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CHARACTERISATION AND EVALUATION OF CARROT GERMPLASM

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TABLE OF CONTENTS

1. INTRODUCTION

1.1. General part on the aspects of gene banks and genetic resources held in gene bank

Biodiversity is foundation for our agricultural systems. It can be stated that loss of biodiversity presents serious threat to agriculture and livelihood of mankind. It becomes global imperative to conserve biodiversity and use it wisely in the future. Biodiversity is the main source for current crop improvement regarding yield, quality, resistance to pests and diseases and for adaptation to changing environmental conditions, like global warming. For lot of people it is also direct source of food. Without biodiversity our ecosystems, entire planet's biosphere, cannot function.

Biological diversity, as variation, is present in all plant species and it is expressed on three levels: genetic diversity (variations in genes and genotypes); species diversity (species richness); and ecosystem diversity (communities of the species and environment).

The main reason of great genetic erosion is the replacement of local varieties by high yielding commercial ones. Successful creation of new varieties and restriction of seed trade only to certified one, cause disappearance of landraces and old cultivars of most crops in Western Europe.

FAO estimated that 75% of genetic diversity in agricultural crops has been lost since 1900 (Mohamad and Zakri, 2001).

For centuries crop ancestors and other crop wild relatives have been used for crop improvement. Most of the crops modern varieties contain some genes which derived from wild relatives. The natural populations of many species of crop wild relatives are increasingly at risk primarily by habitat loss, degradation and fragmentation. Big climate change has significant impact on species distribution through suitable habitat reduction. It is likely that within fifty years, climate will cause many important species of crop wild relatives to be threatened with extinction. Genetic resources are non-renewable and it is essential to work on their conservation at all levels: species, genepool or ecosystem level. There is an urgent need to identify priority species and areas for conservation and to

develop strategies to ensure that the rich genetic diversity of crop wild relatives is protected for the benefit of future generations.

There are different approaches for biodiversity conservation for genetic resources protection. They can be divided in the following main groups:

- On farm management – present maintenance of crop species on the farm or in the garden. Big number of plant genetic resources, especially of minor crops, can be managed as a part of agricultural production system. It is so called "conservation through use". Main advantages of such conservation are:
    - Ongoing process of evolution and crop adaptation to the environment
    - It allows continued selection of superior material by farmers according to their needs and preferences
    - Helps in preservation of indigenous knowledge and promotes farmers' participation in conservation program
    - It creates necessary backup for genebank collection
    - Provides natural laboratories for agricultural research
- *In situ* conservation – (*in situ* = on site) is maintenance and wild plant populations usage in their natural habitats with their continuous evolution without human help. This conservation method benefit from existing interaction between different species and other environmental components (like pests and disease). *In situ* dynamic co-evolution is driven purely by environmental pressure and leads to greater diversity and better adapted germplasm.

  In situ conservation is mostly carried out through establishment of protected areas such as national parks and nature reserves. Biodiversity work in such areas should be focus on the maintenance of diversity and crop wild relatives

  Main advantages and disadvantages of in situ and ex situ conservation are listed in Table 1.(Tao, 2001b).

Table 1. Relative advantages and disadvantages of *ex situ* and *in situ* conservation

Conservation strategy	Advantage	Disadvantage
Ex situ	1. Greater diversity of target taxon can be conserved as seeds. 2. Easy access for evaluation for resistance to pests and diseases. 3. Easy access to plant breeding and other forms of utilization. 4. Little maintenance once material is in long-term conservation.	1. Freezes evolutionary development in relation to environmental changes. 2. Genetic diversity is potentially lost with each regeneration cycle.
In situ	1. Dynamic conservation in relation to environmental changes. 2. Permits species/pathogen 3. Applicable to many "recalcitrant" species. 4. Requires long-term active supervision and monitoring. Less genetic diversity can be conserved in any single location.	1. Materials not easily available for utilization. 2. Vulnerable to natural and maninteractions and co-evolution. directed disasters, e.g. fire,vandalism. 3. Appropriate management regimes poorly understood.

• *Ex situ* conservation – (*ex situ* = off site) takes place outside the natural habitat or production system in facilities specifically created for that purpose. Based on the species characteristics *ex situ* conservation can be realized in form of whole plant in field genebanks; seeds in seed genebank; certain other parts of the plant such as roots, dormant buds, pollen, explants, or DNA (Tao, 2001b). Its goal is not only to conserve biodiversity but also to enable breeders, farmers and researchers to use existing materials. *Ex situ* conservation may represent a last opportunity for many species and varieties that would disappear as their habitats are destroyed or replaced by modern varieties (Mohamad and Zakri, 2001).

Main methods of *ex situ* conservation are:

- Seed genebank is storing of orthodox seeds at low moisture content and at subzero temperature. Currently it's the most conventional and widely used method for biodiversity conservation. This method will be explained in details later in this work since it is good, relatively cheap and most spread way for conservation of seed material from *Umbelliferae* family.
- *In vitro* storage for conservation uses tissue culture. This method is used for:
  - Species with vegetative propagation
  - Species with recalcitrant seeds

        - Wild species which seed production is very low
    - DNA storage is one of the future technologies for conservation of plant genetic resources which need to be further proved and developed through research work.
    - Pollen storage by conservation of pollen grains in appropriate conditions is possible for some species, it enable their use for crossing with living plants. Pollen longevity for different species can be in range between few minutes up to years. Longevity depends on taxonomic status of the plant and on environmental conditions (Barnabas and Kovacs, 1997).
    - Field genebank presents materials collecting and planting in orchard or field of another location. Field genebank is used for perennial plants like: species with recalcitrant seed, with low seed production, species preferable stored as clonal material, species with long life cycle for generation of breeding material. They are used for conservation of species like: cocoa, rubber, coconut, coffee, banana, onion and garlic etc.
    - Botanical garden in general holds living collection and usually have other supportive facilities like seed banks and units for tissue culture. In many cases botanical gardens are focused on the conservation of wild, ornamental and endangered species.

- Complementary conservation – combination of different conservation actions, which together lead to an optimum sustainable use of genetic diversity existing in targeted genepool, in the present and future. Different conservation approaches which are already mentioned have their advantages and disadvantages. If they are used in complementary manner they provide the most effective system of conservation. Complementary conservation strategy depends from species that is conserved, local infrastructure and human resources, number of accession in certain collection, geographic site and potential use of conserved germplasm.

1.1.1. Seed viability

It is good to know classification of different seed storage behaviors before appropriate seed storage method is defined. First publication referring the seed storage behavior has

been published by Ssu-Hsien Chia in 353 AD in China, and it already recognized two methods of seed storage: cereals seed should "not to store in warm and damp environment"; while Chinese chestnut "the fresh seed should be packed in damp soil placed in the house, and during transportation the seeds should be packed in a leather bag, because chestnut seeds will die when exposed to the sun and wind".

Later in modern time, classification has been based on seed response during storage on moisture content and temperature (Roberts, 1973) in two groups: orthodox seeds, which can be dried to low moisture content of 2-5%; recalcitrant which can not survive desiccation below 12% of moisture. Later authors (Farrant et al., 1988) (Bonner, 1990) introduced one more class of the seeds behavior, intermediate one. Overall they suggested three groups of seed performance during storing: orthodox, recalcitrant and intermediate.

- **Orthodox seeds** can be dried without damage to low moisture content, and over a wide range of environments, their longevity increases with decreases in seed storage moisture content and temperature (Roberts, 1973). Some major characteristics of seeds with orthodox storage behavior and its influence on the seed viability will be explained further.

  Main focus is to identify species for which hermetic storage on -18^{o}C at low moisture content is feasible (FAO and IPGRI Genebank standards, 1994), species whose seeds can be stored successfully for long period under IPGRI preferred condition for long-term seed storage.

  Regarding cold storage of orthodox seeds, it can be damaged at subzero temperatures due to the ice crystallization of free water (Leopold and Vertucci, 1989), especially during cryopreservation.

  Ability of orthodox species to tolerate desiccation to low moisture content is closely related to the seed developmental stage and can be influenced by seed production environment. Through many studies is found that once orthodox seeds start with germination they begin to lose desiccation tolerance and became very sensitive to the moisture loss (McKersie & Stinson, 1980) (Koster & Leopold, 1988)

Fully hydrated orthodox seeds have tendency to germinate, but if this can be prevented by maintaining seeds in dormant condition, they can remain viable for many years (Villiers, 1974) (Villiers, 1975).

One of the main characteristic of orthodox seeds is possibility to use equation for seed viability prediction. Equation is the following (Cromatry *et al.* 1990):

$$V = Ki - P/10^{Ke - Cw.\log m - Ch.t - Cq.t.t}$$

V = final viability

Ki = initial seed quality

P = storage time in days

Ke, Cw, Ch and Cq = constants

m = seed moisture content at storage

t = storage temperature

Above mentioned equation demonstrated importance of initial quality of the seeds (Ki) which are included into genebank accession. Little difference in initial quality of seeds can drastically influence seed storage viability as it can be seen in Table 2. (Tao, 2001a). That is the factor which must be closely followed when accession enters into genebank collection, to ensure long time seed viability.

Table 2. Importance of initial quality on longevity of seeds

Initial quality (%)	Storage time (Years)	Lost storage time (%)
99	95	–
98	55	43
95	11	89

Note: Longevity of onion seeds with various initial quality was calculated by the seed longevity equation as given earlier as: MC = 7%, storage temperature = – 10°C, final viability = 85%, Ke = 6.975, %Cw = 3.47, Ch = 0.004 and Cq = 0.000428.

In the Appendix is a table which make correlation between fruit and seed characteristics with seed storage behavior. There can be seen that scizokarp as fruit of *Umbelliferae* family belong to the group of orthodox seeds.

- **Recalcitrant seeds** cannot be dried without damage, when dried it viability is first slightly reduced as moisture is lost, but then begins to decline considerably at a certain so called "critical moisture content" (King and Roberts, 1979) (King and Roberts, 1980). There is no suitable method to keep viability of recalcitrant

seeds over the long term. This is because they cannot be dried, neither they can be stored at subzero temperatures, because then they would be killed by freezing injury due to ice formation. In addition, some tropical recalcitrant seeds are also damaged by chilling injury at temperatures of 10-15^{o}C and below. The longevity of recalcitrant seeds is short from a few weeks to a few months (King and Roberts, 1979) (King and Roberts, 1980). Desiccation tolerance in recalcitrant seeds increases during seed development on the mother plant, although drying to low moisture content does not occur during maturation (Hong & Ellis, 1990).

- **Intermediate seed** is category between two major groups of orthodox and recalcitrant. This category contain a small number of species which can stand desiccation to 10-12% of moisture content, but prefer also warmer temperatures of 10-21^{o}C e.g. Arabic coffee (Hong & Ellis, 1992) for a long term conservation.

In general, and King and Roberts (1980) have tried to made association between plant ecology and seed storage behavior. By this hypothesis orthodox seeds originated from plants used to occasional or seasonal drought in which desiccation tolerance of seeds is crucial for it survival and ensures regeneration of the species. Recalcitrant species usually originated from moist or aquatic ecosystems in which seeds are exposed to high humidity during seed development and maturation. Recalcitrant species do not occur in the nature in arid habitats, desert and savannas.

Also general characteristic of recalcitrant and intermediate seeds storage behavior, which are adapted to tropical lowlands, tend to show chilling injury a 10-15^{o}C. At the same time seeds of species adapted to the high altitudes are able to tolerate exposure to the lower temperatures.

Having in mind that *Umbelliferae* family has seeds with orthodox behavior during storing, further will be explained in more details *ex situ* conservation in genebanks as way to save biodiversity of the species from this family.

1.1.2. Gene bank

Establishment of genebanks has been conventional solution for conservation of plant genetic resources, *ex situ* conservation. It is estimated that genebanks and other institutes in Europe maintain a total of over 2 million accessions, which is approximately one third of the global *ex situ* germplasm holdings.

FAO since early seventies has been working on the improvement of national capabilities in ex situ conservation of plant genetic resources. It included development of agreements and network activities with institutions which accepted primary responsibility for long term conservation of gremplasm of particular species in their base collection. Particular attention was on development of standards applicable to the wild, forest tree and crop species.

Genebank standards were focused on seeds with orthodox storage characteristic, species whose seeds are not injured with desiccation and which longevity is drastically improved by reducing seed storage moisture contend and temperature.

Genebank standards are important to set up targets for responsible institution on the national levels. Having in mind that resources in certain countries are limited, standards should enable curators in genebanks to reach pragmatic compromises in a way that even under not ideal operating conditions they should insure that collection have not be placed in jeopardy. The aim has been to store as many accessions as possible in the acceptable manner.

Considering all mentioned above, standard for genebank were specified on:

- Acceptable – in many cases minimal but still adequate (short term).
- Preferred – higher and safer standards.

Based on the research results of seed storage and archeological findings, which proved that certain species retain viability for several centuries, for acceptable standards were considered seed moisture content of around 5% and storage temperature of about +5^{o}C.

Genebank should hold different collections:

1. The base (core) collection which is set of distinct accessions, regarding genetic integrity, as close as possible to original sample and preserved for the long time in the future. Base collection can be dispersed among several institutions, especially with development of biodiversity networks. Seeds should not be distributed

directly to the users from the base collection. Acceptable condition for base collection seeds are temperature under zero and seed moisture of 3 - 7%, preferred temperature of -18^{O}C and moisture 3 - 7%.

2. Active collection contains accessions which are immediately available for multiplication and use; from this collection users get seeds for their needs. Since this kind of collection is accessed frequently, it can be hold even under medium term storage conditions. In fact active collection should be kept in condition which will ensure accession viability at least 65% for 10 to 20 years.

Seeds should be preserved under best possible condition before storage in order to maintain high level of viability of gremplasm for base and active collection. Before introduction to the collection seed should pass drying process to the acceptable level of moisture and should be clean (free of weed seeds, pests and disease).

1.1.3. Availability of accessions and information

Genebank should ensure that all accessions are available for potential use in crop improvement or for other studies. One of the major objectives of Plant Genetic Resources (PGR) conservation is to enable immediate or future use of genetic diversity. Since the future need are still unknown, it is important to conserve the widest range of genetic diversity (Hodgkin and Debouck, 1992). PGR program should promote and facilitate the use of conserved material through:

- Maintenance of healthy and easy accessible material which is adequately characterized and evaluated.
- Proper documentation with relevant information inside.

Future potential involvement of new germplasm in the improvement of crops breeding material can be demonstrated by: a) razing the genetic ceiling b) decreasing vulnerability to biotic and abiotic stress c) new developmental pathways and ecological adaptations (Kannenberg and Falk, 1995).

It is also important that material that gets out from the genebank is followed by proper documentation and necessary information. In the time of increased international

exchange of material, it is essential to establish a certain amount of uniformity in accession data collection.

1.1.4. Characterization and evaluation

Taxonomic status and evolutionary relationship between and inside species can be partially explained through collection and conservation of genetic material. Other important role of genebank accessions is that they have been used for the improvement of existing crops varieties. For successful usage of collected material it is important to know all the desirable characteristics available in germplasm accessions. That is possible only through systematic evaluation of present germplasm (Rao, 1980). Characterization and evaluation have two major functions:

- Characteristics that are recorded for certain accessions can be used as descriptors for accession. Those accession diagnostic characteristic can be used later to check material genetic integrity over a number of years of conservation.
- Characterization and evaluation enable results recording for number of agronomic characteristics which will be useful to the users in order to identify accessions with desirable traits needed for crop improvement.

Considering that most of traits which are described during characterization are morphological, this part should be done by person that manages the germplasm material. On the other hand evaluation should be done by users, if possible by multidisciplinary teams with breeders, agronomist and pathologists included. Efficient techniques designed to differentiate among accessions would determine potential value of germplasm. As final users of the product, farmers should be included at some stage of evaluation process.

Goal of characterization and evaluation is basically to describe an accession with its various attributes – morphological, physiological, agronomic, biochemical, cytological and reaction to various stresses (biotic/abiotic). They also should help to curator in identifying of accessions with desirable genes or genotypes and, in general, they provide information about the diversity of the available collection. The task of describing or using germplasm is relatively easier if it can be described in terms of genes and alleles than in terms of phenotypic expressions, but this is rarely possible. Because of their value in crop improvement, evaluation descriptors, although contributing to some extent to

identifications of an accession, are more interesting than characterization descriptors. In general, effective evaluation is possible when there is close institutional and personal interaction between curators from one side and breeders or other crop improvement scientists on the other side. It is also very important that potential breeding objectives are reflected in evaluation programs (Riley *et al.* 1996a; 1996b).

1.2. Present networks on vegetable genetic resources evaluation

Collaboration has been present traditionally among the entities of community responsible for genetic resources in Europe. That was on line with principles outlined in Food and Agriculture Organization of the United Nations (FAO) agreement named International Undertaking on Plant Genetic Resources for Food and Agriculture (PGRFA) in 1983, which now turned into Treaty (Europe report, 2000).

European Coordination Program on Crop Genetic Resources (ECP/GR) was established in 1980 on the basis of the recommendations of FAO, United Nations Development Program (UNDP) and as such is one of the oldest programs of genetic resources networking. One of its initial objectives was to create system which will promote direct contact between national institutions and programs related to genetic resources preservation. Nowadays ECP/GR goal is promotion of joint activities, among the members, in field of germplasm documentation, collecting expeditions, characterization and evaluation of germplasm and in encouraging exchange of up to date information on existing material (European network report).

Most significant achievement of ECP/GR is development of European central crop databases for 57 species, groups of species or genera of crop plants. Those databases have grown steadily through the different phases of the program to become comprehensive inventories of germplasm which is held in genebanks.

Currently main objectives of ECP/GR are the following:

- Facilitate the long term *in situ* and *ex situ* conservation of plant genetic resources.
- Assist in the increased utilization of plant genetic resources in Europe.
- Encourage cooperation between all stakeholders, including Non Governmental Organizations and private breeders.

- Strengthen cooperation between all plant genetic resources programs in Europe and work on the integration of non member countries.
- Support planning of joint programs with development of cooperative project proposals which would be submitted to the agencies.
- Encourage the shearing of conservation responsibilities for Plant Genetic Resources for Food and Agriculture (PGRFA) in Europe
- Increase awareness of the importance of PGRFA activities including conservation and sustainable use.
- Seek for collaboration with other relevant regional and global initiatives/institutions.

In last several years goal of ECP/GR to establish and standardize data basis for number of species was shifting toward work on characterization and evaluation of existing accessions and improvement in the distribution of related documentation. As a result of that ECP/GR focus shifting, main priorities for the coming period are:

1. Characterization and evaluation of germplasm with use of new technologies
2. Task sharing
3. In situ and on farm conservation
4. Documentation and information

All mentioned actions are coordinated for 38 member countries through system of nine networks. Each network can carry out main programs in the framework of twelve existing network groups or ad hock meeting. Current ECP/GR networks are for: Cereals; Forages; Fruits; Oil and Protein Crops; Sugar, Starch and Fibre Crops; Vegetables; Documentation and Information; *In situ* / On farm Conservation and inter Regional Cooperation. Networks were build up from number of Working Groups that work on particular species or group of species. Each Working group contains member countries.

Vegetables network, the network of our further interest, was established in 1999. In year 2000 ECP/GR vegetables network starts with extension of collaboration activities for wider range of crops. The scope of interest for the new network was on: *Solanaceae*

(tomato, pepper and eggplant); *Cucurbitaceae* and Leafy Vegetables (lettuce, spinach and chicory) and establishment of mentioned groups were realized in the following three years. Later on, new working groups were established to reach the current number of the following six: *Allium*, Brassica, Cucurbitis, Leafy Vegetables, *Solanaceae* and Umbellifer Crops.

Main achievement of Vegetable network in recent years is progress on ECP/GR crop databases, and more available data related to the crops characterization and evaluation (http://www.ecpgr.cgiar.org/Networks/Vegetables/vegetables.htm) (accessed 15th March 2008).

Main focus of the network in the near future will be on:

- Tracing duplicates – goal is to define methodology among working groups in order to be able to trace duplicate accessions in European collections, based on analysis of passport data. It should help to establish procedure to identify priorities among holders of duplicates for there further regeneration and conservation.
- Safety – duplication – ensure that existing accessions could not be lost in the future throughout:
    - Identification of "black box" storage offers.
    - Identification absence of safety – duplicate arrangements among working group members.
    - Encouraging each member to improve level of safety – duplication.
- Wild species management – to support the study of wild genepool taxonomy in order to clarify relationship between genomes of wild relatives and cultivated species, having on mind that this finding should be used for breeding and GR managing.

Main activities of Working Groups that are members of Vegetable Network would be briefly presented.

Allium Working Group was one of the original six Working Groups developed during the initial phase of ECP/GR and it was established in 1982.

Major Group achievement was creation of European Allium database (EADB) which is available on the internet. Database contains four passport fields and FAO/IPGRI multi crop passport descriptors and it is maintained by the Horticulture Research International, Genetic Resources Unit (HRIGRU) in Wellesbourne, UK. This database holds information for 8426 accessions of five major Allium crops and wild taxa from 17 institutions (13 countries) and the Nordic Gene Bank. Group also developed and published in 2001 characterization descriptors for Allium crops which are used by genebanks of member countries.

In the coming period Allium group would focus on:

- Duplication in collections – it is important to identify duplicates in collections of genebanks of member countries and to reduce number of accessions that improves cost efficiency of those institutions.
- Safety duplication – as a way of shared responsibilities for germplasm already saved in genebanks. Safety duplication is established between certain member institutions, but must be further improved and on line with funding limitations.
- Taxonomy – studies to check taxonomic identity for wild *Allium* taxa.

Brassica Working Group was established at 1991.

From the very beginning the group recognized importance of European database for Brassica species (Bras – EDB) and work on it creation. Database has passport data for almost 20,000 accessions which are saved in 36 collections. Base is on the internet and it is (http://documents.plant.wur.nl/cgn/pgr/brasedb/) (accessed March 10, 2008) facilitated by Centre for Genetic Resources, the Netherlands.

Currently on going activities of Brassica are related to the:

- Safety duplication – at the beginning of the Working Group CGN in Netherlands and Horticulture Research International (HRI), Wellesbourne, UK have maintained responsibility for International *Brassica* Base Collections since the 1980s and are reciprocally safety-duplicating the accessions conserved. The concept of a "black box" arrangement, whereby the genebank of origin holds the responsibility for the quality of the stored material and its regeneration when required, is considered the most cost effective method of safety duplication.

- Regeneration guidelines – important issue since studies made by Group demonstrated unexpected changes of genes frequencies during normal regeneration process. In order to avoid further problem in the future, till specific guidelines for specific species are not developed, Group suggested general procedure for all Brassica species:
    - Use not less then 50 plans per accession
    - Strictly controlled pollination (inside or very short distance between plant in the open)
    - Priority is on the regeneration of unique accessions of national origin.
- *In situ* conservation – of wild relatives from Brassica family in order to save its diversity, especially from Mediterranean region. This study should be strongly supported by governments.

Cucurbits Working Group was founded in year 2000 as a part of Vegetables Network. It was developed with main idea to focus on the conservation of cucumber, melons, watermelon, gourds, pumpkin, zucchini and other minor crops.

Main achievement of the Group was development of Central Cucurbits Database (ECCUDB) (http://www.comav.upv.es/eccudb.htmlwhich) (accessed March 10, 2008) contains information of passport data for 27,492 accessions in accordance with FAO/IPGRI Multi Crop Descriptors list.

Group is currently working on further improvement of:

- Safety duplication – should be for each collection under long-term conservation conditions in arrangement with other genebank facilities on the "black boxes" arrangements.
- Regeneration guidelines and primary characterization – still need to be expanded. Development of minimum descriptors list for melon, cucumber, watermelon and pumpkin is in the progress. Harmonization of regeneration protocols also should be finished in near future.

Leafy Vegetables Working Group was established in year 2000. Since then Group managed to establish International *Lactuca* Database (ILDB) with passport information

for all collections of *Lactuca* species worldwide. As of 2003, based on the experience with ILDB, Group started with development of similar databases for spinach and chicory. Group activities for the coming period would be in the following directions:

- Characterization and evaluation – should be fully applied in the coming years. At the moment for three major crops was agreed minimum list of characterizations descriptors with the task to develop common IPGRI descriptors. Evaluation work on several diseases was done and records were presented in databases of genebank which were involved in the whole process.
- Regeneration – status of regeneration and proper protocols were developed and applied by genebanks which generally do not have problems with germplasm regeneration.
- Safety duplication – genebanks with proper facilities for long-term storage were identified and it is on going process of material exchange between institutions.
- Duplication – since percentage of duplication is very high within lettuce collection, detection of Most Original Samples (MOSs) is important task in front of the Group. Detection of duplicates in Lactuca collection would improve efficiency during search for valuable traits and lower the cost for genebanks.
- Conservations and management of wild relatives – although wild relatives of lettuce and *Cichorium* are very common in Europe, only small number of accessions are available. Broader action for collection of wild *Lactuca*, *Cichorium* and spinach is necessary.

Solanaceae Working Group was also established in 2000. It deals with cultivated and wild relatives of *Solanum* sp., *Capsicum* sp., *Lycopersicon* sp., *Physalis* sp., *Cyphomandra* sp. All mentioned species originated from other continents than Europe.
Until now Working Group developed five databases for each of the genera mentioned above and they are maintained by Group member institutions. Compilation of passport data was done for each species separately and it should be adapted to the FAO/IPGRI Multi-Crop Passport Descriptors list. Current number of accessions held in Europe is estimated on around 5,000 for eggplant, 13,000 for pepper and 23,000 for tomato.
Solanum Working Group set following objectives in front of them in the coming period:

- Compile inventories of Solanaceae germplasm (passport data) for eggplant, tomato, pepper and some minor crops such as *Cyphomandra* sp. and *Physalis* sp.
- Identification of duplication level among various collections
- Develop harmonized descriptors and protocols for primary characterization.
- Produced common protocols for seed regeneration and storage since currently partners are using different protocols.
- Identify the taxonomic status of wild species, accessions which are present in genebanks.

Umbellifer Crops Working Group is group of our main focus since research that would be discussed in this thesis was done under umbrella of this group.

Umbellifer Working Group was officially established in 1998 and apart from *Daucus* (carrot) it interest focused on the following crops and taxa : *Anethum* (dill), *Apium* (celery), *Carum* (caraway), *Chaerophyllum* (chervil), *Coriandrum* (coriander), *Foeniculum* (fennel), *Pastinaca* (parsnip) and *Petroselinum* (parsley).

Working Group agreed to set the following objectives:

- Integration of the activities of the Group with the EU funded GEN RES carrot keeping in mind that the EU project is only focused on carrot and not on the other Umbellifer genera.
- Improvement of collaboration with non ECP/GR countries of eastern Europe.
- Database development.
- Collecting landraces of Umbellifer crops in Mediterranean and eastern Europe regions
- Definition of responsibilities for crop accession maintenance. Samples that are not considered priority by certain genebanks could be transferred to different partners willing to maintain them.

Based on the mentioned objectives Umbellifer Working Group has been running certain activities, some of them still need time and efforts to be finalized. All those activities can be grouped as follows:

- Proper Documentation development – through the establishment of European Umbellifer Database (EUDB) which has been developed and maintained by

Genetic Resources Unit at Horticulture Research International (HRIGRU), Wellesbourne, UK. The database has been limited to passport data stored in the FAO/IPGRI Multi-Crop Passport Descriptors format and it contains data for 8,426 accessions for nine major Umbellifer crops and wild taxa. EUDB is searchable database available on the internet on the address: http://www2.warwick.ac.uk/fac/sci/whri/research/gru/ecpumbel/ (accessed March 5, 2008).

During 1998, with support of some Group members were developed minimum characterization descriptors for carrot and they have been printed as IPRGI Descriptors for Wild and Cultivated Carrot (IPGRI, 1998).

- Collecting - since 1997 24 collecting expeditions were organized by the Polish Gene Bank, in collaboration with national genebanks in Greece, Moldova, Ukraine, Slovakia, Turkey and USDA. Expeditions have been organized in many countries like Greece, Turkey, Syria, Poland, Slovenia, Portugal etc. During those expeditions have been collected 2121 accessions, 533 of them were accessions of 7 Umbellifer species.
- Identification of duplicates – Group recognized problem of duplicated within partner institutions. It was agreed to work on the identification of duplicates and also to identify the Most Original Sample (MOS) for the wild taxa accessions inside European collection. This work is in progress and should provide essential information for the future collections. MOS definition would be also important for the potential in situ projects.
- Safety duplication – importance have been distinguished by the Working Group. For purposes of safety-duplication of all genetic resources accessions a number of the partner institutes offered to use their facilities under bilateral "black box" agreements. As outcome, several bilateral agreements are now in place.
- Regeneration – assistance to the Vavilov Institute in St. Petersburg (VIR) in regeneration of germplasm, especially of landraces of carrot. Four national programs (France, Italy, Poland and UK) have been very successful and managed to regenerate over 150 accessions. Produced bulk seed has been returned to the VIR for further long term conservation.

- Taxonomy – and identification of invalidated material is important for the efficiency of genebank and it proper management. Having that on mind it is important to improve cooperation's between genebanks and taxonomic experts in order to exclude from the *Umbelliferae* collections materials which are not belonging to that family. Further identification study should be applied in the future in this field.
- Research - several partner institutions have been collaborating with University of Wisconsin, USA, in developing the molecular characterization of carrot and related wild *Daucus* taxa. Number of accessions from genebanks has been assessed for genetic diversity through usage of RAPD technique.

  In the Czech Republic there is research on the bactericidal effects of essential oils. Research work at the N.I. Vavilov Research Institute of Plant Industry, St. Petersburg includes projects on disease and pest resistance, CMS and increasing the content of specific biochemical components of the plant product.

Species from family *Apiaceae* (known also as *Umbelliferae*) are generally herbaceous plants which are growing in temperate and boreal regions. The botanical family Umbelliferae consists of around 250 genera and about 2800 species (Rubatzky, *et al*., 1999).

Main morphological characteristic of this family is the inflorescence with convex or flat-topped flower cluster in which individual pedicels are arising from the same apex. From that umbrella like shape of inflorescence comes old family name *Umbelliferae* since on the Latin umbel means sunshade. Characteristic of certain number of species from *Apiaceae* family is their unique biochemistry which provides distinctive flavour and aroma to the various plant parts.

Considering variety among *Umbelliferae* species they are plants with annual, biannual and perennial growth characteristics.

Existing diversity range also gives possibilities for different usages of Apiaceae plants. Some of them play important role in mankind history like poison hemlock *Conium maculatum*, a rare alkaloid-containing plant, well known for the poisoning of Socrates.

It was also interesting usage of *Azorella* genus as a primary fuel source for the highland natives in the Andean mountains (Hodge, 1960) due to the characteristic compact fibrous structure.

Great variety of Umbellifers vegetables have been grown for their raw consumption of edible roots, tubers, stems, petioles, leaves, flowers, fruits and seeds. Other species are used for extraction of essential oils, carotenoids and other compounds. Some of the crops such as carrot, celery, parsnip, cilantro and arracacha can be considered among the major vegetables in certain regions. Others are gathered from the wild or produced locally on a smaller scale, mainly in home gardens. In general, vegetable umbellifers aim in nutrition is to provide micro-nutrients, since majority of them have low caloric contribution (Rubatzky, *et al*., 1999). With a broad mixture of flavouring and textural characteristics umbellifers are used as a direct food and as additives that enhance the enjoyment of other foods. Many umbellifers during the history used to be cultivated or gathered from the wild and applied as folk medicines, although generally not used in contemporary medicine.

Major vegetable crops from Apiaceae family according to the plant part that is used in consumption can be divided in the following groups:

- Root crops – are primarily grown for their edible storage root and tuber portions, but also, some of them, have secondary usage of edible foliage or seeds for condiment. Together with carrot as the most important root crop, in this group also belong: parsnip, celeriac, arracacha, Hamburg parsley and many other small scale crops. Their importance and production varies from region to region. While carrot is grown world wide, celeriac for example is mainly known and produced in northern and central Europe; arracacha in South America.
- Foliage crops – are cultivated for usage of edible leaves, petioles and stems for fresh consumption or as cooked potherb. Apart from parsley and celery other relatively important species are: cilantro (coriander) and Florence fennel. Other crops of regional importance are: salad chervil, Japanese hornworth, angelicas etc.
- Seed crops – are mainly grown for production of seeds which contain specific essential oils and compounds used for condiment and flavouring purposes. Foliage of some of the seed crops can be used as flavouring for various dishes and

salads. Major crop in this group are: coriander, caraway, fennel, anis etc. Like in the other groups many other specie are used regionally and produced on a small scale.

In general interest for Apiaceae family of public and scientists increased in last few decades together with the increase of carrot usage and it production. Most of the phases of crop production, it processing and nutritional value have been closely examined through the series of studies (Rubatzky, *et al*., 1999).

1.3. Carrot

Carrot (*Daucus carota* L., var. *sativus* Hoffm.) is the major vegetable from Apiaceae family which is cultivated worldwide. Carrot origin and first traces of cultivation around the world was subject of lot of studies. Vavilov (1951) reported wide distribution of weedy species *D. carota* var. *carota* in Afghanistan and Turkestan (Central Asia) and suggested that origin of the Asian cultivated carrots was the Inner Asiatic Centre. On the other hand Vavilov estimated that the origin of western cultivated carrots is in the Asia Minor Centre, primarily Turkey. According to Heywood (1983) the Himalayan Hindu Kush region of Afghanistan is the primary centre of diversity for eastern carrots.

In general, cultivated carrots can be separated into two types:

- Eastern/Asiatic carrots (var. *atrorubens* Alef.) with reddish purple (anthocyanin-containing) or yellow roots, pubescent leaves which give a grey-green appearance. They have a tendency for early flowering and can be considered as annual crop.
- Western carrots (var. *sativus* Hoffm.) have orange, yellow, red or white roots, less pubescent green leaves, and lower tendency to bolt without extended exposure to low temperatures. These carrots are biannual crops.

The origin of western carrots has been studied extensively, but little evidence for carrot cultivation exists before the 10th century when it was grown in Iran and Arabia. As of 12^{th} century carrot production spread in Europe.

Although yellow carrots described in Iran and Arabia had culinary quality inferior to purple-rooted carrots, they eventually replaced purple carrots in Europe.

The origin of western yellow and purple carrots is well accepted, but on the other hand the development of western orange types is less clear. Banga (1963) proposed that orange-rooted carrots originated in Holland as result of selection from yellow carrots. Other researcher suggested that orange types are coming from the combination of inevitable hybridization with wild carrot and selection (Heywood, 1983). Mentioned hybridization could occur in the Anatolian region of Turkey where (Mackevic, 1929) (Banga, 1963), cultivated carrot diversity was especially great. Nowadays orange-rooted carrots do exists in carrot germplasm in Turkey (Simon, 1996). Whether that was the case in 12th century it is not known.

1.3.1. Carrot distribution

Carrot is the most important crop in Umbelliferae family. It is cultivated in whole world, but main areas of production are in Europe and Asia. Production of carrot continues to increase and in 2005. reached amount of 26 million tons (2001. 19,5 million). Area for carrot production was in 2005 around 1,174,000 ha. All mentioned just increase importance of carrot as vegetable crop. As illustration of increase can be used the fact that export of carrot enabled certain countries to gain around 600 million dollars in 2005. All mentioned is result of further promotion of carrot as a crop with good nutritive characteristics. Increase of carrot consumption on big markets like Chinese one cause higher demands for this crop worldwide.

Major producer in the world is China with 8,4 million tons and with income of 100 million dollars is the biggest exporter of carrot. Other big producers are: Russia (1,8 million tons), United States of America (1,6). In the European Community biggest producers (2005 data) were: Poland (0,9 million), United Kingdom (0,83), France (0,72), Italy (0,59) and Spain (0,57). Major exporters within EU countries were Nederland with income of 70 million dollars and Italy with income of 60 million dollars.

Presented date demonstrated importance of carrot for Italian agriculture. Amount of produced carrot in Italy is rather stable around 0,58 million tons (date 2001. and 2005.). Major region for carrot production in Italy are Abruzzo, Sicilia and Emilia Romagna. In those three region was produced almost 70% of total Italian production. The biggest

production was in Abruzzo in amount of 0,15 million tons. Two Italian regions with significant contribution in carrot production are: Lazio and Veneto.

1.3.2. Carrot botany

All carrot types are having same number of chromosomes 2n=18. Carrot is biannual crop which in the first year develop storage root and leaf rosette, while in the second year form leaves and floral stalk. On the floral stalk are placed umbel like inflorescences which later gives fruit – schizocarp.

Among genera in Umbelliferae family, *Daucus* is one of the largest with great diversity. It is estimated that *Daucus* genera have around 25 species. The majority of those species originated in the Mediterranean region, north Africa, Europe and Central Asia.

All Daucus species were grouped into two aggregates:

- subsp. *agg*. *gingidium* with white flowers and present on continental part. Other subspecies from this group are: *gummifer, commutatus, hispanicus, hispidus*
- subsp. *agg*. *carota* at costal region with white-rose flowers. Subspecies from this aggregate are also: *maritimus*, *major* and *maximus*.

Immediately after emergence carrot seedlings demonstrate clear determination between hypocotyl with cotyledonary node at the end, and thick taproot without lateral roots.

From emergence cotyledons, after 10-15 days is developed the first true leaf. Throughout the growing season carrot leaves are growing in interval of about 10-15days. Usually in the first year the carrot plant develops up to 15 leaves on the compressed stem which is just above the ground, in a way that internodes are not clearly visible.

Alternate and compound leaves are developed on the expanded petiole and they form a basal rosette. Their leaf blade is two to three pinnate and green. The new leaves expand centripetally in a spiral within the basal clasping of previous petioles.

Carrot storage root is composed of outer parenchymatous phloem and inner xylem (core) filled by vascular tissues with cambium sections joining together in a cylinder. Carrots with larger portion of phloem relative to that of the xylem are considered as one with higher consumption quality. Some cultivars have characteristic small xylem region, but

even them are not “coreless”. Coreless appearance root of some varieties occur since the xylem tissues are very similar in colour intensity to the phloem.

Carrots used for production can have orange, yellow, red, purple and white-fleshed roots. Orange and yellow root colour comes from α- and β-carotene as major pigments. Xanthophylls are carotenoids largely responsible for yellow root colour, while lycopene gives red colour, and anthocyanins are responsible for purple root colour. β -Carotene often may represent 50% or more of the total carotenoid content, and usually is about twice that of α-carotene. Since carotenoid synthesis proceeds from proximal to distal tissues, their content is not uniformly distributed in the root. Phloem tissues usually contain more carotenoids than the xylem. Varieties with white roots are without pigment.

