



*Brassica 2000*

***3<sup>rd</sup> ISHS International Symposium  
on Brassicas***

***12th Crucifer Genetics Workshop***

held at

**Horticulture Research International  
Wellesbourne, CV35 9EF, UK**

**5<sup>th</sup>-9<sup>th</sup> September 2000**

**ABSTRACTS**



Please note that first authors only are listed in the Contents. The postal address is given for the first author only. Please refer to list of delegates for additional contact information.

Edited by Graham J. King, Horticulture Research International

Abstracts of the 3<sup>rd</sup> ISHS International Symposium on Brassicas and 12th Crucifer Genetics Workshop, Wellesbourne, Sept 5-9 2000. Horticulture Research International, Wellesbourne. 2000

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**Opening Symposium**

**Contested innovation: what lessons may be drawn from public controversies on GMOs?**

Pierre-Benoit Joly

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This paper analyses the failure of the introduction of GM crops in Europe. It challenges the idea that this failure is due to the influence of the public irrationality on decision processes. Instead, it focuses on the inability of the regulatory system to manage complex situations which result from the limits of sound science in contexts of uncertainty and controversy. It argues that the way out of the currently blocked situation requires more open and participatory processes of innovation and technological assessment, based on the normative stance provided by the precautionary principle.

**Opening Symposium*****Brassica* Vegetables and Human Health:  
Glucosinolates in The Food Chain**

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Although some of the glucosinolates have long been known to exert antinutritional effects in animals, there is an increasing body of evidence to suggest that breakdown products derived from this complex group of compounds provide a potentially important source of anticarcinogens in human foods. Coupled with the epidemiological evidence for a protective effect of *Brassica* vegetables against a variety of human cancers, these findings provide a strong motive for the manipulation of glucosinolate levels in vegetables for human consumption. Recent progress in our understanding of the genetic basis of glucosinolate biosynthesis make this strategy a practical possibility, but before it can be exploited commercially, both the safety and the health benefits of increased glucosinolate consumption must be unequivocally established. To achieve this it will be necessary to establish the levels of intact glucosinolates and breakdown products ingested from vegetables harvested, stored and processed under commercial conditions. The quantities and types of glucosinolate breakdown products reaching various regions of the intestinal mucosa must be quantified, together with their availability for interactions in the gut and the systemic tissues. Finally it is essential to estimate the dose-response relationship between glucosinolate breakdown products and selected biological end-points in humansubjects, so that both the safety and benefits can be properly assessed. Many of these issues have recently been explored in an integrated multidisciplinary project funded by the European Union.

**Sustainable production, Agronomy & Diseases****Genetics of disease resistance in *Arabidopsis* to crop pathogens**

Eric B. Holub

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*Arabidopsis* is generally resistant as a species to most foliar crop pathogens. This species level resistance to non-native pathogens could potentially provide durable resistance for crop improvement if examples can be found which are amenable to genetic and molecular analysis. Genetic analyses of genotype or race specific resistance (associated in crops with “boom and bust” of resistant cultivars) is beginning to reveal likely components of species level resistance. RPS4 and RPS5 are pathogen “surveillance” or so-called R-genes (naturally variable resistance genes) which were isolated from *Arabidopsis* and provide components of resistance to legume pathogens (*Pseudomonas syringae* pv. *psis* and *P.s.phaseolicola*, respectively). Another example, EDS1, is a gene that is required for transducing defense responses triggered by a major subclass of R-genes (called TIR-NBS-LRR). Mutation in EDS1 renders a plant fully susceptible to isolates of *Albugo candida* (white rust) from *Brassica oleracea*., whereas wild *Arabidopsis* appears to be universally resistant as a species to this parasite. Such examples are not sufficient for explaining species level resistance because redundancy is likely at levels of both pathogen detection (R-genes) and at downstream defense responses defined by genes such as EDS1. For instance, eds1 mutants retain residual resistance to *Peronospora parasitica* (downy mildew) from brassicas. Additional defense pathways will be discussed including NDR1--mediated resistance thought to be triggered by a different subclass of R-gene (LZ-NBS-LRR), and an alternative salicylate-independent source of downy mildew resistance.

**Sustainable production, Agronomy & Diseases****Cytological events associated with infection of *Crambe* spp. by *Leptosphaeria maculans***

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The first true leaves of 14-day-old plants of six *Crambe abyssinica*, four *C. hispanica*, and two *C. glabrata* accessions were inoculated with GL-11, a *gusA* positive isolate of *Leptosphaeria maculans*. Inoculations were performed by puncturing the leaves with a needle and depositing a droplet of pycnidiospore suspension on each wound site; control plants were inoculated with sterile distilled water. Thirteen days after inoculation, the inoculated leaves were removed and a section of tissue around each wound site was excised and processed for *gusA* expression. Tissue samples were then decolourized in boiling ethanol and cleared in saturated chloral hydrate. Cleared tissue samples were examined for browning and then stained for lignin or callose. Leaf tissue of all the *Crambe* accessions was generally resistant to infection by *L. maculans*. Lesions were less than 2 mm in diameter for all but one accession, and there was no pycnidium formation in any of the lesions. Browning was restricted to cells bordering wound sites in all the control samples. More extensive browning, extending beyond the wound site and the hyphal front, was present in all samples inoculated with GL-11. More browning was evident in accessions of *C. abyssinica* than in accessions of the other two species. A lignification zone two to three cells wide surrounding the region of browning was present in control samples of all the *C. glabrata* and *C. hispanica* accessions; this lignification zone was not evident in control samples of most accessions of *C. abyssinica*. No lignification zone was observed in any of the samples inoculated with GL-11. More callose was present in control samples of *C. abyssinica* than in control samples of the other two species; the same was true for samples inoculated with GL-11.



**Sustainable production, Agronomy & Diseases****Resistance to *Xanthomonas campestris* pv. *Campestris* in *Brassica* spp**

Vicente, Joana G.

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Six races of *Xanthomonas campestris* pv. *campestris* (X.c.c.) have been identified on the basis of their reactions on a series of differential *Brassica* genotypes. Only races 1 and 4 are of major importance world-wide in *B. oleracea*. The relationship between X.c.c. races and *Brassica* cultivars has been explained on the basis of gene-for-gene interactions, with four avirulence genes in the pathogen matched by four resistance genes in the host. Searches for resistance in collections of *B. oleracea* (C genome) were mainly unsuccessful. However, resistance to races 2, 3 and 5, which are rather rare races, was present in a number of accessions. Resistance to race 3 is controlled by a single dominant gene in several lines. Partial resistance to races 1 and 3 in the line Badger Inbred 16 is quantitative and recessive. In *B. rapa* (A genome) and *B. napus* (AC) resistance to race 4 is widespread. This resistance is controlled by a single dominant gene. The gene Xca4 was mapped in the *B. napus* A-genome indicating that the gene originated from *B. rapa*. Resistance to races 1, 3 and 4 was found in *B. nigra* (B genome), *B. carinata* (BC) and *B. juncea* (AB). This resistance appears to be dominant. A different class of resistance, conferring resistance to all races was also observed in these three species, suggesting a B genome origin. Preliminary results indicate that this potential race non-specific resistance is recessive in *B. carinata*. Transference of genes to *B. oleracea* will be attempted via interspecific crosses and/or transformation.

**Genomics****Comparative physical mapping and gene expression analysis of segments of the genome of *Brassica oleracea* var *alboglabra* that are homoeologous to sequenced regions of chromosomes 4 and 5 of *Arabidopsis thaliana***

Ian Bancroft

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The brassicas include the group of crops most closely related to the key model plant *Arabidopsis thaliana*. They therefore represent an obvious first target for the application of the genome sequence and functional genomics data being generated from *Arabidopsis*. The brassicas also provide an excellent opportunity to study the mechanisms effecting genome evolution in plants. To support work in these areas, we have constructed and characterized a new BAC library (the JBO library) using genomic DNA from *Brassica oleracea* var. *alboglabra* and the transformation-competent vector pBIBAC2. We have used the library to analyze the extent of conservation of triplicated (paralogous) segments of the *B. oleracea* genome relative to their homoeologous regions in the genome of *Arabidopsis*. The relatively large size of the inserts in the JBO BACs (average 145kb) was crucial to our near-complete representation in BAC contigs of 6 regions of the genome of *B. oleracea*. Details will be presented of the divergence we have observed in gene content, gene order and gene spacing between the 2 regions of the genome of *Arabidopsis* and their corresponding 6 regions in the genome of *B. oleracea*. Data will also be presented from preliminary analyses of the sequence divergence and differential expression of some of the genes contained in these regions.

**Genomics****Integrating the molecular cytogenetic and genetic maps in *Brassica oleracea***

Sue Armstrong

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Linking genetic maps constructed with molecular markers to the corresponding physical maps is an important goal in the investigation of crops. The relationship between these maps is reviewed, and we illustrate our approach by the analysis of the short arm of chromosome 4 of the model dicotyledonous plant, *Arabidopsis thaliana* L. We show that it is possible to produce a detailed molecular cytogenetic map by placing multicopy and unique sequences in relation to euchromatic and heterochromatic segments. This map correlates well with the molecular physical map (sequence map), but as expected show differences to the genetic map. For *Brassica oleracea* L., as a first step we have produced a partial karyotype based on the combination of chromosome size, arm ratios and the location of repeated 45S rDNA, 5S rDNA and pericentromeric heterochromatin sequences by fluorescence *in situ* hybridisation (FISH). We are currently using BACs, cosmids and RFLPs, with known genetic map positions, as FISH probes to identify additional linkage group associated markers for the chromosomes. The construction of a definitive karyotype, with all linkage groups assigned to our cytogenetic map is approaching completion.

**Quality, Functional Foods & Health**

**Enhancing the anticarcinogenic activity of broccoli**

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In this paper, I will summarize the on-going programme at the John Innes Centre to develop commercial broccoli cultivars with enhanced anticarcinogenic activity. I will firstly summarize information about the genetic regulation of glucosinolate biosynthesis obtained from studies of *Brassica* and *Arabidopsis*, and describe both transgenic and non-transgenic approaches to enhancing specific glucosinolate in broccoli. I will then discuss a marker-assisted breeding programme which has introgressed segments of the genome of *B. villosa*, a wild member of the *B. oleracea* n=9 complex, into broccoli and the assessment anticarcinogenic activity of the breeding lines through the use of cell culture assays. I will then conclude by discussing initial human dietary intervention studies with the novel broccoli lines.

**Quality, Functional Foods & Health****Quality and technological characterisation of two cauliflower hybrids (*Brassica oleracea* L. convar. *botrytis* L.)**

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The quality and technological characteristics and comparisons between two different hybrid cauliflowers were studied. The two hybrids were Nautilus (early classification, harvest at 85 days from transplanting) and Artemis (intermediate classification, harvest at 100 days from transplanting). They were harvested near Rome in December 1999. The analysis of the quality of the product was about physical-morphological, commercial characteristics and domestic preservation (8 days at 8-12°C). These included: dimensions, weight, volume, volumic mass and consistency (resistance force to penetration) of the cauliflower head and the cut strain of the stem. The two hybrids had very similar morphological values (average weight 1 kg), but only the 73% of Nautilus and the 63% of Artemis reached the optimum weight for retail (0.7 - 1.4 kg). At the harvest, the values of the consistency of the head of cauliflower and the cut of the stem are higher for Artemis than for Nautilus, but after 8-days of conservation, the reduction of the consistency was lower for Nautilus (-10%), than for Artemis (-44%). For Nautilus, inverse correlation was found between consistency reduction and cauliflower weight, while Artemis showed inverse correlation between consistency reduction and cauliflower volumic mass. It is very important to best explore the cause of these behaviours to direct the selection and agronomic practice to improve quality and technological characteristics of cauliflower. This also could be usefull in the resolution of the harvest mechanisation problem due to scarce uniformity of commercial maturity.

**Quality, Functional Foods & Health****Identification of genes and signalling pathways that control leaf senescence in *Brassica* and *Arabidopsis***

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Leaf senescence is a key step in the development of annual and perennial crops during which photosynthetic activity decreases and a highly controlled dismantling of the cellular components occurs. As a result, a large proportion of the constituents of previously green cells is transported from the senescing material and eventually is stored in the developing seeds. The efficiency of senescence is therefore important for the success of subsequent generations. As well as occurring as part of the developmental process, senescence like symptoms are also induced in response to many different environmental stresses such as pathogen infection, temperature changes, shading, water availability, ozone and UV-B. Also, senescence like symptoms are induced in plants after harvest. Postharvest deterioration of vegetable material is visible as a yellowing of green tissue which is accompanied by loss of nutritional quality. If we could understand the mechanisms by which plants regulate senescence then it would be possible to manipulate the process in crop plants to improve their yield, appearance and quality. Current research is aimed at the identification and characterisation of genes and pathways that are involved in the regulation of senescence in *Brassica* and also in comparing the senescence parameters that occur during developmental, stress induced and postharvest senescence. *Arabidopsis* genomic resources are being used to investigate the function of several senescence enhanced regulatory genes.

**Genetic improvement****Role and structure of R&D in a modern seed company**

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This is a period of major changes, in which Seed companies are involved in the same way as other businesses. Development of new technologies is speeding up the movement, and powerful supermarket chains and emerging consumer lobbies are putting more and more pressure on producers. The Seed Industry is now in the centre of this maelstrom and must adapt or disappear. The constitution of large global groups is a consequence of both the internationalization of the markets and the need for new biotechnologies, giving them a chance to remain leaders on their markets, and develop their sales in emerging markets . The costs involved are huge and this is why less and less companies can afford to work by themselves. This trend started more than a decade ago, and will continue till there are probably less than ten large players left on the seed market. These Groups have different development strategies, depending on their history or management objectives. Be it horizontal, vertical , or sometimes mixed, the goal of the organization is to create varieties and increase market shares on target markets worldwide. Research and Development are driving the movement, and it is from their capacity to innovate and fine tune their markets that Companies will thrive or decline. Consumer concerns are becoming more and more important and Seed Companies need to include ethics, health and environmental issues in their development approach.

**Genetic Improvement****Enhanced marker-assisted breeding in *Brassica* crops using microsatellites**

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The Agriculture and Agri-Food Canada Saskatoon Research Centre is developing *Brassica* microsatellite markers in association with a consortium of 14 industrial partners. A number of libraries highly enriched for a variety of simple sequence repeats have been developed using several *Brassica* species and a variety of restriction enzymes. The high capacity DNA sequencing program is expected to yield approximately 1500 microsatellite markers and accurate estimates of the level of polymorphism and the degree of robustness of the markers are being established. Genetic mapping in the well-characterised and highly polymorphic N-fo-61-9 (Parkin et al. 1995; Genome 38: 1122-1131) and N-o-72-8 (Sharpe et al. 1995; Genome 38: 1112-1121) populations is being used to determine the genomic locations of microsatellite loci. The microsatellite markers will make possible a six-fold increase in the efficiency of marker-assisted breeding over RFLP markers. These markers will be applied to a variety of breeding applications, including, marker-accelerated backcrossing, QTL analysis, population development and genetic diagnostics.



**Genetic improvement****Developmentally "aberrant plants" in F1 hybrid of  
*Brassica oleracea***

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Over the last fifty years, F1 hybrids have been developed to improve the homogeneity of *Brassica* crops. In these varieties, male sterility (mainly CMS) allows the production of 100% hybrid seed. Nonetheless, under field conditions, we observed aberrant plants that were unsuitable for harvest in F1 hybrid. The phenotypes of these plants principally involve modification of three characters: shape, size and leaf thickness. We observed similar developmental perturbations in a range of different cultivars (hybrids, lines and populations). Between 5% and 21% aberrant plants were observed in crops of the same hybrid observed over a 4 year period. We were not able to identify agronomical parameters (seed origin, period of planting, locality) that could be correlated with the frequency of aberrant plants. Aberrant plants responded in different manners to in vitro propagation, either remaining aberrant, altering their phenotype or recovering a normal phenotype. Analysis of ploidy level by flow cytometry did not reveal any abnormalities linked to the observed phenotypes. Inheritance of the aberrant phenotypes was variable. Progeny obtained by selfing aberrant plants included (i) families of 100% normal plants, (ii) families showing mendelian segregation of the phenotype, (iii) mixtures of aberrant plants with numerous phenotypes and normal plants and (iv) families of 100% aberrant plants. Further studies have been engaged to identify agronomic factors that favour the appearance of aberrant phenotypes and to understand the underlying genetic mechanism. The susceptibility of different genetic backgrounds to the development of the aberrant phenotypes will also be considered.

**Genetic Improvement****Elucidation of genes controlling downy mildew resistance in broccoli**

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Downy mildew is one of the most destructive diseases of broccoli and other *Brassica oleracea* L. crops. This fungal disease, caused by the biotrophic parasite *Peronospora parasitica* (Pers. Fr.) Fr. has worldwide distribution. Fungicide application can provide control of downy mildew in broccoli. However, the use of resistant broccoli cultivars is an alternative control method that could provide a practical, long-term, and environmentally-benign means to limit damage due to this disease. We have an ongoing program to develop doubled-haploid (i.e., homozygous) lines of broccoli that express high levels of downy mildew resistance and that may serve as inbred parents for development of resistant F1 hybrids. Among our developed lines, we have identified doubled-haploid lines that are susceptible at the cotyledon stage but highly resistant at the true-leaf stage and others that are highly resistant at cotyledon as well as true-leaf stages. Our long term goal is to elucidate genes for downy mildew resistance. To study inheritance of the different downy mildew resistance phenotypes we have identified, resistant lines were crossed to a susceptible line, and conventional (e.g., F2 and backcross) and doubled-haploid populations were developed from the resulting F1 hybrids. These populations were evaluated for response to inoculation with *P. parasitica*. Inheritance of true-leaf stage resistance was determined to be controlled by two complementary dominant genes. This mode of inheritance was confirmed in both conventional and doubled-haploid populations. A RAPD marker linked to the true-leaf stage resistance has been identified and sequenced. Initial tests indicate that cotyledon stage resistance exhibits dominance.

**Sustainable production, Agronomy & Diseases****Temperature influences on the plant development of different maturity types of cauliflower**

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Cauliflowers can be harvested for twelve months of the year in the UK. This is done by growing in production areas with slightly different temperature conditions and by using different maturity types of cauliflower. The latter produce crops maturing in early summer, summer/autumn and winter. The information presented in this paper is taken from a compilation of studies of the growth and development of a number of varieties in each of these maturity types over more than twenty years. This work has in particular studied the numbers of leaves produced, the timing of curd initiation, and the conditions satisfying the vernalization requirement of the crop. For each maturity type information is presented showing the duration of juvenility, the timing of curd induction, the numbers of leaves produced at curd initiation, the optimum temperatures of vernalization and the duration of vernalization. The contrast in growth of these types is interesting. Early summer crops have the shortest period from planting to curd initiation and initiate curds at the lowest numbers of leaves. Winter cauliflower crops have the longest period from planting to curd initiation and produce much higher numbers of leaves. The data also show that early summer cauliflowers initiate curds at lower ambient temperatures than winter cauliflowers and that the optimum temperature for vernalization of all types is relatively high, ranging from 9 to 14°C. Indications are that under optimum conditions the duration of vernalization increases from early summer cauliflowers to winter cauliflowers. Summer/autumn cauliflower characters are intermediate between early summer and winter types.

**Sustainable production, Agronomy & Diseases****Empirical models for harvest date prediction in broccoli (*Brassica oleracea* L. var. *italica* Plenck)**

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Broccoli is characterised by fluctuations in product supply. Therefore, harvest date prediction and programming continuity of supply is essential. Although crop growth and developmental models have been described in the literature, increased research on empirical models for harvest date prediction is needed. Here, data from 16 broccoli crops of 4 cultivars (Compacta, Comanche, Green Valient and Marathon), carried out all year round for two years, in three locations representative of the most important areas of broccoli production in Portugal, showed that a cold requirement was not detected for any of these cultivars and that, the time from planting to spear initiation could be described through a linear relationship between the reciprocal of time to spear initiation and the mean air temperature. From spear initiation to crop maturity the best empirical description was attained by using a quadratic relationship between thermal time, accumulated after spear initiation above 0°C, and spear growth, although previous work to predict the duration of spear growth from initiation, for an early cultivar (Mercedes), was found to be closely related to an exponential model. From transplanting to harvest, the duration of crop growth can then be determined by combining the model for spear initiation with that for spear growth. From a field sampling of spear diameter and using long term average temperature data, the thermal time required for a spear to reach a certain diameter can be converted into the expected days to maturity. In addition, yield can be predicted by the linear relationship between spear diameter and spear fresh weight.

**Monitoring and control of *Plasmodiophora brassicae* in spring oilseed brassicas**

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In the 1980's, severe attacks and subsequent marked yield reductions from clubroot infections were observed in Swedish oilseed crops. In this study, the presence of clubroot in soil samples from 190 fields was assessed using a bioassay based on baiting the soils with *Brassica campestris* spp. pekinesis (Chinese cabbage) cv Granaat. Clubroot was detected in 148 (78 %) of the fields. Subsequent testing showed that disease incidence was significantly decreased after *Brassica* crops ceased to be grown. Fitting the formula for radioactive decay to the data gave a half-life of 3.6 years for a field with 100 % infestation. The level of infestation declined to below the detection level after a period of 17.3 years. In field experiments where increasing amounts of infested soil were supplied as soil inoculum prior to planting, linear relationships between yield of oilseed rape and disease incidence ( $R^2=0.90$ ), and between yield and soil infestation ( $R^2=0.94$ ) were found. A clear positive linear relationship between disease incidence and degree of infestation was also found ( $R^2=0.87$ ). In field tests of partly resistant cultivars of spring oilseed turnip (*B. campestris*), multiplication of clubroot was moderate. The average disease severity index (DSI) after harvest ranged between 3.6 and 4.4 for the partly resistant lines, as compared to 15.3 for the susceptible control cultivar. A partly resistant cultivar could preferably, according to these results, be integrated in the crop rotation where the soil infestation level gives a DSI of less than 10. In another experiment, in search of control measures for *P. brassicae*, the influence of different non-*Brassicae* plant species on the soil survival of the pathogen was evaluated. PCR based methods were developed for detecting DNA in soils naturally infested with *P. brassicae* at levels of soil inoculum higher than  $DSI=21$ . For a sequenced region of DNA from three Swedish *P. brassicae* isolates, we found full identity, although they differed from an isolate sequenced in the UK by a few nucleotides.

**Sustainable production, Agronomy & Diseases****Genome analysis of the blackleg fungus *Leptosphaeria maculans*: characterisation of virulence and mating type genes**

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*Leptosphaeria maculans* causes blackleg disease of oilseed *Brassica* crops including canola (*B. napus*) worldwide. This fungus is amenable to genetic analysis as it is haploid, outcrossing, can be readily transformed and has a small genome size (35 Mb). Its chromosomes are of a size range (0.7 to 3.5 Mb) and number (15) for optimal resolution by electrophoretic karyotyping. We are developing a genetic map using Amplified Fragment Length Polymorphic (AFLP) markers and have mapped the mating type gene and a virulence gene that enables *L. maculans* to attack all Indian mustard (*B. juncea*) cultivars tested (more than 90). We are hybridising cloned AFLP markers to blots of *L. maculans* chromosomal DNA so that linkage groups can be assigned to particular chromosomes. The virulence locus is on a chromosome sized 1.85 Mb and flanked by two markers about 20 cM either side, whilst the mating type locus is on a chromosome sized 2.6 Mb and co-incident on an AFLP marker, which has amino acid sequence similarity to the High Mobility Group (HMG) domain of the mating type genes of other fungi, and does not hybridise to DNA of isolates of opposite mating type. We are also sequencing Expressed Sequence Tags (ESTs) of *L. maculans* with the aim of identifying genes involved in disease (pathogenicity genes). Of the 120 characterised so far, 30% have open reading frames with no homologies to genes currently in the databases. About 50% have significant sequence similarities to genes from other fungi with roles in metabolism, signalling, transport and disease and toxin production. Candidate pathogenicity ESTs will be examined for their ability to elicit host defence responses and expression patterns during infection.

**Sustainable production, Agronomy & Diseases****Using *Arabidopsis* as a model to identify useful resistance genes in *Brassica***

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Studies in *Arabidopsis* have revealed numerous loci coding for resistance to the fungal pathogens *Peronospora parasitica* (downy mildew) and *Albugo candida* (white blister). We have shown that syntenic relationships between *B. oleracea* and *Arabidopsis* are conserved in the regions containing orthologues of the RPP1 (Recognition of *Pe. parasitica*) resistance genes from *Arabidopsis*. The same pathogens cause serious diseases of *Brassica* crops. We have screened a large *Brassica* germplasm collection to identify sources of resistance to these two pathogens. Five sources of resistance to each pathogen have been identified and crosses made to rapid cycling *Brassica oleracea*. Two sources of resistance, one for each pathogen, segregated in a simple 3:1 manner. Bulk segregant analysis, using AFLP, has been performed using these two crosses to identify markers linked to these two genes. A mapping interval has been established for the *A. candida* resistance and two markers linked to the *P. parasitica* resistance have been identified. The recombinants identified are being used to increase the stringency of the selection for closely linked AFLP markers. The syntenic relationships of the map locations of these genes with *Arabidopsis* are being established. This will provide information as to the relationship of functional resistance to these pathogens in the *Arabidopsis* and *Brassica* genomes. In addition, the *Arabidopsis* genome will provide markers that are being used to reduce the mapping interval in *Brassica* and may provide candidate resistance genes.

