes of glutenin polypeptides will also be applied as well as modern chromatographic (RP-HPLC) techniques for quantifying individual polypeptides. This work will be carried out in conjunction with project 2.4 carried out at Partner 17.

d. Based on the results obtained attempts will be made to devise rapid and simple small scale tests that can be used at the early stages in breeding programmes to identify wheats with gluten characteristics appropriate for superior bread-making quality.

e. Data accumulated from the processing requirements, and biochemical and rheological properties of UK and Continental European wheat varieties will be assembled into a wheat quality data base including both white and wholemeal bread. This will form a significant contribution to a wider dossier on wheat varieties in the UK and Continental Europe, that will be of benefit to the EC generally. Data generated by other ECLAIR partners on common sample sets including those from subprogramme C could be included.

It is hoped and intended that the data base will expand as new varieties are tested. The data base will be a significant deliverable from the processing requirements subprogramme within the ECLAIR AGRE 0052 programme.

### Possible benefit to European Industry and Agriculture

The investigations will lead to a better definition of the type of processing properties required in newly bred wheat varieties. This may lead to final elimination of non-EC wheat imports, to still greater use of the EC wheat crop to elimination of the need for gluten supplementation and to provision of cheaper yet still high quality flours to EC processors.

### Manpower

Partner 14                      62 man-months

### Interdependence with other tasks

See 'Diagram of Interdependence'

### Duration

48 months from month 1 to month 48

<b>A.2.2 - Wholemeal bread</b>
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### Starting point

Whereas most research has been devoted to improving wheat varieties in terms of white-bread making quality, current consumers trends throughout the EC show a growing preference for consumption of wholemeal breads. There are also indications from work done in the USA that the wholemeal bread-making quality of different wheat varieties may not be predicted accurately by making quality assessments of white flours milled from these same varieties. Furthermore assessments of the bread-making potential of wheat varieties in many countries do not include tests using wholemeal bread-making procedures.

### Objectives

To define the factors that affect wholemeal bread-making quality of flours, distinguishing between effects of milling procedures on flour performance and those resulting from the distribution and composition of anatomical components.

### Experimental approach

a. Earlier work has established the key milling procedures for producing optimum wholemeal flour, and the most appropriate processing conditions for bread-making for EC wheats. The range of, and the principles governing, the variability of flours from wheats of different regions of the EC and other sources (e.g. North America) will be examined and compared with those exhibited by the corresponding white flours.

b. Overlapping with these studies techniques will be developed for separating wholemeal into well-defined fractions such as endosperm, bran and germ. Baking experiments or dough expansion experiments will be carried out involving interchanging fractions between wheat samples of good and poor quality. This will lead to the identification of anatomical components in which the factors responsible for variation reside.

c. The next steps to identify the biochemical factors within the anatomical components that may be responsible for variation in quality. To this end fractions will be extensively characterized using a series of advanced biochemical techniques (*e.g.* HPLC, differential pulse polarography). The research should establish the biochemical basis of variation in wholemeal bread-making quality and thereby indicate how methods might be devised for differentiating good and poor quality wheat samples and possibly indicating what targets should be set in breeding for good wholemeal bread-making performance.

#### Possible benefit to European Industry and Agriculture

Identification of factors responsible for variation in wholemeal bread-making quality will lead to better methods of raw material selection as well as to an enhanced ability to select varieties during plant breeding. A long term benefit may be an increased consumption of wholemeal bread which is generally recognized as being of significant benefit in relation to long-term health.

#### Manpower

Partner 14                      41 man-months

#### Interdependence with other tasks

See 'Diagram of Interdependence'

#### Duration

48 months from month 1 to month 48

**Task A.2.3 - EVALUATION OF TECHNOLOGICAL  
FUNCTIONALITY OF WHEAT FLOURS AND PROTEIN  
FRACTIONS IN BAKED PRODUCTS (Partners 08, 07M)**

Starting point

The widespread use of EC wheats in the production of breads and other baked products is hampered by a number of factors. Quality and variation in quality are two important causes, inadequate definition of exact requirements is without doubt a third important factor. It is necessary to define precisely the type of wheat a baking industry needs for each country, for each type of product. Today this is virtually impossible due to a lack of knowledge on the factors which govern the technological ability of a flour for a given application. Current research has shown the importance of certain flour constituents (proteins, lipids, non-starch polysaccharides) and their interactions. It is not known however which are the important interactions for a certain type of product and a certain type of manufacturing process. In parts 2.1 and 2.2 attention is given to two important applications of wheat: whole wheat breads and breads from four blends. In this part this work will be extended to biscuits.

Objectives

During next years our aim will be **the definition of an optimal biscuit flour.**

Experimental approach

We plan the following tasks:

1. On the important parameters we have already identified we will precise the ranges we need to make a good quality biscuit.
2. To improve our understanding of the relations between ash content, pentosan content, damaged starch content, granulation and extraction rate.

This will be facilitated by closer collaborations with other partners of subprogramme already working on milling quality (task A.1.1 - Partners 17 (TNO) and 14 (FMBRA))

3. To improve our understanding of relations between quality of protein and quality of biscuits. Relationships between mixing conditions and nature of interactions involving proteins will be determined using sequential extraction procedures, gel protein determination and high-pressure liquid chromatography (SE-HPLC).

Collaborations with Partner 14 (FMBRA) on gel protein would help us for achieving this task.

4. Test of some samples coming from Subprogramme C (from the North-Western Europe network only).

Possible benefit to European Industry and Agriculture

Improvement of the knowledge of the process. Due to this fact a possibility to increase productivity, quality and creation of new products.

### Manpower

Partner 07M	16 man-months
Partner 08	42 man-months

### Deliverables

- Collection of flour samples;
- Rheological characters of flours and flour blends;
- Technological characters of flours from lab scale and pilot scale experiments;
- Related product quality characters;
- Definition of processing requirements with special reference to biscuits and breads.
- Relation between molecular size distribution of proteins in flour and its dynamics during mixing and biscuit quality.

### Interdependence with other tasks

See 'Diagram of Interdependence'

### Duration

48 months from month 1 to month 48

**Task A.2.4 - PROCESSING PROPERTIES OF FLOUR BLENDS.  
PREDICTION AND IMPROVEMENT (Partner 17)**

### Starting point

Apart from problems with processing properties as discussed in task A.2.1, it proves to be very difficult to standardize and control quality. Effects of climate and cultivation strongly influence the properties and availability of EC wheats. These EC wheats have to be blended to obtain flours with the desired bread-making quality. It has proved to be virtually impossible to predict and optimize the processing properties of a blend from year-to-year on the basis of the properties of its components. Dough properties are in large part determined by interactions between gliadins, glutenins on the one hand and hemicellulose, starch and lipids on the other.

### Objectives

Task A.2.4 has therefore the following aims:

- To improve the knowledge of gluten composition in relation to dough functional characteristics in order:
- To improve the quality of EC flour blends in terms of dough (processing) properties;
- To compensate for year-to-year variations in flour quality.

### Experimental approach

a. Three EC grown wheat varieties (A, B, C) which are normally used in flour blends for bread-making will be selected on the basis of differences in rheological properties and/or on the basis of advice from flour millers. Biochemical characterization will comprise analysis of glutenins and gliadins (RP-HPLC, electrophoretic techniques), endogenous enzyme activities (amylase, ascorbic acid dehydrogenase, oxido-reductase, protease), non-starch polysaccharide composition and content and other standard parameters. Biochemical processes occurring during mixing and resting will be studied in detail. Physico-chemical analysis will consist of rheological characterization (Brabender Extensograph, Farinograph, Bohlin rheometer).

b. Two series of flour blends will be prepared. Each time taking one flour (B) as base and flour A or C as the blending flour. Blends will be characterized in terms of bread-making quality and rheological properties. Using statistical analysis these data will be corroborated with blend biochemical or physico-chemical properties thereby identifying which biochemical features control processing properties.

c. Some flour components identified in (b) will be isolated on a large scale. For this purpose a pilot starch/gluten separation system, developed at Partner 17 and recently published will be used in combination with large scale protein fractionation (BioPilot) techniques developed at Partner 17.

d. Isolated fractions will be used to study systematically the changes in the processing properties of the base flour after addition of the different flour or flour components. In



this way confirmation can be obtained of the results of the statistical studies in part b. The results from parts b and d will be used to design a predictive model for the processing properties of flour blends.

e. In the last part of the study the model will be validated through the analysis of a number of commercial flour blends. The model will be used to predict year-to-year variations in flour processing properties on the basis of biochemical and/or physico-chemical characteristics. Furthermore attempts will be made to improve further processing properties of flour following a blending strategy as dictated by the model developed in d).

#### Possible benefit to European Industry and Agriculture

The combined results of the fundamental and applied studies will provide a sound basis for understanding the effects of blending and gluten addition on dough properties. This will in turn provide new and better ways of controlling and improving processing properties in a selective and directed way. Also valuable information will be obtained with regard to the possibilities of emerging new wheat varieties and requirements towards future wheat varieties.

#### Manpower

Partner 17                      59 man-months

#### Deliverables

- Collection of wheat varieties and flour samples, well characterized biochemically and physico-chemically;
- Physico-chemical characters of doughs prepared from a series of binary flour blends; insight in properties of gluten matrix and its development;
- Isolated flour components and wheat protein fractions;
- First statistical model relating rheological properties of the constituent flours;
- Results of confirmation / extension experiments using blends of a base flour with isolated fractions;
- Prediction model;
- Results of validation experiments using commercially blended flours.

#### Interdependence with other tasks

See 'Diagram of Interdependence'

#### Duration

48 months from month 1 to month 48

**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)**

Without doubt one of the main problems limiting the use of EC wheats is the lack of knowledge on processing requirements for specific uses. Most of the current understanding of processing requirements is related to white bread-making. And even this understanding is very limited as discussed in Section 2.1. With regard to other bread formulas (particularly wholemeal bread, but also French, Italian and British formulations) and their characteristics differences in terms of required wheat characters no common understanding exists. In order to use and adapt EC wheats for this wide range of applications it is vital to obtain this understanding by relating processing requirements to physico-chemical wheat parameters. It is well accepted that rheological techniques can play a central role in obtaining this understanding. However, the equipment generally used for this purpose (*i.e.* Brabender Farinograph, Extensograph, Chopin Alveograph) lacks standardization and in all cases gives data on combinations of physical parameters instead of enabling the calculation of single physical parameters. This severely limits their use other than for local standardization purposes and quality control.

In part 2.5 the work described in section 2.3. is further extended to samples obtained from subprogramme C.

Further, next to Italian bread formulas attention is given to new classes of baked products, leavened sweet bakery products containing high amount of sugar and lipids and to products containing sour starters.

#### Experimental approach

- a. Collection and preparation of samples. Wheat samples are obtained from the participants of subprogramme C. Samples need to be cleaned, milled and characterized on common parameters (protein, ash, falling number, etc.).
- b. Rheological measurements. Flour samples will be measured using dynamic oscillatory measurements enabling the determination of storage and loss moduli ( $G'$  and  $G''$  respectively). This work also contains evaluation of small scale sample preparation procedures (mixing time, effect of mixing energy, rest time, etc.).
- c. Technological evaluation. Selected samples will be evaluated in a number of baking tests. Protocols will be developed to evaluate the suitability of wheats for the preparation of sweet leavened products, breads and products with lyophilized lactobacilli under highly standardized conditions. Products will be also evaluated in terms of sensorial properties and keeping quality.
- d. Exchange and testing of samples. In other sections of chapter 2. 'Applications of wheat products' samples of wheat are tested and characterized extensively. A number of these samples will be exchanged and evaluated.

e. Correlation of analytical, rheological and technological data. Results of tasks b, c and d will be statistically evaluated to identify specific relations between wheat analytical characters (information obtained from subprogramme C), flour rheological characters and data from the technological evaluation. Special attention will be given to identify these factors which are related to specific differences between products (*i.e.* where do processing requirements for sour doughs differ from processing requirements for sweet leavened products ?).

#### Possible benefit to European Industry and Agriculture.

A clear view on processing criteria in terms of wheat quality characters enables optimization of the use of current EC wheat varieties and stimulates the development of varieties better adapted to industrial requirements.

#### Manpower

Partner 04                      72 man-months

#### Deliverables

- Collection of flour samples;
- Small scale rheological testing protocol;
- Rheological characters of flours samples;
- Small scale technological testing protocol;
- Technological characters of flours from baking tests;
- Related product quality characters;
- Correlation of data;
- Definition of processing requirements with special reference to sweet bakery products and products with sour starters.

#### Interdependence with other tasks

See 'Diagram of Interdependence'

#### Duration

48 months from month 1 to month 48

**Task A.2.6 - INTERACTIONS BETWEEN SELECTED MICRO-ORGANISMS AND WHEAT FLOUR COMPONENTS AND THEIR APPLICATION TO IMPROVE BREADMAKING PROCESSES (Partner 12)**

Starting point

In the near future both in the EC and in the USA the market for fermented products will grow rapidly. The European consumer welcomes these products which are often marketed as having a higher nutritional value and good organoleptic. Still, little is known of the complex interaction between micro-organisms and wheat flour components. This interaction is of vital importance since flour components can promote, modulate or even inhibit metabolic activities of micro-organisms. Project 2.6. is directed at understanding the interaction between micro-organisms and wheat flour doughs. Results will furnish valuable background for a better understanding of complex wheat flour dough systems, and will allow the development of a rational scientific basis for improving baked products.

Objectives

The selection of micro-organisms with suitable functional properties and the establishment of optimized and controlled process conditions, are the key to assure the production of variety bread with the level and uniformity of quality required at present. In this sense the knowledge of the interaction between selected micro-organisms and wheat flour components is of relevant importance for a controlled fermentation.

Experimental approach

Studies will be carried out in model systems and standard doughs prepared with Spanish and EC selected wheat flours from the project, selected pure micro-organisms and commercial yeast.

a. Biochemical changes in flour components and fermentation metabolites.

This part of the project is aimed at studying the effect of metabolic activities of micro-organisms, yeast and lactic acid bacteria, from sour and bread doughs, on various flour components. The effects on flour carbohydrates and nitrogenous fractions, as well as fermentation catabolites closely related to dough behaviour will be studied systematically, using different biochemical techniques.

b. Specific enzyme activities.

Enzyme activities, mainly proteinase activities, are of great importance in bread-making. Flour endogenous enzymes as well as those from selected micro-organisms will be studied for their positive or deleterious actions. This will lead to controlling the quality of baked products from wheat.

### c. Changes in functional characteristics.

The effects of micro-organisms, derived from biochemical activities, on the fermentative and rheological properties of model doughs, as well as on bread characteristics will be studied in relation to process conditions.

#### Possible benefit to European Industry and Agriculture

A good knowledge of the role of micro-organisms during wheat bread dough fermentation as well as of the interactions with white flour components will also allow to direct and control bread-making processes. This will without any doubt contribute to the production of high quality bread. This is expected to promote an increase in bread consumption and therefore wheat utilization. The study of enzyme activities is of interest in connection with the use of Spanish wheat in other EC countries.

#### Manpower

Partner 12                      72 man-months

#### Deliverables

- Collection of purified micro-organisms;
- Effects of micro-organisms on flour components;
- Effects of micro-organisms in dough systems;
- Information on biochemical changes occurring during dough fermentation;
- Evaluation of possible relationships between biochemical changes and functional properties;
- Improved definition of processing requirements; insight in possibilities of using EC wheat in fermented dough systems.

#### Duration

48 months from month 1 to month 48

## **Subprogramme B: FUNCTIONAL COMPONENTS AND THEIR INTERACTIONS**

### **B.1 - COMPONENT INTERACTIONS**

#### **Task B.1.1 - PURIFICATION AND CHARACTERIZATION OF GLUTEN SUBFRACTIONS (Partners 07M, 07N, 19, Subcontractors 22, 23)**

##### Starting point

Depending of the different subtasks, see separate flow sheets for the different partners involved in this task.

##### Objectives

- Purification and characterization of whole gluten, gluten subfractions and protein subunits of different genotypes;
- Study on the conformational and functional properties of the individual gluten proteins and the interaction of the different proteins and other wheat components.