In general root shape of many carrot cultivars is conical, while the extent of taper varies between cultivar types. Shapes of cultivar types can be cylindrical, round or various intermediates between those two. Root diameter may be in range between 1 and 10 cm at the widest portion. Storage root lengths range from 5 to more than 50 cm in length, although for most of cultivars is between 10 and 25 cm. Root shoulder also divert in shape and can be: square-like, slightly rounded, conical and flat.

During the second production year stem’s uplifted conical meristem is capable to produce stem elongation and an inflorescence (Borthwick *et al*., 1931). Grown of floral stalk is slow at the beginning of the season. Later, floral stem greatly elongate and branches.

The major characteristic of the *Umbelliferae* is their compound umbel (umbrella-like) inflorescence. Umbellets are formed of flowers, where pedicels of each flower radiate from a common point. The umbellets in turn arise on pedicel rays originating from the apex of the inflorescence stalk. At the end of main floral stalk is developed primary, so called “king” umbel. The branches that are terminating from the main stem are secondary umbels, and depending on further growth and stem branching, third, fourth and even higher order umbels can be formed. These umbels are developed later and they are progressively smaller. Each umbel may contain even certain number of umbellets, and each carries as many as 10 to 30 flowers.

Carrot flowers are small and white or occasionally greenish white or light yellow in colour. They are usually bisexual and consist of a five-lobed calyx, five petals and

stamens, and a two-celled inferior ovary. Typically anthers dehisce and stamens fall before the stigma becomes receptive.

Floral development is protandrous and centripetal. Usually, flowers first open at the periphery of the primary umbel. About a week later the process begins on the secondary umbels, to be followed a week or more later in the tertiary umbels. The flowering period of individual umbels usually ranges from 7 to 10 days. It means that plant can be in the process of flowering for 30–50 days. The distinctive umbels and floral nectaries attract insects and enable cross pollinations between the different plants.

The fruit that develops is a schizocarp consisting of two mericarps, each mericarp being an achene or true seed. Mericarps are small, longer than they are wide, and make up the longitudinal hemisphere of the fruit. After drying it is easy to separate paired mericarps. Premature separation (shattering) before harvest is undesirable because it can result in seed loss. Mature brown seeds are flattened on the commissural side that faced the septum of the ovary. The opposite side has five longitudinal ribs. Spines protrude from some ribs. These are removed usually by abrasion during milling and cleaning. Seed also contain oil ducts and canals. Seed size variation is expressed through the weight of 1000 seeds and it can be in a range of 0,85 and 1,25 g. Seeds can be conserved for 3-4 years with minimum germination rate of 65%.

1.3.3. Carrot cultivation

In Italy are cultivated different carrot varieties and they can be hybrids of F1 generation, imported varieties or some local cultivars.

Apart from variety some other environmental factors are important and can be limitations in carrot production.

Climate has great influence on crop production, and temperature is its most important factor. Carrot as crop that is grown in temperate climate can stand lower temperatures. For germination it is necessary to have temperatures over 10°C. Optimum temperatures for normal carrot development are between 20-25°C. Long period of temperature over 25°C or under 15°C can limit carrot colour development. Higher amount of carotene is produced when days with moderate temperatures (15°C) are combined with cold nights

(7°C) (Simon and Wolff, 1987). After the growth of storage root, carrot can resist and survive on the freezing temperature down to -3°C (Tesi, 1994).

Another important factor for carrot production is the soil. Carrot can grow satisfactorily in a wide range of soils. It is important that soil has good drainage characteristics and with low level of salinity, less then 1%. Optimum soils are deep, friable, fertile and relatively high in organic matter. Alluvial and sandy and light sandy loams are desirable where early cropping is important. Such soils usually are better drained and aerated, warm rapidly, and can be tilled relatively soon after rain or irrigation with less compaction and damage of soil texture. Soil pH should be between 6 and 7 for optimal carrot growth. Light-textured soils are preferred for fresh market carrots in order to facilitate harvesting, and to produce smooth root surfaces

For successful carrot production soil moisture content throughout the season is essential. Carrot can benefit from an evenly distributed and adequate moisture supply during growing season. The most critical moisture requirement for carrot occurs during root storage tissue enlargement and photosynthate accumulation. Peaks in water supply are causing different deformation in shape of carrot roots which are not standard to the market requests, difficult to sell. For example insufficient moisture cause appearance of thinner and longer roots, on the other hand in water saturated soils roots are easy cracking.

Carrots, when grown in a wide range of soil water concentrations, usually will produce marketable roots. According to White (1992) high water concentrations can reduce yields more than do low water concentrations.

Soil native fertility, the previous cropping history, soil type, organic matter, pH, rainfall and other production factors influence on moment of application, fertilizer composition and the way of it application.

Residual effects of previous crops are important in carrot production and fertilization strategies should take into account this influence and that of rotations. Although carrot prefers certain amount of organic matter in the soil, it should not be grown just after application of organic fertilizers. Higher amount of organic matter inside the ground can cause root deformations (forked roots), with undesirable odor. Manure should be used for

the crop that is grown before the carrot on the certain field, it would provide enough time for manure decomposition.

For 100 kg of carrot plant, amount of uptake nutrients is 0,3-0,5 kg of nitrogen (N), 0,1-0,2 kg of phosphorus (P_2O_5) and 0,5-0,6 kg of potassium (K_2O). Observed crop performance and experience, especially when supported by the results of soil and tissue testing, are valuable for determinations on the amount and ways of fertilizer applications. General recommendation, in order to integrate consumption of nutrients of organic and mineral origin, is to use complex fertilizers before seed sowing, integrated with N side dressing during the season, if necessary. For a majority of field conditions, fertilizer levels commonly used should be in a range: 75-150 kg/ha of N; 25-125 kg/ha of P_2O_5 and 0-175 kg/ha of K_2O (Rubatzky et al., 1999).

Chessin and Hicks (1987) reported that a higher nitrogen rate (336 kg/ha) increased nitrate content into the roots, and they recommended that high levels of nitrogen should be avoided in carrot production since the nitrate content in that product could increase. Cserni *et al*. (1989) also proved that high application of nitrogen rates (320 kg/ha) increased carrot root NO_3 content above the permitted level for infants (400 p.p.m.). However, unless very high nitrogen rates are used, this is considered unlikely.

Phosphorus content in carrots was found to be higher when band applied, whereas potassium content was not influenced by application method, and simply increased with higher application levels.

In Europe carrot can be grown as a major crop in summer or as second crop in winter. For spring carrot production sowing of seeds is done in April-May with usage of varieties which have semi long and long roots. Varieties with similar characteristics are used for winter carrot production, and seed sowing should be done in August-September. Nowadays seed sowing is usually done by pneumatic sowing machines which enable precise sowing according to the previous set up. That drastically decrease usage of seeds per hectare, from 4-6 kg/ha for manual sowing down to 1,5-2 kg/ha, and decrease production cost. Carrot is usually grown in bands with three-six rows per band. Distance between the rows is 8-10 cm and among bands around 30-35 cm.

Carrot roots should be harvested before full maturity in order to avoid possible hardness of roots core part as result of increased development of fibrous tissues. ‘Harvest stage’

may be an appropriate term to identify carrot roots judged suitable for harvesting, rather than mature or ripe. Therefore, determination of harvest suitability will vary according to cultivars, intended use, production season, market conditions and other factors. Carrots are often harvested before achieving their full potential size, weight or marketable yield.

Carrot genetic background has a large influence on growth rate and the time required to reach the harvesting stage. For example, cultivars of early developed 'Amsterdam Forcing' type can be harvested in less than 70 days after planting, occasionally even earlier. Other cultivars, in order to achieve the development considered suitable for harvest, may require a growth period of 150 days or more (Rubatzky et al., 1999).

Carrot growing period till harvesting generally can be: till three months for early development varieties with short roots, over 4 months for varieties of medium root length in summer production and around 5-7 months in autumn carrot production.

Cultivars with longer growth periods generally produce larger and heavier roots and more often are grown for processing or long-term storage. Later-planted carrots generally have better and longer storage characteristics. Those with shorter growth periods are typically grown for fresh market use.

Interpretation of what may be considered good yields will vary with the type of production. Depending fro the variety and type of production carrot yield can be in range of 25–30 t/ha till 100 t/ha.

Nowadays carrot harvesting is mechanized with usage of different equipment. Harvesting is divided in three phases: roots removing from the soil, removing of the leaves, and loading of roots into containers.

Following harvest, fresh market carrot roots, with or without attached foliage are transported from the field in bulk containers or wagons to packing sheds. There they are unloaded, often into a water tank to reduce impact damage and remove attached soil. Bundles of carrots with attached foliage are then washed with clean water. Having been graded before bundling they can be packaged directly into containers.

Carrots which are packed into polyethylene bags, tick 0,05 mm, can be conserved in fridges on 0°C and relative humidity of 90-95 % for 4-6 months (Tesi, 1994)

Carrot protection from pests and disease is necessary in order to have healthy product which will be competitive on the market. It is important to develop proper strategy for

crop protection, taking into account the most common pests and diseases. Great number of diseases is developed in the very humid conditions, with high density crop, low aeration between plants and bad crop rotation. Most present diseases on plant that appear in mentioned conditions are: alternaria (Alternaria porri f. sp. Dauci), peronospora (Plasmospora crustosa) and (Erysiphe heraclei). In such environment roots are damaged by Rizoctonia violacea, Scelrotinia, Alteranria radice. Among bacteria is important impact from Erwinia carotovora and Xantomonas carotae.

From fauna the biggest problem are provoked by nematodes which are causing roots deformations. Some insects are also damaging carrot during growing: carrot fly (Psilla rosae) in the larval stage is attacking roots, aphids as major vectors for carrot viruses (Cavariella aegopodii) etc.

1.3.4. Carrot market normative

Different carrot varieties enable existence of great diversity in the market.

Important carrot characteristic for further division and quality evaluations are:

- Root length
- Root shape (cylindrical, conical, Obovate, oblong)
- Shape of the root neck (shoulder like, rounded, conical, flat)
- Root tip shape (blunt, rounded, pointed)
- Root colour (orange, red, yellow, pink)
- Ratio between core part and outer parenchyma (ration should be high in favor of core part)
- Uniformity of the colour of core and parenchyma

According to the Italian quality normative (d.m. 21 luglio 1962 e regolamento Cee 9721/89) are recognized three quality categories: extra, I and II. In extra category are grouped roots with colour typical for the variety, without any root damage and optimal characteristics which present superior product. In the I category are classified entire roots with slight deformations, little damages and with homogeneous presence of characteristic colour. Roots in this grade can have slight green colour on the root neck.

Carrots are calibrated in accordance to the weight and the root diameter and grouped in juvenile and normal group. Juvenile carrots and small varieties can be with or without leaves with minimum caliber of 10 mm or weight of 8 g, and maximum of 40 mm diameter and weight of 150 g. For carrot harvested in normal conditions, at "harvesting" stage, minimum are: 40 mm diameter or 150 g for extra category, and 20 mm or 50 g for I and II category. It is important to use mechanical calibration in order to obtain uniform material and avoid situation to have differences between roots above 30 mm or 200 g.

Good quality carrots have normal shape, smooth surface, without signs of disease, insect's attacks, with intense colour. They should be but not to hard and slightly sweet (Gorini, 1990).

1.3.5. Carrot uses

Fresh carrots are used raw in salads and as a snack food for their sweet, flavourful taste and crunchy texture. Usual cooking methods of fresh carrots preparation include steaming, boiling, baking and stir-frying. Although the storage root is the primary product, young tender foliage can be used as a stir-fried potherb, and as salads content in China and Japan. For these purposes, the foliage is produced from high density plantings and harvested at an early growth stage before significant root enlargement.

A recent application of what is called lightly processed technology involves peeling and shaping of root segments to resemble small carrots. The root segments, usually ranging between 3 and 7 cm in length are processed with machines by abrasive peeling which smooth surfaces and rounds-off cut ends which results in a product having the appearance of a small 'baby' carrots. Those "baby" carrots are used for different fresh and cooked preparations.

Considerable volumes of carrots are processed as canned, frozen, dehydrated, and juice products. Small roots may be processed intact. Large roots are diced or cut into slices, strips and other shapes. For use in infant food preparations carrots can be puréed. Carrot juice consumption is increasing worldwide; it can be pure carrot or part of multivitamin juices. Thin slices of carrots can be deep fried for snack food use.

2. EXPERIMENTAL WORK

Experimental work was done under umbrella of project: The Future of European Carrot: a programme to conserve, characterize, evaluate and collect carrot and wild relatives (GENRES carrot project).

It is well known that continued development of certain crop depends upon breeders and other scientist's ability to have access to characterized and well documented genetic resources collection. Objective of the mentioned project was to provide high quality germplasm collection and to support applied and pure research related with it.

Project has listed objectives as it follows:

- To draw up minimal descriptors for carrot and wild taxa based on existing formats (IPGRI descriptors).
- Design and build database for *Daucus* collection.
- Coordinate Genetic Resource Center for *Daucus* in the EU.
- To characterise germplasm collection for agreed minimal descriptors and incorporate data in European Daucus Database.
- Develop preliminary core collection using passport and characterisation data.
- Evaluate collections for characters important to growers, processors and consumers.
- Use characterisation data to rationalize collections and increase efficiency of conservation and utilization.
- Identify the gaps in existing germplasm and organize collections of cultivated and wild material.

From listed goals, part related to evaluation and characterisation of existing accession in germplasm collection was realized by Department of Agro-environmental Science and Technology at University of Bologna.

Part of the work that was realized by the research group in Bologna is included in this study.

Main objectives of this study are the following:

1. To study the variability of root shape in a germplasm carrot collection. Based on obtained data, discuss the performances of root shape indexes and try to individuate the one that in best manner summarise root shape.
2. Examine the genotype x environment interaction on carrot germplasm.
3. Sensory evaluation of 20 selected accessions from available germplasm collection.

2.1. Materials

This research took place in period of four year since 2000 till 2003 as part of GENRES carrot project. Accessions used for this study were received collected from genebanks, and their source and number were:

- Institute National of Horticulture: **INH,** 20 accessions
- Federal Research Center for breeding of cultivated plants: **BAZ,** 12 accessions
- Horticulture Research International: **HRI,** 75 accessions
- Nordic Gene Bank: **NGB** 5, accessions
- Two local varieties from South of Italy

Apart from the mentioned accessions, the following commercial varieties were included into the experiments: Amsterdam, Autumn King, Bolero, Nikki 1, New f1, Parmex and Rubrovitamina

Since this study contain three parts and all listed accession were not used in all three parts, more detailed explanation of used materials will be explained for each part separately.

2.2. Carrot root shape

2.2.1. Introduction

Root shape is an important carrot characteristic, especially as reference for determination of different varieties.

Importance of the carrot root appearance is demonstrated in the following fields:

- Carrot root shape is a relevant trait for cultivar identification and taxonomic determination.
- Root appearance can be used as factor during selection and breeding process in development of varieties with desired root shape, especially from the market point.
- Carrot root shape can determines consumer's affection and its consumption in general. As an example, while European consumers seem more attracted by cylindrical roots, on the other side, North Americans are preferring long conical ones. Particular shapes, like round small roots of Paris market types, are appreciated in niche markets.
- Carrot root shape is sometimes affected by environmental conditions.

Considering everything mentioned above, it was normal to include shape as one of the factors into Carrot descriptors, which were established by IPGRI (IPGRI, 1998). To the root shape are dedicated descriptors from 7.4.14 to 7.4.21.

Descriptor (7.4.14) try to establish general shape categories (Figure 2.1.) in integration with attribution to cultivar group types for better precision (7.4.19) presented in Figure 2.2.

Figure 2.1. General root shape; 7.4.14 IPGRI carrot descriptors (IPGRI, 1998)

1. Round
2. Obovate
3. Obtriangular
4. Oblong
5. Tapering

99. Other

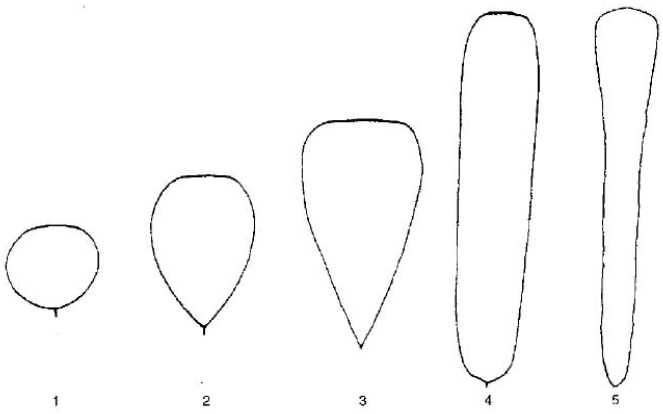
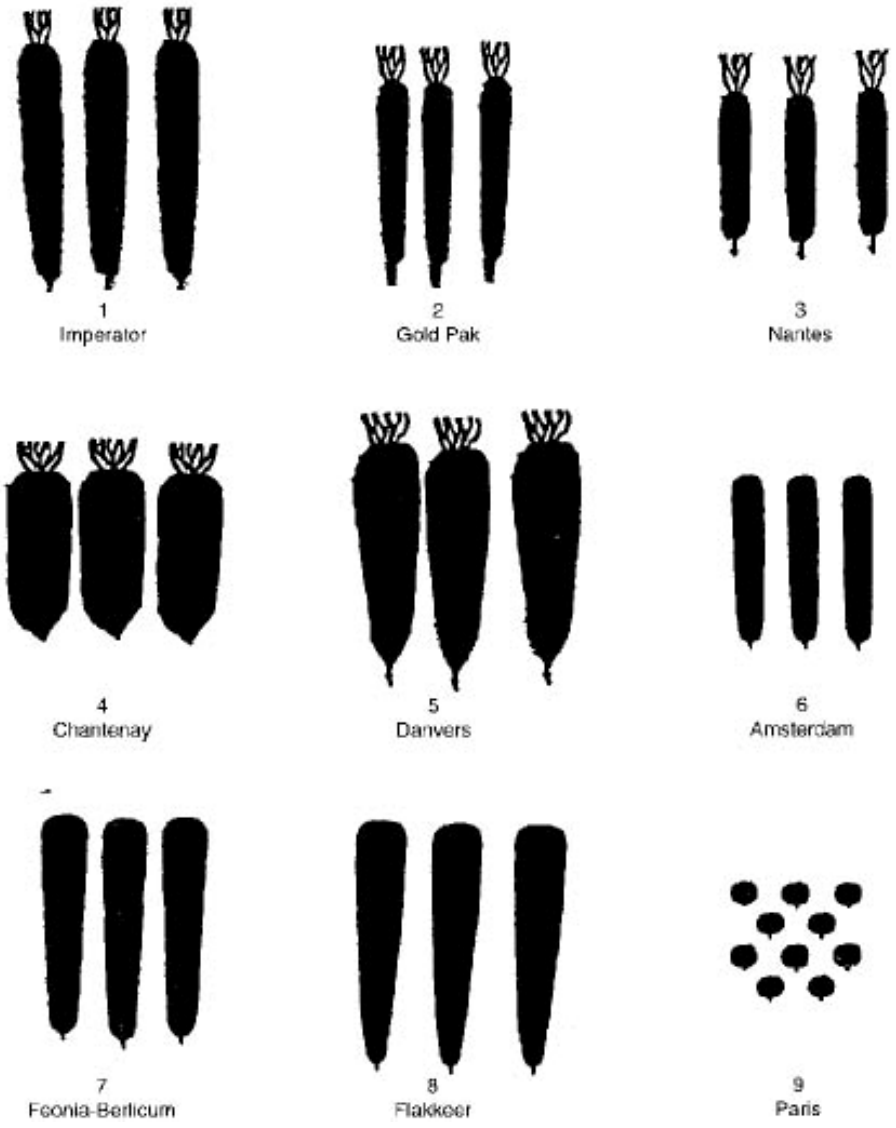


Figure 2.2. Root type grouping; 7.4.19 IPGRI carrot descriptors (IPGRI, 1998)



IPGRI descriptors also include:

- Uniformity of root shape (7.4.15), in range 3 for low to 7 for high uniformity
- Root shoulder type (7.4.16) as flat; flat to rounded; rounded; rounded to conical; conical and other
- Root tapering (7.4.20) in range from absent; slight; intermediate to acute
- Root tip/end shape (7.4.21) as blunt; rounded; pointed and other.

It is evident that the two most important descriptors of root shape (7.4.14 and 7.4.19) establish the main categories, but these do not correspond to a real numerical continuum of shape types.

Shape descriptors, in numerical terms, have been a rather classical topic of some research, developed from the seventies of the last century.

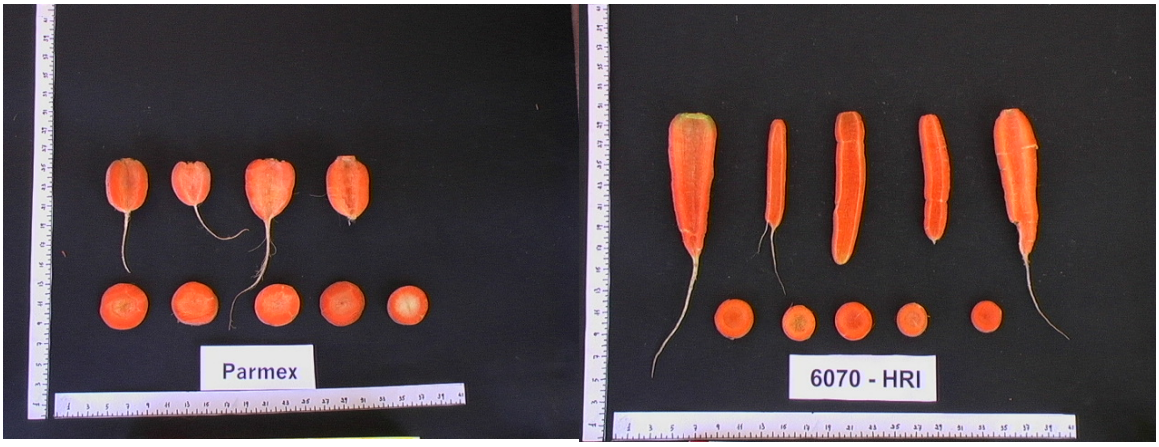
Initially, root weight (W) has been generally used as an estimation of root volume, in the hypothesis of a specific density close to 1 g cm^{-3} and not relevant variation between varieties. Further on, simple theoretical approach was the approximation of root shape between the two extremes of a cylinder and a cone. As outcome of such theory was developed first shape index:

$$C = W/ (p (D/2)^2 L)$$

C represents the ratio between the root volume, estimated from root weight and the volume of a cylinder with base diameter equal to root diameter (D) and height equal to root length (L). C root index was defined by Bleasdale and Thompson (1963), cited in Dowker *et al*., 1976 and Benjamin and Sutherland, 1989.

Not taking into account facts connected to particular shapes of root shoulder and tip, C index can vary between 1 (perfect cylinder) and 1/3 (perfect cone). It is rather clear that C represents well the attribute "cylindricality" of a root, actually it represents the amount by which a root geometrically deviates from a cylinder with the same main dimensions. But from a visual perception, normal C values correspond to rather different root types, e.g. varieties Parmex and HRI 6070 with same C value of 0,75 presented in Figure 2.3.

Figure 2.3. Root shape of varieties with similar C index values



According to Dowker *et al*., (1976) C index is under great influence of environmental conditions, indicating same tendency for the carrot root shape.

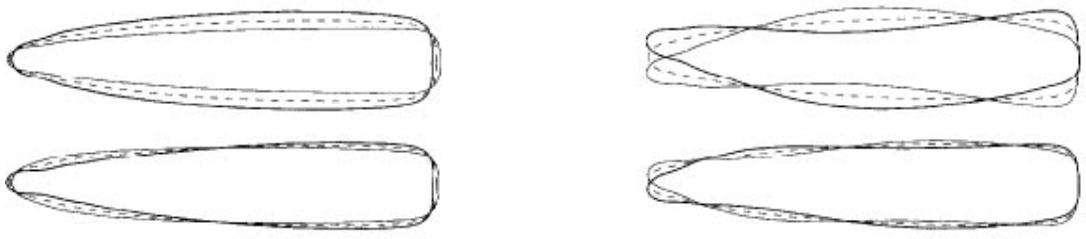
Another approach to define root shape was by using of ratio between root length and diameter; this index has been also used as a shape index of carrot (Dowker *et al*., 1976). Contrary to C index, the range of L/D is more difficult to define, with same indication about environmental influence on its value.

Other approaches in root shape descriptions have been based on allometric relationships. Benjamin and Sutherland (1989) studied the direct and logarithmic relations between root diameter, length and weight. They found good linear relations between logarithm of diameter and logarithm of weight, which enabled prediction of root diameter based on its weight. Good linear relation between root length and diameter was detected, as well.

Some authors came up with a more complex way of root shape definition. Snee, 1972 based his approach on repeated measures of diameters (five positions) along root length for shape reconstruction. By combination of repeated measurement of root diameters and core shape angle Umiel *et al.*, 1972 managed to describe root and root tip shape.

Recently, a method to acquire basic images and their subsequent processing to the contour of digital shapes has been developed, based on the principles of image analysis. Those digital shapes were further processed by means of numerical methods, and therefore transformed into values used for the statistical analysis (Iwata *et al.*, 1998; Grabov *et al*., 2005). Such approach enables precise root reconstruction considering not only its shape, but also positive and negative variations (Figure 2.4.) under influence of factors such as soil conditions and water amount.

Figure 2.4. Root shape variation under environmental conditions (Iwata *et al.*, 1998)



The need to find a unique index which will summarise the main features of root shape is not realised yet. A contribution towards that goal will be given in this study, which rather ample range of root shapes was made available by exploring a germplasm collection.
In this research focus will be on:

- investigation of root shape variability in a collection of carrot germplasm,
- usage the complex of data to try to discuss the performances of root shape indexes and try to locate the individual ones better summarising root shape,
- individuation of representative indexes would be done starting from the already discussed indexes, developed during the 70 and the 80 of the last century, together with indexes beyond that list.

2.2.2. Materials

For this research was used data collected in four year period 2000–2003 of evaluation of European carrot, as a part of big project. Accessions used for this study were received collected from genebanks, and their source and number were:

- Institute National of Horticulture: **INH,** 20 accessions
- Federal Research Center for breeding of cultivated plants: **BAZ,** 12 accessions
- Horticulture Research International: **HRI,** 75 accessions
- Nordic Gene Bank: **NGB** 5, accessions
- Two local varieties from South of Italy

Apart from the mentioned accessions, the following commercial varieties were included into the experiments: Amsterdam, Autumn King, Bolero, Nikki 1, New f1, Parmex and Rubrovitamina
Test varieties used for this study were:

- Amsterdam and Bolero – with long and cylindrical root shape.
- Autumn King and Rubrovitamina – long and conical roots
- Parmex – with short and conical shape.

2.2.3. Methods

For the mentioned five test varieties, results from years 2000 and 2001 were used for preliminary checking of indexes performance and further fitting of relations. Major indexes of our interest were:

- **RLD** – is ratio between root length (L) and diameter (D).
- **RWVCYL** - is representing ratio W/VCYL where W is root weight and VCYL present cylinder with base equal to diameter (D) and height is equal to the root length (L).
- **RWVCYLD** - is ration W/VCYLD with weight (W) and VCYLD representing cylinder with base and height equal to the root diameter d.
- **RDW3** - geometrical ratio between root diameter d and weight W^3
- **RVTRCONVCYLD** - is ratio VTRCON/ VCYLD where vtrcon is volume of trunk of cone with base and height equal to diameter; vcyld same as for the second index listed.

2.2.4. Discussion and indexes comparison:

In this part will be discussed characteristics of mentioned indexes in details.

The first step of data processig was regression analysis on the five test varieties from years 2001/02, in order to assess relations between certain index and root volume/shape.

After that each index was calculated for all accessions as a base for varietals' comparison, and index variability within variety was evaluated. Finally the effect of certain factors: year, variety and their combination; on the indexes performance, and root shape in general was analyzed by means of the analysis of variance and LSD test.

2.2.4.1. RLD index

This index represents root elongation and it was one of the indexes used from the beginning for the root shape definition. It represent ratio between root length (L) and root diameter (D)

$$RLD = L/D$$

Very short and nearly isodiametric root may have values as low as 1, whereas upper limit is more difficult to define, with 7-8 as typical values of elongated roots. In the Table 2.1. are presented average values of this index for the test varieties.

Table 2.1. Range of RLD mean values for the five test varieties

	Amsterdam	A. king	Bolero	Parmex	Rubrovitamina
RLD	6,7-8,6	4-6,5	5,5-8	1-1,3	4,7-8

From Table 2.1. can be seen that values of 1-1.3 defines short conical shape (Parmex like); values between 4 and 8 are attached to the long conical roots and values between 5.5 and 8.6 are often associated with cylindrical shape. It is obvious that for the long conical and cylindrical roots, exist an overlapping range of 5.5-8, which make difficult application of RLD index for clear root shape definition. In that range it is difficult to define root shape just on the basis of RLD value, so some other indexes, measures should be included for more precise shape explanation. RLD values for all test varieties in experimental years and three interesting examples of other accessions are listed in the Table 2.2

Table 2.2. Values of RLD index for test varieties (+ three interesting accessions) in all experimental years

Variety	Year	N of cases	Minimum	Maximum	Range	Mean	Std. Error	Standard Dev	C.V.
Amsterdam	2000	358	1,93	15,14	13,21	6,721	0,11	2,11	0,314
Amsterdam	2001	151	3,47	22,48	19,01	7,823	0,19	2,35	0,301
Amsterdam	2002	194	2,26	18,73	16,47	6,899	0,20	2,72	0,394
Amsterdam	2003	50	5,04	13,12	8,08	8,632	0,27	1,90	0,220
A. king	2000	502	0,60	12,54	11,94	3,918	0,05	1,04	0,265
A. king	2001	181	2,47	22,09	19,62	5,682	0,16	2,11	0,371
A. king	2002	123	1,17	13,92	12,75	5,902	0,21	2,31	0,392
A. king	2003	50	4,32	9,62	5,30	6,495	0,18	1,28	0,197
Bolero	2000	186	2,78	14,43	11,65	5,573	0,11	1,45	0,260
Bolero	2001	157	4,25	22,30	18,05	7,474	0,14	1,79	0,239
Bolero	2002	50	5,63	10,70	5,07	8,048	0,20	1,45	0,180
hri 10176	2002	42	1,58	20,07	18,49	8,041	0,71	4,62	0,574
hri 11169	2002	75	2,74	11,15	8,42	5,944	0,25	2,19	0,369
hri 11503	2002	91	1,83	15,42	13,59	5,975	0,27	2,61	0,436
Parmex	2000	232	0,66	2,67	2,01	1,178	0,02	0,33	0,280
Parmex	2001	149	0,61	3,01	2,40	1,282	0,03	0,36	0,281
Parmex	2002	123	0,55	1,84	1,29	0,995	0,02	0,21	0,216
Rubrovitamina	2000	424	1,75	10,68	8,92	4,728	0,05	1,07	0,226
Rubrovitamina	2001	167	2,90	10,22	7,32	5,641	0,10	1,33	0,235
Rubrovitamina	2003	50	5,45	11,35	5,90	8,046	0,19	1,34	0,167
Cycle	lsd (p=0.05)				**	0,222			
Genotype	lsd (p=0.05)				**	0,124			
Cycle x Genotype	lsd (p=0.05)				**	0,334			

Some examples of misleading values for the RLD that can give wrong indication about root shape are presented in Table 2.2. First example are values of varieties Bolero and HRI 10176 in 2002 (8.04). They have same values, which should lead to the conclusion that like Bolero, HRI 10176 variety also has long cylindrical root. But from the figure 2.5.a it can be seen that variety HRI 10176 has rather conical shape more similar to the test variety Autumn King.

Another example are varieties HRI 11169 and HRI 11503 both with a RLD of 5.97 , which are in the group of long conical varieties, but the shape of the latter one is more close to cylindrical shape than the first one (Figure 2.5.b). That can not be seen from the values of RLD index.

Coefficient of Variation (CV) for five test varieties is presented in Table 2.2. Apart from varieties with long conical root, others have small variation in CV values between the years of experiment. Variety Rubrovitamina has CV variation in range of 0,17 to 0,24 while Autumn King has much higher differences in CV, from 0,20 to 0,39. This indicates low variations of RLD index among the years for majority of test varieties.

Figure 2.5.a Root shape of varieties with similar RLD values

Figure 2.5.b Root shape of varieties with similar RLD values

On the other hand, analysis of variance shows different results (Table 2.3.). Considering year of experiment as factor it can be seen that it has statistically significant influence on the RLD index. That suggests important influence of environment on the L/D ratio and root shape in general. Variety influence is also statistically significant (Table 2.3.), and it can be understandable having in mind all the different shapes present among accessions.

Table 2.3. Analysis of variance for RLD index

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Year	692,25	1	692,25	316,350	0,0000
Variety	8180,77	4	2045,19	934,622	0,0000
Year*Variety	202,05	4	50,51	23,084	0,0000
Error	5461,89	2496	2,19		

Interaction between those two factors causes significant differences in RLD values and carrot root.

Significant difference in the values of RLD index for two mentioned factors and their interaction was confirmed after performing the LSD test on the same data (Table 2.2). It just confirms environmental influence on RLD mean values and carrot root shape.

2.2.4.2. RWVCYL

RWVCYL is the second index of interest of this study. It represent ratio:

$$RWVCYL = W/VCYL$$

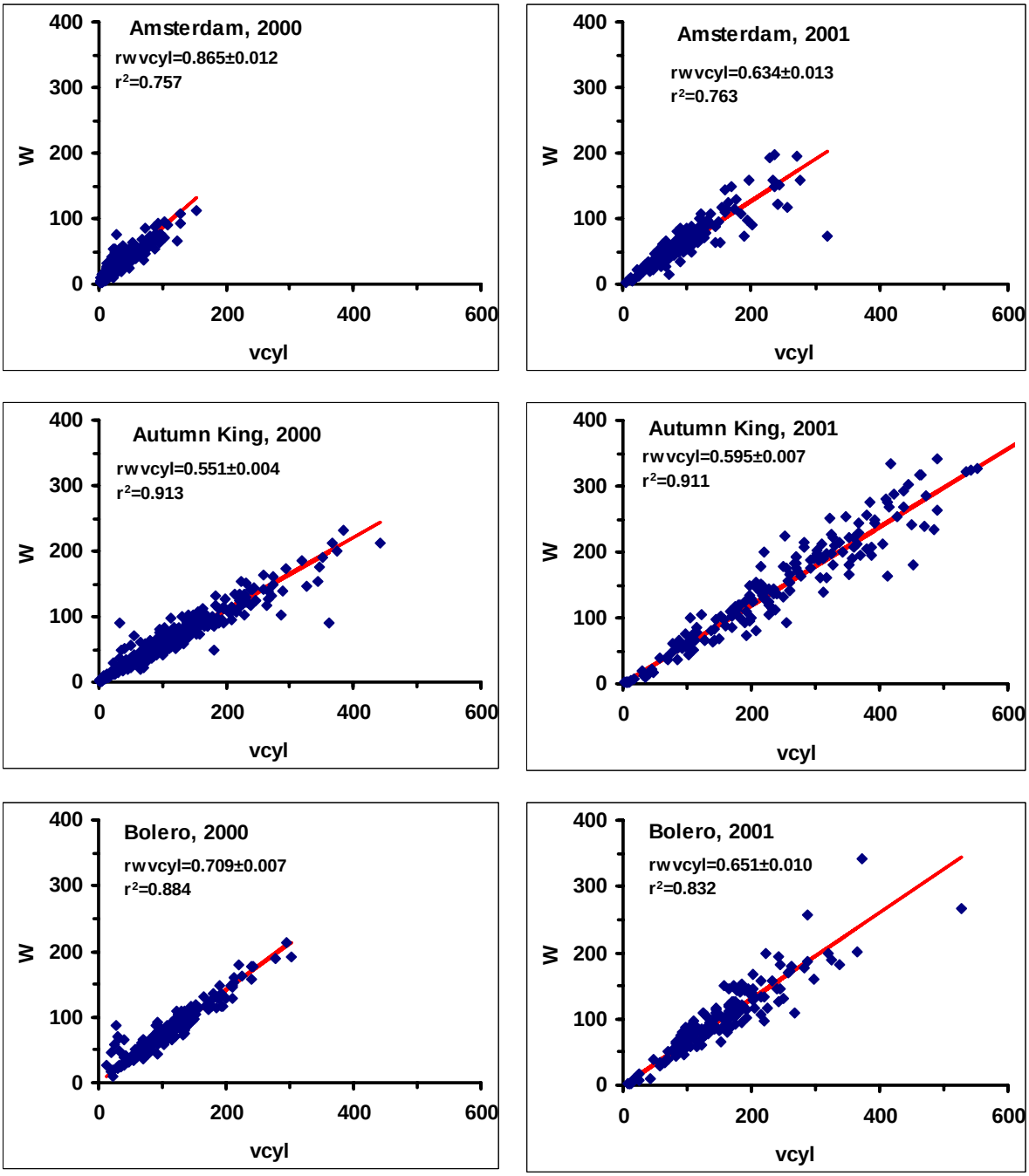
Where W is the root weight and VCYL is the volume of the cylinder with base equal to the root diameter (D) and height is equal to the root length (L). This index is equivalent to the shape index C (Bleasdale and Thompson, 1963).