**Transgenics****DrCP1, a putative developmental cysteine protease in Crucifer root maggot (*Delia radicum*): Target for inactivation?**

Dwayne Hegedus

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All higher organisms express a vast suite of proteases that are involved in a diverse array of functions ranging from digestion to cell signaling and development. In virtually all instances, proteolytic activity must be temporally and spatially coordinated in order to carry out the prescribed function without affecting the integrity of host tissues. The inactivation of insect digestive proteases as a pest control strategy has met with some success and, as such, has been the primary focus of insect protease research. Conversely, insect proteases involved in more complex developmental processes have received little attention despite playing an equally vital role in the insect's life cycle. During an investigation of the proteolytic components of the *D. radicum* midgut we isolated an abundant cDNA encoding a cysteine protease, subsequently termed DrCP1. Previously, other researchers had found DrCP1 homologues in *Sarcophaga* (SpCP1) and *Drosophila* (DmCP1), however its precise function has not been determined. Northern blot analysis revealed that DrCP1 is expressed in all developmental stages including eggs, larvae, pupae and adults. Interestingly, Western blot analysis confirmed this observation but also showed that DrCP1 exists almost exclusively as an inactive 37 kDa proenzyme precursor. Employing "in-gel" separation and analysis of protease isoforms we demonstrated that a single 26 kDa cysteine protease, which corresponded to the activated enzymatic form, was present specifically in mid to late third instar larvae. No activity was observed in second instar larvae or pre-pupae.



**Transgenics****Evolution of chromosome structure in backcross generations of interspecific hybrids between transgenic oilseed rape and wild radish: effect of the insertion site?**

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Studies in various species have shown that homoeologous recombinations are not randomly distributed along chromosome arms. They pointed out separately different genetic factors that may shape this distribution (physical position, amount of homoeology, ...). These results suggest that the probability for a locus to be transferred into the genome of a recipient species depends on its original position within the genome of the donor species. We want to test this hypothesis by surveying the rate of introgression of different markers evenly distributed over the oilseed rape genome into the wild radish genome. Advanced generations of backcrossing interspecific hybrids between male sterile oilseed rape (7 independent herbicide tolerant transgenic F1 obtained from the same parental lines) and wild radish (as the recurrent parent) are currently produced. Here we report the evolution of the chromosomal structure in the interspecific hybrids produced at the different backcross generations. Interestingly, the expected "BC1" chromosomal structure was barely observed and the different "BC1" populations (each originating from one of the 7 transgenic F1 respectively) displayed different patterns of chromosomal structure. These differences softened at the next 'BC2' generation, whether the hybrids were treated with herbicide, or not. Only one line (named 235.3) remained different due to the fact that most of the plants had a higher chromosome number. In the "BC3" generation, the reduction in the number of chromosomes went on in the different hybrids and was more pronounced in the herbicide untreated populations. The line 235.3 still displayed a significantly higher number of chromosomes. These results will be discussed with respect to the assessment of transgene dispersal and the transfer of desirable genes from wild species into domestic ones.

**Transgenics****Gene Expression and Insect Control in Transgenic Broccoli Carrying a *Bacillus thuringiensis* cry1Ab Gene with the Chemically Inducible PR-1a Promoter**

F, Jun

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Although the current resistance management strategy for crops expressing *Bacillus thuringiensis* (Bt) genes is based on high expression combined with refuges, it is important to broaden the options for resistance management. To that end, we are studying inducible expression of Bt transgenes. We produced 49 transgenic broccoli (cv. Green Comet) plants carrying a synthetic cry1Ab Bt gene under the control of the chemically inducible PR-1a promoter from tobacco. Almost all transgenic plants showed substantial or complete control of neonate diamondback moth larvae, regardless of whether the transgene was induced or not. Ten lines were selected for detailed study via northern and western analysis and insect bioassays. Primary transformants expressed the cry1Ab gene and gave complete control of diamondback moths (*Plutella xylostella*) when treated with the chemical inducers INA (2, 6, dichloroiso-nicotinic acid) and BTH (1, 2, 3-benzothiadiazole-7-carbothioic acid S-methyl ester); however, leaves treated with water alone were also partially or completely protected from insect damage. Transgenic progeny of the primary transformants had a higher degree of inducibility. Many progeny lines continued to exhibit partial or complete insect control without induction, but two lines produced cry1Ab mRNA or Cry1Ab protein and showed insect control only when induced. The relevance of these results to resistance management strategies is discussed.

**Genomics****Genomic organisation and sequence analysis of replicated loci in *B. oleracea***

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*Brassica* genomes possess a more complex organisation than that of the related crucifer *Arabidopsis*. As such they are ideal for studying the properties and behavior of crop genomes. Our group is carrying out systematic and detailed analysis of developmental gene families in *Brassica oleracea*, including MADS-box, AUX-1 and small heat shock protein. We are comparing locus and species variation in coding and non-coding sequences. Contrasting patterns of sequence variation have been observed in 5' regulatory regions of MADS-box and HSP-17 loci. The ramifications of locus replication in crop brassicas will be discussed in the context of developmental and environmental adaptations of regulatory genes. The higher order interactions which are possible may be studied through networks of genes which interact through their direct transcriptionally activation. There is a need for development of appropriate methods, in the context of increased genome complexity, for analysis and hypothesis testing when assigning gene function. In particular, with the availability of functional genomics tools such as DNA chips, there is an urgent requirement for development of methods which distinguish accurately between transcripts arising from replicated loci.

**Genomics****Changes in mitochondrial and chloroplast genome structure accompanied with cytoplasmic male sterility induced by protoplast fusion and chimera synthesis**

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We obtained cytoplasmic male sterility (CMS) by intergeneric protoplast fusion between normal radish and cabbage, and by artificial chimera between cabbage and komatsuna in crucifer. By the analyses of mitochondrial genes, orf138 which is the typical indicator gene of radish CMS were found in the progenies from both materials. However, CMS expression was very different from that of ogura type. Orf138 is originally included in *Brassica campestris*, *B. oleracea* at low copy numbers, and regulated at the level. However, the genome which included orf138 changed to dominant type by cell fusion and chimera synthesis with other many genes located on the same genome. Out of 16 mt genes in both CMSs, 12 genes are the same as those of ogura radish. PCR experiments clearly showed the existence of the stoichiometric shift in mitochondrial genome with RFLP analysis. As our inductive CMSs express more or less chlorosis in young seedlings, we further analysed chloroplast genome structure by using the rice chloroplast full genome sequences. In those, one 8kb B-3 clone shows the different RFLP pattern between CMSs and mother materials in chloroplast genome. It is very interesting the correspondence CMS with cytoplasmic genome changes in two different origins such as intergeneric hybrid and interspecific chimera, suggesting the existence of the same regulating mechanism CMS expression. Those two types of the CMS are very stable without pollen sac. Thus, those are very hopeful materials for hybrid breeding of *Brassica campestris* and *B. oleracea*.

**Genomics****Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* chromosomes**

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Where synteny can be established with the model genome, gene identification and studies on chromosomal gene organization in crop species may be greatly facilitated. We have undertaken systematic comparative mapping of *A. thaliana* (n=5) and *Brassica oleracea* (n=9) genomes. *Arabidopsis* gene probes comprising all five chromosomes were selected. These were hybridised to the *B. oleracea* mapping population such a way identifying regions of the genome corresponding to the targeted *A. thaliana* chromosomal regions. All regions of the *A. thaliana* genome investigated were found to be homologous to 2-4 distinct regions in *B. oleracea* genome. We have enlarged the existing *B. oleracea* RFLP map with 249 loci corresponding to cDNA probes from the *A. thaliana* genome sequencing programme. Our results indicate some extend of conservation of chromosome collinearity at the micro and macro levels. This suggests that DNA sequence data from *A. thaliana* will be of direct use in the identification and isolation of genes in *Brassica* crop species. The studies confirmed earlier observations, that there are many segmental duplications in the *Brassica* genomes reflecting their polyploidal origin.

**Genomics****The genetic control of resistance to turnip mosaic virus in *Brassica***

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The *Brassica* A genome carries a number of genes that confer resistance to different spectra of the twelve distinct pathotypes of turnip mosaic virus (TuMV). Genetic mapping is identifying the genomic positions of individual resistance genes. This information will allow gene pyramiding and the marker-assisted selection of durable resistance to TuMV in *Brassica*. The genetic dissection of resistance determinants in near-isogenic lines carrying defined resistance genes will facilitate the biological evaluation of different resistance mechanisms. In combination with the ability to construct hybrid virus genomes carrying defined segments of two or more TuMV isolates, this represents an excellent system for resolving the molecular biology of pathogenesis and host resistance for an economically important crop pathogen. High resolution mapping of *Brassica* genes for resistance to TuMV is also bringing cloning of these genes within reach.

**Transgenics****Genetic constraints to the transformation of *Brassica oleracea***

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A major constraint to the efficiency of *Agrobacterium*-mediated transformation is the interaction of the bacterial strain and plant genotype. In the course of optimising an *A. rhizogenes*-mediated co-transformation system for vegetable brassica approaches to removing genetic constraints have been developed. This has been achieved through *rol* gene induction of 'hairy-roots' in seedling explants and subsequent screening of roots for expression of *gus* and *gfp* reporter genes. This allows the detection of transformation events without the need for selection using antibiotic or herbicide marker genes. Phenotypically normal transgenic plants are recovered in the second generation following segregation of Ri T-DNA from vector T-DNAs. Bacterial constraints were studied by comparing the efficiency of transgenic hairy root production induced by four *Agrobacterium* strains, representing combinations of two virulence plasmids and two chromosomal backgrounds. Transformed roots were produced from explants of all tested genotypes, although differences in transformation competence, ranging from 5-52% of explants with transgenic roots, were detected. The basis to genotypic variation is not known but competence for transformation is heritable. Easy to transform genotypes were identified from doubled haploid lines derived from F1 cultivars with poor transformation efficiency. Little is known about the plant host factors involved in T-DNA transfer and integration. To locate genetic regions controlling transformation efficiency 73 lines of a doubled haploid mapping population were screened for transgenic root production and easy to transform lines identified.

**Transgenics****Evaluation of cauliflower transgenic for resistance to *Xanthomonas campestris* pv. *Campestris***

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Four cauliflower cultivars were transformed with binary vectors containing two types of antibacterial peptides. The shiva protein is a synthetic analogue of cecropin B from the Giant silk moth, while the magainin II peptide was isolated from the African clawed frog. Both chimeric genes were modified for plant codon usage and include a 35S promoter, the 5' leader sequence from AMV, the signal peptide from the tobacco PR-S gene to target export of the protein to the intercellular space and a NOS terminator sequence. In addition, both vectors contained a NPTII (NOS-NPTII-NOS) gene for selection of transgenic cells. Both *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens* methods were used to obtain transgenic plants. Molecular assays including PCR and RT-PCR has confirmed the presence and expression of the introduced genes. Southern analysis has been completed on 13 independent lines with copy number ranging from 1-4. Plants from 12 independent transgenic lines from four cultivars have been transferred to a contained greenhouse. Plants are healthy with excellent seed set. In vitro segregation assays on seven lines indicate a 3:1 ratio for most lines as expected for a single active insertion site of the T-DNA. In some *A. rhizogenes*-derived lines a mixture of kanamycin resistant seedlings with normal and Ri phenotypes were obtained indicating independent segregation of the Ti and Ri T-DNA. PCR analysis will be used to confirm this observation. In vitro bacterial assays using crude leaf extracts have confirmed increased resistance to the bacterial pathogen *X. campestris* pv. *campestris*. The results of in vivo plant assays will be presented.



**Transgenics****Ecological consideration of transgenic oilseed rape releases in Austria: vegetation-ecological and genetic analysis**

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Ecological behaviour and regional aspects of feral crops and allied species are decisive for risk assessments of GMOs. In Austria, the release of transgenic crops is still prohibited by law. In order to give a comprehensive overview about the current situation, the present study was commissioned by the Austrian federal ministry of female affaires and consumer safety. In the case of oilseed rape emphasis was primarily put on investigations of 23 *Brassicaceae* species in Austria which all belong to the tribe Brassiceae. Field work was carried out during spring, summer, and autumn 1998 and 1999 with a focus on species' frequency of occurrence. Potential cross breeding partners of oilseed rape were investigated on fields and ruderal sites (railways, roadsides, areas adjacent to cultivated farmland, etc.) all over Austria. The vegetation-ecological studies were combined with genetic analyses. The main intention of this study was the discrimination of *Brassicaceae* genera, species and cultivars. So far, no method has been described to characterize *B. napus* cultivars. In order to explore the homogeneity of wild oilseed rape populations, 352 plants were collected from 145 different agricultural and ruderal sites. Additionally, 19 *Brassica napus* cultivars grown on Austrian fields during the last ten years were included in the experiments. Nine microsatellite markers were used to characterise *Brassica napus* cultivars and distinguish them from feral oilseed rape. Eight individuals each were tested within 14 selected populations showing a high genetic variability between and within oilseed rape populations. These results clearly show that feral oilseed rape is able to form stable communities outside cultivation. However, it was not possible to trace the feral populations to cultivars grown in Austria during the last ten years. Due to their close relationship to oilseed rape, the frequency of occurrence, and the overlapping flowering time, feral *Brassica napus*, *Brassica rapa*, *Sinapis arvensis* and *Raphanus raphanistrum* were classified as superior critical for crossbreeding with oilseed rape in Austria.

**Genetic Improvement****Preliminary results obtained with a novel method of hybrid production - 'Pair-cross' hybrids.**

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Any plant can be considered to be an F1 hybrid between two theoretical inbred lines. Crossing two plants together is, therefore, the equivalent of producing a double-cross hybrid. The sporophytic self-incompatibility system of brassicas can be used to produce such a hybrid from the progeny of two single plants. Selfing a single plant, which should be a self-incompatible heterozygote, produces progeny of which half are homozygotes. The two types of homozygotes produced can cross pollinate each other. This allows a multiplication to be made. The progeny produced are all heterozygotes and, therefore, self- and cross-incompatible. Crossing two such lines together produces a 'pair-cross' hybrid. Preliminary work with kale and turnips has produced lines with suitable pairs of S-alleles that enable the system to work. Experimental pair-crosses have also been made with kale to examine the degree of heterosis that could be produced with such hybrids; dry matter yields of up to 25% higher than the highest parent cultivar have been obtained. The advantages and disadvantages of the proposed method will be discussed.

**Genetic Improvement****Intergeneric transfer and introgression of nematode resistance from *Raphanus sativus* into the *Brassica napus* genome**

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The beet cyst nematode (*Heterodera schachtii*) causes major damage to plant crops in temperate climates and is difficult to control effectively because of the long persistence of its spores in the soil. Oilseed rape (*Brassica napus* L.), while not seriously affected by this pest, possesses very little natural resistance and thus acts as an efficient host for the perpetuation of nematode populations. Consequently, it is generally unsuitable for crop rotations with highly susceptible crops like sugar beet. To overcome this, intergeneric crosses were made between spring oilseed rape and nematode-resistant oilradish (*Raphanus sativus*) genotypes, using embryo rescue to overcome incompatibility barriers. In three backcross generations, highly resistant progeny with a minimal *Raphanus*-genome component were identified by genomic in situ hybridisation (GISH). Among the early backcross offspring was a BC3 plant with a monosomic addition chromosome, and the resistant BC4 offspring from this individual included a rapeseed-like plant with a normal *B. napus* karyotype and no visible *Raphanus* chromatin. This indicates that the resistance has been recombined into the *B. napus* genome on a small chromosome introgression. Selfing progeny from this and other candidate plants will now be used to develop rapeseed lines containing the resistance on a stable introgression with minimal genetic drag.

**Genetic Improvement****Breeding *Brassica* vegetable crops in Yugoslavia**

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Vegetables from the *Brassica* group are highly appreciated and much consumed in Yugoslavia. Cabbage (*Brassica oleracea* var. *capitata*) is the traditional, economically most important member although production and consumption of cauliflower, Broccoli, Brussels sprouts, kale and kohlrabi is steadily increasing. In recent years breeding of *Brassica* group vegetables gets more attention due to the increased interest and demand for this vegetables on the green market and for processing. *Brassica* breeding is performed at few different approaches. One of them is improvement of National collection located in the Centre for Vegetable Crops in Smederevska Palanka. This collection is still recovering from a row of unfortunate events happening in recent years among which the flood in the summer 1999 brought the most damage. National *Brassica* collection was established with the aim to preserve autochthonous *Brassica* species of our country (for example *B. elongata* or other species from *Brassicaceae* family) as well as to try to use them in breeding programmes. One of the most important tasks is to create early cabbage hybrids. So far, many perspective lines of cabbage and other *Brassica* vegetables have been created and characterized. Creating new cultivars through a combination of conventional with new, recently evolving methods such as tissue culture and genetic engineering is another aspect of our breeding efforts, which we currently intensively investigate. These techniques could help us to overcome incompatibility barriers, which exist in *Brassica* species. Another aspect that we consider very important is constant search for resistance to diseases and pests. Increased tolerance is observed in some of our domestic populations of cabbage, which is basis of our conventional breeding programme.

**Genetic Improvement****Improved evaluation of novel genetic resources in crop plants**

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Crop domestication and the development of modern plant breeding methodologies over the past century has produced highly productive varieties. However as a result from crossing genetically related modern species, genetic variation of the crops has been reduced. Wild and unadapted germplasm is a valuable source of new genetic variation that could be utilized to improve important quantitative traits. Two sets of chromosome segment substitution lines have been produced in *Brassica napus* using a marker assisted backcrossing technique. They are valuable to evaluate the potential benefit of less adapted germplasm to crop breeding programs and for accelerating the incorporation of novel alleles into crop varieties. Substitution lines containing isolated chromosome segments of wild donor genotype in the genetic background of a modern variety allow the detection of beneficial donor alleles separated from undesirable portions of donor genotype. However the cost of producing substitution lines is considerable and to be accessible to the plant breeders, the process must be cost effective. Using data from both sets of substitution lines we are developing mathematical models describing frequency distribution of genotypes in the later generations of backcrossing programs. The model will be used to predict the optimum population sizes and selection strategies for the later stages of gene introgression programs. It is hoped that the development of mathematical models will optimise the designs of marker assisted backcrossing programs and thereby reduce the cost.

**Genetic Improvement****Blackleg resistance gene introgression from *Brassica nigra* occurred through homoeologous recombination**

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Interspecific hybridization allows to introduce into oilseed rape genome genes of interest, such as resistance to blackleg which is one of the most damaging disease of oilseed rape. One resistant recombinant line ( $2n=38$ ) was produced from the selfed progeny of a disomic addition line ( $2n=40$ ) containing one pair of *B. nigra* chromosomes carrying resistance gene(s) to blackleg in the 'Darmor' genetic background. Its meiotic behavior was disturbed. On one hand, a selection for meiotic stability was applied in the following generations of selfing and backcross. In all the cases, the *B. nigra* isozyme locus, Pgi-2B, appeared totally linked to the resistance gene. The isozyme analysis of the selfed progeny of a stable resistant plant revealed that the *B. nigra* introgression replaced the homoeologous region of the A genome. However, the homozygous resistant plants had a poor vegetative development. On the other hand, the initial recombinant mother-plant was crossed with one of the oilseed rape varieties used to establish our reference oilseed rape genetic map, 'Yudal'. The stability of the F1 resistant hybrids allowed to produce two backcross progenies, one with 'Yudal' and the other one with 'Darmor' as recurrent susceptible parents. Genetic mapping confirmed the location of *B. nigra* introgression on the DY5 (A genome) linkage group but also indicated that the missing A genome fragment replaced the homoeologous region of the C genome on the linkage group DY15. The meiotic stability and the male fertility were not affected by the different chromosome rearrangements. The present work indicates that the introgression and chromosome rearrangements occurred only between the homoeologous regions.

**Quality, Functional Foods & Health****Glucosinolate genetics and nutritional quality of horticultural brassicas**

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The anticarcinogenic activity of broccoli has been associated with the presence of methylsulphinylalkyl glucosinolates. Many other botanical forms of *B.oleracea*, and all forms of *B. rapa* (eg Chinese cabbage and Pak Choi), do not contain these glucosinolates, but contain alkenyl and hydroxyalkenyl glucosinolates, which are not associated with anticarcinogenic activity in cell culture assays. In this paper, I describe the genetic regulation of glucosinolate side chain structure, and the mapping and cloning of the 'GSL-ALK' gene, which regulates the conversion of methylsulphinylalkyl to alkenyl glucosinolates. I conclude by discussing strategies to improve the quality of horticultural *Brassica* crops via both genetic modification and marker assisted breeding.

**Quality, Functional Foods & Health****Atmosphere modification extends the shelflife of fresh-cut leafy Asian brassicas**

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Many leafy Asian brassicas are common constituents of fresh-cut pre-packaged salads. Brassicas in use include pak choy (*Brassica rapa* var. *chinensis*), tatsoi (*B. rapa* var. *rosularis*), mizuna and mibuna (*B. rapa* var. *nipposinica*), choy sum (*B. rapa* var. *parachinensis*) and Chinese mustard (*B. juncea*). At retail temperatures (e.g. 10°C) however, postharvest shelflife of these vegetables can be severely curtailed by leaf yellowing. A series of studies are documented outlining the effect of oxygen and carbon dioxide modification on the shelflife of the above vegetables, in a view to using this information for modified atmosphere package design. The youngest fully-expanded leaf was excised from plants four weeks after germination. Leaves were stored in perforated polyethylene bags under continuous flow atmospheres at 10°C. Treatments consisted of a matrix of carbon dioxide (0-15%) and oxygen (0.5-21%) atmospheres. Shelflife was consistently limited by leaf yellowing for all vegetables except mizuna, where off-odours limited shelflife at carbon dioxide concentrations greater than 10%. Both low oxygen and enhanced carbon dioxide levels significantly delayed yellowing. Generally, the most effective oxygen concentration for all vegetables was 0.5-1%O<sub>2</sub>, with shelflife declining as oxygen concentration increased. Optimum carbon dioxide concentrations varied between vegetables, with concentrations of 2-15%CO<sub>2</sub> for choy sum, 5-15%CO<sub>2</sub> for pak choy and tatsoi, and 2-5%CO<sub>2</sub> for mizuna, extending shelflife most significantly. Oxygen and carbon dioxide significantly interacted for all vegetables except tatsoi. Shelflives attained under oxygen (1-21%O<sub>2</sub>) were generally increased by enhancing the level of carbon dioxide.



**Quality, Functional Foods & Health****Long life' broccoli: Factors affecting the post-harvest performance of broccoli heads.**

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Broccoli is a major vegetable *Brassica* crop world-wide. In the UK the demand for fresh broccoli has increased significantly over the last 10 years. However, crops of the same cultivar treated in the same way after harvest, vary in the time taken for heads to become unmarketable. Loss of quality of harvested heads is due to yellowing of the buds and/or loss of turgor resulting in 'wilted' heads. The lack of predictability in post harvest performance causes the major retailers to specify a product 'shelf-life' of only two days, with a further two days to 'best consumed by' in an attempt to ensure a quality product to the consumer. Poor post harvest performance results in significant waste to the retailer and consumer. Generally the UK crop is despatched on the day of harvest. Variation in post harvest performance between crops of the same cultivar may therefore be due to pre-harvest crop culture and environment. We have investigated the effect of crop culture on shelf life of cv Marathon and shown that environmental factors do influence the time taken for both bud yellowing and loss of turgor of harvested broccoli heads to occur. We have also demonstrated genotypic effects by selecting doubled haploid lines derived from F1 broccoli cultivars which differ significantly in their post harvest performance. In addition we have shown that genes expressed during leaf senescence in *Brassica napus* are also expressed during senescence of broccoli buds. Previous reports had indicated that lipid peroxidation was an early event in senescence of broccoli heads. However, we have shown that although breakdown of membrane lipids occurs in harvested broccoli heads this appears to be by beta oxidation releasing energy for respiration rather than by peroxidation

**Genetic improvement****A Picture is Worth a 1000 Words**

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The description of an accession in a genetic resources collection would be reasonably comprehensive if it ran to one thousand words. However, the information relating to individual genetic resource accessions is stored electronically in agreed international formats and often in coded form. One of the objectives in genetic resources conservation is to maintain the broadest spectrum of variation for a given crop or taxon for conservation and utilisation. The fact that many individual accessions are themselves extremely variable, e.g. landraces makes a standard scoring system difficult to use by gene bank works and difficult to interpret by potential users. Technological advances in image capture and storage now make it possible for visual information to be stored alongside the data used to score morphological and other traits. Using a scanner and digital camera we are routinely gathering images of the plants representing the HRIGRU accessions. The images and related data are stored in a database linked directly to the Genetic Resources Unit data management system. Currently images have been collected and stored for more than 10% of the GRU accessions. For *Brassica* accessions we aim to store multiple images including whole plant, leaf inflorescence/flower, pods and if appropriate marketable product.