##### Experimental approach

Gluten fractions will be prepared and characterized using the following lines which are currently available.

- i. Siccio lines with altered compositions of HMW subunits (from P. Payne, Cambridge)
- ii. Double and triple translocation lines, in which the short arms of chromosomes 1A / 1B, 1A / 1D, 1B / 1D and 1A, 1B, 1D have been replaced with short arm of chromosome 1R of rye. These should contain a glutenin fraction consisting solely of HMW subunits of glutenin (triple translocation) or HMW subunits / LMW subunits encoded by only one genome (from K. Shepherd, Adelaide).
- iii. A series of related lines with one, two, three, four, five or six HMW subunits of glutenin (from G. Lawrence, Sydney).

A series of procedures for the production of defined gluten subfractions has been established. Gluten prepared from dough is initially extracted with aq. 70 % ethanol followed by aq. 50 % propan-1-ol. Although these fractions contain mainly monomeric gliadins, they also contain some polymeric glutenins that are separated from the gliadins by gel filtration chromatography on a column of Sephacryl S300 in 2 % acetic acid - 8M urea. The gliadins from these two extractions can then be combined. The polymeric glutenins present in these fractions probably differ in their solubility as a result of differences in their subunit composition and Mr's (smaller polymers that are

rich in LMW subunits being most readily soluble). These two fractions, the insoluble gluten residue (consisting of high Mr polymers enriched in HMW subunits) and the gliadins will be analysed by quantitative SDS-PAGE under reducing conditions to determine their subunit compositions, and their amino acid composition. They will also be supplied to collaboration for NMR and FTIR spectroscopy and for rheological studies. Their Mr distributions will also be determined by sedimentation equilibrium ultracentrifugation. Individual gliadin and glutenin proteins will be purified using established procedures (solvent extraction precipitation by pH, ion-exchange and gel filtration chromatography, RP-HPLC). A novel approach will be used to study their non-covalent interactions with each other and with dough components. This is sedimentation ultracentrifugation. Single proteins and mixtures will be studied under a range of conditions of protein concentration, solvent composition and pH, and temperature. This procedure is extremely sensitive to changes in shape and aggregation behaviour, and will give new information on which gluten and dough components interact non-covalently, and on the forces that stabilize these interactions. The latter may include hydrogen bonds, hydrophobic interactions and electrostatic forces.

Series of functional subfractions of gluten will be prepared by a procedure based on differential solubility of proteins. Those fractions will differ by their glutenin polymer compositions. A combination of ultracentrifugation and SE-chromatography will be also assayed in order to provide gluten subfractions with a low polydispersity of polymer sizes.

The quantities prepared will be compatible with the analysis of their biochemical and rheological properties. All the subfractions will be characterized by their protein contents, their gliadin and glutenin subunit compositions (2 x ID electrophoresis) and their polymerization/aggregation state (SE-HPLC in dissociating medium).

Novel HMW or LMW glutenin subunits as well as gliadins affecting gluten viscoelastic properties and identified in subprogramme C will be purified and characterized (amino acid composition and sequence, aggregative behaviour).

Studies will be carried out on HMW and LMW glutenin fraction and aggregates using the recently developed technique of reductive isoelectric focusing. The structure and homologies (determined by polyclonal antibodies) of the proteins will be determined in relation to bread-making quality using wheat lines including those lacking storage protein genes at the *Glu-1*, *Glu-3* and *Gli-1* loci. For instance, at partner 22, gluten aggregates were characterized by free-flow isoelectric focusing. Native (unreduced) proteins were extracted from wheat flour or gluten in 50% aqueous propan-1-ol and fractionated by free-flow isoelectric focusing (IEF) in the same solvent. The IEF fractions contained an extremely dispersed population of relatively small polymeric proteins with different HMW glutenin subunit composition as determined by two-step one-dimensional SDS-PAGE. Most polymeric molecules comprised both  $\alpha$ -type and  $\gamma$ -type HMW subunits of glutenin and some polymers contained new gluten polypeptides similar in mobility to HMW subunits of glutenin. Small quantities of HMW glutenin subunits in single polypeptide form were also detected. Moreover, the LMW subunits B and C of glutenin were found in polymeric molecules that comprise HMW glutenin subunits.

Another approach for fractionating gluten subfractions is adsorption chromatography on controlled pore glass beads. Adsorption chromatography on 2000 Å controlled pore glass beads, performed by frontal analysis in the absence of any detergent/chaotropic agent, was found to be capable of highly reproducible separation of the acetic acid-soluble unreduced proteins from wheat gluten. Glutenin polymers of molecular weight over  $10^7$  Da free from both monomeric proteins and starch were recovered. Furthermore, a number of lower molecular weight polymers differing in both molecular weight and subunit composition, along with monomeric proteins, were obtained. The polymers retained their ability to make up the gluten. Gluten proteins were adsorbed onto the glass with a sequential and competitive mechanism, which seems mainly due to hydrophobic interactions.

At Partner 23, old cultivars and landraces of common wheat from different countries will be also analyzed by established one- and two dimensional electrophoretic techniques and by RP-HPLC (reverse phase high performance liquid chromatography) in order to detect new allelic variants at both the *Gli-1* and *Glu-1* complex loci. The analyses will be extended to other species of the genus *Triticum*, exploiting the variation for storage proteins in these species and the possibility of incorporating them in advanced breeding lines.

Biochemical characterization will be carried out on single purified polypeptide components of the gluten complex. Purification will be performed by chromatographic techniques (preparative RP-HPLC, size-exclusion or ion-exchange) and characterization, in terms of amino acid composition and N terminal amino acid sequences will be achieved, establishing direct relationships between structure of these polypeptides and their possible role in determining bread-making properties.

#### Manpower

Partner 07M	36 man-months
Partner 07N	18 man-months
Partner 19	72 man-months
Subcontractor 22	81 man-months
Subcontractor 23	80 man-months

#### Deliverables

- Samples of wheat of selective genotypes;
- Purified gluten and subfractions for other partners in the same subprogramme.

#### Interdependence with other tasks

There is an interdependence with the tasks B.1.2, B.1.3, B.1.5 and B.2.5 from this subprogramme and with the two other subprogrammes.

#### Duration

48 months, but depending on the different subtasks (see flow chart).



**Task B.1.2 - PHYSICO-CHEMISTRY AND FUNCTIONALITY OF WHEAT PROTEINS (Partners 07M, 07N, 15)**

### Starting point

Depending on the different subtasks, see separate flow sheets for the different partners involved in this task.

### Objectives

- Study of the physico-chemical properties of gluten and subfractions of gluten from wheat of different genotypes;
- Correlation of those properties with the functional properties of wheat to find a possible relation between wheat protein components and wheat functionality in *e.g.* starch/gluten separation industry.

### Experimental approach

The physico-chemical and functional properties of gluten subfractions prepared as described above will be studied. Much of the work will be focused on the aggregation behaviour and on the rheological (viscoelastic) properties of gluten proteins in function of their HMW or LMW glutenin subunit compositions. Therefore the samples will be selected among the near isogenic lines and substitution lines available in the programme. However some investigations will be also carried out on the different gliadin types ( $\alpha$ ,  $\gamma$  and  $\omega$ ).

Studies on protein interactions will be carried out on simplified systems comprising a small number of identified protein components. Hydrophobic interactions of gliadin components and of glutenin polymer fractions will be determined by TNS-binding (ultrafiltration). The mobility of the gluten proteins and their interactions will be investigated also by ESR spectroscopy. Hydrated proteins will be probed and labelled with spin probes that differ by their sizes and their binding specificity (binding on SH or NH<sub>2</sub> groups). We will try to prepare "macromolecular spin probes" by labelling pure gliadins. They could be added to glutenin polymer fractions.

The aggregation behaviour of gluten subfractions will be studied in model system. Their aggregation state in dissociating solvent will be studied by SE HPLC and by quasi-elastic light scattering in order to gain some information about the real size of the glutenin polymers. The formation of the aggregates will be followed by turbidimetry in conditions of solvent perturbation.

The rheological properties of the gluten subfractions and of purified gliadins and glutenin polymers will be examined. Dynamic and creep measurements will be carried out on hydrated proteins. Those assays will be carried out in varied conditions. Namely the effect of the temperature will be studied in a range of 10-80°C. The role of SH groups and SS bonds will be also particularly considered in specific experiments on partially reduced and on alkylated gluten proteins. In each case aggregation state will

be characterised. The rheological properties of monolayers of gluten proteins will be also studied.

Finally an attempt will be made to relate aggregation behaviour, rheological properties and prolamin compositions.

At Partner 07M, accurate quantitation of sequentially extracted LMWG subunits will be carried out from samples differing in quality characteristics, using image analysis of 1D (SDS-PAGE, A-PAGE and IEF) patterns. The question is raised as to whether differences in quality potential of the various alleles are due to structural differences or to quantitative differences only.

Investigation of the Mr distribution (analytical SE-HPLC) of protein aggregates extracts by various solvents of LMWG. Most attention will be paid to the 100-800 kDa range in relationship to quality (extensibility and elasticity) characteristics.

Distribution of each LMWG and HMWG allelic type in the different parts of the elution curve (and in the insoluble residue) using preparative SE-HPLC. Estimation of the functionality of each subunit by its contribution to the different sizes of aggregates, or by the modification of the Mr distribution as a result of various denaturing treatments.

Purification of LMWG subunits (notably those that will be shown to be functional markers by their contribution to large aggregates) by sequential extraction or precipitation, cation-exchange chromatography, or preparative IEF. Alternatively, blotting of LMW spots from 2D electrophoresis gels will be attempted in view to determine the N-terminal amino acid sequence of some LMWG subunits.

See also the experimentation approach of B.1.5, the work at Partner 15, which also deals with the tasks dealt with in this subtask.

### Manpower

Partner 07M	36 man-months
Partner 07N	54 man-months
Partner 15	24 man-months

### Deliverables

- Purified gluten and subfractions and information about the functionality of them for other partners in the same subprogramme;
- Quantitation of gluten subunits.

### Interdependence with other tasks

There is an interdependence with the tasks B.1.2, B.1.3, B.1.4, B.1.5 and B.2.5.

### Duration

48 months, but depending on the different subtasks (see flow chart).

<p><b>Task B.1.3 - GLUTEN HYDRATION AND INTERACTIONS OF GLUTEN PROTEINS WITH OTHER COMPONENTS (Partners 15, 16)</b></p>
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### Starting point

Depending on the different subtasks, see separate flow sheets for the different partners involved in this task.

### Objectives

- Elucidation of the mechanism of gluten hydration;
- Study of the mechanisms which play a role in the interaction processes between gluten and other components.

### Experimental approach

$^1\text{H}$  NMR relaxation time measurements will be carried out on freeze-dried glutes which have been rehydrated in  $\text{D}_2\text{O}$ .  $\text{D}_2\text{O}$  is used so that NMR signal arises solely from the non-exchangeable protons of the protein, with no interfering water resonance. Studies of longitudinal, transverse and rotating frame relaxation in hydrated biopolymers have been relatively uncommon although such methods have been widely used to obtain information on the dynamics of synthetic polymers. In dry gluten only 10% of the protons can be described as mobile (*i.e.* have  $T_2 > 100\mu\text{s}$ ); when fully hydrated this figure is increased to 70%. The relaxation behaviour of the mobile component is complex: it cannot be simply resolved into a small number of exponential components. Accordingly, the development of the mobile component will be studied as the amount of hydrating water is increased.

Transverse water relaxation in biopolymer systems is dominated by exchange of water protons with the OH and NH groups of polysaccharides and proteins. In gluten exchange with the  $\text{NH}_2$  protons on glutamine side chains will be particularly important. The exchange will be studied by CPMG spin-echo experiments on hydrogen and deuterium, by varying the inter-pulse spacing and/or static magnetic field strength.

Hydration of gluten will be studied by measuring water relaxation following the addition of starch and non-starch polysaccharides (pentosans). This will allow an assessment of the relative importance of the different components in the absorption of water by flours. The phase behaviour of the phospholipids in doughs can be monitored by high power  $^{31}\text{P}$  NMR spectroscopy.

2D NMR and FTIR/Raman spectroscopy will be used to identify structural elements in short synthetic peptides which represent the repeated peptide sequences known to be important determinants of elasticity. This will complement recent CD studies of the peptides and work on antibody binding to gluten proteins.

See also the experimental approach of B.1.5, the work at Partner 15, which also deals with the tasks dealt with in this subtask.

### Manpower

Partner 15	12 man-months
Partner 16	48 man-months

### Deliverables

Fundamental knowledge about the interactions of wheat components in dough.

### Interdependence with other tasks

There is an interdependence with the task B.1.1, B.1.5, B.2.3, B.2.4 and B.2.5 of this subprogramme, and with the subprogrammes A and C.

### Duration

48 months, but depending on the different subtasks (see flow chart).

**Task B.1.4 - THE ROLE OF MINOR PROTEIN COMPONENTS  
ASSOCIATED WITH STARCH GRANULES (Partner 14)**

Starting point

The minor protein component of wheat starch granules has been characterized as at least ten polypeptides by earlier work at Partner 14. Some of these, integrated into the granule, are probably enzymes of starch biosynthesis. Others are on the surface, and the 15k component has been named "friabilin" because of its association with friable, or soft, endosperm texture. The earlier work had shown a linkage between friabilin on starch and the Hardness gene on chromosome SDS. This led to the hypothesis that friabilin might be the product of this gene, and therefore a good biochemical marker for soft endosperm texture.

Initial work in this programme has discovered that:

- (i) operational friabilin on the granule surface contains several 15k species. One of these has been purified and called friabilin (basic) because it has a very high isoelectric point.
- (ii) a monoclonal antibody against friabilin (basic) reacts with several similar proteins, designated "immunofriabilins" in whole endosperm of both hard and soft breadwheats, but this group is absent from durum wheats. The antibody has been used to develop a commercial test for adulteration of durum wheat by bread wheat.
- (iii) the structural gene(s) for immunofriabilin are on chromosome SDS, but are not the same as the Hardness gene.
- (iv) friabilin (basic) is similar in many ways, including N-terminal amino-acid sequence to detergent soluble "Triton X-114 proteins" of flour discovered under Task B.1.5.
- (v) operational friabilin on starch granules is associated with starch granule surface lipids.

Objectives

To establish the role(s) of starch granule protein in relation to functional properties of wheat.

To devise a predictive test for endosperm texture on the basis of starch granule surface components for use in wheat breeding and at mill intake.

Experimental approach

Endosperm texture is of fundamental importance to most uses of wheat, and therefore work will continue on this aspect of starch granule proteins as first priority. The failure of the initial simple friabilin hypothesis has pointed to several derivative hypothesis

about the nature of the product of the hardness gene. These will be explored in order to discover the true molecular basis of endosperm texture by means of:

- (i) Structural studies of friabilin (basic) and other components of operational friabilin, to establish their relationship to each other, to the various immunofriabilins of endosperm, and to the TX-114 proteins of Task B.1.5.
- (ii) Examination of the lipid binding properties of friabilin species, in comparison to those of TX-114 proteins.
- (iii) Studies of the linkage of starch granule surface lipids to operational friabilin and to the Hardness gene, using existing special genetic lines and relevant new ones produced under Task C.9.

#### Possible benefit to European Industry and Agriculture

Successful outcome of the work will have profound implications for the supply, either through conventional breeding or bioengineering, of wheats with new or improved end use properties and thus increased usage of EC wheats.

#### Manpower

Partner 14                      78 man-months

#### Deliverables

Fundamental knowledge on the role of starch proteins in wheat, which has an impact on both plant breeding and functionality and use of EC wheat.

#### Interdependence with other tasks

There is an interdependence with the tasks B.1.5 and C.9.

#### Duration

48 months, but depending on the different subtasks (see flow chart).

## **Task B.1.5 - LIPID INTERACTIONS (Partners 07N, 15)**

### Starting point

Depending on the different subtasks, see separate flow sheets for the different partners involved in this task.

### Objectives

- Study of the wheat lipid composition and behaviour in dough with non-invasive methods;
- Study on the mechanisms which play a role at the interaction processes between wheat lipids and other components.