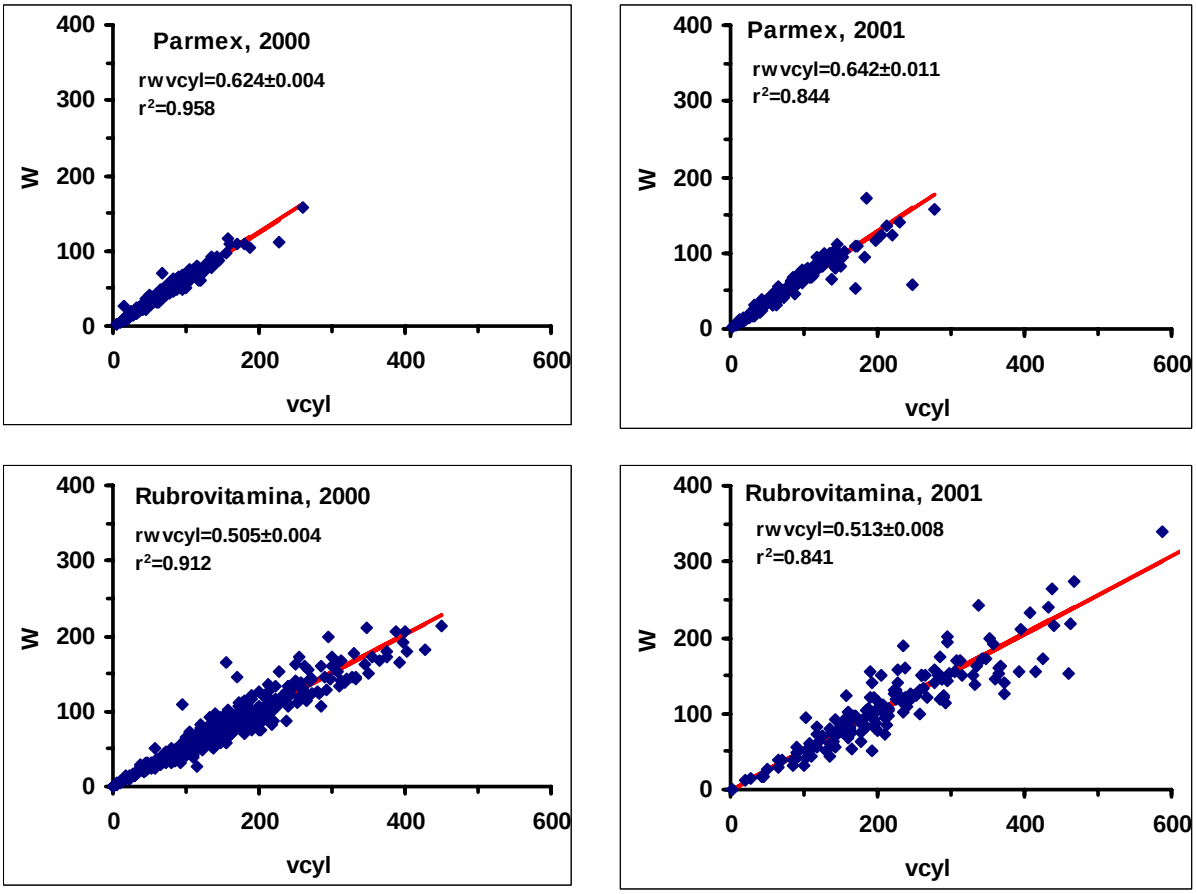
On the data obtained in the first two years (2000 and 2001) of experiment from the five test varieties, was performed regression analysis in order to check possible linear relation between root volume (estimated from W) and volume of the cylinder that contain the root. Analysis was done based on the equation:

$$W = RWVCYL * (p(d2/2)2*l) = RWVCYL *VCYL$$

The slope of linear relation between estimated volume and the volume of the cylinder (VCYL) that contain root, with measured diameter (D) and length (L), for all test varieties are presented in scatter diagrams in Figure 2.6. All varieties demonstrated linear relation between estimated volume and cylinder volume. RWVCYL values can give indication about carrot root shape, defining it cylindricality. This index should have values in range of 1/3, for perfectly conical root shape, till 1 for cylindrical roots.

Figure 2.6. Estimated root volume in relationship with volume of cylinder which contain root (RWVCYL)





From figure 2.6 can be seen that long conical varieties had bit lower RWVCYL (Rubrovitamina 0,51 and Autumn King 0,55 – 0,59) and scattered of point was grater than in cylindrical (Amsterdam, Bolero) or short conical (Parmex) varieties. R square values of around 0,85 and higher confirms, even for those two varieties, that at least 85% of estimated root volume can be explained by volume of cylinder that contain the root.

In the Table 2.4. can be seen that RWVCYL mean values are in the range of 0,35 – 1. It is expected that lower values are explaining conical roots, wile higher values are describing cylindrical roots. Still some examples show that RWVCYL can lead to the wrong expectations.

Table 2.4. Values of RWVCYL index for test varieties (+ one characteristic variety) in all experimental years

Variety	Year	N of cases	Minimum	Maximum	Range	Mean	Std. Error	Standard Dev	C.V.
Amsterdam	2000	358	0,46	2,87	2,41	1,023	0,02	0,38	0,367
Amsterdam	2001	151	0,21	0,99	0,79	0,667	0,01	0,14	0,213
Amsterdam	2002	194	0,20	1,17	0,96	0,732	0,02	0,22	0,297
Amsterdam	2003	50	0,52	1,53	1,02	0,879	0,03	0,23	0,263
A king	2000	502	0,25	2,85	2,60	0,609	0,01	0,16	0,268
A king	2001	181	0,26	0,95	0,69	0,596	0,01	0,12	0,200
A king	2002	123	0,17	0,90	0,73	0,440	0,02	0,17	0,397
A king	2003	50	0,28	0,70	0,42	0,471	0,02	0,11	0,227
Bolero	2000	186	0,44	3,10	2,66	0,789	0,02	0,32	0,406
Bolero	2001	157	0,22	0,96	0,75	0,652	0,01	0,13	0,206
Bolero	2002	50	0,63	1,56	0,94	0,931	0,03	0,22	0,232
hri 6070	2002	55	0,27	1,03	0,75	0,751	0,02	0,13	0,177
Parmex	2000	232	0,47	1,95	1,48	0,634	0,01	0,11	0,176
Parmex	2001	149	0,23	0,96	0,72	0,689	0,01	0,10	0,149
Parmex	2002	123	0,52	0,99	0,46	0,747	0,01	0,09	0,122
Rubrovitamina	2000	424	0,24	1,14	0,90	0,529	0,00	0,09	0,175
Rubrovitamina	2001	167	0,27	0,92	0,65	0,514	0,01	0,10	0,196
Rubrovitamina	2003	50	0,22	0,56	0,34	0,372	0,01	0,07	0,200
Cycle	lsd (p=0.05)				**	0,030			
Genotype	lsd (p=0.05)				**	0,017			
Cycle x Genotype	lsd (p=0.05)				ns	0,045			

One example RWVCYL for varieties Amsterdam, HRI 6070 and Parmex in 2002 is very similar (range from 0,72 for Parmex till 0,79 for Amsterdam) and can give indication of shape similarities for those three varieties (long and cylindrical), while in reality from the Figure 2.7. it is obvious that Parmex is short and conical, HRI 6070 is longer and conical, while Amsterdam is long and cylindrical.

Figure 2.7. Root shape of varieties with similar RWVCYL values

In the Table 2.5. are presented intervals of RWVCYL mean values for the test varieties. It shows that lowest index values of 0,42-0,6 are for long conical varieties (Autumn king and Rubrovitamina), medium of 0,5-0,7 for short conical Parmex and highest for long cylindrical (Amsterdam and Bolero). One of the reasons for shape misleading based on

the values of RWVCYL can be in the fact that certain range of RWVCYL 0,5-0,7 can be attached to all the shapes mentioned above. That drastically decrease RWVCYL precision in root shape description, what was demonstrated through the examples above.

Table 2.5. Range of RWVCYL mean values for the five test varieties

	Amsterdam	A. king	Bolero	Parmex	Rubrovitamina
RWVCYL	0,6-1	0,43-0,6	0,6-1	0,5-0,7	0,4-0,52

In the Table 2.4. is also presented Coefficient of Variation for all accessions. It show that for some varieties was recorded higher variation between the years, like Bolero has CV's of 0,206 in 2001 up to 0,406 in year 2000.

In order to check possible source of variation, two factors Variety and year of experiment were taken for the analysis of variance. Data for all test varieties was taken into consideration for the analysis and results are present in the Table 2.6.

Variation of the RWVCYL between the years is statistically significant and it can be expected that this index is under different influenced of environment in each year of experiment (Table 2.6.).

There is also significant difference in the values of RWVCYL index among analyzed varieties (Table 2.6.). That can be expected having on mind different shapes of five test varieties.

Interaction between the year of experiment realization and variety also has significant influence on the size and shape of the carrot roots which reflects on the values of RWVCYL index.

Table 2.6. Analysis of variance for RWVCYL index

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Year	4,54	1	4,54	112,416	0,00000
Variety	26,64	4	6,66	164,890	0,00000
Year*Variety	11,08	4	2,77	68,593	0,00000
Error	100,83	2496	0,04		

Further analysis of mean comparison for the RWVCYL index was done by LSD test. According to this test (Table 2.4.) for variety as a factor, there is significant difference among the means for different varieties on both probability levels. Influence of the year

when experiment was done also has significant influence on the final value of RWVCYL index, and root shape as outcome. Combination of year and variety as factors does not cause significant difference in the mean values of this index.

As a resume for RWVCYL index, can be stated from the previous discussion that this index with values in range 0,3 - 1 can help in definition of the carrot root shape. Index values between 0,5 and 0,7 are common for three different shapes, and that can lead to the wrong indications regarding carrot root shape. RWVCYL has significant variations from year to year and that should be taken into consideration when this index is applied.

2.2.4.3. RWVCYLD

Another index interesting for this study is RWVCYLD which represent ration between root volume and volume of the cylinder with base and height equal to the root diameter. It is calculated by the following formula:

$$RWVCYLD = W / VCYLD$$

In the equation W represents root volume, based on the weight and VCYLD, volume of the cylinder with base and height equal to the root diameter.

In order to define relation between root volume and volume of the cylinder with height equal to the root diameter, regression analysis was done for five test varieties.

Equation used for regression analysis was:

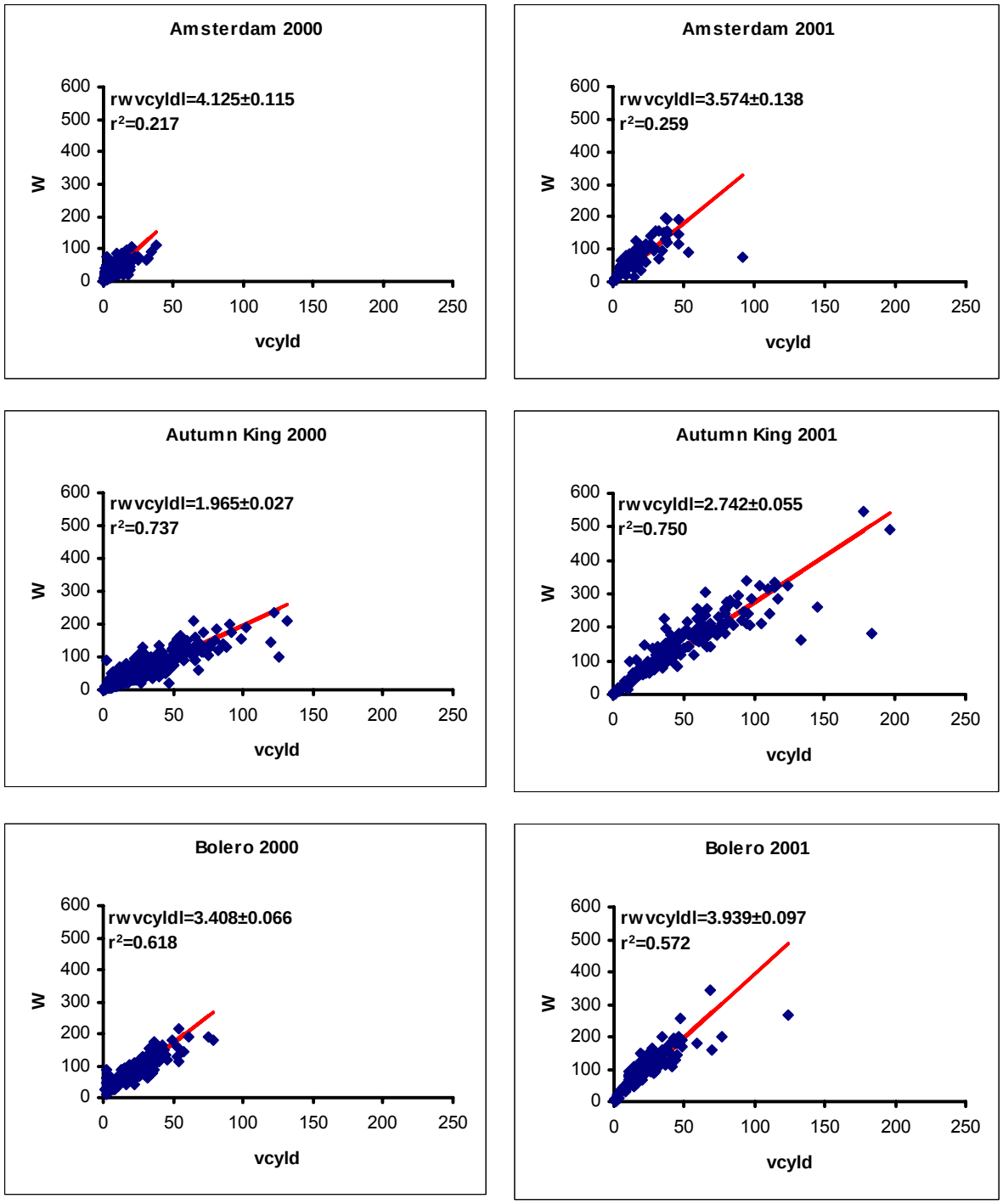
$$W = RWVCYLD * (p(d2/2)2*l) = RWVCYLD * VCYLD$$

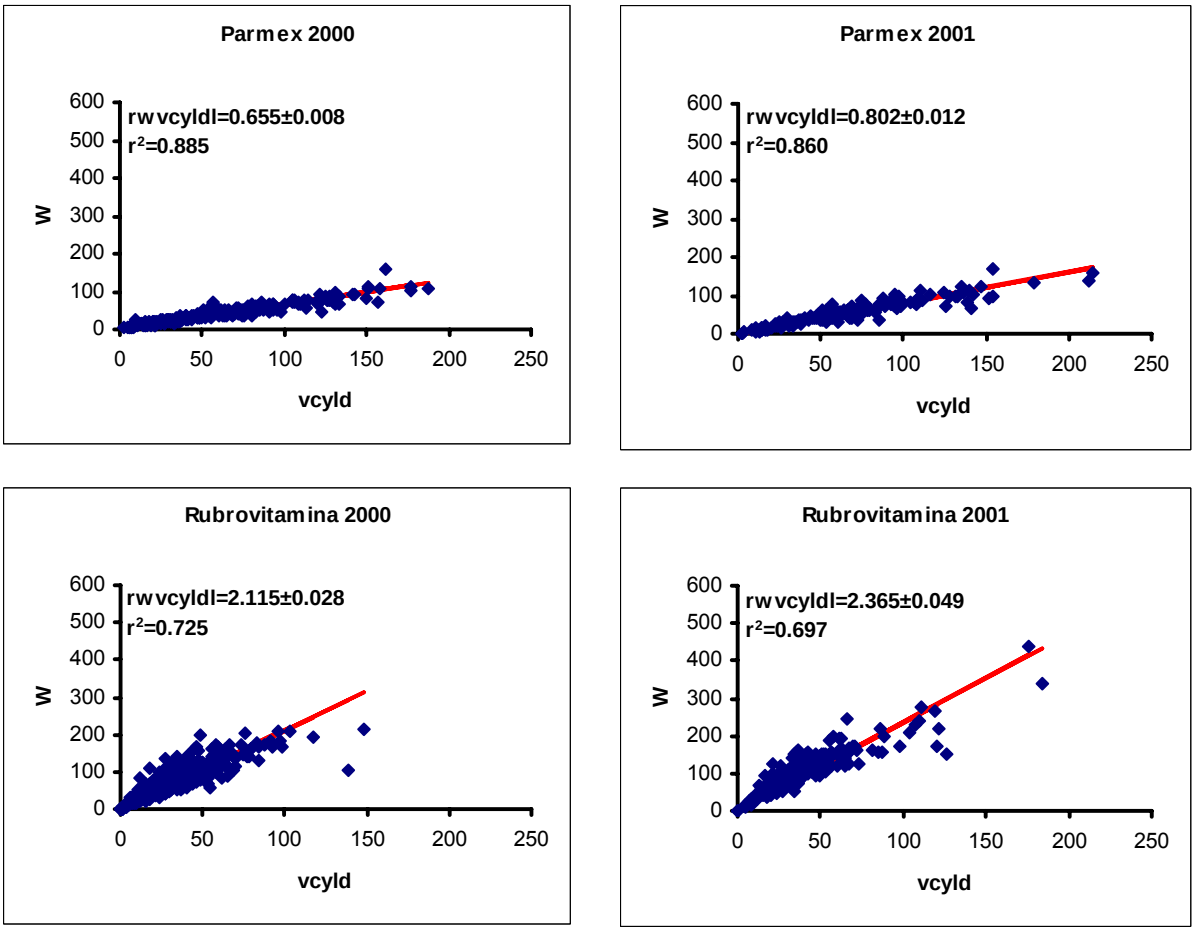
On the scatter diagrams (Figure 2.8.), for all test varieties in year 200/01, are presented slopes of relationship between the root volume and volume of the cylinder with base and height equal to the root diameter.

Linear relation was recorded for all test varieties, with highest values for variety Amsterdam, from 3,5 till 4,1, and it can be seen that dispersion of scatter points is generally wider in year 2001 for all varieties, especially Autumn King and Rubrovitamina.

R square value is very low for Amsterdam variety, 0,22 – 0,25 and indicates that only one quarter of variations in root volume can be explained by volume of the cylinder with all dimensions equal to the root diameter. For other four varieties, value of r square is over 0,6 and in that case can be estimated that cylinder volume can be good indication for the root volume, providing solid indications about it shape.

Figure 2.8. Estimated root volume in relationship with volume of cylinder with base and height equal to the root diameter





Based on the root cylindricality, RWVCYLD index can give indication about it shape. Values of RWVCYLD for five test varieties may be in the range of 0,73 and up to 7,64 (Table 2.7.).

In general, higher values (higher than 3,5 - 4) of RWVCYLD suggest that root is more cylindrical, while lower values indicates conical roots. The lowest values of RWVCYLD, under 1,0 are indicating conical and short root shape.

As a test for precision of RWVCYLD index, examples used for the previous indexes were included in the Table 2.11. and their values were used as root shape indicators.

Table 2.7**.** Mean values of RWVCYLD index for test varieties (+ five characteristic accessions) in all experimental years

Variety	Year	N of cases	Minimum	Maximum	Range	Mean	Std. Error	Standard Dev	C.V.
Amsterdam	2000	358	1,12	35,94	34,82	7,295	0,25	4,79	0,657
Amsterdam	2001	151	0,80	11,60	10,79	5,201	0,14	1,75	0,336
Amsterdam	2002	194	1,91	11,70	9,78	4,651	0,10	1,41	0,304
Amsterdam	2003	50	3,17	17,90	14,73	7,613	0,40	2,84	0,373
A king	2000	502	0,42	35,74	35,32	2,452	0,08	1,76	0,719
A king	2001	181	0,98	14,22	13,25	3,318	0,09	1,25	0,376
A king	2002	123	0,89	4,87	3,97	2,304	0,06	0,66	0,286
A king	2003	50	1,65	5,33	3,68	3,051	0,13	0,91	0,299
Bolero	2000	186	1,94	44,73	42,78	4,723	0,32	4,39	0,929
Bolero	2001	157	1,73	8,60	6,87	4,809	0,10	1,25	0,259
Bolero	2002	50	3,73	16,39	12,66	7,641	0,39	2,78	0,363
hri 10176	2002	42	1,17	7,88	6,71	2,583	0,23	1,47	0,571
hri 11169	2002	75	1,19	3,46	2,27	2,137	0,06	0,50	0,233
hri 11503	2002	91	1,24	4,70	3,47	2,741	0,08	0,76	0,277
hri 5784	2002	97	0,59	3,08	2,49	1,667	0,04	0,41	0,244
hri 6070	2002	55	1,98	6,16	4,17	3,994	0,16	1,19	0,298
Parmex	2000	232	0,39	2,92	2,53	0,739	0,02	0,23	0,315
Parmex	2001	149	0,42	1,36	0,94	0,862	0,01	0,18	0,208
Parmex	2002	123	0,49	1,63	1,14	0,734	0,01	0,15	0,205
Rubrovitamina	2000	424	0,77	7,34	6,57	2,508	0,04	0,77	0,306
Rubrovitamina	2001	167	1,20	6,00	4,80	2,885	0,07	0,84	0,291
Rubrovitamina	2003	50	1,88	5,31	3,43	2,984	0,11	0,78	0,260
Cycle	lsd (p=0.05)				**	0,364			
Genotype	lsd (p=0.05)				**	0,203			
Cycle x Genotype	lsd (p=0.05)				**	0,547			

The first test for RWVCYLD precision was comparison of the values for varieties Parmex, Amsterdam and HRI 6070. Their values were drastically different so for Parmex that was in range 0,7 – 0,85 suggesting short conical shape (Table 2.7.); for Amsterdam was between 4,7 and 7,6 (Table 2.7.) indicating long cylindrical roots and for HRI 6070 was 4, between two mentioned (Table 2.7.) indicating long conical to cylindrical roots.

Figure 2.9.a Root shape of varieties with similar RWVCYL values and different RWVCYLD index

Photos of roots, for those varieties which are presented in Figure 2.9.a, confirm that indications based on RWVCYLD index were correct.

Figure 2.9.b Root shape of varieties with similar RLD values and different RWVCYLD index

Another test of RWVCYLD index was performed on the varieties Bolero and HRI 10176. From the Table 2.7. can be seen that mean RWVCYLD value for Bolero was in a range 4,7 to 7,6 clearly suggesting long and cylindrical root shape. At the same time variety HRI 10176 has RWVCYLD of 2,5 indicating long conical root shape. Figure 2.9.b shows that indication based on the RWVCYLD value was proper.

Third test for RWVCYLD index accuracy was based on the results for HRI 11169 and HRI 11503 varieties. HRI 11169 has mean RWVCYLD value of 2,1 indicating long conical roots with big diameter. At the same time for variety HRI 11503, RWVCYLD mean value was 2,7 suggest that it root shape is long and conical (Table 2.7.). On the Figure 2.11. can be seen difference in shape of mentioned two varieties, where HRI 11169 has conical but more robust root, while HRI 11503 has more cylindrical shape.

Figure 2.11. Root shape of varieties with similar RLD values and different RWVCYLD index

In the Table 2.8. are presented intervals of RWVCYLD mean values for all test varieties in years of experiment. As expected, the highest values in interval of 4,5 – 7, for RWVCYLD index were recorded for long cylindrical varieties Amsterdam and Bolero. On the other hand short conical Parmex has the lowest index values under 1. Table 2.8. clearly demonstrates that RWVCYLD mean values were grouped into different intervals for different root shape. Those values for different root shape groups are not overlapping, that enables that RWVCYLD index gives indications about carrot root shape with much precision. In general, RWVCYLD index can also give indication for the shapes on the border of main groups like index value of 3,5 suggest long conical to cylindrical root shape, and values of around 2 (like in the presented variety HRI 11169) indicate conical robust root in diameter.

Table 2.8. Range of RWVCYLD mean values for the five test varieties

	Amsterdam	A. king	Bolero	Parmex	Rubrovitamina
RWVCYLD	4,5-7	2,5-3,3	4,5-7,5	0,7-0,85	2,5-2,9

Coefficient of Variation (CV) for all test accessions was also presented in the Table 2.7. For varieties Amsterdam, Autumn King and Bolero was recorded much higher CV in year 2000 then for other years (Table 2.7.), when it was very similar. Almost same tendency has variety Parmex with much lower differences in CV value among 2000 end other years (Table 2.7.). Only from the recorded values in year 2000 it can be concluded that RWVCYLD as index depend a lot from the year of the growing and it environmental conditions. In other years, and in case of variety Rubrovitamina, difference of CV values was very low, indicating that this index has small variations between the years and it was not a lot under environmental influence.

Variety, year of experiment and their possible interaction were taken into consideration for the analysis of variance related to all five test varieties. Data obtained by analysis of variance is presented in the Table 2.9.

Between the years variation of RWVCYLD is not statistically significant, that further supports already mentioned thesis that this index is not depending a lot from the environment which can differ from year to year. This indicate that RWVCYLD index has stable values and can be used as reliable indicator of root volume = root shape prediction.

Significant difference in the values of RWVCYLD index among analyzed varieties (Table 2.9.) is expected having on mind diversity of root shapes of five test varieties. It also enables clear differentiation among the varieties with different roots.

Combination of mentioned factors, time of experiment realization and variety, also has significant influence on the carrot root shape, according to the analysis of RWVCYLD index values.

Table 2.9. Analysis of variance for RWVCYLD index

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Year	8,97	1	8,97	1,525	0,2170
Variety	6910,02	4	1727,50	293,706	0,0000
Year*Variety	580,80	4	145,20	24,686	0,0000
Error	14680,80	2496	5,88		

For the influence of variety and year of experiment as factors that influence mean values of RWVCYLD index, statistical analysis was also done by LSD test.

Variety as a factor cause significant difference among RWVCYLD varieties means on both level of probability (Table 2.7.). Unlike analysis of variance, this statistical test show that year of the experiment, actually environmental conditions in those years have significant influence on the RWVCYLD mean values and root shape. Interaction between two mentioned factors has also significant influence on the RWVCYLD values (Table 2.7.).

From everything presented so far can be stated that RWVCYLD index has values in range from under 1 up to 8 and more, and it can be good indicator for definition of the carrot root shape. Since variation of RWVCYLD index between the years is not significant, according to analysis of variance, it can be reliable signal of root shape, because it is not under environmental influence.

2.2.4.4. RDW3

RDW3 index is developed on the basis of direct relation between diameter and root weight (Benjamin *et al*., 1989). Instead usage of log relations, like in Benjamin *et al.* studies, this index is based on the geometrical relation of mentioned factors. Equation for index calculation is the following:

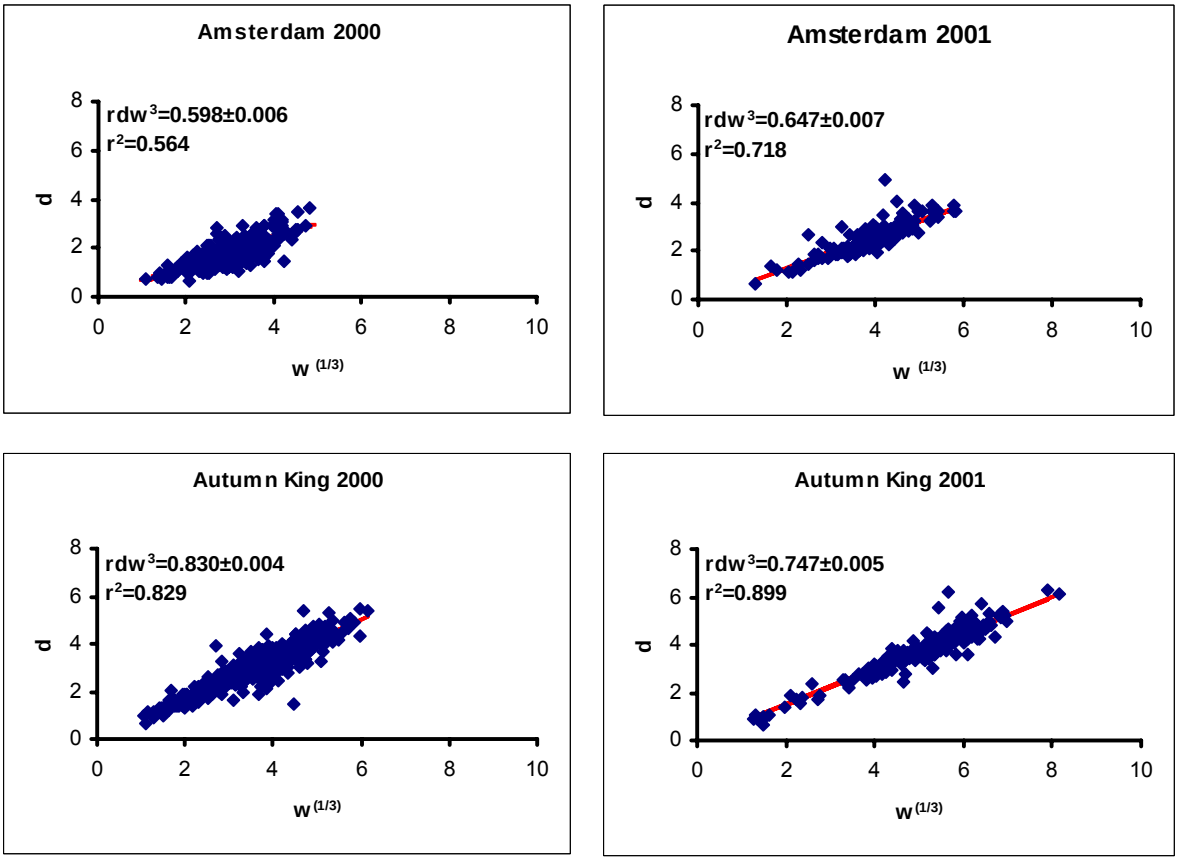
$$RDW3 = D * W^{(1/3)}$$

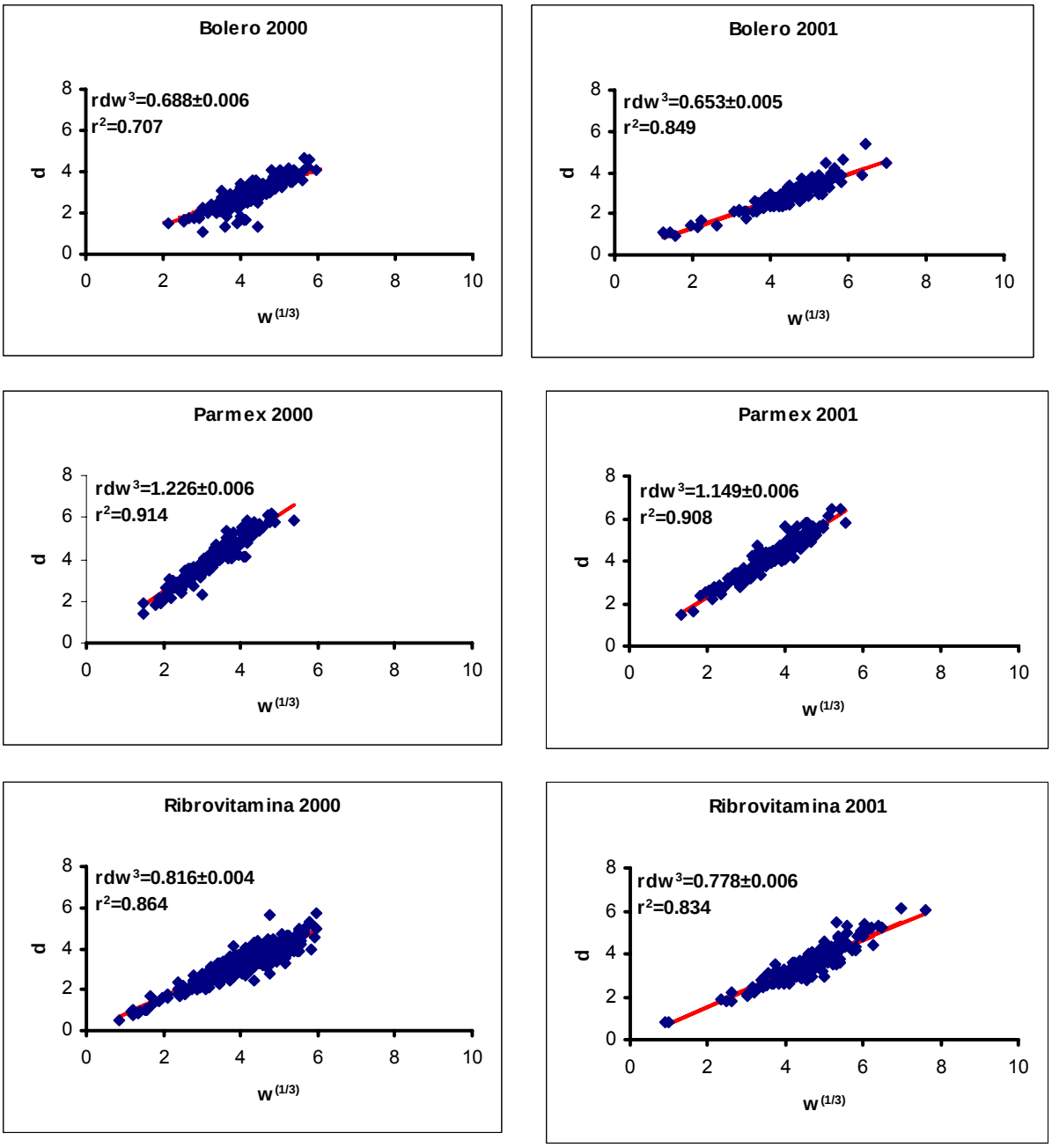
In function D is diameter value, W is the root weight.

Data from the year 2000 and 2001 for all test varieties was statistically analyzed by regression analysis. Analysis was done with aim to define possible linear relation between root estimated volume based on diameter and volume of cube with base and height equal to the root diameter.

Figure 2.11. contain scatter diagrams for all five test varieties in years 2000/01. All diagrams shows existence of linear relation between estimated root volume and volume of the cube with bases equal to the root diameter. Among the test varieties Amsterdam has the lowest correlation of mentioned volumes with RDW3 of 0,598 up to 0,647. From the diagrams, can bee seen that varieties Amsterdam and Autumn King have greater scatter of points than other three varieties. Apart from variety Amsterdam with lower r square, other varieties have it value over 0,7. It indicates that in more than 70% of cases root volume can be estimated on the basis of cube volume with basis equal to the root diameter.

Figure 2.11. Estimated root volume in relationship with volume of cube with d value as a base





Values of RDW3 index are dispersed in range of 0,6 to 1,2. Lower values are characteristic for varieties with long cylindrical shape of the root, while higher RDW3 values (1,2) are typical for short and conical roots.

Mean RDW3 values for the test varieties and five more varieties which were presented in discussion for the previous indexes were presented in the Table 2.10.

To check the precision of RDW3 index we will use same varieties like for the previous indexes as examples. Comparison of RDW3 mean values in 2002 for varieties

Amsterdam, HRI 6070 and Parmex shows different result and can give better indications about root shape. Amsterdam has the lowest RDW3 of 0,66 that indicates long cylindrical root. Variety HRI 6070 has value of 0,69 which is indicates long root in between cylindrical and conical shape, close to the later one. Finally Parmex has distinctively higher RDW3 index of 1,21 and clearly indicate short conical root. Expected shapes are very much on the line with real one presented on the Figure 2.12.

Figure 2.12. Root shape of varieties with similar RWVCYL values and different RDW3 index

Table 2.10. Mean values of RDW3 index for test varieties (+ five characteristic accessions) in all experimental years

Variety	Year	N of cases	Minimum	Maximum	Range	Mean	Std. Error	Standard Dev	C.V.
Amsterdam	2000	358	0,33	1,04	0,72	0,600	0,01	0,11	0,181
Amsterdam	2001	151	0,48	1,17	0,69	0,645	0,01	0,09	0,144
Amsterdam	2002	194	0,48	0,87	0,40	0,663	0,00	0,07	0,103
Amsterdam	2003	50	0,41	0,74	0,32	0,565	0,01	0,06	0,112
A king	2000	502	0,33	1,44	1,11	0,832	0,00	0,10	0,118
A king	2001	181	0,45	1,09	0,65	0,742	0,01	0,07	0,097
A king	2002	123	0,64	1,12	0,49	0,834	0,01	0,08	0,092
A king	2003	50	0,62	0,92	0,30	0,761	0,01	0,07	0,093
Bolero	2000	186	0,31	0,87	0,56	0,684	0,01	0,08	0,123
Bolero	2001	157	0,53	0,90	0,37	0,652	0,00	0,06	0,092
Bolero	2002	50	0,43	0,70	0,27	0,565	0,01	0,06	0,114
hri 10176	2002	42	0,54	1,03	0,49	0,833	0,02	0,12	0,150
hri 11169	2002	75	0,72	1,02	0,31	0,852	0,01	0,07	0,080
hri 11503	2002	91	0,65	1,01	0,36	0,789	0,01	0,08	0,099
hri 5784	2002	97	0,75	1,30	0,55	0,927	0,01	0,08	0,087
hri 6070	2002	55	0,59	0,86	0,27	0,698	0,01	0,08	0,108
Parmex	2000	232	0,76	1,49	0,73	1,216	0,01	0,09	0,077
Parmex	2001	149	0,98	1,45	0,47	1,150	0,01	0,08	0,072
Parmex	2002	123	0,92	1,37	0,45	1,211	0,01	0,07	0,059
Rubrovitamina	2000	424	0,56	1,18	0,63	0,812	0,00	0,08	0,094
Rubrovitamina	2001	167	0,60	1,02	0,42	0,775	0,01	0,07	0,094
Rubrovitamina	2003	50	0,62	0,88	0,26	0,763	0,01	0,06	0,080
Cycle	lsd (p=0.05)				ns	0,013			
Genotype	lsd (p=0.05)				*	0,007			
Cycle x Genotype	lsd (p=0.05)				**	0,020			

Figure 2.13. Root shape of varieties with similar RLD values and different RDW3 index

Another example of RDW3 values precision is expected shape for varieties Bolero and HRI 10176. RDW3 index for Bolero was in the range 0,56-0,68 (Table 2.10.) indicated cylindrical and long root, another variety HRI 10176 has value of 0,83 suggesting the long conical root shape. Figure 2.13. just confirms indications obtained by RDW3 index.

Last example of RDW3 index accuracy is results for HRI 11169 and HRI 11503. Value of 0,79 for HRI 11503 suggest that it root shape is long and conical. At the same time RDW3 index for HRI 11169 was 0,85 indicating also conical and long roots, but more robust (wider diameter). Figure 2.14. demonstrate that RDW3 index give precise suggestions for shape of carrot roots.

Figure 2.14. Root shape of varieties with similar RLD values and different RDW3 index

Table 2.11. contain intervals of RDW3 mean values for the test varieties in all years of experiment. Lowest index values are recorded for long cylindrical varieties Amsterdam and Bolero, and short conical Parmex has the highest index values of 1,2. It can be seen that there is no interval of overlapping index values which can be potentially attached to the more root shapes. Each main root shape is clearly defined with RDW3 interval and it gives reliable indication about real shape. Even more, in general, interval between 0,6 and 0,7 suggest shape between long cylindrical and conical roots, while range 0,83-1,2 indicate medium – short conical and robust roots.

