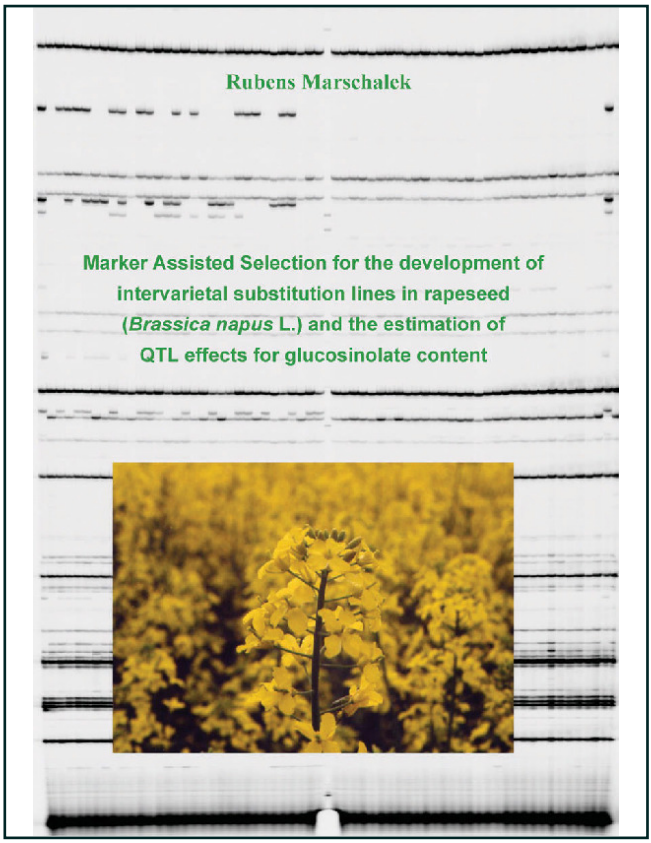
Rubens Marschalek (Autor)

Marker Assisted Selection for the development of intervarietal substitution lines in rapeseed (Brassica napus L.) and the estimation of QTL effects for the glucosinolate content



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In the beginning God created
the heavens and the earth.
Genesis 1:1 (Bible)

1. INTRODUCTION

Our earth is about 4,600 million years old and about 3,500-4,000 million years ago the first organic molecules have got the ability to reproduce and so the first unicellular organisms have arisen (Welter-Schultes & Krätzner, 1999). Nevertheless, flowering plants only arose later, about 80 or 90 million years ago (Goth, 2002), during the cretaceous period. Looking through this period it is also possible to find the first fossils of many insect groups, modern mammal and bird groups. The Cretaceous was thus the time in which life, as it now exists on Earth, came together (Welter-Schultes & Krätzner, 1999; Museum of Paleontology – Uni. of California, 2003)

According to Allard (1999) there is an agreement that humanlike creatures had evolved in Africa by about 3 to 4 million years before the present (b.p.), although new reports (Ziegler, 2002; Gibbons, 2002) show that it may have happened earlier (6 to 7 million years b.p.). The genus *Homo* seems to be appeared around 2 million years ago (Leakey and Walker, 1997) being the ancestor of all modern people probably an early *Homo erectus* in Africa who lived at least 1.8 million years ago (O'Neil, 2003). Nearly all specialists on human origin agree that “anatomically” modern humans originated relatively recently, perhaps about 200,000 – 270,000 years b.p. (Allard, 1999; O'Neil, 2003; Bräuer, 2003), and that the modern human traits are not older as 100.000 years (Bräuer, 2003). Concerning the activities of humans however, the agricultural economies developed only about 15,000 to 10,000 years ago (Allard, 1999).

Humans started with quite simple agricultural activities, and at the same time they started to interfere definitively in the way some species evolved. That means, humans activities started to change dramatically the fate of other species, or even changed the evolution of such species. Plants were affected directly by humans since that their necessities imply in the search for some traits in the plants. In this way humans have, for a long time maybe unconsciously or empirically, used selection to obtain from the nature what they were looking for. History proves that they have succeeded and nowadays we are using different and quite extraordinary methods to still following persistently our aims.

Some of the important plant traits show a clear discrete variation. Such traits are called qualitative traits since different classes can be easily distinguished. Other characters show a continuous variation and are called quantitative traits.

It remained to Johannsen, Nilsson-Ehle, and East to provide, early in the twentieth century, convincing evidence that alleles of Mendel's particulate "factors" or "elements", now called genes, were responsible not only for discretely inherited characters but also for continuously varying characters. It is important to emphasize that both, continuous and sharply discontinuous variation are observed in many characters and this establishes that the distinction between qualitative and quantitative characters is not clear-cut (Allard, 1999). Generally it could be said that qualitative traits are controlled by one or few genes, while quantitative characters are typically controlled by many genes, usually termed quantitative trait loci (QTL), and they are usually much more affected by the environment than the qualitative ones.

To breed new and better plant genotypes we should improve our knowledge about the inheritance of desired traits. Regarding this aspect two points are interesting to consider nowadays: identification and localization of genes responsible for the characters and estimation of the genotypic effects of the alleles found at these loci. These are questions to be solved in all important agricultural species.

Rapeseed is one of the more important oil crops of the world and today, after a decline of over 2 million ha in the last two years, world rapeseed area has regained 1.3 million ha in 2002/03 (Fapri, 2002), giving a total of about 26 million ha, which is representing in 2002 10.1% of the harvested area from oilseed crops (FAO, 2003). It is estimated that the grown area will increase annually by about 0.3% (Fapri, 2002). The world production of rapeseed in 2002/03 is estimated to be around 31,604,000 t (Oil World, 2003). The most important oil seed crop nowadays is soybean but in the temperate zones rapeseed is more important. A better understanding of the genetics and inheritance of characters in rapeseed is therefore an important and desired aim of plant breeding science.