### Experimental approach

Interfacial behaviour of various wheat flour components in correlation with baking quality.

This subprogramme project is set up to study the interfacial behaviour of various wheat flour components, like the gluten, lipid and hemicellulose fractions, at the dough-air interface in the gas bubble present in the dough. The components will be acquired from associates in section B of the ECLAIR programme, or will be isolated according to the same methods and from the same flours as used by these associates.

The surface tension (free surface energy), during the static and dynamic measurements, will be measured by means of the Wilhelmy plate method. Techniques used to measure the dynamic interfacial behaviour are:

- A Langmuir trough, where the dynamic interfacial behaviour will be measured near the equilibrium surface tension, by making small changes in the size of the surface and measuring the response of the system to these changes by means of the surface tension;
- An overflowing cylinder by which means a continuous expanding surface is created. This method mimics the expansion of the surface of the gas cells during the rising of the dough;
- A free falling film apparatus to determine the stability of a surface formed by the different components and reconstituted doughs;
- Also, the drainage, disproportioning and coalescence of gluten foams will be studied;
- A plan parallel plate viscosimeter (rheometer) will be used to determine shears in (reconstituted) dough systems. (For this study, a rheometer has been bought);
- Standard analytical puploave baking tests will be done. Baking performance will be compared to the analytical results, gained by the methods described in this subprogramme project and of the other associates, to study the correlation between the different parameters.

### Manpower

Partner 07N	36 man-months
Partner 15	24 man-months

### Deliverables

Fundamental knowledge on the composition and behaviour of wheat components, especially lipids, in dough, which has an impact on dough processing

### Interdependence with other tasks

There is an interdependence with the tasks B.1.1, B.1.2, B.1.3, B.2.4 and with the subprogrammes A and C.

### Duration

48 months, but depending on the different subtasks (see flow chart).



## **B.2 - DYNAMICS OF DOUGH DEVELOPMENT (Partner 16)**

### Starting point

Depending on the different subtasks, see separate flow sheets for the different partners involved in this task.

### Objectives

To study the behaviour and interactions of wheat components in doughs and baked goods with the aid of antibodies against the different components and microscopic techniques.

### Experimental approach

#### **Subtask B.2.1 - Development of Microscopic Techniques for Examining Bread Doughs**

A panel of highly characterized anti-gluten monoclonal antibodies is already available, some of broad and others of narrow specificity. One of these has already been successfully used to probe bread ultrastructure using a combination of electron microscopy and immunogold labelling. This approach will be extended to examining doughs, to follow changes in the distribution and integrity of gliadin structure during dough development. Aspects covered will be the effect of different work inputs during dough mixing to reach peak development, over-mixing of dough causing dough breakdown and the effect of relaxation on over-worked doughs.

#### **Subtask B.2.2 - Characterization of Polyclonal and Monoclonal Antibodies to Wheat Pentosans**

For use as probes, antibodies (already raised using proven technology) will be characterized in terms of binding to various types of pentosan. Fractions of the pentosans will be prepared which differ in their degree of branching, associated proteins, or cross-linking. Once their specificity is defined, the anti-pentosan antibodies will then be used to characterize the pentosan composition (in terms of features such as degree of cross-linking) in flours of known technological performance. Structural features of the pentosans will then be related to the bread-making quality of wheat flours.

### **Subtask B.2.3 - Role of Pentosans in the Structure of Dough and Baked Products**

Anti-pentosan antibodies of known specificity will then be used to probe pentosans *in situ* in doughs developed as described in task B.2.1 in addition to baked products such as bread using immunogold labelling techniques in conjunction with electron microscopy. This will enable the distribution and changes in the structure of the pentosans to be studied during dough development and baking.

### **Subtask B.2.4 - Study of Interactions between Proteins, Pentosans and Lipids in Doughs and Baked Products**

The anti-gluten antibodies and those to the pentosans will be labelled with different sizes of colloidal gold. This will allow both the proteins and the pentosans, to be probed simultaneously in doughs as described in task B.2.1 and enable their interactions to be studied during dough development using electron microscopy. The effect of adding different types of lipid such as phospholipids and solid fats on the binding of antibody probes to dough will also be examined, in order to gain an insight into putative lipid/protein or lipid/carbohydrate interactions.

### **Subtask B.2.5 - Effects of Heat and Mechanical Work**

The input of heat or mechanical work is known to profoundly affect gluten rheology. To date only very simple NMR experiments have been carried out on heated gluten. This showed changes in the mobile to immobile polymer ratio but did not show the expected hysteresis effects. This was probably because these experiments measured transverse relaxation and thus responded only to very slow motions of the polymers. It is proposed to use spin lattice relaxation times in the laboratory frame, rotating frame and local dipolar fields to examine the range of polymer motions present. A particularly important feature of these measurements is that they are sensitive to the morphological distribution of motions within the material and have been used to detect the local slowing of motions caused by disulphide bonds between polymers. In order to examine the effects of mechanical work it is proposed to develop methods for putting controlled amounts of mechanical work into samples needed for NMR experiments. Glutens and dough from good and bad baking quality wheats will be examined as well a purified and enriched protein fractions which are available only in small quantities.

#### Manpower

Partner 16

54 man-months

### Deliverables

Fundamental knowledge of the distribution, composition, behaviour and changes in this behaviour of wheat components in dough and baked goods.

### Interdependence with other tasks

The knowledge obtained in those subtasks shows interdependence with the other subprogrammes (A and C) and especially the subtasks B.1.2, B.1.3, B.1.5.

### Duration

48 months, but depending on the different subtasks (see flow chart).

## **SUBPROGRAMME C - BIOCHEMICAL- GENETICS AND PHYSIOLOGY**

### **Task C.1 - MULTILOCAL EXPERIMENT OF ADVANCED LINES AND VARIETIES AND PRODUCTION OF SAMPLES IN CONTROLLED CONDITIONS**

The evaluation of yield potential and expression of quality attributes (technological characteristics) of wheats in different environments represents the necessary premise to all other activities envisaged in the Programme. In order to gather such information, a network of variety trials carried out with a rigorous and uniform methodology in wheat growing areas will be established.

#### **Subtask C.1.1 - Sub Network 1: Southern Europe (Partners 02, 03, 04, 05, 06, 07C, 09, 18; Subcontractors 24, 25)**

#### Starting point

The environment (soil, climate, agronomic practices, diseases, etc.) and the interaction genotype  $\times$  environment exert a strong influence on the expression of technological quality. The consistency of quality for most existing European wheat cultivars is insufficient because it is affected by agronomic and climatic factors. Variation in protein content and rheological properties among seed samples of the same wheat cultivar from different environments can be as large as that among cultivars grown in one environment.

A set of varieties from Italy, France, Spain and Portugal has been grown in trials for two years in order to evaluate their potential and stability in terms of production, quality and response to late nitrogen application.

#### Objectives

To produce seed/flour samples to be used in subprogrammes A and B, to study the stability parameters of quality expression, to identify environments and agronomic practices favourable for enhancing quality expression.

#### Experimental approach

The Southern Europe Network involves Italy, Spain, Portugal, France. This sub network is especially devoted to wheats with high bread-making quality. These genotypes will be well adapted to the growing conditions prevailing in Southern Europe.

- Locations: 2-5 locations for each country for a total of 8-20 locations/year;
- Varieties or advanced lines: 25;
- Experimental design: balanced lattice with 3 replications;
- Agronomic practices: the best agronomic practices for the conditions of Southern Europe with minor modifications according to specific environmental requirements (two levels of nitrogen manure). The final aim will be to discard the use of pesticides and growth regulators. The statistical elaboration of the results is centralized and the data are available at the end of each year;
- Grain quality is evaluated with the following analyses:
 

On each plot	( protein content (%) ( SDS sedimentation value (ml) ( hectolitre weight (g).
Sample from a mixt of the replications	( rheological tests (alveograph, farinograph) ( baking tests ( other tests required by the industry (work at ( laboratories 04, 05, 06).

### Manpower

Partner 02	31 man months
Partner 03	27 man months
Partner 07C	15 man months
Partner 09	5 man months
Partner 18	36 man months
Subcontractor 24	47 man months
Subcontractor 25	49 man months

### Deliverables

On September-October of each year, seed/flour samples will be delivered to participants in subprogrammes A and B for technological/biochemical analyses. Documents regarding rheological analyses, agronomic/ physiological results and statistical elaboration will be available by the end of each year.

### Interdependence with other tasks

This task will provide input for tasks outlined in subprogrammes A and B.

### Duration

48 months from month 1 to month 48

**Subtask C.1.2 - Sub Network 2: North-Western Europe (Partners 04, 05, 06, 07C, 08, 09, 18)**

Starting point

See subtask C.1.1.

Objectives

Besides the study of stability parameters of quality expression, the aim of this experimentation will be the definition of selection criteria to identify stable genotypes for quality expression in highly diversified growing conditions and the evaluation of yield potential.

Experimental approach

France will be the main area for developing this network. Medium to high quality wheats will be evaluated. These genotypes will be well adapted to the growing conditions prevailing in North-Western Europe.

- Locations: 8-10 locations in France;
- Varieties or advanced lines: 15 to 25;
- Experimental design: balanced lattice with 3-4 replications;
- Agronomic practices: see before;
- Grain quality is evaluated with the following analyses:
 

On each plot	( protein content (%) ( SDS sedimentation value (ml) ( hectolitre weight (g).
Sample from a mixt of the replications	( rheological tests (alveograph, farinograph) ( baking tests ( other tests required by the industry (work at ( laboratories 04, 05, 06, 08).
- Growing conditions: fungicide (treated - non treated);
- Measurements: yield and yield component, TKW, % N, harvest index for N;
- Observations: climatic conditions (rainfall, temperature, energy, P.E.T.).

Manpower

Partner 07C	5 man months
Partner 09	14 man months
Partner 18	36 man months

Deliverables

See subtask C.1.1.

Interdependence with other tasks

See subtask C.1.1.

Duration

48 months from month 1 to month 48

## Task C.2 - GENOTYPE x ENVIRONMENT INTERACTION

### Subtask C.2.1 - Ecophysiological Approach of the Genotypic Expression (Partners 07C, 09, 18)

#### Starting point

See task C.1

#### Objectives

The objectives of the research are to determine the environmental factors affecting yield and bread-making quality and to study the accumulation kinetics of endosperm protein fraction in relation to the environmental variations and to obtain a better understanding of the variations in quality expression, the methodology will be based on the study of mechanisms involved in dry matter yield elaboration. This approach should reveal the environmental factors which are responsible for yield and quality variations.

In any specific environment, this analytical approach should make it possible to determine if the genotypic potential is expressed and in the case of a negative answer which are the limiting factors in yield and quality expression.

#### Experimental approach

For this ecophysiological study different approaches will be used:

- Description of the environmental conditions in relation with the growing stages of the plant: What are the climatic conditions at each developmental or growing stage ? Is there any limiting factors for a particular stage ?
- Description of the dry matter elaboration: for some well-defined critical developmental stages dry matter is evaluated. The dynamic evolution of total dry matter and of the ratio between different organs could be a precise indication of some disturbance in the growth and development of the whole plant;
- Description of the grain filling period and maturation;
- Four or more cultivars with contrasting;
- Observations on spikes and stems from flowering to maturity;
- Accumulation kinetics for dry matter, protein and endosperm protein fractions.

#### Manpower

Partner 07C	10 man months
Partner 09	20 man months
Partner 18	10 man months

Deliverables

The results of research on kinetics of dry matter accumulation will be available by the end of each year. Seed/flour samples will be delivered to participants for further studies.

Interdependence with other tasks

This task will provide input for tasks outlined in subprogrammes A and B. In particular, subtasks C.2.2, C.1.1 and C.1.2 are strongly linked to this task in terms of experimental methodology, cultivars, and interpretation of results.

Duration

48 months from month 1 to month 48

**Subtask C.2.2 - Experimentation in a Controlled Environment (Partners 09, 18)**

Starting point

See task C.1.

Objectives

To complete the multilocal experiment it is worthwhile, in order to obtain a better understanding of the quality expression, to grow wheat genotypes in environments where the growth factors are controlled more precisely. Nitrogen dynamics will be studied using  $^{15}\text{N}$ -labelled fertilizers and a range of fertilization methods will be developed.

Experimental approach

Two approaches are proposed:

a) To grow genotypes in controlled climatic conditions during grain filling and maturation some of the genotypes studied in the theme I should be grown in micro plots. Then, from a well-defined plant population (canopy), different climatic conditions will be applied during grain filling and maturation.

The consequences for quality expression are recorded and the physico-chemical and biochemical studies proposed by the biochemists Partners are applied.

Finally, it should be possible to relate the observed characters to a genetic basis.

b) From a multilocal experimentation involving some of the genotypes already included in other experiments (theme D), the aim is to propose a protocol designed to:



- evaluate and compare the quality potential of the varieties;
- assess the regularity of quality expression.

The programme will cover three cereal seasons.

Tests will be performed on well-known experimental sites under climatic conditions representatives of the North-western Europe on the one hand, and Southern Europe on the other. The varieties studied (supplied by the other subprogramme Partners) will be compared to accepted standard samples of agronomic and technological characteristics. Production factors - excepted for nitrogen manure - will be optimized during each phase of the experiment. In particular, this includes the dates and density of sowings, fungicide protection and possibly straw shorteners.

With regard to nitrogenous manure, a range of fertilization methods (including amount and division) will be systematically developed. Nitrogen dynamics will be monitored using plot fertilized with  $^{15}\text{N}$ -labelled fertilizers. Weather stations located at the test site will measure the main climatic parameters affecting wheat growth and development.

Samples from the test plots will be submitted for the technical tests and analyses performed by the various laboratories taking part in this study, with particular attention paid to protein constituents.

Interpretation of results (agronomy plus laboratory analyses) will be largely based on statistical multi-dimensional analysis techniques.

#### Manpower

Partner 09	20 man months
Partner 18	26 man months

#### Deliverables

Documents regarding the results of this research will be available by the end of each year. Seed/flour samples will be delivered to participants in the project for further studies.

#### Interdependence with other tasks

Task C1 provides samples for this subtask. Moreover, task C.1 and subtask C.2.1 are strongly linked to this task in terms of experimental methodology and interpretation of results. This task will provide input for tasks in subprogrammes A and B by request.

#### Duration

48 months from month 1 to month 48

### **Task C.3 - EXPERIMENTATION ON POPULATIONS FOR BREEDING (Partners 02, 03)**

#### Starting point

The advantage of producing synthetic populations lies in the creation of genetic pools characterized by a high concentration of favourable alleles contributing positive agronomic and quality traits. From synthetic populations, superior lines can be isolated by either conventional breeding procedures or more rapid methods such as SSD and anther culture.

#### Objectives

The experimentation will produce new synthetic populations originated from already established synthetic populations containing 'good quality' alleles coding for endosperm proteins affecting bread-making quality. Moreover, small samples or populations selected for quality will be supplied to co-operators. Biochemical tools and technological microtests studies in the other subprogrammes will be experimented to screen synthetic populations.

#### Experimental approach

- The participants confer the already existing synthetics;
- Field trials are carried out in order to test the potential of the populations in terms of production and bread-making quality;
- Two more cycles of intermating will be carried out using Chemical Hybridizing Agents;
- Field screening of segregating populations for diseases resistance and agronomic traits started already and will continue;
- Electrophoretic analyses and technological microtests will be applied to select superior progeny.

#### Manpower

Partner 02	32 man months
Partner 03	16 man months

#### Deliverables

Small samples of synthetic populations selected for quality will be supplied to co-operators from each cycle of the Synt. Results of the technological/agronomic evaluation of synthetic populations will be available by the end of each year.

#### Interdependence with other tasks

This task will provide input for tasks C.1 and C.8 and gives the possibility to evaluate microtests devised in subprogramme A.

#### Duration

48 months from month 1 to month 48

<p style="text-align: center;"><b>Task C.4 - GENETICS OF LMW GLUTENIN SUBUNITS (Partners 07M, 07C)</b></p>
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### Starting point

Recent results clearly indicate that variation for LMW glutenin subunits is primarily responsible for differences in gluten viscoelastic properties in both durum and bread wheat. LMW are the least characterized group of wheat proteins because of the difficulty to study them by one-dimensional electrophoresis. Moreover, the positive effects of certain LMW glutenin subunits and HMW glutenin subunits on bread-making quality were found to be additives.

