

GenRes Carrot Newsletter No. 3

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Project News and Information

The third annual project meeting was held in Thessaloniki, Greece. The weather was variable with some nice bright sunshine generally when we were indoors, but we also had very high winds and heavy rain, which made the northern European partners feel very much at home. We toured the facilities of the Greek Gene Bank and took the opportunity to look at the wild *Daucus* taxa collected by Stelios Samaras in collaboration with Phil Simon, University of Wisconsin, Madison. The group discussed whether we could extend the availability of wild *Daucus* taxa by including collecting within the current GENRES project. The answer to this question will depend on the availability of funds within our existing programme budget.

It is encouraging to see that the project is providing the basis for additional work by partner institutes, particularly for PhD projects with examples of taxonomic studies in Angers and analytical characterisation in Bologna.

The 2 German partners are both involved in the movement of the genetic resources collections from the Federal Centre for Breeding Research on Cultivated Plants (BAfZ), Braunschweig to the Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben.

This is a major task and we wish them all success in this very critical exercise.

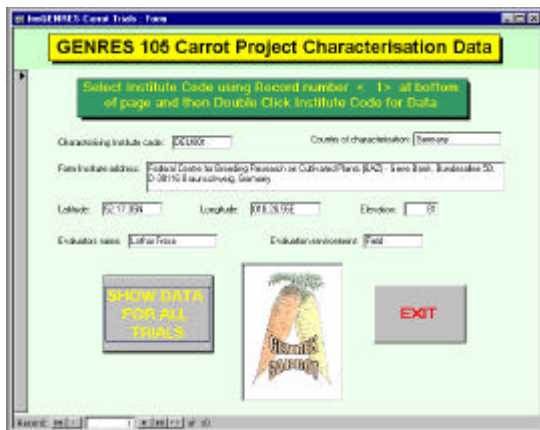
The INH Angers group was involved in the very interesting analysis of molecular diversity in cultivated and wild carrot as published in the Journal of the American Society of Horticultural Science [127(3) 383-391]. The paper concluded that the diversity in the *Daucus*/carrot gene pool is extensive, but is a genetically non-structured composition. The study failed to find clear alliances or subgroups within 124 accessions representative of wide provenance and morphotype, other than the distinction between the wild and cultivated groups. These conclusions are extremely interesting and need to be considered very closely from a carrot/*Daucus* genetic resources perspective.

News from HRI Genetic Resources Unit

The project partners have collected their characterisation data in the format agreed at the 1st project meeting in Angers. These data have been forwarded to HRIGRU either as Excel files or Access tables. Over recent months we imported the data into an Access database transferring the partner data sets into individual database tables. The objective in the final year of the project is to make these data available on the Internet accessible via the project web pages. We have designed a simple Access form allowing a user to select all the data in the characterisation database or to view the data of individual partners.

A draft version of the form was distributed to partners and demonstrated during the project meeting in Thessaloniki resulting in some extremely valuable suggestions on

improvements to make the form more "user-friendly".



The data will be made available on the Internet in a read-only format. The addition of new data from the 3rd and 4th year's partner characterisation trials will be simple to add to the partner specific tables in the database.

We have "advertised" the activities of the project by exhibiting a poster at various technology transfer meetings for growers and at meetings at HRI Wellesbourne.



The GENRES project European Carrot & *Daucus* Inventory is available on the project web site located on the HRI Server at the URL below the title.

News from the Federal Centre for Breeding Research on Cultivated Plants (BAfZ), Quedlinburg

The 2002 programme comprised the evaluation of 100 accessions in a parallel laboratory test and semi-field test analogous to the evaluation in 2001. Seeds of carrot accessions were ordered from the partners of HRI, NGB, BAZ-GB and GGB. 25 accessions obtained from the GGB were propagated this year, because too small seed probes were obtained. An evaluation of the GGB material is planned for next year. In 2002 we tested 60 accessions from HRI and each 20 from the NGB and BAZ-GB. Between January and May 2002 both inocula I 89001 and I 189 (see newsletter No.2) were propagated under in vitro conditions. Approximately 6.5 litre of the inocula I 89001 and 11.6 litre I 189 were harvested. The aggressiveness of both inocula were controlled within the cotyledon test analogous 2001.