**Genetic improvement****RFLP Mapping of pod-shatter resistance in *Brassica***

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Pod-shatter is an agronomically undesirable trait present in all *Brassica napus* crops. The susceptibility of *B. napus* pods to shattering when ripe results in significant potential losses in yield which are minimised by agricultural practices such as swathing and desiccation. Even so a sizeable proportion of the potential yield can be lost and the shed seeds contribute a massive load to the soil seed bank. Resistance to pod shatter has not been found in conventional *B. napus* varieties. In previous investigations a synthetic *B. napus* population was generated from crosses between *B. rapa* var. *chinensis* and *B. oleracea* var. *alboglabra*. This population showed variation in pod shatter resistance. Two of the most promising lines were crossed with the *B. napus* cultivar Apex to produce F1 populations (POSH1 and POSH2). POSH1 and POSH2 were selfed and F2 plants grown in field plots at three locations. These field populations were assessed for agronomic traits including pod-shatter resistance. At the same time a population of doubled haploid plants has been produced by microspore culture. These doubled haploids are being used to generate an RFLP map that will be used to identify markers associated with the pod-shatter resistance trait and introgress the trait into commercial backgrounds using marker assisted breeding.

**Genetic Improvement****Recombination between B- and C-genomes in a *B. napus* / *juncea* hybrid .**

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The frequency of homoeologous exchange between B- and C-genome chromosomes under meiotic conditions designed to relax chromosome pairing, was examined in an allotetraploid (AA'BC) *Brassica* line generated by hybridising a primary synthetic doubled haploid (DH) *B. napus* with a primary synthetic DH *B. juncea*. To optimise the detection of recombinants, each parent was resynthesised from previously-mapped DH or inbred lines of the appropriate *Brassica* diploid species. Chromatids transmitted by the female gametes of the allotetraploid were analysed in "F1" progeny recovered by embryo rescue. Analysis of RFLP loci covering the eight *B. nigra* (B-genome) and the nine *B. oleracea* (C-genome) linkage groups (LGs) revealed infrequent disturbances to both recombination and assortment. In total, 339 C-genome and 286 B-genome LGs were analysed. About 90% of the time parental LGs were transmitted to approximately half the gametes, reflecting normal pairing and disjunction in most meioses. However, occasional nondisjunction in both the resynthesised parents was implied: in *B. napus* by the transmission of one of the *B. oleracea* LGs to 72% of the gametes, and in *B. juncea* by transmission of one of the *B. nigra* LGs to 90% of the gametes, and by the complete lack of transmission of another of the *B. nigra* LGs to any gamete. In the allotetraploid meioses nondisjunction resulted in approximately 2% of the gametes inheriting additional copies of a given LG. Linkage groups with apparent terminal deletions were also infrequently detected in both the allotetraploid and its progeny. Well defined B/C recombinant LGs (manifested by the concerted loss/gain of *B. nigra*/*B. oleracea* alleles) were detected in only six gametes (2%) and in each case recombination had occurred between regions of homoeology. Thus, homoeologous recombination between B- and C-genome chromosomes was detectable, but its frequency was lower than the approx. 10% level detected between the more closely related A- and C-genome chromosomes under similar meiotic conditions.

**Genetic improvement**

**Allele Specific Diagnostics for Use in a  
Self-incompatible *Brassica napus* Hybrid Breeding  
Program**

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A *Brassica napus* hybrid breeding program, based on self-incompatibility alleles introgressed from *Brassica rapa* and *Brassica oleracea*, has been established at the University of Guelph. This poster describes the development of allele specific PCR primers based on polymorphisms within the S-locus glycoprotein (SLG) of the introgressed alleles. These primers will form the basis of a diagnostic system for use in the SI breeding program and for assessment of hybridity levels in hybrid seed lots.

**Genetic Improvement****Identification of Molecular Markers Associated with Black Rot Resistance in Cole Crops**

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Black rot (*Xanthomonas campestris* pv. *campestris*) is one of the most serious diseases of cole crops throughout the world, particularly during warm, damp seasons. Current host plant resistance available in commercial cultivars is complicated and inadequate. A more resistant source of black rot resistance from a *Brassica carinata* accession (previously identified a *B. napus* PI 199947) has been introgressed to broccoli using protoplast fusion. Molecular polymorphisms have been identified between black rot resistant and black rot susceptible plants using 800 oligonucleotide primers. Segregating F2 populations from black rot resistant broccoli and black rot susceptible cauliflower crosses have been generated, and the populations have been screened for phenotypic resistance and marker segregation. Molecular polymorphisms have been compared to disease severity ratings, and the association of polymorphisms with resistance to black rot in seedlings and mature plants has been studied.

**Genetic improvement****Changes in mitochondrial and chloroplast genome structure accompanied with cytoplasmic male sterility induced by protoplast fusion and chimera synthesis in *Brassicaceae***

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We obtained cytoplasmic male sterile (CMS) lines by artificial chimera between red cabbage and Komatsuna (*B. campestris*), and by intergeneric protoplast fusion between radish (*Raphanus sativus*) and cabbage (*Brassica oleracea*). Two types of different CMSs have stably maintained the radish CMS-specific *orf138* in the mitochondria, although parental materials in both CMSs are normal plants. As it is very difficult to explain the mechanism of recombination within each cytoplasmic genome, the change in quantitative regulation was thought to be due to the copy number of heteroplasmic genomes or genes that were originally present. RFLP and PCR analyses support the existence of stoichiometric shifts in mitochondrial and chloroplast genomes or genes. In order to determine the stoichiometric shifts of cytoplasmic genomes accompanying CMS induction, RFLP patterns among mother plants and the resulting CMSs were compared using known chloroplast and mitochondrial gene probes. RFLPs in the two CMSs were very similar to those in Ogura CMS radish. PCR cycle experiments also supports our theory.

**Genetic improvement**

**Genetic diversity in Japanese rapeseed and swede (*Brassica napus* L.) germplasm based on RAPD markers.'**

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Genetic diversity among 59 rapeseed varieties including 37 Japanese and 22 exotic accessions, and 37 swede varieties including 15 Japanese and 22 exotic accessions were estimated using random amplified polymorphic DNA (RAPD) markers. A total of 83 polymorphic bands amplified by 27 PCR primers were used to calculate a Dice coefficient, which is necessary for both cluster and principal component analysis. Overall, the varieties examined were classified into three major groups. The first group included Japanese rapeseed varieties of early maturity type and old local type. The second group contained rapeseed varieties which have been cultivated in north Japan and exotic, the third mostly swede accessions with small genetic diversity. These results show that Japanese *Brassica napus* germplasm is divided into 3 kinds of groups that consist of two rapeseed groups and local swede varieties. We discuss the relationship of Japanese *Brassica napus* germplasm on the basis of genetic background.



**Genetic Improvement**

**Inheritance of a mutant with apetalous flowers in  
oilseed rape (*Brassica napus*)**

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The inheritance of an apetalous mutant induced by EMS was investigated. The apetalous character was inherited by two nuclear genes which showed an interaction with the cytoplasm. In preliminary field trial with segregating lines from a cross between the apetalous mutant and normal cultivars no significant effect of the apetalous type on grain yield was observed.

**Genetic Improvement****Plant-to-plant variations of Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> contents in two *Brassica* species differing in salinity tolerance**

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*Brassica juncea* (L.) Czernjaew. "Common Green", which is relatively salt-tolerant and *Brassica rapa* L. "Sani", which is relatively salt-sensitive, were grown in a continuous aerated hydroponic system with 125 mol m<sup>-3</sup> Na for 10 days. Salinity induced significant increase of Na<sup>+</sup> content in fully expanded leaves but decrease of K<sup>+</sup> and Ca<sup>2+</sup> contents. *B. rapa* "Sani" accumulated less Na<sup>+</sup> and greater K<sup>+</sup> and Ca<sup>2+</sup> than did *B. juncea* "Common Green". Plant-to-plant variations were significant in leaf area, biomass and ion contents in the presence of salinity. The correlation coefficient between ion parameters and plant growth parameters were lower. Coefficient of variation (%) was 27.33 and 36.01 for dry weights of *B. juncea* and *B. rapa* respectively, 20.39 and 23.09 for K<sup>+</sup>, 19.15 and 26.81 for Ca<sup>2+</sup>, 15.58 and 13.60 for Na<sup>+</sup>. The results indicate that there is potential to develop salinity tolerance based on plant-to-plant variation in *Brassica* species.

**Genetic improvement****Genetic purity assessment in hybrid *Brassica* crops using molecular markers**

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Hybrid varieties of crops offer advantages to the grower (increased vigour, yield and uniformity) and to the breeder (intellectual property protection, guaranteed return seed sales). To benefit from these advantages, there is a need to ensure the purity of the hybrid; non-hybrid individuals in a seed lot could be a potential source of germplasm allowing Essential Derivation from the hybrid variety by competitor breeders, as well as reducing the vigour and yield advantage of the hybrid, while hybrids with a parent of the wrong line, will not have the varietal characteristics and full heterotic advantage of the correct hybrid. Current methods to evaluate hybrid seed lots involve growing plants for morphological observation of uniformity, but as this generally takes a full growing season, the test is retrospective of the release of seed to growers. A molecular test could offer purity information on seed lots at the time of production, which would be available at the point of sale. This research has addressed the usefulness of DNA based molecular markers for the assessment of purity in hybrid varieties of two brassicas - Oilseed rape (*Brassica napus*) and white cabbage (*Brassica oleracea*). Both crops have been investigated with AFLP and microsatellite markers, in addition, oilseed rape has been investigated using traditional morphological assessment. Heterogeneity within the inbred parental lines of oilseed rape has been found to be a major problem. Markers that discriminate between parental lines (thus allowing hybrids to be detected) which are also uniform within the parental lines (so that offtypes within the hybrid can be correctly identified) were very rare in the oilseed rape lines investigated. White cabbage shows much greater parental uniformity and a higher level of polymorphism in the marker sets used in this project, and could be considered a model crop for the development of this approach.

**Genetic Improvement**

**Oligosaccharide effects on *Brassica oleracea*  
microspore embryogenesis**

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To study oligosaccharide effects on in vitro cell development, microspore culture was used as a model. 18 molecules were tested: 12 oligosaccharides -degree of polymerisation range between 3-10- and some of their respective polymers. Final yield of microspore-derived embryos was greatly enhanced by one family of oligosaccharides when supplied in combination to heat stress. Furthermore, we observed that the higher the sulphation level of the molecule, the higher the embryogenic response: a two-fold increase in embryo numbers was observed in the most reactive treatment. Experiments showed that these molecules were most active at 2 concentrations: 170 nM and 850 pM. Kinetic studies also showed that a 30 minute-treatment is enough to stimulate embryogenesis. Our results so far hint to an oligosaccharide signal triggering the microspore embryogenesis response. A specific saccharide pattern seems necessary, together with sulphate groups. Molecular and biochemical analysis are planned to increase our understanding of the embryogenesis program progress following an oligosaccharide signal.

**Genetic Improvement****Plant Regeneration of Rapid Cycling *B. juncea* Containing  $\rho$ Anand $\rho$ ± Cytoplasmic Male Sterility (CMS) through Plant Tissue and Protoplast Culture**

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Several types of Cytoplasmic male sterility (CMS) are known in *Brassica* spp. and related genera. New types of Cytoplasmic male sterility in *Brassica* species would be useful for F1 hybrid seed production. The  $\rho$ Anand $\rho$ ±CMS derives from the wild species *Brassica tournefortii*. Rapid cycling *Brassica* stocks have been developed recently. They show a considerable shortened life cycle, allowing much easier and faster study of the genetics of new traits. Leaves and stems of Rapid cycling stock of *B. juncea* (CrGC4-3) were used as experimental materials. Very high plant regeneration rate (85%) was found in the Kao & Michayluk medium supplemented with plant growth regulators when leaf segments were cultured as explants. No regeneration was observed when stem explants were used. Protoplasts were isolated from leaves using mixtures of two enzymes (1% Cellulysin and 0.5% Macerozyme) in 0.4M Mannitol and 50mM CaCl<sub>2</sub> · 2H<sub>2</sub>O. Macrocalli were transferred on plant regeneration medium containing 2mg/l BAP, 2mg/l Zeatin, and 0.5mg/l NAA. After 60 days of culture, regenerated plantlets were obtained.

**Genetic Improvement****Production and Characterization of Cytoplasmic Male Sterility (CMS) Somatic Hybrids in *Brassica* Species**

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In order to develop valuable and stable cytoplasmic male sterility (CMS) as well as disease resistant materials, many different fusion experiments were performed using various sources of CMS derived from diverse *Brassica* species. Inbred lines of *B. oleracea*.var capitata, *B. campestris*. ssp. *pekinensis*, *B. oleracea*.var. *italica*, *B. juncea* and Rapid cycling of *B. juncea* and *B. rapa* with n and cytoplasmic male sterility (CMS) were used as protoplast fusion materials. Many somatic hybrids were obtained from protoplast fusion combinations of *B. campestris* x *B. oleracea*, *B. campestris* x *B. oleracea*, *B. campestris* x *B. oleracea* with Ogura CMS. Calli were obtained from combinations of *B. juncea* x *B. rapa* with nand CMS, and *B. campestris* x *B. juncea* with and CMS. We also obtained somatic hybrids between *B. oleracea* with CMS and *B. campestris* with CMS. For the identification of somatic hybrids, molecular biological analysis such as PCR and Southern blotting, flow cytometric analysis, pollen production and viability, plant and flower morphology, and cytological analysis were performed. Genomic in situ hybridization (GISH) showed very diverse patterns in the chromosome constitution depending on the fusion combinations and genotypes used in the experiments. The first and second progenies were obtained from two of fusion combinations of *B. campestris* x *B. oleracea* and their phenotypes were mostly like *B. oleracea*

**Genetic Improvement****Construction of doubled haploid mapping population in Chinese cabbage**

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For studying morphology and constructing genetic linkage of Chinese cabbage, a doubled haploid mapping population was developed. F1 anthers obtained from the cross between Chiifu type 401-42 and Kenshin type 402-43 lines of Chinese cabbage were cultured, and 1,418 plants were regenerated from the calli derived from anthers. Three hundred and twenty eight out of 1,418 plants survived after the hardening. The ploidy level of 328 plants was determined, and 146 diploid plants (approximately 44.5%) were selected by phenotype. Seeds obtained from the 133 out of 146 diploid plants were used for evaluating morphological traits. Seventy-five lines out of 133 lines were evaluated with parents for 33 traits including the total plant weight. The field experiment followed a randomized complete block design with three replications. Ten plants in the middle per line were collected. The mean of three replica was used for data analysis. The molecular map spanning 2,695 cM (10 linkage groups) was constructed using two PCR-based marker systems, AFLP and RAPD. QTL mapping using morphological traits and RFLP mapping using 200 markers previously mapped in several *Brassica* species are in process now.

**Genetic Improvement****Development of novel resynthesised *Brassica napus* as genetic resource for rapeseed**

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Due to substantial progress in breeding and cultivation practice, *Brassica* crop species have become one of the world-wide most important sources of oil and vegetables. During the last couple of years a shift to hybrid cultivars has been accomplished in the breeding of oilseed rape (*B. napus* L.) due to the availability of effective hybridisation systems. However, the present breeding material is very closely related and intensive quality breeding has contributed additionally to limit the genetic base of oilseed rape. In order to develop divergent germplasm - representing further improvements of seed yield, resistance against diseases and pests as well as important seed quality traits - *Brassica* genetic resources can be utilised through resynthesis of *B. napus*. In the course of previous experiments we developed high-erucic acid rapeseed performing interspecific crosses between *B. rapa* ssp. *trilocularis* ('Yellow Sarson') and different cauliflowers (*B. oleracea* convar. *botrytis* var. *botrytis*). The progeny were analysed for seed characteristics, such as oil, protein and glucosinolate content as well as fatty acid composition. In the course of field evaluations phenotypic traits - such as plant height, different leaf characters, days to flowering, flowering period, time of maturity and vegetation period as well as seed yield components (e.g., number of pods/plant, number of seeds/pod, thousand-seed weight) - were assessed showing a wide variation. Due to its inferior agronomic performance and seed quality - as compared to current double-low breeding material - the establishment of a new gene pool based on artificial *B. napus* is limited and has to be considered under more long-term perspectives. One strategy to exploit novel *B. napus* in rapeseed improvement with minimum losses neither of seed quality nor genetic divergence will be our resynthesis experiments using zero-erucic *B. oleracea* forms, which we have identified as a novel source of a gene conferring low erucic acid content to *Brassica* seed oils.



**Genetic improvement**

**Genetic linkage map of chinese cabbage based on  
Random Amplified Polymorphic DNAs**

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A molecular genetic linkage map of *Brassica campestris* L. (syn. *B. rapa*) was constructed based on the segregation of 99 RAPDs markers from eight-four 10-base random primer using DNA samples extracted from F2 population of Turnip (*Brassica campestris* L. ssp. *rapifera*) X chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*). this genetic linkge map covered 1632.4 cM genome with 16.5 cM mean interval between flanking markers and defined thirteen linkage group, in which the logest linkage group is 267.5 cM with 20.6 cM mean interval and the shortest linkage group is 62.2 cM with 15.6 cM mean interval. the size and distribution of linkage group in this map is similar to other RFLP maps and consistent with karotype data of *B. campestris* L.

**Genetic improvement**

**Breeding New CMS Line of Heading Chinese Cabbage**

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It's well to know that male sterile application is a good approach for combining F1 hybrid, and it has many advantage comparing with self-incompatible line in *Brassica* genus, such as getting 100% rate of F1 hybrid, no-requirement of pollen on budding stage. Since we have introduce the cytoplasm of polCMS into heading chinese cabbage, we find that this male sterility is caused by cytoplasm-nucleus interaction, for some good type of heading chinese cabbage such as overlap-round type or bamboo-type ect, which have restorer gene in it and can't use this CMS trait directly. in this paper, according to the genetic characters of this CMS, we design two procedures similar to *Allium cepa* Formula, the restorer gene RF has been removed from the donor parents and breeding a new CMS line, which has high and stable sterility and its combining ability are been testing.

**Genetic Improvement****High-precision interspecies transfer of restorer genes**

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Ogura cytoplasmic male sterility has had a large impact on vegetable *oleraceae* crops by providing a reliable method of hybrid seed production. It has also begun to have an impact on oilseed *Brassica* production. In oilseeds, unlike in most vegetable crops, restoration of male fertility in the hybrid is a key element. Restoration in *B. napus* without associated effects on seed vigour and quality has been difficult to obtain. We have chosen to overcome this by using natural homoeologous recombination to transfer a small, defined segment of *Raphanus* into the equivalent region on the *Brassica A* genome. To transfer this small segment, accurate comparative maps of the *Raphanus* and *Brassica A* genomes are needed to identify which chromosome segments are primary homoeologues. Interspecific hybrids with relaxed control of homologous pairing are also necessary to allow homoeologous recombination between the *Raphanus* and *Brassica* genomes to occur. We are well advanced in the development of these technologies, including the construction of the first high density map of the entire *Raphanus* genome. This map is based on RFLP probes already densely mapped in the *Brassica A* and *C* genomes which has allowed comparative mapping amongst these species. A number of Ogura restorer genes have been mapped in *Raphanus* and we are in the process of determining which are key in the restoration of *B. napus* with Ogura cytoplasm. The necessary interspecific hybrids have been developed to allow for the transfer of the specific *Raphanus* segment. This planned approach for interspecific gene transfer holds out the prospect for the development of a completely robust system for the production of male fertile *B. napus* hybrids for *Brassica* oilseed production.

**Genetic Improvement****Development of *Brassica* Microsatellite Markers**

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Microsatellites have proven to be one of the most effective tools for marker-assisted breeding and diversity studies. With new techniques for enriching and pre-screening libraries, it is now possible to produce greater numbers of microsatellite markers. These techniques have been applied to develop a large set of microsatellite markers in *Brassica* species. Libraries from *Brassica napus*, *B. oleracea*, *B. rapa* and *B. nigra* showed approximately 85% enrichment for the motifs CT, CA and tri-nucleotide repeats. Approximately 1300 clones have been sequenced from the four libraries. Duplication of sequences within the libraries ranged from 18-39% and 420 primer pairs were designed from the flanking regions of the unique microsatellites. Primers from all species were screened on 1.8% agarose and 65% showed a product between the expected range of 100-300bp, in at least one species. The band producing primer pairs have been used for polymorphism screening on polyacrylamide gels on 14 lines from *B. napus*, *B. oleracea*, *B. rapa* and *B. nigra*. Results thus far show that approximately 40% are polymorphic in *B. napus*, with polymorphism being evident in the remaining species. The informative microsatellite markers are to be mapped on a highly polymorphic population of *B. napus* previously used to produce a dense RFLP map. Microsatellite loci will also be positioned on genetic maps of *B. oleracea* and *B. rapa*.

**Genetic Improvement****Effects of sex and genotype on recombination in  
*Sinapis alba***

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*Sinapis alba* L. (white or yellow mustard) shows considerable promise as an alternative crucifer oilseed and protein crop for dry temperate climates. It possesses many beneficial characteristics such as drought and heat tolerance, pest resistance and a short growing season, however seed oil content is relatively low (less than 30%). We have generated the first genetic linkage maps of *S. alba* using *Brassica* RFLP markers. As *S. alba* is an outcrossing species and susceptible to inbreeding depression, we employed a crossing strategy which maximised the degree of heterozygosity in the mapping populations. Highly heterozygous parents were crossed reciprocally to produce two complementary F1 mapping populations (F1-1 and F1-2). Initial mapping was carried out on a small F1 population (128 individuals) using 160 probes previously used to generate maps in other *Brassica* species. On the basis of these maps, 80 probes were selected to provide an even coverage of the genome and these were used to produce maps in large reciprocal populations (188 F1-1 and 194 F1-2 individuals). The large size of the mapping populations allowed statistically robust comparisons of the rate and distribution of recombination in male and female meioses in two distinct genetic backgrounds over the entire *S. alba* genome. The observed differences have implications for mustard breeding. Single-seed descent is being used to develop inbred lines for the field testing of quality and yield characters. Characterising these lines with RFLP probes will allow the mapping of important QTL, particularly those controlling oil and protein content.

**Genetic Improvement****Molecular phylogenetic relationships of *Brassica* and allied genera**

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Coding region of *rbcL* gene of cpDNA and plastid subtype ID sequence (PS-ID) were sequenced to study phylogenetic relationships of *Brassica* and allied genera. The sequence length of the *rbcL* was 1296bp in all species, and that of PS-ID varied from 122 to 135bp among species. The phylogenetic trees based on *rbcL* and PS-ID showed similar results. In both trees, *Brassica* and allied genera were divided into two lineages. One is *nigra* group, which was composed of *B. nigra*, *B. fruticulosa*, *B. tournefortii*, *Hirschfeldia incana* and *Sinapis* sp., and another is *campestris/oleracea* group composed of *B. campestris*, *B. oleracea*, *Diplotaxis erucoides*, *Eruca sativa* and *Raphanus sativus*. These results are consistent with those found in cytological, RFLP and restriction site variation of cpDNA studies.

**Genetic Improvement****Genomic changes in intergeneric hybrids between  
*Brassica napus* and *Orychophragmus violaceus***

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The crucifer *Orychophragmus violaceus* (OO,  $2n=24$ ) possesses agronomically valuable traits such as high palmitic acid (14.3%) and high linoleic acid (53.2%) in its seed oil. Twenty-two F1 plants between *Brassica napus* (AACC,  $2n=38$ , ) and *O. violaceus* were produced and classified on the basis of morphology, meiotic behaviour, and RAPD banding profiles. Type I plants ( $n=5$ ) had traits belonging to both parents and  $2n=29$  chromosomes, instead of the expected number ( $2n=31$ ). 62.8% of the pollen mother cells (PMCs) had a pairing configuration of  $1\text{III} + 9\text{II} + 8\text{I}$ , while the remaining PMCs had  $10\text{II} + 9\text{I}$ . The plants had 97.6-98.8% of the RAPD bands specific to the *B. napus* parent, but only 9.2-11.7% of the bands specific to the *O. violaceus* parent. Type II plants ( $n=3$ ) showed morphological traits not observed in the parents. The plants had  $2n=35$  ( $17\text{II}+1\text{I}$ ),  $2n=36$  ( $17\text{II}+2\text{I}$ ), and  $2n=37$  ( $18\text{II}+1\text{I}$ ) chromosomes, and exhibited 94.1-95.3% of the *B. napus*-specific bands and none of the *O. violaceus*-specific bands. Type III plants ( $n=14$ ) were morphologically similar to *B. napus*, and had 95.3-99.4% of the *B. napus*-specific bands and none of the *O. violaceus*-specific bands. Eleven Type III plants were meiotically studied. Seven plants had  $2n=38$  chromosomes ( $19\text{II}$ ). One plant was a *B. napus*-like haploid with  $2n=19$  chromosomes. The remaining three plants had  $2n=37$  ( $18\text{II}+1\text{I}$ ),  $2n=38$  ( $17\text{II} + 4\text{I}$ ), and  $2n=39$  ( $18\text{II}+3\text{I}$ ) chromosomes. Chromosome fragments were observed in the majority of the F1 plants. Based on the results, it was suggested that genomic changes such as chromosome elimination followed by duplication had occurred in the hybrids between *B. napus* and *O. violaceus*.