Table 2.11. Range of RDW3 mean values for the five test varieties

	Amsterdam	A. king	Bolero	Parmex	Rubrovitamina
RDW3	0,6	0,7-0,8	0,6	1,2	0,7-0,8

Coefficient of Variation for certain varieties (Table 2.10) is pretty stable, with small value differences among the years. It suggests that RDW3 index does not depend from environmental conditions.

Analysis of variance was done also for RDW3 index of all five test varieties, taken into consideration possible differences among years and varieties as two main factors.

Unlike previous findings, analysis of variance shows that values of RDW3 were significantly different from year to year (Table 2.12) suggesting significant environmental influence on the carrot root shape.

With different shapes of test varieties it was expected to have significant differences in RDW3 index values among test varieties. That was proven by the analysis of variance and can be seen in Table 2.12. Year of experiment and variety through their interaction also have significant influence on the RDW3, actually on root shape and root size (Table 2.12.).

Table 2.12. Analysis of variance for RDW3 index

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Year	0,67	1	0,67	85,488	0,0000
Variety	72,69	4	18,17	2333,100	0,0000
Year*Variety	1,18	4	0,30	37,947	0,0000
Error	19,44	2496	0,01		

In order to define influence of mentioned factors on the RDW3 index even further, LSD test was done. Outcome of test is presented in Table 2.10., and it shows that variety as factor does not cause significant difference of rwd3 values. When year of experiment was taken into consideration, difference in mean values was recorded, but it is not significant and suggests sleight environmental influence on the value of RDW3. On the both level of significance it was not recorded significant difference in mean RDW3 values as result of interaction between variety and year of experiment.

From everything mentioned above about RDW3 index can be concluded that it can be used as a good indicator for the carrot root shape. Since it has clearly defined value intervals for different root shapes (0,5-0,68 for long cylindrical; 0,7-0,8 for long conical; 1,2 for short conical) it can be used with great precision in root shape description. Like other indexes, RDW3 variation can be significant between years, depending from the environmental conditions.

2.2.4.5. RVTRCONVCYLD

Base for development of RVTRCONVCYLD index was idea to cut the root from the crown at the distance that is equal to the root diameter. Volume of trunk of cone created by that action should be compared with the volume of cylinder which contains it.

Calculation of the RVTRCONVCYLD was done by the following equation:

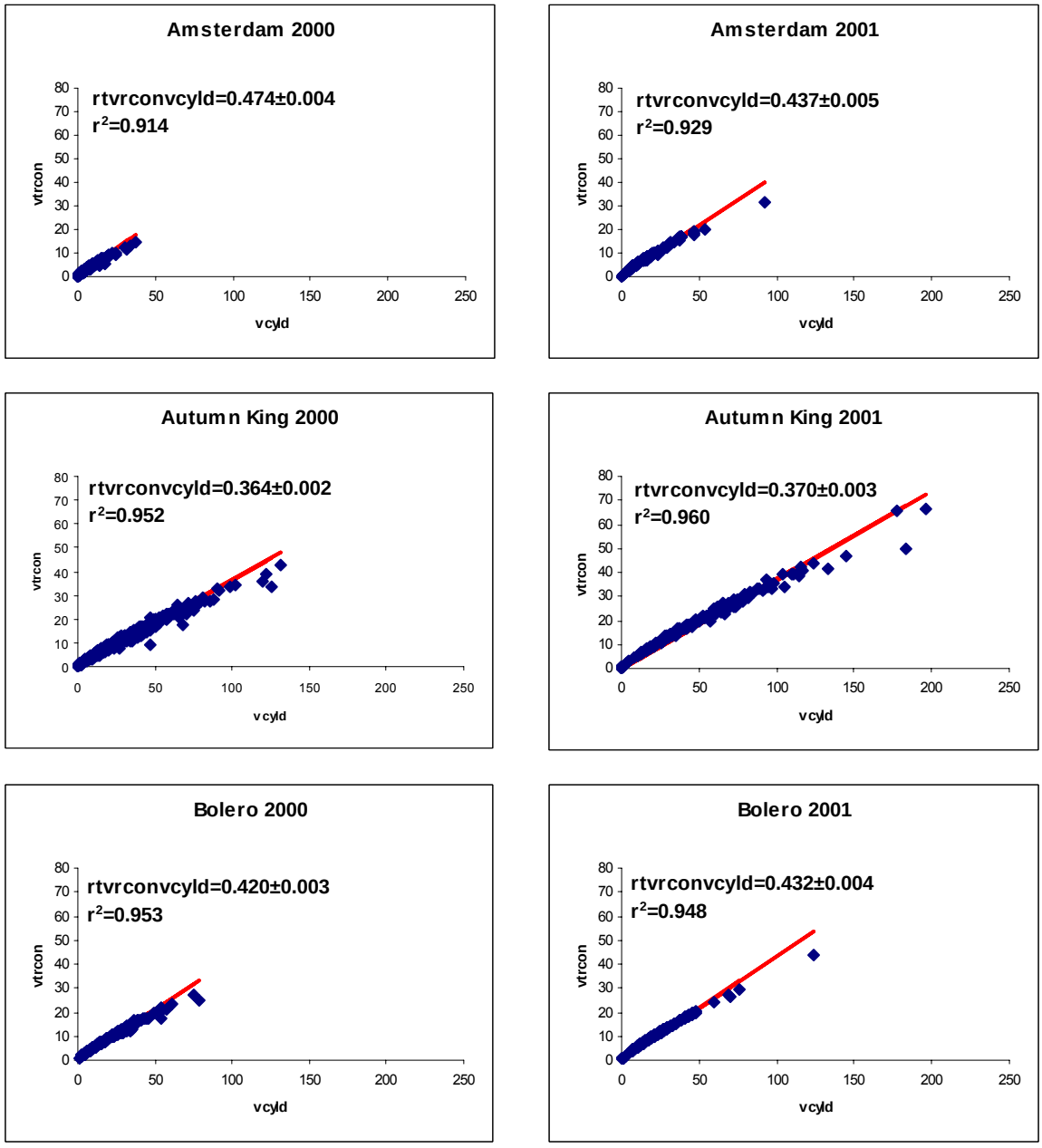
$$RVTRCONVCYLD = VTRCONV * VCYLD$$

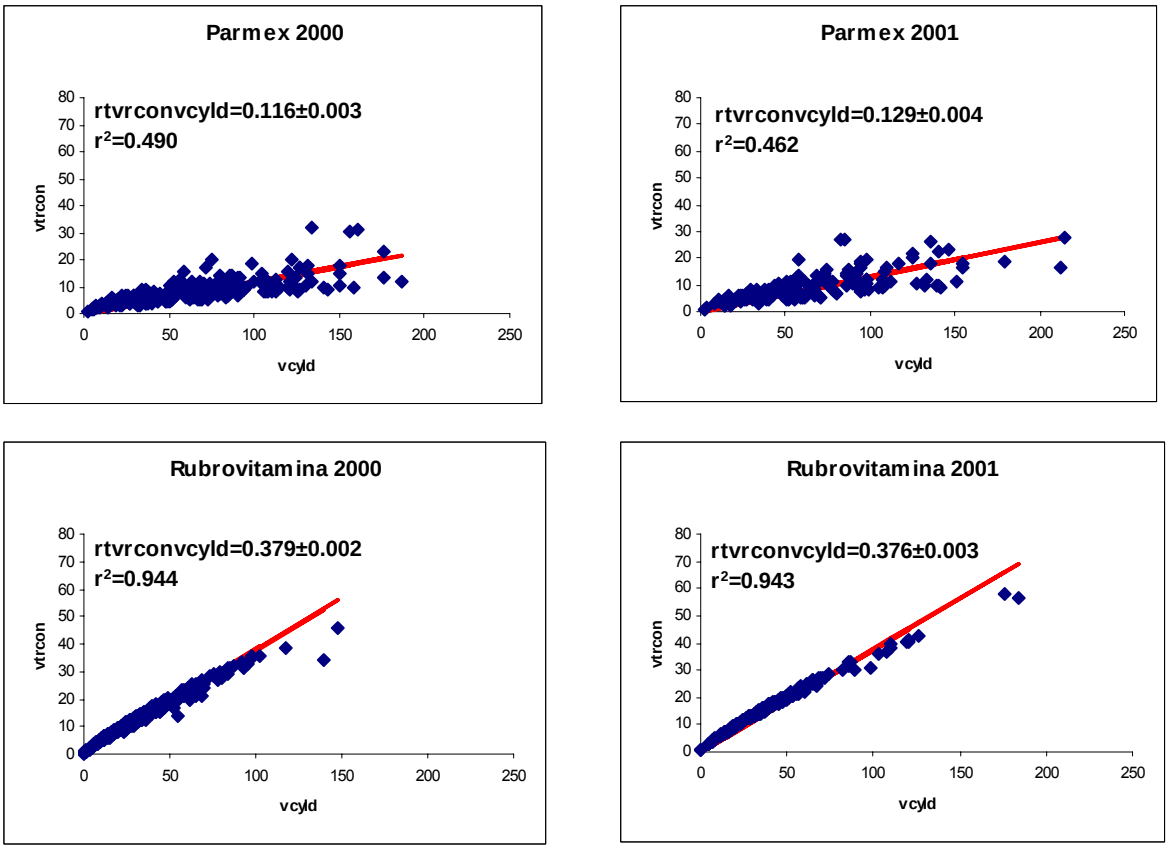
In the function VTRCONV presents volume of the trunk of cone with both bases and height equal to the diameter and VCYLD is the volume of cylinder with base and height same as the root diameter.

Regression analysis was done for all five test varieties in order to analyze possible linear relation between volume of the cone with all dimensions equal to the root diameter and volume of the cylinder that contain it.

On the Figure 2.15. are presented scatter diagrams with distribution of RVTRCONVCYLD index for all five test varieties. Apart from the Parmex variety, all other diagrams shows existence of strong linear relation between trunk of cone with dimensions same as diameter and cylinder with dimension equal to the root diameter. Variety Parmex has lowest RVTRCONVCYLD index 0,11 – 0,13 and also very low r square values 0,46 - 0,49.

Figure 2.15. Estimated root volume in relationship with volume of cube with D value as a base





Mentioned r square values for Parmex variety suggest that only 46 – 50% of all variations were define with this regression model. In case of other four varieties r square are indicating that over 90% of variation was covered by this regression slope.

Greater scatter of points and their wider distribution was recorded for Parmex variety (Figure 2.15.). That higher variation can be explained by the fact that Parmex has wider diameter then other varieties, it can varies a lot and directly had an influence on the point distribution around equation slope for RVTRCONVCYLD.

Values of RVTRCONVCYLD index are in range of 0 to 1 measuring cylindricality of the root. Higher values, closer to 1, are characteristic for varieties with long cylindrical shape of the root, while lower RVTRCONVCYLD values (0,15 – 0,18) are typical for short and conical roots (Table 2.13.).

Mean values of RVTRCONVCYLD test varieties and four more varieties which were discussed for the previous indexes were presented in the Table 2.13.

Table 2.13. Mean values of RVTRCONVCYLD index for test varieties (+ four characteristic accessions) all experimental years

Variety	Year	N of cases	Minimum	Maximum	Range	Mean	Std. Error	Standard Dev	C.V.
Amsterdam	2000	358	0,31	1,23	0,93	0,624	0,01	0,13	0,205
Amsterdam	2001	151	0,34	1,31	0,97	0,538	0,01	0,11	0,196
Amsterdam	2002	194	0,40	1,01	0,62	0,649	0,01	0,09	0,137
Amsterdam	2003	50	0,40	0,74	0,34	0,579	0,01	0,06	0,102
A king	2000	502	0,19	1,06	0,87	0,433	0,00	0,09	0,196
A king	2001	181	0,27	1,27	1,00	0,446	0,01	0,13	0,295
A king	2002	123	0,21	1,07	0,86	0,519	0,01	0,13	0,244
A king	2003	50	0,38	0,57	0,19	0,466	0,01	0,04	0,089
Bolero	2000	186	0,32	0,86	0,55	0,474	0,01	0,08	0,161
Bolero	2001	157	0,35	1,03	0,68	0,501	0,01	0,09	0,177
Bolero	2002	50	0,46	0,69	0,23	0,569	0,01	0,06	0,098
hri 10176	2002	42	0,31	1,26	0,95	0,635	0,04	0,25	0,395
hri 11169	2002	75	0,32	0,88	0,56	0,511	0,01	0,13	0,249
hri 11503	2002	91	0,31	0,81	0,49	0,528	0,01	0,10	0,197
hri 6070	2002	55	0,46	1,07	0,61	0,644	0,02	0,12	0,182
Parmex	2000	232	0,06	0,48	0,42	0,157	0,01	0,08	0,487
Parmex	2001	149	0,06	0,47	0,41	0,163	0,01	0,07	0,445
Parmex	2002	123	0,08	0,53	0,44	0,196	0,01	0,08	0,423
Rubrovitamina	2000	424	0,25	1,29	1,05	0,440	0,00	0,09	0,215
Rubrovitamina	2001	167	0,31	0,92	0,61	0,437	0,01	0,07	0,167
Rubrovitamina	2003	50	0,40	0,60	0,19	0,502	0,01	0,04	0,089
Cycle	lsd (p=0.05)				ns	0,014			
Genotype	lsd (p=0.05)				*	0,008			
Cycle x Genotype	lsd (p=0.05)				ns	0,022			

Testing of RVTRCONVCYLD index as shape indicator was done by comparison of estimated root shape based on the index value with root shape on the photos made during the experiment.

The first test took into consideration varieties HRI 10176, Amsterdam and HRI 6070. From the Table 2.13. can be seen that all mentioned varieties in the year 2002 had similar RVTRCONVCYLD value, around 0,64. Based on that result, all mentioned varieties should have long roots with cylindrical shape. In the reality (Figure 2.16.) variety HRI 10176 has conical shape, Amsterdam has cylindrical shape, and HRI 6070 is between mentioned two varieties.

Figure 2.16. Root shape of varieties with similar RVTRCONVCYLD index values

Another example of RVTRCONVCYLD index accuracy are results for HRI 11169 and HRI 11503 varieties. Those varieties have similar values of RVTRCONVCYLD, around 0,52 (Table 2.13.), indicating similar conical root shape. Figure 2.17. show mentioned similarities in the root shape, but differences like cylindrical shape for HRI 11503, can not be detected by RVTRCONVCYLD index, especially not so precise like it was case with RWVCYLD and RDW3 indexes.

Figure 2.17. Root shape of varieties with similar RVTRCONVCYLD index

Range of mean RVTRCONVCYLD values for the test varieties in all years of experiment is shown in the Table 2.14. It demonstrated that RVTRCONVCYLD value is in the range of 0,15 to 0,6. Since this index is based on comparison of the volume for trunk of cone, with basis equal to the diameter of the root, and volume of the cylinder which contain that cone, it higher values explains cylindrical roots and vice versa.

Table 2.14. Range of RVTRCONVCYLD mean values for the five test varieties

	Amsterdam	A. king	Bolero	Parmex	Rubrovitamina
RVTRCONVCYLD	0,6	0,45-0,5	0,5-0,55	0,15-0,2	0,45-0,5

It can be seen from Table 2.14. that varieties, with long cylindrical roots like Amsterdam and Bolero, have the highest RVTRCONVCYLD value of 0,5 – 0,6 (Table 2.20.). Completely opposite results of 0,15 to 0,2 index value were recorded for short and conical Parmex variety. Index values of 0,45 – 0,5 were calculated for long and conical roots like Autumn King and Rubrovitamina. Range in RVTRCONVCYLD values of 0,45

– 0,6 can be difficult for application for root shape indication, since varieties from group with conical and group of cylindrical roots can have results in that range. That makes difficult usage of this index for precise determination of carrot root shape between long conical and long cylindrical group.

Influence of environmental conditions on the carrot plant/root can be indicated by values of Coefficient of Variation for test varieties (Table 2.13.) in different years.

CV of all test varieties was different from year to year, suggesting that values of RVTRCONVCYLD are very much under environmental influence.

Analysis of variance for the data obtained from all test varieties in different years was focused on the major factors, year and variety, and their influence on the RVTRCONVCYLD variations end influence of environment.

In the case of the first factor, year of experiment, it was recorded statistically significant difference among the years (Table 2.15.). That is clear indication for possible environmental influence on the carrot root diameter, volume and shape.

Difference among varieties as a second factor of analysis of variance for RVTRCONVCYLD is statistically significant. That was expected knowing diversity of the root shapes for the test varieties.

Interaction of mentioned factors, year of experiment and variety also has significant influence on the on root shape and volume according to the influence on the RVTRCONVCYLD index (Table 2.15.).

Table 2.15. Analysis of variance for RVTRCONVCYLD index

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Year	0,04	1	0,04	4,410	0,0358
Variety	37,40	4	9,35	999,258	0,0000
Year*Variety	0,83	4	0,21	22,146	0,0000
Error	23,35	2496	0,01		

Influence of variety and year of production on the RVTRCONVCYLD index was also checked by LSD test. Opposite from analysis of variance, in LSD test variety as a factor did not cause significant difference of RVTRCONVCYLD means among different varieties for both probability levels (Table 2.13.). Year of experiment, similar to the RDW3 index, has significant influence on means of the RVTRCONVCYLD on 0,05

probability level. Influence of year as a factor was not statistically significantly on the 0,01 probability level. Interaction between variety and year did not have statistically significant influence on RVTRCONVCYLD index values (Table 2.13.).

Regarding RVTRCONVCYLD index can be resumed that is the one of indexes which is based on the comparison of two volumes, volume of the trunk of cone with basis equal to the root diameter, and volume of the cylinder that contain mentioned cone. Values of this index goes in the range of 0,15 to 0,6 (for our test varieties) and higher it is, more cylindrical shape has the carrot root. On the basis of RVTRCONVCYLD value it is hard to predict precise root shape if the value falls into interval 0,45 to 0,6 since that number can be dedicated to the long conical, but also to the long cylindrical roots. Influence of the environment in different years is significant on the RVTRCONVCYLD value, making it less reliable in root shape predictions.

2.2.4.6. Principal component analysis

2.2.4.6.1. Introduction

Principal component analysis was done as a final study of existing data with the idea to reduce number of variables (indexes) by deriving factors which are linear combinations of individual variables.

Principal component analysis defines correlation between original variables (indexes) and principal components (factors) and it results are presented in the Table 2.16. Into consideration were taken three major factors, since they cumulatively explains 90% of variations (Table 2.16.).

Table 2.16. Correlation between original variables and principal components

INDEX	1	2	3
L	-0,39038	0,64308	-0,60123
D	0,69398	0,32541	-0,54900
RLD	-0,91129	0,10839	0,15222
RWVCYL	0,04323	-0,86814	-0,47637
RDW3	0,86367	0,15638	0,34200
RWVCYLD	-0,75825	-0,47180	-0,34396
RVTRCONVCYLD	-0,89744	-0,07326	0,34854
RWVCYLRDL	0,67012	-0,59248	0,09283
Variance explained	50,52052	23,60398	15,97942
Cumulative %	50,52052	74,1245	90,10392

From Table 2.16. it can be seen that the first component has positive correlation with:

- RDW3 (index which is low for long cylindrical roots and high for short conical roots)
- RWVCYLRDL (the ratio between root weight and volume of the cylinder, multiplied by d/l)
- D (root diameter)

On the other hand this first factor is negatively correlated to:

- RLD (the length / diameter ratio)
- RWCYLD (indicates the cylindricality, but divided by L/d)
- RVTRCONVCYLD (higher for long roots)
- L slightly correlated (root length)

The first component, explaining 50.5% of variability, contains both a positive relation to diameter and to conic shape. This factor seems to be associated, at high positive values with short and large conic roots.

The second factor explains 23.6% of total variability. Second component is correlated positively to:

- L root length (L)
- D root diameter (slightly)
- RWVCYL (cylindricality it is low for conic roots; negative correlation indicates conic roots at the positive side)
- RWCLD (same correlation as factor 1, but lower values)
- RWVCYLRDL (higher for long roots)

This component therefore is rather clearly related to long and conically shaped roots.

The third component which explains other 16% of variability is positively correlated to: RDW3 and RVTRCONVCYLD.

This factor demonstrated negative correlation with: L, D, RWCYL, and RWVCYLD. Its meaning is not very immediate, but perhaps it seems connected (on negative side) with big, long and cylindrical shaped roots.

2.2.4.6.2. Principal component 1

Range of mean values of the first principal component has been presented in the Table 2.17.

In general Factor 1 has values in a range from negative to positive one. Positive one should be related, as it was mentioned, to the short and large conical root, e.g. variety Parmex with mean value of around 2,1. This factor should have negative mean values for long cylindrical roots, e.g. Amsterdam variety with values of -0,7 to -1,1. Problem can be for the values of -0,45 to -0,20 since then is unclear if negative value is due to the small diameter, or shape somewhere between conical and cylindrical or because length of the root.

Table 2.17. Range of mean values for factor 1 of test varieties for all experimental years

	Amsterdam	A. king	Bolero	Parmex	Rubrovitamina
factor 1	(-1,12)-(-0,67)	(-0,19)-(0,36)	(-1)-(-0,25)	(2)-(2,2)	(-0,43)-(0,2)

To test precision of factor 1, several examples were used and compared with photos of the chosen varieties.

Table 2.18. Mean values of factor 1 for test varieties (+ four interesting accessions) all experimental years

Variety	Year	Statistic	N of cases	Minimum	Maximum	Range	Mean	Std. Error	Standard Dev	C.V.
Amsterdam	2000	Factor 1	358	-4.77	1.23	6.00	-0.870	0.04	0.79	-0.904
Amsterdam	2001	Factor 1	151	-3.35	1.12	4.47	-0.717	0.05	0.57	-0.798
Amsterdam	2002	Factor 1	194	-2.20	0.73	2.92	-0.676	0.03	0.47	-0.690
Amsterdam	2003	Factor 1	50	-2.44	0.11	2.55	-1.129	0.07	0.50	-0.441
A king	2000	Factor 1	502	-3.97	3.25	7.22	0.363	0.02	0.46	1.269
A king	2001	Factor 1	181	-3.65	1.46	5.11	-0.031	0.04	0.55	-17.580
A king	2002	Factor 1	123	-1.66	1.84	3.50	-0.027	0.05	0.50	-18.816
A king	2003	Factor 1	50	-0.96	0.39	1.34	-0.194	0.05	0.33	-1.713
baz 56341	2002	Factor 1	31	-2.31	0.80	3.11	-0.796	0.13	0.70	-0.881
Bolero	2000	Factor 1	186	-4.93	0.91	5.84	-0.255	0.05	0.65	-2.538
Bolero	2001	Factor 1	157	-2.67	0.56	3.24	-0.597	0.03	0.39	-0.650
Bolero	2002	Factor 1	50	-2.22	-0.18	2.04	-1.051	0.07	0.48	-0.455
hri 10305	2002	Factor 1	72	-1.23	1.50	2.73	0.009	0.06	0.52	57.078
hri 11169	2002	Factor 1	75	-1.08	0.96	2.04	0.018	0.06	0.48	27.202
hri 11503	2002	Factor 1	91	-1.56	1.20	2.76	-0.129	0.05	0.52	-3.997
Parmex	2000	Factor 1	232	0.72	3.11	2.39	2.175	0.03	0.47	0.214
Parmex	2000	Factor 3	232	-1.19	2.02	3.21	0.807	0.03	0.45	0.556
Parmex	2001	Factor 1	149	0.72	3.07	2.35	2.082	0.03	0.41	0.198
Parmex	2002	Factor 1	123	0.82	3.32	2.50	2.220	0.04	0.43	0.192
Rubrovitamina	2000	Factor 1	424	-1.36	1.66	3.02	0.201	0.02	0.39	1.936
Rubrovitamina	2001	Factor 1	167	-0.94	0.98	1.92	0.019	0.03	0.39	21.004
Rubrovitamina	2003	Factor 1	50	-1.21	0.22	1.43	-0.436	0.04	0.31	-0.706
Cycle	lsd (p=0.05)						0.079			
Genotype	lsd (p=0.05)						0.044			
Cycle x Genotype	lsd (p=0.05)						0.119			

First example from accessions was variety BAZ 56431 which has very negative 1st factor –0,80 (Table 2.18.), due do its length or cylindricality. It suggests that this variety should

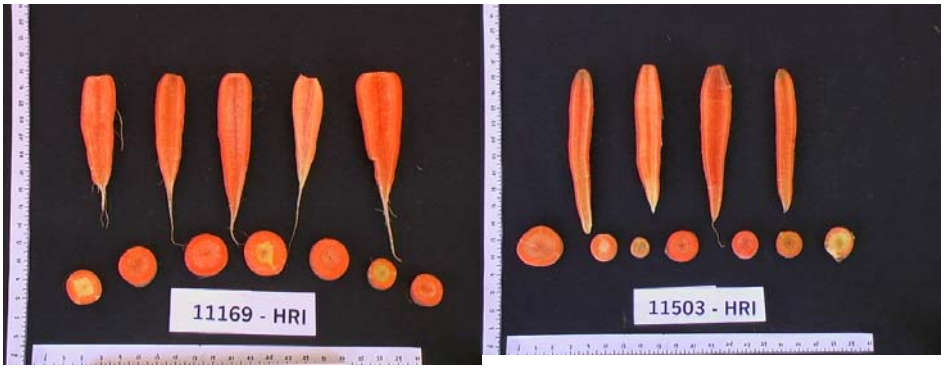
have long cylindrical root. From Figure 2.18. can be seen that high negative value for factor 1 is related less to the length, but more to the low root diameter.

Another tested variety was HRI 10305. In the Table 2.18. can be seen vary low positive value for factor 1 of 0,009 indicating that root is conical or short, but still positive for this factor. Photo of this variety from Figure 2.18., proves that indication was good, and looks like positive value of factor one was also related to the wider root diameter.

Figure 2.18. Root shape of varieties used for testing of factor 1

Final checking of factor 1 precision was done on the varieties HRI 11169 and HRI 11503. Variety HRI 11169 has slightly positive factor 1 (0,018) since it has conical shape or wider diameter. From factor values can be suggested that HRI 11169 has long conical root and large one, as a result of possibly higher diameter values. Photo on Figure 2.19. of HRI variety supports this indication. Variety HRI 11503 has negative value for factor 1 on the level of – 0,13. Since factor 1 has slightly negative value, prediction for root shape of this variety should be cylindrical and medium length root. It looks like this is good identification of the HRI 11503 root shape if it is compared with photo from Figure 2.19.

Figure 2.19. Root shape of varieties used for testing of factor 1



Coefficient of Variation for factor 1 varies a lot from year to year (Table 2.18.).

Highest CV differences for factor 1 was recorded for Autumn King (-18,8 to 1,27) and Amsterdam (-0,44 to -0,90). Other varieties have lower CV differences and variations between the years, when factor 1 is taken into consideration.

That indicates possible environmental influence on the factor values and on the root shape, as final outcome.

Analysis of variance for the data obtained from all test varieties in different cycles was focused on the major factors, year and variety, and their influence on the factors 1 and 2 variations.

Influence of year of experiment on the factor 1 was tested by analysis of variance (ANOVA) and LSD test. Year caused statistically significant difference of factor 1 in ANOVA (Table 2.19.) and LSD test (Table 2.18.). Those findings are indicating important environmental influence on the factor 1 mean values and it variations in different years.

From Table 2.19. can be seen that Factor 1 was significantly different, for different carrot varieties. That significant influence on factor 1 mean values was confirmed through LSD test (Table 2.18.). Those results are confirming great variations among carrot test varieties in the root diameter, shape and length.

Interaction of production cycle and varieties also demonstrated significant influence on the factor 1 of principal component analysis and on the root shape (Table 2.19.).

Table 2.19. Analysis of variance for principal component 1

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Year	15,11	1	15,11	54,243	0,0000
Variety	1908,53	4	477,13	1713,060	0,0000
Year*Variety	20,62	4	5,16	18,510	0,0000
Error	695,21	2496	0,28		

From the above discussion can be resumed that factors 1 can be used for solid indication on the carrot root shape and length and largeness in most of the cases. Still some examples prove that main problem with factor usage is in the fact that they are related, positively or negatively, to the more then one root characteristic (index). Then in the cases like intervals for factor 1 and values of -0,45 to -0,20 it is difficult to identify

source of certain value. Is that value based on the root shape (cylindricality), or diameter, or length? The root shape prediction is not straight forward based on the results for factor 1, it requests also different combining of shape, length and diameter.

2.2.4.6.3. Principal component 2

Factor 2 should have higher values for the varieties with longer conical roots. As en example can be used variety Rubrovitamina where factor 2 values gives good indication (values in range 0,12 to 0,92) (Table 2.20.). On the other side variety with similar shape, Autumn King has range of mean values for factor 2 from -0,39 to 0,52. It shows that factor 2 is not so precise and reliable in root shape definition. Variety Parmex has very low negative factor 2 since it has short roots, while varieties Bolero and Amsterdam have negative values due to the cylindrical shape.

Table 2.20. Range of mean values for factor 2 of test varieties for all experimental years

	Amsterdam	A. king	Bolero	Parmex	Rubrovitamina
factor 2	(-2)-(-0,36)	(-0,39)-(0,53)	(-1,4)-(-0,07)	(-2)-(-1,12)	(0,11)-(0,92)

Performance of factor 2 for root shape indication, was tested through several examples.

Table 2.21. Mean values of factor 2 for test varieties (+ four interesting accessions) all experimental years

Variety	Year	N of cases	Minimum	Maximum	Range	Mean	Std. Error	Standard Dev	C.V.
Amsterdam	2000	358	-8.64	0.05	8.69	-2.046	0.07	1.36	-0.666
Amsterdam	2001	151	-1.96	1.54	3.49	-0.360	0.05	0.57	-1.574
Amsterdam	2002	194	-2.92	1.06	3.98	-1.140	0.06	0.82	-0.718
Amsterdam	2003	50	-3.59	0.24	3.83	-1.213	0.12	0.85	-0.701
A king	2000	502	-8.70	1.98	10.68	-0.396	0.03	0.75	-1.890
A king	2001	181	-2.09	1.85	3.94	0.204	0.04	0.52	2.566
A king	2002	123	-2.02	1.48	3.50	0.165	0.07	0.77	4.688
A king	2003	50	-0.38	1.51	1.89	0.530	0.06	0.45	0.846
baz 56341	2002	31	-1.60	1.31	2.91	-0.014	0.10	0.55	-38.091
Bolero	2000	186	-10.10	0.24	10.35	-0.803	0.09	1.25	-1.557
Bolero	2001	157	-1.20	1.08	2.28	-0.078	0.04	0.45	-5.809
Bolero	2002	50	-3.88	-0.18	3.70	-1.407	0.12	0.82	-0.582
hri 10305	2002	72	-2.42	1.45	3.87	0.419	0.08	0.71	1.694
hri 11169	2002	75	-1.48	1.29	2.78	0.384	0.08	0.66	1.717
hri 11503	2002	91	-1.98	1.18	3.15	-0.171	0.08	0.77	-4.524
Parmex	2000	232	-6.07	0.05	6.13	-1.121	0.04	0.56	-0.497
Parmex	2001	149	-3.89	1.35	5.24	-1.219	0.05	0.66	-0.540
Parmex	2002	123	-4.41	-0.62	3.78	-2.068	0.06	0.64	-0.307
Rubrovitamina	2000	424	-2.14	1.18	3.32	0.117	0.03	0.53	4.496
Rubrovitamina	2001	167	-1.15	1.42	2.56	0.469	0.04	0.45	0.967
Rubrovitamina	2003	50	-0.03	1.61	1.64	0.923	0.05	0.36	0.385
Cycle	sd (p=0.05)					0.122			
Genotype	sd (p=0.05)					0.068			
Cycle x Genotype	sd (p=0.05)					0.183			

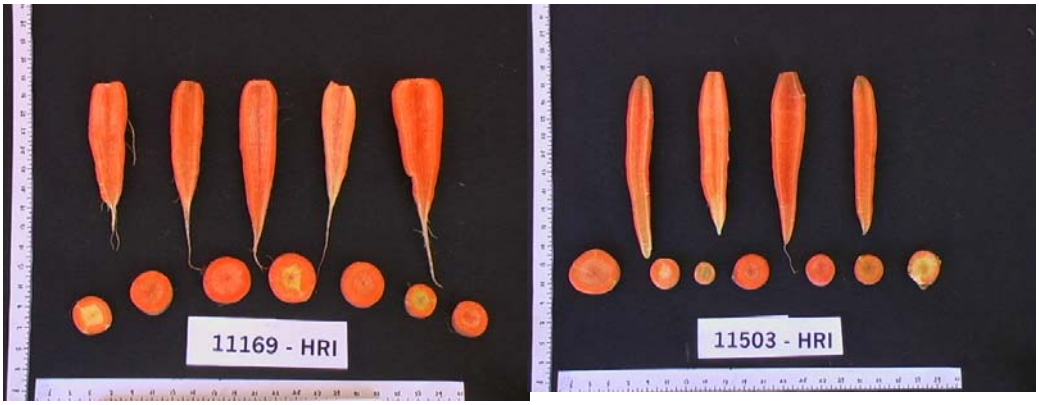
Accessions BAZ 56431 was the first example which has very slightly negative factor 2, which suggests that this variety should have long conical root. Figure 2.20. confirms that indication.

Another tested variety was HRI 10305 with factor 2 on a level of 0,42 and indicates that this is long conical root. Photo on Figure 2.20. confirms it conical shape, but the length is more medium than long.

Figure 2.20. Root shape of varieties used for testing of factor 2

Last test of factor 2 precision was done with varieties HRI 11169 and HRI 11503. Variety HRI 11169 has positive factor 2 of 0,38 (Table 2.21.). From factor value can be suggested that HRI 11169 has long conical root. Photo on Figure 2.21. of HRI variety supports this indication. Variety HRI 11503 has negative value for second factor on the level of -0,17. Having in mind negative value, prediction for root shape of this variety should be conical and medium length root. It looks like this identification of the HRI 11503 root shape is not so precise, it looks more cylindrical on photo on Figure 2.21.

Figure 2.21. Root shape of varieties used for testing of factor 2



Influence of variety and year of production was analyzed by analysis of variance. The second principal component was under influence of experimental cycle and variety. That influence was statistically significant for both, year and variety as factors. That shows important influence of environment and genotype characteristics on the carrot root (Table 2.22.). This hypothesis was also confirmed by the results of LSD test (Table 2.21.) with statistically significant differences in mean values of second factor.

Relation between year of experiment and varieties has statistically significant influence on the second factor of principal component analysis according to the analysis of variance (Table 2.22.) and LSD test (Table 2.21.). It is clear that the same influence could be expected for the shape of carrot root.

Table 2.22. Analysis of variance for principal component 2

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Year	221,72	1	221,72	335,733	0,0000
Variety	759,96	4	189,99	287,686	0,0000
Year*Variety	176,03	4	44,01	66,636	0,0000
Error	1648,39	2496	0,66		

Above discussion is not in favor of factor 2 usage in root shape indications. It has uncertainty since it is has more than one component that influence on it value. It also has great interval of values, which can be attached to the completely different root shapes.

2.2.4.6.4. Principal component 3

This part will cover possible usage of principal component 3 in definition of carrot root shape. According to the relationship of factor 3 with variables (indexes) of principal component analysis, it should be positively related to RDW3 and RVTRCONVCYLD.

Ranges of mean values of the factor 3 for test varieties are listed in Table 2.23. That range of factor 3 values goes from negative to positive one. Negative values should be related to the long cylindrical roots, Amsterdam and Bolero like. In the Table 2.23. can be seen that mentioned two varieties have factor 3 values from -1,3 to -0,31. Positive factor 3 values are recorded for short and conical roots (e.g. Parmex); on the border of negative and positive values are varieties with conical root of different length and root diameter. It is hard to define root shape for the varieties with values of third factor in

interval – 0,98 to -0,30 because it is difficult to determine the source of low values. It can be as result of small diameter, shortness, cylindricality of the root.

Table 2.23. Range of mean values for factor 1 of test varieties for all experimental years

	Amsterdam	A. king	Bolero	Parmex	Rubrovitamina
factor 3	(-1,2)-(-0,31)	(-0,98)-(0,59)	(-1,33)-(-0,88)	(0,4)-(1,3)	(-0,74)-(1,3)

This factor was used as a tool to identify root shapes of certain chosen varieties.

Table 2.24. Mean values of factor 3 for test varieties (+ four) all experimental years

Variety	Year	N of cases	Minimum	Maximum	Range	Mean	Std. Error	Standard Dev	C.V.
Amsterdam	2000	358	-7.19	1.47	8.67	-0.739	0.06	1.20	-1.623
Amsterdam	2001	151	-2.22	1.77	3.99	-0.793	0.05	0.67	-0.849
Amsterdam	2002	194	-1.19	1.77	2.95	0.311	0.05	0.63	2.020
Amsterdam	2003	50	-3.87	0.12	4.00	-1.281	0.11	0.79	-0.619
A king	2000	502	-7.65	2.45	10.09	0.119	0.03	0.74	6.280
A king	2001	181	-3.16	2.12	5.28	-0.987	0.07	0.88	-0.895
A king	2002	123	-1.39	2.10	3.49	0.591	0.06	0.68	1.153
A king	2003	50	-1.43	0.25	1.68	-0.507	0.07	0.50	-0.995
baz 56341	2002	31	-0.46	2.45	2.91	0.818	0.16	0.89	1.086
Bolero	2000	186	-9.06	0.89	9.94	-0.889	0.07	1.00	-1.124
Bolero	2001	157	-2.56	2.44	5.00	-1.116	0.06	0.74	-0.661
Bolero	2002	50	-3.13	-0.21	2.92	-1.330	0.09	0.66	-0.493
hri 10305	2002	72	-1.47	2.28	3.75	0.576	0.11	0.90	1.566
hri 11169	2002	75	-1.13	2.49	3.62	0.525	0.10	0.84	1.603
hri 11503	2002	91	-0.68	1.85	2.53	0.477	0.07	0.64	1.346
Parmex	2000	232	-1.19	2.02	3.21	0.807	0.03	0.45	0.556
Parmex	2001	149	-0.78	2.00	2.78	0.487	0.04	0.49	1.012
Parmex	2002	123	0.11	2.55	2.44	1.341	0.04	0.40	0.301
Rubrovitamina	2000	424	-1.79	2.02	3.80	-0.176	0.03	0.63	-3.577
Rubrovitamina	2001	167	-2.20	2.71	4.91	-0.743	0.05	0.65	-0.872
Rubrovitamina	2003	50	-1.45	0.29	1.74	-0.344	0.05	0.37	-1.073
Cycle	lsd (p=0.05)					0.120			
Genotype	lsd (p=0.05)					0.067			
Cycle x Genotype	lsd (p=0.05)					0.180			

The first accessions for test of factors precision was variety BAZ 56431 with value of 3rd factor of 0,81 specifying the conical roots shape (more Parmex like). From factor 3 value can be expected conical root with medium length and long diameter for BAZ 56431 variety. Photo from Figure 2.22. does not confirms suggested root shape for this variety, especially in root diameter part.