Laboratory test

Three test series out of four are finished. Generally a large variation of the resistance reaction was observed between and within the accessions. Plants without disease symptoms could not be found but a large number of plants expressed weak *Alternaria* symptoms, only. Four accessions were classified as 'resistant', 57 accessions were classified as 'tolerant' and 44 accessions as 'moderate tolerant' so far (Fig.1). The laboratory test will be completely finished by end of December.

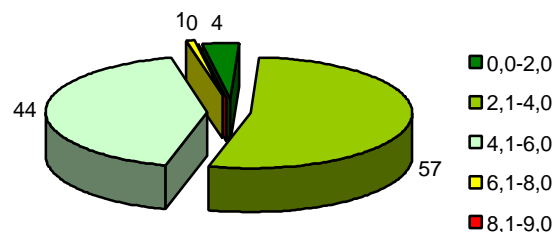


Fig. 1: Evaluation of the carrot accessions with the laboratory test (based on three test series)

Semi-field test

The semi-field test was exactly performed under a plastic tunnel as described in 2001. Generally the accessions showed a large variation of resistance reaction as in the laboratory tests, but the expression of the *Alternaria* symptoms were much stronger in the semi-field test. Only a few individual plants without symptoms indicating 'resistance' could be found. The expression of the *Alternaria* symptoms was generally stronger in comparison to the semi-field test in 2001. Some accessions were completely destroyed by *Alternaria* and were susceptible to secondary infection by *Erwinia* root rot. No accession was classified as 'resistant' or 'tolerant', only 19 accessions were 'moderately tolerant', 83 accessions were scored as susceptible and 4 as highly susceptible (Fig.2).

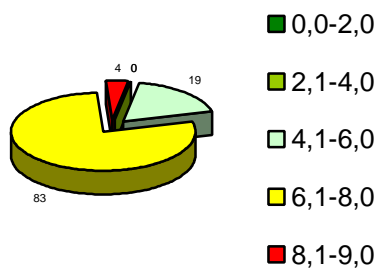


Fig.2: Evaluation of the carrot accessions in the semi-field test 2002

More detailed results of the *Alternaria* evaluation 2002 will be prepared for the third technical report.

We are now preparing a new test cycle. The thirty best accessions from years 2001 and 2002 will be tested together with the thirty best accessions from the Mathilde Briard in Angers.

News from the Federal Centre for Breeding Research on Cultivated Plants - Gene Bank, Braunschweig

Characterisation

As in the previous years 25 accessions were sown together with the 3 standard varieties in the field. In summer very heavy rain showers flooded the experimental fields. Compared to non-root crops the carrots survived well and they were harvested on 10th September 2002. The percentage of root splitting (8.6 – 45.8%)

was determined. 2 x 15 non-split roots were used to measure root weight (71 – 149 g.), length (5.1 – 17.8 cm) and diameter (28 – 45mm). Shape and colour traits were determined using longitudinal sections of 2 x 10 roots. In total, 17 characters have been counted, measured or scored. Standardised photos were taken of the leaves, entire roots and the longitudinal root sections. Only little variation was detected with respect to colour characters, while there was more variation in shape and yield traits.

Amongst the material tested the old variety "Bauers Kieler Rote" (DAU 069 from the IPK genebank) differed with respect to the colour intensity and variation pattern (Fig. 3) from the rest. This variety has been selected by W. Bauer around 1954 and was grown for baby food. (http://www.luhn-gmbh.de/ueber_uns/chronik.htm)



Fig. 3: Bauers Kieler Rote

Seed multiplication

Accessions were cultivated for seed increase in small isolation greenhouses, as well as under isolation tents. The seed yield of accessions grown under tents ranged between 37 and 272g. This year a wild carrot accession collected in the region of Ardabil, North Iran was grown for the first time for seed increase. The plant type is similar to "*Daucus grandiflorus*" as shown in the image in the rare book collection of Missouri Botanic Garden at the following URL (<http://ridgwaydb.mobot.org/mobot/rarebooks/page.asp?relation=QK387D4T1&identifier=0597>). This taxon is not mentioned in the "Federal Information System Genetic Resources" system (<http://www.bigflora.de/>) in either the species or the synonym list. Some of the plant traits are shown below.