### Objectives

The research will determine (i) the genetic variability for B, C, and D groups of LMW glutenin subunits, (ii) the genetic linkage between loci coding for gliadins and glutenin on group 1 chromosomes and (iii) the effects of both LMW and HMW glutenin subunits on gluten properties.

### Experimental approach

Research will focus on genetic diversity and allelic variants of B and D groups of LMW glutenin subunits amongst a large collection of both hexaploid and tetraploid wheats from Europe and Mexico. Allelism and linkage among the loci coding for LMW glutenin subunits and gliadins on the group 1 chromosomes will be studied using a new two-dimensional electrophoretic technique which separates gliadins and glutenins on the same gel.

Each subunit of allelic group of LMW glutenin will be given an average quality score based on its effect on baking quality. The possible interest of some of the LMW allelic types in bread wheat breeding for specific technological attributes (alveograph parameters: W, P, G; baking test) will be also investigated.

Statistical analyses will be applied to a group of European bread wheat cultivars sharing the same HMW glutenin subunit composition as well as segregating populations from crosses between some of them in order to determine the effects of variation for LMW glutenin subunits on rheological characteristics of dough.

An accurate study of the specific effect of individual subunits or allelic groups of LMW glutenin subunits on gluten viscoelasticity will be carried out by analysing random progeny of various crosses between cultivars selected for their appropriate electrophoretic compositions, allowing to test different allelic types within a given HMW glutenin composition and vice versa.

Near-isogenic lines, substitution lines, null forms lacking the genes coding for LMW subunits, wild hexaploid and tetraploid species will be also considered.

Manpower

Partner 07C	54 man months
Partner 07M	12 man months

Deliverables

Documents regarding the results of both genetic and technological analysis be available by the end of each six-month reporting period.

Interdependence with other tasks

This task is strongly linked with tasks C.5, C.7, C.8 (see also 'Diagram of Interdependence') and also to task B.1.1 and B.1.2 in subprogramme B.

Duration

48 months from month 1 to month 48

**Task C.5 - GENETIC AND TECHNOLOGICAL ASPECTS OF HMW  
GLUTENIN SUBUNITS, HMW-ALBUMINS AND S-PROTEINS  
(Partners 03, 07C)**

Starting point

Some albumin/globulin proteins were reported in gluten and HMW-albumins were found to affect bread-making quality. Albumin-like S-protein have high affinity for polar lipid suggesting that there may be important in bread-making quality (see task C.4 for LMW glutenin subunits and task B.1.5 for lipid-protein interactions).

Objectives

The genetic variability for HMW glutenin subunit composition, the genetic structure of HMW-albumins and S-proteins, and the relationship with gluten viscoelastic properties are the objectives of this research.

Experimental approach

Lines, populations and cultivars of *Triticum* species (diploid, tetraploid and hexaploid wheats) from available collections will be screened for novel HMW glutenin subunits by SDS-PAGE. Variation in HMW-albumins and S-protein composition will be also studied on the same plant material.

The genetic analysis of structural genes controlling new glutenin polypeptides, HMW-albumins and S-proteins will be carried out on F<sub>2</sub> segregating progeny from crosses between parents with contrasting protein composition; the genetic linkage with morphological and physiological characters will be studied as well. The F<sub>3</sub> and F<sub>4</sub> progeny will be analysed for bread-making quality by the SDS-sedimentation test, microtests for direct quality assessment (gluten viscoelasticity) and alveograph test. Technological and biochemical analyses on random progeny will be carried out by laboratories 07M and 22.

Moreover, ancient wheat populations of primitive farming as well as diploid, tetraploid and hexaploid wheat species from many parts of the world will be screened for novel subunits. Research will also focus on isolation of spontaneous null forms and mutants showing duplication and overproduction of glutenin subunits.

The association between genetic variability for storage protein composition and functional quality will be studied in 36 intervarietal substitution lines (see task C.7). Also, crosses between intervarietal substitution lines will be carried out and quality tests will be applied to genotypes showing recombination at level of individual chromosome pairs. Electrophoretic analyses (A-PAGE and SDS-PAGE-) will be carried out on single grains and the agronomic characteristics of the progeny will be considered.

Manpower

Partner 03	30 man months
Partner 07C	24 man months

Deliverables

See task C.4.

Interdependence with other tasks

This task is strongly connected with tasks C.4, C.7, and C.8 (see also 'Diagram of Interdependence') and also with task B.1.5 in subprogramme B (lipid interactions).

Duration

48 months from month 1 to month 48

**Task C.6 - PRODUCTION OF LINES AND NEAR-ISOGENIC LINES  
WITH DIFFERENT HMW-GLUTENIN SUBUNIT AND GLIADIN  
COMPOSITIONS, AND OF NULL FORMS  
(Partners 03, 07M; Subcontractor 25)**

Starting point

Near-isogenic lines and null forms are an excellent material for studying the relationship between storage protein composition and gluten properties. They provide large amount of seed for rheological analysis and bread-making tests and allow to study the effects of genotype  $\times$  environment interaction on quality.

Objectives

The aims of these researches are (i) to study the effects of storage protein composition on bread-making quality using near-isogenic lines grown in replicated plots and (ii) to introduce new alleles coding for LMW glutenin subunits and gliadins in European wheat germplasm.

Experimental approach

Near-isogenic lines with contrasting HMW-glutenin subunits (encoded by *Glu-B1* and *Glu-D1* loci) and gliadins (*Gli-B1* and *Gli-D1* loci) have been obtained from crosses between biotypes of the Italian cultivar Alpe. These lines will be grown in replicated plots and analysed for quality by rheological and baking tests.

Null forms lacking *Gli-A1*-, *Gli-B1*- and *Gli-D1*- encoded gliadins and LMW-glutenin subunits have been isolated in the Italian common wheat cultivars S. Pastore and Alpe. Near-isogenic lines lacking one, two, or three loci will be produced. The effects of these mutations on bread-making quality as well as on gluten structure will be determined using rheological tests, baking tests and free-flow isoelectric focusing (in connection with subprogramme B).

Some of the new alleles (and mutant genes) will be transferred into the genome of Italian spring wheat Costantino so that the effects on yield and bread-making quality can be assessed in comparison with cv. Costantino.

Several alleles for all the *Gli-1* loci have been found in Canada Western Red Spring (CWRS) cultivars with strong elastic gluten. The genes for three of them will be transferred into the genome of the Italian spring cv. Costantino by repetitive back crossing. Their effects on yield and bread-making quality will then be assessed in comparison with the recipient cultivar. The starting material is BC2.

Moreover, the good quality LMW-2 subunits from type-45 durum wheat cultivars will be transferred into the genome of common wheats. Their effects on bread-making quality will then be assessed by rheological tests. The starting material is BC2.

### Manpower

Partner 03	40 man months
Partner 07M	12 man months
Subcontractor 25	10 man months

### Deliverables

Documents regarding the results of these researches will be available by the end of each six-month reporting period. Seed/flour samples for biochemical/technological analyses will be provided to co-operators.

### Interdependence with other tasks

This task provides seed/flour samples for task C.1.1 and for biochemical/ technological researches in subprogrammes A and B.

### Duration

48 months from month 1 to month 48



## **Task C.7 - CHROMOSOMAL LOCATION OF STORAGE PROTEIN GENES, CHROMOSOME INTERACTION ON PROTEIN SYNTHESIS AND DEVELOPMENT OF NEW GERMPLASM (Partner 07C)**

### Starting point

Chromosome substitution lines have been widely used for genetic, biochemical and agronomic-physiological studies. The 36 substitution lines used for this task have been developed for analysing the effects of several alleles coding for storage proteins and of their interactions on bread-making quality.

### Objectives

The objectives of this task are (i) to learn more about variability and expression of genes coding for storage proteins in wheat, (ii) to develop new genetic material, and (iii) to study the relationship between protein composition and gluten quality.

### Experimental approach

Thirty-six intervarietal chromosome substitution lines in which individual chromosome pairs of the homeologous groups 1 and 6 of the recipient variety Courtot have been replaced by their homologues from six donor varieties (V23, Cappelle, Magnif 27, Magdalena, Prinqual and Azteca) will be used in the study of endosperm protein genetics, particularly chromosomal location, allelic variation and gene linkage for HMW and LMW glutenin subunits and gliadins. Regulation of genes expression associated with chromosome interactions will be also studied. From the thirty-six intervarietal chromosome substitution lines, new genetic material (R.I.L., Recombinant Inbred Lines) will be developed for genetic and technological studies as well as for synthetic populations. New genotypes showing recombination at level of individual chromosome pairs will be obtained by crossing the intervarietal substitution lines. The doubled haploid techniques with anther culture in addition to controlled hybridization and natural selfing will be used for fixing the new genotypes.

### Manpower

Partner 07C 87 man months

### Deliverables

The results of these researches will be available by the end of each six-month reporting period. Seed/flour samples will be provided to co-operators.

### Interdependence with other tasks

This task is connected with tasks C.4 and C.5 and provides input in task C.1.1.

### Duration

48 months from month 1 to month 48

## **Task C.8 - BIOCHEMICAL MARKERS FOR SCREENING EARLY GENERATIONS (Partner 07C)**

### Starting point

The relationship between individual HMW subunits of glutenin and gluten quality is now sufficiently well known to estimate the contribution of this group of proteins to bread-making qualities of varieties and to introduce HMW glutenin subunit composition as a biochemical marker for screening superior progeny into traditional breeding programmes.

Since allelic variation for LMW glutenin subunit was found to have a strong influence on gluten viscoelastic properties, further investigations are needed to estimate the contribution of these proteins to bread wheat quality.

### Objectives

The aim of this research is to develop a quality score based on the relationship between some LMW subunits of glutenin and quality as determined by biochemical and rheological tests.

### Experimental approach

A Quality index based on storage protein composition (HMW and LMW glutenin subunits, gliadins) will be developed to screen early generations for good bread-making quality. Each subunit or allelic group of LMW glutenin will be analysed and the average quality score reflecting its effect on bread-making quality will be proposed.

### Manpower

Partner 07C 40 man months

### Deliverables

This task will provide a quality index during the last two six-month reporting periods.

### Interdependence with other tasks

This task will receive inputs from tasks C.1, C.2, C.4, C.5, C.6 and C.7 (see also 'Flow-Diagram C.8').

### Duration

48 months from month 1 to month 48

**Task C.9 - SOMACLONAL VARIATION FOR FACTORS AFFECTING  
BREADMAKING QUALITY (Partner 02)**
Starting point

Regeneration of adult plants from wheat embryo or anther culture is now a routine procedure in many laboratories around the world. Regenerated plants may differ from the initial variety or line in a number of characters. This somaclonal variation can be a new and powerful source of genetic variation for plant breeding. Somaclonal variants lacking *Gli-B1* and *Glu-B3* loci were identified in co-operation with partner 23 (Prof. Lafiandra) and multiplied.

Using NIRA we found that in some of these variants the grain hardness was reduced compared to the original varieties.

Objectives

The quality of somaclonal variants will be evaluated using rheological and biochemical tests.

Experimental approach

Seeds of progenies of regenerated plants deriving from different genotypes will be analysed in order to find out whether there are changes in seed storage proteins and grain texture, and the cause of these changes. Variants will be screened in the field to assess their agronomic value and the quality of their progeny will be checked to make sure that it is stably inherited. Partner 14 (Dr. Greenwell, task B.1.4) found a relation between the reduction in grain hardness of some of the variants and an increase in their starch friabilin. On the other hand, there is a couple of variants showing decreased grain hardness but no starch friabilin increase. Such variants will be studied in more detail.

Manpower

Partner 02	33 man months
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Deliverables

- Information on the effect of *Gli-B1* and genes on bread-making quality.
- Information about the relation between grain hardness measured by NIRA and amount of starch friabilin.
- Study of a “mutation” that seems to disconnect starch friabilin amount from grain texture.

Interdependence with other tasks

This task is connected with task C.6 and will provide input for task C.1.

Duration

48 months from month 1 to month 48

## Task C.10 - SPROUTING RESISTANCE (Partner 17)

### Starting point

Sprout damage is one of the main factors causing loss of quality in wheat thereby severely affecting farming profitability. Current methods to detect sprouting (Falling number test) are unsuitable for breeders and farmers.

### Objectives

This project is aimed at developing a new methodology based on factors related to dormancy (plant hormones, wheat germ agglutinin). This will lead to (i) the design of a reliable test for screening breeding stocks with sprouting resistance, (ii) the development of a rapid test to monitor the early changes of sprouting in the field and (iii) the development of a rapid test to ascertain the extent of sprouting damage of wheat. Genetic variability for dormancy will be evaluated and genetic resources will be produced having high levels of dormancy and adaptation to Northern European parts. This resource will be made available to breeders.

### Experimental approach

After screening of current breeding stocks, varieties and other genetic resources by conventional germination tests, the sources showing good performance will be combined with breeding stocks having good adaptability to Northern European environment. After multiplication, this material will be supplied to breeders. This part of the project will be carried out in co-operation with breeders.

In order to define the immunochemical bases of the assay, different tests for factors related to dormancy (*e.g.* monoclonal antibodies against abscisic acid and wheat germ agglutinin) and for amylase isoenzymes (PhastSystem electrophoresis and substrate overlay techniques) will be evaluated using a range of wheats with different falling numbers. Criteria are speed, reliability and sensitivity. The selected methods will be tested on breeding stocks and variety trials and the results will be compared with germination tests and conventional sprout damage assessment. Special attention will be given to variation in sprouting resistance between individual kernels since this may affect the reliability of the test. As a result of this study, a fast immunochemical screening procedure will be developed. Work will comprise the preparation and testing of purified antibody-fluorescent probe conjugates which will be used for the development of a kit for breeders and farmers. The assay will be developed along the lines of the Carlsberg Seed Tester (immunofluorescent detection on single seeds plus fast detection by image analysis). With help of an assay, the farmers will be able to estimate the potential sprouting risk when delaying the harvest. Also, the wheat breeders will be able to screen segregating lines for sprouting resistance, even in the absence of adverse weather conditions.

### Manpower

Partner 17

54 man-months

### Deliverables

The results of these researches will be available by the end of each six-month reporting period. Seed/flour samples will be provided to co-operators. In particular, this task will provide germplasm with genetic variation for sprouting resistance, conventionally assayed varieties and developed germplasm, inhibitory extracts, biochemically characterization of extracts and results of evaluation of marker testing.

### Interdependence with other tasks

This task will provide input (seed and chemicals) to co-operators. In order to facilitate the exchange of material, each co-operator will list the material he is willing to make available to the other research groups giving detail about its main characteristics and the amount of seed. This list will be circulated to all research groups. It is intended that, as far as the homozygous material is concerned, the originating breeder maintains all his rights and allows it to be used only for the studies included in the ECLAIR project.