After extraction of the oil, which is the most valuable seed component, the resulting meal is an important protein source for animal feed. Since some components present in the meal are detrimental to animal nutrition, like glucosinolates, it still remains a breeding aim to develop varieties with lower glucosinolate content. Glucosinolates are nitrogen- and sulphur-containing natural plant products which have different biological effects, ranging from antimicrobial and cancer preventing function to inflammatory and goitrogenic activities or antithyroid activity (Wittstock and Halkier, 2002). The goitrogenic activities appear since the glucosinolates of rapeseed meal increase iodine requirement in animals (Schöne, 1999).

Regarding to the plant itself, the benefits of glucosinolates in the defences against insects and pathogens should not be neglected (Wittstock and Halkier, 2002).

The negative effects of the antinutrients in rapeseed meal can be reduced or eliminated by plant breeding, proper processing or a combination of breeding and processing (Jensen, 1999). With respect to glucosinolates, the wide spread growing of double low rapeseed (<25 µmol glucosinolates/g seed) has greatly reduced the negative effect of glucosinolates on animal performance and health. However, even the double low rapeseed varieties are only used in restricted amounts to monogastric animals (Sørensen, 1988, cited after Jensen, 1999). Therefore, in spite of having been a trait submitted to breeding efforts since long time, it is still a breeding aim to have varieties with low glucosinolate content. Older forms of rapeseed have a glucosinolate content above 80 µmol/g in the seed. Presently cultivated low-glucosinolate forms of oilseed rape have less that 25 µmol/g of seed. The first low-glucosinolate cultivar was the Canadian spring cultivar Tower released in 1974, which contained alleles for low glucosinolate content derived from the Polish fodder rape cultivar Bronowski (Campos de Quiroz and Mithen, 1996).

Six QTL for glucosinolate content have been identified until now in rapeseed, three of them have been reported to have an important effect while the other 3 showed only smaller effects (Uzunova *et al.*, 1995; Weißleder, 1996; Fischer and Ecke, 1997; Gül, 2002). Despite of the use of low glucosinolate genotypes in modern breeding programmes it seems to be common that genotypes appear, through segregation, which carry higher levels of glucosinolates than each of the parents (pers. com. H.C.Becker). Also Rücker and Röbbelen (1994) reported that even in crosses between genotypes with less than 20 µmol/g seed, sufficient genetic variation is present enabling selection. All this indicates that a more detailed study about the inheritance of glucosinolates in rapeseed would be very useful to better understand and control this character. In this way, the glucosinolate inheritance could be used as a model for better understanding of other traits.

To reach a better level of knowledge on the rapeseed genetics and more specifically about the inheritance and effects of the glucosinolate genes, as a model for other traits, some aspects should be considered, i.e. identification and localization of genes and estimation of the phenotypic effects of such genes using intervarietal substitution lines. An intervarietal substitution line carries a single segment of a donor genotype, in an otherwise genetic background of one recurrent genotype. A complementary set of intervarietal substitution lines represents the whole donor genome divided into a limited number of distinct segments, each carried by a different intervarietal substitution line in a common genetic background. Trying to improve information about the localization and effects of genes related

to the glucosinolates in rapeseed the specific aims of the present work consists respectively of two points:

1) **The development of substitution lines as a tool to study with more detail the QTL:** using the backcross method and molecular markers to develop a complementary set of intervarietal substitution lines. These lines will be used in mapping and studying different traits.

2) **Investigate glucosinolate inheritance:** estimation of the effect of the alleles of 6 QTL (according to the literature, responsible for the seed glucosinolate content in *Brassica napus* L.) based on field data.

1.1 The subject species: rapeseed (*Brassica napus* L.)

Rapeseed is classified as:
Order: Capparales
Family: Brassicaceae – Mustard family
Genus: Brassica L. – mustard
Species: *Brassica napus* L. – rapeseed
(USDA-Natural Resources Conservation Service, 2002)

Brassica napus L. is an allopolyploid with 19 pairs of chromosomes (n=19), derived from the A and C genomes of *B. rapa* and *B. oleracea*, respectively. It is an allopolyploid, which means an organism originated from a combination of two or more sets of chromosomes derived from different parental species. Brassica crops consists of six species, of which *B. nigra* (n=8; B genome), *B. oleraceae* (n=9; C genome) and *B. rapa* (n=10; A genome) are diploid monogenomic species. The other three, *B. carinata* (n=17), *B. juncea* (n=18) and *B. napus* (n=19; AACC genome) are species which evolved in nature through hybridization between any two of the diploid species. All three genomes are partially homologous; the genetic information in all three genomes is similar, only its organization and distribution on the chromosomes is different (Prakash, *et al.*, 1999 in Gómez-Campo, 1999).

Brassica napus has a genome with approximately 1,129 to 1,235 Mbp. (Prakash, *et al.*, 1999 in Gómez-Campo, 1999). More recent studies show *Brassica napus* genome having 1,127 Mbp (1C), corresponding to 1.15 pg (1C) (Bennett and Leitch, 1995; Bennett and Leitch, 2001). C-value measures the amount of DNA in the haploid genome of an organism (MayHospi.com, 2000).

Brassica napus is not known to occur truly wild in nature though it often occurs as an escape. The first reference to rapeseed (*B. napus*) was by Dodoens (1578, cited after Gómez-Campo 1999). As a crop it appeared around the year 1600. Cultivation of rapeseed