Fig. 4: Traits of a *Daucus* accession collected in the region of Ardabil (Iran)

News from the Scottish Agricultural Science Agency, Edinburgh

Characterisation

Characterisation of cultivars for DUS tests follow UPOV guidelines defining internationally agreed characteristics and test methods. For all registered or protected varieties official descriptions are compiled from the considerable morphological data collected during the tests. The *Umbellifer* crops tested are Carrot, Parsnip, Celery and Parsley.

Characterisation of wild or landrace material is useful to gain a better understanding of both the range of character expression in species and the genotype/phenotype relationship in cultivars. For characterisation of both cultivated and wild/landrace material in this project we have followed the IPGRI guidelines.

During years two and three of the GENRES project, SASA completed its characterisation commitments by scoring 157 accessions, comprising 127 cultivated types and 30 wild types or landraces. Data recorded in 2000 was presented in the 2001 report and data for 2001 will be included in the 2002 report. All root samples were split into their different phenotypes and photographed. These

photographs have been digitised and will be made available to the project in 2003.

Development of Imaging Systems for characterisation of carrot and parsnip

The development of two digital imaging systems, IMAGIN and VISOR, to aid characterisation of both cultivated and wild material has been jointly undertaken by Biomathematics and Statistics Scotland (BioSS) and SASA.

IMAGIN

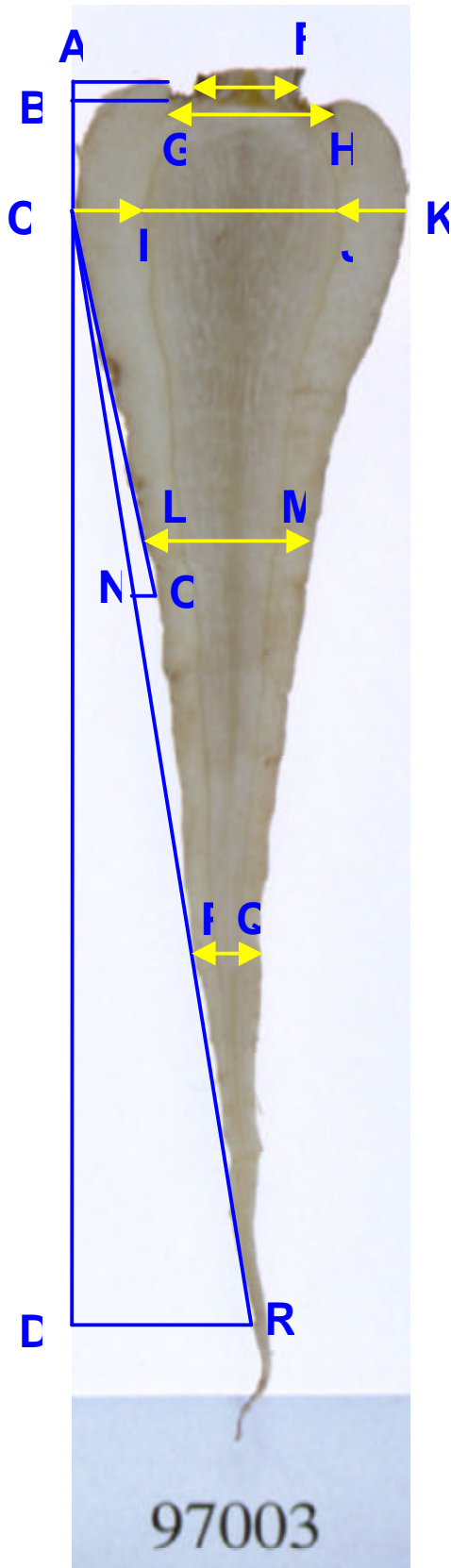
The objective of this project is to automate the measurement of morphological characteristics from digital images, the results of which can be used to discriminate material submitted for DUS tests (Green *et al* 2001). The measurements are also used to describe population samples. The main input has been targeted on Pea, but recent unpublished work has been undertaken on Parsnip (see image for characters measured).