### Duration

48 months from month 1 to month 48

## **7. WORK PLANNING CHART**

### **SCHEDULE**

### **INTERDEPENDENCE OF TASKS**





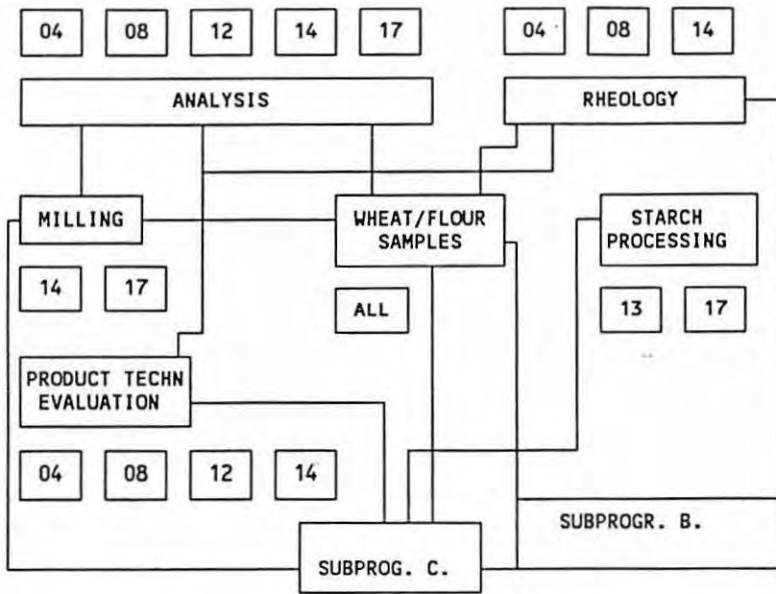


SUBPROGRAMME A : INDUSTRIAL PROCESSES

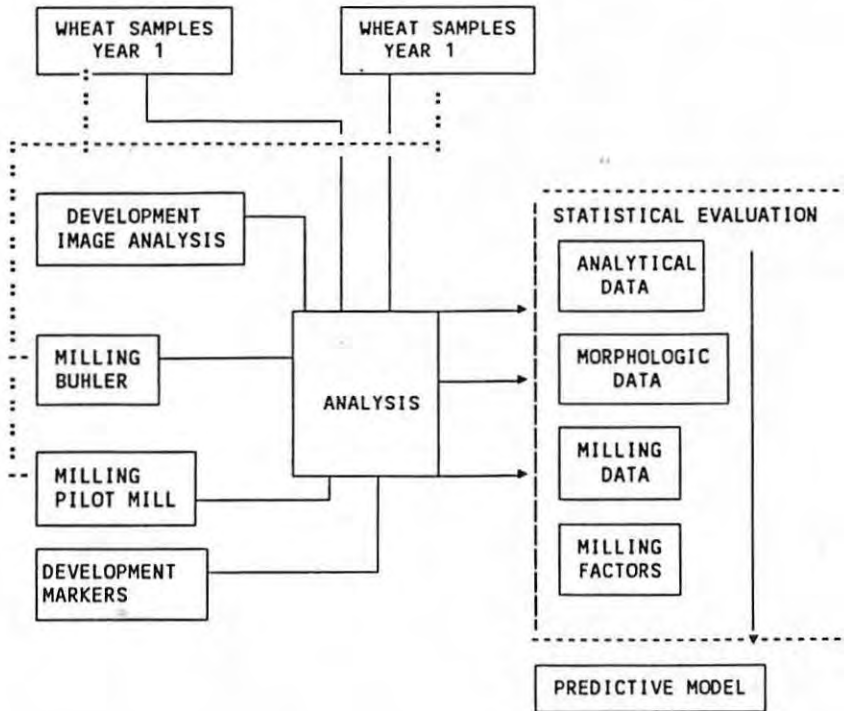
TASKS	SUBJECT	04	07M	8	12	13	14	17	y 1	y 2	y 3	y 4	TOTAL /TASK
A.1	Industrial Processes												
A.1.1.	Milling Quality						18	21	4 4	8 8	5 4	3 3	39
A.1.2	G-S separation												
A.1.2.1	Use of Enzymes							16	2 2	2 2	2 2	2 2	16
A.1.2.2	Characteristics of <b>Gluten</b>					36			6 6	6 6	6 6		36
A.2	Application of Wheat Products & evaluation												
A.2.1/2	White bread						62		13 13	9 9	3 6	6 3	62
	Wholemeal bread						41		6 10	5 5	7 3	2 3	41
A.2.3	Baked products		16	42					2 6	6 6	10 10	10 8	58
A.2.4	Flour blends							59	8 8	5 12	7 7	6 6	59
A.2.5	Sweet bakery	72							2 4	12 12	14 14	12 2	72
A.2.6	Interactions of <b>microorganisms</b>				72				9 9	12 11	11 11	5 4	72
SUB TOTAL		72	16	42	72	48	121	96	52 62	65 71	61 59	48 33	455



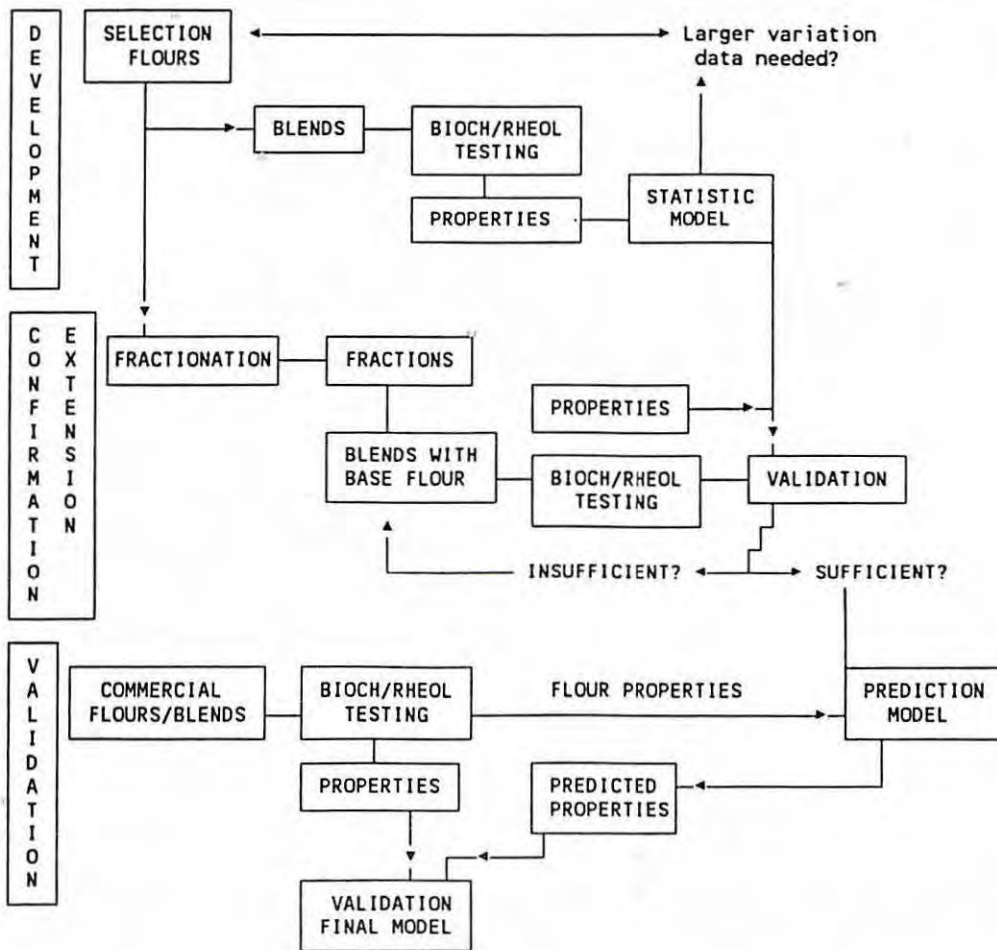
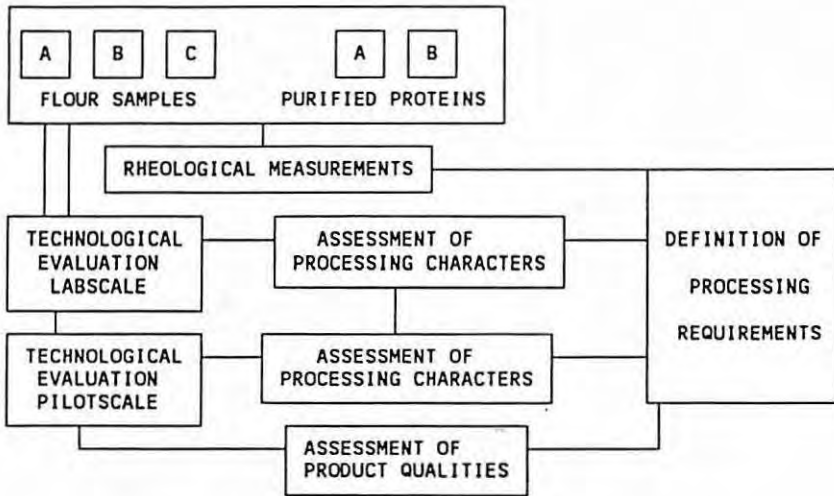
INTERDEPENDENCE OF TASKS - SUBPROGRAMME A



TASK A 1.1

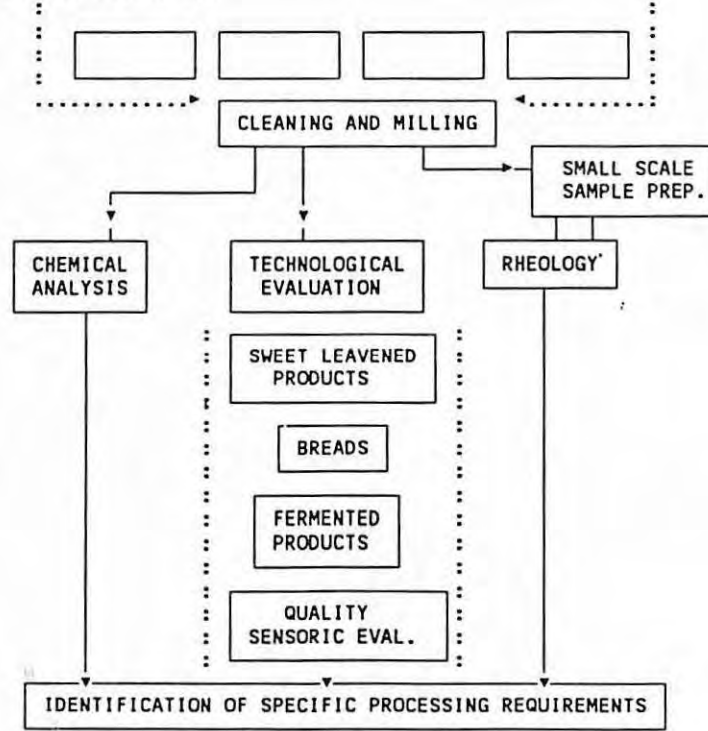




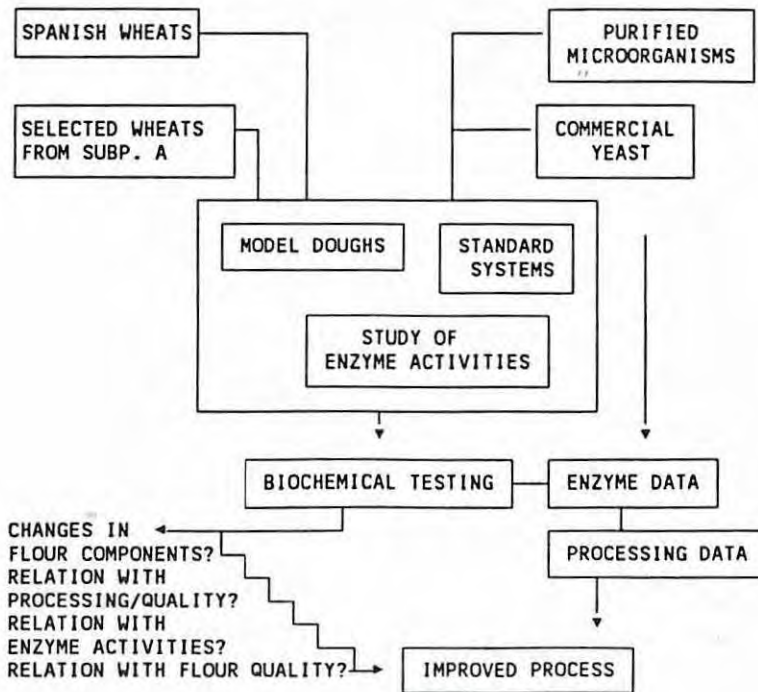


WHEAT SAMPLES SUBPR. C/A

TASK A.2.5



TASK A.2.6

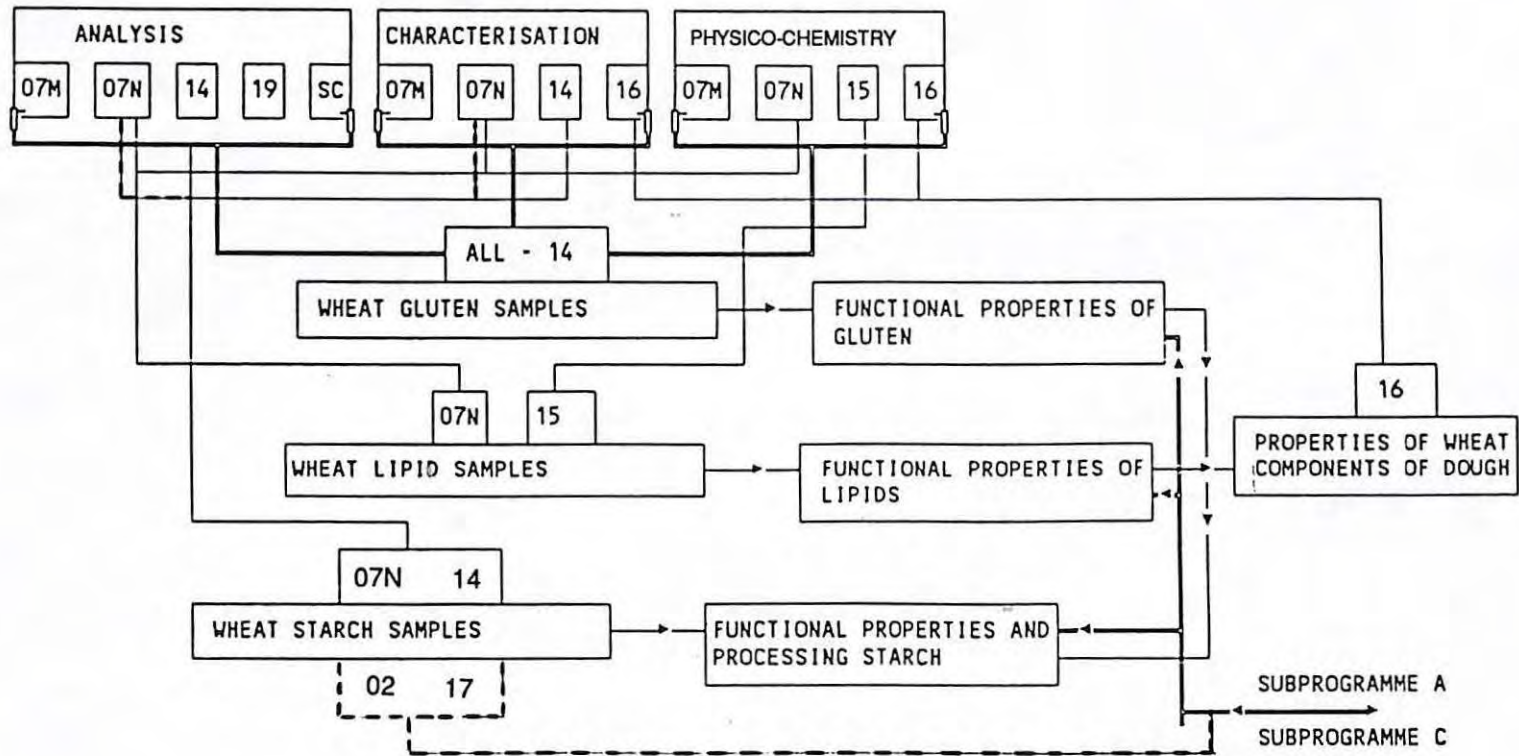




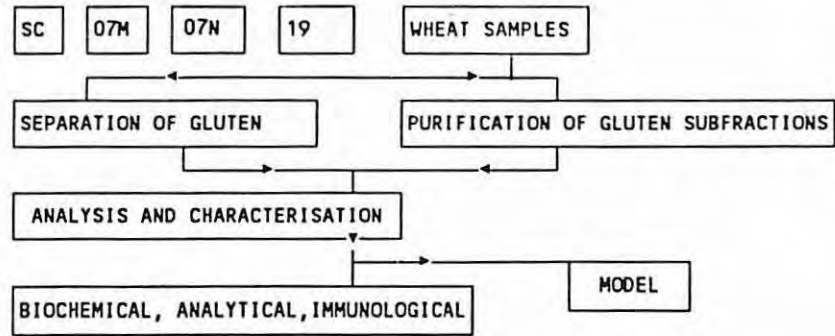
SUBPROGRAMME B : FUNCTIONAL COMPONENTS AND THEIR INTERACTIONS

TASKS	SUBJECT	07 M	07N	14	15	16	19	22	23	y 1	y 2	y 3	y 4	TOTAL /TASK
B.1	Component Interactions													
B.1.1	Gluten subfractions	36	18				72	81	80	45 45	38 44	26 32	29 29	287
B.1.2	Wheat proteins	36	54		24					4 12	19 19	21 21	9 9	114
B.1.3	Gluten proteins				12	48				6 6	8 8	8 8	8 8	60
B.1.4	Proteins and Starch			78						9 9	9 11	12 12	12 4	78
B.1.5	Lipid interactions		36		24					10 16	10 10	8 2	2 2	60
B.2	Dynamics of Dough					54				6 6	6 12	6 6	6 6	54
B.2.1	Microscopic techniques													
B.2.2	Antibodies pentosan													
B.2.3	Pentosans - role													
B.2.4	Protein-lipid-pentosans													
B.2.5	Heat & Mechanical effects													
SUB TOTAL.		72	108	78	60	102	72	81	80	80 93	90 104	81 81	66 58	653