**Genetic Improvement****The sclerotinia stem rot infection process in *Brassica napus***

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The objective of the research was to compare the rate of colonization of young and senescing petals of *Brassica napus* by the stem rot fungus, *Sclerotinia sclerotiorum*. Petals (1 and 7 days old) of spring cultivars CréSOR, Ebony, Topas, and Westar were selected at random from five plants of each cultivar and inoculated with a droplet of ascospore suspension of *S. sclerotiorum* clone 321. Inoculated petals were placed in a humidity chamber and incubated at room temperature for 5, 16, 24, 48, 72, and 96 hours. Necrotic cells were observed 16 to 24 hours post-inoculation (hpi) in inoculated 1-day-old petals; necrosis was associated with the penetration of petal cells by germinating ascospores and mycelium, and infection cushion formation was observed 72 hpi. Necrotic cells were not observed in inoculated 7-day-old petals; mycelium growth and infection cushion formation were observed 17 and 24 hpi, respectively.



**Genetic Improvement****Analysis of chlorophyll A and chlorophyll B content in shoot culture of cabbage under paraquat treatment**

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Throughout experiments about possible tolerance to free radicals creating chemical such as total herbicide paraquat, we have investigated influence of paraquat on chlorophyll a and b content in shoot culture of cabbage. Paraquat was applied in medium for shoot culture growth, at four different concentrations (0.5, 2.0, 10.0, 50.0 mg l<sup>-1</sup>). Four genotypes of cabbage developed through breeding program from some of our domestic populations were used in this experiment. Genotypes were chosen according to data about possible tolerance to diseases and pests in the beginning population from which selection process started. Content of chlorophyll a and b was measured and from these data we have established analysis of variance, coefficient of phenotype variation and wide sense heritability, correlation and regression analysis. Wide sense heritability coefficient had higher values for chlorophyll a content (0.77) than for chlorophyll b (0.35). All genotypes did not respond the same way to the paraquat treatment. Significant correlations among measured parameters were established at two examined genotypes, and they had a significant regression as well. Similarities and differences in chlorophyll a and b content changes under paraquat treatment were examined. We have tried to investigate the causes for different profiles of chlorophyll decrease among four genotypes and we have discussed role of structural and regulatory polygenes involved in chlorophyll a and b determination. Treatment at 2.0 mg l<sup>-1</sup> paraquat has shown to be some point of convergence of chlorophyll a and b content, since concentrations higher than that have shown to be too toxic to let any significant differences to be expressed among examined cabbage genotypes.

**Genetic improvement*****Brassica* vegetable research in India**

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As the majority of the Indian population is vegetarian in nature, vegetables play an important role in Indian diets, as they contain most of the vitamins, minerals, carbohydrates & protein etc. *Brassica* vegetables are cultivated throughout world including in India. Sungro Seeds Limited, one of the leading vegetable seed company in India is involved to develop superior hybrids and varieties suitable for different agro climatic conditions in India and also for export purpose. So far as *Brassica* vegetable research at Sungro is concerned, we have been able to develop hybrids of cabbage, cauliflower & radish which are acceptable in varying climatic zones of India at affordable prices. As a result of intensive research, exclusively in vegetable brassicas. Sungro has been able to occupy highest (approx. 20%) market share in India.

**Genetic Improvement****Expression of ME-leaN4 in desiccation-tolerant microspore-derived embryo of *Brassica napus* and *Brassica campestris***

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ME-leaN4 is a late embryogenesis abundant (LEA) gene isolated from microspore-derived embryos of *Brassica napus* cv. Topas which have been induced desiccation tolerance by ABA. The ME-leaN4 gene which is ABA-inducible is thought to be involved in desiccation tolerance in microspore-derived embryos. In situ hybridization and immunocytochemistry showed that the ME-leaN4 mRNA accumulated in cotyledonary shoot meristem and hypocotyl, specially in provascular tissues of desiccation-tolerant embryos, but not in desiccation-intolerant embryos. The ME-leaN4 protein expressed throughout tissues of desiccation-tolerant embryos and especially accumulated in provascular and epidermis tissues. In zygotic embryos, the ME-leaN4 protein also accumulated in the similar tissues in maturation stage of embryos, but not in early stage. Our results indicate that the expression pattern of ME-leaN4 is resemble in desiccation-tolerant microspore-derived embryos and zygotic ones.

**Genetic Improvement****Molecular characterization of genetic identities and relationships of *Brassica rapa* L.**

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*Brassica rapa* L. (n=10, A genome) is grown both for oil and vegetables. It exhibits enormous morphological variability and a correspondingly high number of infraspecific taxa. All cultivated n=10 *Brassica* species are treated as a single species (ca.7-8 subspecies). Various data (morphology, geography, isozymes and nuclear RFLPs) have indicated a division of *B. rapa* into "Western" and "Eastern" groups, corresponding to two independent centres of origin. The primary centre proposed as Europe (turnip and oilseed rape) from which Asian oilseed types were putatively derived and the second as China (Asian vegetables). The objective of the study was to use random amplified polymorphic DNA markers (RAPDs) to test the validity and genetic relatedness of the infraspecific taxa and to test the affiliation of four members of the *B. rapa* cytodeme. A total of 68 accessions were examined: subspecies *chinensis*, *dichotoma*, *narinoso*, *nipposinica*, *oleifera*, *pekinensis*, *perviridis*, *rapifera*, *sylvestris*, and *trilocularis*), *B. perviridis* *B. purpuraria*, *B. ruvo*, and *B. septiceps*, including *B. juncea* as an outgroup. Using 7 primers, 70 markers were scored each of two individuals per accession. The data was analysed using the NTSYS and the UPGMA clustering method. The Asian material showed the greatest variability. Accessions of *oleifera*, *rapifera* and *ruvo* formed a "European" cluster as expected, whereas several clusters were observed for the Asian subspecies. Most infraspecific taxa formed single clusters with the exception of *chinensis*, *pekinensis* and *dichotoma*. Asian oilseed, *trilocularis*, was not closely associated with the European cluster, whereas *dichotoma* formed two groups, one accession clustering with *trilocularis* and the remainder with the European cluster. Additional relationships will be discussed.

**Genetic Improvement****Electronic Guide to Wild Germplasm of *Brassica* and Allied Crops (tribe *Brassicaceae*, *Brassicaceae*) 2nd Edition**

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This electronic publication provides an update to the 1993-94 "Guide to the wild germplasm of *Brassica* and allied crops (Tribe Brassicaceae, family Cruciferae) Parts I to V". The Guide has been expanded to include taxonomic, geographical and ecological data on an additional 18 species, with updates on a total of 235 species in 49 genera. Over 1400 chromosome number reports are given, including 380 original citations for both wild and crop species. The section on inter-specific and inter-generic hybridization includes 240 original references. The Guide has been proven to be useful for both public and industry plant breeders, in providing direction for future genebank needs for these crops and for assisting biotechnologists and breeders wishing to utilize these genetic resources in their research programs. It is also an extremely valuable resource for regulators concerned with the possibility of gene flow between transgenic cruciferous crops and wild relatives not only in Canada but on a global basis. Economically, the crop brassicas display enormous diversity and are used as a source of oil, vegetables, mustard condiments, and fodder. The genera *Raphanus* and *Sinapis* are also of major importance. Several species are important weeds, e.g. *Sinapis arvensis* (wild mustard) and *Raphanus raphanistrum* (wild radish), representing both a potential source of germplasm and agricultural problems. An understanding of the genetic potential of wild relatives in the Tribe Brassicaceae is critical for the establishment of long-term breeding programs of these crops. Many of the wild species have potential value as industrial oils (*Crambe*, *Eruca*), value-added products, or as host systems for molecular farming. Many wild relatives possess resistance to disease and insect pests and tolerance of cold, salt and drought conditions.

**Genetic Improvement****Genetic diversity in *Brassica carinata* and *B. juncea* based on molecular AFLP markers**

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Cultivated species *Brassica carinata* (n = 17, BBCC) and *B. juncea* (n = 18, AABB) are amphidiploids derived from diploid taxa, *Brassica rapa* (n = 10, AA), *B. nigra* (n = 8, genome BB), and *B. oleracea* (n = 9, genome CC). *Brassica carinata* or Abyssinian mustard, primarily an oilseed crop, is believed to have originated in the Ethiopian plateau, as a cross between wild-growing *B. nigra* and cultivated kale-like forms of *B. oleracea*. There is little differentiation into various crop types and genetic levels are predicted to be low. *Brassica juncea*, Indian or brown mustard is grown in North America and Europe for condiment use, on the Indian subcontinent for seed oil and the Far East as a vegetable. The species is very variable, and includes oleiferous, semi-oleiferous, rapiferous and leafy types. Genetic diversity is expected to be high. It seems likely that *B. juncea* may have arisen more than once as a result of hybridization; with proposed origin(s) in the Middle East, west Asian region and China. The objective of the current study was to evaluate the utility of amplified fragment length polymorphisms (AFLPs) to compare levels of genetic diversity in *B. carinata* and *B. juncea*, to test for multiple origins in each species, and to test the validity and genetic relatedness of infraspecific taxa (vars. *juncea*, *multiseptis*, *rapifera* and *tsa-tsai*) proposed for *B. juncea*. Samples include ca. 100 accessions of each species. An initial screening with 30 primer pair combinations was conducted on *B. carinata* in order to select primers with a high relative multiplex ratio. A subset of 10 primer pairs, each of which gave 100-150 loci per pair, was selected. Data from both manual and automated analyses using a LICOR sequencer will be compared. Initial data for *B. carinata* indicated that less than 10% of the amplified loci were polymorphic. Comparative data for *B. juncea* will be presented.

**Genomics****Organisation & allelic analysis of the LAX gene family  
in *Brassica oleracea***

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As part of a project to characterise agriculturally important traits in the crucifer AUX1 gene family, we are currently identifying and characterising locus-specific and gene-specific alleles of the LAX (Like AuX1) gene family. We aim to determine if *Brassica* BoLAX genes will complement the homologs in *Arabidopsis thaliana* mutants. Analysis of gene families in the crop species *Brassica* is complicated by the typical triplication of large segments of the genome when compared with the closely related crucifer *Arabidopsis*. In an attempt to understand the extent of functional specificity or redundancy at different loci we are isolating sequences from *B.oleracea*. Detailed genetic and physical comparative mapping and sequence analysis is being carried out prior to determining the effect of both over- and under-expression of the members of this gene family in *Brassica napus*. This will allow us to identify any locus specific differences in gene structure, coding sequence, regulatory (promoter) sequences, and/or gene function.

**Genomics****Construction of a genetic map for the *Brassica oleracea* genome based on ESTs from the *A. thaliana* genome project.**

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Studies concerned the construction of *Brassica oleracea* chromosomal map with the use of known DNA coding sequences (ESTs) as probes from a genome of the model plant, *Arabidopsis thaliana*. ESTs from all five *A. thaliana* chromosomes were applied. Mapping was carried out with use of a set of 67 lines F2 derived from the cross combination of collard (*B. oleracea* var. *acephala*) x cauliflower (*B. oleracea* var. *botrytis*). As probes, 214 cDNA clones of *A. thaliana* were chosen, among them 164 clones with high sequence homology to known genes. Linkages and loci chromosomal order were evaluated with a help of MapMaker 3.0. As a result of mapping, all together 249 loci were added to previous chromosomal map of *Brassica oleracea*. The map covers 9 basic linkage groups. Many homeologous chromosomal segments among *B. oleracea* and *A. thaliana* were established. Molecular probing of the *B. oleracea* genome confirmed previous suggestions that most short chromosomal segments in *B. oleracea* and *A. thaliana* display similar linear organization.



**Genomics****Towards a contiguous physical map of the *Brassica C* genome: A valuable resource for the *Brassica* community**

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The *Brassica C* genome plays an important role in both arable agriculture and horticulture, containing various vegetable, oilseed and mustard crops. Due to the close relationship with *Arabidopsis thaliana* it is ideally suited for the transfer of fundamental knowledge derived from this species into crop plants. A more detailed understanding of the genomic organisation within *Brassica* will also provide information on the role and ramifications of replicated loci, particularly with respect to transcription. The development of a physical map has been highlighted by the UK *Brassica* research community as being vital for such work, together with characterisation of gene function and marker assisted selection. As part of a UK consortium, at HRI we are in the process of developing a physical map of the *Brassica C* genome using large scale fluorescent fingerprinting of BAC clones. Anchoring fingerprinted contigs to both the *Arabidopsis* genome and the genetic maps of *Brassica* will provide an invaluable resource for the interchange of functional genomics resources between *Arabidopsis* and *Brassica*. Initial results show the feasibility of this approach and its usefulness to the whole *Brassica* research community

**Genomics****Integrating the genetic and physical maps of *Brassica oleracea***

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Linking genetic maps constructed with molecular markers with the corresponding cytogenetic maps is an important goal in the investigation of crops such as *Brassica oleracea* L. As a first step we have produced a partial karyotype for *B. oleracea* var. *alboglabra*, based on the combination of chromosome size, arm ratios and the location of repeated 45S rDNA, 5S rDNA and pericentromeric heterochromatin sequences by fluorescence *in situ* hybridisation (FISH) (Armstrong *et al.*, 1998, Physical mapping of DNA repetitive sequences to mitotic and meiotic chromosomes of *Brassica oleracea* var *alboglabra* by fluorescence *in situ* hybridisation. Heredity 81: 666-673). The mapping of one 45S rDNA and the 5S rDNA loci to LG04 of a *B. oleracea* genetic map (Sebastian *et al.*, 2000, An integrated AFLP and RFLP *Brassica oleracea* linkage map from two morphologically distinct doubled-haploid mapping populations. Theor Appl Genet 100: 75-81) has enabled us to associate chromosome 2 with this linkage group. We are currently using BACs, cosmids and RFLPs, with known genetic map positions as FISH probes to identify markers for the remaining chromosomes (e.g. Vicente and King, 2000, ). Characterisation of disease resistance gene-like sequences in *Brassica oleracea* L. Theor Appl Genet (in press)). The construction of a definitive karyotype, with all linkage groups assigned to our cytogenetic map is approaching completion.

**Genomics****Blackleg resistance gene introgression from *Brassica nigra* occurred through homoeologous recombination**

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Interspecific hybridization allows to introduce into oilseed rape genome genes of interest, such as resistance to blackleg which is one of the most damaging disease of oilseed rape. One resistant recombinant line ( $2n=38$ ) was produced from the selfed progeny of a disomic addition line ( $2n=40$ ) containing one pair of *B.nigra* chromosomes carrying resistance gene(s) to blackleg in the 'Darmor' genetic background. Its meiotic behavior was disturbed. On one hand, a selection for meiotic stability was applied in the following generations of selfing and backcross. In all the cases, the *B. nigra* isozyme locus, Pgi-2B, appeared totally linked to the resistance gene. The isozyme analysis of the selfed progeny of a stable resistant plant revealed that the *B. nigra* introgression replaced the homoeologous region of the A genome. However, the homozygous resistant plants had a poor vegetative development. On the other hand, the initial recombinant mother-plant was crossed with one of the oilseed rape varieties used to establish our reference oilseed rape genetic map, 'Yudal'. The stability of the F1 resistant hybrids allowed to produce two backcross progenies, one with 'Yudal' and the other one with 'Darmor' as recurrent susceptible parents. Genetic mapping confirmed the location of *B. nigra* introgression on the DY5 (A genome) linkage group but also indicated that the missing A genome fragment replaced the homoeologous region of the C genome on the linkage group DY15. The meiotic stability and the male fertility were not affected by the different chromosome rearrangements. The present work indicates that the introgression and chromosome rearrangements occurred only between the homoeologous regions. The implication in breeding programs will be discussed.

**Genomics****A consensus linkage map for rapeseed (*Brassica napus* L.) integrating three individual genetic maps derived from distinct DH populations**

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A framework consensus map for rapeseed (*Brassica napus* L.) was constructed from the integration of three DH mapping populations derived from three crosses: (i) a cross between a French winter rapeseed cultivar, 'Darmor.bzh', and a Korean spring line 'Yudal'; (ii) a cross between two winter cultivars 'Darmor' and 'Samourai'; (iii) a cross between two spring cultivars 'Stellar' (Canadian) and 'Drakkar' (French). Several sources of genetic markers were used: isozymes, RFLPs, RAPDs, and AFLPs. A total of 992 different markers were mapped on a least one mapping population, of which 538 were included in the consensus map and 253 were common to at least two populations. Markers were distributed over 19 linkage groups, thus reflecting the basic chromosome number of rapeseed and covered 2429 cM (K), which was close to the mean confidence interval estimates of genome length [2127 – 2480] cM. Markers were evenly spaced on the entire genome even if for several linkage groups, both RAPD and AFLP markers were not uniformly distributed. A higher recombination rate was observed in the population resulting from the cross 'Stellar' x 'Drakkar'. A translocation was identified between the map derived from the cross 'Stellar' x 'Drakkar' and the two others. The consensus approach allowed to map a larger number of markers, to obtain a near complete coverage of the rapeseed genome, to fill number of gaps and to consolidate linkage groups of the individual maps.

**Genomics****Use of RAPD markers to evaluate the genetic variability of Sicilian wild populations of *Brassica***

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*Brassica* comprises very variable species from the morphologic and genetic point of view. Among these species, the Sicilian populations of *Brassica* sect. *Brassica*, related species to kale crops (*Brassica oleracea*), form a complex group. The genetic relationships among fifteen populations occurring in Sicily and one from Calabria, representing the whole populament, have been investigated using random amplified polymorphic DNA (RAPD) markers. This essay carried out with 22 arbitrary primers, generated 236 polymorphic fragments, 21 of which were specific of single populations (mainly of *B. insularis*, *B. incana*, *B. macrocarpa*). Jaccard's genetic distances were computed and the phylogenic tree was established using UPGMA algorithm. The dendrogram obtained showed four branches grouping: a) *B. incana* populations; b) *B. insularis* and *B. macrocarpa*, populations of small islands around Sicily; c) *B. rupestris* populations d) *B. villosa* populations. Only one population of *B. rupestris* (*B. rupestris* subsp. *brevisiliqua*) was clustered to *B. villosa* group. The classification obtained is discussed with regard to the morphological, ecological and geographical data.

**Genomics****A new gene for a Lon protease in *Arabidopsis thaliana***

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A method applied on the design of consensus PCR primers allowed us to identify a new gene possibly encoding a lon protease. Interestingly, two genes encoding lon proteases had already been identified in the genome of this plant, whereas the completely sequenced genome of yeast carries only one gene for this protein which is required for normal mitochondrial function. Furthermore, only one lon protease gene seems present in the human genome. The genes previously identified in the *Arabidopsis* genome by Sarria et al (The Plant Cell, 1998, 10:1217-1228) and Murray et al (Plant Physiol, 1998, 116(2):868) encode respectively a mitochondrial targeted protein and a non targeted protein (Barakat et al, Plant Mol Biol., 1998, 37:141-154). They are both located on chromosome V (respectively mapping at ca 60 cM and ca 100 cM). We will show results indicating that the new identified gene is located on chromosome III and encodes a protein more closely related to the mitochondrial targeted lon protease than the non targeted one. It is specifically expressed in some tissues of the plant, particularly in reproductive organs. We intend to characterise further this new gene, including the possible targeting of its peptide product to mitochondria and to gather clues in order to elucidate the reason why higher plants seem to need more genes for this function than seems sufficient for yeast and mammals.

**Genomics*****Brassica* chromosomes and seed colour**

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Most oilseed rape (*Brassica napus*,  $2n=38$ , genomes AACC) cultivars are black-seeded. The progenitor species *B. rapa* ( $2n=20$ , AA) has either yellow or black seeds, while known *B. oleracea/alboglabra* ( $2n=18$ , CC) cultivars have black seeds. To determine which chromosomes of the C genome are carriers of seed colour genes, *B. rapa-alboglabra* monosomic addition lines ( $2n=21$ , AA+1C chromosome) were produced from a *B. napus* resynthesized from yellow-seeded *B. rapa* and black-seeded *B. alboglabra*. Eight of nine possible such lines have been developed so far. Three of the eight *B. alboglabra* chromosomes influenced seed colour. *Brassica rapa* plants carrying chromosome 1 of *B. alboglabra* produced only brown seeds. These brown seeds gave rise to plants that produced either yellow or brown seeds. On the other hand, *B. rapa* plants carrying chromosome 4 or another as yet unidentified *B. alboglabra* chromosome produced a mixture of yellow and brown seeds. The yellow seeds gave rise to yellow-seeded plants, while the brown seeds gave rise to plants that yielded a mixture of yellow and brown seeds. Thus the three *B. alboglabra* chromosomes induce brown seed colour in two ways. When *B. rapa* is a carrier of chromosome 1, a maternal control of seed colour is manifested in that only brown seeds are produced. The presence of chromosome 4 or the hitherto unidentified chromosome of *B. alboglabra* however effectuates an embryonal control of seed colour, resulting in the production of both yellow and brown seeds. The yellow seeds give rise to plants that lack the extra chromosome yielding only yellow seeds, while the brown seeds give rise to plants carrying the extra chromosome producing a mixture of yellow and brown seeds. Consequently both maternal and embryonal control of seed colour contribute to the black-seeded phenotype of oilseed rape.

**Genomics****Characterisation of MADS-box gene expression in the cauliflower curd**

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The edible part of cauliflower (*Brassica oleracea* var. *botrytis*) consists of thousands of proliferating apical meristems, arrested at the inflorescence stage of floral development. Previously an *Arabidopsis* double mutant for the MADS box genes CAL and AP1 was shown to possess a similar heading phenotype. We have characterised CAL and AP1 loci in cauliflower and broccoli and studied their interaction in a cross between these two varieties. From the results of this we have proposed a model in which recessive alleles of each of these loci can explain the different heading phenotypes obtained in this cross. Comparing further the expression patterns of other MADS box genes in *Arabidopsis*, we predict that additional members of this family might also be expressed in cauliflower curds. To investigate this, a cauliflower cDNA library was prepared and surveyed for MADS box gene expression. A number of different MADS box genes was identified, including four different paralogues of one gene previously characterised in *Arabidopsis*. To compare the general pattern of expression of the paralogues found in cauliflower to the expression of the genes found in *Arabidopsis*, Northern blot analysis has been carried out. By developing specific probes for each of the different paralogues, to be used in situ hybridisation, we hope to obtain more specific expression data. Where probes are unable to distinguish paralogues from different loci, PCR locus-specific assays, will be employed. The expression data will enable us to test the hypothesis that paralogues at different loci in the cauliflower genome are differentially expressed. Following the work described above, complementation studies will determine the ability of different (over-expressed) paralogues in rescuing *Arabidopsis* mutant phenotypes.



**Genomics****Construction and Characterization of Bacterial Artificial Chromosome Library in Chinese Cabbage**

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Bacterial artificial chromosome (BAC) system commonly used for developing large insert libraries has been a useful tool for the genomic research in plants. In the present study, a Chinese Cabbage inbred line and BAC system have been adapted for constructing *Brassica A* genomic library. Protoplasts obtained from mesophylls were used as a source for high molecular weight DNA. The pCUGIBac1 donated from Clemson University Genomics Institute (USA) was used as a vector. As the genome size of Chinese Cabbage is 770 Mb, the HindIII BAC library consisting 20,736 clones which contain 115 kb average insert size is estimated for 3 fold coverage of the whole genome. The BAC library was stored in 96 and 384-well format in freezing medium at -70°C. The further study for characterization and the increasing genome coverage of the BAC library is in process now.