Green FN, Horgan GW, Mann AD, McCarthy L; and Roberts, AMI. 2001. The Development of an automated image measurement system to discriminate among cultivars of Peas (*Pisum sativum* L.) submitted for Distinctness Uniformity and Stability tests. *Scottish Agricultural Science Agency. Science Review 1997-2000.* ISBN 0 7559 0287 4.

VISOR

The objective of the VISOR project was to demonstrate how composite aspects of a whole image, including shape and colour patterns, can be used to discriminate between varieties and to match them with most similar material in an image library. (Davey *et al* 1997 and Horgan *et al* 2001).

Carrot cultivars, representing a wide diversity of types, were photographed and the success of matching field material with a library of images, was assessed. Various statistical tools have been used (Horgan 2001), including those which compare root shape by standardising size, and the comparison of average root images which are derived from the photographed population sample.



Davey JC, Horgan GW, Talbot M. 1997. Image analysis: A tool for assessing plant uniformity and cultivar matching. *Journal of Applied Genetics* **38A**: 120-135.

Horgan GW, Talbot M. & Davey JC. 2001 Use of statistical image analysis to discriminate carrot cultivars. *Computers and Electronics in Agriculture* **31**, 191-199.

Horgan GW. 2001. The statistical analysis of plant part appearance – a review. *Computers and Electronics in Agriculture*. **31**: 169-190.

News from Institut National d'Horticulture, Angers

Morphological characterisation

51 accessions (Guérande, Touchon, St Valéry and Nantaise à forcer) were sown in two replicates of 4 metres (100 seeds/metre) in the INH field. Material was sown on the 26th June 2002. Foliage characters were recorded on the 3rd October, while harvest and root characterisation was on the 23rd October. Climatic conditions were rather mild and the growing was normal. Characterisations were realised in good conditions. The minimum characterisation descriptors defined by all partners were recorded. Among all the accessions observed; single typical root types of St Valéry and Guérande were found. In contrast, no typical Touchon was identified. The Nantaise à forcer studied grown in 2002 were not typical of the variety, but other accessions previously characterised were typical.

Molecular characterisation of the Jaune du Doubs trial

On several occasions, molecular markers have proved their efficiency for the identification of redundant accessions. Partners of the GENRES Carrot project decided to evaluate the opportunity to use molecular markers as a complementary tool to morphological markers for the identification of carrot duplicates accessions. As a model of analysis, 25 presumed duplicate accessions of « Jaune du Doubs » were obtained. Only accessions that were not distinguished on a morphological basis were submitted to molecular analysis. Whatever the strategy employed, the crucial question was to

These two projects are related in that images from the IMAGIN project could be analysed to derive average images which could then be used to select the most similar material.

determine the threshold to declare whether two accessions were duplicates or not. For this purpose, we used a strategy based on the comparison between intravarietal and intervariatal genetic distances. In order to save time and limit costs, DNA extractions were realised on 4 to 8 bulks of 5 individuals per accession and bulks were analysed with 75 AFLP markers. An additional set of 7 bulks was extracted from one accession to provide true replicates. With the exception of the true duplicates, all the accessions were clearly identifiable. Based on these results, we have proposed a general strategy for the identification of carrot duplicates in a paper submitted for publication.

Alternaria dauci trial 2002

The trial was performed as previously described in GENRES Newsletters 1 & 2. Cultivars Bolero and Presto were used as positive and negative controls. An accession of *Daucus halophilus* used in BAZ-ZG, Quedlingburg was also introduced in the trial. The weather conditions were very favourable for symptom development. The mean disease scores were rather high in comparison with previous years. The disease range among accessions varied from 5 to 9 (maximum index) at the final scoring date (02/11/02).

Revision of *Daucus* Taxonomy combining both Morphological and Molecular studies

INH is responsible for the establishment and the maintenance of the French *Daucus* seed bank. Within the framework of this programme, a new taxonomic study was initiated in 2002 in order to avoid misidentification in *Daucus* collections. Some *Daucus* species are easily recognisable due to their specific characters, such as *Daucus muricatus*, with particularly large seeds in comparison to other species (see below).



Other species are far more difficult to differentiate, this being particularly true within the 'carota complex'. According to Small (1978), the lack of notable development of breeding barriers between species is probably responsible for the absence of easily recognisable groups in the complex.