Variety HRI 10305 was further used for testing of third factor, which has value of 0,58 as signal of conical shape and wider diameter and medium length. Figure 2.22. confirm mentioned prediction, it shows maybe little bit longer and wider roots.

Figure 2.22. Root shape of varieties used for testing of factor 3

Factor 3 has lower CV in general, but still with some differences. CV difference of factor 1 means was highest for variety Amsterdam in interval of -1,62 till 2,01. Recorded variations are result of environmental influence on the values of factor 3, and on the root shape also.

Data from all test varieties in different cycles was statistically analyzed by usage of analysis of variance. Analysis was interested t check the influence of different years/cycles of production and varietals influence on the factor 3.

Third factor was statistically significantly different in the different years of the experiment (Table 2.25.). Statistically significant difference between the factor 3 means was recorded by application of LSD test on factor 3, on both probability levels (Table 2.24.). Those finding are indicating significant environmental influence on factor 3means, throughout the different cycles.

Statistically significant difference for 3rd factor means was identified for the variety as a component which has influence on it. That statistically significant difference was proven by analysis of variance (Table 2.25.) and by LSD test (Table 2.24.). Considering variety of carrot root shapes, that could be expected outcome of analysis.

Correlation between production year/cycle and varieties has statistically significant influence on the third factor of principal component analysis; same finding was obtained by analysis of variance (Table 2.25.). Same influence of year and variety combination could be expected for the shape of carrot root.

Table 2.25. Analysis of variance for principal component 3

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Year	107,61	1	107,61	168,934	0,0000
Variety	580,06	4	145,02	227,656	0,0000
Year*Variety	79,79	4	19,95	31,314	0,0000
Error	1589,93	2496	0,64		

To resume above discussion, factor 3 does not prove to be efficient tool for root shape predictions. Like for the usage of factor 1 and 2, problem appears from the fact that more than one, positive or negative, index correlation is included into factors. In that case it is very complex to define the reason behind value of certain factor (shape, diameter).

2.2.5. Conclusion

From the discussion of obtained result for the certain indexes is obvious differentiation in their precision and variations related to the different experimental cycle or variety. Briefly are listed main characteristic of all indexes and factors which were subjects of this study.

- **RLD** – not so precise index; interval of values which can be assigned to the different root shapes; environmentally dependent.
- **RWVCYL** - not so precise index; interval of values which can be assigned to the different root shapes; environmentally dependent.
- **RWVCYLD** – very precise index; interval of values is clearly distinguished among different root shapes; good definition of shapes on border of main groups; environmentally little dependent.
- **RDW3** - very precise index; interval of values is clearly distinguished among different root shapes; good definition of shapes on border of main groups; environmentally dependent.
- **RVTRCONVCYLD** - not so precise index; interval of values which can be assigned to the different root shapes; environmentally dependent.
- **factor 1** - not so precise index; interval of values which can be assigned to the different root shapes; environmentally dependent.

- **factor 2** - not so precise index; interval of values which can be assigned to the different root shapes; environmentally dependent.
- **factor 3** - not so precise index; interval of values which can be assigned to the different root shapes; environmentally dependent.

From the above list of index performance can be seen that best results were obtained for indexes RWVCYLD and RDW3. Those two indexes provide better results in root shape identification than well known L/D and C indexes and can be suggested for the further use. Another advantage of those two indexes is also fact that it is easy to obtain necessary data, since for their calculations are needed root weight and root diameter.

2.3. Analysis of Carrot root quality characters

2.3.1. Introduction

2.3.1.1. Carotenes

Carotenes are natural pigments widespread in the nature among the: plants, algae, fungi and bacteria. Inside the cell, they are placed in chloroplasts and chromoplasts and other cell organelles. They belong to the bigger group of pigment called carotenoids.

Carotenoids are classified as follows:

- Carotenoid hydrocarbons are known as carotenes and contain specific end groups. Lycopenes have two acyclic end groups; β-carotene has two cyclohexene type end groups.
- Oxygenated carotenoids are known as xanthophyls. Examples of these compounds are a) zeaxanthin and lutein (hydroxy), b) spirilloxanthin (methoxy), c) echinenone (oxo), and d) antheraxanthin (epoxy).

The biosynthetic sequence of the carotenoids in plants is as follows:

phytoene → phytofluene → ζ-carotene → neurosporene → lycopene → γ-carotene and β-carotene. Each enzymatic step from phytoene to lycopene adds one double bound to the molecule, resulting in lycopene, which is a symmetrical molecule containing 13 double bonds. The biosynthetic step after lycopene involves enzymatic cyclization of the end groups, which results in γ-carotene (one beta ring) and β-carotene (two beta rings). The addition of oxygen to the molecule leads to the formation of xanthophylls (Goodwin, 1980).

Major carotenes that are present in carrot plants are: α, β, γ and δ-carotene, others are in minor amounts. α and β-carotene are pigments responsible for yellow and orange colour. Most of the time β carotene may present more than 50% of all carotenes, and its amount is usually two times higher than α carotene.

Xanthophylls are accumulated in small amounts inside orange carrot varieties, while in the yellow one their quantity is much higher.

The most important physiological function of carotenoids is their capacity to serve as a precursor of vitamin A in animals, and this ability has forty different carotenoids. Recent

statistic shows that carotene from vegetables contributes 70% of dietary vitamin A on a world wide basis.

Carotene of major importance is β-carotene which is present in almost all vegetables and fruits, and it is very important precursor of vitamin A. During digestion carotenes in food are subjected to the action of different enzymes (esterases, lipases). Most of carotenoids are cleaved to retinoids (vitamin A), or to a lesser degree, absorbed intact (Simpson, 1983). If the organism has sufficient amount of retinol, β-carotene stays in original form playing important antioxidant role in organism tissues. Real vitamin A can be located in food of animal origin like batter, eggs etc. If consumption of vitamin A goes over 5000 units per day, that can have negative effect on organism. From those reasons nutritionist are preferring usage of β carotene, with antioxidant activity, and food that contain it.

Mentioned role of carotene as antioxidant is also important one, and for the orange carrot that ability does not decrease during storage period (Alasalvar et al, 2005). Peto *et al* (1981) suggested that β-carotene might be the primary anticancer agent in fruits and vegetables. Higher dietary intake of β-carotene has inverse relationship on various cancers, predominantly one of the aero digestive tract (van Poppel, 1996). It has same influence on coronary heart disease (Gey, 1993).

Vitamin A is important factor in human growth and immunity. Daily needs for vitamin A of an adult person are around 600-700 μg, having in mind rate that for 1μg of vitamin A are needed 6 μg of β carotene or 12 μg of some other carotenoid. According to Heinonen (1990) carrot cultivars, in most of the cases, contains 1200 – 2300 μg/100 g of provitamin A in root fresh matter, providing enough pigment to satisfy daily needs.

Configuration of β carotene that is most stable and dominantly present is *trans* isomer, but certain amount of *cis* form is also existing. Exposure to the high temperature, light, presence of acids can cause isomerisation. Isomerisation can happened during cooking and carrot processing, it cause formation of *cis* isomers which have lower vitamin A precursor activity (Nyambaka et al., 1995).

Carotenes are important in the food processing industry as colorants and antioxidants; they can have medicinal application in curing from photosensitive disease and in cancer prevention.

Most of variation in carotene amount inside carrot root is due to several factors: genotype, developmental stage, temperatures during the growing, fertilization, light exposure.

Genotype is the first factor of limitation for the quantity of carotene inside the root tissue. Variety can not produce carotenes beyond it potential and capacity. Some varieties can have much higher amount of carotene, in comparison with others.

Carotene distribution inside the root is not uniform. Carotene formation is much higher in older tissues in comparison with young one. That is way amount of carotene is decreasing longitudinally from the upper root part to the tip. Usually phloem root part has more carotene than xylem. During the maturation, carotene accumulation is raising inside root, improving it colour intensity (Gabelman, 1974). Throughout that period, colour difference between root upper part and tip is decreasing. Parallel with growth of carotene synthesis, proportion of α and β-carotene is changing, at the initial stage balance was moved toward α carotene. Later, in the mature stage that balance changed in favour of β carotene which is present in amount of around 60% of all carotenes, while α-carotene is on the level of 20% (Banga and De Bruyn, 1964; Gabelman, 1974).

Through the first plant developmental stage, until the mid growing period, synthesis of carotene is very low. In the second phase they have been created much faster to reach the highest concentration in harvesting period.

Environmental factors like normal water content in soil can reduce amount of carotene, while more fertilizer applications can increase synthesis of these pigments. For optimum increase of carotene amount it was recommended fertilization of 80 to 150 kg ha $^{-1}$of nitrogen. Optimum temperature for carotene synthesis is in the range of 15 up to 21^{o}C (Rubatzky *et al*., 1999).

2.3.1.2. Carbohydrates

Carrot has medium – high energetic value (47 cal/100g) with balanced content of carbohydrates (8 – 9%). Those carbohydrates are increasing nutritional value of the carrot and they are giving sweet taste to this vegetable. That sweet taste if preferred consumers characteristic. Major function of the root, as a storage plant part, is to reserve compounds with energetic value for later need in the plant development and inflorescence formation.

Sugar amount inside the root is genotype characteristic and it divers among different varieties.

Root and leaves have balanced amount of those compound, their quantity can be partly influenced by environmental factors like plant density on the field and exposure to the light. Dry matter amount in the root can be under great influence of plant density, it decreases with higher density (Hole *et al.*, 1983).

Accumulation of glucose and fructose is dominant during the initial growing period of the root. That is connected with high activity of enzyme invertase in that growing stage. Amount of sucrose is increasing later, in mature plant. Capability of parenchyma tissue to stores sucrose enables drastically increase of it concentration inside carrot root (Rubatzky *et al*., 1999).

Although sugars amount depend a lot from variety characteristic and environmental factors, in general in the mature root two thirds of total carbohydrates amount should be sucrose (Phan and Hsu, 1973). Sugars distribution inside the root is not equal. Usually parenchyma of phloem has more carbohydrates per dry matter than root core part. Similar is sucrose distribution, less present in core part (Phan and Hsu, 1973).

Environmental also has important influence on sugars amount and composition. Rosenfeld (1998) suggested that higher temperature influences on carrot root length and amount of glucose. Under higher temperature root length is shorter and amount of glucose is decreasing. According to the same author higher temperature have positive influence on the sucrose amount, while lower temperature keep in content on low to medium level. Fertilization as environmental factor, up to level of 140 kg/ha may influence on the increase of carbohydrates synthesis in certain varieties (Hochmuth *et al.*, 1999).

2.3.1.3. Nitrates

The changes in agriculture, food processing, urbanization, and industrialization have had an impact on the accumulation of nitrate in the environment. Intensive agricultural production has consumed an increasing amount of nitrogen-based fertilizers leading to increasing the exposure of man and animals to significant nitrate levels in food, feed, and water.

Nitrate may be incorporated into plants grown in soil with unusually high levels of this chemical. Nitrate decomposition and formation to nitrite is done by certain microorganisms in soil, water and the alimentary tract. Thus, the concern with nitrate in the environment is related in part to its conversion by biologic systems to nitrite. Methemoglobinemia is caused by high levels of nitrite, or indirectly from nitrate, in humans. It results in difficulties in the oxygen transport system of the blood. Cases numbering in thousands have been reported, mostly involving poisoning in infants (Menzer, 1993). Generally, from nutritional and toxicological point of view, nitrates are non harmful compounds for adult person, but can be harmful for kids.

On the other hand, nitrates and nitrites are involved in the production of nitrosamines (chemical carcinogens) in food by the reaction of nitrite with secondary amines (Fine, 1980; Hardisson *et al.*, 1996).

From all mentioned reasons World Health Organisation recommended maximum nitrate consumption on 237 mg of NO_3 per day, for adult person with 65 kg.

The need to measure the nitrate and nitrite levels in different food, and subsequently control them, becomes evident when a higher standard of living for the population at large is to be achieved. This has been established in numerous works carried out in different countries (Hardisson *et al.*, 1996).

Around 70% of nitrates that are present in the food are originating from the vegetables. Amount of nitrogen differ from one crop to other and it is also related to the genotype, environmental conditions and cultural practices in their production.

For most of the crops nitrate amount is decreasing with plant maturations. Quantity of nitrate is diverse for different plant parts e.g. carrot leaves are containing around 1300 mg of NO_3 per kg of fresh matter, while root xylem has 450 mg and phloem contains 70 mg.

Content of accumulated nitrates depends from plant exposure to the light, since that factor stimulate actions of nitrate reductase. In general, crops which have been grown in period of the year with less light intensity and shorter days (autumn and winter) are containing more nitrates.

Soil can be important source of nitrate uptakes by plants. Nitrate absorption is related to the soil water amount, amount of this nutrient and time of NO_3 availability.

Soil with higher water content is generally enabling plants to better absorb existing NO_3.

In the experiment on the carrot was recorded positive relation among increase of nitrate

amount in the soil and carrot production. That positive reaction was up to level of 100 kg/ha of nitrate. Higher amount of nitrogen in soil can not be absorbed by plant, and it even can have negative influence on crop, plants are more sensible on pest attacks, roots are more sensible during storage and they contain more nitrates (Borin and Satin, 1994).

It is important to have information about soil conditions and it nutrient availability before crop production and fertilizer application, in order to use those resources in the most economical and productive way.

The highest need for nitrogen carrot plant has in the period of fast growing, middle of growing period. Initial vegetation stage and at the end of growing period requirements for nitrogen are low, and in some cases to much NO_3 can cause product damages.

2.3.2. Materials

For this research was used data collected in three year period 2001–2003. Accessions used for this study were received / collected from genebanks, and their source and number were:

- Institute National of Horticulture: **INH,** 10 accessions
- Federal Research Centre for breeding of cultivated plants: **BAZ,** 4 accessions
- Horticulture Research International: **HRI,** 31 accessions
- Nordic Gene Bank: **NGB** 8, accessions
- Two local varieties from South of Italy

Apart from the mentioned accessions, the following commercial varieties were included into the experiments: Amsterdam, Autumn King, Bolero, Nikki 1, New f1, Parmex and Rubrovitamina.

Since whole research was realized under umbrella of GENRES carrot project it has some limitation regarding evaluation of possible genotype x environment interaction.

Main scope of the project was to characterise and evaluate as much accessions as possible, in order to have valuable information about existing germplasm in genebank collections. Those accessions were collected by end of the last and beginning of this century, that fact just enforces importance of the fast evaluation process. With such limitation it was not possible to grown same accession for few years in different environmental conditions in order to recheck it performance and obtained results. In most

of the cases one accession get the chance to be grown, end later evaluated for qualitative and quantitative characteristics only in one growing cycle.

Some exception from that rule are mentioned before, and based on their repeated result we do analysis in this part of study about main characteristics.
Experiments took place in the following manner:

- Experiment summer and autumn 2002 included following accessions:
    - Institute National of Horticulture: **INH,** 8 accessions
    - Federal Research Centre for breeding of cultivated plants: **BAZ,** 2 accessions
    - Horticulture Research International: **HRI,** 25 accessions
    - Nordic Gene Bank: **NGB** 7, accessions
    - Two local varieties from South of Italy
    - Commercial varieties: Amsterdam, Autumn King, Nikki 1 and Parmex

  Experiment was established in Bellaria region on sandy soil. Summer cycle was started with sowing at 2nd of May and finished with harvesting in period August – September. Autumn cycle started at 23rd of July and finished till 6th of December.
- Experiment summer 2001 and autumn 2003 included following accessions:
    - Institute National of Horticulture: **INH,** 1 accession
    - Federal Research Canter for breeding of cultivated plants: **BAZ,** 2 accessions
    - Horticulture Research International: **HRI,** 3 accessions
    - Commercial varieties: Amsterdam, Autumn King, Bolero, Parmex and Rubrovitamina

  In both years, experiment was performed in Bellaria region on sandy soil. In 2001 experiment was done in summer growing cycle, wile in 2003 it was done in autumn goring period.
- Experiment summer and autumn of 2002 and autumn 2003 was done with following accessions:
    - Institute National of Horticulture: **INH,** 1 accession
    - Horticulture Research International: **HRI,** 3 accessions
    - Nordic Gene Bank: **NGB** 1, accessions
    - Commercial varieties: Amsterdam, Autumn King, Parmex and news f1.

Plants for all three experiments were grown in Bellaria region, on typically sandy soil. In 2001 experiment was done in summer growing cycle, wile in 2003 it was done in autumn goring period. Summer cycle in 2002 started at 2nd of May and finished with harvesting in period August – September. Autumn cycle in 2002 started at 23rd of July and finished till 6th of December. Autumn 2003 production cycle started at 26th of June and harvesting was done by first week of October.

2.3.3. Methods

Determination of certain compound was done in the following manner:

- Carotenes were extracted from fresh matter into mixture of acetone and hexane (40/30) and determined by RP-HPLC-DAD analysis. Obtained results for carotenes were: total carotenes amount, amount of α and β-carotene.
- Through the analytical analysis was determined amount of: sugars, glucose, fructose and sucrose. Sugars were extracted in ethanol and further through processes prepared for Gas chromatography. Their determination was done by GC-FID.

2.3.4. Discussion

In the Table 3.1. are listed all varieties which were included in carrot quality characters analysis

Table 3.1. List of varieties used in all three years of experiment

1	Amsterdam	17	hri 3936	32	inh 13	48	Parmex
2	Aut king	18	hri 3966	33	inh 15	49	baz 56367
3	baz 56341	19	hri 3998	34	inh 16	50	baz 69563
4	baz 56355	20	hri 5784	35	inh 18	51	Bolero
5	hri 10168	21	hri 6070	36	inh 19	52	hri 3937
6	hri 10220	22	hri 7301	37	inh 20	53	hri 4007
7	hri 10225	23	hri 7893	38	locita 1	54	hri 6519
8	hri 10233	24	hri 8080	39	locita 2	55	inh 1
9	hri 10246	25	hri 8081	40	ngb 13936	56	Rubrovitamina
10	hri 10305	26	hri 8095	41	ngb 13945	57	hri 6102
11	hri 10468	27	hri 8116	42	ngb 13946	58	hri 6760
12	hri 10520	28	hri 8125	43	ngb 13949	59	hri 7801
13	hri 11163	29	hri 8394	44	ngb 13951	60	inh 14
14	hri 11169	30	inh 11	45	ngb 13955	61	news f1
15	hri 11503	31	inh 12	46	ngb 7748	62	ngb 2399
16	hri 3838			47	Nikki f1		

2.3.4.1. Carotenes amount in fresh matter

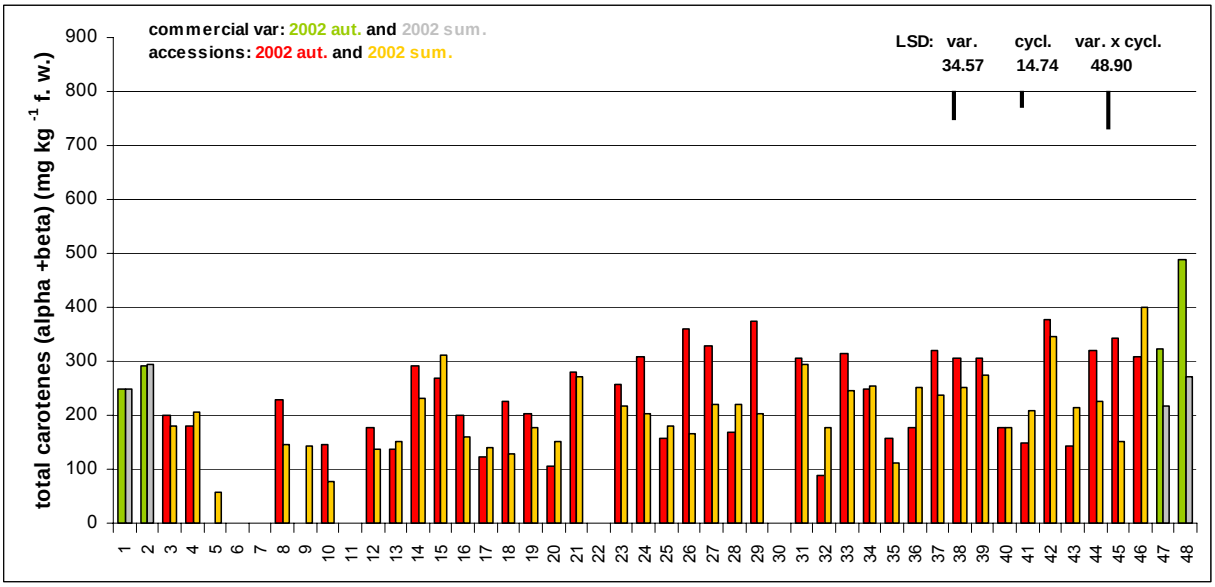
This part covers carotenes quantity and quality characteristics (like α and β-carotene ratio; retinol equivalent) expression in carrot roots under influence of variety, growing cycle and their combination as main factors.

2.3.4.1.1. Total carotenes amount in carrot fresh matter

Results obtained during the summer and autumn 2002 experiments are illustrated in Figure 3.1.

Total carotenes varies from 0 (accession No. 6, 7) to 492 mg kg^{-1} for accession No. 48, clearly demonstrating significant differences among genotypes. Growing cycle has important influence on the carotenes amount, which was generally higher in autumn period for the majority of accessions. Genotype x environment interaction was significant (Figure 3.1).

Figure 3.1. Total carotenes amount in experiment held in summer and autumn of 2002 for 48 accessions



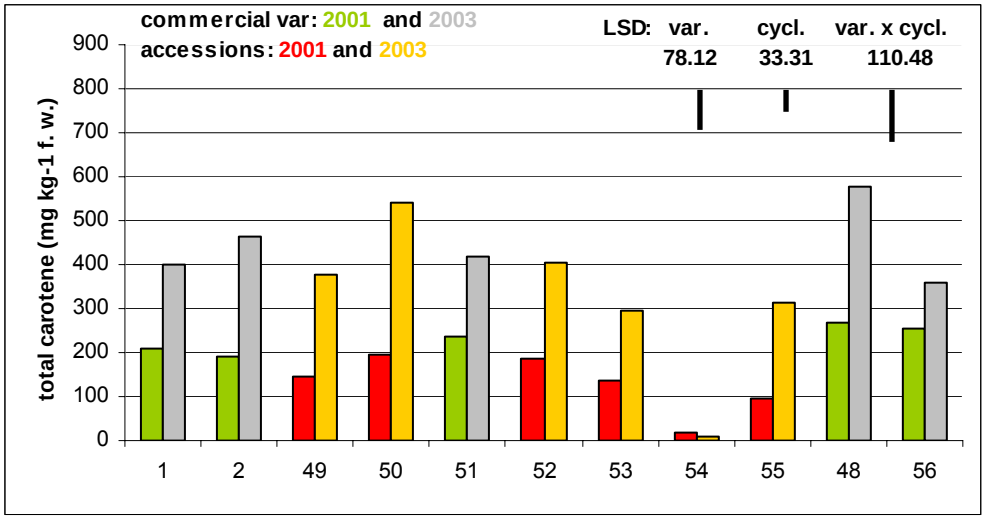
According to the different influence by variety x cycle interaction accessions can be grouped on:

- The ones having higher carotene amount in autumn cycle like No. 8, 24, 26, 27, 29, 48. The highest total carotenes content in this group was recorded for variety 48. The biggest difference between summer and autumn cycle in means of total carotenes was obtained for accessions 26, 27, 29 and 48.
- Accessions with small non significant variation in total carotenes quantity between summer and autumn growing period e.g. accessions No. 1, 2, 15, 21, 31 and 42. Particularly interesting are accessions 21 and 31 with total carotene amount similar to commercial varieties and, especially accession No. 42 (over 360 mg kg^{-1} of carotenes) with results even higher than varieties 1 and 2.

For the experiment held in 2001-2003 results are illustrated in Figure 3.2. Total carotenes, ranging from 8 to 575 mg kg^{-1} clearly indicates significant differences among genotypes.

Most accessions had two times higher carotene amount in autumn growing period of year 2003, in comparison with summer growing cycle of 2001. None of the accessions, however, demonstrate particularly favorable characteristic, with respect to commercial varieties, to be considered for further study.

Figure 3.2. Total carotenes amount in experiment held in summer of 2001 and autumn of 2003 for 11 accessions



In Figure 3.3. are illustrated results for 9 accessions common for the two cycles in year 2002 and autumn period of 2003. Variety, as a factor, cause significant difference among accessions carotene amount. Total carotenes amount varies from 18 (No. 58) up to 800 mg kg^{-1} (No. 60).

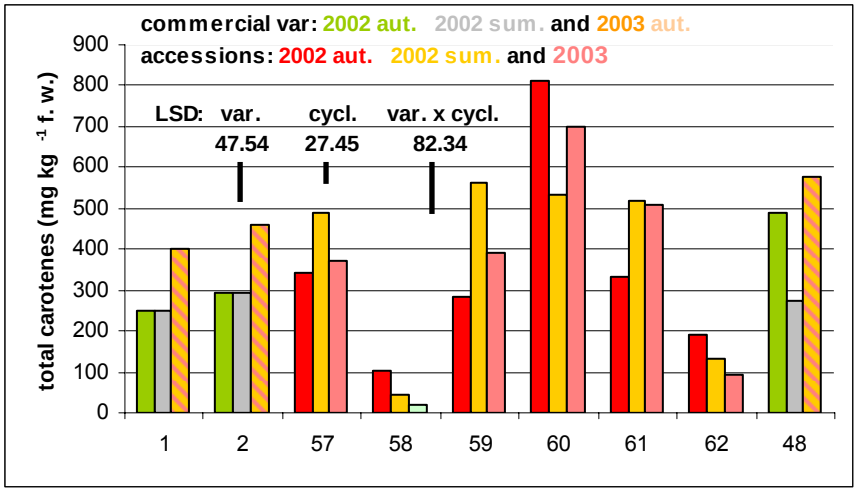
Genotype x environment interaction had significant influence on carotenes amount. As result of interaction different combination of carotenes distribution in certain varieties and in particular growing period were recorded. Those different combinations can be grouped like:

- Accessions with higher carotenes amount in autumn cycles, of year 2002 or 2003 like No. 48, 60. Particularly interesting is accession 60 with high total carotene amount of 700 and 800 mg kg^{-1} in autumn cycles and 535 mg kg^{-1} in summer of 2002.
- Accessions with higher carotenes quantity in summer growing period e.g. No. 57 and 59. Interesting accessions with carotene amount around 500 mg kg^{-1} during summer growing cycle
- Accessions with overall lower variation in carotenes amount throughout different cycles e.g. 1, 2 and 61. Accession 61 should be studied further, since it shows

results on the same level, or even slightly better, than commercial varieties in carotenes amount variation in fresh matter.

Accessions No. 58 and 62 have low total carotenes content in all growing cycles.

Figure 3.3. Total carotenes amount in experiment held in summer and autumn of 2002 and in autumn of 2003 for 9 accessions



In general, amount of carotenes is characteristic for certain genotype. Under influence of environmental factors it can be significantly different from one growing cycle to another one. In this study, carotenes total amount was lower in summer growing periods. Genotype x environment interaction causes different combinations in total carotenes amount. Particularly interesting accessions were No. 21, 31, 42 and 61 which have pretty stable amount of total carotenes in different growing cycles (summer; autumn) and they should be subject of new researches. Further study should also include accessions with tendency to have more total carotenes in summer period e.g. No. 57 and 59. And finally accession No. 60 has the highest amount of carotenes (around 800 mg kg^{-1}) in autumn cycle, and should be further checked.

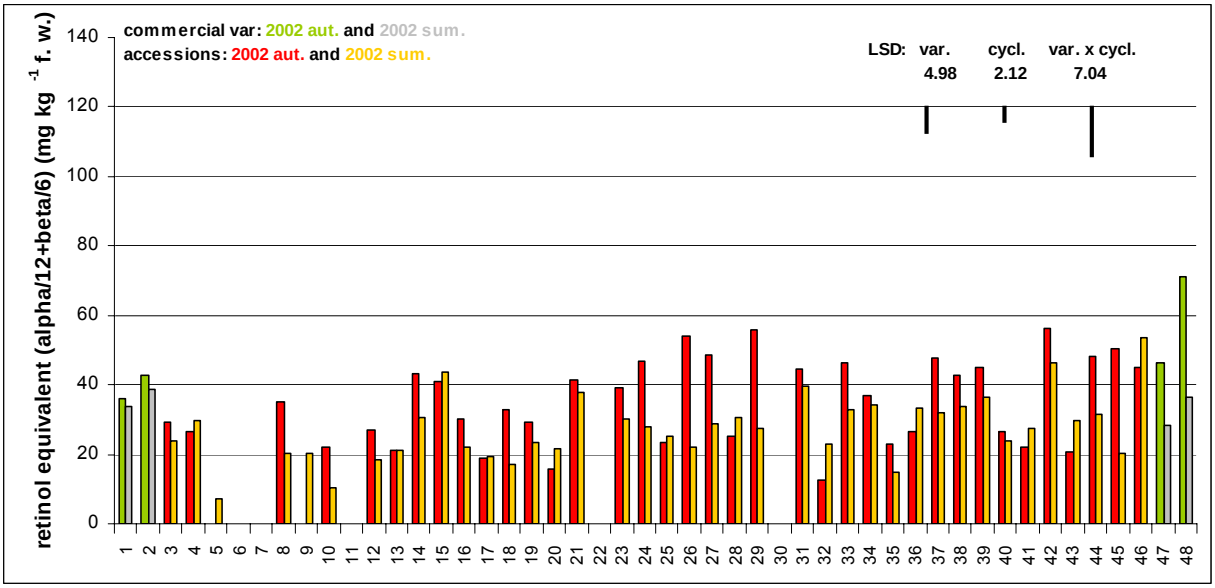
2.3.4.1.2. Retinol equivalent

Retinol equivalent is calculated on the basis of α and β-carotene amount in carrot root. Calculation was done according to the ratio α-carotene/12 and β-carotene/6. Their joined amounts were transformed into retinol equivalent, representing total carotenes available as provitamin A.

Retinol equivalent of the 2002 summer and fall cycles is shown in Figure 3.4.

All three major factors: variety, growing cycle and their interaction have significant influence on retinol equivalent. Significant difference between genotypes is demonstrated by range of retinol equivalent which goes from 0 to 70 mg kg^{-1} (accession No. 48).

Figure 3.4. Retinol equivalent in experiment held in summer and autumn of 2002 for all 48 accessions



In general, retinol equivalent was significantly higher in roots e grown in autumn than in summer cycle.

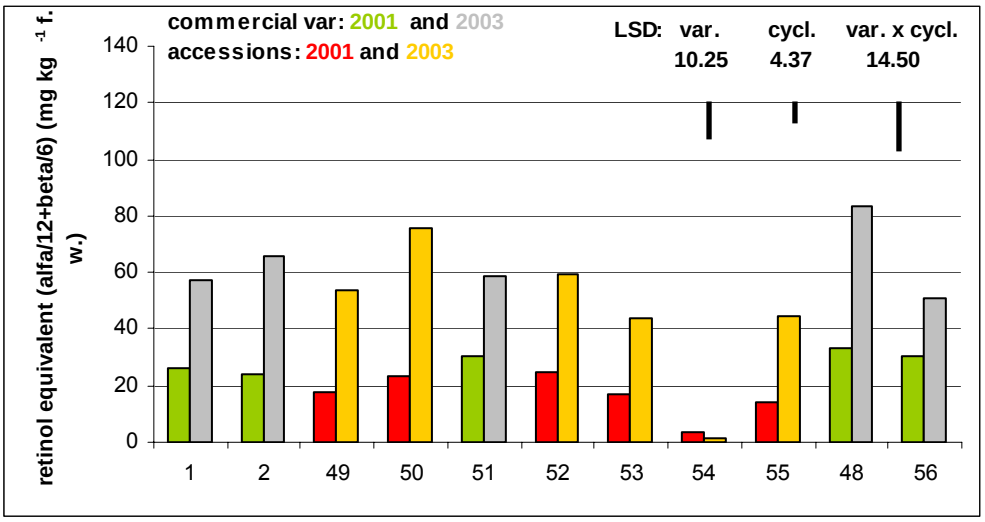
The most interesting is examination of interaction between variety and growing cycle for the identification of accessions with favorable characteristics expressed in certain growing period or with least character variations throughout different cycles.

Biggest variation in favor of autumn cycle was recorded for varieties: 24, 26, 27 and 29. Opposite tendency, higher retinol equivalent in summer trial was recorded for small number of accessions like in case of No. 32, 43 and 46 (Figure 3.4); the last two of particular interest, together with 31, for their high summer retinol equivalent amount, higher that the test variety Parmex (n. 48). . Several accessions have higher retinol equivalent in autumn cycle in comparison with commercial varieties. Those accessions are: No. 26, 29, 37 and 42 and they have equivalent around 50 mg kg^{-1} and above. It is interesting that some of commercial varieties, 47 and 48 also had significantly higher retinol equivalent in autumn time, while other two, No. 1 and 2 have stable amount of α and β-carotene, when they are converted into retinol equivalent.

Comparison of same accessions through different growing periods was also done for autumn of year 2001 with summer of year 2003, and summer and autumn period of 2002 with autumn cycle of 2003.

Figure 3.5. reports retinol equivalent for the accessions common to the 2001 and 2003 trials. All accessions have demonstrated significantly higher amount of retinol equivalents in the autumn cycle of year 2003.

Figure 3.5. Retinol equivalent in experiment held in summer of 2001 and autumn of 2003 for 11 accessions



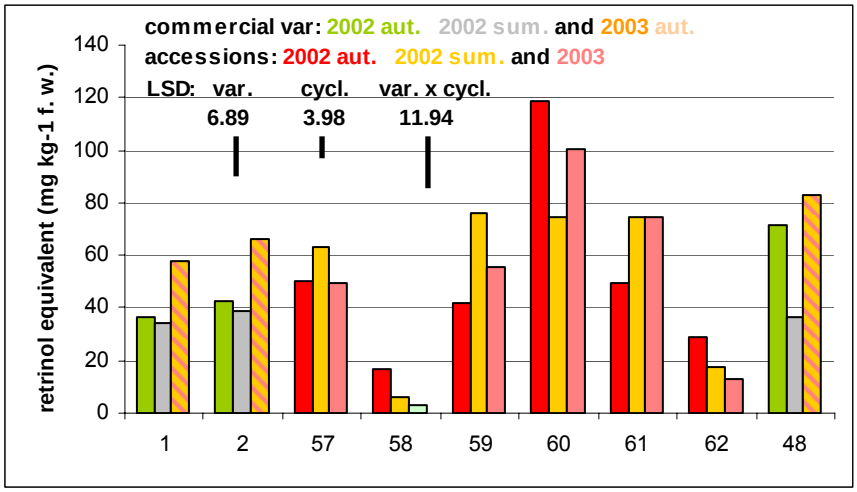
Apart from accessions 54 and 56, others have 2-3 times higher retinol in autumn cycle with respect to summer 2001, thus confirming the trends of the 2002 trials. Accession No. 50 in 2003 autumn cycle, reached a retinol equivalent close to 80. On the other side accession 54 has vary low equivalent.

For the comparison in trials held in year 2002 and autumn 2003 were included 9 accessions. Their retinol equivalent in mentioned cycles is illustrated in Figure 3.6. Here we can see three groups of accessions with specific behaviour due to the variety x cycle interaction.

- First group, of No. 1, 2, 48, has the lowest retinol equivalent in the summer cycle of 2002 and the highest in autumn of 2003.
- Second group with accessions No. 57, 59 has the highest equivalent in summer period in 2002, and especially accession 59 is interesting, with values over 75.
- Third group of accessions include No. 58, 60 and 62 with the highest score in autumn of 2002. Accession 60 is particularly interesting since in autumnal cycles it has retinol equivalent around 100, much higher than commercial varieties.

Also interesting are accessions 57 and 61, because of their rather stable retinol equivalent, although not particularly high.

Figure 3.6. Retinol equivalent in experiment held in summer and autumn of 2002 and in autumn of 2003 for 9 accessions



Commercial varieties that have been grown in all cycles (No. 1, 2, 48) were under significant environmental (cycle) influence and their retinol equivalent depended from growing season, being much lower in summer growing cycle.

As a summary, retinol equivalent in carrot is genetic/variety characteristic which is also under great environmental influence. Variety x growing cycle interaction as well demonstrated significant effects on the retinol equivalent in carrot roots. Summer growing cycles were characterized by lower retinol equivalent in years 2001 and 2002 in comparison with autumn period. Exceptions from that rule are accession No. 57, 59 and 61, which can be subjects of new studies. Accessions No. 57 and 61 are also interesting since they had retinol equivalent on similar level in different growing periods (summer; autumn) and in different years. Further research on accession 60 could be useful having on mind that it has the highest retinol equivalent in both autumn growing cycles.