**Genomics****Recent Progress of Chinese Cabbage Genome Research in Korea**

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Chinese cabbage, which is the typical representative species of *Brassica A* genome, is one of the most widely used crops as dried, pickled, or cooked vegetable. For genome research of Chinese cabbage, we had applied 3 different strategies; genetic and physical mapping, and molecular cytogenetics. For genetic mapping of Chinese cabbage, AFLP and RAPD map has been developed with 89 doubled haploid lines from the F1 of North China ('Chiiifu' type) x South China ('Kenshin') type. A total of 498 polymorphic markers (460 AFLPs and 38 RAPDs) were generated using 40 AFLP primer combinations and 28 RAPD primers. The map included 389 marker loci in 10 linkage groups (LGs). The total linkage distance was of 2,695 cM with an average interval of 7.4 cM. Now, we are constructing the RFLP map using over 200 probes mapped already to several *Brassica* species. We had analyzed 33 morphological characteristics for QTL analysis last fall, and plan to analyze more characteristics during this spring. For construction of fine mapping population, we are selfing by SSD methods using the recombinant inbred lines whose number of population is 150 F3 and 500 F2. For genome researches such as physical mapping and map based cloning, a HindIII BAC library is being constructed using pCUGIBac1 donated from Clemson University Genomics Institute (USA), as cloning vehicles. We had constructed and confirmed about 20,736 clones which average insert size was about 115 kb insert size. The BAC library is estimated for 3 fold coverage of the whole genome. The further study for the increasing genome coverage of the BAC library is in process now. These BAC clones are now using for generating the physical contig map, BAC-end sequencing, and SSR marker developments.

**Genomics****Sensitivity of PCR method for detection of CMS ogura trait in *Brassica napus* L.**

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The detection of CMS ogura trait in *Brassica napus* L. is now possible using PCR method. For further studies of this method the sensitivity test was done. Two individual plants - with and without CMS ogura trait - were chosen and the seed samples were collected. Then combined DNA samples were prepared using different ratios of plant material of each type (seedlings were used for DNA extractions). This was the simulation of DNA contamination caused by the presence of one or more foreign seeds mixed with the proper seed sample. Another set of DNA samples was prepared by mixing of CMS ogura (+) and CMS ogura (-) DNA samples or by diluting them. This was done to simulate the effects of DNA sample contamination that could happen after extraction and also to find the lowest detection level for CMS ogura trait. For all prepared DNA samples PCR reactions were performed using constant reaction parameters and reagents. Obtained results that are presented here make it possible to calibrate the method for detection of the CMS ogura trait.

**Genomics****Genome collinearity as a tool for technology transfer between the model plant *Arabidopsis thaliana* and *Brassica* crops**

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We are developing a detailed picture of genome wide collinearity at both the genetic and molecular level between the genomes of the model crucifer *Arabidopsis thaliana* and *Brassica* crops. This information will indicate where to find potential *Arabidopsis* homologues of *Brassica* genes controlling traits of interest, the identification of such candidate genes in *Arabidopsis* allows us to use reverse genetics to confirm the function of such genes in the model plant before further analysis in the crop. The regions of collinearity observed between the model plant *Arabidopsis* and *Brassica* crops also provides a wealth of *Arabidopsis* molecular markers which are easily transferred to the *Brassica* genomes. Such markers can aid in the map based gene cloning of *Brassica* genes and assist in the manipulation of such genes by marker assisted breeding. We have recently extended our study of genome collinearity to a diverse range of crucifer species including; *Sinapis*, *Moricandia*, *Raphanus* and *Crambe*, this extensive comparative mapping has given us insights into the evolutionary pathway of the crucifers, specifically the consequences of polyploidy in the establishment of a species.

**Genomics****Orthology assignment in *Brassica oleracea* and *Arabidopsis* by comparative sequencing**

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We sequenced the region corresponding to the ABI1-Rps2-Ck1 segment on chromosome 4 of *A. thaliana* in *B. oleracea*, using the Rps2 gene as a probe to isolate cosmid clones. As in *A. thaliana*, the *B. oleracea* homolog to this gene, BoRps2, is present in single copy. We detected two orthologous segments on chromosomes 4 and 7 of *B. oleracea* corresponding to these genes. High nucleotide identity between the coding sequences and low identity in spacers, promoters and exons was the rule for these two segments. In the first orthologous segment, a synteny break between Rps2 and Ck1 was observed due to the presence of a N-myristoyl transferase gene (N-myr) in *B. oleracea*. Homologs to the N-myr gene in *Arabidopsis* are on chromosomes 2 and 5. One of these homologs is associated to a Ck1 gene in *Arabidopsis* (chromosome 5), which indicates that this is the ancestral gene arrangement. Inspection of this segment by DNA amplification in other *Brassica* species reveals the same *B. oleracea* gene arrangement. A general survey of the different tribes in the *Brassicaceae* family is in progress to estimate the age of the synteny break. There are at least four Ck1 (casein kinase) homologs in *Arabidopsis*. Orthologs to two of these genes present on chromosomes 4 and 7 in *B. oleracea* were partially sequenced. We have now detected two other Ck1 orthologs for this gene and a fifth homolog in a broccoli BAC library. Complete sequencing of these genes is now underway. Microsynteny studies based on sequencing accurately reveals gene content and structure and will therefore permit a survey of genome structure in other crucifers. The complexity of the genomic regions observed in the present study demonstrates that broad based hypotheses of genomic origin and structure based on conventional genetic mapping

**Genomics****Development of microsatellite markers for the analysis of genetic diversity in rapeseed (*Brassica napus* L.)**

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To identify microsatellites, also called simple sequence repeats (SSRs), size-fractionated libraries of total DNA of two winter rapeseed varieties, 'Mansholt's Hamburger Raps' and 'Samourai', were produced. These libraries were screened three times for the presence of GA and CA SSRs. Positive clones were sequenced to identify microsatellites with more than 7 repeats. The sizes of identified SSRs range between 16 and 132 nts. In agreement with Kresovich et al. (1995) GA-SSRs were found to be 4,5 fold more abundant than CA-SSRs. In these screenings 6 AT-, 2 T- and 3 three-base-motives were also found and used in the polymorphism studies. In total 67 perfect, 50 dispersed and 2 compound SSR repeats were investigated. Flanking primer pairs for PCR amplification were designed for these 119 microsatellites. All of them were tested in the winter rapeseed varieties 'Mansholt' and 'Samourai' and their F1 and 97 primer pairs gave reproducible and clearly separated amplification products with 1 to 5 fragments per primer pair. The average number of 1.7 amplification products per primer pair shows that most microsatellite primers produce only one or two bands. Therefore they are easy to score. All 97 SSRs were characterised in an array of 34 *Brassica* varieties. In all they show 142 different loci with 64% polymorphism: while 51 loci were monomorphic 91 polymorphic loci could be identified with two to twelve alleles and an average of 3.9 alleles per locus. The length differences between alleles vary between 2 and 69 nts. Some microsatellite markers show different behaviour in winter and in summer rapeseed that will be discussed as well as their utility in variety identification or the calculation of genetic distances.

**Genomics****Biological Clocks in *Arabidopsis thaliana* and *Brassica oleracea***

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The circadian clock is an endogenous ubiquitous feature of all eukaryotes as well as cyanobacteria. The clock confers a period of approximately 24 hours on the organism and is vital in co-ordinating various physiological and biochemical pathways such as the timing of photosynthesis etc. Clock genes have been identified previously in *Arabidopsis* using Quantitative Trait Loci (QTL) analysis. Such a study is done using RILs (Recombinant Inbred Lines), derived from different accessions of *Arabidopsis*. Assaying for circadian period of the plants is done by analysing rhythmic leaf movement, using automated video imaging and computer software programs. A similar, but more advanced technique will be used on a *Brassica oleracea* mapping population derived from cauliflower x Brussels sprout double haploid lines. This will enable us to locate putative QTL for circadian period in *Brassica*. This project will study the colinearity of clock genes, known to exist in *Arabidopsis*, with those identified in *Brassica*. Of particular interest to the project is FLC (Flowering Locus C). FLC a MADS box gene, known to be involved both in flowering time and the circadian period of *Arabidopsis*. Homologues of FLC have been identified in *B. oleracea* var. *alboglabra* A12Dhd genomic DNA using PCR, and positive genomic BoFLC clones have been obtained from A12Dhd BAC libraries. Future work will aim to identify not only additional circadian genes from a plant genetic system independent of *Arabidopsis*, but also characterise the homologues of BoFLC and understand its role in the *Brassica* circadian clock.

**Genomics****Functional analyses of disease resistance genes in *Brassica oleracea* using conserved synteny with *Arabidopsis***

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White blister (*Albugo candida*) and downy mildew (*Peronospora parasitica*) are fungal pathogens that can cause severe damage to *Brassica* crops. The aim of this research is locate functional resistance genes against these pathogens in *Brassica oleracea*. To do this we are using the conserved syntenic relationships between *B. oleracea* and the closely related crucifer *Arabidopsis thaliana*, in which over 20 resistance loci for these pathogens have been described. Resistance to both pathogens has been identified in *B. oleracea* from various sources. These have been crossed into rapid cycling brassicas and mapping populations developed. Five resistance genes were identified that recognised each pathogen. These segregated either as single dominant or recessive genes. Two crosses were developed further to map a dominant resistance gene to each pathogen. AFLP analysis was used to identify markers linked to the resistance genes. A mapping interval was constructed for both genes. By using the linked AFLP bands that were also polymorphic in the standard mapping populations, these genes were located on the *B. oleracea* genetic map. The white blister resistance mapped to the top of linkage group 2 and the downy mildew resistance to the bottom of linkage group 2. Further efforts were focussed on the white blister resistance. Markers from the *B. oleracea* genetic map linked to the white blister resistance gene were mapped in *Arabidopsis*. This showed that the gene lies in a region of discontinuity with the *Arabidopsis* genome. Currently we are working on more closely defining the relationship between the *B. oleracea* and *Arabidopsis* genomes in the region of the white blister resistance gene. In addition we are creating a larger mapping population and identifying further AFLP markers linked to the resistance gene.



**Genomics****Molecular Genetic Regulation of Early Floral Development in *Brassica oleracea***

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*Brassica oleracea* is a morphologically diverse species that includes such crops as cauliflower, broccoli, cabbage, Brussels sprout and kohlrabi. Recently, a project has been initiated to construct a complete physical map of *B. oleracea* that will be anchored to the closely related *Arabidopsis* genome. These features, together with the replicated nature of the *B. oleracea* genome relative to *Arabidopsis*, makes *B. oleracea* an excellent model for the application of *Arabidopsis* genomics and developmental biology in a more complex crop system. We are studying the early stages of floral development in *B. oleracea*, with a focus on a group of homologues of *Arabidopsis* genes that have key roles in this process. In the context of the replicated *B. oleracea* genome, this group of genes comprise an interacting regulatory network. In addition, these genes are candidates for some of the morphological variation seen in *B. oleracea*. For example, we have produced a simple genetic model in which allelic variation at loci of two of these genes accounts for the segregation of phenotypes obtained in a cross between cauliflower and broccoli. We are particularly interested in the nature and implications of locus replication in this group of genes. We are therefore characterising each locus with respect to its sequence and map position and will use locus-specific probes to determine their individual expression patterns. We are also starting to characterise the expression profiles conferred by promoter::GUS fusions for each of the replicated loci of a selected member of this group of genes in transgenic *Arabidopsis* and *B. oleracea*. In combination, these analyses will enable us to correlate the expression data with sequences conserved between individual *B. oleracea* loci and their *Arabidopsis* homologues and give us greater insight into the regulatory mechanisms of these genes. We will report on our progress to date

**Genomics*****Arabidopsis* Genome Sequencing and Analysis at TIGR**

Chris Town

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At TIGR, we have recently completed the sequence of chromosome 2 of *Arabidopsis thaliana* as two gap-free assemblies (contigs) of 3.6 Mb and 16 Mb, respectively. Chromosome 2 represents 15% of the genome and encodes 4,037 genes, 48% of which have no predicted function. Approximately 250 tandem gene duplications were found in addition to large-scale duplications of approximately 0.5 and 4.5 Mb between chromosomes 2 and 1 and between chromosomes 2 and 4, respectively. Sequencing of nearly 2 Mb within the genetically defined centromere revealed a low density of recognizable genes and a high density and diverse range of vestigial and presumably inactive mobile elements. With the completion of chromosome 2, we have started sequencing regions of chromosomes 1 and 3, in conjunction with other groups in the *Arabidopsis* Genome Initiative. In parallel with the high throughput sequencing, additional work is aimed at a more complete analysis of the *Arabidopsis* genome. We discovered a large fraction of an intact mitochondrial genome near the centromere of chromosome 2. We are currently exploring the evolutionary history of this insertion by analysis of other ecotypes as well as by sequencing the mitochondrial genome from Columbia. Other work is aimed at integrating orphan and unanchored contigs into the genome. Fiber-FISH is being used to investigate the relationship between isolated contigs near CEN3, and hybridization and sample sequencing are being used to determine whether totally unanchored contigs are in fact part of the *Arabidopsis* genome.

**Genomics****Genome organisation in two regions of the *Brassica oleracea* genome compared with colinear regions of the *Arabidopsis thaliana* genome**

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The imminent availability of the complete DNA sequence of the *Arabidopsis* genome provides a large pool of data which may be exploited within the various vegetable, oilseed and mustard *Brassica* crops. A particular challenge is the ability to map-base clone major and quantitative trait loci in *Brassica* species, aided by interpolation of map and sequence information from the *Arabidopsis* genome. Colinearity has been observed previously between the two genomes over regions covering as much as 30cM in *A. thaliana*, and a high proportion of loci in *A. thaliana* seem to be present in at least three copies in the *Brassica* genome. We selected two defined and contrasting regions of the *B. oleracea* genome to establish the relationship with orthologous sequences in *A. thaliana*. Our approach contrasted with that of previous colinearity studies within the cruciferae. We have used a combination of RFLP probes, SSR and other PCR markers from *Brassica* to establish the corresponding position of loci in *Arabidopsis*. With an inverted duplication covering much of *B. oleracea* linkage group O6 marker order was reasonably well conserved with a region of *A. thaliana* chromosome I. Within a short section of linkage group O3 colinearity was observed with corresponding short regions of four of the *Arabidopsis* chromosomes.

**Genomics*****BrassicaDB: a Brassica genome database***

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*BrassicaDB* is a database of genetic mapping, DNA sequence and bibliographic information that is currently focused on *Brassica napus* and *Brassica oleracea*. Developed at the John Innes Centre (JIC) it is publicly available via the World WideWeb at the UK CropNet server (<http://synteny.nott.ac.uk>). It is our aim to serve the global *Brassica* research community through providing added value to contributed data, such as results of BLAST analyses and links to *Arabidopsis* genome information. We also aim to offer greater functionality through a newly developed user interface and associated displays. *BrassicaDB* will be the principal repository of physical mapping data generated by the new UK *Brassica* genomics initiative.

**Genomics****Resistance to turnip mosaic virus in *Brassica***

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Turnip mosaic virus (TuMV), a member of the Potyvirus genus, is one of the most important viruses that infects field grown vegetable crops. Its wide host range includes economically important *Brassica* crops e.g. cabbage, broccoli, swede, spring and winter oilseed rape. The symptoms induced in TuMV-infected plants range from chlorotic spots to severe stunting and necrotic lesions, which significantly reduce the marketability of such plants. It also reduces crop yield and can cause plant death, sometimes destroying crops completely. The non-persistent, stylet-borne manner in which TuMV is transmitted by aphids causes insecticides to be ineffective, as brief probes are enough to transmit the virus. This, along with the wide host range of TuMV, makes it extremely difficult to control. Natural plant resistance offers the most effective and environmentally friendly way of controlling this virus. To use plant resistance efficiently in combating TuMV we are characterising sources of resistance in terms of genetic inheritance and interaction with different TuMV isolates. Markers linked to resistance genes are being identified and mapped. This will enable the breeding of crop varieties with combinations of genes that confer resistance to a broad spectrum of TuMV isolates using marker-assisted selection.

**Quality, Functional Foods & Health****Development of a microspore-derived embryo selection protocol for accelerated-desaturase fatty acid mutants of *Brassica napus* L.**

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Mutations in the fatty acid synthesis pathway were induced by exposing freshly isolated microspores to long wave UV light. Microspore-derived embryos underwent maturation at 35C to elevate the levels of saturated fatty acids deposited in the cotyledons for easier identification of low saturate profiles. Initially, HPLC (High Performance Liquid Chromatography) analysis of embryo fatty acid profiles was only minimally successful, as the embryos emerging from heat shock were very small and the levels of fatty acids extracted were often below the limits of detection. To optimize HPLC analysis, the effect of embryo sub-culturing (transfer to fresh liquid medium) and age upon transfer to 35C on the number of extractable embryos was investigated. The optimum sub-culturing age and density varied between the four microspore donor lines, while increasing the 35C transfer age increased the number of extractable embryos for all lines.

**Quality, Functional Foods & Health****Screening local cultivars of cauliflower to *Peronospora parasitica***

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Downy mildew (*Peronospora parasitica*) is a serious disease of vegetable brassicas in several countries. Seedlings are very susceptible to this pathogen. The use of resistant cultivars would be an economical, reliable and environmental friendly method of managing this disease in alternative to frequent fungicide treatments. In this work thirty-four Italian local cultivars of violet cauliflower were screened in comparison to fifteen commercial cultivars at cotyledon stage using two downy mildew isolates from Italy and Portugal. Symptoms were scored seven days after inoculation using a scale based on host plant response (necrosis) and on pathogen growth (sporulation). The combination of the two responses provided seven host-pathogen phenotypic interaction classes ranging from resistant plants, which showed light necrotic fleck and no sporulation, to susceptible plants, which showed diffuse necrosis and heavy sporulation. There were significant differences in the resistance to downy mildew between cultivars but no differences between the two *P. parasitica* isolates. Some accessions showed high resistance to downy mildew so that they can be considered an interesting starting material to set-up resistant cultivars.

**Quality, Functional Foods & Health****Suitability of violet cauliflower curds to freezing**

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In the Eastern areas of Sicily violet cauliflower is grown all year round by several local cultivars while the production of the white curded cultivars is marginal. The local markets, in fact, request usually violet curds as consequence of their distinctive qualitative traits which are appreciate for preparing typical dishes. In the frame of the activities related to a programme devoted to the improvement of violet cauliflower we assess quality characteristics of some local cultivars in relation of their possible utilisation for industrial processing and in particular for freezing. Eight local cultivars, characterised by different typologies of curds, were evaluated in comparison to two commercial ones white curded. The curds were harvested from plant grown in summer-winter cycle. The parameters registered were curd weight, diameter, colour, texture, number and size of sub-curds. Curds were underwent the freezing process beforehand, mechanical decoring simulation was carried out and the average florets size distribution was evaluated. The samples were then boiling water blanched (120") and frozen in an air blast pilot tunnel (I.Q.F. system) at -48°C, air speed: 4ms-1. On fresh, blanched, frozen and stored (12 months at -20°C) cauliflowers, ascorbic acid content, colour attributes (L\*a\*b\* reflectance), DM%, pH and titratable acidity were measured. The results shows differences among the material tested after both blanching and freezing treatments mainly in relation to colour and ascorbic acid content.



**Quality, Functional Foods & Health****Survey of glucosinolates in Sicilian wild and cultivated *Brassicaceae***

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In the frame of the activities carried out to exploit Sicilian local cultivars of brassicas, we focused our attention to some of the potential health compounds of various local cruciferous crops. These compounds are of interest to improve the quality of the produce with the aim to develop new cultivars capable of providing functional foods able to prevent disease. In this context, we surveyed for presence of specific glucosinolates in local cultivars of broccoli, cauliflower, kale, and in some wild species widespread in Sicily. Various commercial cultivars were included as controls. Total glucosinolates from leaves of 10 week-old seedlings were extracted in methanol and converted into their desulfo analogs by sulfatase treatment. HPLC separation of desulfoglucosinolates was accomplished in an acetonitrile gradient according to the procedure of Kraling et al (1990; Plant Breed 105:33-39). Glucosinolate composition varied extensively among species and crops of the same species, such as cauliflower, broccoli and kale. Cultivar variation for glucosinolate profiles was also observed for some of the crops. For example, Sicilian cultivars of cauliflower with coloured curds displayed a high content of glucosinolates, glucoraphanin in particular, compared to white curd commercial cultivars. Also for some wild species had a high content of other glucosinolates.

**Quality, Functional Foods & Health****Mapping of a mutation for high oleic acid content in seed oil of *B. napus* L.**

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For nutritional as well as technical reasons there is increasing interest in vegetable oils with a high oleic acid (HO) content. Advantages of this monounsaturated fatty acid are its high nutritive value with respect to a reduction of LDL cholesterol in blood plasma and its concomitantly positive impact in the prevention of arteriosclerosis, as well as the lower oxidative susceptibility, better oil stability and thus longer shelf life of the product. Two winter oilseed rape mutant lines, '7488' and '19661', with a high oleic acid content in the seed oil were characterised phenotypically. It was found that in both mutant lines the HO trait was monogenically inherited. Segregation analysis in F2 seeds from a cross between '7488' and '19661' showed the two mutations to be allelic. A comparison of seed, leaf and root fatty acid composition indicated that *fad2*, the oleic acid desaturase located in the endoplasmic reticulum, is affected by the mutation. In a bulked segregant analysis three AFLP markers linked to this mutation were detected and localised on the genetic map of *Brassica napus*. The most closely linked AFLP marker, E32M61-141, has a distance of 3,7 cM to the mutant locus and may be suited for marker assisted selection of the HO genotype. Based on the data of an alignment of different linkage maps of *Brassica napus* it can be concluded that the AFLP markers are near the locus of one copy of the *fad2* gene in the rapeseed genome, confirming the hypothesis that *fad2* is the gene affected in the mutants.

**Quality, Functional Foods & Health**

**Residual Characteristics of Endosulfan in Japanese Radish and Cabbage**

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Recently, it is clarified that a part of pesticides used in crop production might have the action of endocrine disrupter, and the vegetable safety is apprehended. Endosulfan, applied as a insecticide in vegetables, is one of them. Then, the residual characteristics of endosulfan was investigated in Japanese radish and cabbage. As the result, the endosulfan concentration in the crop at the harvest time decreased under the agricultural chemical registration standard, even in the intensive spraying condition also assumed in the usage standard in either vegetables. Still, the degree of residual is different in the culture condition, and the concentration of endosulfan in the open-field culture were detected higher than in the plastic greenhouse culture. The difference in the growth rate of crop seemed to be related to this. And, in the forage of Japanese radish, it was higher than the root several times, and it was judged that the we must mind the fact in the utilization. In addition, the spraying at the growth later stage, when the root is exposed in the air, might raise the residual. These results are expected to be usefull for the proper usage of pesticides in vegetable production.

**Quality, Functional Foods & Health****Retardation of senescence by ascorbic acid in Japanese radish cotyledons**

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In edible Japanese radish seedlings, cotyledon senescence, such as degradation of chlorophyll, is an important factor causing loss of quality. The relationship between senescence of cotyledon of Japanese radish and changes in levels of antioxidants was investigated by monitoring the levels of malondialdehyde, chlorophyll, ascorbic acid,  $\alpha$ -tocopherol in cotyledons, and the percentage of electrolyte leakage from cotyledonary discs. The content of malondialdehyde, which reflects lipid peroxidation, increased 3 days after sowing paralleling the increase in electrolyte leakage and chlorophyll loss.  $\alpha$ -Tocopherol content increased linearly 2 to 10 days after sowing, whereas the ascorbic acid content increased rapidly, reaching a maximum 3 days after sowing and decreasing thereafter. An increase in malondialdehyde was accompanied by a concomitant decrease in ascorbic acid content. The ascorbic acid content increased significantly when the cotyledonary discs were administered L-galactono-1,4-lactone, an immediate precursor of ascorbic acid, which suppressed changes in malondialdehyde and chlorophyll contents. These results suggest that ascorbic acid has an important role for suppression of lipid peroxidation and chlorophyll loss in cotyledons of Japanese radish. The relationship between ascorbic acid content in cotyledons of Japanese radish and temperature or light intensity during growth was also investigated.

**Quality, Functional Foods & Health****Quality and technological characterisation of two cauliflower hybrids (*Brassica oleracea* L. convar. *botrytis* L.)**

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The quality and technological characteristics and comparisons between two different hybrid cauliflowers were studied. The two hybrids were Nautilus (early classification, harvest at 85 days from transplanting) and Artemis (intermediate classification, harvest at 100 days from transplanting). They were harvested near Rome in December 1999. The analysis of the quality of the product was about physical-morphological, commercial characteristics and domestic preservation (8 days at 8-12°C). These included: dimensions, weight, volume, volumic mass and consistency (resistance force to penetration) of the cauliflower head and the cut strain of the stem. The two hybrids had very similar morphological values (average weight 1 kg), but only the 73% of Nautilus and the 63% of Artemis reached the optimum weight for retail (0.7 - 1.4 kg). At the harvest, the values of the consistency of the head of cauliflower and the cut of the stem are higher for Artemis than for Nautilus, but after 8-days of conservation, the reduction of the consistency was lower for Nautilus (-10%), than for Artemis (-44%). For Nautilus, inverse correlation was found between consistency reduction and cauliflower weight, while Artemis showed inverse correlation between consistency reduction and cauliflower volumic mass. It is very important to best explore the cause of these behaviours to direct the selection and agronomic practice to improve quality and technological characteristics of cauliflower. This also could be usefull in the resolution of the harvest mechanisation problem due to scarce uniformity of commercial maturity.