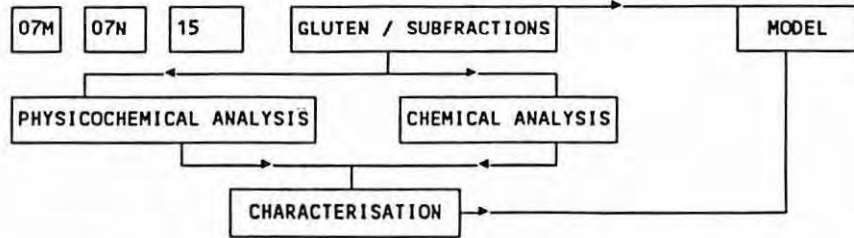
### INTERDEPENDENCE OF TASKS - SUBPROGRAMME B



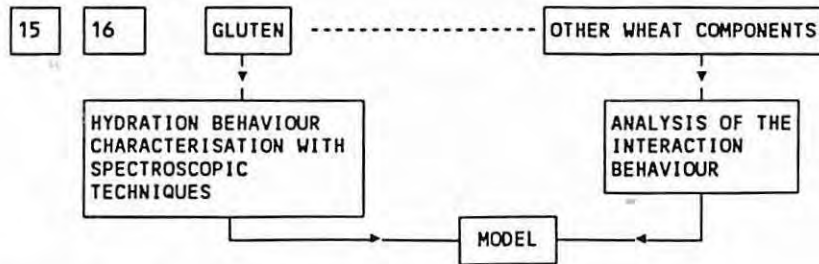
**TASK B. 1.1.**



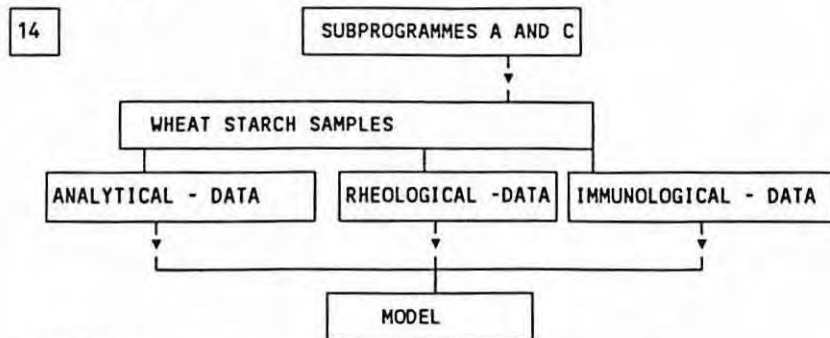
**TASK B. 1.2.**



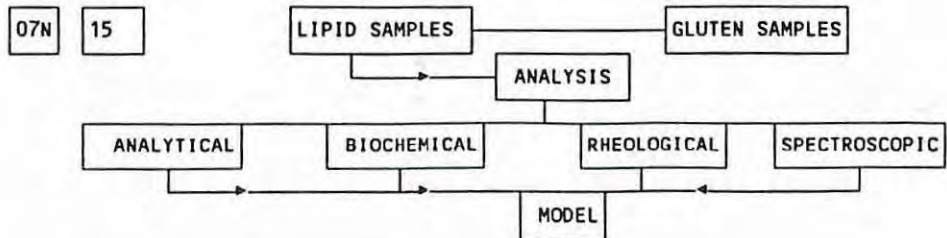
**TASK B. 1.3.**



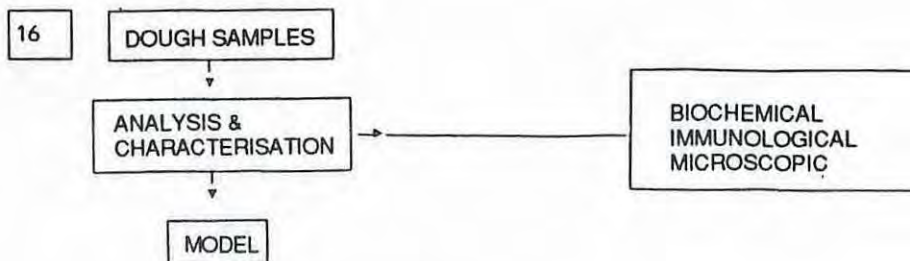
**TASK B. 1.4.**



**TASK B. 1.5.**



**TASK B. 2**

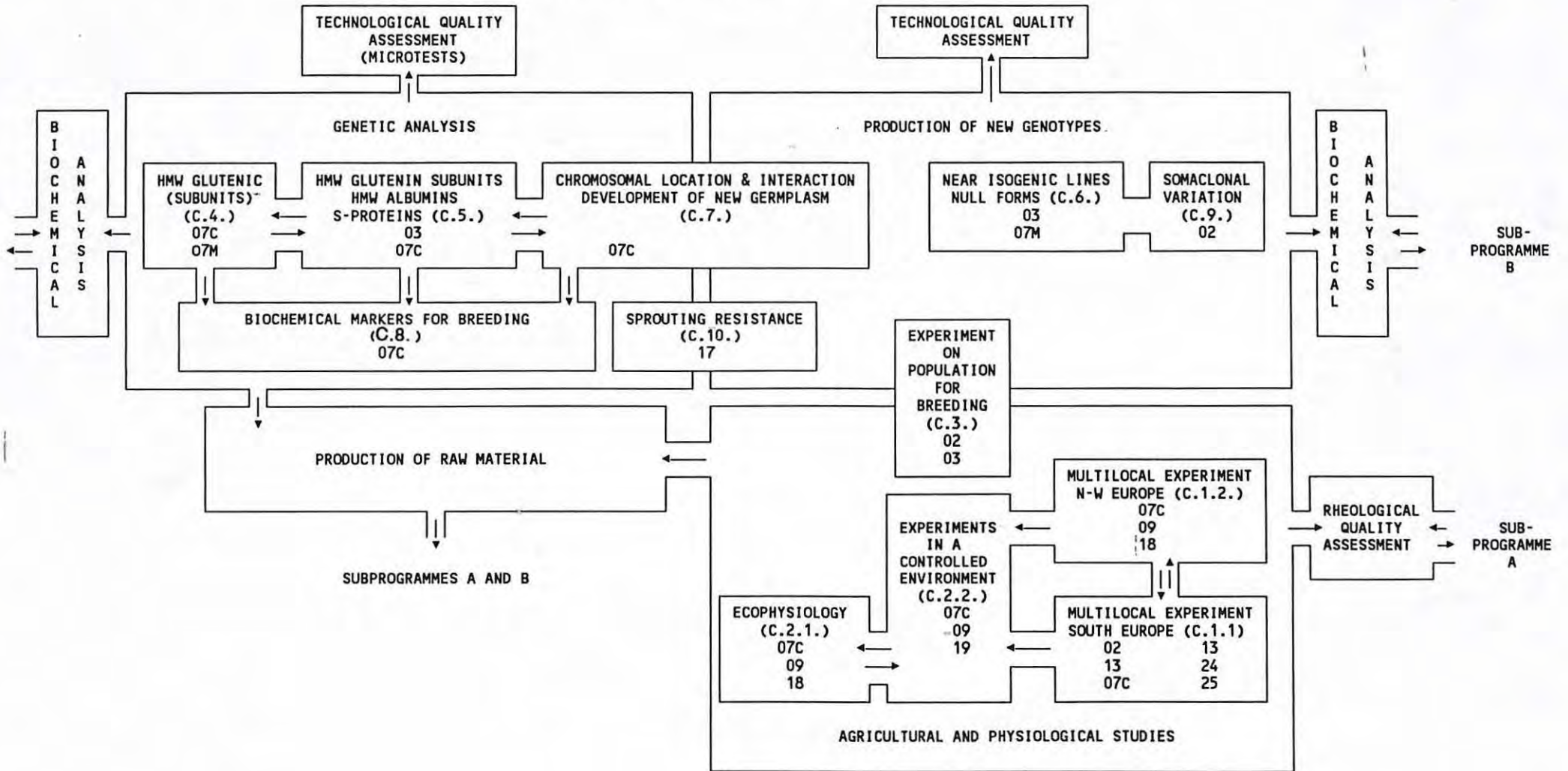


SUBPROGRAMME C : BIOCHEMICAL, GENETICS AND PHYSIOLOGY

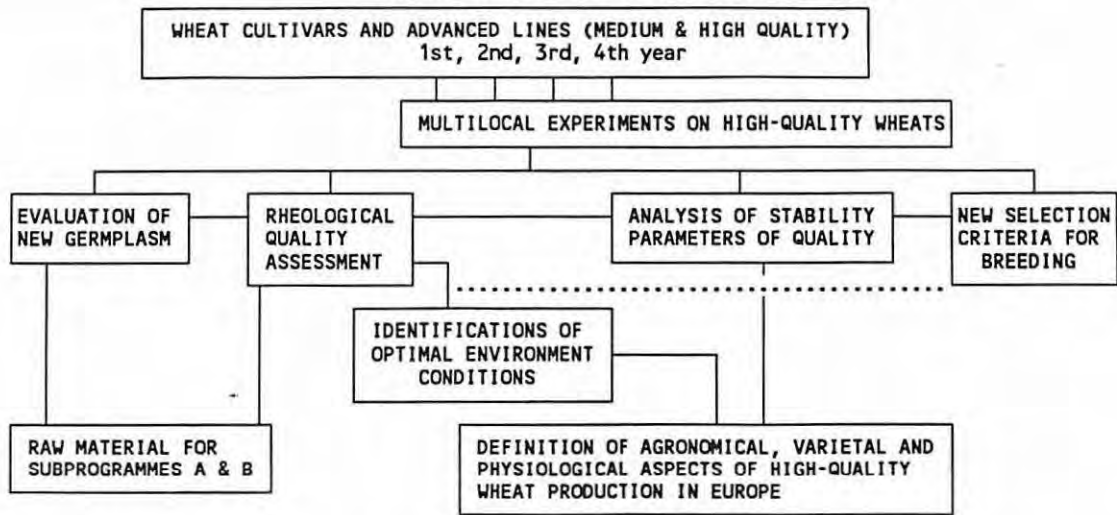
TASKS	SUBJECT	02	03	07C	07 M	09	17	18	24	25	y 1	y 2	y 3	y 4	TOTAL /TASK
C.1	Multilocations and Controlled conditions														
C.1.1	Southern Europe	31	27	15				36	47	49	30 30	30 30	30 30	20 5	205
C.1.2	Northern Europe			15		14		36			10 10	10 10	10 10	4 1	65
C.2	GxE interaction														
C.2.1	Ecophysiological			10		20		10			6 6	6 6	6 6	3 1	40
C.2.2	Controlled Environment					20		26			10 10	5 5	5 5	4 2	46
C.3	Breeding Populations	32	16								6 6	6 6	6 6	6 6	48
C.4	LMW subunit genetics			54	12						12 12	12 12	9 9	- -	66
C.5	HMW & protein genetics		30	24							6 6	6 12	12 6	6 -	54
C.6	Lines & isogenic lines		40		12					10	6 6	6 12	12 12	4 4	62
C.7	Storage protein genes			87							7 8	10 15	20 15	6 6	87
C.8	Biochemical markers			40							- -	- -	10 10	10 10	40
C.9	Somaclonal Variation	33									5 6	6 6	6 4	- -	33
C.10	Sprout resistance						54				9 10	7 7	7 7	4 3	54
SUB TOTAL		96	113	265	24	54	54	108	47	59	107 110	109 126	138 125	67 38	800



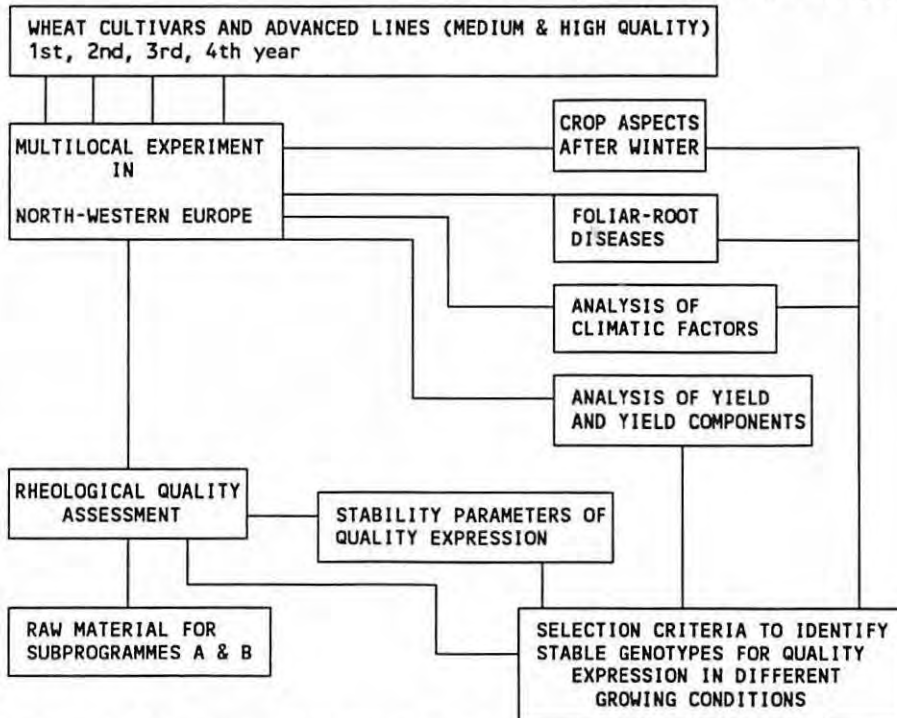
DIAGRAM OF INTERDEPENDENCE OF TASKS - SUBPROGRAMME C



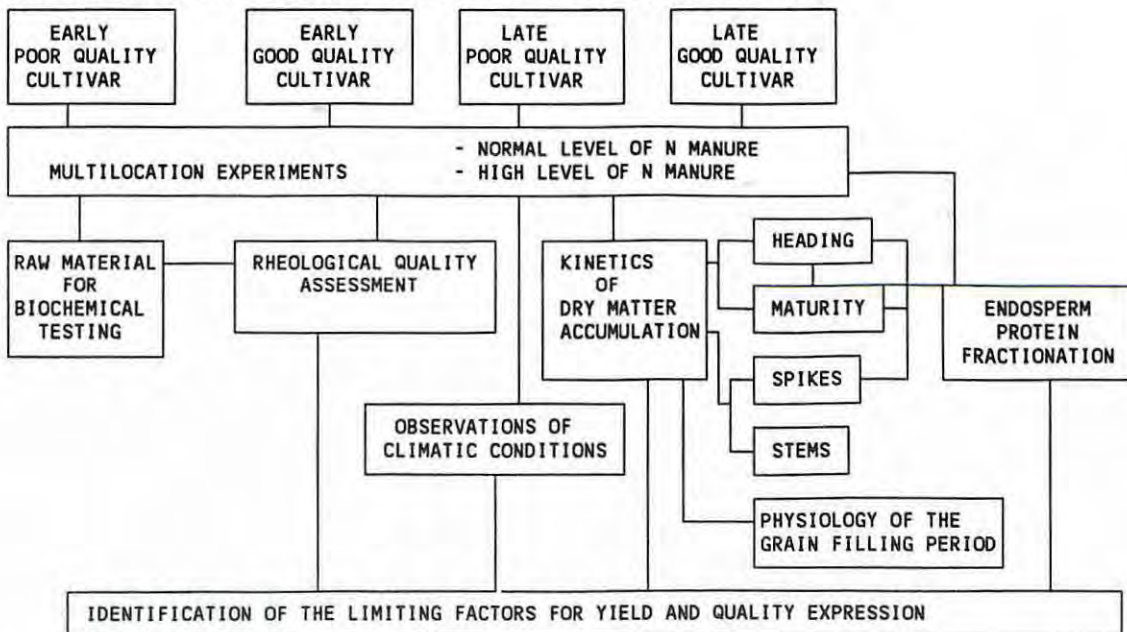
**TASK NO. C 1.1. SUBNETWORK 1 (South Europe)**