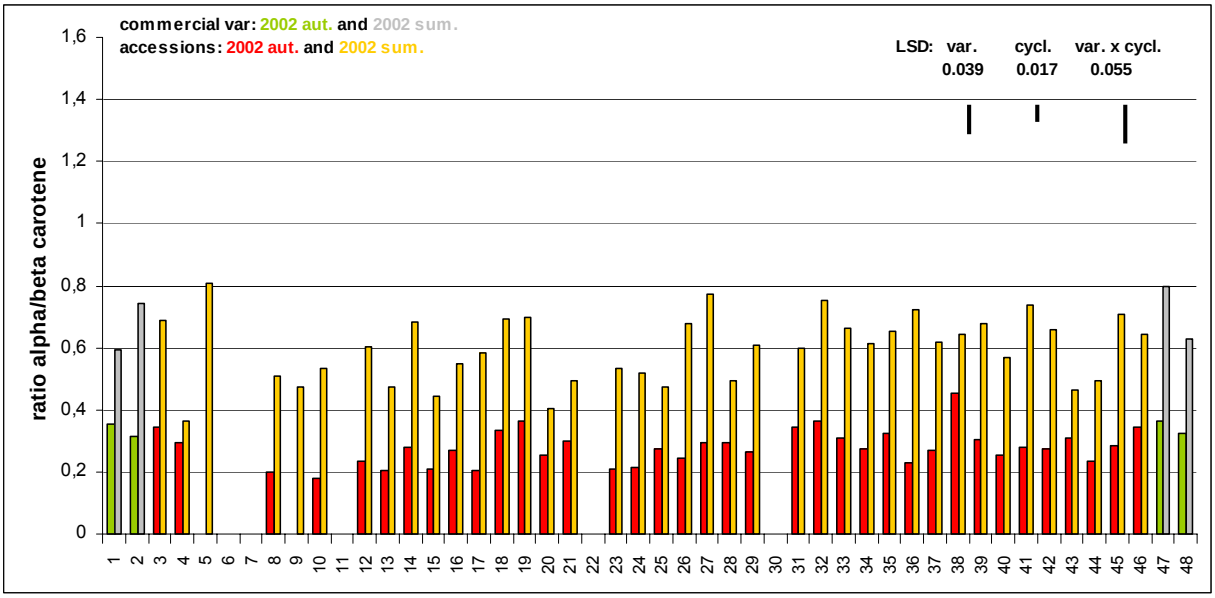
2.3.4.1.3. Ratio between alpha and beta carotene amounts in carrot fresh matter

Ratio between alpha and beta carotene is also an indicator for carotenes effectiveness as provitamin. Since beta carotene has conversion rate to provitamin A in ratio β-carotene/6 it is two times more effective than alpha carotene with ratio α-carotene/12.

First group of 48 accessions which were grown in summer and autumn of 2002 have recorded alpha to beta carotene mean ratio as it is illustrated in Figure 3.7. Alpha to beta

carotene ratio differs significantly between genotypes. In the presented results it value is in interval between 0.18 up to 0.81. High ratio between carotenes was especially recorded in summer of 2002 and it is in comparison to the autumn one two to three times higher. That indicates growing cycle as factor which causes significant difference in the relative amount of alpha carotene i and in alpha/beta ratio as an outcome. Accession which did not duplicate alpha/beta ratio in summer trial were accessions No. 4, 38 and 43.

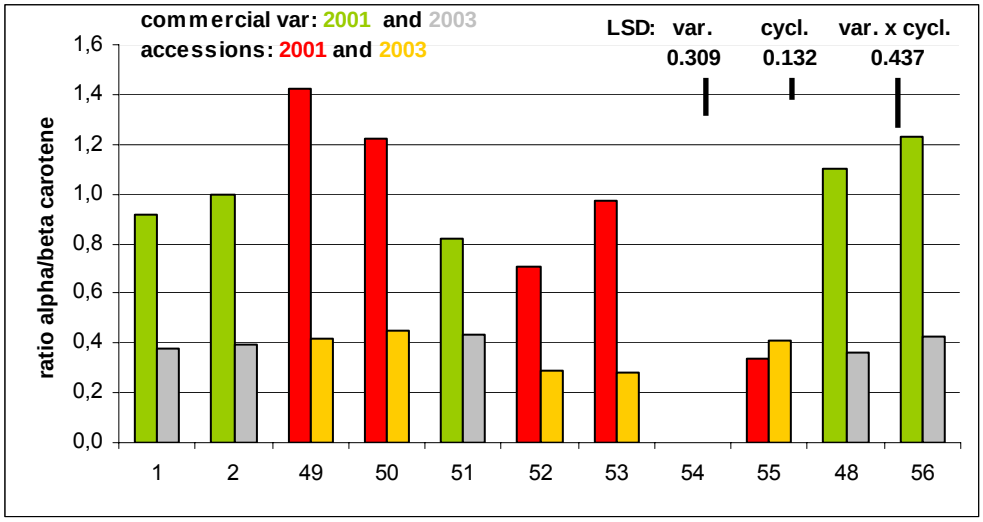
Figure 3.7. Alpha and beta carotene ratio in experiment held in summer and autumn of 2002 for 48 accessions



Ratio between alpha and beta carotene in trials held in summer of year 2001 and autumn of 2003 also was significantly related to the certain variety. Ratio range in those trials had an wider range than in 2002, from 0.28 to 1.42 (Figure 3.8.). Values higher than one were recorded for accessions No. 49, 50, 48 and 56. The majority of other accessions have significantly higher alpha to beta carotene ratio in summer of 2001, under environmental influence. The only exception from that rule was accession No. 55 which has lower alpha to beta carotene rate in summer growing cycle. At the same time this accession in both cycles kept carotene ratio under 0.4.

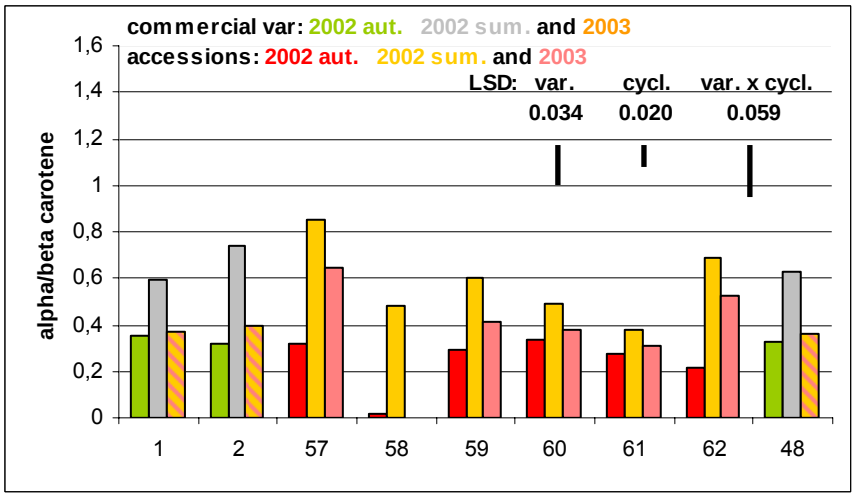
Alpha to beta carotene ratio among accession was significantly different in trials that were held in two seasons during 2002 and in autumn of 2003. Figure 3.9. illustrates that the lowest ratio value was almost zero (No. 58 autumn of 2002) and the highest was 0.84 (No. 57 summer 2002). Environment has significant influence on alpha to beta carotene ration since all accessions have higher ratio in summer cycle of year 2002.

Figure 3.8. . Alpha and beta carotene ratio in experiment held in summer of 2001 and autumn of 2003 for 11 accessions



Variety x cycle interaction causes two main behaviors of experimental accessions. One accession group has big difference in alpha to beta carotene ratio between summer and autumn cycles. That group is made of accessions No. 1, 2, 57, 58, 62 and 48 where accessions 57, 58 and 62 are the ones with biggest variations. Another group of accessions has smaller variations between the growing cycles in alpha to beta carotene ratio like No. 60 and 61. Accession 61 is particularly interesting because its ratio is under 0.4.

Figure 3.9. Alpha and beta carotene ratio in experiment held in summer and autumn of 2002 and in autumn of 2003 for 9 accessions



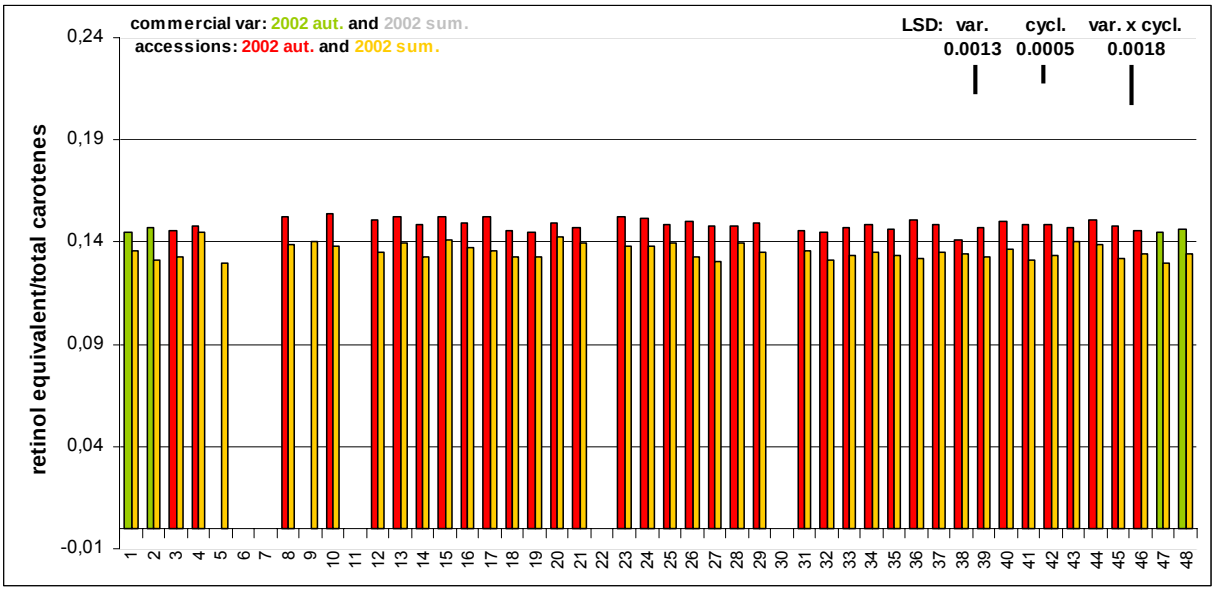
As a whole, the ratio between alpha to beta carotene was under strong environmental control, being almost always higher in summer that in fall cycle. The varieties differed

especially in nether reaction to the environment, the majority of them following the general illustrated pattern. Cases for potential further study are accessions No. 57, 58 and 62 with lower variation in α to β carotene ratio between the summer and autumn cycles.

2.3.4.1.4. Ratio of retinol equivalent and total carotenes in carrot fresh matter

Ratio between retinol equivalent and total carotenes amount is connected to the efficiency of conversion of total carotenes into provitamin A. Higher ratio means better effectiveness of carotenes which are present in carrot fresh matter.

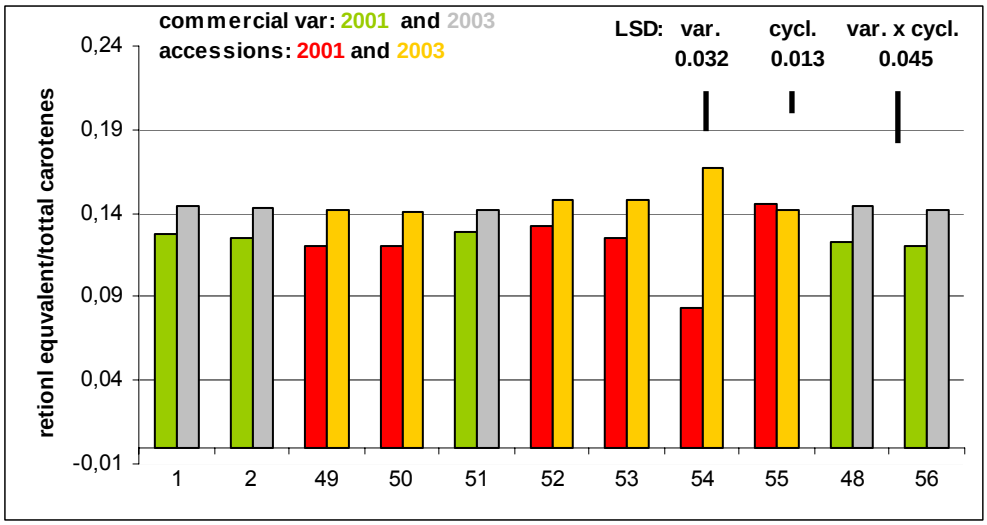
Figure 3.10. Retinol and total carotenes ratio in experiment held in summer and autumn of 2002 for 48 accessions



Results of comparison between the 2001 and 2003 experiments are illustrated in Figure 11. Also in this case, higher carotenes effectiveness was in autumn cycle. Difference in effectiveness among the two cycles was very similar for the majority of the 9 tested accessions. Two exceptions are:

- Accession No. 54 which has bigger difference between autumn and summer carotenes effectiveness than other accessions.
- Accession No. 55 which has better retinol/total carotenes ratio in summer cycle, with summer value the highest among all 9 accessions.

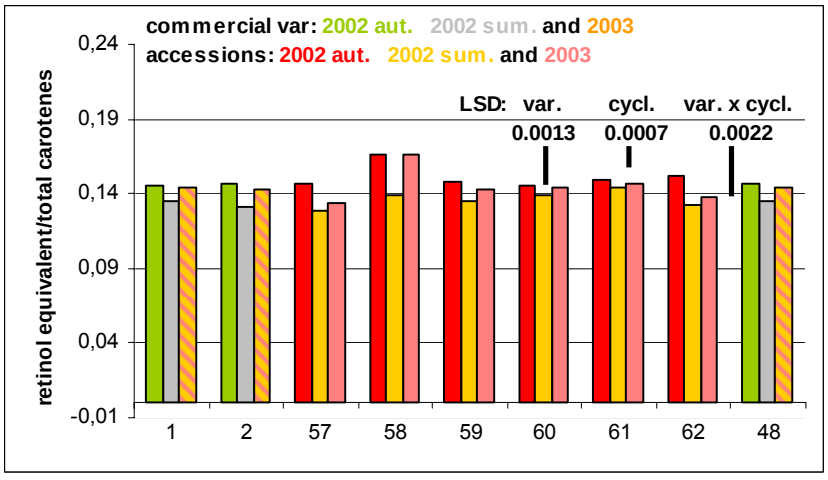
Figure 3.11. . Retinol and total carotenes ratio in experiment held in summer of 2001 and autumn of 2003 for 11 accessions



Retinol / total carotenes ratio for experiments in 2002 (both cycle) and autumn 2003 for 9 accession is shown in Figure 3.12. There difference in carotenes effectiveness among accessions is significant and it is in range of the 0.128 to 0.165. Also in this case, lower values were registered in summer cycle, for all 9 accessions

Genotype x environment interaction creates different combinations in retinol carotenes ratio. As a result of combinations, in summer cycle accessions No. 58, 60 and 61 have the best effectiveness of present carotenes. Accessions 1, 2, 57, 59, 62 and 48 have almost the same levels of carotenes effectiveness in all three cycles. Interesting result in retinol carotenes ratio had accession 58 with it highest values for the autumn of 2002 (0.165) and 2003 (0.167).

Figure 3.12. Retinol and total carotenes ratio in experiment held in summer and autumn of 2002 and in autumn of 2003 for 9 accessions



To summarize, the ratio between retinol and total carotenes is important as an indicator of carotenes effectiveness as provitamin. I. Most accession have shown similar trend in ratio values in different growing cycles, in line with behavior of commercial varieties. Only accession No. 58 has slate higher/better carotenes effectiveness in autumn cycles and it should be good base for further studies. Summer grown carrots, besides having generally lower total carotenes and retinol equivalent that autumn grown, also demonstrated lower conversion efficiency. This character was also rather stable, with slight, although significant, genotype x environment interaction.

2.3.4.1.5. Conclusion on carotene amount and effectiveness

All examined characters can be considered as genotype characteristics since their values are closely related to certain accessions.

Characteristics are also under significant environmental influence in a way that:

- Total amount of carotenes in carrot fresh matter is lower in summer cycles which are not in line with finding of other authors (Simon and Wolff, 1987) findings about carotene increased amount under influence of higher temperature.
- Retinol equivalent is generally lower in summer cycle, indicating lower amount of carotenes, and especially β carotene in fresh matter. That assumption was proved by α to β carotene ratio which was much higher in summer time.
- Ratio between alpha and beta carotene is higher in summer cycles and causes lower retinol equivalent and lower effectiveness of present total carotenes.
- Carotenes effectiveness ratio is lower in summer growing periods and this finding corresponds with the fact that retinol equivalent was lower in summer.

Genotype x environment interaction causes significant differences in characteristics behaviour in trials. This interaction also indicated certain accessions with favourable characteristics, and accessions with stable behaviour, under particular environmental conditions. In Table 3.2. are listed characteristics related to carotenes in carrot fresh matter, with accessions that show interesting behaviour.

Table 3.2. Carrot - carotenes characteristics with favourable behaviours and list of accessions

Characteristic	Favourable behaviour	Accessions of interest
Total carotenes	Higher total carotenes in summer cycle	57; 59
	Stable amount of total carotenes in different growing cycles	21; 31; 42; 61
	Highest total carotenes amount	60
Retinol equivalent	Higher equivalent obtained in summer cycle	57; 59; 61
	Similar level of retinole equivalent in summer and autumn cycle	57; 61
	Highest retinol equivalent	60
α/βcarotene ratio	Small variation in ratio for different growing cycles	60; 61
Retinol equivalent/total carotenes	Best ration between ratinol equivalent and total carotenes	58

Based on accessions characteristics (Table 3.2.) some of them should be further studied, since they have demonstrated interesting behavior in variety x cycle interaction. One of the most interesting accession No. 61 has stable retinol equivalent (even slightly higher in summer time) and total carotenes in different growing cycles. It also has pretty stable alpha to beta carotene ratio. Having al mentioned in mind, this accession should be studied in the future. Particularly interesting accession is No. 60 with highest total amount of carotenes and highest retinol equivalent and stable alpha to beta carotene ratio. That accession also has stable amount of nitrates in different growing conditions. Other accessions like 57, 59 21, 31, 42 and 58 may also be worth reconsideration.

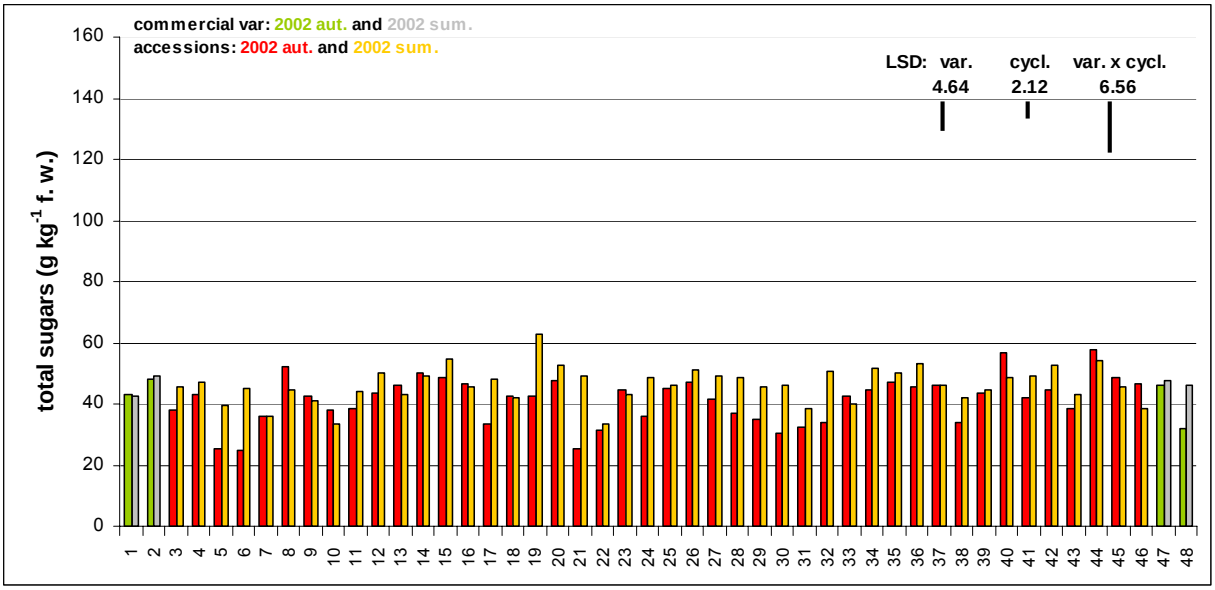
2.3.4.2. Sugars amount in carrot fresh matter

This part explores sugars quantity and their qualitative characteristics (like simple sugars/sucrose ratio; ratio between glucose and fructose) inside the carrot roots under influence of different factors: variety, growing cycle and their combination.

2.3.4.2.1. Total sugars amount in carrot fresh matter

Amount of sugars was also recorded during experiment that was held in summer and autumn of 2002. Recorded results (g kg^{-1}) for 48 accessions are illustrated in Figure 3.13. It is shown significant difference in sugars amount among different accessions. Cycle of growing has also significant influence on total amount of sugars. In average, sugars content was higher in summer growing period.

Figure 3.13. Total amount of sugars in experiment held in summer and autumn of 2002 for 48 accessions

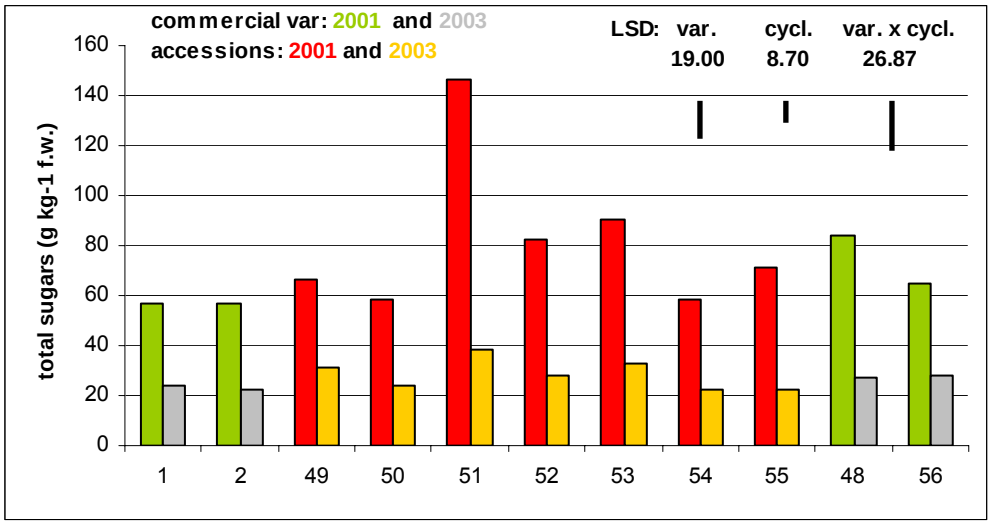


Genotype x environment interaction causes significant differences. As an outcome of mentioned interaction they are:

- Group of accessions with higher sugar amount recorded in summer growing period. The biggest difference between the summer and autumn growing cycle have accession No. 5, 6, 21, 32 and 19 with the highest overall sugar quantity of 62 g kg^{-1}.
- Group of accessions with stable behavior in both growing season when amount of sugars has been considered. This group is made of commercial varieties No. 1, 2 and 48 and of accessions like 7, 13, 14, 23, 24, 37 and 45 with values between 40 and 50 g kg^{-1}.
- Small group of accessions that have more sugars in autumn growing period and those accessions are: 8, 40 and 44. Accession 44 has the highest amount of sugars (58 g kg^{-1}) among all varieties that have been grown in autumn period.

In the experiment that was held in summer 2001 and autumn of year 2003 amount of sugars was closely related to the varieties and growing cycle (Figure 3.14.). All 11 accessions have much higher sugar content in summer of 2001 and it is 2 to 3 times more than in autumn of 2003. The highest difference was recorded for accession No. 51 with sugars total quantity in summer cycle of 147 g kg^{-1}, which is absolute maximum for all accessions, and that fact can be potentially useful. However, remaining none accessions did not demonstrate particularly favorable characteristic, with respect to commercial varieties, to be considered for further study.

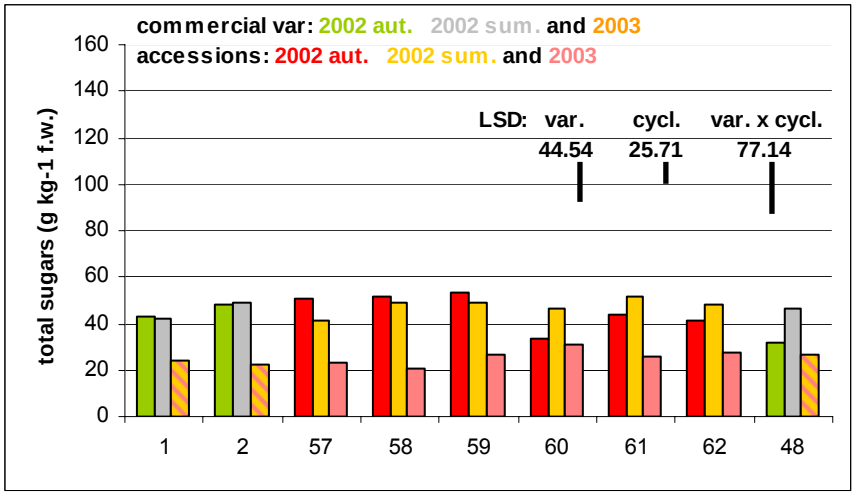
Figure 3.14. Total amount of sugars in experiment held in summer of 2001 and autumn of 2003 for 11 accessions



Lake in the previous discussed experiments, in trials that were held in two cycles of 2002 and in autumn 2003, sugars quantity was typical genotype characteristic. Sugars amount significantly was related to the growing cycle (Figure 3.15.). For all accession lowest sugar amount was recorded in autumn growing period of 2003. In general, in summer cycle of 2002 was recorded slightly higher content of sugars in comparison with autumn of 2002.

Interaction of varieties and growing cycles create different combinations of sugars amount. First group of accessions with No. 1, 57, 58 and 59, has higher sugar quantity in summer period, and that difference was not big. Other accessions like No. 60, 61, 62 and 48 have more sugars in autumn 2002 cycle and that difference was more obvious.

Figure 3.15. Total amount of sugars in experiment held in summer and autumn of 2002 and in autumn of 2003 for 9 accessions



To resume, sugars quantity in carrot is genotype characteristic, but it can be under great environmental influence. Summer growing cycles were characterized with higher sugars amount in years 2001 and 2002 in comparison with autumn period. In year 2001 that difference was more obvious.

Variety x growing cycle interaction as well demonstrated significant effects on sugars quantity in carrot roots. As a result of interaction of two factors, certain accessions have characteristic behaviour. Particularly interesting accessions are one with stable amount of sugars in different environmental conditions like No. 14, 37 and 45. It should be stressed that their sugar amount was on the level of commercial varieties. Accessions 8, 40, 44, 60, 61 and 62 have higher sugar quantity in autumn cycles and they should be taken into consideration for further research and evaluation.

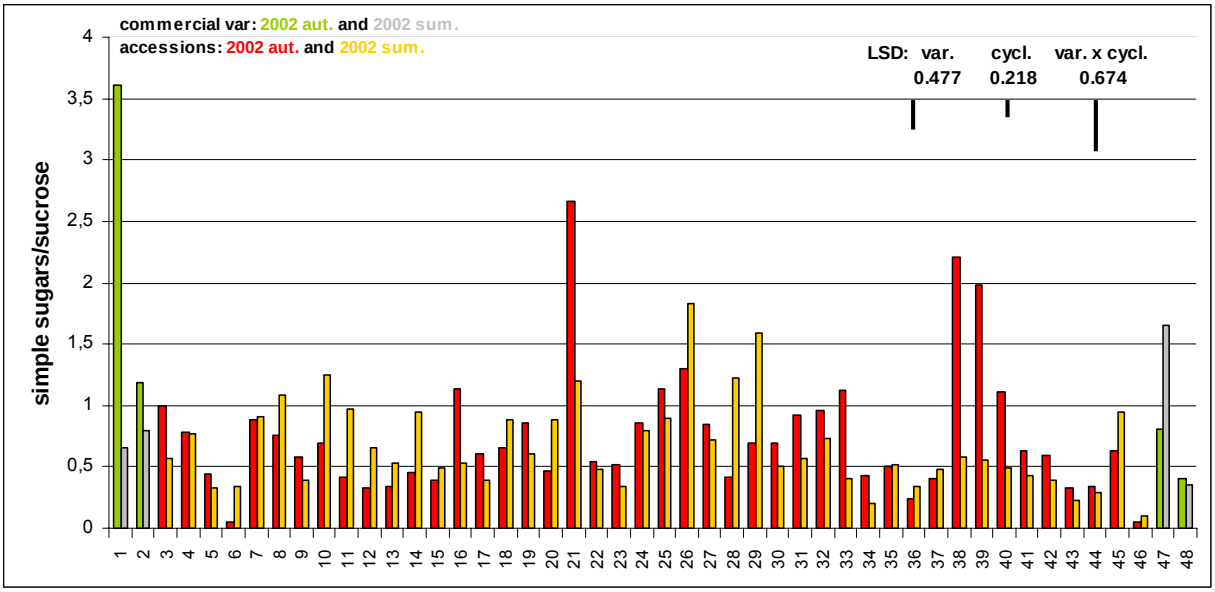
2.3.4.2.2. Relationship between monosaccharides and sucrose and ratio among monosaccharides

Ratio between monosaccharides and sucrose in experiment held in summer and autumn of 2002 are illustrated in Figure 3.16. Monosaccharides to sucrose ratio should be in general under 1, end in this study we can see some accessions with ratio even over 2 and 3.6.

Relationship among simple sugars and sucrose significantly varies among different accessions. Genotype x environment combination causes accessions behaviours that can be grouped in two major groups. In the first group are accessions that have higher amount

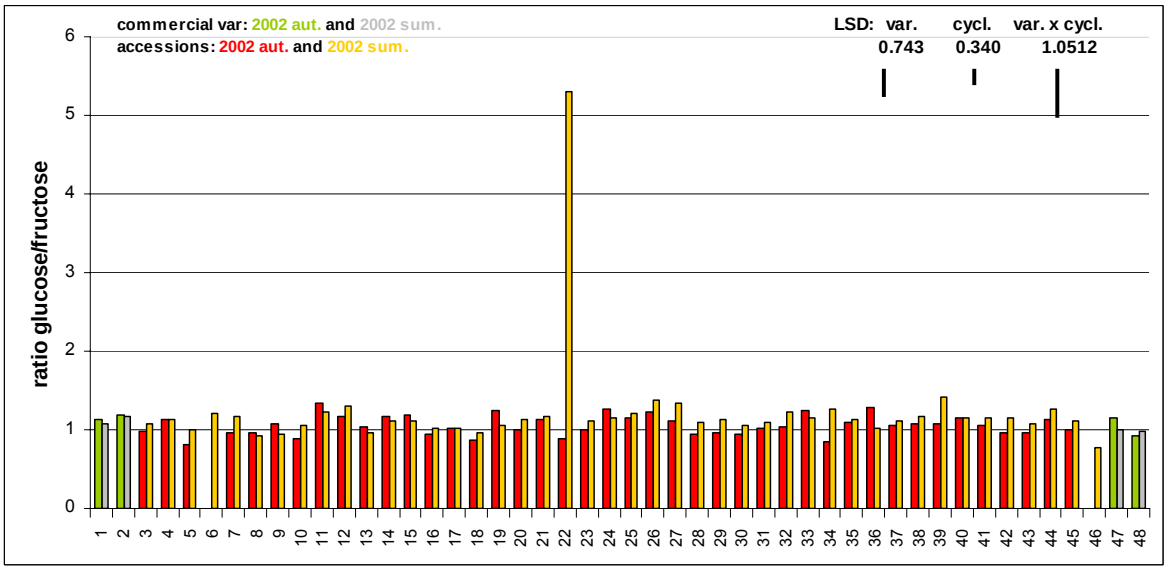
of monosaccharides in autumn growing cycle, and those accessions are: 16, 21, 38, 39 and 1 with the highest ratio of 3.6. Second group is made of accessions which have more monosaccharides in summer growing cycle like 8, 18, 26, 28, 29 and 47.

Figure 3.16. Ratio between monosaccharides and sucrose in experiment held in summer and autumn of 2002 for 48 accessions



At the same time, those accessions did not show major differences in glucose/fructose ratio among different growing seasons (Figure 3.17.). Exceptions with higher ratio in summer were accessions 34, 39 and especially accession 22 with extremely high value in summer 2002 (over 5).

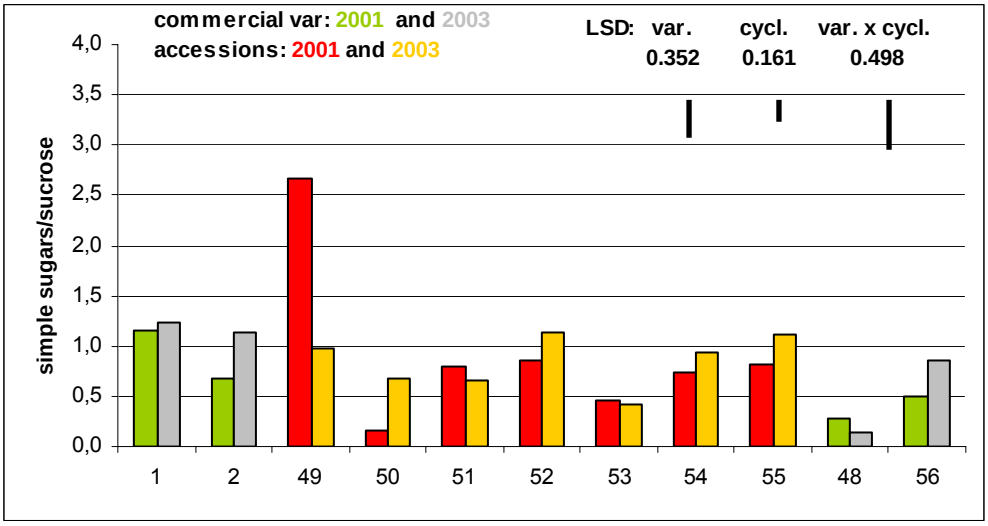
Figure 3.17. Ratio between glucose and fructose in experiment held in summer and autumn of 2002 for 48 accessions



When the result from experiment in summer of 2001 and autumn 0f 2003 are taken into consideration (Figure 3.18.), it is clear that monosaccharides/sucrose ratio is variety related characteristic. Growing period has significant influence on the monosaccharides amount in comparison to the sucrose and it is in average higher in summer cycle of 2001. Variety and growing cycle interaction are dividing accessions in three groups:

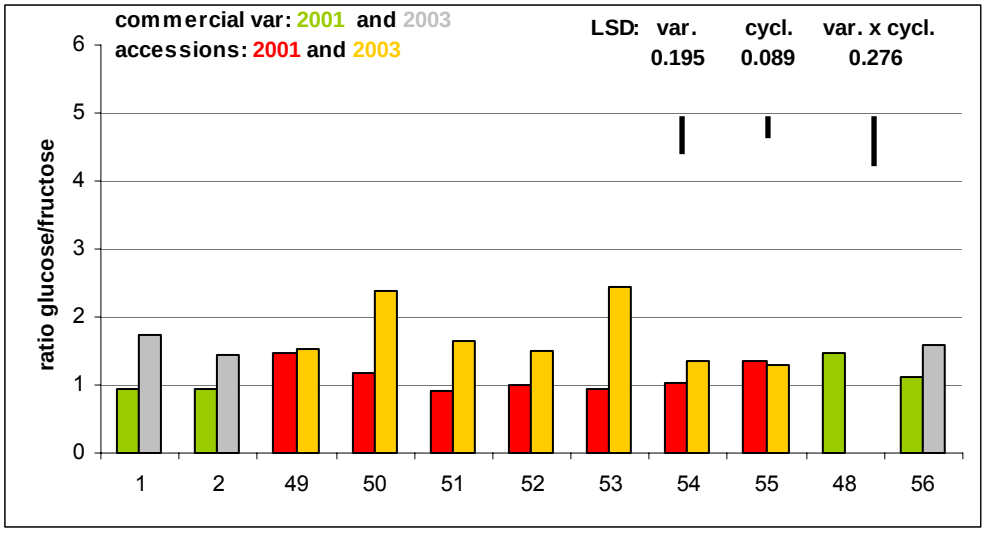
- Group with higher ration in monosaccharides favour in summer growing period and in this group are No. 51, 48 and 49 which have extremely high ratio in summer time, over 2.5. It is interesting that accession 49 keep the same balance between glucose and fructose (Figure 3.19.) in both growing cycles.
- Group of accessions which have more monosaccharides in autumn period. That group is made of accessions No. 2, 50, 52, 54, 55 and 56. Here is interesting to stress that all accessions are having higher glucose to fructose ratio in autumn cycle (Figure 19.), indicating that majority of monosaccharides increase is related to glucose.
- Accessions No. 1 and 53 which have stable balance among simple sugars and sucrose. Still internal monosaccharides balance is moved toward glucose, ratio is around 2, in autumn growing period (Figure 3.19.).

Figure 3.18. Ratio between monosaccharides and sucrose in experiment held in summer of 2001 and autumn of 2003 for 11 accessions



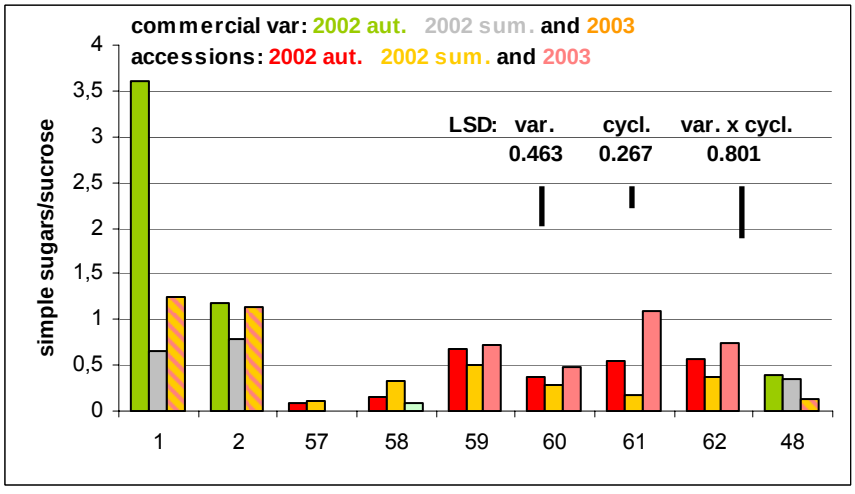
Considering glucose to fructose ratio in experiment from 2001 summer and 2003 autumn, can be seen important influence of variety on it value. It is also clear environmental influence which causes higher ratio in glucose favour in autumn growing cycle. Apart from 55 that was the case for all other accessions.