**Quality, Functional Foods & Health**

**Molecular and biochemical characterisation of post harvest senescence in broccoli**

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Molecular and biochemical analyses are being used to study post harvest senescence in broccoli (*Brassica oleracea*). Once harvested the product quality of broccoli deteriorates rapidly and the shelf life is limited by a visual yellowing of the florets, caused by degradation of chlorophyll in the sepals of the florets. We are investigating gene expression in broccoli florets after harvest and during storage with the aim of identifying and characterising genes involved in the regulation of post harvest senescence. We are investigating the timing of gene expression and correlating this with the biochemical changes that occur. Once genes have been identified as having an important role in senescence there is the possibility of manipulating post harvest changes thus improving quality.

**Quality, Functional Foods & Health****Fresh or cooked: two organically cultivated "brassicas" as an important source of vitamin C**

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The organic or ecological cultivation's, free of chemical applications, lead to a healthy food production and contribute to keep a less contaminated environment. Crucifers constitute an important source of Vitamin C, each day more appreciate substance, due to its beneficial properties: prevention of infarcts, cancer, infection and antioxidant power. The objective of this trial was to evaluate the content of Vitamin C (AOAC) of two crucifers organically cultivated: white cabbage (*Brassica oleracea*, var. *capitata*) cv. Brunswick enriched with compost of Californian red worms (vermicompost) and broccoli (*Brassica oleracea*, var *italica*) hybrid Legacy from Asgrow with incorporation of green of oat, compared to controls cultivated on soil. Vitamin C was determined on fresh and 20 minutes cooked material. The fresh material presented significant differences (Tukey 0.05) in the organically grown cultivars with regard to the controls: white cabbage cultivated with vermicompost presented a 32% more vitamin C, with an average content of 51 mg/100 Fw; broccoli grown with incorporation of oat green presented a 36% more vitamin c with a medium content of 120 mg/ 100 Fw. After cooking most content of vitamin C was in the broth :70% for white cabbage and 45% for broccoli. The observed values present the organic cultivation as an positive alternative for an sustainable production and corroborate both species of *Brassica* as good sources of vitamin C, which in case of consumed cooked has a very important content of vitamin in the broth.

**Quality, Functional Foods & Health****Identifying genes controlling synthesis of aliphatic glucosinolates in *B. oleracea* for map-based cloning**

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HPLC analysis of glucosinolates was performed on 86 recombinant inbred (RI) lines of collard &#61620; cauliflower and 57 RI lines of collard &#61620; broccoli. After comparison of the glucosinolate profiles of individual plants in each line, we inferred the presence of one gene controlling side-chain desaturation of aliphatic glucosinolates segregating in both RI populations. Another gene controlling the synthesis of 3-carbon side-chain glucosinolates, such as glucoiberin, sinigrin, and hydroxy-propyl glucosinolate, also segregated in the collard &#61620; broccoli RI population. Our results indicate that the synthesis of 3-carbon and 4-carbon side-chain glucosinolates is controlled by independent genes. Further, the genes controlling side-chain desaturation and hydroxylation can act on both 3-carbon and 4-carbon side chains. In order to attempt to clone the genes inferred above, we developed a simplified high throughput marker system called Sequence-based Amplified Polymorphism (SBAP) to search for markers closely linked to the genes of interest. We have found three markers closely linked to the gene for desaturation. After sequencing of these markers and performing BLAST analysis, we found that the sequence of one marker matched with the sequence of an *Arabidopsis* BAC clone. This clone is located in the region where a gene controlling side chain desaturation (gls-alk) was mapped by Mithen and Campos (1996, Entomol Expt. & Applic 80:202). From a broccoli BAC library we picked two clones containing this marker. These BAC clones will be used to develop new markers, which will be used to screen larger segregating populations. For the gene controlling synthesis of 3-carbon side chain glucosinolates, we have found several SBAP makers.



**Quality, Functional Foods & Health****The Potential of Brassicas for Phytoremediation**

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Caesium (Cs) is an alkali metal with chemical properties similar to potassium. It has no known role in plant nutrition and is not toxic to plants at the micromolar concentrations occurring naturally in soil solutions. However, two radioisotopes of Cs ( $^{134}\text{Cs}$  and  $^{137}\text{Cs}$ ) are of environmental concern due to their relatively long half-lives, emissions of beta and gamma radiation during decay and rapid incorporation into biological systems. These isotopes arise from the testing of thermonuclear weapons and discharges from nuclear installations. There is considerable interest in remediating sites contaminated by Cs radioisotopes by phytoextraction and, since the produce from contaminated areas enters the food chain, the introduction of 'safe' crops that do not accumulate Cs. Caesium enters plants through the root symplast and must cross the plasma membrane of root cells at least twice before it can be transported to the shoot. We have inferred the molecular identities of Cs<sup>+</sup> transporters in the plasma membrane of root cells from recent molecular-biological and electrophysiological studies and incorporated these into a theoretical model to predict their contributions to Cs<sup>+</sup> influx under natural soil conditions. The inward-rectifying K<sup>+</sup> (KIR), outward-rectifying K<sup>+</sup> (KOR) and voltage-insensitive cation (VIC) channels are all permeable to Cs<sup>+</sup> and, by analogy with their bacterial counterparts, it is likely that 'high-affinity' K<sup>+</sup>/H<sup>+</sup> symporters (KUP) also transport Cs<sup>+</sup>. Modelling has suggested that, under natural conditions, (i) VIC channels mediates most (30 to 90 %) of the Cs<sup>+</sup> influx, with KUP transporters mediating the remainder, (ii) that Cs<sup>+</sup> influx through KIR channels is negligible and (iii) that stelar KOR load Cs<sup>+</sup> into the xylem. We have corroborated these conclusions by characterising Cs fluxes in *Arabidopsis* mutants lacking the dominant root KIR (AKT1) or stelar KOR (SKOR1). This information can be used to direct the genetic modification or conventional breeding of *Brassica* for phytoremediation.

**Sustainable production, Agronomy & Diseases****Identification of RAPD markers of downy mildew (*Peronospora parasitica*) resistance gene in Broccoli (*Brassica oleracea var italica*)**

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Downy mildew, caused by the oomycete *Peronospora parasitica*, is a significant problem, in vegetable brassicas during the seedling stage and in established crops. Nowadays, molecular markers are widely used in plant breeding, especially for screening individuals which own interesting gene(s): Marker Assisted Selection (MAS). Our project aims at finding markers linked to a mildew resistance gene in broccoli, in order to apply the MAS strategy to broccoli and cauliflower breeding. The usual pathogenicity test consisted in inoculating seedlings, leading the susceptible ones to death (no plant material for a later molecular study). Therefore, we first worked on an in vitro pathogenicity test, in order to obtain plant tissue from susceptible plants and also to analyse several Pp isolates per plant. We produced an F2 population, each plant of which was analysed for its resistance to 6 Pp isolates, using our in vitro test. We found that the resistance to these isolates was controlled by one dominant gene. In order to identify markers linked to this resistance locus, we are using the Bulk Segregant Analysis (BSA) associated with the RAPD technique. The genetic distances between the RAPDs found that way and the resistance gene will be determined by analysing individually the F2 population with each marker.

**Sustainable production, Agronomy & Diseases****Phenotype and cytological characterisation of cotyledon resistance to *Peronospora parasitica* in Couve Tronchuda (*Brassica oleracea* sp. *tronchuda* ssp. *tronchuda*)**

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The downy mildew (*Peronospora parasitica*) is an important disease that attacks the cole crops in the nurseries as well as the adult plants in the field. The importance of studying the disease in Portugal is related with the fact that there are Portuguese land races that are good sources of resistance to *P. parasitica* and could be used in breeding programmes. On the other hand, downy mildew provides an interesting case study of specificity, as the fungus occurs on a wide variety of wild hosts as well as on cultivated species (Lucas et al., 1995). There is evidence that within host species of this fungus, specificity may be determined by genotype-specific interactions consistent with a gene-for-gene system (Lucas et al., 1988). A detailed analysis of the resistance of Couve Tronchuda (*B. oleracea* ssp. *tronchuda*) to *P. parasitica* is under study. The inoculation tests of 3 pathogen isolates (two Portuguese and one UK) on 95 host accessions, at the cotyledonary stage, revealed a range of interaction phenotypes including: 1) Resistant (R) showing necrotic flecking on the upper surface of the cotyledon restricted to the infection area without sporulation; 2) R/S similar to the previous one but with the production of few conidiophores on the lower surface of the cotyledons; 3) Susceptible (S) showing no necrosis but otherwise abundant sporulation on the infected area. A cytological study was made for a better understanding of what differentiates the referred interaction phenotypes. Seven days old seedlings of Couve Tronchuda "Algarvia" were inoculated with 10 µl droplets of a Portuguese isolate of the fungus on the cotyledons. The inoculated cotyledons were sampled at 4, 6, 8, 10, 17, 24 and 48h after the inoculation and prepared using the whole cotyledon clearing and staining technique to study the development of fungal infection and the host responses.

**Sustainable production, Agronomy & Diseases****Preseowing soaking treatment to seeds with nitrates and its effect on some physiological parameters and yield attributes of *Brassica* spp.**

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In the present investigation the seeds of *Brassica juncea* L.Czern & Coss Var. Kranti were pretreated either with distilled water or with solutions of Potassium Nitrate, Magnesium Nitrate and Calcium Nitrate (15 mM Nitrate) for 24h and were sown in the field at early, middle and late period of its growing season; in control sets seeds were sown directly to the field without providing any type of pre-treatment. All the salts containing nitrate were found to improve the parameters like plant height, number of leaves, leaf area, leaf area index, leaf nitrogen, leaf nitrate reductase activity and yield attributes like appearance time of first and 50% flower, number of pods/plant, pod weight, pod length, number of seeds/plant, 1000 seed mass, seed yield/ plant, pod nitrogen, seed nitrogen and yield/ha as compared to control and distilled water treated sets; only oil content of seeds were found more in later two sets. Further, the cationic part of nitrate containing salts were also found to impose their specific impact on the above mentioned parameters. Sowing time of the seeds has shown its influence on the crop growth and yield. Late sowing of the crop has represented low level of all the above mentioned parameters as compared to early and middle sown sets. However, the plants raised from the nitrate treated sets sown in late period showed more yield in respect to the plants raised from the control and distilled water treated sets sown in early period of its growing season. The over all result has been discussed in the light of the mechanism of action of nitrate, used during the pre-treatment period before seed sowing.

**Abundance and relative importance of caterpillars attacking *Brassica oleracea* (convar. *acephala*) in northwestern Spain**

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Crops belonging to the genus *Brassica* are affected by several lepidopterous pests causing considerable economic loss. In northwestern Spain, where these crops are widely grown, there is not any study about the importance of pests attacking brassicas. The objectives of this work were: i) to assess the abundance and relative importance of pests, and ii) to study the varietal response to damage under natural infestation. The attack by cabbage caterpillars was recorded on 15 open-pollinated cultivars of *Brassica oleracea* at two locations in northwestern Spain. Genotypes were evaluated in a randomized complete block design with four replications at two sowing dates (early and late). Ten plants from each plot were randomly sampled at 3-week intervals from April to December. Data were: percentage of attacked plants and leaves (young and adults) and number of larvae and eggs of each species. Damage of each plot was rated on a 9-point scale from 1 (wholly damaged) to 9 (no injury). We found mainly three lepidopterous species: the cabbage moth, *Mamestra brassicae* (L.); the imported cabbageworm, *Pieris rapae* (L.); and the large cabbage white butterfly, *Pieris brassicae* (L.). Of the 16,141 larvae recorded, 71.2% were the cabbage moth, 17.1% the imported cabbageworm, 9.1% the large cabbage white butterfly, and 2% other lepidopterous pests. The highest number of larvae of cabbage moth were found from June until the end of September. Early and late plantings had a similar damage and the highest attack was recorded for both locations towards the end of July.

**Sustainable production, Agronomy & Diseases**

**Development of management practices for onion thrips, *Thrips tabaci* Lindeman, in cabbage grown in New York State**

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In 1999, we evaluated timed applications of dimethoate 4 E.C. (a.i. dimethoate, Helena) for thrips control in four processing cabbage varieties which had varied levels of thrips susceptibility. There were two planting dates (1 May and 1 June) to relate thrips damage to thrips abundance in the field. 'Transam' is a tolerant variety and dimethoate sprays were unnecessary. In the first planting, thrips damage in 'Hinova' and 'Upton' (moderately susceptible varieties) was reduced to acceptable levels by spraying from cupping to harvest. However, in the second planting thrips damage was reduced to acceptable levels in 'Hinova' and 'Upton', without dimethoate sprays. 'Genesee' is a very susceptible variety and dimethoate was ineffective at reducing thrips damage. Thrips damage was related to thrips abundance in cabbage and thrips flights activity.

**Sustainable production, Agronomy & Diseases****Interaction between non-homologous portuguese isolates of *Albugo candida* and *Brassica oleracea***

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The interaction of five non-homologous portuguese isolates of *A. candida* (four isolated from *B. rapa* – Ac506, Ac508, Ac509 and Ac510, and one from *Raphanus sativus*) in forty *B. oleracea* accessions from different geographic origins was evaluated at the cotyledonar stage. Some accessions presented susceptibility to the non-homologous isolates of *B. rapa*, mainly head cabbage ‘Large Blood Red’ and savoy cabbage ‘Brusselse Winter’. These accessions exhibited mean levels of infection higher than 20 and 46.7% respectively, independently of the *B. rapa* isolate tested. The isolates Ac508 and Ac510 revealed higher pathogenicity in the *B. oleracea* accessions tested than isolates Ac506 and Ac509. The isolate from *R. sativus* was the less pathogenic for the *B. oleracea* accessions tested. The kale ‘Verza San Giovanni’ was the accession that exhibits higher susceptibility to this isolate with 20.7% of infected plants. Non-homologous isolates of *B. rapa* and *R. sativus* were able to colonize some *B. oleracea* host accessions, which means that it is important to study the interaction and the variability between different *Brassica* accessions and isolates, and to review the concept of "races" of *A. candida* to formae speciales.

**Sustainable production, Agronomy & Diseases****Black rot resistant broccoli derived from protoplast fusion followed by sexual crosses**

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Black rot, caused by *Xanthomonas campestris* pv. *campestris*, is one of the most serious diseases of crucifer vegetables. Methods to control this disease are limited. An extremely resistant accession (PI 199947) was identified in *B. carinata*, a non-vegetable crucifer species that is difficult to cross with *B. oleracea*. In order to introduce this resistance source to *B. oleracea*, Hansen and Earle (1995) fused protoplasts of PI 199947 and rapid cycling *B. oleracea*. They obtained resistant somatic hybrids and two generations of backcross progeny. To obtain broccoli-type plants with high levels of resistance, we have done further backcrosses to broccoli 'Green Comet' as well as several generations of selfing. Evaluation and selection in each generation were done both by greenhouse inoculation of seedlings at Cornell and by field inoculation of plants at Reed's Seeds, Cortland, NY. Plants were generally less resistant in the greenhouse assays than in the field. Resistance in field inoculations was not always well correlated with greenhouse inoculation. However, plants that scored as highly resistant in the seedling assay were also highly resistant when transplanted to the field and later inoculated there. In this case, there was a high correlation between seedling resistance and field resistance ( $r=0.81$ ,  $n=20$ ). In one line (designated 98 11B) about 50% of plants were completely free from the disease in seedling inoculation and uniformly resistant in the field. This line also has desirable horticultural characteristics with a good broccoli head. To date, six highly resistant broccoli lines (4 closely related and two other more distinct ones) have been released to seed companies. This project demonstrates the value of protoplast fusion for introgression of useful traits.



**Sustainable production, Agronomy & Diseases*****Brassica* collections of the Centre for Conservation and Breeding of the Agrodiversity of the Polytechnic University of Valencia**

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The Center for Conservation and Breeding of the Agrodiversity of the Polytechnic University of Valencia holds more than 6000 accessions of vegetables crops. Cruciferae family is represented by 500 accessions, 362 of which are from *Brassica oleracea* and 76 of *Raphanus sativus*. All accessions have been collected in Spain, mainly in Catalonia and Valencia Communities. Samples are desiccated with silicagel and stored in a germplasm cold-storage room at 3°C and 5-6 % of relative humidity. Forty accessions of cauliflower (*Brassica oleracea* var. *botrytis*) and 41 of broccoli (*Brassica oleracea* var. *italica*) have been characterized, according to the Descriptors for *Brassica* and *Raphanus* published by the IPBGR (IPBGR, 1990). Other characteristics of interest from the commercial point of view have also been included. Insect proof cages have been used for regenerating each individual accession in order to avoid cross-pollination with other accessions. All passport and characterization data are computerized. A catalogue "Collection of cauliflower and brocculi seeds", in which passport and characterization data of the characterized accessions appear, has been recently published (Nuez et al. 1999). Accessions have been grouped according to the following characteristics: relative time to maturity, head cover from subtending leaves and leaf angle. Within each group several origin, plant, leaf and head characteristics are presented. Similarities of the main groups established with the most known commercial varieties has been underlined in order to make easier the use of the catalogue. The participation of the Polytechnic University in the EU Project "*Brassica* collections for broadening agricultural use" will favour the characterization of the whole collection and will facilitate their use by breeders.

**Sustainable production, Agronomy & Diseases****The impact of calcium on restance in *Brassica* genotypes to *Plasmodiophora brassicae* Wor. (Clubroot)**

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The European Clubroot Differential (ECD) Series is a set of resistant and susceptible genotypes whose responses to infection by wild type collections of *Plasmodiophora brassicae* Wor., the causal agent of clubroot disease, have been well characterised internationally. A series of controlled environment experiments will be reported that studied the effects of changing the calcium and nitrogen environments in the root zone on subsequent pathogenesis by *P. brassicae*. The resistance responses of selected ECD genotypes inoculated with *P. brassicae* were altered where calcium concentrations in the root zone increased. Populations of resting spores of *P. brassicae* extracted from galls of *Brassica* genotypes exposed to increased calcium changed in their capacity to elicit disease symptoms in some ECD hosts. Inoculating these hosts with miztures of wild type and calcium enhanced resting spores indicated that changes in the pathogenic abilities of *P. brassicae* result from varying the nutrient environment. It is apparent that calcium and nitrogen affect the proportions of *P. brassicae* phenotypes within a collection of resting spores. Additionally there may be a direct interaction between these elements and the expression of host resistance. The implications of these results will be discussed in relation to the expression of both host resistance and pathogen fitness as modified by components of the environment.

**Sustainable production, Agronomy & Diseases****Resistance to *Alternaria brassicicola* in transgenic broccoli expressing a *Trichoderma harzianum* endochitinase gene**

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Broccoli plants (cv. Green Comet) expressing a *Trichoderma harzianum* endochitinase gene were obtained by *Agrobacterium tumefaciens*-mediated transformation. Primary transformants (T0) and selfed progeny (T1) were characterized for transgene presence and expression and for resistance to the fungal pathogens *Alternaria brassicicola* (leaf spot) and *Sclerotinia sclerotiorum* (Sclerotinia rot). PCR and Southern blot analysis confirmed the presence of the endochitinase gene. Endochitinase activity was measured by a fluorometric assay. Endochitinase activity of mature T0 plants in soil was 15 to 37-fold over controls; levels of expression in comparable T1 plants were higher (97 to 208-fold). Expression levels in polyploid broccoli transformants were not different from diploids. All plants with higher endochitinase levels than non-transgenic controls showed the expected 42 KDa endochitinase band in Western blot analysis, whereas no band was detected in the controls. Inoculation of detached leaves of T0 broccoli with *A. brassicicola* revealed no statistical difference between transgenic plants and controls, although some reduction in lesion size correlated with endochitinase levels. In contrast, when T1 seedlings were inoculated with *A. brassicicola*, the mean disease ratings of all transgenic lines were significantly lower than controls. Levels of resistance did not correlate with levels of endochitinase activity. Control of *A. brassicicola* in the transgenic seedlings was comparable to that seen with sub-optimal levels of fungicide (Bayleton), but no synergism between transgene and fungicide was detected. Endochitinase- transgenic broccoli did not show enhanced tolerance to *S. sclerotiorum*.

**Sustainable production, Agronomy & Diseases****Introgression of resistance to *Alternaria* leaf spot from *Sinapis alba* to *Brassica oleracea* via somatic hybridization and backcrosses**

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Leaf spot caused by *Alternaria* ssp. can significantly reduce yield and quality of *Brassica* vegetables. Highly resistant materials are not available within *B. oleracea*, but are found in *Sinapis alba*, a crucifer species difficult to cross with *B. oleracea*. The goal of this work was introgression of resistance from *S. alba* to broccoli via protoplast fusion. We produced a large number of somatic hybrids between *S. alba* and rapid cycling *B. oleracea* and confirmed them as hybrids by morphological characters, nuclear DNA measurements, and isozyme analysis. Embryo rescue was required to obtain the first backcross progeny after pollination of the hybrids with 'Green Comet' broccoli. Further generations of backcross or selfed progeny were produced by hand bud pollination. Mist-chamber seedling assays and detached leaf assays were used to identify resistance (0-9 scale). Among 51 somatic hybrids, 17 were highly resistant with disease ratings of 0 or 1, compared to those of the fusion partners *S. alba* (1) and *B. oleracea* (9). Nine BC1 plants were obtained from crosses between the most resistant hybrids and 'Green Comet'. Two of these remained highly resistant. SB1-4-1-1, a BC1 plant highly resistant after repeated inoculations, served as the basic material for the next generation. After two generations of backcrosses, some plants became partially fertile with more morphology more like broccoli. Four highly resistant broccoli lines have been recovered from the BC2-S1-BC1 population by repeated evaluation and selection. These lines may be evaluated in a field test in the summer of 2000.

**Sustainable production, Agronomy & Diseases****RAPD and AFLP markers linked to *Peronospora parasitica* resistance gene in broccoli**

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The linkage of random amplified polymorphic DNA (RAPD) and of amplified fragment length polymorphism (AFLP) markers with a single dominant gene for resistance to downy mildew (*Peronospora parasitica* [Pers. ex. Fr.] Fr.) of broccoli (*Brassica oleracea* var. *italica*) is investigated using the "bulked segregant analysis". The tightness of the linkage between molecular markers and the resistance gene are presently being estimated using an F2 population obtained crossing resistant and susceptible lines of broccoli. Four RAPD markers, apparently linked to the resistance gene because showing a clear tendency to co-segregate with resistant phenotype, were identified. These markers are now being cloned and sequenced in order to design specific PCR primers for their conversion to sequence-characterized amplified region (SCAR) markers. Twenty-six *EcoRI/MseI* primer combinations have already been used for identification of AFLP markers. However, the linkage of the resistance gene to the several polymorphic markers distinguishing between DNA bulks of resistant and susceptible plants still requires an extensive confirmation among the F2 population. The identification of DNA markers tightly linked to the downy mildew resistance gene will be useful for marker-assisted selection (MAS) in breeding programs and for location and isolation of this gene

**Sustainable production, Agronomy & Diseases****Quantitative genetic analysis of seed vigour and pre-emergence seedling growth traits in *Brassica oleracea* L.**

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The ability of seeds to germinate and establish seedlings in a predictable manner under a range of conditions has a direct contribution to the economic success of commercial crops, and should therefore be considered in crop improvement. We measured traits associated with seed vigour and pre-emergence seedling growth in a segregating population of 105 doubled haploid *B. oleracea* lines. The germination traits measured were mean germination times for unstressed germination, germination under water stress or germination after a heat treatment; and conductivity of seed leachate. The seedling growth traits measured were seed weight, seedling growth rate, and seedling size at the end of the exponential growth phase. There were some correlations, notably between germination traits and between seed weight and seedling growth. Heritability of the various traits was typically in the 10 -15% range, with heritability of conductivity and mean germination time under water stress 25 and 24% respectively. Collectively the results indicate that germination and seedling growth are under separate genetic control. Quantitative trait loci (QTL) analyses were carried out on all measurements and revealed significant QTL on linkage groups O1, O3, O6, O7 and O9. We suggest that genes at these loci are important in determining predictable seed germination and seedling establishment in practice.

**Molecular and biological variation in turnip mosaic virus from wild brassicas**

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Turnip mosaic virus (TuMV) was isolated from wild *Brassica oleracea* populations growing at four coastal sites in the UK, three in Dorset and one in North Yorkshire. Isolates were separated from other co-infecting viruses by plant passage and checked for the absence of other viruses by electron microscopy. The incidence of TuMV at the four sites was compared. Using four lines of *B. napus* capable of differentiating twelve pathotypes of TuMV, the Dorset isolates were all found to belong to the most common pathotype found in the Europe. RT-PCR amplification and sequencing of coat protein genes of five randomly selected isolates from each site and analysis of homologies between isolates, both at the nucleotide and predicted amino acid sequence levels, has been carried out. A lower level of sequence variation was detected at one of the Dorset sites compared to the other two. The maximum level of variation found between the isolates from Dorset, was approximately half the maximum level found world-wide. In contrast, a number of isolates from the Yorkshire site showed higher levels of variation than found at the Dorset sites.