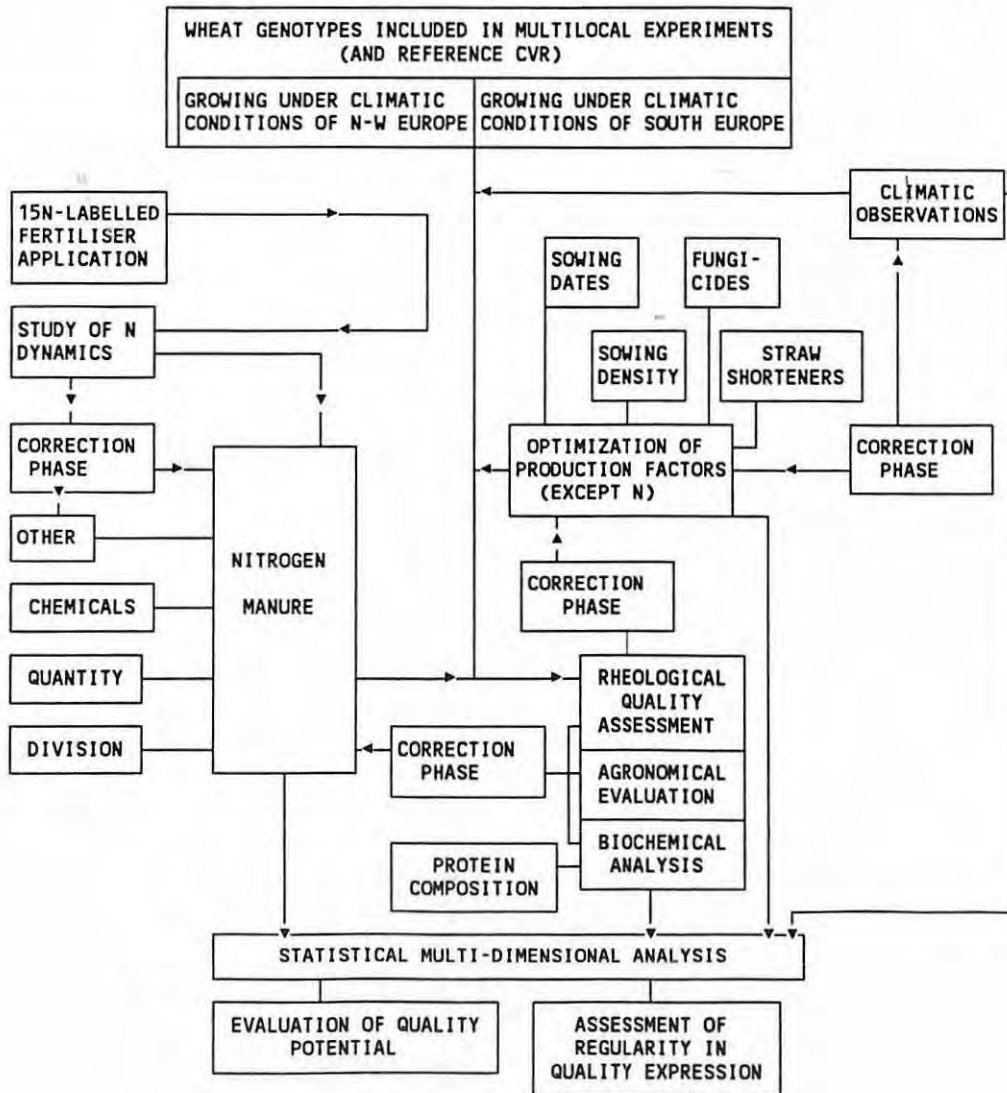
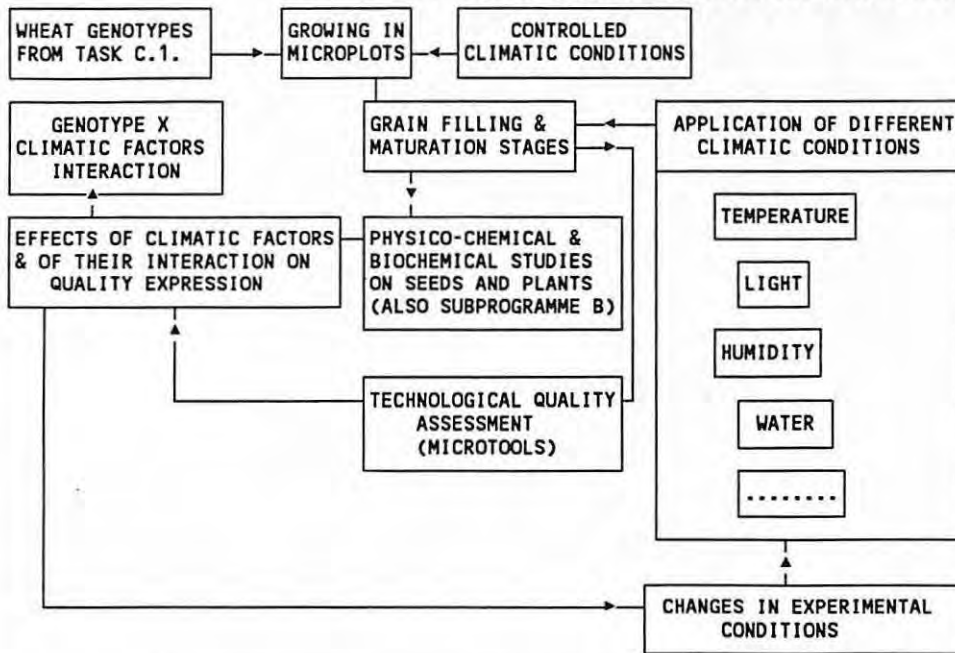
**TASK C.1.2. SUBNETWORK 2 (North-Western Europe)**