During the past 20 years, the comparison of the primary nucleotide sequence of homologous genes has been of great help in the elucidation of the taxonomy of various genera (Soltis *et al.* 1998). Therefore, in the present study both morphological and molecular approaches will be combined. From classifications in the literature (Saenz Lain 1981 & Cullen 1972), 37 taxa were identified for study. The GENRES European seed banks were then contacted in order to acquire germplasm, resulting in 24 taxa being obtained (1 to 16 accessions per taxon). Two replicates of 50 seeds for each accession were grown in the INH field. Morphological and molecular analyses are in progress. A single specimen was used for scans, photos, herbarium specimen and DNA extraction. Molecular analyses will investigate ribosome gene amplification and nucleotide sequence analysis. If ITS (Internal Transcribed Spacers) fail to be informative, other sequences such as IGS (Intergenic spacers) and part of 26S and 18S will be studied. All results will be computerised in a database.

Do you have any *Daucus* taxa that we want to include in our work? We would be very grateful to receive a few seeds of the material listed in Table 1.

Please contact Dominique Dumet at: Dominique.Dumet@inh.fr.

This taxonomy work is supported financially by the 'Ministère de la Jeunesse, de l'Education et de la Recherche' and the 'Bureau des Ressources Génétiques'.

Table 1: Taxa INH want to include in a complete revision of *Daucus* taxonomy

Daucus aureus Desf.
Daucus bicolor Sm.
Daucus capillifolius Gilli
Daucus carota ssp *major* (Visc.) Arc.
Daucus carota ssp *parviflorus* (Desf.) Thell.
Daucus carota ssp *ruseus* (Onno) Heywood & Okeke
Daucus glaber (Forsk.) Thell.
Daucus glaberrimus Desf.
Daucus glochidiatus (Labill.) Fisher & al.
Daucus involucratus (Sm.)
Daucus jordanicus Post
Daucus montanus Humb. & Bonpl. Ex Schult.
Daucus montevidensis Link ex Sprengel
Daucus sahariensis Murb.
Daucus setifolius Desf.
Daucus syrticus Murb.
Daucus tenuisectus Coss.

News from the Greek Gene Bank

Following the year 3 tasks and milestones, 30 accessions of wild *Daucus*, collected in 1999, were sown in 2002 in order to be characterised and regenerated. This material included, 14 accessions of *Daucus carota*, 1 accession of *D. carota* sp *maximus*, 6 accessions of *D. bicolor* and 9 accessions of *D. muricatus*. Besides these, 3 other accessions of Greek land races and the 3 control accessions were sown.

Following last year's bad experience of sowing the wild *Daucus* material directly in pots, all accessions were sown in a nursery and then transplanted into pots and in the field. The accessions were sown in the nursery on the 22nd and 23rd January 2002 and transplanted into pots in April and into the field in May. All of the accessions sown germinated with the exception of 3 *D. bicolor* accessions.

Seed was produced from all of the successful accessions and has been stored in the GGB active collection under the following storage conditions - 2-4^o C and controlled humidity at 30%. In addition, seed was harvested from the last year's wild *Daucus* accessions that had not seeded in 2001, but had been maintained over-winter. The seed

production ranged from 8 to 67g. per accession in *D. carota*, 1 to 88g. per accession in *D bicolor* and 0.5 to 20g. per accession in *D. muricatus*.

Seed was harvested from pots from 6-6-2002 to 17- 9-2002. *D. muricatus* was the earliest and *D. bicolor* the latest seeding species. The seed was also harvested from the field plots from 6-8-2002 to 17-9-2002. All accessions were characterized according the agreed IPGRI descriptor list for wild *Daucus* taxa and the results will be forwarded to the coordinator in January.



The Group studying the collection of wild *Daucus* taxa during the project meeting at the Greek Gene Bank
(photos: Thomas Nothnagel, BAZ)



The "official" Group photo

L to R: Nicos Stavropolis, Hesd of GGB, Stelios Samaras, George Campbell, Mathilde Briard, Filippo d'Antuono, Angela Pinnegar, Dave Astley, Andreas Boerner, Kerstin Olsson, Katarina Wedelsback-Bladh & Thomas Nothnagel

News from the Department of Agro-environmental science and technology, Bologna, Italy

The 2002 program consisted of: a) the characterisation of 66 accessions and their

evaluation for carotene, sugars and nitrate content; b) further analyses and data processing of the 2001 trials.

a) 2002 Characterisation and evaluation.