**Sustainable production, Agronomy & Diseases****Evaluation of *Brassica napus* seed infection by fungal pathogens**

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Several pathogens of oilseed rape can be transmitted by seeds of oilseed rape (*Brassica napus* L.). Seed transmission may play an important role in dissemination of the pathogen worldwide, especially in the case of spreading virulent pathotypes to regions where they are not present. The aim of this work was to conduct a mycological analysis of *Brassica napus* seed infection, with special focus on contamination by *Leptosphaeria maculans/Phoma lingam* - the cause of blackleg of crucifers. Seed samples were obtained from field experiments done in 1997/98 at IPG (Poland) and IACR (UK), which comprised four replicates of five treatments (control and four fungicide spraying regimes) on two cultivars. The field site was inoculated with stem-base debris which showed symptoms of blackleg disease. In England the prevailing *P. lingam* pathotype on inoculum was A (Siro+), whereas in Poland, B (Siro0) was the only pathotype present. For mycological analysis of seeds the deep-freezing blotter method was used following the modified protocol of ISTA. The working sample was 2000 seeds per variant (cultivar &#61620; treatment). Seed samples were highly contaminated with fungi and bacteria. The average infection percentage of the whole seed lot was 34.4 %. There were 15 fungal genera recovered from seed samples. *Alternaria* spp. was the most common genus recovered, followed by *Fusarium* spp. and *Penicillium* spp. Levels of seed infected by *P. lingam* ranged from 0% to 2.5%. Both A and B pathotypes were found, but B was dominant (93% of population on UK seeds). On the basis of these experiments it may be concluded that the probability of dissemination of A pathotype by seeds is much lower than that for the B pathotype. The experiment allowed to assess the effect of fungicide treatment.



**Sustainable production, Agronomy & Diseases****Molecular genetics of turnip mosaic virus**

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Turnip mosaic virus (TuMV) is one of the most important viruses affecting brassicas worldwide. The isolate TuMV UK 1 is typical of many isolates found in Europe in its pathogenicity profile, being unable to infect *Brassica napus* lines N-o-1, R4 and 165. The genetic basis of the resistance to UK 1 is a single dominant gene in each plant line. The gene in line N-o-1 has been mapped and named TuRB01. For plant lines N-o-1 and R4, resistance-breaking isolates of TuMV have been characterised, sequenced, and chimeric viruses made by exchanging segments of the genome into an infectious cDNA clone of TuMV UK 1. Site-directed mutagenesis of the viral coat protein has been carried out to investigate its potential interaction with TuRB01. The cylindrical inclusion protein (CI) region of the genome was identified as important in resistance-breaking on lines N-o-1 (TuRB01) and R4, but not on line 165.

**Sustainable production, Agronomy & Diseases****DNA-based diagnostics of *Xanthomonas campestris* pv. *campestris* in *Brassica* seed.**

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*Xanthomonas campestris* pv. *campestris* is a seed-borne pathogen of horticultural brassicas which can devastate crops such as cauliflower. Early diagnosis of the pathogen can allow appropriate management steps. Current methods are expensive and time consuming involving removal of the seed coat by shaking in water, growth in selective media and then testing of pathogenicity on *Brassica* seedlings. A PCR based test offers the potential for faster and more sensitive detection of the pathogen. The first stage of this project has been to identify diagnostic markers, i.e. DNA sequences present in Xcc but absent or different in other soil and seed-borne micro-organisms. Database searches have revealed a number of potentially useful sequences. Although none are uniquely diagnostic some are more frequently present in Xcc compared to other bacteria. By using a number of diagnostic primers in combination it is thought that any Xcc infection will be identified. Diagnostic primers are to be used in conjunction with an enrichment culture method to determine levels of infection of seed and growing media. The race specificity of the test will be examined using strains of bacteria capable of infecting different *Brassica* varieties.

**Sustainable production, Agronomy & Diseases****Resistance in wild *Brassica* species to *Brevicoryne brassicae* in the field is not reproduced in the glasshouse, but increases with advancing plant age**

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*Brevicoryne brassicae* (the cabbage aphid) is an important pest of horticultural brassicas. Control relies on unpopular synthetic insecticides and concerns over their environmental effects has led to studies on possible use of plant resistance as part of an integrated approach to control. Field data collected on resistance to a number of wild C genome *Brassica* species suggested that there was consistent resistance in *B. incana* and *B. villosa* to *B. brassicae*. In addition to this some moderate resistance was seen in *B. macrocarpa* in the field. In glasshouse experiments this observed resistance was not seen to the same extent in *B. incana* and not found at all in *B. villosa*. However *B. cretica* was shown to possess some resistance *B. brassicae* in the glasshouse. Increasing numbers of plants were found to be resistant at 6 months old compared to 1 month old, suggesting that there may be mature plant resistance in *B. villosa* and *B. cretica*. These results show the importance of environmental influence on expression of resistance, and represent the first record of mature plant resistance to *B. brassicae* in any *Brassica* species as well as the first record of resistance to *B. brassicae* in *B. cretica*.

**Sustainable production, Agronomy & Diseases****Nitrogen fertilization of winter rape (*Brassica napus* L. *oleifera* spp.) cultivars**

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In this research, the effect of nitrogen fertilization on vegetative and generative characters, yield and quality of some rape varieties. For this purpose, experiment was carried out on two years (1995-96/1997-97) on the field of the Agricultural Faculty of Gaziosmanpasa University in Kazova-Tokat/Turkiye. The seeds were sown on October 17 and October 21, in the first and second year respectively. The rows were 30 cm apart. After emergence, plants were thinned as to achieve a 10 cm distance between plants. Plant height varied from 87.57 to 159.40 cm, number of lateral branches from 2.77 to 5.60, number of capsules on main stem from 30.20 to 50.83, number of capsules on lateral branches from 38.57 to 75.80, number of seeds on main stem from 13.50 to 28.20, number of seed on lateral branches from 18.33 to 28.33, 1000 seeds weight from 2.80 to 5.33 g, seeds yield from 740.5 to 2656.0 kg/ha, oil content from 39.40 to 45.50% and stem yield from 1517.7 to 5592.0 kg/ha.

**Sustainable production, Agronomy & Diseases****Studies on necrosis of Turnip mosaic virus (TuMV) infected white cabbage**

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TuMV is one of the most important pathogens of white cabbage (*Brassica oleracea* var. *capitata* L.). The virus can cause yield reductions up to 25% as well as necrotic disease symptoms in susceptible white cabbage cultivars. The impact of TuMV infection in developing necrosis on outer and inner leaves was investigated with different virus detection methods during cold storage of the heads. Three white cabbage cultivars, cultivated under field conditions, were infected with a mixture of 8 TuMV isolates and harvested in autumn. Our results suggested that there exists a correlation between TuMV infection and the occurrence of larger necrotic external and internal lesions (5 to 15 mm) during cold storage. In contrast, small necrotic spots approximately 1 mm in diameter distributed across the head surface (pepper-spotting) and appearing on the leaf midribs (vein streaking) have probably a physiological origin. Other pathogens like Turnip yellow mosaic virus and *Xanthomonas campestris* pv. *campestris* could be detected only very rarely in the necrotic areas and spots. On the other hand using two polyclonal antisera or selected monoclonal antibodies Turnip yellow virus (synonym Beet western yellows virus) was detected with high incidence in the whole heads independently of the occurrence of necrosis. Using DTBIA it was shown that TuMV is unevenly distributed inside of symptomless or necrotic halved heads. Therefore DTBIA was utilised to localise TuMV inside symptomless halved heads. Afterwards samples were taken from areas shown to contain TuMV for application of other detection methods. In the most cases the results of DTBIA could be confirmed by IC-RT-PCR as well as by ELISA.

**Sustainable production, Agronomy & Diseases****Expression of resistance to fungal diseases and turnip mosaic virus in somatic hybrids**

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Clubroot (*Plasmiodiophora brassicae*), leaf spots (*Alternaria brassicicola*, *A. brassicae*, *Phoma lingam*), and the turnip mosaic virus are frequent diseases causing remarkable losses in yield and quality in all culture forms of *Brassica oleracea*. In order to increase variability of expression of biotic resistance somatic hybrids were produced by PEG-induced symmetric and asymmetric fusions to transfer resistance into cabbage (cv. 'Toscama') and cauliflower (cv. 'Korso'). Wild relatives and some other species of the family *Brassicaceae* were used as resistance donors. Of more than 2240 somatic hybrids which were produced by 29 donor/receptor combinations nearly 73% showed resistance reactions to at least one of the involved pathogens. When adequate screening techniques were used (simultaneous inoculation of leaves/segments) hybrids possessing multiple resistance in a two- to fourfold combination could be detected. Chances of finding relevant resistance depended on number of hybrids generated in the fusions. As could be shown in asymmetric fusions with *Sinapis alba*, *Barbarea vulgaris* and *Hesperis matronalis*, transferred resistance to a pathogen in the hybrids might not be corresponded with resistance exhibited by the donor plant. In some cases, hybrids from combinations between *Raphanus* and cabbage, both highly susceptible, showed resistance to *Alternaria* indicating that after fusion of protoplasts new resistance principles could be formed of which the causal relationship is not yet clear. In general, resistance reaction in *Alternaria* pathogens were shown as being very unstable. Many hybrids into which (also variable) resistance of some donors (*Barbarea*, *Sinapis alba*, *Brassica carinata* a.o.) has been transferred.

**Sustainable production, Agronomy & Diseases****Variation in virulence of *Plasmodiophora brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage**

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The differential hosts of Williams (1966) and the European Clubroot Differential (ECD) (Buczacki et al., 1975) have been used commonly to identify populations of *Plasmodiophora brassicae*, which causes clubroot disease in *Brassica* crops. However, some of these hosts showed intermediate and fluctuating scores to most populations from Japan. Therefore, these hosts could not be used to provide a clear classification in Japan. We have tried to clarify the genetic diversity in pathogenicity of *P. brassicae* in Japan using Japanese clubroot-resistant (CR) F1 hybrid (F1) cultivars and lines of *Brassica rapa*. The responses of some CR F1 cultivars were very clear. Four groups were recognized in field populations and isolates derived from a single resting spore, using the CR F1 cultivars. The clear response obtained here may depend largely on the genetic purity of the F1 cultivars. Moreover, pathogenicity of some single-spore isolates obtained was compared with their original field population to these F1 cultivars. The response of the differential hosts suggests that there are several major CR genes in *B. rapa*. It is suggested that pyramiding CR genes would be useful in breeding CR cultivars that can overcome the breakdown of the present CR cultivars of Chinese cabbage.

**Sustainable production, Agronomy & Diseases****Genetic analysis of TuMV resistance in Chinese cabbage**

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Turnip Mosaic Virus (TuMV) occurs one of the major diseases in Chinese cabbage and causes severe yield reduction in South Korea (about 20%). However, due to technical difficulty in control, developing TuMV resistant variety has long been an important issue for stabilizing the yield. To develop new resistant variety, thirty-two Chinese cabbage lines were screened for seven TuMV strains (C1, C2, C3, C4, C5, K1, and K2). Five strains, C1, C2, C3, C4, and C5 used in the present study were donated by AVRDC. Two strains, K1 and K2 were isolated from Chinese cabbage at Seoul National University in South Korea. F1, F2 and BC1 of S1111 (resistant line) and YCA (susceptible line) were genetically analyzed by using K1 and K2 strains which show the most hyper-virulent among tested 7 strains. For K1 strain, fifty six out of 72 F2 plants showed resistance. For K2 strain, fifty-three out of 73 F2 plants showed resistance. These observations indicated that a single dominant gene is involved in resistance to K1 and K2 strain. In addition, the ratio between resistant and susceptible plants on K1 strain was 51 to 48 in back-cross analysis. These results suggest that the resistance to K1 or K2 strain is controlled by a single dominant gene.



**Sustainable production, Agronomy & Diseases****Pathotype specific resistance to downy mildew in *Brassica oleracea* at cotyledon and adult-plant stages.**

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Nine *Brassica oleracea* lines were simultaneously tested at cotyledon stage with two *Peronospora parasitica* isolates, from Portugal (Pt) and from the UK, by depositing a droplet of spore suspension on each cotyledon. Seedlings showing clear susceptible (sporulating) or resistance (non-sporulating) phenotypes to any of the isolates were selected and grown in pots until reaching 15 leaf stage. Then the plants were tested again with the same two isolates inside a greenhouse by spraying four leaves with a spore suspension using the single leaf inoculation technique. According to the combination of cotyledon (CTL) and adult-plant (AP) reaction *B. oleracea* lines were assigned to the following response groups: CTL and AP susceptibility to UK and Pt isolates; CTL and AP resistance to UK and Pt isolates; CTL and AP resistance to the UK isolate and CTL and AP susceptibility to the Pt isolate; CTL susceptibility and AP resistance to UK and Pt isolates. Cotyledon resistance may be associated to adult-plant resistance to the same isolate but there was no example of CTL resistance and AP susceptibility. There was evidence of pathotype specific resistance at cotyledon and adult-plant stages.

**Sustainable production, Agronomy & Diseases****Crucifers as intercrops of different cultivars of cabbage (*Brassica oleracea*, L.): a phytosanitary control in a sustainable agriculture**

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Three cultivars of cabbage (*Brassica oleracea*, L.): red, white and bull-heart and two crucifers as intercrops: Chinese salt-wort (*Brassica juncea*) a leafy vegetable, and white mustard (*Sinapsis alba*, L.) an aromatic specie, were cultivated to evaluate the influence of intercrops as an option for a sustainable fitosanitary control. The use of two crucifers as intercrops associated to cabbage contribute to a greater biodiversity. Similarity with the cultivar and greater bearing could build a physical barrier to the colonisation with some insects besides of offering an alternative in horticultural production. Intercrops were simultaneously sown between rows with each cultivar of field grown-cabbage. Recount of aphids (Number/plant) and evaluation of foliar area caterpillar's damages were carried out each two weeks to determine the influence of intercrops on these attacks (Anova, Tukey 0.05). The content of vitamin C were measured in fresh harvested (AOAC, 1990), big and small heads of white and bull-heart cabbage, to determine the nutritional quality (Chi squared test). The three cultivars presented different results with intercrops: red cabbage presented a significant attack of aphids reducing the effect of intercrops near harvest; for bull-heart and white cabbage both intercrops presented more effectiveness at the moment of harvest. Foliar damage did not present significant differences for red cabbage with intercrops, while bull-heart presented a better performance without intercrops and only white cabbage presented less foliar damage in with white mustard as intercrop. The nutritional quality (content of Vitamin C, did not present significant differences) was not affected by the Intercrops, while yield was affected in white and bull-heart cabbage, which did not adapt to the presence of intercrops.

**Sustainable production, Agronomy & Diseases****RFLP-characterisation of *Plasmodiophora brassicae***

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Parasite-specific DNA-fragments as hybridisation probes were used to establish RFLP markers as well as fingerprints for four single-spore isolates of *Plasmodiophora brassicae*. Sufficient amounts of DNA from the obligate biotrophic parasite were extracted from young diseased roots of *Brassica rapa*. Furthermore PCR markers were analysed and single-spore specific amplification products were sequenced to develop single-spore isolate specific PCR-primers. The localisation of the markers on 13 chromosomal bands separated in pulsed-field-gels is ongoing. These molecular tools were used to characterise a set of isolates originated from an infected root which was inoculated with a mixture of two single-spore isolates (e3 and e6) which clearly differ in their virulence. Spores of this mix-infected root were used to establish 17 oligo-spore isolates, resulting from an inoculation of a *B. rapa* var. Graanat plant with 10 spores, and 40 new single-spore isolates, resulting from an inoculation with only one spore. All oligo-spore isolates revealed to be genetically homogenous according to their molecular fingerprints. Up to now two of these isolates showed new RFLP-bands indicating recombination events during meiosis within the root, which could be explained by a crossing between the original single-spore isolates e3 and e6 or by mutation.

**Sustainable production, Agronomy & Diseases****Immunohistochemical analysis of *Plasmodiophora brassicae* in *Arabidopsis thaliana***

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The obligate biotrophic parasite *P.brassicae* induces galls in the roots of the model organism *A. thaliana*. The ecotype Tsu-0 of *A. thaliana* revealed to be resistant to *P.brassicae* isolate eH. This resistance has been shown to be pathotype-specific, dominant and monogenically inherited, which facilitates the cloning of the gene. The resistance reaction is accompanied by an hypersensitive reaction. Infected cells were surrounded by necrotic boundaries and thereby the infection area is encapsulated. Immunohistochemical staining of cytoskeleton elements (actin and tubulin) of the parasite has been used to detect the pathogen during the very early steps of the infection, when the pathogen succeeded in invasion of the host root in susceptible line or when the resistant line was able to build up a necrotic barrier. Using such lines combined with this staining technique the secondary infection of the pathogen in epidermal cells can be detected four days after inoculation with spores, when mature primary sporangia can be also observed in root hairs. Very young plasmodia with two to four nuclei passing nearly intact cell walls by pseudopodia-like elements filled with actin filaments could often be observed in the susceptible ecotype. Eight to twelve days after inoculation young plasmodia with only few nuclei were mainly found near the central cylinder, presumably within or neighbored to the cells of the pericycle, indicating cell-to-cell movement in direction to the central cylinder in this early phase of infection. Afterwards a symmetric distribution of clusters of later to earlier developmental stages of the parasite within the root from the cortex to the central cylinder were found in *A. thaliana*, indicating spreading in this phase mainly by host cell divisions.

**Sustainable production, Agronomy & Diseases**

**Chromosomal organisation of repetitive sequence elements in *Brassica* genomes**

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The chromosomal distribution of repetitive DNA sequences can be effectively observed by fluorescence in situ hybridisation (FISH). Using this technique we are investigating the organisation of various classes of repetitive DNA, including microsatellite motifs, Ty1-copia group retrotransposons, ribosomal DNA and telomere repeats, at the chromosomal level in *Brassica* genomes. Because repetitive DNA comprises a large proportion of the genome in *Brassica* and other crop plants, an understanding of the physical distribution of repeat sequences within the genome provides important information for molecular mapping and genomic studies. Chromosomal patterns of repetitive sequences also have implications for studies of *Brassica* genome evolution.

**Sustainable production, Agronomy & Diseases****Influence of temperature and daylength on flower initiation of broccoli (*Brassica oleracea* L. var *italica* Plenck).**

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The transition from vegetative growth to flower initiation is one of the most important period of ontogenesis in broccoli (*Brassica oleracea* L. var. *italica*, Plenck.). In cauliflower and, to a lesser extent in broccoli, the stage of curd and spear initiation is based on changes in the size and configuration of the shoot apice, from a flatened to a conical form and when the apical meristem bud widened to approximately 0.6 mm (Salter, 1969). There is few information about the influence of temperature and photoperiod on time to flower initiation of broccoli cultivars, that will allow the prediction of that stage. Therefore, the aim of this study was to evaluate three regimes of temperature (10, 15 and 20 °C) and daylength (8 and 16 h) on flower initiation of broccoli cultivars. The experiments were conducted in environmental growth chambers, using two intermediate cultivars Marathon and Green Valiant and one early cultivar Comanche. A morphological study of the apical meristem was done, from planting to flower initiation, using a destructive dissection technique, every three days. At planting time the mean apical meristem size was between 0.16 – 0.20 mm. Flower initiation did not occur at 20 °C/8 h in all cultivars. At 10 °C/8h conditions there were no differences on time to flower initiation between early and intermediate cultivars, which was 34 days (mean value) after planting. However, at 20 °C/16 h, Green Valiant plants differentiated one month later than 'Comanche' but 'Marathon' did not differentiated, at the same conditions. At 15 °C, flower initiation in 'Marathon' occurred independently of daylength. These results suggests that for flower initiation, Comanche and Green Valiant cultivars are photoperiodically sensitive plants at warmer conditions (20 °C), while 'Marathon' is more sensitive to temperature, requiring low temperature treatment for spear initiation.

**Sustainable production, Agronomy & Diseases****Possible host adaptation in the cabbage aphid,  
*Brevicoryne brassicae***

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Adaptation to host plant species has been reported widely in phytophagous insects. However, the extent to which these adaptations are due to the genetics of the herbivore and its genetic environment (i.e. the plant genotype) or are a response to true environmental effects (i.e. seasonal weather effects on both herbivore and plant) require elucidation. The *Brassicaceae* and *Brevicoryne brassicae* system have been selected as a model system to test the hypothesis that adaptation to heterogeneity in their environment in asexually reproducing plant herbivores is driven by host plant diversity. Samples of *B. brassicae* are being collected from different plant genotypes. A suite of microsatellite and AFLP markers are being developed to examine genetic variation in the population. Correlations between the presence of particular aphid genotypes and particular host plants are being investigated to determine whether there are host plant adapted genotypes of this aphid. Potentially host-adapted clones of *B. brassicae* will be established from the different plant genotypes at the end of the season and be genotyped. These clones will be used in controlled laboratory experiments in which they will be switched between plant genotypes to determine the relative intrinsic rate of increase,  $r_m$ , a measure of their fitness. These experiments will determine the effect of host plant genotype on the herbivore and support the findings from the field experiments. These findings will have implications for to the deployment of plant varieties resistant to insects, either through genetic modification or conventional breeding.

**Sustainable production, Agronomy & Diseases****Identification and origin of races of *Xanthomonas campestris* pv. *campestris***

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Six races of *Xanthomonas campestris* pv. *campestris* (X.c.c.) were defined based on the reaction of differential cultivars of *Brassica* spp. derived in part from the series of Kamoun et al. (1992). The numbering of the races was modified to accommodate additional races and to allow for a rational interpretation of the interaction of pathogen races and host differentials based on a gene-for-gene relationship. A model based on the interaction of four avirulence genes in the pathogen races and four matching resistance genes in the differential hosts is proposed. A collection of X.c.c. isolates, together with isolates received as related pathovars (X.c. pv. *aberrans*, X.c. pv. *armoraciae*, X.c. pv. *incanae*, X.c. pv. *raphani*) of cruciferous hosts, were inoculated onto a differential series of *Brassica* spp. to determine both pathogenicity to brassicas and race type. Most isolates were identified as X.c.c., with races 1 and 4 being predominant. Other races were rare. Some isolates from brassicas and other cruciferous hosts were non pathogenic or very weakly pathogenic on the differential series and could not be race typed. A number of these showed clear evidence of pathovar-like specificity to certain ornamental crucifers. PCR assays that differentiate races and isolates of X.c.c. are being developed.



**Sustainable production, Agronomy & Diseases****The turnip mosaic virus (TuMV) / *Brassica* pathosystem**

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Turnip mosaic potyvirus is a very important pathogen affecting brassicas worldwide. Extreme forms of resistance to TuMV have been identified in the A genome of *Brassica rapa* and *B. napus*, however no such extreme forms of resistance have been found in the C genome of *B. oleracea*. Evidence for a gene-for-gene relationship between TuMV and the *Brassica* A genome is being established. The serotypic, molecular and biological variation in TuMV worldwide is being studied. Plant resistance genes and the viral determinants of virulence / avirulence are being characterised and mapped. Attempts are being made to clone resistance genes, and they are being moved from the A to the C genome of *Brassica* non-transgenically. Strategies to provide durable resistance to TuMV are being developed.

**Transgenics****Production of broccoli with insect resistance genes**

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Transgenic broccoli has been obtained after *Agrobacterium tumefaciens* mediated transformation of seedling explants from cultivar Shogun. Hypocotyl and cotyledonary petioles were cocultivated with strain EHA105. The T-DNA of the binary vector contained a NPTII (NOS-NPTII-NOS) gene for selection of transgenic plants and a chimeric gene for avidin production. Avidin is a potent insecticidal gene which acts by tightly complexing biotin (vitamin H) and inhibiting the activity of biotin-requiring enzymes. The avidin construct was under the control of the 35S promoter with a OCS terminator. In addition, a vacuolar signal sequence was present to target the peptide to the vacuole to ensure plant biotin is not affected. In vitro shoot and root growth on selective levels of kanamycin confirmed plants were transgenic. Molecular assays have confirmed gene presence. Protein expression and insect feeding assays will be conducted to determine if there is sufficient gene expression for production of insect resistant plants.