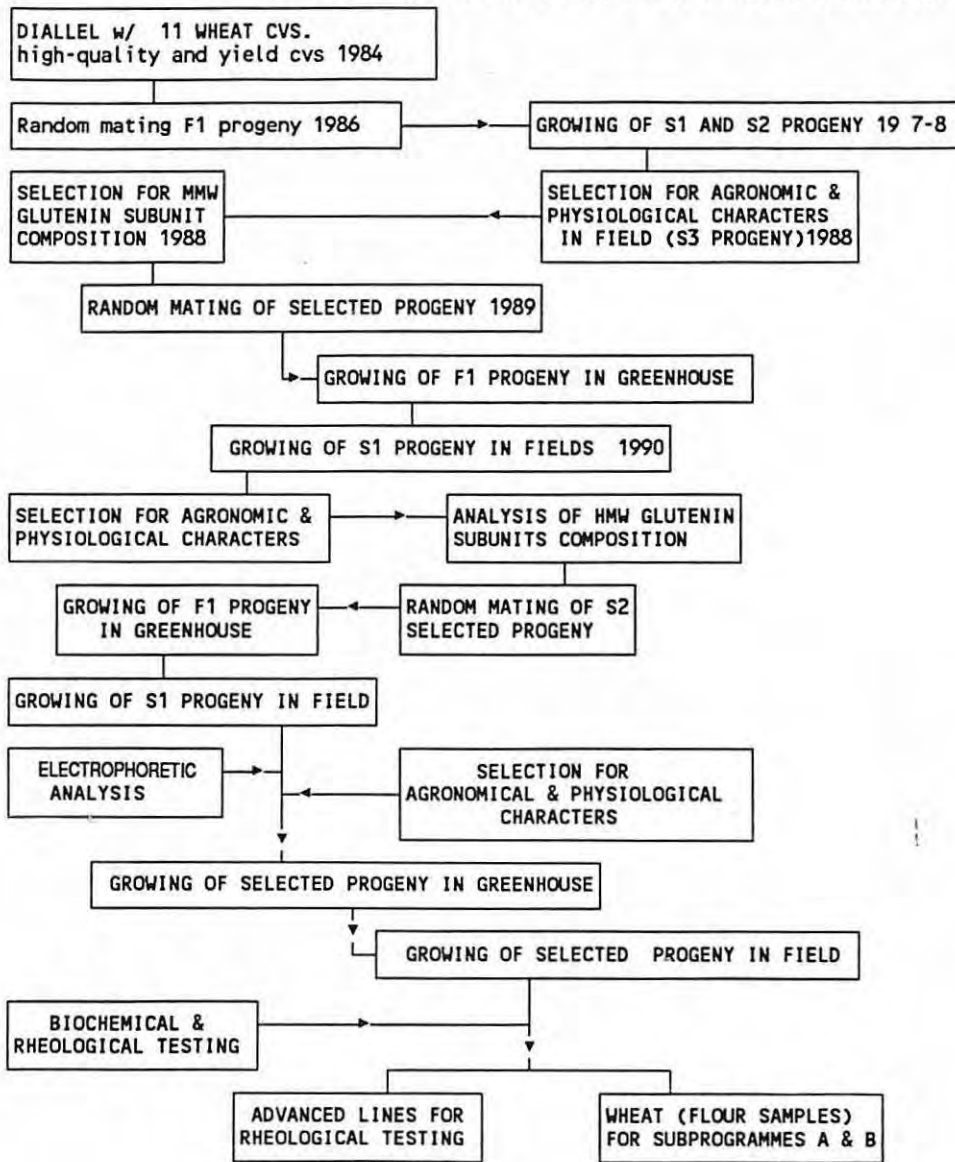
**TASK NO. C.2.1. ECOPHYSIOLOGICAL APPROACH OF THE GENOTYPIC EXPRESSION**



TASK NO. C.2.2 EXPERIMENTATION IN A CONTROLLED ENVIRONMENT

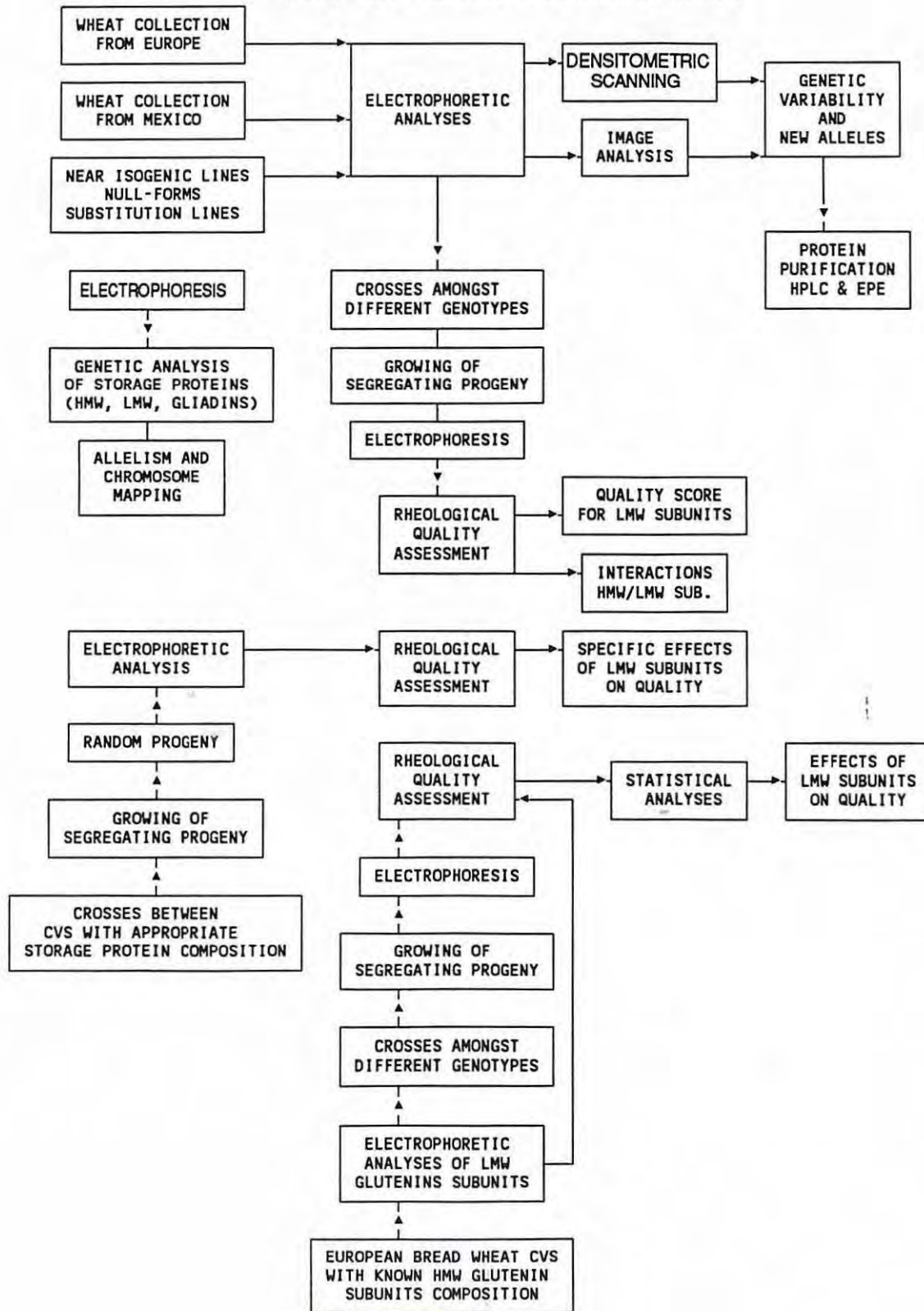


TASK NO. C.3. EXPERIMENTATION OF POPULATIONS FOR BREEDING

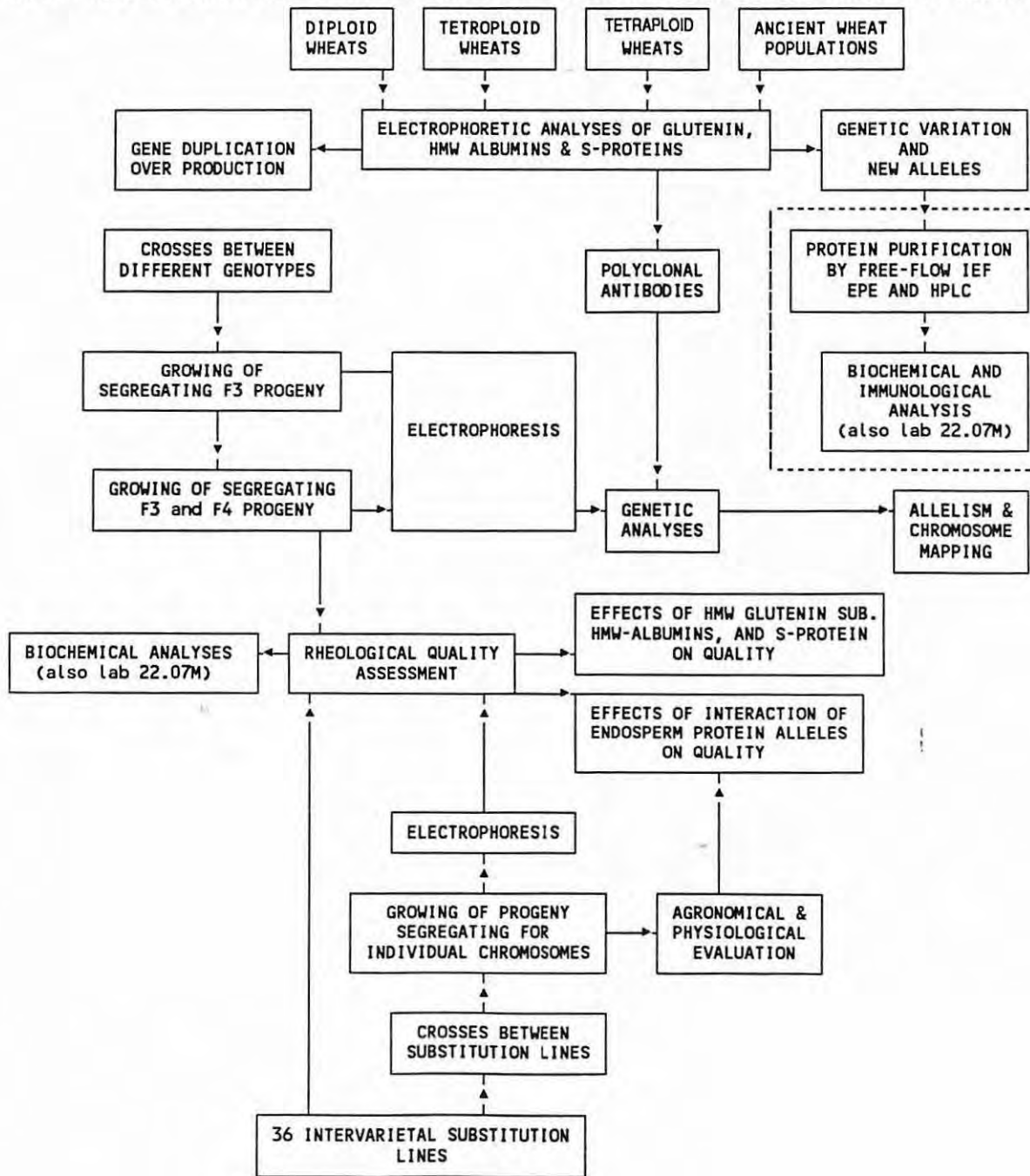




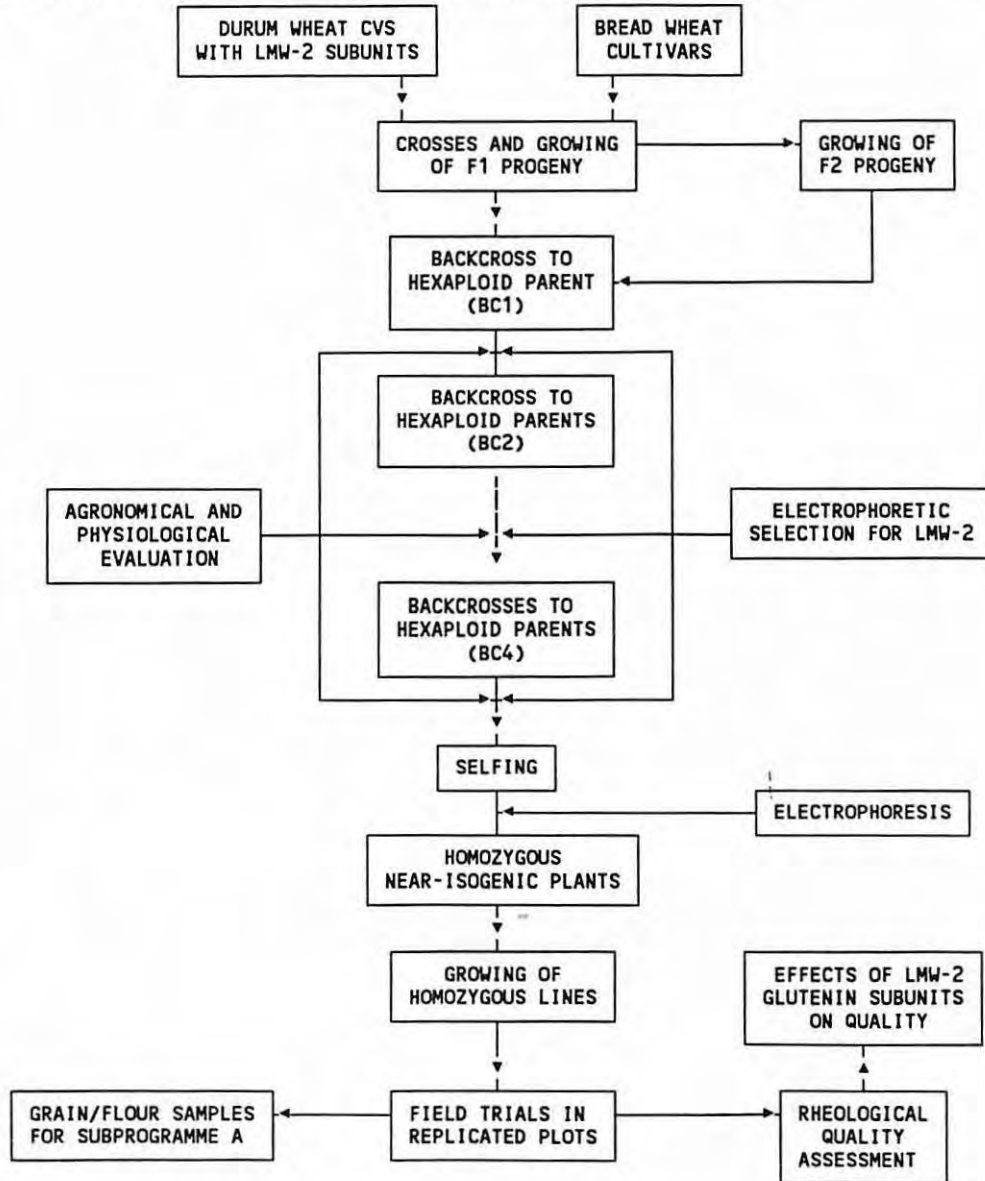
TASK N° C.4. GENETICS OF LMW GLUTENIN SUBUNITS



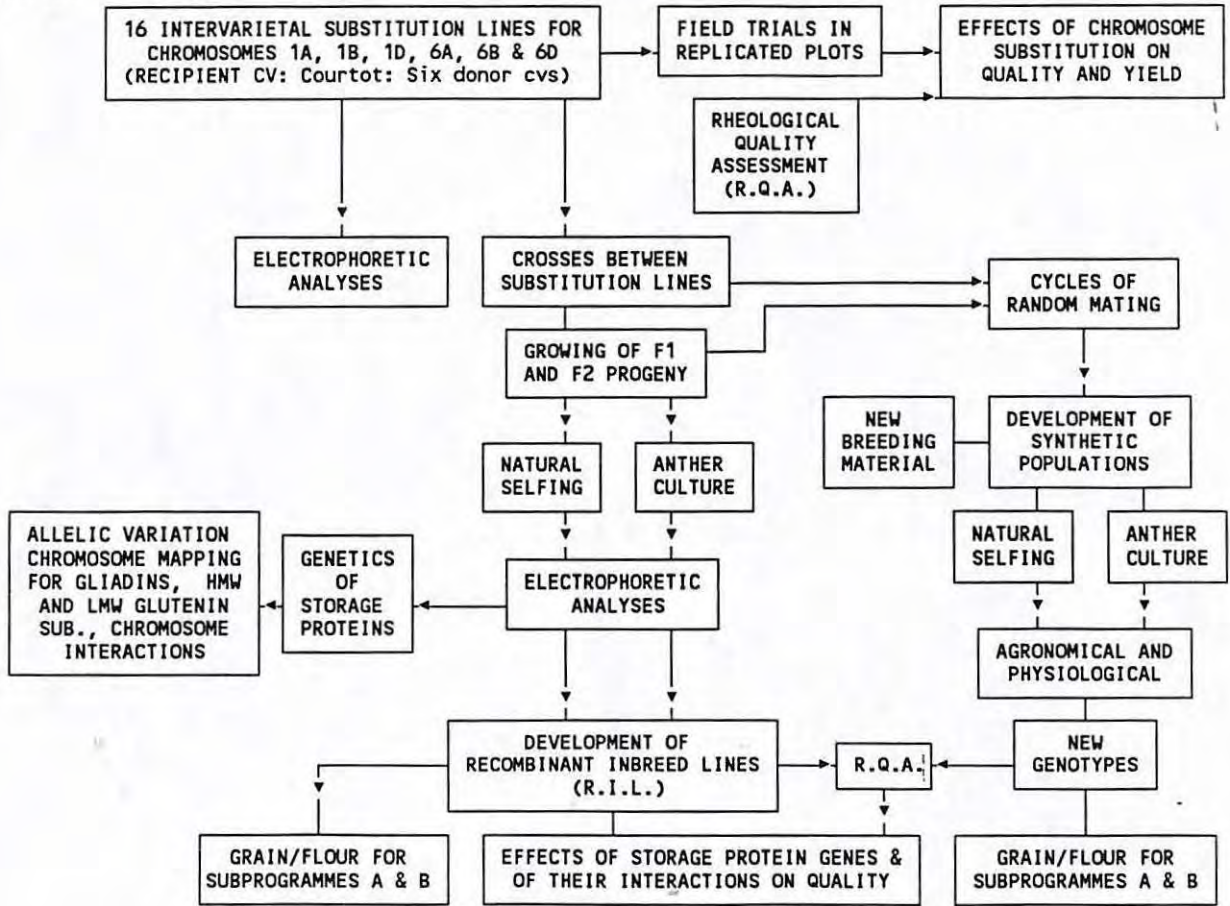
TASK N° C.5. GENETIC AND TECHNOLOGICAL ASPECTS OF HMW GLUTENIN SUBUNITS HMW ALBUMINS AND S-PROTEINS



**TASK N° C.6. PRODUCTION AND TECHNOLOGICAL EVALUATIONS OF NULL FORMS, AND NEAR-ISOGENIC LINES WITH DIFFERENT COMPOSITIONS FOR HMW GLUTENIN SUBUNITS, LMW GLUTENIN SUBUNITS AND GLIADINS**

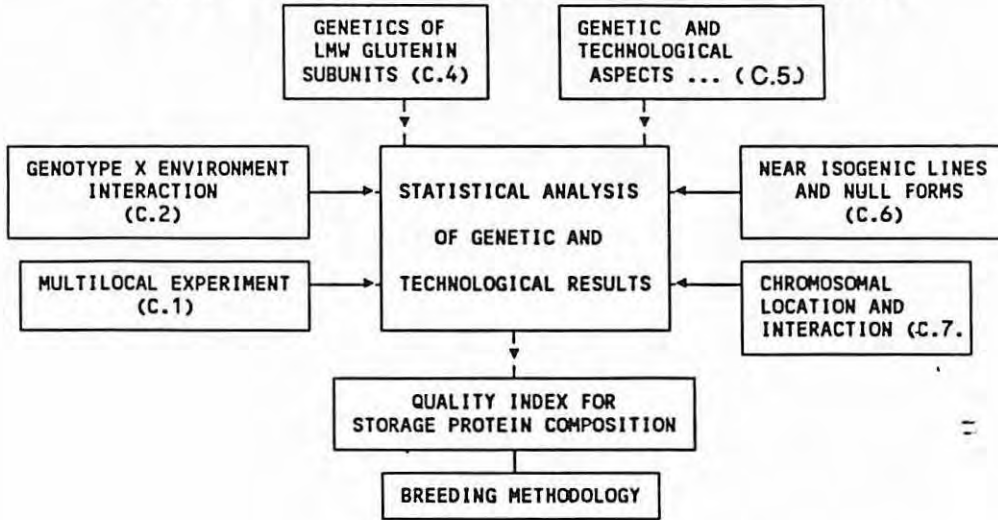


**TASK N° C.7 CHROMOSOMAL LOCATION OF STORAGE PROTEIN GENES, CHROMOSOME INTERACTION ON PROTEIN SYNTHESIS AND DEVELOPMENT OF NEW GERMOPLASM**

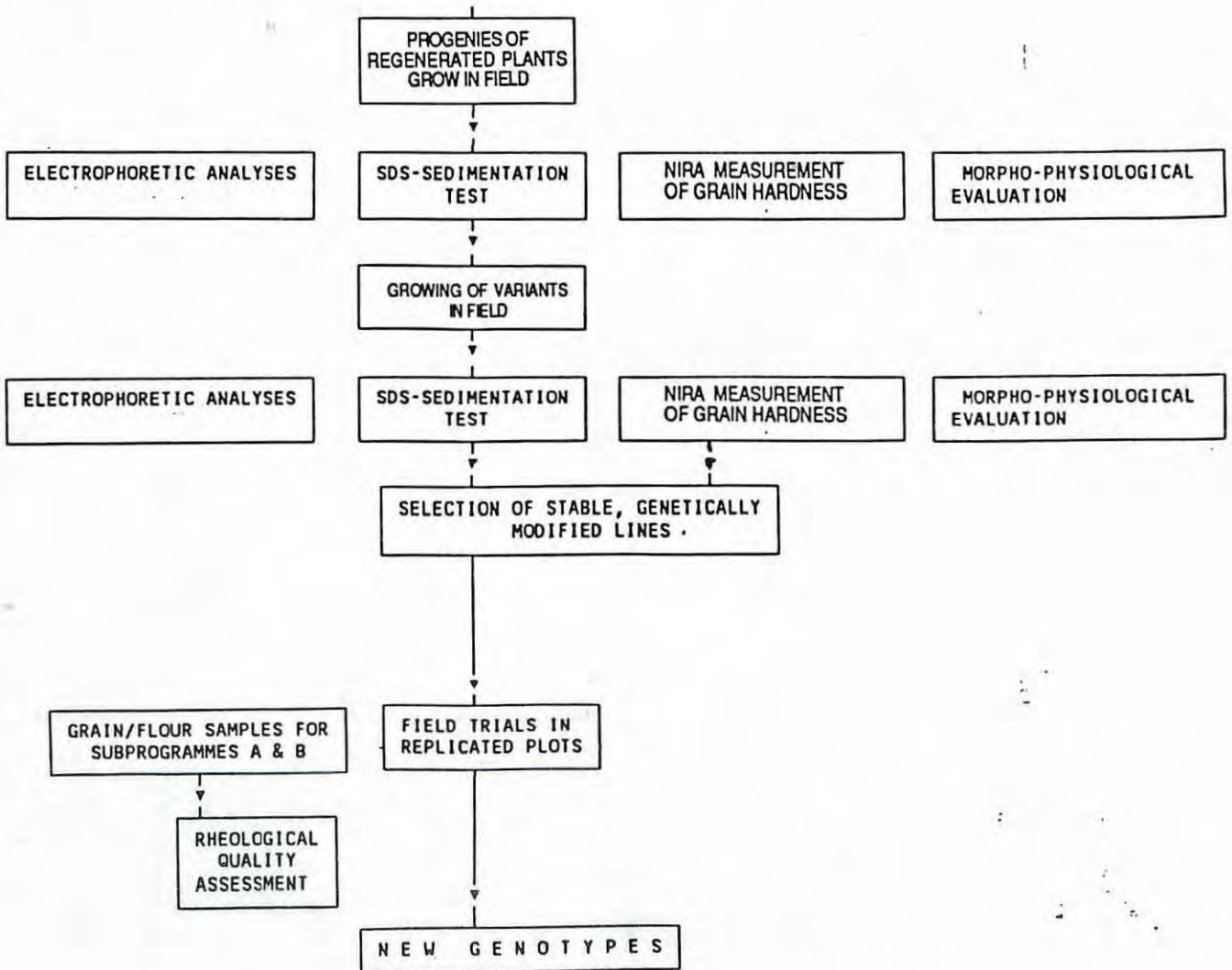




**TASK N° C.8 BIOCHEMICAL MARKERS FOR SCREENING EARLY GENERATIONS**



**TASK N° C.9. SOMACLONAL VARIATION FOR FACTORS AFFECTING BREAD MAKING QUALITY**



TASK C.10 SPROUTING RESISTANCE