Accessions were received from INH, Angers (10), BAZ, Braunschweig (2), HRI, Wellesbourne (37), Nordic gene bank (10), two were local types of southern Italy. Parmex, Autumn King, Amsterdam, News F1 and Nikki F1 were added as control commercial varieties. The seeds were sown at Bellaria (RN) on a typical coastal sandy soil, on 2 May 2002. Unfortunately, heavy rains and waterlogging caused high mortality and very poor plant stand in many accessions. Roots were harvested on 6 August and samples were taken for characterisation and evaluation. A second sowing was made on 23 July 2002.

Digital photographs were taken during the characterisation of the roots from the first sowing. Chemical analyses are in course. Plants of the second sowing are being harvested at the moment of writing. As for the previous year, a wide range of variation was observed. External colour was orange (34 accessions), orange with few yellow or white roots (14), mixed orange/yellow (2), yellow (1), purple (2), white (1), with the other samples mixed at various degrees. Six accessions were completely flowered at harvest, whereas bolters were presented in high percentage in 10 other accessions.

The two accessions obtained from southern Italy were both orange rooted types, but they really look like rather primitive local populations (see figure).

Miss Manuela Drei graduated in Food Science and Technology on October 15, 2002, discussing a thesis entitled "Caratterizzazione analitica di germoplasma di carota" (Analytical characterisation of carrot germplasm), obtaining 110/110 mark, based on the work carried out within the RESGEN programme.

b) Further work on the 2001 accessions.

Also thanks to the commitment of a student working for her thesis, it has been possible to evaluate the 2001 accession

also for volatile oil content and composition. The volatile oil was extracted by static headspace sampling and determined by GC/MS. Quantification was done by calibration curves with pure standards. Essential oil content ranged from 0.025 to 3.21 mg g⁻¹ fresh weight. Caryophyllene was the more abundant component, with average content of 0.6 g g⁻¹ essential oil, and exceeding 0.5 g g⁻¹ in about 70% of the accessions. Accessions with very low percentage of this component were present as well. Terpinolene ranged second for its average content (0.17 g g⁻¹), and very high content in some accessions (up to 0.73 g g⁻¹). Many minor components were present with average amounts of 0.01-0.05 g g⁻¹, but were abundant in some accessions. As a whole, these analyses indicate interesting variation in essential oil content and composition, that may deserve future consideration.

Multivariate analysis on the analytical characters of the accessions evaluated in 2001 allowed a preliminary, rather artificial grouping of the accessions. A group mainly characterised by short, conical roots, also had high average carotene content and high terpinolene, among essential oil components. A group characterised by low carotene, had also average high nitrate content. Another group was differentiated by its high average bisabolene content.

A poster was presented at the 6th scientific meeting of the Italian Horticultural Science; citation: Moretti A., Neri R., D'Antuono L. F., 2002. Valutazione di pigmenti, zuccheri ed acidi organici in germoplasma di carota. In: Pandolfi S., Parlanti M.V., Libori F. (eds.), Atti VI giornate scientifiche SOI, 22-25 Aprile 2002, Spoleto., 443-444. (English title: Pigments, sugars and organic acid evaluation of carrot

News from the Nordic Gene Bank

At the Nordic Gene Bank we have characterised 26 accessions during 2002. The carrot accessions were sown on 21st May and then harvested at the end of October. The summer offered dry and hot