Figure 3.19. Ratio between glucose and fructose in experiment held in summer of 2001 and autumn of 2003 for 11 accessions



Results of monosaccharides to sucrose ratio in 2002 and 2003 experiments are illustrated in Figure 20. It can be seen that ratio is in the range of 0 to 3.6 and it diverse form one accession to another.

Figure 3.20. Ratio between monosaccharides and sucrose in experiment held in summer and autumn of 2002 and in autumn of 2003 for 9 accessions

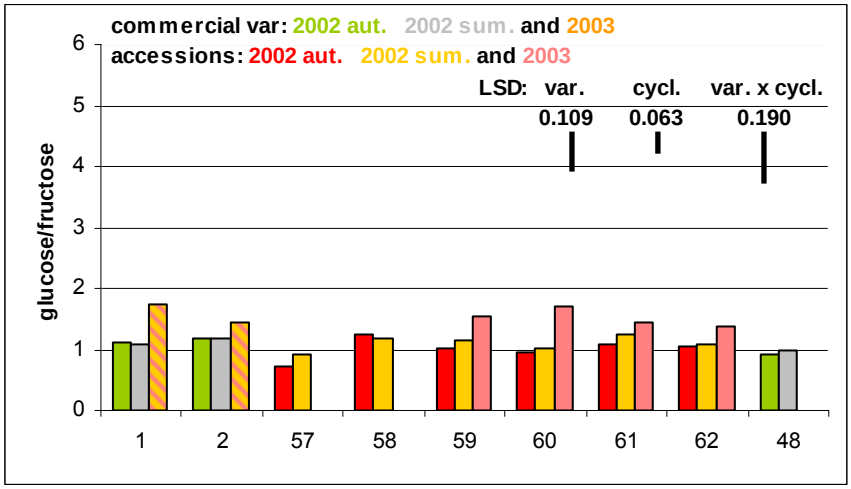


Influence of growing period was significant, causing higher ratio in favour of monosaccharides in autumn cycles for both years 2002 and 2003, with highest difference in case of accession 1. Exceptions are accessions 58 and 48, but their ration was very low, under 0.5 in all growing conditions.

Accessions 59, 60, 61 and 62 have the highest monosaccharides to sucrose ratio in autumn period of 2003 (Figure 3.20.). At the same time their balance among glucose and fructose has been move in glucose favour (Figure 3.21.). It suggests that increase in monosaccharides to sucrose ratio is mainly related to increase of glucose content.

For experiments held in 2002 and autumn of 2003 glucose to fructose ratio varies in range of 0 to 1.75 (accession No.1). In general it has lowest values in autumn of 2002 and the highest in autumn of 2003. Accessions No. 1, 2, 58, 60, 62 and 48 have similar tendency. Their glucose to fructose ratio was stable in both growing cycles in year 2002. Than in year 2003 that balance is moved in glucose favour.

Figure 3.21. Ratio between glucose and fructose in experiment held in summer and autumn of 2002 and in autumn of 2003 for 9 accessions



2.3.4.2.3. Conclusion on sugars amount in fresh matter

All examined characters are closely related to the particular accessions and can be considered as genotype characteristics.

Significant environmental influence on characteristics related to sugars in a way that:

- Total sugar amount was higher in summer growing cycle.
- Monosaccharide to sucrose ration was slightly higher in autumn growing period.

- Glucose to fructose ratio has higher values in autumn; balance is moved toward glucose formation.

Genotype x environment interaction causes significant differences in characteristics appearance in experiments. This interaction also indicated certain accessions with stable behaviour and some accessions with favourable characteristics in particular environmental conditions. Table 3.3. contains list of characteristics related to sugars in carrot fresh matter, with accessions that show interesting behaviour.

Table 3.3. Carrot - sugars characteristics with favourable behaviours and list of accessions

Characteristic	Favourable behaviour	Accessions of interest
Total sugars	Higher total sugars in autumn cycle	8; 40; 44; 60; 61; 62
	Stable amount of total sugars in different growing cycles	14; 37; 45
	Highest total sugars amount	51
Monosaccharides to sucrose ratio	Higher ratio obtained in summer cycle	26; 29
	Similar ratio in summer and autumn cycle (under 1)	37; 44

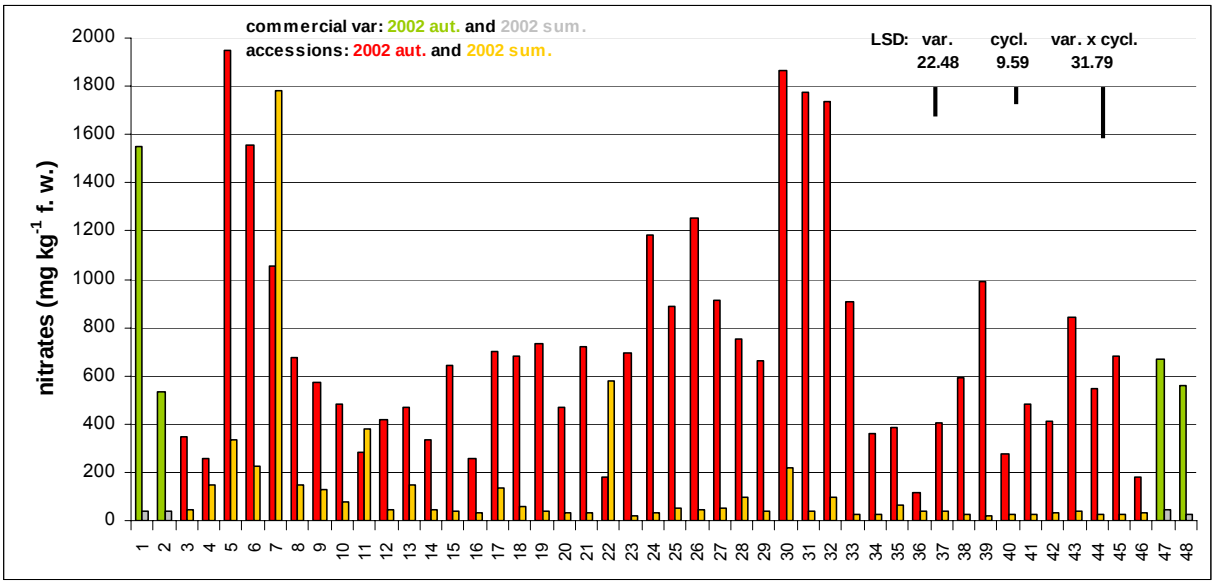
Listed accessions that have interesting results and should be studied in the future are one with higher sugar amount in autumn cycle (No. 8, 40, 44, and 62) together with one that has stable sugar quantity (14, 37 and 45).

2.3.4.3. Nitrate amount in fresh matter

In the 2002 experiment it was possible to compare 48 accessions in summer and autumn growing seasons. Obtained mean amounts of nitrates are presented in Figure 3.22.

The effects of all the experimental factors: variety, growing cycle and their interaction had significant effect on nitrate content. The examination of the variety x cycle interaction is of particular interest to detect particularly suitable accessions to certain growing periods or with overall favorable characteristics.

Figure 3.22. Nitrate amount in experiment held in summer and autumn of 2002 for all 48 accessions



Autumn grown carrots had substantially higher nitrate content. This finding goes in line with the results of Graifenberg (1993), who indicates that in periods with colder weather and shorter days, nitrate reductase is less active and amount of nitrate is increasing inside carrot roots. There are two exceptions from this general rule, accessions 22 and 7. Accession 22 has almost tree times more nitrates in summer period, on the other side variety 7 has really high amount of nitrates, over 1500 mg kg^{-1}.

Other varieties with high nitrate content in autumn cycle were: 5, 6, 24, 26, 30, 31 and 32. Accessions 36, 40 and 46 have low nitrate amount in both growing seasons (less than 300 mg kg^{-1}).

It is interesting to note that commercial varieties had generally very low nitrate content in summer cycle, whereas their nitrate contents increased in autumn cycle. On the contrary, some accessions were rather interesting since they kept low nitrate contents also in fall: among these n. 3, 4, 36, 40 and 46 were particularly interesting.

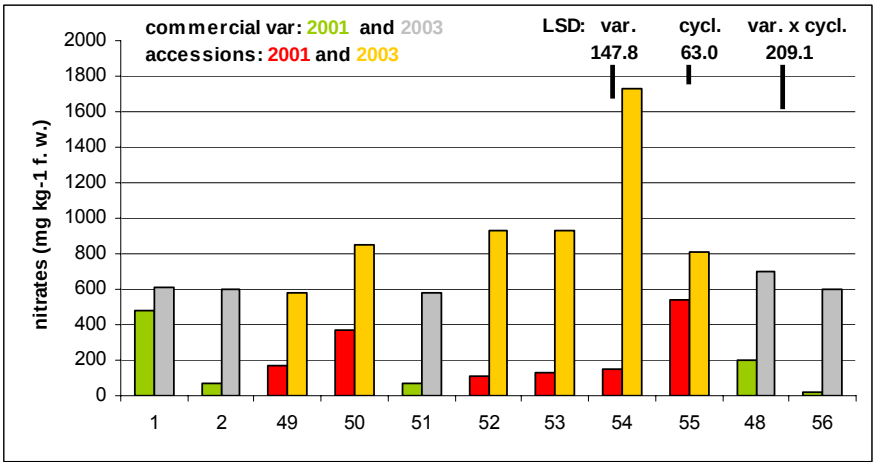
Other cases in which the same accessions were compared through different growing cycles were year 2001 in summer and 2003 in autumn cycle, and summer and autumn cycles of 2002 with autumn cycle of 2003.

Figure 3.23. reports nitrate content of accessions common to summer 2001 and 2003 autumn cycles. All 11 accessions have higher amount of nitrates in 2003 autumn cycle.

Apart from accession 1, which has similar nitrate content in both cycles, others have 3 to 8 times higher nitrate amount in year 2003. This finding is confirming the ones from year

2002, with similar differences in nitrates amount for summer and autumn growing period. The biggest difference between two cycles has accession 54 (less then 200 mg kg^{-1} in 2001 and over 1700 mg kg^{-1} in 2003). In this case, no particular interest of the accessions included, with respect to commercial varieties, was detected.

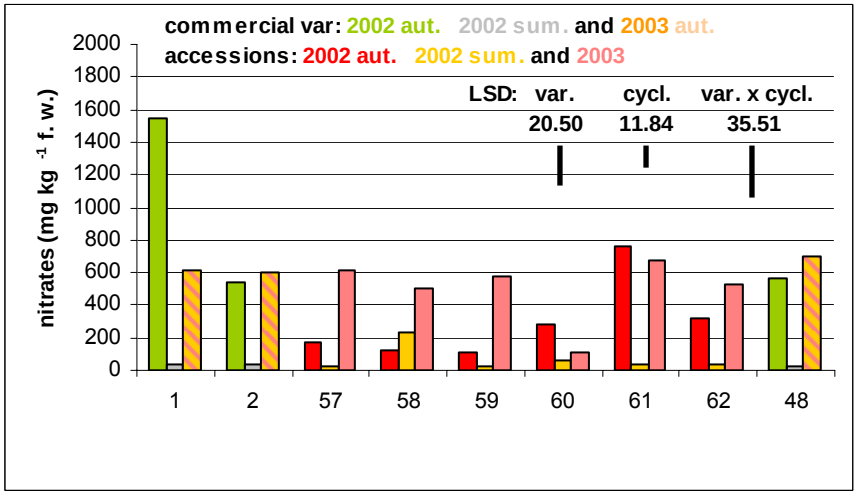
Figure 3.23. Nitrate amount in the 11 accessions common to the 2001 and 2003 autumn cycle experiments.



Variety, cycle of growing and their interaction, as experimental factors, demonstrated significant influence on nitrate content inside carrot roots in treatments from 2001 and 2003.

The nitrate content of accessions common to the two 2002 cycles and 2003 are illustrated in Figure 3.24. Most accessions had higher nitrate amount in year 2003, exceptions are accessions 1 and 61 (maximum in autumn 2002). Accession 58 and 60 are interesting since their nitrate content was less than 500 mg kg^{-1} in all three cycles.

Figure 3.24. Nitrate amount in experiment held in summer and autumn of 2002 and in autumn of year 2003 for 9 accessions



Further comparison of results for three commercial varieties: 1, 2 and 48, which were used as standards and grown in all cycles (summer 2001; summer, autumn 2002 and 2003) shows that the most constant nitrogen amount in autumn cycles have varieties 1 and 2 (between 500 – 600 mg kg^{-1} of f.w.). They were not so constant during summer growing cycles in 2001 and 2002, when in general nitrate amount was lower for most of accessions.

To summarize the discussion on nitrates amount in carrot root, it is clear that their amount is variety/accession related characteristic. Period of experiment alone, and coupled with genotype has important influence on nitrates quantity. Nitrates quantity in summer growing period was significantly lower for majority of accessions. Accessions that can be interested for further study, since they have low nitrates amount in all growing cycles are: 30, 40, 46, 58 and 60. Among the commercial one, varieties 1 and 2 have the lowest variations in nitrate quantity expressed only in autumn growing period.

Based on discussed accessions characteristics it is obvious that some of them should be considered as good base for further research. From discussed accessions two are particularly promising:

- Accession 60 has stable nitrates amount in different growing cycles and highest carotene amount together with higher sugars in autumn cycle.
- Accession 61 with stable carotene quantity in different environmental conditions and more sugars in autumn growing period.

Other accessions that have interesting behaviors for certain quality characteristics, already mentioned for particular character, should be studied in the future as a potential source of crop improvement.

2.4. Carrot sensory evaluation

2.4.1. Introduction

The commercial market for food, including carrot is increasing and at the same time becoming more competitive. Ensure good quality of product is the first condition for entrance and survival on such market. It is also important to know costumer needs and preferences to deliver product with asked characteristics. Product sensory quality have important role in fulfillment of costumers desires.

Carrots hold importance place in European diet because of it high dietary values as a major β carotene source. The sensory quality of carrots is especially important for fresh consumption and it may vary from delicious sweet flavour to an unpleasant bitter taste. In general, quality of raw material had substantial effect on the sensory quality agricultural products.

One way to improve the sensory quality of raw carrots is to ensure suitable and uniform raw materials. That is difficult to obtain since genetic variation and environmental conditions largely influence the sensory characteristics of carrot raw material.

Selecting of carrot cultivars with desirable color, taste and aroma characteristics is a useful instrument for good quality product. The most direct tool to check developed variety, or check potential of unknown varieties and breeding lines, in terms of market preferences, is through sensory evaluation.

Sensory evaluation is a scientific technique that uses human senses (i.e. taste, smell, sight, touch and hearing) to judge and evaluate the quality of foods and materials. It involves both trained panelists and consumers. Trained panel evaluations are used to detect and describe the organoleptic characteristics of food and non-food products. Consumer tests provide an indicator of the acceptability of a product.

Important sensory carrot attributes: sweet odor, sweet taste, juiciness, hardness and crispness. Characters like crispness and juiciness are usually associated with food freshness by consumers (Filion and Kilcast, 2000).

As it was already mentioned, environmental and growing conditions during carrot production are important considerations when assessing processed product quality. Soil

type and root maturity at harvest can impact sensory quality, as bitter flavors are more prevalent in carrots grown in muck and sandy soils versus loam soils. Additionally, reducing sugars predominate in early root development as sucrose becomes the prevalent sugar near horticultural maturity (Simon, 1985).

Growing, handling and storage conditions affect the level of carrot volatiles as well as the content of sugars and carotenoids. Consumer's likings for carrots are generally agreed to be correlated to perceive sweetness. (Fjelsenden *et al.* 1983; Martens *et al.* 1983).

Climatic conditions can influence carrot flavor since cooler than average temperatures, low relative humidity, short days, and long nights promote maximum sugar production (Simon *et al.* 1982).

In fresh carrot the non-volatile chemical constituents (sugars and amino acids) are preliminary responsible for the taste sensation.

Carrots grown at high temperature were less sweet and bitterer than those grown at low temperature, although high temperatures produced carrots with the highest sugar content.

It is well known that certain substances, especially bitter-tasting substances, may interact with or mask the effect of other substances.

Simon *et al.* (1980) stress importance of sugars and terpenes for raw carrot flavor. He suggested that sweetness and overall acceptance are enhanced by sugars and reduced by volatile compounds. The upper root part more sugars, but more terpenes, not sweet sensation.

Simon *et al.* (1980) and Rosenfeld *et al.* (2002) identified terpenes (α-terpinene, β-myrcene, trans-caryophyllene, γ-terpinene) as volatile compounds responsible for bitter taste and thus suppressed the perception of sweet taste in carrots.

2.4.2. Materials

For this research 20 accessions from the autumn production cycle of year 2003 were used. This research was small part of big scope project which was related to the evaluation of European carrot. Accessions used for this study were received from genebanks, they have been evaluated on different characteristics, and the most interesting ones were chose for sensory evaluation program. Source and number of accessions was:

- Institute National of Horticulture: **INH,** 2 accessions

- Federal Research Center for breeding of cultivated plants: **BAZ,** 3 accessions
- Horticulture Research International: **HRI,** 8 accessions
- Nordic Gene Bank: **NGB** 1, accession

Apart from the accessions, the following commercial varieties were included into the experiments: Amsterdam, Autumn King, Bolero, Nikki 1, Parmex and Rubrovitamina

2.4.3. Methods

Plant material of 20 accessions was collected and prepared for panel test. Three panel sessions were held where students and personnel of the university participate after a very short training.

They were asked to indicate their appreciation for the following characteristics:

- External characteristics: general acceptance, root shape, external colour and internal colour
- Sensory evaluation – taste characteristics: global acceptance, crispiness, toughness, fibrousness, aroma and sweetness.

For evaluation of external characteristic were used 20 accessions, on the other side for sensory evaluation were used 16 accessions.

Results obtained through panel sessions were used for further analysis.

2.4.4. Discussion

2.4.4.1. External and internal sensory characteristics

During trial held in 2003 were taken into consideration 20 accessions for sensory analysis of external carrot characteristics. Factors considered for external carrot evaluation were: global acceptance, visual shape, external root colour and internal root colour. Members of panel have to give their opinion (score) for each of mentioned characteristics. Score was in range from 1 to 100 and it was in accordance with level of acceptance by each panel member.

Obtained results for particular accessions are listed in Table 4.1. and illustrated in Figure 4.1. by radar type data. Illustrations were done for 4 genotypes on all external characteristics.

Table 4.1. External sensory evaluation factors (acceptance) for 20 accessions

accession	general	shape	colour	
			external	internal
baz 352	54,4	46,2	61,4	62,3
baz378	47,4	54,3	50,8	39,8
baz 428	43,3	36,7	51,5	56,0
hri 6760	52,1	65,5	48,8	43,7
hri 6519	43,1	73,4	34,8	32,3
news f 1	77,6	75,6	78,1	79,7
inh 1	78,8	79,4	81,9	78,8
inh 14	62,7	52,9	65,9	69,7
hri 3937	52,6	58,5	53,2	44,7
hri 7801	76,7	70,1	80,5	78,0
Parmex	37,4	35,2	40,4	44,7
Amsterdam	70,1	69,4	64,1	71,4
ngb 2399	40,5	34,2	52,9	36,9
Aut. king	75,6	72,0	72,1	79,0
hri 13404	41,6	58,0	47,5	29,5
Bolero	76,8	71,3	75,0	77,3
hri 3838	63,5	51,6	67,0	68,3
hri 4007	46,5	75,7	62,4	27,3
hri 6102	81,4	80,0	80,8	87,1
Rubrovitamina	78,6	81,4	77,9	76,5
significance	**	**	**	**
lsd p=0.05	9,9	8,7	8,7	7,8
lsd p=0.01	13,0	11,4	11,5	10,2

From Table 4.1 it can be seen that significant differences among accession where detected on all characters. Results presented in table regarding general appearance are in range of 37.4 for Parmex variety to 81.4 for HRI 6102 accession. By further analysis of results obtained for this character it is obvious that lowest score was recorded for

accessions with short conical roots, like Parmex, BAZ 428. Other source for low general level of acceptance is colour of the root, e.g. HRI 6519 with very low general score but high result in shape of 73.4 and little bit higher than 30 for colour characters, since this accession has yellow root.

On the other side, accessions with highest score for general acceptance are the ones with long conical to cylindrical roots. Highest score for this characteristic was recorded for accession HRI 6102 with long, conical orange roots.

Shape as external character for many accession has similar values to those of general acceptance. Still in the cases of non orange colored root, shape value can be higher, like for purple HRI 6760.

Colour as external factor influenced a lot the panelist perception of certain accessions. Accessions with orange colour and slight difference in parenchyma and core colour had the best results in external and internal colour ratings. Accession with non orange colour like purple HRI 6760, yellow HRI 6519 and yellow to orange HRI 13404 have low values for both, external and internal colour. Low colour values are also recorded for the accessions with short conical roots like Parmex and NGB 2399. Great decrease in internal colour appreciation in comparison with external one was recorded for the accessions with great difference between parenchyma and core colour e.g. HRI 4007.

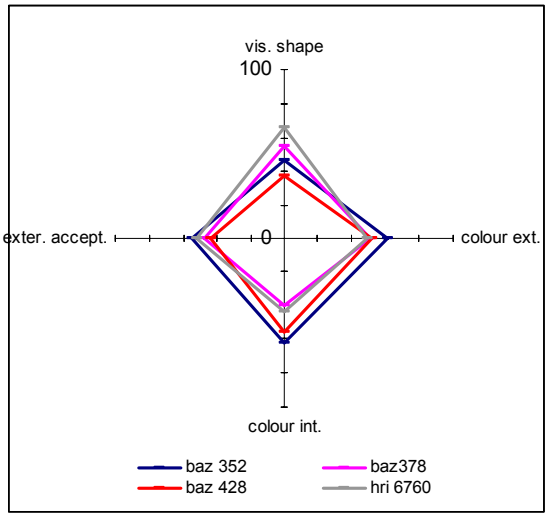
In general, panel members preferred accessions with long and cylindrical to conical roots. It was also favorable that root have similar external colour to the one of core part. Accessions with short conical roots and yellow, purple or other non typical orange colour evaluated at lover level. Figure 4.1 has interesting illustrations for certain varieties. From that figure it is obvious that Parmex has very low values for all four characters. Then accessions HRI 6519, HRI 13404 and HRI 4007 have same data distribution on the radar illustration. Only difference is little bit lower level of external colour for HRI 6519.

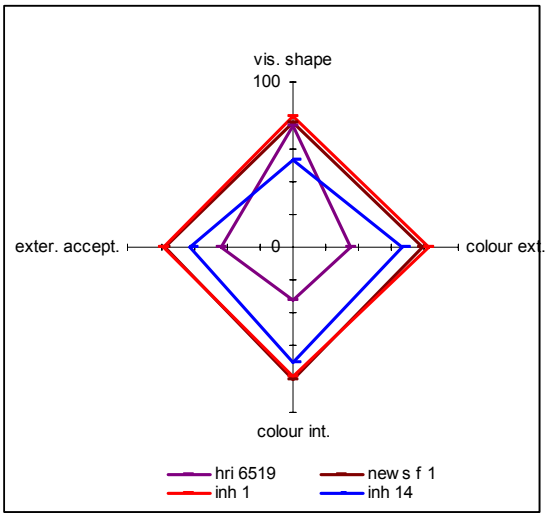
The true sensorial characters for evaluation were: crispiness, toughness, fibrousness, aroma, sweetness and internal acceptance. The obtained results are presented in Table 4.2. and in Figure 4.2. In this part of sensory evaluation were included 16 accessions since for other four not enough plant material was available.

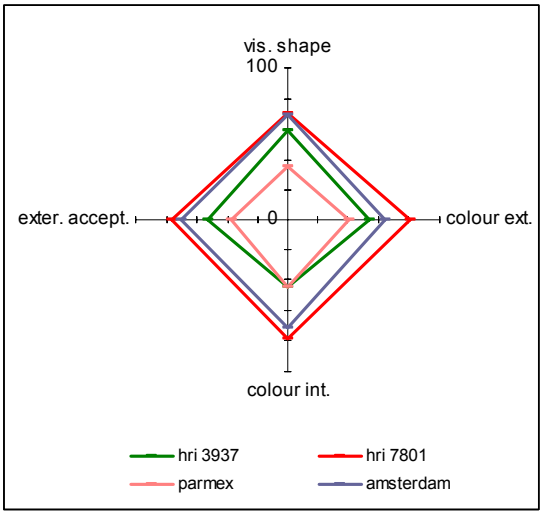
Taking into consideration crispiness, its value goes in range of 60 to 82.5 (Bolero) (Table 4.2.). By comparison of crispiness results, for certain accessions, with internal acceptance it is indicated that accessions with low crispiness score had a tendency to low score for internal acceptance. So, accession HRI 6519 with minimum value for crispiness of 60 has

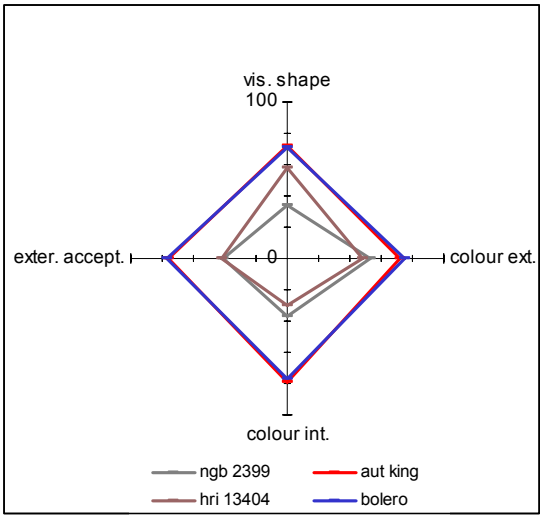
acceptance value of 36. At the same time Bolero variety has crispiness level at 82.5 and internal acceptance at value of 72 (Figure 4.2.).

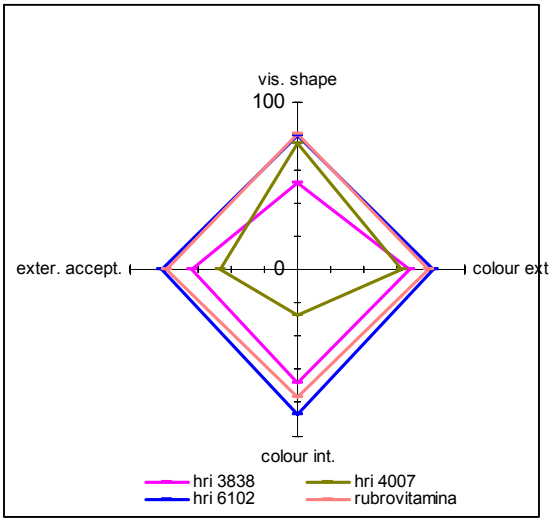
Figure 4.1. External sensory evaluation factors (acceptance) for 20 accessions











Toughness has smaller range of values, between 59.2 and 72.1. For this character the highest and the lowest score were recorded for the same accessions as for crispiness, Bolero and yellow HRI 6519.

Fibrousness rating had much lower values in range from 42,5 (Bolero) up to 57.9 (HRI 6102). This factor in general has opposite behavior from crispiness and toughness. Accessions with higher first two characters usually have lower record for fibrousness e.g. Bolero with high values for crispiness and toughness has the lowest value for fibrousness. It looks like fibrousness has inverse relationship with internal accession acceptance.

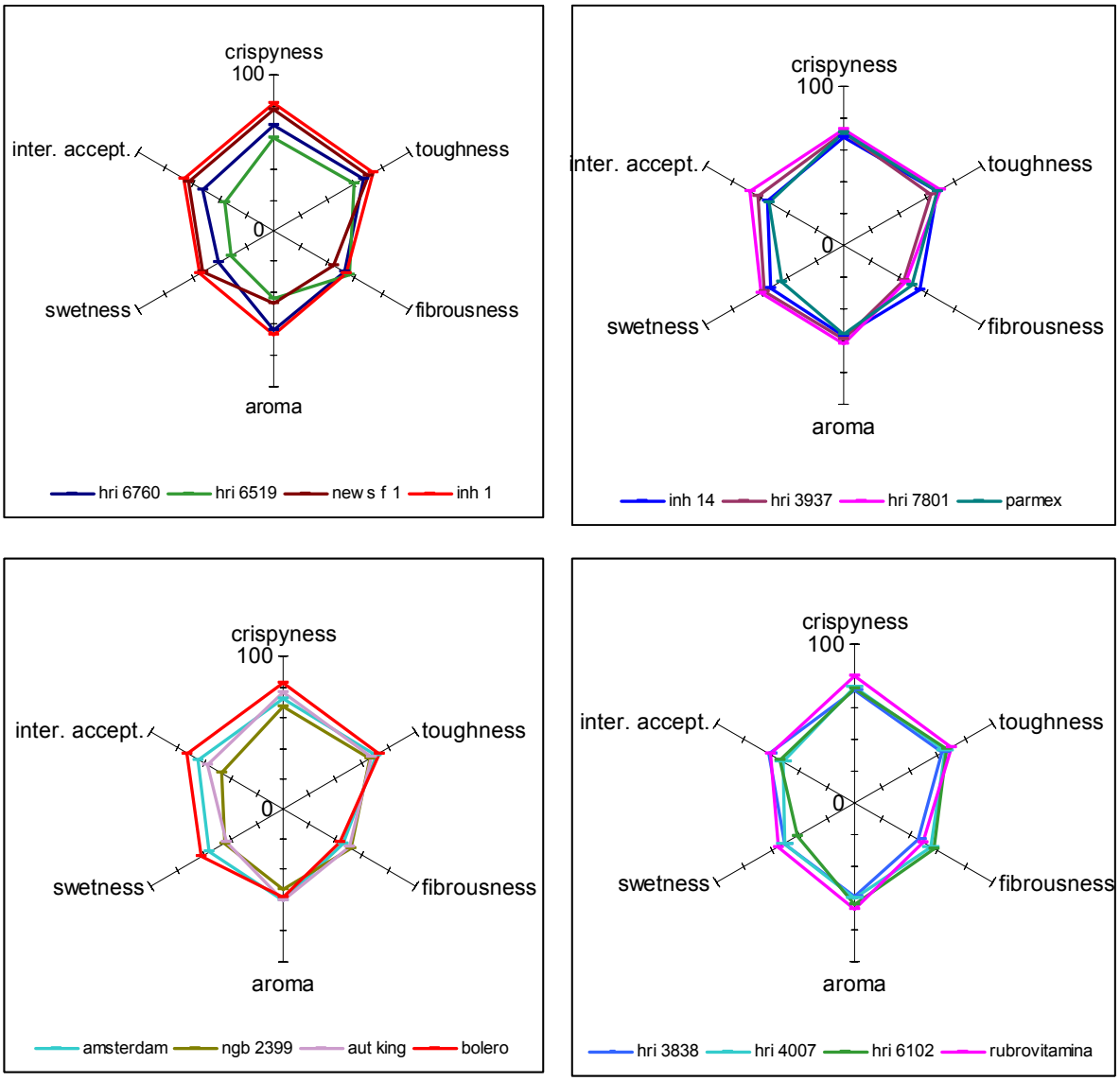
Table 4.2. Sensory evaluation factors (tasting) for 16 accessions

accession	crispiness	toughness	fibrousness	aroma	sweetness	internal acceptance
hri 6760	67,7	66,2	52,1	64,0	40,4	52,7
hri 6519	60,0	59,2	56,8	43,7	31,7	36,0
news f 1	78,0	69,4	44,4	46,2	52,5	62,9
inh 1	81,4	73,3	53,8	66,5	54,6	67,1
inh 14	67,7	67,8	55,6	57,3	53,3	56,5
hri 3937	71,3	63,2	43,7	59,1	58,3	62,0
hri 7801	73,1	70,4	44,9	61,9	60,5	67,8
Parmex	70,8	68,0	49,6	56,3	45,1	53,7
Amsterdam	71,7	70,2	45,7	59,8	55,7	64,4
ngb 2399	66,7	65,4	51,9	52,7	44,0	46,8
Aut. king	76,2	67,3	49,8	59,5	43,0	56,9
Bolero	82,5	72,1	42,5	57,5	61,8	72,0
hri 3838	70,6	63,4	46,3	58,6	51,2	61,8
hri 4007	73,3	66,1	56,5	60,4	50,7	52,2
hri 6102	72,1	67,4	57,9	63,7	42,4	54,4
Rubrovitamina	79,6	69,8	49,9	67,2	56,4	60,8
significance	**	*	**	**	**	**
lsd p=0.05	7,5	7,7	8,5	9,4	9,5	9,0
lsd p=0.01	9,8	10,1	11,2	12,4	12,5	11,8

Aroma intensity rating was the lowest for accession HRI 6519 and reason for it can be low intensity, or maybe undesirable aroma. Variety Rubrovitamina has the most acceptable aroma with value of 67.2.

One of the characters with the highest variation among the varieties is sweetness with range of ratings between 31.7, for the already mentioned HRI 6519, and 61.8 for Bolero. In Figure 4.2 accessions with overall good scores are well evident, like: Bolero (high values in crispiness, toughness, aroma and low score on fibrousness). On the other side are examples like HRI 6102 with lower level of crispiness, and especially sweetness but high value for fibrousness.

Figure 4.2. Sensory evaluation factors (tasting) for 16 accessions



Internal acceptance is illustrated in Figure 4.2. and it has clearly low value for varieties: HRI 6519, NGB 2399, but from the graph can be also seen their low sweetness level.

2.4.4.2.1. Relationship between external acceptance and three external factors

For the relationship analysis between external acceptance and other external factors was used path coefficient analysis. Path coefficient is a statistical analysis in which the correlation between a Y (dependent variable) and x (independent variables) is divided in a direct effect (this is the standardised multiple regression coefficients of each x on y) and an indirect effect (the effect via the correlation with other x).

Table 4.3. "Path coefficient" analysis between external acceptance (dependent variable: y) and the three external sensory evaluated characters (independent variables: x). Overall r^2=0.99**, n=20.

External sensory characters	**r** [(1)]	**Effects (*direct*[(2)], indirect)**			**Correlations between variables** [(3)]		
		shape	*ext. colour*	*int. colour*	*shape*	*ext. colour*	*int. colour*
shape	0.70 **	*0.32***	0.14	0.25	1	0.58**	0.42°
external colour	0.93 **	0.19	*0.24***	0.51	0.58**	1	0.86**
internal colour	0.92 **	0.13	0.20	*0.59***	0.42**	0.86**	1

(1) correlation coefficient between y and individual xs
(2) direct effect: path coefficients or standard multiple regression coefficients
(3) correlations between xs
significances: ** p≤0.01; p≤0.05; p≤0.10; ns : non significant

According to the path coefficient analysis, all three external sensory characters have positive relation to external acceptance. The direct effect of all the three characters was significant. However, the high correlation coefficients had an important component of indirect effect, thanks to the demonstrated mutual positive correlation between the three characters. Only in case of internal colour the direct component was prevalent.

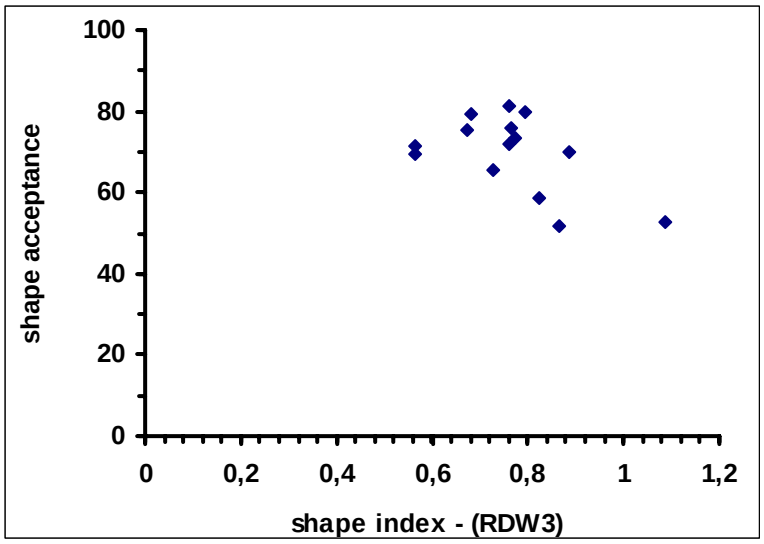
2.4.4.2.2. Relations between shape appreciation and shape index

Among known shape indexes it was not applied numerical regression as indicator of relationship between the shape index and consumer shape appreciation. Still in case of shape index RDW3 is possible to find certain relation. RDW3 is a shape index which presents volume of the cube with all three bases equal to the root diameter. Index values of: around 0,6 are indicating long cylindrical roots, around 0,8 long conical roots and values around 1 and higher are indicating short conical roots.

In the scatter diagram (Figure 4.3) it can not be seen any numerical regression, but it is shown that RWD3 values under 0,8 and especially around 0,8, cause acceptance of around 70-80, while RDW3 values over 0,8 are influencing on the drop of consumer shape acceptance to 50-60.

From the mentioned results it can be indicated that panellists preferred long roots with cylindrical to slightly conical shape. Those roots also have medium to large root diameter.

Figure 4.3. Relation between RDW3 index values (as shape indicator) and consumer shape appreciation



2.4.4.2.3. Relations between perception of colour and its determinants

The relations between external and internal colour perception and colorimetric characters (L: lightness; hue and saturation) were investigated.

Table 4.4. "Path coefficient" analysis between external colour perception (dependent variable: y) and colorimetric characters Lightness (L) and saturation (independent variables: x). Overall r^2=0.84**, n=14.

external colour	**r** [1]	**Effects (*direct* [2], indirect)**		**Correlations between variables** [3]	
		l	*saturation*	*l*	*saturation.*
L	-0.28 ns	*-0.92***	0.64	1.00	0.59*
saturation	0.54*	-0.54	*1.08***	0.59	1.00

[1] correlation coefficient between y and individual xs
[2] direct effect: path coefficients or standard multiple regression coefficients
[3] correlations between xs
significances: ** p≤0.01; p≤0.05; p≤0.10; ns : non significant

"Path coefficient" analysis indicates that correlation (r) between lightness and external colour is not significant and that with saturation is slightly positive (Table 4.4.). However, path coefficient better stresses the real functional relations, that the direct relation between both lightness and saturation, to external colour perception is significant, when it is combined in the multiple regressions. Lightness has strong negative relation to external root colour perception and saturation strong positive. Those two

factors are mutually positively related, and in that way indirect effect on external colour partially hides the direct effects, especially for lightness.