**Transgenics****Improving transformation efficiency in vegetable  
*Brassica* crop types.**

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The efficiency of transformation is frequently limited by genotypic constraints, necessitating the identification of easy-to transform genotypes. An *Agrobacterium rhizogenes*-mediated co-transformation system was used to determine the efficiency of transgenic root production in seedling explants of eight commercial cultivars of *B. oleracea*, representing four crop types. The proportion of inoculated explants with transgenic roots ranged from 1.4 to 58% between cultivars. On this basis easy-to and difficult-to transform cultivars were identified. Components of genetic variation can be fixed in doubled haploid (DH) lines derived from heterozygous material, such as F1 cultivars. The efficiency of transgenic root production was established for DH lines derived by anther culture from Hawke and Trixie, representing easy-to and difficult-to transform F1 cultivars respectively. Genetic factors controlling transgenic root production segregated in DH lines. For two of the 23 Hawke lines screened transgenic root production from inoculated explants was increased by over 100%; transformation was also reduced by over 60% in two lines. Transgenic root production was increased by over 400% in explants of three lines derived from Trixie. Fixing variation for transformation competence provides ideal experimental material for investigating the genetic basis of genotypic variation. This understanding will broaden the application of transformation in genetic improvement programmes.

**Transgenics****Modelling genotypes' and cropping system's influence on gene flow in rapeseed**

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In Europe, numerous cultivars of oilseed rape (*Brassica napus* var. *oleifera*) of different qualities, especially for fatty acid profiles, are currently developed. Already oilseed rape of three kinds for fatty acid profiles are commercialised : classical, erucic and low-linolenic rape. However, because of gene flow through pollen and seeds, the purity of the harvest is hard to guarantee. The aim of GeneSys (Colbach et al., 1996, 2000) is to model the influence of cropping systems on gene flow from an oilseed rape variety including a specific gene to other varieties in a small agricultural region over the years. GeneSys models on each plot each year the number of plants and seeds per m<sup>2</sup> and the proportion of each genotype. This model takes into account volunteer rapes, evolution of the seed bank, pollen dispersal, rape populations in the field borders, the cropping techniques used, etc. Once the validation is over, this model will be a tool to determine which cropping situations are most susceptible of harvest contaminations by surrounding rapeseeds. It will then be possible to find which rape characteristics and which cropping system are necessary to diminish the contamination risks in this situation. This will be illustrated in the influence of the volunteer population and its management on the contamination for a specific field pattern or in the influence of seed and pollen productivity on the contamination of fields with non resistant crops.

**Transgenics****Success of hybridisation between oilseed rape (*Brassica napus*) and wild radish (*Raphanus raphanistrum*) varies among individuals.**

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One of the main concerns linked to the release of transgenic crops is the possibility of transgene escape towards weeds and wild relatives. Interspecific hybridisation would allow a few wild plants to pick up new characteristics such as insect and herbicide resistance. This would give new advantages to these wild plants which could result in more troublesome *Brassica* weeds in oilseed rape fields and other crops, and also change the present species composition in wild habitats. Interspecific hybridisation between oilseed rape (*Brassica napus*) and its wild relatives as wild radish (*Raphanus raphanistrum*) has been shown to occur spontaneously. The within-population polymorphism of wild radish for interspecific hybridisation with two cultivars of oilseed rape was investigated by hand crossing experiments. Wide variability occurred from plant to plant for the ability to form interspecific hybrids. Different barriers are identified. The relevance of these results to the question of the gene flow control between the crop and its wild relatives will be discussed.

**Transgenics****Factors Influencing on the High Efficient Plant Regeneration and Genetic Transformation in *Brassica* Vegetable Crops**

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Many steps are involved in the high and stable genetic transformation in crops. Especially, Chinese cabbage (*Brassica campestris*) has been known to be difficult to obtain high frequency transformants. Many different explant types such as cotyledon, hypocotyl, leaf, and petiole of many different genotypes of the Chinese cabbage and other *Brassica* vegetables were used. Several pre-incubation conditions for improving high shoot regeneration were established using plant growth regulators and liquid medium supplemented with high level of the sucrose (4%). To improve the genetic transformation rate in Chinese cabbage, the effects of acetosyringone and tocopherol were examined, and an addition of 10mg/L of acetosyringone during selection procedure, indeed, improved the number of the plants transformed and time to shoot induction about one week, though it varied depending on the genotypes. Herbicide resistant gene (bar) and DTx-A gene with anther-specific promoter of Chinese cabbage have been introduced into Chinese cabbage, broccoli, and mustard. Progenies of these transformants were confirmed for their stable expression based on PCR, Southern blotting, Northern blotting, and bioassay.

**Transgenics****Plant Regeneration and Genetic Transformation of Radish (*Raphanus sativus*) using bolting related gene RsCO**

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Radish is known as a very difficult crop to be regenerated. Explants were cotyledons and hypocotyls of seedling grown in MS medium for 5-6 days. The basal medium for shoot regeneration was MS added with 2% sucrose and 0.8% agar. Various levels of BAP and NAA combined with different degree of silver nitrate were tested. Shooting took place in the medium supplemented with BAP 2.5-5.0 mg/L and NAA 0.5-1.0 mg/L. To induce the late-bolting type of the radish cultivars, we introduced RsCO gene in anti-sense direction into the radish cultivar 'Jinju DaePyeong' which is known as a very early bolting cultivar under the normal field growing condition. We obtained several transgenic plants, and progenies of these plants were tested for stable integration of the gene. T0 plant showed 15-30 days of delay in bolting under the normal field condition compared with the control plants. Northern blotting also confirmed its expression based on NPTII gene as a probe. PCR analysis of T0 showed the 3:1 ratio of segregation. Further characters of bolting and horticultural traits have been in the process.

**Transgenics****Study of the *in vitro* culture in *Crambe***

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*Crambe* is an annual species and belongs to the Cruciferae. *In vitro* culture of *Crambe* should enhance breeding efficacy and constitutes a prerequisite for the use of *Crambe* genotypes as a genetic sources via genetic transformation or somatic hybridization. Cotyledonary petiole explants and hypocotyls were evaluated for *in vitro* regeneration response by screening on MS basal media with different combinations of phytohormones. Only with hypocotyls on MS medium with 4.5  $\mu\text{M}$  Zeatin and 0.5  $\mu\text{M}$  naphthalenacetic acid (NAA) multiple shoots were produced. Depending on genotypes shoot regeneration ranging in efficiency up to 61%. Leaf segments were cultured on MS basal medium with 8.8  $\mu\text{M}$  - 22.2  $\mu\text{M}$  6-benzylaminopurine (BA) and 2.6  $\mu\text{M}$  NAA and also multiple shoot induction were observed. The shoots were subcultured and propagated on MS medium with 10.7  $\mu\text{M}$  NAA. After the rooting on the same medium the regenerated plantlets were transferred in the greenhouse. There the plants developed normally flowers and seeds. Plant regeneration from isolated protoplasts could be obtained on MI medium (Li & Kohlenbach, 1982) supplemented with 2.6  $\mu\text{M}$  NAA; 2.2  $\mu\text{M}$  2,4-dichlorophenoxyacetic acid (2,4-D) and 2.2  $\mu\text{M}$  BA. This medium is suitable for protoplast division and microcallus formation. When protoplast derived colonies of callus were transferred onto Nitsch medium (Nitsch & Nitsch, 1969) and MS-based medium plantlets could be regenerated. Only *Crambe hispanica* showed a good ability for plant regeneration. The frequency was 3.7%. The possibility to regenerate shoots allows to use these systems for successful *Agrobacterium*-mediated gene transfer and somatic hybridization.



**Transgenics****Control of Gene Expression in Cauliflower (*Brassica oleracea* var. *botrytis*) with the tCUP Gene Expression System**

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A cryptic, constitutive, TATA-less promoter was recently isolated from tobacco by T-DNA tagging. It is 2200bp in length and is comprised of a short TATA-less core promoter region with an initiator (*Inr*) sequence, an upstream region, and a leader sequence. This promoter is known as tCUP (tobacco, constitutive promoter) and it is capable of activating gene expression in a wide variety of angiosperm and gymnosperm plants. The objective of this study was to evaluate in cauliflower the core promoter region, upstream region and mRNA leader region of tCUP and also to determine whether the addition of a 35S TATA box sequence would improve gene expression. It was determined that there were enhancer elements in the upstream region between positions -197 and -62 (relative to the transcriptional start site) and when deleted resulted in a significant decrease in gene expression. It also appears that the core promoter region is found between -62 and +30. The mRNA leader sequence was found to be crucial for maintaining high levels of gene expression and its removal resulted in a three-fold decrease in gene expression; thus, it appears that there are translational enhancers present in the leader region. When the leader sequence was added to the minimal 35S promoter, there was a 2.5 fold increase in gene expression. The addition of a 35S TATA sequence to minimal tCUP promoter sequence (promoter truncated from 5' end to -62) at the -30 position resulted in a 3 fold increase in gene expression. Finally, the deletion of the sequence surrounding the transcriptional start site (-12 to +32 deleted) resulted in a significant decrease in activity, thus suggesting that the *Inr* region is crucial for tCUP promoter activity in cauliflower. The results show that the tCUP gene expression system may be an effective modular promoter for genetic engineering of cauliflower.

## **Development of clubroot resistant plant using anther culture in Chinese cabbage**

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Clubroot disease is one of the major diseases in Chinese cabbage in Northeastern Asia. In order to develop clubroot resistant variety, anthers of ten resistant varieties were cultured. All doubled haploid (DH) lines obtained from anther culture exhibited resistance. The crosses between DH and susceptible line showed resistance as well. F<sub>2</sub> generation of 10 resistant varieties segregated into resistant (CR) and susceptible (SP) plants. The segregating ratio between CR and SP was 3 : 1 in 8 out of 10 varieties, and two other varieties showed a skewed segregation. This result indicates that clubroot resistance is possibly controlled by a single dominant gene. In F<sub>3</sub> generations selected from F<sub>2</sub> that exhibited resistance, seven out of ten varieties were segregated, while the other three were not segregated at all. For further study, AFLP for cloning the clubroot resistance gene is in process now.

## Comparative analysis of genetic factors controlling clubroot resistance in *Brassica oleracea* and in *Brassica napus*

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Clubroot, caused by the obligate biotroph *Plasmodiophora brassicae*, is one of the most damaging diseases of cruciferous crops in the world. The development of resistant cultivars is considered now as a need to control this disease for all *Brassica* species. In *B. oleracea*, *B. napus* and *B. rapa*, a large variability of qualitative and quantitative resistance systems, under oligo- or polygenic control, have been described. The objective of this investigation was to compare the location, the effects and the specificity of resistance conferred by the resistance genes identified in *B. oleracea* with those of *B. napus*. Genetic control and mapping of loci implied in resistance to two single spore isolates of *P. brassicae* (Pb137-522 and K92-16) were studied in the F2/3 progeny of the cross resistant kale x susceptible broccoli (*B. oleracea*) and in the DH progeny of the cross resistant winter rapeseed x susceptible spring rapeseed (*B. napus*). In *B. oleracea*, high level expression of quantitative resistance against Pb137-522 and K92-16 isolates was controlled respectively by six and five QTLs. Four QTLs were common to both isolates, of which a major-QTL explaining 60-70% of the variance. In *B. napus*, high level of resistance to isolate Pb137-522 was conferred by a major-gene, Pb-Bn1, and a minor-QTL. Partial quantitative resistance to K92-16 isolate resulted from the association of two additive QTLs, one of them explaining 19% of the variance, was mapped at the same position as the major gene Pb-Bn1. Epistatic interactions between regions with or without additive effects were detected in *B. oleracea* and *B. napus* for both isolates. Isolate-non-specific and isolate-specific genetic factors having major- or minor-effect on resistance were detected in both species. Work is in progress to determine if some of these genetic factors are common to the two species. To construct durable resistances, several gene combinations can be envisaged thereby associating either loci implied in the different resistance systems or loci of different *Brassica* species.

**RFLP-characterisation of *Plasmodiophora brassicae***

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Parasite-specific DNA-fragments as hybridisation probes were used to establish RFLP markers as well as fingerprints for four single-spore isolates of *Plasmodiophora brassicae*. Sufficient amounts of DNA from the obligate biotrophic parasite were extracted from young diseased roots of *Brassica rapa*. Furthermore PCR markers were analysed and single-spore specific amplification products were sequenced to develop single-spore isolate specific PCR-primers. The localisation of the markers on 13 chromosomal bands separated in pulsed-field-gels is ongoing. These molecular tools were used to characterise a set of isolates originated from an infected root which was inoculated with a mixture of two single-spore isolates (e3 and e6) which clearly differ in their virulence. Spores of this mix-infected root were used to establish 17 oligo-spore isolates, resulting from an inoculation of a *B. rapa* var. Graanat plant with 10 spores, and 40 new single-spore isolates, resulting from an inoculation with only one spore. All oligo-spore isolates revealed to be genetically homogenous according to their molecular fingerprints. Up to now two of these isolates showed new RFLP-bands indicating recombination events during meiosis within the root, which could be explained by a crossing between the original single-spore isolates e3 and e6 or by mutation.'

**Advances in the integrated control of clubroot in Australia**

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A fully adjustable machine was developed to incorporate liquids and/or solids to a depth of 15-20cm in two 23cm wide bands along transplant rows. Three products, a fungicide (fluazinam), a fertiliser (calcium cyanamide) and lime (calcium oxide) were used to determine the commercial potential of this method of application. Preplant incorporation of fluazinam into the soil in bands was found to be the most effective method of applying this product. Plants grown in soil treated in this way developed significantly less clubroot than plants treated with fluazinam applied using either a spot drench (100 ml/plant) or a continuous spray over the plants immediately after transplanting. The banded soil incorporation treatment required 80% less water (2500 L/ha reduced to 500 L/ha) and was more reliable and effective in a range of soil types than the high volume drenches currently used. Treatment of a reduced area (approximately one third of the broadcast area) in this way reduced the amount of lime and fertiliser product used by approximately two thirds without reducing product efficacy. Where the cost of treatment is high (eg. calcium cyanamide broadcast at 1 t/ha, costs approximately A\$1600/ha), this represents a considerable saving and has increased the profitability of the treated crop by up to 100% compared to a crop treated with a broadcast application of the same product. Soil residues were also reduced by treatment of a reduced area by band incorporation. A reduction in the use of lime is particularly important in some parts of Australia where potatoes follow crucifers in rotation, as potatoes are more susceptible to common scab (*Streptomyces scabies*) and powdery scab (*Spongospora subterranea*) at high soil pH. There is potential for this method of application to be used to apply a wide range of products to control *Plasmodiophora brassicae* and other root pathogens in row crops.

**Studies of *Plasmodiophora brassicae* (Clubroot) in India**

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Clubroot disease, caused by *Plasmodiophora brassicae* has become an increasingly devastating problem in *Brassica* vegetable and oil seed crops grown in Bengal over the past decade. The oil seeds *B. juncea* (Indian mustard - Rai) and yellow sarson (*B. rapa* cv Benoy) and leafy vegetable *B. juncea* subsp. *rugosa* (Rayo) are highly susceptible in the Darjeeling hills. Further south substantial clubroot development has been recorded on Rayo and in cabbage and cauliflower crops whereas Rai has not been infected. Mapping studies are in progress to establish the extent of variations in physiological races of *P. brassicae* using the European Clubroot Differential Series (ECD). Knowledge of the distribution of virulence will permit more effective deployment of tolerance amongst current oil seed and vegetable *Brassica* types. The effectiveness of crop rotations involving non - hosts such as pea and potato in diminishing disease pressure is also being investigated. Results from both areas of research will be reported

**Analysis of diversity in Nordic Swedes.**

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Swede (*Brassica napus* var. *napobrassica* (L.) Rchb.) is a fairly young cultivar. Sweden and other Nordic countries are regarded as secondary centre of evolution. It serves as forage and feed for farm animals and particularly in the Nordic countries it formerly contributed to human diet. Swedes are thus a crop with particular interest for the nordic countries. The Nordic Gene Bank holds 104 accessions of swedes and 50 of these have been analysed by isoelectric focusing and staining for PGM- and GPI activities. The results are currently being analysed.

**Studies on Ethiopian mustard (*Brassica carinata* Braun): germplasm variability and genetics of quality and agronomic traits**

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Ethiopian mustard (*Brassica carinata* Braun) although is agronomically robust, has still several shortcomings: high erucic acid, high glucosinolates, low oil and low protein contents. Several inbred lines with contrasting levels of the respective traits were developed from a germplasm and intercrossed. The F<sub>1</sub>'s were field tested and lab-determinations were made in Goettingen. The material has generally exhibited a tremendous amount of variation; oleic acid varied from 5 to 34% and erucic acid from 6 to 51%. The high-oleic genotypes exhibited not only low erucic but also higher linoleic (25%) and considerably lower (8%) linolenic acids. The F<sub>2</sub> seeds of the cross with the lowest erucic acid which was a cross between two low-erucic lines segregated into three distinct classes of low- (6-12%), intermediate-low and/or high- (18-32%) and high- (36-42%) erucic acid with a statistically acceptable digenic segregation ratio of 1:14:1. The mean dominance ratios of gsl, oil and protein were found below unity indicating a clear-cut case of partial dominance of the genes involved.



**Introgression of *B.napus* genome into restorer lines for CMS *ogura* estimated with the use of molecular markers**

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The object of research were double low restorer lines of CMS *ogura*. These lines were derived as a result of crosses between double low CMS *ogura* lines and starting restorer line with glucosinolate content of about 60  $\mu\text{M/g}$  of seeds. Low glucosinolate recombinants selected in F<sub>3</sub> and F<sub>4</sub> generations were examined for presence or absence of isozyme PGI-2 marker. All recombinants which had lost the PGI-2 alleles were characterized by low glucosinolate content in range 4.8 - 18.8  $\mu\text{M/g}$  of seeds. These recombinants were investigated also with RAPD markers linked with restorer gene to determine the radish genome introgression in oilseed rape.

Poster  
**Genetic improvement**

**P**

## **New varieties in Swede and Cabbage based on microspore derived lines**

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Instead of producing homozygous lines in *Brassica* by self-pollination, it can be done by microspore culture. Microspore culture represents a time-saving method and it works very well in old Norwegian cabbage varieties and swede. Microspore culture has made it possible to carry out field experiments with F1-hybrids 2-3 years after the breeding programme started. In Swede it is not possible to produce F1-hybrids based on self-incompatibility, but because swede is self-fertile, it is possible to produce new varieties that are pure lines, and microspore culture seems to be an important tool for the production of pure lines

## **Clubroot**

### **Current Status of Clubroot Detection in Australia**

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Clubroot, caused by *Plasmodiophora brassicae*, is the most serious disease of vegetable Brassica crops in Australia. A range of control strategies (prevention to eradication) have been developed which vary in efficacy and. Optimal use of these strategies will only occur when growers can accurately predict inoculum levels and use this information to select an appropriate (i.e. cost effective) management approach. Current methods are reliant on bioassays that are time- and labour-intensive and often require successive plant generations to detect low-levels of inoculum. Collaborative research between Agriculture Victoria Knoxfield and RMIT University is: 1) developing a rapid and reliable method to detect and quantify *P. brassicae* in soil, water and plant tissue. 2) developing a rapid alternative to ECD screening of *P. brassicae* isolate collections. A PCR protocol has been used successfully to detect clubroot in naturally infested soil, water and plant material (Faggian *et al.*, 1999). With this tool, clubroot outbreaks in Brassica nurseries were traced to a number of sources, including: Irrigation and bore water, Dust, Seedling trays. However, detection was difficult in some soils (i.e. poor sensitivity). To investigate possible reasons, over thirty commonly used agricultural chemicals (pesticides, fumigants, fertilisers and wetting agents) were added to PCR tests and were shown to have no effect on the sensitivity. Experiments are continuing to identify and remove inhibitors from soil DNA samples. Early experiments indicate the TaqMan® system for quantitative PCR may be a useful tool for estimating clubroot inoculum levels. Such an assay would form the basis of a predictive-farming system to reduce chemical use and increase grower profits. Rapid alternatives to ECD pathotyping of *P. brassicae* are being investigated through the use of such techniques as microsatellite-primed PCR (MP-PCR). MP-PCR uses primers directed at microsatellites to amplify reproducible profiles. Thus far, one microsatellite primer has given a similar level of discrimination of pathotypes to the ECD series. MP-analysis may therefore be a viable alternative to ECD's for identifying different pathotypes for resistance screening trials.

**Multiplexing Sequence Tagged Site  
Microsatellites (STSMs) for Distinctness,  
Uniformity and Stability Testing in Oilseed and  
Forage Rape (*Brassica napus*)**

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Before being marketed within the EU, newly bred varieties of crop plants must go through statutory testing to determine their eligibility for inclusion in the National List of Varieties. Part of this testing consists in showing that new varieties are Distinct from others known to exist and that they are Uniform and Stable in the characteristics used to distinguish them. These characters identify varieties uniquely and allow plant breeders to protect their intellectual property rights through Plant Breeders' Rights schemes. Currently, phenotypic characters are used in DUS testing. However, the potential of different DNA profiling techniques as a tool for DUS testing is being investigated and is proving useful for variety identification. Sequence tagged sites microsatellites have shown technically simpler and more robust than other DNA profiling methods. Nevertheless, for the wider application and acceptability of microsatellites as the genotyping marker of choice in DUS testing, it is essential to develop high throughput marker kits that can be used successfully in different laboratories irrespectively of the technologies used. The objective of this project was to develop and use multiplex STSMs sets for maximising the efficiency of variety testing. STSMs sets used for multiplexing were developed in a previous BBSRC project. Pooled DNA from leaves of 10 varieties of oilseed and forage rape was amplified by the PCR. Amplification products were separated by gel electrophoresis on the ABI Prism 377 Sequencer. The alleles present in each variety at 15 loci were recorded. The potential of the 3 multiplex primer sets and single primer sets to test distinctness between varieties was assessed by cluster analysis and expressed using separation coefficient rates (s). All varieties could be uniquely identified using STSMs data from single multiplex sets (multiplex set 1 and 3). Multiplex set 2 could identify 80% of the varieties uniquely. Separation

coefficients of single STSMs primers varied from 0 to 80%. Cluster analysis produced from 3 multiplex STSMs data brought together spring oilseed rape types in one group, winter oilseed rape types in another group and the forage rape variety in a third group. Results from the cluster analysis were confirmed by PCO analysis. This gives a certain level of confidence that results from STSMs data reflect actual, genetically based relationships between varieties. Uniformity within some varieties was assessed using multiplex STSMs sets by analysing DNA from individual plants. Preliminary data from the uniformity study showed that some varieties were non-uniform at specific loci, the level of polymorphism detected depending on the variety examined.

**Genetic improvement**

**Analysis of diversity in Nordic Swedes**

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Swede (*Brassica napus* var. *napobrassica* (L.) Rchb.) is a fairly young cultivar. Sweden and other Nordic countries are regarded as secondary centre of evolution. It serves as forage and feed for farm animals and particularly in the Nordic countries it formerly contributed to human diet. Swedes are thus a crop with particular interest for the nordic countries. The Nordic Gene Bank holds 104 accessions of swedes and 50 of these have been analysed by isoelectric focusing and staining for PGM- and GPI activities. The results are currently being analysed.

Poster

**p108**

**Genetic improvement**

**Studies on Ethiopian mustard (*Brassica carinata* Braun):  
germplasm variability and genetics of quality and agronomic  
traits**

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Ethiopian mustard (*Brassica carinata* Braun) although is agronomically robust, has still several shortcomings: high erucic acid, high glucosinolates, low oil and low protein contents. Several inbred lines with contrasting levels of the respective traits were developed from a germplasm and intercrossed. The F<sub>1</sub>'s were field tested and lab-determinations were made in Goettingen. The material has generally exhibited a tremendous amount of variation; oleic acid varied from 5 to 34% and erucic acid from 6 to 51%. The high-oleic genotypes exhibited not only low erucic but also higher linoleic (25%) and considerably lower (8%) linolenic acids. The F<sub>2</sub> seeds of the cross with the lowest erucic acid which was a cross between two low-erucic lines segregated into three distinct classes of low- (6-12%), intermediate-low and/or high- (18-32%) and high- (36-42%) erucic acid with a statistically acceptable digenic segregation ratio of 1:14:1. The mean dominance ratios of gsl, oil and protein were found below unity indicating a clear-cut case of partial dominance of the genes involved.

Poster

**p109**

**Genetic improvement**

**Introgression of *B.napus* genome into restorer lines for CMS  
*ogura* estimated with the use of molecular markers**

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The object of research were double low restorer lines of CMS *ogura*. These lines were derived as a result of crosses between double low CMS *ogura* lines and starting restorer line with glucosinolate content of about 60  $\mu\text{M/g}$  of seeds. Low glucosinolate recombinants selected in F<sub>3</sub> and F<sub>4</sub> generations were examined for presence or absence of isozyme PGI-2 marker. All recombinants which had lost the PGI-2 alleles were characterized by low glucosinolate content in range 4.8 - 18.8  $\mu\text{M/g}$  of seeds. These recombinants were investigated also with RAPD markers linked with restorer gene to determine the radish genome introgression in oilseed rape.



Poster

**p110**

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**Clubroot**

**xc06**

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