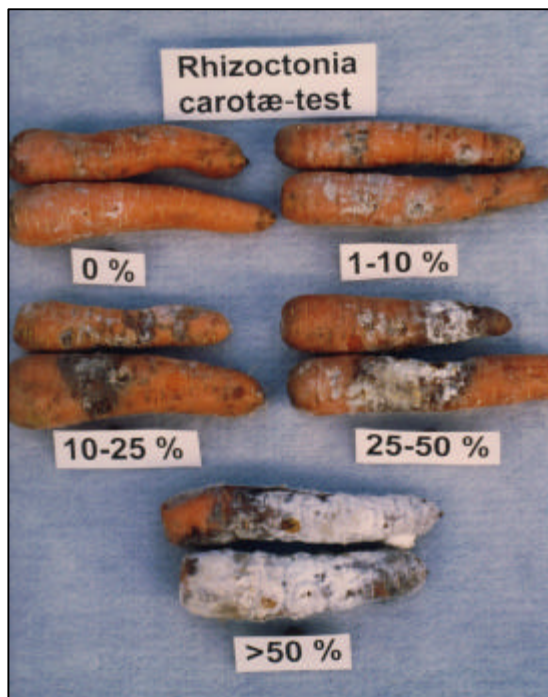
weather during a long period in July and August in the south of Sweden, where the material was grown.

All of the accessions included in the trial were Swedish and Danish varieties divided between following variety groups.

Variety group
Amsterdammer
Chantenay
Feonia
Flakkeer
Nantes
Pariser
Touchon

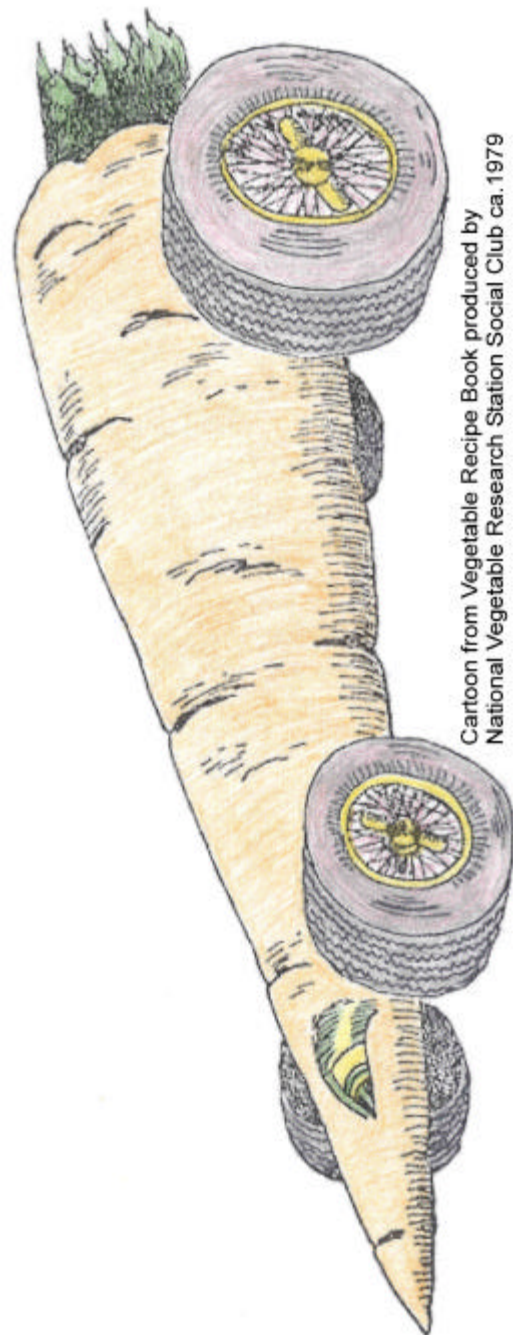
During the autumn, the material was evaluated for quality characters as alpha, beta and gamma carotene contents, as well as sugar and nitrate content. The results show considerable variation within the material for all the characters.

Last year we evaluated 37 accessions for the storage diseases, Sclerotinia rot, Crater rot and Liquorice root rot. The results showed an interesting range of variation within the tested material. We have continued the disease evaluation tests on the 26 accessions grown this year. The results for these trials will be ready in the beginning of January.



Note: it is interesting to see how carrots are represented in some publications, for instance the HRIGRU group found an old recipe book produced by the Sports & Social Club members of the NVRS at Wellesbourne. The current S & S Committee gave permission for us to reproduce the **Carrot Cartoon** below attached to recipes for Cream of Carrot Soup and Carrot Jam - we may print the recipes in our final newsletter!!!

Happy New Year



Edited by Dave Astley at HRIGRU funded by GenRes 105 project & the UK Department for Environment, Food & Rural Affairs