While lightness had no correlation with carotene content, saturation as factor was positively correlated with total carotenes (0.68**), α carotene (0.69**) and β carotene (0.64*) and α/β carotene (0.69**). So the content of carotenes affects colour saturation, and this in turn is perceived as positive fact for colour appreciation.

Colour lightness, on the contrary is not favourably affecting external colour sensory appreciation. In evaluated samples, this was positively related to colour saturation, but it was independently of carotene content.

This positive correlation lowered the effect of colour saturation on external colour appreciation.

The pattern of internal colour in relation to its determinants was very similar. Internal colour also had a direct relation to carotenes, and especially with α carotene (r=0.57**).

2.4.4.3.1. Relations between internal acceptance and internal sensory evaluated characters

It is interesting to define, if possible, the influence of internal sensory characters (crispiness, toughness, fibrousness, aroma and sweetness) on the overall internal acceptance. Table 4.5 reports the results of “path coefficient analysis" for these relationships. The results show that crispiness, toughness, aroma and sweetness had positive correlations with overall sensory acceptance, whereas fibrousness negatively affected acceptance.

Internal acceptance seemed to be a rather complex resultant of individual sensory perception. In fact, direct effects of single characters were generally rather low. In particular, crispiness and toughness affected acceptance by a substantial indirect effect, due to their mutual positive relation. Aroma was also positively related to the sweet taste perception, thus affecting overall acceptance by an indirect effect through sweetness.

Sweetness had the highest positive direct relation to acceptance.

On the other hand fibrousness had a direct negative relation to acceptance. Even more, its negative correlation to acceptance is enforced by its negative correlation to sweetness: this means that fibrousnesses negatively affected the perception of sweetness, a positive character for acceptance. In order to further define the relations among internal sensorial characters and overall acceptance, principal component analysis was carried out.

Table 4.5. "Path coefficient" analysis between internal acceptance (dependent variable: y) and the four internal sensory evaluated characters (independent variables: x). Overall r^2=0.93**, n=16.

Internal sensory characters	**r**	**Effects (*direct* [2], indirect)**					**Correlations between variables [3]**				
		crispiness	*toughness*	*fibrousnesses*	*aroma*	*sweetness*	*crispiness*	*toughness*	*fibrousness*	*aroma*	*sweetness*
crispiness	0.80**	*0.17*	0.18	0.14	0.08	0.24	1.00	0.81**	-0.42ns	0.46°	0.67**
toughness	0.77**	0.14	*0.22*	*0.10*	0.08	0.23	0.81	1.00	-0.29ns	0.49°	0.63**
fibrousness	-0.69**	-0.07	-0.07	*-0.33*	0.01	-0.23	-0.42	-0.29	1.00	0.05ns	-0.65**
aroma	0.48°	0.08	0.11	-0.02	*0.17*	0.14	0.46	0.49	0.05	1.00	0.39ns
sweetness	0.89**	0.11	0.14	0.22	0.06	*0.36*	0.67	0.63	-0.65	0.39	1.00

[1] correlation coefficient between y and individual xs
[2] direct effect: path coefficients or standard multiple regression coefficients
[3]. correlations between xs
significances: ** p≤0.01; p≤0.05; p≤0.10; ns : non significant

Principal component analysis defines correlation between original variables (characters) and principal components (factors) and it results are presented in the Table 4.6. Two factors were taken into consideration because they cumulatively explain 82% of variations (Table 4.6).

Table 4.6. Correlation between original variables (characters) and principal components

INDEX	1	2
CRYSPYNESS	0.8982	0.0761
TOUGHNESS	0.8677	0.2155
FIBROUSNESS	-0.5863	0.7454
AROMA	0.5746	0.6803
SWEETNESS	0.8768	-0.2387
Variance explained	3.0023	2.1277
Cumulative %	60.047	82.601

The first component (PC1), explains about 60% of total variance. This component is positively related to crispiness, toughness, aroma and sweetness, and negatively to fibrousnesses. It looks like the first component clearly represents acceptance.

The second component (PC2) is positively correlated to fibrousness and aroma and it explains 22.5 % of total variance. It seems that PC2 represents a negative component for acceptance, thus revealing a negative component of aroma. Indeed, taken alone, it does not have any relation to internal acceptance (r2 = 0.03 ns). However, including it in a multiple regression on internal acceptance, together with PC1, both PC have significant contribution:

The obtained standardised equation is:

Internal acceptance= 0.948 PC1 (**) -0.159 PC2 (°)

So this analysis seems to individuate a negative component of acceptance associated not only with fibrousness, but also with certain aspect of aroma perception. These two determinants seem however to act rather independently, although associated in the same principal component, since the two character are not mutually correlated.

2.4.4.3.2. Relations between aroma, acceptance and volatiles.

Explanation of the effect of some volatile were obtained exploring the relation between internal acceptance, other sensory characters and the relative content of specific volatile components.

The perception of aroma was slightly correlated with some components of the essential oil, either positively or negatively. The attempt of finding more explanatory relations by means of multiple regression and path analysis did not give relevant results.

This just confirms the complexity of aroma perception, and its possible involvement in determining both positive and negative sensory notes.

Therefore, an attempt was done to directly relate acceptance to individual essential oil components, also including aroma.

A final relation was obtained in which aroma was not retained:

sensory acceptance= 55.6 + 1.1 sabinene + 5.8 p-cymene –7.2 γ-terpinene –43.9 borneol +3.5 β-selinene

Path coefficient analysis of this relation is reported in Table 4.7.

The correlation between acceptance and essential oil components was negative, except for β-selinene. However, path analysis revealed that sabinene and p-cymene had positive direct relation to acceptance. At the same time, the relative content for both of them was positively related to that of γ-terpinene and borneol, which were strongly negatively related to acceptance. Therefore, the indirect effect of this correlation determined an overall negative relation of sabinene and p-cymene to acceptance.

β-selinene content, on the contrary, was negatively related to all the other components, and retained an overall positive effect on acceptance.

These results seem to confirm the complexity of the relation between acceptance and essential oil composition. The apparently positive effect of some components (sabinene and p-cymene) appears scarcely exploitable, because of their association to components with marked negative effects on acceptance (borneol and γ-terpinene).

Only β-selinene seems to be a potential useful component for positive selection (Table 4.7).

Table 4.7. "Path coefficient" analysis between internal acceptance (dependent variable: y) and some essential oil components (independent variables: x). Overall r^2=0.97**, n=16.

essential oil components	**r** [1]	**Effects (*direct* [2], indirect)**					**Correlations between variables** [3]				
		sabinene	p-cymene	γ-terpinene	borneol	β-selinene	sabinene	p-cymene	γ-terpinene	borneol	β-selinene
sabinene	-0.60*	*0.49***	0.41	-0.45	-0.83	-0.22	1	0.39ns	0.42ns	0.81**	-0.59*
p-cymene	-0.49°	0.19	*1.05***	-1.00	-0.49	-0.24	0.39	1	0.94**	0.48°	-0.64**
γ-terpinene	-0.61*	0.21	0.98	*-1.07***	-0.53	-0.21	0.42	0.94	1	0.52*	-0.55*
borneol	-0.87**	0.39	0.50	-0.55	*-1.02***	-0.20	0.81	0.48	0.52	1	-0.53*
β-selinene	0.55*	-0.29	-0.67	0.59	0.54	*0.37***	-0.59	-0.64	-0.55	-0.53	1

[1] correlation coefficient between y and individual xs
[2] direct effect: path coefficients or standard multiple regression coefficients
[3]. correlations between xs
significances: ** p≤0.01; p≤0.05; p≤0.10; ns : non significant

Further contribution to the understanding of acceptance was given by analysing the relation with the other main sensory determinant of acceptance (sweetness) and essential oil components. These results are illustrated in table 4.8.

Table 4.8. "Path coefficient" analysis between sensory acceptance (dependent variable: y), sweetness and borneol relative content of essential oil (independent variables: x). Overall r^2=0.93**, n=16.

		Effects (*direct* [(2)], indirect)		**Correlations between variables [(3)]**	
	r [(1)]	sweetness	borneol	sweetness	borneol
sweetness	0.89**	*0.54***	0.36	1.00	-0.79**
borneol	-0.87**	-0.42	*-0.45**	-0.79	1.00

[(1)] correlation coefficient between y and individual xs
[(2)] direct effect: path coefficients or standard multiple regression coefficients
[(3)]. correlations between xs
significances: ** p≤0.01; p≤0.05; p≤0.10; ns : non significant

As already illustrated, sweetness was strongly positively correlated with acceptance, and borneol relative content strongly negatively. Path analysis brought out interesting results by including these two variables. Sweetness and borneol relative content are significantly negatively related. So it seems that borneol negatively affects sweetness perception. By this mechanism, the direct effect of sweetness, although already positive and significant, is enhanced by a further positive indirect effect of low borneol, via the negative relation of sweetness to borneol itself.
Among the components of essential oil, borneol seems therefore to be one of the more negatively critical in determining sensory acceptance.

2.4.5. Conclusion

Sensorial evaluation is a tool to get more information about consumer preferences for foods. It can be used in market acceptance predictions for new varieties with different characteristics. In this study was used to evaluate potentials of accessions with interesting results in other aspects (high sugar content, high carotenes content, different root colour) and to try to analyze and understand triggers for consumer's acceptance of one accession and in refusing of another.

Sensory evaluation included several characteristic which can be grouped in two groups: external and sensorial (tasting).

General external acceptance is positively related to all three factors (shape, external and internal colour) and to their mutual positive correlation.

Sensorial characters have more complicated interrelation. This study suggest that crispiness, toughness and especially sweetness as characters are in positive relation with internal acceptance. At the same time fibrousness and some volatile compounds related to carrot aroma have negative relation with internal acceptance. Precise and clear identification of compounds with negative influence on internal acceptance was hard to be obtaining due many interrelations. Still seems that compound like borneol, γ-terpinene, sabinene and α-pinene have negative influence on overall acceptance.

This study shows that general external acceptance was good in case of long cylindrical roots with typically orange colour which is almost equal for parenchyma and core part.

Considering sensorial acceptance, accessions with sweet taste, good crispiness and toughness were preferred by consumers. On the other side, accessions with strong fibrousness character and considerable amount of borneol and γ-terpinene were not appreciated.

This study included well known commercial varieties, but also some genebank accessions which were evaluated. Through the sensory evaluation process few "unknown" accessions have shown interesting results. Those accessions are HRI 6102 and INH 1 and INH 14. Their potential future usage should be further explored.

4. CONCLUSIONS

Throughout the history crop ancestors and other crop wild relatives have been used for constant crop improvement. Most of the crops modern varieties contain some genes which derived from wild relatives.

Still the natural populations of many species of crop wild relatives are increasingly at risk primarily due to the habitat loss or it degradation. Big climate change has significant impact on species distribution through suitable habitat reduction.

It is important to remember that genetic resources are non-renewable and it is essential to work on their conservation at all levels: species, genepool or ecosystem level.

Apart from collection of new germplasm, and it conservation, it is important to know more about characteristics of accessions which are already present in genebanks and aveliable for different research programs.

Main aim of this study was to characterise and evaluate current carrot collection in European genebanks.

Three main direction of study were:

- Evaluation of available accessions on the basis of root shape as important external characteristic. Usage of divert material for determination of most reliable indexes for root shape definition and indication.
- Usage of available plant material for its qualitative and quantitative evaluation on the basis of main carrot root compounds like carotenes, sugars and nitrates. At the same time study potential genotype x environment interaction and influence of accessions behavior in different growing cycles.
- Sensory evaluation of accessions with interesting previous results obtained during characterisation process.

When root shape was taken into consideration, evaluated accessions have great diversity of shapes from short conical to long cylindrical. That diversity was very useful base for further exploration of known shape indexes in order to find one that in the best manner can explain this carrot root external characteristic. Several indexes were calculated for all accessions and their precision in root shape prediction was tested. Through this study, two indexes performed very well in terms of precision and stability under different environment, and they can be suggested for further usage in carrot root shape studies.

Those indexes are RWVCYLD and RDW3. The first one is actually ratio between estimated root volume (based on root weight) and volume of the cylinder with base and height equal to the root diameter. It actually represents root cylindricality.

The second one is based on the direct relation of root diameter and it weight.

Both indexes were very precise in root shape identification, more precise than wieldy used L/D and C index; low environmentally dependent.

Parallel study of major carrot compounds inside fresh matter and their quantity in different environmental conditions indicated possible genotype x environmental interaction. As a result of that interaction appears great number of accessions with interesting behaviors in different environmental conditions. Particularly interesting, even for further research, were accessions with stable amount of certain compound (carotene, sugar and nitrate) in different growing cycles or accessions with increase in compound quantity under unfavorable environmental conditions. Two maybe most interesting accessions were:

- NGB 2399 with stable carotene quantity in different environmental conditions and more sugars in autumn growing period.
- NGB 13936 with very low amount of nitrates and higher amount of sugars in autumn growing cycle.

Other genotypes that were mentioned in discussion part are also good potential for further research and wider usage.

In this study, sensorial evaluation was used as a tool to get more information about consumer preferences for foods. Actually it was used to evaluate potentials of accessions with interesting results in other aspects (high sugar content, high carotenes content, different root colour) and to try to analyze and understand triggers for consumer's acceptance of one accession and in refusing of another.

For evaluations have been taken into consideration several characteristic which are grouped in two major groups: external and sensorial (tasting).

Finding was that general external acceptance is positively related to all three factors (shape, external and internal colour) and to their mutual positive correlation. According to this study, general external acceptance was good for accessions with long cylindrical roots with typically orange colour which is almost equal for parenchyma and core part.

When sensorial characters are considered, it was obvious that their interrelation is more complicated. Study suggest that crispiness, toughness and especially sweetness as characters have positive influence on internal acceptance. At the same time fibrousness in combination with some volatile compounds, related to carrot aroma, have negative relation with internal acceptance. Precise and clear identification of compounds with negative influence on internal acceptance was difficult. Still looks that compounds like borneol, γ-terpinene, sabinene and α-pinene have negative influence on overall acceptance.

Through the sensory evaluation process some "unknown" accessions have demonstrated interesting results. Those accessions are HRI 6102 and INH 1 and INH 14. Their potential further usage should be explored in the future.

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APPENDIX

Table 5.1. Relative advantages and disadvantages of the various conservation methods

	Method	Advantage	Disadvantage
Ex situ	Seed storage	• Efficient and reproducible. • Feasible for medium- and long-term storage. • Wide diversity of each target taxon conserved. • Easy access for characterization and evaluation. • Easy access for utilization. • Little maintenance once material is conserved.	• Problems storing seeds of 'recalcitrant' species. • Freezes evolutionary development, especially, that related to pest and disease resistance. • Genetic diversity may be lost with each regeneration cycle (but individual cycles can be extended to periods of 20–50 years or more). • Restricted to a single target taxon per accession (no conservation of associated species found in the same location).
	In vitro storage	• Relatively easy long-term conservation for large numbers of 'recalcitrant', sterile or clonal species. • Easy access for evaluation and utilization.	• Risk of somaclonal variation. • Need to develop individual maintenance protocols for most species. • Relatively high-level technology and maintenance costs.
	DNA storage	• Relatively easy, low-cost of conservation.	• Regeneration of entire plants from DNA cannot be envisaged at present. • Problems with subsequent gene isolation in association with phenotypes.
	Pollen storage	• Relatively easy, low-cost of conservation.	• Need to develop individual regeneration protocols to produce haploid plants; further research needed to produce diploid plants. • Only male genetic material conserved.
	Field genebank	• Suitable for storing material of 'recalcitrant' species. • Easy access for characterization and evaluation. • Material can be evaluated while being conserved. • Easy access for utilization.	• Material is susceptible to pests, diseases and vandalism. • Involves large areas of land, but even then genetic diversity is likely to be restricted. • High maintenance cost once material is conserved.
In situ			
	Genetic reserve	• Dynamic conservation in relation to environmental changes, pests and diseases. • Provides easy access for evolutionary and genetic studies.	• Materials not easily available for utilization. • Vulnerable to natural and man-directed disasters, e.g. fire, vandalism, urban development, air pollution, etc.
	On-farm	• Appropriate method for 'recalcitrant' species. • Allows easy conservation of a diverse range of wild relatives. • Possibility of multiple target taxa reserves. • Dynamic conservation in relation to environmental changes, pests and diseases. • Ensures the conservation of traditional landraces of field crops.	• Appropriate management regimes for genetic conservation poorly understood. • Requires high level of active supervision and monitoring. • Limited genetic diversity can be conserved in any one reserve. • Vulnerable to changes in farming practices. • Appropriate management regimes for genetic conservation poorly understood. • Requires the maintenance of traditional farming systems and possibly payment of incentives. • Only limited genetic diversity can be conserved in any one location, so requiring multiple farms and regions for effective conservation. • Depends on the ongoing goodwill of farmers and their descendants.

Table 5.2. Amounts of compounds (ratio) in carrot fresh matter; lsd test for variety, cycle and cycle x variety as factors (2001 summer & 2003 autumn trials)

	total carotenes mg kg^{-1} f.w.	retinol equivalent mg kg-1 f.w.	α/β carotene g g^{-1}	retinol equ/tot carot.	total sugars g kg-1 f.w.	monosacch/ disacch	glucose/f ructose	nitrates mg kg-1 f.w.
Cycle								
2001 summer	188	23,74	0,897	0,124	75,04	0,854	1,112	210
2003 autumn	377	54,18	0,349	0,146	27,26	0,843	1,538	810
significance	**	**	**	ns	**	**	ns	**
lsd (p=0.05)	78	10,25	0,309	0,032	19,00	0,352	0,195	148
Genotype								
Amsterdam	272	36,78	0,738	0,133	45,89	1,185	1,203	547
Aut king	299	40,75	0,753	0,132	48,32	0,792	1,072	334
baz56367	260	35,51	0,921	0,132	54,93	2,103	1,499	375
baz69563	366	49,63	0,837	0,131	44,48	0,368	1,665	609
Bolero	296	39,90	0,690	0,133	110,44	0,745	1,148	324
hri3937	259	36,44	0,568	0,138	63,94	0,955	1,160	519
hri4007	215	30,43	0,627	0,137	61,90	0,436	1,697	528
hri6519	14	2,28	0,000	0,125	46,28	0,798	1,146	939
inh1	205	29,29	0,374	0,144	46,63	0,965	1,319	675
Parmex	422	58,04	0,732	0,134	65,19	0,231	0,973	451
Rubrovitamina	289	37,45	0,962	0,128	52,21	0,616	1,283	307
significance	**	**	*	ns	**	ns	ns	**
lsd (p=0.05)	33	4,37	0,132	0,013	8,70	0,161	0, 089	63
Cycle x Genotype								
2001 summer								
Amsterdam	208	26,38	0,921	0,127	56,67	1,157	0,941	483
Aut king	190	23,85	0,994	0,125	56,87	0,678	0,945	71
baz56367	143	17,39	1,427	0,121	66,75	2,667	1,484	171
baz69563	194	23,42	1,221	0,121	58,26	0,163	1,187	368
Bolero	235	30,41	0,820	0,129	146,65	0,788	0,902	71
hri3937	187	24,79	0,709	0,132	82,02	0,865	0,991	109
hri4007	135	16,95	0,972	0,126	90,65	0,451	0,944	126
hri6519	19	3,19	0,000	0,083	58,40	0,733	1,041	149
inh1	96	13,98	0,335	0,146	71,06	0,822	1,342	544
Parmex	267	32,89	1,101	0,123	84,33	0,277	1,459	199
Rubrovitamina	253	30,59	1,232	0,121	64,51	0,496	1,127	18
2003 autumn								
Amsterdam	400	57,56	0,374	0,144	24,35	1,243	1,726	610
Aut king	462	66,10	0,393	0,143	22,67	1,133	1,453	597
baz56367	377	53,64	0,415	0,142	31,30	0,976	1,527	578
baz69563	539	75,85	0,452	0,141	23,81	0,677	2,382	850
Bolero	416	58,89	0,431	0,142	38,03	0,660	1,639	577
hri3937	403	59,74	0,286	0,148	27,78	1,135	1,499	928
hri4007	296	43,90	0,282	0,148	33,16	0,421	2,451	931
hri6519	8	1,37	0,000	0,167	22,04	0,926	1,355	1729
inh1	313	44,59	0,414	0,142	22,20	1,108	1,295	805
Parmex	576	83,19	0,364	0,144	26,91	0,139	0,000	703
Rubrovitamina	360	51,16	0,424	0,142	27,61	0,857	1,596	595
significance	**	**	*	ns	**	ns	ns	**
lsd (p=0.05)	110	14,49	0,437	0,045	26,87	0,498	0,276	209

Table 5.3.a Amounts of compounds (ratio) in carrot fresh matter; lsd test for variety, cycle and cycle x variety as factors (2002 summer & 2002 autumn trials) I part

	total carotenes mg kg^{-1} f.w.	retinol equivalent mg kg-1 f.w.	α/β carotene g g^{-1}	retinol equ/tot carot.	total sugars g kg-1 f.w.	monosacch/di sacch	glucose/fructose	nitrates mg kg-1 f.w.
Cycle								
2002 summer	212	31,35	0,244	0,127	41,36	0,811	1,016	729
2002 autumn	185	25,03	0,543	0,121	46,31	0,691	1,202	117
significance	**	**	**	ns	**	**	**	**
lsd (p=0.05)	35	4,980	0,039	0,0013	4,641	0,477	0,7433	22
Genotype								
Amsterdam	250	35,02	0,473	0,140	42,70	2,135	1,099	794
Aut king	293	40,77	0,527	0,139	48,74	0,987	1,184	286
baz56341	189	26,41	0,516	0,139	41,77	0,781	1,030	196
baz56355	206	29,72	0,365	0,145	47,14	0,774	1,125	148
hri10168	29	3,71	0,405	0,065	32,29	0,381	0,904	1141
hri10220	0	0,00	0,000	0,000	34,80	0,197	0,605	891
hri10225	0	0,00	0,000	0,000	36,15	0,891	1,064	1420
hri10233	179	26,10	0,386	0,144	47,76	0,955	0,931	410
hri10246	86	12,06	0,283	0,084	41,75	0,482	1,011	351
hri10305	110	16,38	0,356	0,146	35,80	0,972	0,978	278
hri10468	0	0,00	0,000	0,000	41,27	0,698	1,287	333
hri10520	158	22,70	0,420	0,143	46,89	0,492	1,235	231
hri11163	145	21,18	0,340	0,146	44,49	0,435	1,000	310
hri11169	261	36,99	0,481	0,141	49,72	0,700	1,140	191
hri11503	290	42,44	0,325	0,147	51,66	0,437	1,151	341
hri3838	181	25,98	0,409	0,143	46,13	0,835	0,977	144
hri3936	132	18,99	0,392	0,144	40,83	0,498	1,018	420
hri3966	167	23,39	0,549	0,138	42,29	0,769	0,909	370
hri3998	187	25,77	0,564	0,137	52,44	0,734	1,147	386
hri5784	129	18,80	0,330	0,146	50,17	0,675	1,070	250
hri6070	275	39,50	0,395	0,143	37,35	1,929	1,150	376
hri7301	0	0,00	0,000	0,000	32,37	0,511	3,093	380
hri7893	238	34,67	0,371	0,145	43,74	0,423	1,052	357
hri8080	255	37,31	0,366	0,145	42,42	0,829	1,208	608
hri8081	169	24,40	0,373	0,144	45,46	1,012	1,180	472
hri8095	264	38,21	0,461	0,142	48,96	1,564	1,300	650
hri8116	274	38,52	0,533	0,139	45,39	0,782	1,222	480
hri8125	194	27,78	0,394	0,143	42,95	0,825	1,020	423
hri8394	288	41,62	0,436	0,142	40,09	1,144	1,044	348
inh11	0	0,00	0,000	0,000	38,06	0,596	1,002	1042
inh12	300	42,22	0,469	0,140	35,52	0,741	1,062	906
inh13	132	17,92	0,556	0,138	42,20	0,847	1,125	917
inh15	274	38,23	0,522	0,139	41,12	0,762	1,192	466
inh16	251	35,62	0,444	0,142	48,02	0,314	1,060	190
inh18	134	18,84	0,490	0,140	48,51	0,515	1,115	225
inh19	214	29,98	0,476	0,141	49,36	0,287	1,147	76
inh20	278	39,77	0,444	0,142	46,06	0,441	1,082	220
locita1	272	37,32	0,566	0,137	37,86	1,397	1,124	310
locita2	291	40,87	0,491	0,140	43,94	1,266	1,241	505
ngb13936	177	25,29	0,410	0,143	52,66	0,799	1,147	150
ngb13945	179	24,75	0,509	0,140	45,40	0,532	1,105	253
ngb13946	363	51,30	0,465	0,141	48,70	0,493	1,049	220
ngb13949	178	25,40	0,388	0,144	40,59	0,272	1,020	441
ngb13951	273	39,82	0,365	0,145	55,98	0,316	1,193	287
ngb13955	247	35,34	0,498	0,140	47,11	0,789	1,057	355
ngb7748	345	48,35	0,463	0,141	42,46	0,074	0,387	107
Nikki f1	269	37,32	0,580	0,137	46,80	1,230	1,084	358
Parmex	380	53,91	0,477	0,140	38,99	0,379	0,956	291
significance	**	**	**	ns	**	*	*	**
lsd (p=0.05)	15	2,12	0,017	0,001	2,12	0,218	0,340	10

Table 5.3.b Amounts of compounds (ratio) in carrot fresh matter; lsd test for variety, cycle and cycle x variety as factors (2002 summer & 2002 autumn) II part

	total carotenes mg kg^{-1} f.w.	retinol equivalent mg kg-1 f.w.	α/β carotene g g^{-1}	retinol equ/tot carot.	total sugars g kg-1 f.w.	monosacch/di sacch	glucose/fructose	nitrates mg kg-1 f.w.
Cycle x Genotype								
2002 autumn								
Amsterdam	249	36,15	0,352	0,145	42,88	3,613	1,128	1549
Aut king	292	42,80	0,314	0,147	48,15	1,183	1,193	534
baz56341	200	29,10	0,344	0,145	38,13	0,992	0,979	349
baz56355	179	26,49	0,294	0,148	43,10	0,782	1,124	258
hri10168	0	0,00	0,000	0,000	25,14	0,437	0,810	1947
hri10220	0	0,00	0,000	0,000	24,67	0,054	0,000	1558
hri10225	0	0,00	0,000	0,000	36,19	0,879	0,958	1057
hri10233	229	35,02	0,201	0,153	52,26	0,761	0,955	675
hri10246	0	0,00	0,000	0,000	42,41	0,575	1,070	570
hri10305	145	22,27	0,182	0,154	38,06	0,693	0,895	479
hri10468	0	0,00	0,000	0,000	38,46	0,419	1,346	285
hri10520	178	26,89	0,236	0,151	43,51	0,332	1,173	417
hri11163	138	20,99	0,204	0,153	46,04	0,346	1,037	469
hri11169	292	43,33	0,281	0,148	50,22	0,448	1,173	337
hri11503	270	41,04	0,209	0,152	48,52	0,387	1,181	645
hri3838	201	29,95	0,267	0,149	46,81	1,135	0,942	259
hri3936	123	18,80	0,203	0,153	33,66	0,604	1,015	703
hri3966	227	33,08	0,335	0,146	42,53	0,651	0,860	680
hri3998	202	29,27	0,362	0,145	42,32	0,857	1,245	735
hri5784	105	15,79	0,255	0,150	47,69	0,462	1,007	469
hri6070	280	41,32	0,299	0,147	25,43	2,656	1,138	723
hri7301	0	0,00	0,000	0,000	31,21	0,547	0,887	178
hri7893	258	39,28	0,208	0,152	44,36	0,511	0,995	695
hri8080	307	46,71	0,215	0,152	36,10	0,860	1,268	1184
hri8081	158	23,46	0,272	0,149	44,96	1,133	1,152	890
hri8095	360	54,21	0,243	0,150	46,94	1,301	1,230	1254
hri8116	328	48,45	0,294	0,148	41,59	0,845	1,111	911
hri8125	169	25,00	0,296	0,148	37,16	0,421	0,947	750
hri8394	373	55,69	0,264	0,149	34,86	0,693	0,957	660
inh11	0	0,00	0,000	0,000	30,14	0,693	0,944	1867
inh12	307	44,62	0,342	0,145	32,41	0,919	1,025	1774
inh13	88	12,70	0,362	0,145	33,85	0,958	1,030	1734
inh15	315	46,35	0,310	0,147	42,39	1,118	1,239	910
inh16	248	36,89	0,275	0,149	44,58	0,429	0,853	358
inh18	157	22,91	0,326	0,146	46,94	0,508	1,091	385
inh19	176	26,64	0,231	0,151	45,70	0,237	1,281	116
inh20	320	47,60	0,270	0,149	46,28	0,405	1,056	403
locita1	305	42,95	0,451	0,141	33,72	2,211	1,077	592
locita2	306	45,13	0,304	0,147	43,38	1,976	1,076	988
ngb13936	177	26,57	0,252	0,150	56,88	1,108	1,146	274
ngb13945	149	22,17	0,279	0,148	41,86	0,634	1,053	483
ngb13946	378	56,25	0,275	0,149	44,81	0,590	0,955	408
ngb13949	142	20,88	0,311	0,147	38,24	0,322	0,968	845
ngb13951	320	48,30	0,235	0,151	57,88	0,341	1,129	547
ngb13955	341	50,60	0,285	0,148	48,73	0,636	1,006	682
ngb7748	308	44,86	0,342	0,145	46,35	0,051	0,000	182
Nikki f1	322	46,50	0,363	0,144	46,08	0,812	1,159	669
Parmex	487	71,24	0,325	0,146	31,82	0,403	0,923	559

Table 5.3.c Amounts of compounds (ratio) in carrot fresh matter; lsd test for variety, cycle and cycle x variety as factors (2002 summer & 2002 autumn) III part

	total carotenes mg kg^{-1} f.w.	retinol equivalent mg kg-1 f.w.	α/β carotene g g^{-1}	retinol equ/tot carot.	total sugars g kg-1 f.w.	monosacch/di sacch	glucose/fructose	nitrates mg kg-1 f.w.
2002 summer								
Amsterdam	250	33,89	0,595	0,136	42,53	0,657	1,069	39
Aut king	295	38,75	0,741	0,131	49,33	0,792	1,175	39
baz56341	179	23,73	0,687	0,133	45,40	0,569	1,081	43
baz56355	206	29,72	0,365	0,145	47,14	0,774	1,125	148
hri10168	57	7,43	0,809	0,129	39,43	0,325	0,997	336
hri10220	0	0,00	0,000	0,000	44,94	0,340	1,211	224
hri10225	0	0,00	0,000	0,000	36,10	0,903	1,171	1782
hri10233	146	20,16	0,510	0,139	44,75	1,084	0,916	146
hri10246	144	20,10	0,472	0,140	41,09	0,389	0,952	131
hri10305	76	10,49	0,531	0,138	33,55	1,251	1,060	77
hri10468	0	0,00	0,000	0,000	44,08	0,976	1,229	380
hri10520	137	18,51	0,604	0,135	50,26	0,653	1,296	46
hri11163	153	21,37	0,476	0,140	42,94	0,524	0,963	150
hri11169	231	30,65	0,682	0,133	49,21	0,951	1,106	46
hri11503	311	43,84	0,441	0,141	54,80	0,487	1,121	37
hri3838	161	22,01	0,550	0,137	45,45	0,534	1,012	29
hri3936	141	19,18	0,581	0,136	48,00	0,391	1,021	138
hri3966	128	16,93	0,692	0,133	42,05	0,887	0,957	60
hri3998	177	23,43	0,698	0,132	62,55	0,612	1,049	38
hri5784	153	21,81	0,405	0,143	52,66	0,887	1,132	32
hri6070	271	37,67	0,491	0,139	49,28	1,201	1,161	30
hri7301	0	0,00	0,000	0,000	33,52	0,474	5,299	582
hri7893	218	30,05	0,533	0,138	43,11	0,336	1,108	19
hri8080	202	27,92	0,517	0,138	48,74	0,799	1,147	32
hri8081	181	25,33	0,474	0,140	45,96	0,891	1,209	54
hri8095	167	22,21	0,679	0,133	50,98	1,828	1,369	45
hri8116	219	28,60	0,772	0,130	49,18	0,719	1,334	49
hri8125	220	30,56	0,492	0,139	48,74	1,229	1,093	95
hri8394	204	27,54	0,608	0,135	45,33	1,595	1,131	37
inh11	0	0,00	0,000	0,000	45,98	0,499	1,060	218
inh12	294	39,82	0,596	0,136	38,63	0,564	1,098	37
inh13	177	23,13	0,751	0,131	50,56	0,736	1,220	99
inh15	246	32,81	0,663	0,133	39,86	0,406	1,145	23
inh16	254	34,36	0,613	0,135	51,46	0,200	1,267	23
inh18	110	14,77	0,653	0,134	50,09	0,522	1,139	64
inh19	253	33,32	0,721	0,132	53,03	0,338	1,013	36
inh20	237	31,94	0,617	0,135	45,85	0,477	1,109	38
locita1	250	33,56	0,643	0,134	41,99	0,584	1,171	28
locita2	275	36,61	0,679	0,133	44,50	0,557	1,406	22
ngb13936	176	24,02	0,568	0,137	48,45	0,490	1,149	25
ngb13945	208	27,33	0,738	0,131	48,94	0,429	1,156	24
ngb13946	347	46,36	0,656	0,134	52,60	0,395	1,142	32
ngb13949	213	29,93	0,465	0,140	42,93	0,222	1,071	37
ngb13951	225	31,34	0,494	0,139	54,07	0,290	1,258	27
ngb13955	152	20,08	0,710	0,132	45,50	0,942	1,107	28
ngb7748	400	53,58	0,645	0,134	38,58	0,097	0,775	33
Nikki f1	217	28,14	0,797	0,130	47,52	1,649	1,009	48
Parmex	272	36,58	0,629	0,134	46,17	0,356	0,990	23
significance	**	**	**	ns	**	**	**	**
lsd (p=0.05)	49	7,04	0,055	0,002	6,56	0,674	1,051	32

Table 5.4. Amounts of compounds (ratio) in carrot fresh matter; lsd test for variety, cycle and cycle x variety as factors (2002 summer: 2002 autumn & 2003 autumn)

	total carotenes mg kg^{-1} f.w.	retinol equivalent mg kg-1 f.w.	α/β carotene g g^{-1}	retinol equ/tot carot.	total sugars g kg-1 f.w.	monosacch/disacch	glucose/fructose	nitrates mg kg-1 f.w.
Cycle								
2002 autumn	344	50,78	0,272	0,149	44,11	0,846	1,034	489
2002 summer	350	47,50	0,605	0,136	47,26	0,404	1,104	58
2003 autumn	392	56,01	0,379	0,145	25,39	0,623	1,026	546
significance	**	**	**	**	**	*	*	**
lsd (p=0.05)	48	6,89	0,034	0,001	44,54	0,463	0,110	21
Genotype								
Amsterdam	300	42,54	0,440	0,142	36,58	1,838	1,308	733
Aut king	350	49,22	0,483	0,140	40,05	1,036	1,274	390
hri6102	401	54,29	0,605	0,136	38,24	0,071	0,546	268
hri6760	54	8,64	0,166	0,157	40,79	0,188	0,811	285
hri7801	413	57,94	0,439	0,142	43,03	0,629	1,234	237
inh14	683	98,01	0,401	0,143	36,78	0,378	1,230	149
News f1	455	66,52	0,322	0,146	40,41	0,602	1,248	490
ngb2399	139	19,90	0,479	0,141	39,02	0,560	1,171	296
Parmex	445	63,67	0,439	0,142	34,97	0,299	0,637	428
significance	**	**	**	**	**	ns	*	**
lsd (p=0.05)	27	3,98	0,019	0,001	25,71	0,267	0,063	12
Cycle x Genotype								
2002 autumn								
Amsterdam	249	36,15	0,352	0,145	42,88	3,613	1,128	1549
Aut king	292	42,80	0,314	0,147	48,15	1,183	1,193	534
hri6102	342	50,08	0,318	0,147	50,51	0,095	0,717	169
hri6760	103	16,98	0,014	0,166	51,97	0,149	1,253	127
hri7801	284	42,00	0,296	0,148	53,25	0,672	1,020	111
inh14	814	118,70	0,334	0,146	33,32	0,368	0,943	276
News f1	335	49,82	0,273	0,149	44,10	0,553	1,080	761
ngb2399	193	29,22	0,219	0,152	41,02	0,577	1,045	319
Parmex	487	71,24	0,325	0,146	31,82	0,403	0,923	559
2002 summer								
Amsterdam	250	33,89	0,595	0,136	42,53	0,657	1,069	39
Aut king	295	38,75	0,741	0,131	49,33	0,792	1,175	39
hri6102	490	63,04	0,848	0,128	41,14	0,117	0,920	22
hri6760	42	5,87	0,485	0,139	49,40	0,335	1,181	228
hri7801	563	76,18	0,604	0,135	49,43	0,500	1,156	24
inh14	535	74,56	0,489	0,139	46,10	0,292	1,029	64
News f1	521	74,80	0,383	0,144	51,75	0,165	1,236	31
ngb2399	131	17,37	0,691	0,133	48,28	0,367	1,085	35
Parmex	272	36,58	0,629	0,134	46,17	0,356	0,990	23
2003 autumn								
Amsterdam	400	57,56	0,374	0,144	24,35	1,243	1,726	610
Aut king	462	66,10	0,393	0,143	22,67	1,133	1,453	597
hri6102	372	49,75	0,648	0,134	23,06	0,000	0,000	614
hri6760	19	3,09	0,000	0,167	21,01	0,080	0,000	499
hri7801	391	55,64	0,416	0,142	26,41	0,714	1,526	576
inh14	701	100,76	0,379	0,144	30,93	0,475	1,719	107
News f1	510	74,94	0,311	0,147	25,40	1,087	1,429	678
ngb2399	95	13,09	0,527	0,138	27,78	0,737	1,382	533
Parmex	576	83,19	0,364	0,144	26,91	0,139	0,000	703
significance	**	**	**	**	**	*	**	**
lsd (p=0.05)	82	11,94	0,058	0,002	77,14	0,801	0,190	36